metabolism. Because of the ease with which specimens can be collected, stored and transported nail analyses may prove to be a valuable tool in large surveys, particularly in areas remote from laboratory facilities.

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Some Factors Influencing Mycobactin and Salicylic Acid Production in *Mycobacterium smegmatis*

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In response to an environment deficient in iron, *Mycobacterium smegmatis*, in common with other mycobacteria, produces salicylic acid extracellularly and a mycobactin intracellularly (Snow, 1965; Ratledge & Winder, 1966; White & Snow, 1968; Antonie & Morrison, 1968). Both compounds are iron-chelators and their productions are possibly related, as salicylic acid forms part of the larger mycobactin molecule (Snow, 1965). Before beginning a detailed examination of their biosyntheses, it seemed prudent to examine the effects of various factors on the production of these two compounds so that optimum conditions for their accumulation could be determined, and also to see if both have similar patterns of control.

M. smegmatis was grown for 6 days on glycerol (20 ml.), L-asparagine (5 g.), KH_2PO_4 (5 g.) and

MgSO_4 (0.4 g.), with NaOH added to pH 6.8. Precautions were taken to exclude trace elements from the glassware and water. Mycobactin was extracted and assayed by the methods of White & Snow (1968). Without supplementation the basal medium yielded 1.04–1.77 g. dry wt. of cells/l. with different batches of medium on separate occasions.

The effects of varying the concentrations of Fe^{2+} , Zn^{2+} , EDTA, citrate and salicylate on concentrations of mycobactin and salicylic acid have so far been examined. Preliminary results show that addition of Zn^{2+} (0.46 $\mu\text{g./ml.}$) stimulates mycobactin production from about 2 $\mu\text{g./mg.}$ dry cells to 20–30 $\mu\text{g./mg.}$ but has little effect on salicylic acid. This is an unexpected finding in contrast with *Mycobacterium phlei* (Antonie & Morrison, 1968). The effect produced by Zn^{2+} was not altered by the addition of EDTA (10 $\mu\text{g./ml.}$) or citrate (up to 2 mg./ml.).

Addition of Fe^{2+} (up to 6.25 $\mu\text{g./ml.}$) together with Zn^{2+} (0.46 $\mu\text{g./ml.}$) to the basal medium suppressed only salicylic acid production. However, if citrate (1 mg./ml.) or salicylate (20 $\mu\text{g./ml.}$) was also added then mycobactin production was simultaneously suppressed. This effect was more pronounced with citrate than with salicylate, though neither compound when added alone was an effective inhibitor of mycobactin synthesis. The formation of mycobactin could be entirely eliminated by the dual addition of citrate and Fe^{2+} at 1 mg./ml. and 2.5 $\mu\text{g./ml.}$ respectively.

The results so far suggest that an extracellular iron-chelator is needed before adequate transport of iron to the cell interior can occur. The cell presumably attempts to meet this need by forming and excreting salicylic acid.

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