

## Reply to Dr. Convit's Letter to the Editor

## TO THE EDITOR:

In preliminary experiments, we extracted phospholipids with pyridine in different mycobacterial strains exactly following the technical procedure described by Campo-Aasen and Convit <sup>(1)</sup> and subsequently used by Fisher and Barksdale <sup>(3, 4)</sup>. In spite of this, substantially fewer acid-fast cells were repeatedly observed in some mycobacterial species after pyridine treatment than in parallel control smears. The results of subsequent tests in 32 strains of 18 mycobacterial species in which, as stated in our paper, smears were fixed by heat instead of Bouin's solution did not differ from the former and essentially corresponded with the findings of Skinsnes, *et al.* <sup>(5)</sup>. The paper by Convit and Pinardi <sup>(2)</sup> cited by us says, in connection with the effect of pyridine on acid-fastness, Baker's staining for phospholipids, and fluorochrome staining that "Of all other known mycobacteria, only *M. leprae* completely loses its ability to be stained by the above three methods after 2-hour treatment with pyridine." However, the selection of mycobacteria in the three earlier studies to which the authors refer was limited to only a small number of the more than 25 well-defined mycobacterial species and, in our opinion, was not sufficiently representative to allow the conclusive statement quoted above to be based thereon.

Prior to submitting our paper for publication, we had sent it to Dr. Convit, asking for his comments, and fully agree with his remark, repeated in his Letter to the Editor, that our technic differed in many re-

spects from the method used by him. The differences especially relate to the fixation of material (smears from leprosy patients had been forwarded to us after heat fixation, biopsy specimens in formalin) and also to the staining itself, which we performed by the modification of Ziehl-Neelsen's method usual here. However, we have pointed out both of these changes in the Methods section and, moreover, introduce the summary conclusions drawn from our experiments with the phrase, "Under the conditions used . . . ."

—M. Slosarek, R.N.Dr., C.Sc.

Research Assistant  
Institute of Hygiene and Epidemiology  
100 42 Prague  
Srobarova 48  
CSSR

## REFERENCES

1. CAMPO-AASEN, I. and CONVIT, J. Identification of the noncultivable pathogenic mycobacteria *M. leprae* and *M. lepraemurium*. *Int. J. Lepr.* **36** (1968) 166–170.
2. CONVIT, J. and PINARDI, M. E. Leprosy. Confirmation in the armadillo. *Science* **184** (1974) 1191–1192.
3. FISHER, C. A. and BARKSDALE, L. Elimination of the acid-fastness but not the gram positivity of leprosy bacilli after extraction with pyridine. *J. Bacteriol.* **106** (1971) 707–708.
4. FISHER, C. A. and BARKSDALE, L. Cytochemical reactions of human leprosy bacilli and mycobacteria. Ultrastructural implications. *J. Bacteriol.* **113** (1973) 1389–1399.
5. SKINSNES, O. K., CHANG, P. H. C. and MATSUO, E. Acid-fast properties and pyridine extraction of *M. leprae*. *Int. J. Lepr.* **43** (1975) 339–347.