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, K_2HPO_4 0.5 g, $ZnSO_4$ 0.04 g, $MgSO_4$ 0.4 g, Tween 80 10 ml and glycerol 40 ml. Adjust to pH 7.0 with NH_4OH, 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, $(NH_4)_2SO_4$ 2 g, thioctic acid 0.1 g, ferric ammonium citrate 0.05 g and $MgSO_4$ 0.1 g. Adjust to pH 6.0 with KH_2PO_4, 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 O_2 tension for electron transport. Low O_2 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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