Water Soluble Palmitic Acid-Methylated Cyclodextrin Complex; A Substrate Oxidized by *Mycobacterium leprae*

TO THE EDITOR:

Mycobacterium leprae does not grow axenically on substrates which other mycobacteria use for growth, multiplication, and virulence. A search for decades for the oxidizable substrates for *M. leprae* resulted in the findings by Franzblau ([2]) and Franzblau and Harris ([3]) that palmitic acid is oxidized by *M. leprae* and that the reaction contributes to ATP formation and synthesis of PGL-I. Ishaque ([4]) confirmed these results and provided direct evidence that, indeed, *M. leprae* oxidizes palmitic acid through the tricarboxylic acid cycle and the electron-transport chain with oxygen as the terminal electron acceptor. These results can be considered as an important advance toward *in vitro* cultivation of *M. leprae* since palmitic acid, as oxidized, is a potent source of energy and a rich source of carbon. The key to *in vitro* cultivation of any microorganism is, of course, the incorporation into the media of an energy and carbon source. For *M. leprae* cultivation, palmitic acid could be such a substrate.

However, palmitic acid is insoluble in water and being hydrophobic in nature it remains in solid state in media, thus biologically not readily available. In biological systems, in culture media, and for metabolic studies, substrates insoluble in water—palmitic acid in particular—were used as finely dispersed crystals, suspensions, or liposomes. Bar ([1]) was the first to incorporate lipophilic substrates into media with hydrophobic substances as a fine suspension. Kato ([5]) used β-cyclodextrin to incorporate palmitates into media for the cultivation of noncultivable mycobacteria. In none of these systems was fatty acid directly available to the cells, since the insoluble substrate must be scavenged from a solid state.

Recognizing the importance of palmitic acid as a carbon and energy source for *M. leprae,* Kato initiated systematic studies to produce a water-soluble formulation of palmitic acid and palmitates as molecular dispersions soluble in water. Recently, Szejtli, *et al.* (personal communication, 1992) reported the preparation of a palmitate-heptakis-2,6-di-o-methyl-β-cyclodextrin complex practically soluble in water. This new formulation was incorporated into media for the cultivation of psychrophilic mycobacteria from *M. leprae*-infected tissues by Kato, *et al.* (personal communication, 1992).

The question thus arises, Can the water-soluble palmitate-methylated cyclodextrin complex serve as an energy source and be as potent as the water-insoluble mother substrate palmitic acid or palmitates? The bioavailabilities of the water-soluble and -insoluble palmitates were compared.

The heptakis-methylated cyclodextrin (DIMEB-CD) was synthesized by Cyclolab Research and Development, Budapest, Hungary. The water-soluble sodium palmitate complex was prepared by Kato (Catherine Booth Hospital, Montreal, Canada) according to Szejtli, *et al.* (personal communication, 1992). The complex was donated as a sterile solution containing 10 mg/ml sodium palmitate. The methylated cyclodextrin was used as a control.

Respiratory studies were conducted to determine whether or not soluble palmitate is oxidized by *M. leprae* bacilli. *M. leprae* bacilli were isolated from the foot pads of athymic nude mice previously infected with *M. leprae,* and purified suspensions were prepared by differential centrifugation in potassium phosphate buffer, pH 6.5. Our results indicated that although insoluble palmitate was oxidized by *M. leprae* suspensions after a lag period of 6–8 hr, soluble palmitate was actively oxidized after a lag period of 2 hr. Similar to insoluble palmitate, soluble palmitate was readily oxidized by *M. phlei* suspensions. Based on the effect of specific inhibitors, it is concluded that soluble palmitate oxidation is mediated through the electron-transport chain of both *M. leprae* and *M. phlei.* The methylated cyclodextrin, when used as a control, did not exhibit any oxidation.

The availability of heat-stable, water-soluble palmitate has made it possible to use palmitate as a substrate in both solid and liquid media. We have prepared solid media and soluble palmitate has been found to

be uniformly dispersed in the media. Clear liquid media can also be prepared by using soluble palmitate. Soluble palmitate does not interfere in spectrophotometric studies as well. It is of further advantage that the water-soluble palmitate complex assures a continuous release of the molecularly dispersed palmitate. This unique way of releasing the active substance results in an improved bioavailability, as is clear by the markedly reduced lag period of oxidation by *M. leprae* and *M. phlei.*

Based on preliminary results, the use of the soluble palmitate complex is highly encouraged for the *in vitro* cultivation trials of *M. leprae* as well as for other biological and metabolic studies.

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Avidin-Biotin Immunoblotting Studies on Reactivity of Leprosy Sera with *Mycobacterium leprae* Antigen

TO THE EDITOR:

Mycobacterium leprae, the causative agent of leprosy, has the most complex structure of all the mycobacteria ([3]). Fewer antigenic components in the *M. leprae* extracts have been shown in comparison with other cultivable mycobacteria by gradient gel electrophoresis ([5]). Crossed-immunoelectrophoresis of *M. leprae* sonicate develòped using sera from leprosy patients has shown about seven or eight antigenic bands ([7]). However, by immunodiffusion about 11 to 12 antigenic components could be detected ([11]). Immunoblotting studies using sera from pooled lepromatous leprosy (LL) patients have revealed about five antigenic bands in the cell-free extract of *M. leprae* ([1]). In this communication, we report an avidin-biotin immunoblotting for analyzing the *M. leprae* antigenic components using sera of leprosy patients.

Collection of serum. Blood samples for the study were collected from untreated leprosy patients attending Central JALMA Institute for Leprosy (CJIL), Agra, India. Patients were clinically classified on the criteria of Ridley and Jopling ([9]). Blood samples from healthy laboratory volunteers served as controls. Sera were separated and stored at −70°C. Normal healthy sera from a non-endemic region for leprosy were kindly provided by Dr. H. D. Engers, World Health Organization, Switzerland.

***M. leprae* antigen.** The cell-free extract of armadillo-derived, purified *M. leprae* ([2]) was kindly provided by Dr. R. J. W. Rees from the IMMLEP (WHO) bank, London.

SDS-Polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE of the *M. leprae* antigens was carried out according to the method of Laemmli ([8]) in 12% homogenous gel along with molecular weight markers