

A Multifactorial Culture Medium with Growth Factors from Leprosy-derived Mycobacteria Proposed in Cultivation Trials for *Mycobacterium leprae*

TO THE EDITOR:

A concept was recently advanced that *Mycobacterium leprae* might be a microbe-dependent microorganism^(6, 7, 8, 9, 10). With ever-increasing knowledge of growth requirements for *M. leprae*, it is acceptable that "*M. leprae* to grow is probably a multifactorial problem" as expressed by Hall, Wheeler and Ratledge⁽³⁾. To fulfill such requirements, I propose the following multifactorial medium in cultivation trials for *M. leprae*.

Prepare iron-free Sauton medium containing in 1 liter of distilled water, asparagine 4 g, citric acid 2 g, K₂HPO₄ 0.5 g, ZnSO₄ 0.04 g, MgSO₄ 0.4 g, Tween 80 10 ml and glycerol 40 ml. Adjust to pH 7.0 with NH₄OH, distribute 200 ml/flask, and autoclave for 30 min. Inoculate with a leprosy-derived strain of *M. phlei*, and incubate for 10 days at 34°C. Autoclave the cultures for 30 min and filter on filter paper while hot.

Dissolve in the filtrate Na thioglycolate 1 g, (NH₄)₂SO₄ 2 g, thioctic acid 0.1 g, ferric ammonium citrate 0.05 g and MgSO₄ 0.1 g. Adjust to pH 6.0 with KH₂PO₄, and complete to 1 liter with added distilled water.

Distribute 12 ml aliquots to each of 25 ml screw-cap tubes and sterilize for 25 min in autoclave. Inoculate with host-grown (armadillo or human) *M. leprae* cells partially purified and treated with 2% NaOH for exactly 25 min. Incubate at 34°C.

This multifactorial medium is based on the following data: Optimal endogenous respiration of *M. leprae* was observed at 34°C and pH 5.8. Respiration was stimulated by SH compounds and yeast extract⁽⁵⁾. Thioctic acid is a potent growth factor in yeast extract^(1, 12). Mycobactin is absent in *M. leprae*⁽⁷⁾. Iron uptake by *M. leprae* is mediated by exochelins⁽³⁾. Two distinct iron transport compounds, mycobactin and exochelin, are necessary to mycobacteria⁽¹¹⁾; both compounds are present in mycobacterium spent Tween 80 culture media filtrates⁽¹¹⁾. Cytochromes in *M. leprae* are

present in a reduced state⁽⁴⁾, suggesting optimal reduced O₂ tension for electron transport. Low O₂ concentration in the host tissues⁽²⁾ suggests that microaerophilic conditions are required to grow *M. leprae*.

Several strains of mycobacteria indistinguishable from *M. leprae* were grown in the above proposed media. Further experiments are necessary before claiming the successful cultivation of *M. leprae*.

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Acknowledgment. These investigations were generously supported by the German Leprosy Relief Association, the Institut Fame Pereo, and Secours aux Lépreux, Canada.

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