An Absolute Requirement for 'Iron Transport Factors' by *Microbacterium lacticum* 8181

We recently published a report on the growth stimulation of *Microbacterium* sp. by 'iron transport factors' such as ferrichrome, coprogen and the terregens factor[1]. These factors acted by decreasing the duration of the lag-phase of growth and were by no means essential. The culture resembled *M. lacticum* more closely than *M. flavum*, but possessed characteristics of both species. We felt it of interest to determine whether authentic species of *Microbacterium* were also stimulated by such factors.

M. lacticum 8180, *M. lacticum* 8181 and *M. flavum* 10340 were obtained from the American Type Culture Collection and grown on slants containing Difco nutrient agar plus 1 per cent Difco yeast extract. After 48-hr. incubation at 37°C., the cells were harvested and washed twice with 0·02 *M* dipotassium hydrogen phosphate. The remainder of the procedure and the composition of the basal medium were as previously described[1]. The 'iron transport factors', ferrichrome[2] and coprogen[3], were used 100 (mμgm./ml.

Of greatest interest was the result observed with *M. lacticum* 8181. As shown in Fig. 1, this strain has an absolute requirement for ferrichrome or coprogen. *M. lacticum* 8180 resembled our isolate[1] in that 'iron transport compounds' merely stimulated lag-phase growth. The growth of the *M. flavum* culture was not influenced by these compounds.

M. lacticum 8181 would appear to be a better organism for assay of 'iron transport factors' than those previously described. Its use is much less laborious and time-consuming than that of *Pilobolus kleinii*[4]. Also, *M. lacticum* requires only half of the incubation time and gives higher optical density values than the recommended *Arthrobacter* cultures[5].

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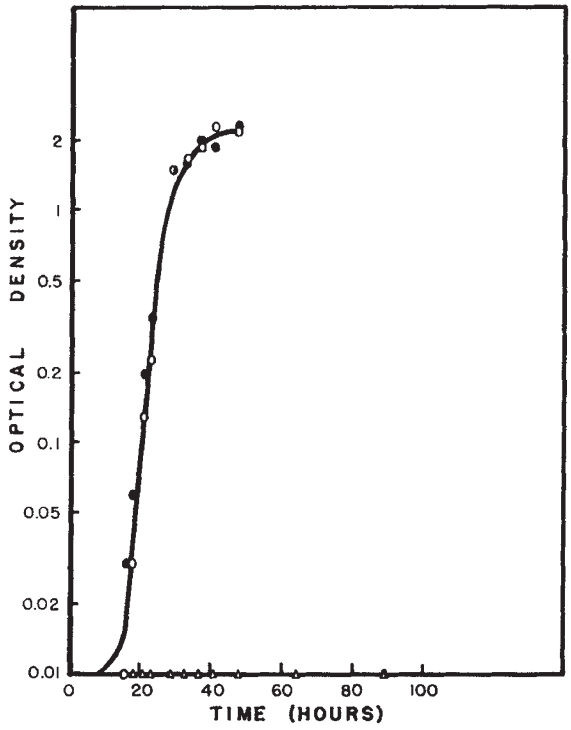

Fig. 1. Growth of *Microbacterium lacticum* 8181 in basal medium (△), in basal plus 100 mμgm. ferrichrome per ml. (●) and in basal plus 100 mμgm. coprogen per ml. (○).

Selective Inhibition of Ribonucleic Acid Synthesis in *Staphylococcus aureus* by Vancomycin

Vancomycin, an antibiotic obtained from *Streptomyces orientalis*, is effective against a number of growing bacteria, particularly those which are Gram-positive. Mutation to resistance towards this compound is of a very low order, and the antibiotic has been shown to be clinically effective against strains of *Staphylococcus aureus* which are resistant to the more common antibiotics.

During investigations on the mode of action of vancomycin (free base) on *S. aureus* it was found that after its addition to a logarithmic-phase culture both nephelos and optical density readings continued to increase for a period of about 60 min., although at a reduced rate. During this same time-interval total cell counts remained constant and plate counts rapidly decreased. Therefore, during the initial stages of its activity, vancomycin inhibits cellular division and yet allows protoplasmic synthesis to continue. No reversal of the action of the antibiotic was obtained by washing vancomycin-treated cells in water or various buffers and no ameliorative effect was found when various concentrations of certain purines, pyrimidines, peptides and metallic ions were added to the growth medium prior to the addition of the vancomycin.

Cells grown in brain heart infusion (Difco) for 21 hr. at 37° C. were harvested by centrifugation. These cells were resuspended in a smaller quantity of fresh medium and incubated until nephelos readings showed a constant increase. The culture was then divided into two parts, to one of which was added vancomycin to a final concentration of 100 μgm./ml. (about 37 μgm./mgm. cell dry weight). The other portion served as a control. Five ml. amounts of culture were removed at various time intervals for analysis. Nephelos readings were taken with a photonephelometer. Protein was determined by the Folin–Ciocalteu method, deoxyribonucleic acid by the diphenylamine reaction after hot perchloric acid extraction, and total nucleic acid by spectrophotometry at 260 mμ and the use of an

Table 1. Effect of Vancomycin on Growth and the Synthesis of Protein, Deoxyribonucleic Acid and Ribonucleic Acid in *Staphylococcus Aureus*

Final concentration of vancomycin (μgm./ml.)	Time after addition of vancomycin (min.)	Protoplasmic growth (nephelos units)	Protein (mgm./5 ml. culture)	Deoxyribonucleic acid (mgm./5 ml. culture)	Ribonucleic acid (mgm./5 ml. culture)
	0	70·5	8·40	0·10	0·93
0	30	76·5	9·20	0·12	1·03
	60	85·5	10·16	0·17	1·13
120	120	87·0	10·56	0·17	1·19
	0	70·5	8·40	0·11	0·93
100	30	76·5	9·20	0·12	0·95
	60	80·0	10·16	0·16	0·95
	120	78·5	9·76	0·16	0·88