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 (7). 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$_2$HPO$_4$ 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$_4$OH, 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$_4$)$_2$SO$_4$ 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$_2$PO$_4$, 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. Autoclave the cultures for 30 min and filter on filter paper while hot.

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 (7). Thioctic acid is a potent growth factor in yeast extract (7,15). Mycobactin is absent in M. leprae (7). Iron uptake by M. leprae is mediated by exochelins (7). Two distinct iron transport compounds, mycobactin and exochelin, are necessary to mycobacteria (11); both compounds are present in mycobacteria spent Tween 80 culture media filtrates (11). Cytochromes in M. leprae are present in a reduced state (7), suggesting optimal reduced O$_2$ tension for electron transport. Low O$_2$ concentration in the host tissues (7) 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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REFERENCES


