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Chemical Analyses of the Cell Wall of the Murine Leprosy Bacillus

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The taxonomy of murine leprosy bacillus, as a species of genus *Mycobacterium*, is not yet established. Based on morphological characteristics during limited growth in vitro, Uyeda (4) argued that this microorganism should not be classified as a mycobacterium, but rather should be in a new genus related to *Nocardia*. However, the chemical composition of the cell wall (arabinose, galactose, and mucopeptide) is qualitatively similar to that of other mycobacteria (1). Since a mycolic acid-arabinogalactan-mucopeptide complex was found to be a common macromolecule in cell walls of mycobacteria (2), this investigation was undertaken to determine whether this characteristic macromolecule is contained in the murine leprosy bacillus.

Murine leprosy bacilli (Hawaian strain) were isolated from mesenteric lepromas of 300 infected mice. Formaldehyde (0.5%)-killed bacilli, free from tissue debris after pancreatin digestion, were disrupted with a Branson sonic oscillator in an ice bath. Cell wall fractions were sedimented by centrifugation at 10,000 × *g* for 30 min after having discarded unbroken cells at 3,000 × *g* for 20 min. To eliminate cytoplasmic fractions, cell walls were digested with pancreatin. Free lipids in cell walls were extracted with acetone, ether, and chloroform successively, by use of reflux. Resultant cell walls were repeatedly treated with 45% aqueous phenol at 65 to 70 C for 30 min each time. The insoluble fraction, sedimented at 30,000 × *g* for 30 min, was washed with acetone and ether, followed by trypsin treatment. The phenol-treated cell wall fraction (50 mg) thus obtained was chemically analysed as described previously (2).

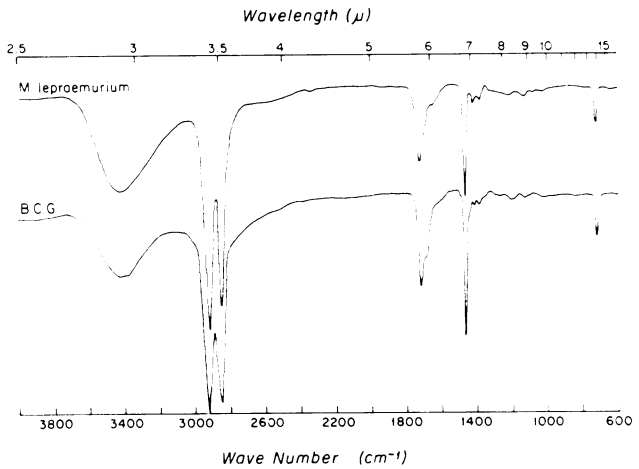

FIG. 1. *Infrared absorption spectrum of mycolic acid fraction obtained from the phenol-treated cell wall of the murine leprosy bacillus. The mycolic acid of BCG was prepared from wax D fraction according to the method of Stodola et al. (5).*

Chemical composition of the phenol-treated cell wall was as follows: total phosphorus, 0.11%; total nitrogen, 2.65%; pentose as arabinose, 15.6%; hexose as galactose, 14.1%: amino sugar as glucosamine, 7.6%; amino acid as alanine, 15.0%; lipid, 33.8%. Neutral sugars were arabinose and galactose. Amino sugars and main amino acids were glucosamine, muramic acid,

alanine, glutamic acid, and α,ϵ-diaminopimelic acid in a molar ratio of 1.00:0.84:2.00:0.97:0.82, respectively. The lipid fraction, obtained after acid hydrolysis (1 N HCl, 10 hr at 100 C) of the phenol-treated cell wall, was treated with 2.5% KOH in methanol-benzene (1:1, v/v) at 50 C for 3 days. Mycolic acid was precipitated from the ethereal solution by adding 2 volumes of ethyl alcohol. Ninety per cent of lipids were recovered in the mycolic acid fraction (melting point, 58 to 60 C) and its infrared spectrum (absorption maxima: 720, 1,470, 2,850, and 2,915 cm^{-1} for methylene group; 1,710 cm^{-1} for carbonyl group; 3,410 cm^{-1} for hydroxy group) is similar to that of the mycolic acid of BCG (Fig. 1). These data indicate that the chemical composition of the phenol-treated cell wall of murine leprosy bacillus is almost identical to that of the mycolic acid-arabinogalactan-mucopeptide complex of mycobacteria. In addition, the presence of mycolic acid, which shows physical properties different from nocardic acids (3), strongly suggests that murine leprosy bacillus belongs to the genus *Mycobacterium*.

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