Mycobacterium X, from Lepromata Cultivated in Tetradecane-DMSO Medium

To the Editor:

It has been known for nearly a century that mycobacteria have a special appetite for petroleum hydrocarbons ([2]). Kallio ([1]) was the first to draw attention to the possibility of using paraffin hydrocarbons in culture media to grow *M. leprae*. The pioneering efforts of Pares ([3]) to cultivate *M. leprae* in media supplemented with "paraffin oil" remained unsuccessful. Meanwhile, it became evident that the medium-length straight-chain hydrocarbon, tetradecane, was the most promising fraction as a source of energy and carbon, even for mycobacteria which had not previously been exposed to this molecule. Such was the background which led to the formulation of a tetradecane-DMSO culture medium, enriched with critically minimal amounts of sheep serum and yeast extract. An unusual strain of mycobacterium was cultivable in this medium from five out of 13 human and armadillo lepromata.

Dimethylsulphoxide (DMSO) 50 ml, KH_2PO_4 8.2 g, Na_2 HPO_4 0.5 g, $(NH_4)_2SO_4$ 2 g, $MgSO_4$ 0.1 g, and yeast extract (Difco) 1.0 g were dissolved in one liter of distilled H_2O. The solution was sterilized in an autoclave for 40 min. Ten ml of this basal solution was distributed aseptically into each 30 ml sterile Corning polystyrene tissue culture flask. Then 0.05 ml tetradecane (USP), 0.05 ml sheep serum, and a 1 cm magnetic bar were added to each flask. Sheep serum was filter sterilized; tetradecane and the magnetic bar were autoclaved.

Flasks were inoculated with approximately 4×10^5 cells of *M. leprae* isolated by centrifugation and a single washing in the above basal solution. The cultures were incubated horizontally at 34°C. The cultures were stirred with a magnetic stirrer for 1 min twice weekly.

Growth was estimated by periodical counting in Ziehl-Neelsen stained preparations. Owing to the two-phase system of the medium, cells were easily washed off the glass slides during the staining procedure. Therefore, glass slides had to be previously coated by dipping in 0.1% solution of neoprene (polychloroprene) in toluol ([4]). The coated slides were dried at 100°C in an oven overnight. Without this procedure practically no cells could be retained for counting on the glass slides.

After a 2-week latency period, the number of acid-fast rods increased logarithmically during a 2 to 4 month incubation period. Bacilli were strongly acid-fast and grouped in small or large clumps. No growth occurred in the heat-killed controls. Löwenstein and Dubos media did not support growth of the inoculated bacilli. Slow-growing subcultures were obtained when cultures were transferred into the homologous tetradecane-DMSO-sheep serum media but not later than from 3 month-old cultures.

The cultures, tentatively designated as *Mycobacterium X*, have the following characteristics. Successful cultivation required a special medium and particular physical conditions. Primary cultures and subcultures were obtained only after heavy inoculum. Five identical cultures were obtained from 13 sources of lepromata from five different geographical locations. All cultures were growing on the surface of a two phase system in maximal contact with atmospheric oxygen. All cultures had identical growth characteristics: slow growth without pigment formation and none of the cultures grew on Löwenstein or in Dubos media. In relatively low concentrations human or horse serum, agar, hyaluronic acid, or gelatin inhibited growth of *Mycobacterium X*. When injected into the foot pads of mice, four tested cultures multiplied in a pattern like that obtained following injection with *M. leprae* from human lepromata.

Further investigations are in progress in order to verify the relationship of *Mycobacterium X* to the pathology of leprosy.

—Laszlo Kato, M.D.

Director of Research
The Salvation Army
Catherine Booth Hospital Centre
4375 Montclair Avenue
Montreal, Canada H4B 2J5

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Respiratory Reflexes (Breath Holding Time) in Leprosy

TO THE EDITOR:

Functional impairment of circulatory reflexes and decreased response of the cough receptors to irritant aerosols in patients with leprosy have been well documented ([2, 4]). In this study we measured breath holding time (BHT) in 35 patients with leprosy and 20 healthy controls. BHT was measured on a low resistance spirometer at end-expiratory level (near functional residual capacity, FRC) and also at the maximal lung volume, i.e., near total lung capacity (TLC). A minimum of three readings was taken at each lung volume. Factors likely to affect the BHT such as motivation, endurance, and training were kept under control in so far as possible.

The patients and control subjects were matched equally as to socio-economic status, age, height, and weight; vital capacity was slightly higher in the control group. The mean BHT in the leprosy patients was 24.0 ± 6.8 seconds at end-expiratory level and 43.8 ± 9.4 seconds near TLC; corresponding values in the control subjects were 18.5 ± 6.1 seconds and 32.3 ± 10.0 seconds, respectively. The difference between the two groups was statistically significant ($p < 0.01$).

Breath holding and the final "break point" represent an interaction of a number of variables such as lung volume, alteration in alveolar-arterial gas tension and pH ([5]). By and large, a prolongation of the BHT indicates impairment of the pulmonary chemosensitivity function. The afferent impulses during a breath hold travel via the pulmonary vagal nerve fibers. In human experiments, blockage of the vagus nerves is followed by prolongation of the BHT ([1]). Histological studies have already shown involvement of the sympathetic and vagus nerves in leprosy ([3]). Therefore, our previous study and the present one are in agreement. We conclude that respiratory reflexes modulated by the vagal nerves are affected in patients suffering from leprosy.

—Suresh K. Malik, M.D., FCCP
—Surrinder K. Jundal, M.D.
—Bhushan Kumar, M.D.
—Surrinder Kaur, M.D.

Department of Chest Diseases and Dermatology
Postgraduate Institue of Medical Education and Research
Chandigarh-160 012, India

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