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Oxidation of Sphingolipids by *Mycobacterium leprae*

TO THE EDITOR:

Mycobacterium leprae is rich in lipids and on a dry-weight basis leprosy bacilli contain up to 40% lipid ([1]). There are several classes of lipids and each class has a specific biological function. The most abundant membrane lipids are phospholipids which serve primarily as structural elements of the membranes. Sphingolipids, an important class of phospholipids, are present in most membranes of animal tissues. Three well-known sphingolipids are sphingosine, sphingomyelin and cerebroside.

Although these lipids seem to be important, nothing is known about their metabolism by *M. leprae*. The key to *in vitro* cultivation of thus far noncultivated *M. leprae* is to find the oxidizable substrate which can be incorporated into the culture medium to achieve its cultivation. Since sphingolipids occur naturally in membranes of animal tissues, it is likely that they are utilized by *M. leprae in vivo* for their growth, multiplication and biosynthetic reactions.

However, like other lipids, sphingolipids (namely, sphingosine, sphingomyelin and cerebroside) are insoluble in water. Therefore, when these sphingolipids are added in the culture media used for cultivation trials of *M. leprae,* they remain immissible and, thus, are not readily available to the bacilli. Szente, *et al.* ([2]) reported a preparation of palmitic acid-heptakis 2, 6-di-*O*-methyl-β-dextrin complex completely soluble in water. Recently, water-soluble preparations of sphingosine, sphingomyelin and cerebroside containing 10 mg of each in a 40% randomly methylated beta cyclodextrin were kindly provided by Professor L. Szente (H-1525 Budapest, P.O. Box 435, Hungary). Crystalline (solid) sphingosine, sphingomyelin and cerebroside were purchased from Sigma Chemical Co., St. Louis, Missouri, U.S.A. It was of interest to know if these water-soluble and -insoluble sphingolipids are oxidized by *M. leprae.*

Oxidation of sphingosine, sphingomyelin and cerebroside was determined by using the standard manometric techniques as described by Umbreit, *et al.* ([3]). During this study, *M. leprae* were isolated from the foot pad lesions of nude mice (athymic) which previously had been infected with human leprosy bacilli, and purified bacillary suspensions were prepared by differential centrifugation in potassium phosphate buffer, pH 6.5.

Our results have shown that whole cell suspensions of *M. leprae* exhibited considerable endogenous respiration. However, the presence of insoluble (about 100 μg per 2 ml) sphingosine, sphingomyelin or cerebroside in cell suspensions showed no enhanced oxygen uptake over endogenous respiration for a period of 24 hr. These results suggested that these sphingolipids were not oxidized by *M. leprae.* However, when soluble sphingosine, sphingomyelin and cere-

broside were the substrates, oxidation did occur, with an induction period of about 8 hr being required. Our results have shown repeatedly that bacillary suspensions preincubated with sphingosine, sphingomyelin and cerebroside for about 8 hr exhibited much higher oxygen uptake than bacillary suspensions without substrates. These results clearly indicated that soluble sphingolipids were oxidized by *M. leprae* after a lag period of 8 hr. It was observed that the oxidation of soluble sphingolipids was completely inhibited by specific inhibitors of the respiratory chain. These observations suggest that the oxidation of soluble sphingolipids is mediated through the electron transport chain of *M. leprae.* When used alone as a control the methylated cyclodextrin solution which was used to solubilize sphingolipids did not show any autooxidation.

These observations suggest that in order to achieve maximal bioavailability of sphingolipids, clear liquid as well as solid media can be prepared using soluble sphingolipids for cultivation trials of *M. leprae.* No substrate is yet known which can be used by *M. leprae in vivo* for their multiplication. Since sphingolipids occur naturally in the membranes of animal tissues, it is likely that such compounds are utilized by *in vivo M. leprae* for their multiplication. Therefore, the use of these water-soluble sphingolipids is encouraged for *in vitro* cultivation trials of *M. leprae.* These sphingolipids are heat stable; thus culture media containing water-soluble sphingolipids can be incubated for a long period of time and growth of *M. leprae* on solid or in liquid media can be evaluated easily.

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