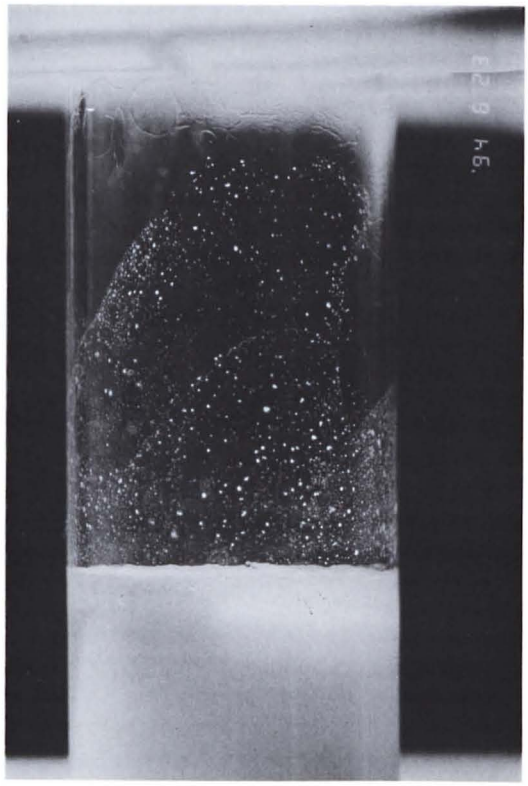
An Unusual Spreading Phenomenon in an Artificial Medium Inoculated with *M. leprae*

TO THE EDITOR:

In 1874 Hansen reported *Mycobacterium leprae* as the causative agent of leprosy. Since that time only very limited multiplication of this mycobacterium has been reported from time to time on artificial media ([3]). Wheeler reported in 1990 that the *de novo* fatty acid synthesis of *M. leprae* is depressed and that *M. leprae* require an exogenous source of fatty acids for growth ([7]). Franzblau described the oxidation of palmitic acid of *M. leprae* with an increased synthesis of phenolic glycolipid-I (PGL-I) ([2]). Kato, *et al.* reported the use of water-soluble complexes of palmitic acid and long-chain alcohols for the *in vitro* cultivation of leprosy-derived psychrophilic mycobacteria ([4]).

At the Armauer Hansen Institute/German Leprosy Relief Association, Wurzburg, Germany, a modified Ogawa medium was developed with the incorporation of different water-soluble fatty acids, water-soluble liposomes, purine precursors and catalase. The egg medium was poured horizontally. Over the remainder of the glass wall a thin layer of agar was deposited. The medium was inoculated vertically with an *M. leprae* suspension (200 μl, 1×10^8/ml) obtained from mouse foot pads previously inoculated *with M. leprae.* The tubes were incubated in a vertical position at 32°C. Three to four weeks after inoculation a spreading phenomenon of the organism appeared in an upward direction (The Figure). The organism appeared to spread upward like a thin film in a wave-like motion from the horizontal inoculated surface of the medium on the glass wall of the tubes, which is suggested to be associated with bacterial growth.

The bacteria which were spreading in an upward direction have been identified using a set of synthetic oligonucleotides [B–16S–27f([6]), B–16S–341r([1]) and B–16S–788r([6]) homologous to broadly conserved sequences of 16S ribosomal RNA. The *in vitro* amplification of the 796 bp fragment of the bacteria with the polymerase chain reaction (PCR) was followed by direct sequencing of the PCR products. A comparison of these sequences with the published sequence resulted in a 100% homology, especially an insertion of 12 nucleotides unique for *M. leprae* at the 5′ end of the 16S rRNA gene was found ([5]).



THE FIGURE. Spreading on the glass wall in a tube inoculated with *M. leprae.*

The significance of this finding is being investigated currently.

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