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 [1] and subsequently used by Fisher and Barksdale [3, 4]. 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.* [5]. The paper by Convit and Pinardi [2] 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 respects from the method used by him. The differences especially relate to the fixation of material (smears from leprous 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 . . . ."

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Considerations on the Treatment of Leprosy

TO THE EDITOR:

In considering the Fifth Report of the WHO Expert Committee on Leprosy (Technical Report Series, 607, Geneva, April 1977); the Heathrow Report (ILEP No. 1, London, August 1977); and the Workshops on Experimental Chemotherapy and Epidemiology, Control and Field Therapy of the XI International Leprosy Congress (November 1978, Mexico), the following observations appear warranted:

1. For the treatment of lepromatous leprosy and for the prevention or treatment of dapsone resistance, 9 drugs are recommended in the above documents.

These drugs may be classified into four groups fundamentally:
a) Drugs with clinically demonstrated, known therapeutic activity in leprosy:
dapsone (oral or by injection)
clofazimine
rifampin
b) Drugs which have been used clinically in the past but which, more recently, have been discontinued because of low therapeutic activity or for toxicity. The value of these drugs is still under investigation:
thiambutosine (Ciba, 1906)
thiacetazone (TB1/698)
sulfamethoxypyridazine
c) A drug which is used clinically, but with doubts about its efficacy:
acedapsone (DADDS)
d) Drugs which have had essentially no controlled clinical trials in leprosy and which are still under assessment:
ethionamide
prothionamide

2. With one or more of the nine drugs mentioned, 11 regimens for the treatment of lepromatous leprosy are recommended, namely:
dapsone alone
rifampin and dapsone (2)
clofazimine and dapsone
ethionamide and dapsone
thiacetazone and dapsone
dapsone by mouth and injection
rifampin and clofazimine
ethionamide and clofazimine (2)
rifampin and ethionamide

With the majority of these regimens there is no therapeutic experience clinically, and the recommendations have been made based either on data obtained in the footpad model of *M. leprae* infection in intact mice or on theoretical considerations.

3. More recent experience with all these regimens has frequently emphasized monitoring the response of treated patients by serial inoculations of bacilli from patients into normal mice. In this regard, it should be pointed out that:
a) Over twenty years was needed to study the anti-leprotic activity of dapsone, and until a few years ago, the proper dosage(5 to 10 mg or 50 to 100 mg daily) was not known. The anti-leprotic activity of clofazimine and rifampin was established after almost ten years of clinical experience. It should be added that during this period little was known about dapsone resistance and drug-sensitive survivors ("persisters").
b) If it took several decades to establish the anti-leprotic activity of dapsone, clofazimine and rifampin, how many decades will it take to know the anti-leprotic activity of 11 different regimens, using 9 drugs, in previously treated patients, considering the additional problems of drug resistance, "persisters," etc.?

4. Strong emphasis has been placed on the model of *M. leprae* infections in the footpads of normal mice in almost all of the more recent experimental and clinical trials dealing with the chemotherapy of leprosy. Bechelli and Guinto [1] have raised fundamental objections to the use of results obtained with this model to make implications for the clinical therapy, epidemiology and control of leprosy. The following points are taken from this work.
a) "It is considered premature to apply laboratory findings to human leprosy before clinical and epidemiological studies have been made in man."
b) "*M. leprae* 'infections' in the footpads of mice, limited at best, die off after reaching a certain level, indicating that mice and human are not alike in their susceptibility."
c) "Judged by their great effectiveness in leprosy in mice, these two measures together—dapsone and B.C.G. vaccination—should by now have accomplished a world-wide reduction in the prevalence of leprosy but there is not evidence that such a reduction has taken place."
d) "It is unquestionably extremely hazardous to extend the results in the mouse to man." (Levy)
e) "It should be appreciated that even the most irregularly-treated of these patients would be considered as grossly over-treated on the basis of the laboratory findings in mice."

If the fundamental validity of the model of *M. leprae* infections in the footpads of normal mice is questioned, then the fundamental validity of many of the assumptions made in the more recent recommendations for the treatment of leprosy may be questioned. Indeed, if the view is taken that the mouse footpad model is completely lacking in validity, then many of the more recently recommended treatment regimens may also completely lack validity. If there is indeed no suitable model to test new antileprosy drugs in animals or *in vitro*, then the many drugs, the many regimens, the revival of old and ineffective drugs, etc., could be interpreted as being completely disorderly, confused and likely to be ineffective. Taken further, this line of reasoning could lead to the conclusion that leprosy control by chemotherapy is unattainable and that it is impossible to predict the future course of leprosy in the world.

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Superoxide Production in PMNs from Leprosy Patients

To the Editor:

In a recent paper (Int. J. Lepr. **46** [1978] 337–441), Dr. O. Rojas-Espinosa reported his results in determining superoxide production of polymorphonuclear leukocytes (PMNs) from patients with leprosy as compared with those from normal individuals. Levels obtained were essentially similar. In addition, he found no significant difference between superoxide production of PMNs from patients with standard lepromatous leprosy and that of PMNs from patients with reactional leprosy (RLL). The author analyzed his results and compared them with those previously reported by us (Clin. Exp. Immunol. **20** [1975] 257–264) as follows: "Goihman-Yahr, *et al.*, found that patients with any type of leprosy, except reactional (RLL) lepromatous leprosy, had normal numbers of NBT-reducing cells. In patients with RLL, the proportion of reducing cells was significantly raised. We did not find a significant increase in the O_2^- levels produced by PMN from patients with RLL when compared with lepromatous patients without reaction."

From these comments, the reader might conclude that Dr. Rojas' results are at variance with ours, at least concerning RLL. This is not the case at all. As I feel that PMN activation is a rather distinctive feature of RLL, the issue should be clarified.

In the method which we used (a modification of Matula and Paterson's, New Engl. J. Med. **285** [1971] 311–317), heparinized peripheral blood is incubated with NBT at 37°C in siliconized excavated glass slides. We found that blood from patients with active RLL had a significantly higher proportion of NBT-reducing PMNs than blood from normal individuals or from any other kind of leprosy patients. We also found that the above was not due to any intrinsic difference between PMNs from RLL patients and those from other persons. Thus, if blood was incubated *in vitro* with endotoxin and NBT, the proportion of NBT-reducing PMNs reached a similarly high level in all groups. We concluded that spontaneous activation (i.e., without incubation with an additional activator) was brought about in RLL patients by some factor, presumably of immunologic nature. Further work has been done in this direction, but it is not germane to the current discussion.

Dr. Rojas-Espinosa employed a method by which PMNs were isolated from peripheral blood and then put in the cold (thereby presumably suppressing any pre-existing metabolic burst). PMNs were then incubated at 37°C with cytochrome and latex particles. The latter are quite capable of inducing an activation comparable to that caused by endotoxin. Dr. Rojas was then