BACTERIOLOGY

Influence of Cobalt and Zinc Ions on the Growth and Porphyrin Production of *Mycobacterium tuberculosis avium*

It was shown by Baisden[1] that the addition of traces of bacillary ash to a synthetic medium prepared from chemically pure constituents was a very effective stimulant for the growth of *Mycobacteria*, and similar results could be obtained with a mixture of fourteen pure inorganic salts. Various other workers have demonstrated the growth-promoting effect of metal ions, especially zinc[2–4] and manganese[5]. A simpler mixture of pure inorganic salts than that used by Baisden has been described recently by Paterson *et al.*[6]. This is as effective an addition to Dorset's[7] medium for growing large quantities of human type bacilli for the production of tuberculin as is Baisden's.

A substantial stimulation of the growth of *Myco. tuberculosis avium* (strain D_4R) has now been observed, using this trace-element supplement of calcium, cobalt, copper and zinc ions. At the same time, organisms grown on the supplemented medium were usually found to be reddish-brown in colour, and a red pigment which fluoresced strongly in ultra-violet light was readily extracted into cold acetone. Following the procedure used by Todd[8] for the extraction of coproporphyrin III from *Myco. karlinski*, hydrochloric acid solutions of the pigment were obtained having very similar light-absorption characteristics in the range 380–630 mμ to those of coproporphyrin. The porphyrin content of a given mass of organisms was assessed by measuring the optical density at 402·5 mμ of the pigment extracted finally into 10 ml. of 20 per cent hydrochloric acid (v/v concentrated acid). The absorption maximum of the Soret band shifted from 402·5 mμ to 401 mμ on dilution of the extract to 0·15 *N* in hydrochloric acid. This is in agreement with the absorption data of Todd[8] and Jope and O'Brien[9].

In these experiments the organisms were grown on the basic Dorset's medium and on the same medium containing the complete trace element supplement of Paterson *et al.*[6]. Three additional groups of flasks were also prepared in which the supplements simply consisted of either 0·27 p.p.m. cobalt or 1·07 p.p.m. copper or 6·04 p.p.m. zinc. After periods of 33–53 days after inoculation, the organisms were harvested, the yields were determined (expressed as bacillary dry weight) and the porphyrin contents of the bacilli estimated (Table 1).

The results of the experiments show that after 45 days incubation a supplement of calcium, copper, cobalt and zinc stimulated the growth of *Myco. tuberculosis avium* three-fold and at the same time the production of porphyrin (which was maximal after 45 days) was increased about 62 times. The effect of adding the same amount of zinc or cobalt to the medium separately was also to stimulate growth and porphyrin production, but in either case the effect was only about half as great as that induced by the full supplement. Copper alone had no effect either on the yield of organisms obtained or on the amount of porphyrin produced. Thus, the stimulating effect of the trace element supplement of Paterson *et al.*[6] on the growth and free porphyrin production of *Mycobacteria* is largely if not solely due to zinc and cobalt.

Table 1. Effect of Cobalt, Copper and Zinc Ions on Growth and Porphyrin Production of *Myco. tuberculosis avium* (Strain D_4R)

Incubation period. (Days at 37° C.)	Additions* to medium	Yield of organisms (gm.)	Amount of porphyrin extracted. Optical density of 10 ml. hydrogen chloride solution at 402·5 mμ
33	Basic medium	10·0	1·100
38		9·3†	0·915
45		7·9†	0·609
48		8·2†	0·273
33	Full supplement of calcium, cobalt, copper and zinc (ref. 6)	16·0	8·70
41		22·3	11·9
45		23·7	38·2
53		19·6	13·8
42	6·04 p.p.m. zinc	17·1	27·6
47		13·7	21·8
49		13·6	23·3
38	0·27 p.p.m. cobalt	18·0	17·1
42		17·9	16·5
47		17·1	19·9
40	1·07 p.p.m. copper	6·6†	0·441
46		8·6†	0·790
52		7·9†	0·534

* Each flask contained 1 litre of a modified Dorset's synthetic medium with or without trace element additives.
† Pellicle had sunk. All others remained floating during the experiment.

The isolation of the porphyrin pigment and its identification as coproporphyrin III will be described elsewhere.

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Antigens of Spheroplast Membrane Preparations from *Escherichia coli* B

Vennes and Gerhardt[1] demonstrated quite conclusively by serological means that no trace of cell-wall antigens remained attached to the protoplast membranes prepared by lysozyme treatment of *Bacillus megaterium*. Because of the differences in the chemical composition of walls of Gram-positive and Gram-negative bacteria it has been suggested that the osmotically fragile, spherical bodies produced from Gram-negative bacteria by lysozyme treatment or induced by penicillin action have only partially lost their cell-wall material[2]. Salton and Shafa[3] have shown that some cell-wall components remain in spheroplasts prepared by the penicillin method from two Gram-negative species. Their results indicated that the lipo-protein components remained attached to the spheroplasts. The wall preparations from the spheroplasts were also agglutinated by a cell-wall antiserum.

In the present investigation spheroplasts of *Escherichia coli* B were produced by the lysozyme–