CORRESPONDENCE

This department is for the publication of informal communications that are of interest because they are informative and stimulating, and for the discussion of controversial matters. The mandate of this JOURNAL is to disseminate information relating to leprosy in particular and also other mycobacterial diseases. Dissident comment or interpretation on published research is of course valid, but personality attacks on individuals would seem unnecessary. Political comments, valid or not, also are unwelcome. They might result in interference with the distribution of the JOURNAL and thus interfere with its prime purpose.

Evidence for the Activity of Rifampin on the Neuropathy of Foot Pad-inoculated Mice with *Mycobacterium leprae*

TO THE EDITOR:

Although the involvement of nerve trunks is the most striking feature in Hansen's disease, little is known about the effects of antimycobacterial drugs on this neuropathy. On the one hand, dapsone, clofazimine, and rifampin have been shown to penetrate the endoneurium ([1]), although in small amounts. On the other hand, solid bacilli have been found in the nerves of patients who have received continuous treatment for many years ([3, 6]), and electrophysiological recordings have demonstrated a motor unit impairment in treated patients ([9]). We report here the improvement of motor conduction velocities (MCV) recorded in the sciatic nerves of foot-pad inoculated Swiss mice treated with rifampin weekly (10 mg/kg *per os*).

The animals were inoculated in the left foot pad with 4.5 to 5.5 $\times$ 10^3 *Mycobacterium leprae* either provided by the laboratory of Professor Pattyn, Antwerp Belgium, or isolated from skin biopsies of sulfone-resistant patients living in the French West Indies. The preparation of *M. leprae* solutions, the inoculations, and the counting procedures for acid-fast bacilli (AFB) were performed according to Shepard's technique ([10]). All of the strains of *M. leprae* used in this study multiplied in the mouse foot pad as quickly as reported by Levy ([7]). Mice treated with rifampin for 1 month after inoculation did not show AFB multiplication for up to 14 months.

The MCV of both sciatic nerves were calculated using the method of Fullerton ([5]). The mice were anesthetized by ether inhalation, and the core temperature of the animals was carefully controlled. A single supramaximal rectangular stimulus (0.1 ms, 30 V, 1 Hz) was applied through a cathode-needle inserted near the nerve trunk, either at the malleolus or the sciatic notch. A bifilar electromyographic needle (Disa 13K80) recorded the potential evoked in the plantar muscles. After amplification ($\times$100, 0.1–30,000 Hz), the latencies of the first positive peak of the potentials were measured on a storage oscilloscope. The distance between the two stimulating needles was measured on the skin of the fully extended limb.

Table 1 compares the results calculated in one group of mice, 14 months following the AFB inoculation, to those of another group, 12 months following the inoculation, but treated with rifampin for 11 months. Although the MCV of the right legs were not different in the two groups, those of the left (inoculated) legs were significantly decreased in the nontreated group compared to the rifampin-treated group. These results suggest the possibility that treatment initiated early would prevent the MCV decrease observed in the inoculated side.

Table 2 gives the results of another set of experiments where AFB multiplied in the left foot pad for 6 months. Rifampin treatment was then initiated and continued for 2 months (9 doses). Six months after in-

TABLE 1. *Sciatic nerve motor conduction velocities.*[a]

Groups	Right sciatic MCV (m/s)	Left sciatic MCV (m/s)
14 months inoculated mice	65.4 ± 13.5 (n = 16)	49.9 ± 15.4[b] (n = 16)
12 months inoculated and 11 months rifampin treated mice	70.9 ± 30.9 (n = 15)	71.1 ± 19.4[c] (n = 15)

[a] Mean results ± one standard deviation.
[b] Significantly less than right sciatic MCV of 14 months inoculated mice, $p < 0.01$, Student's *t* test.
[c] Significantly more than left sciatic MCV of 14 months inoculated and untreated mice, $p < 0.01$, Student's *t* test.

oculation, this group not only showed a highly significant decrease of the MCV in the inoculated legs but also a significant decrease in the other side, compared to normal 8-week-old animals. The significant difference observed between the two sides at this time disappeared after 9 doses of rifampin, but the MCV of both sides remained as low as the values in the 6-months inoculated mice on the noninoculated side.

Since the first signs of bacillation of mouse sciatic nerves were seen 20 months after inoculation with *M. leprae* in foot pads ([2]), the MCV decrease observed bilaterally following 6 months of AFB multiplication must not be due to demyelination resulting from the multiplication of AFB in Schwann cells. Other mechanisms would include auto-immune demyelination ([2]) or endoneurial edema involving an entrapment neuropathy ([9]). However, rifampin given 6 months following the inoculation of *M. leprae* improved the MCV of the inoculated side.

In summary, the inoculation of *M. leprae* into one foot pad of mice resulted in an early asymmetric decrease of the MCV in both sciatic nerves. Initiated 1 month after inoculation, treatment with 10 mg/kg rifampin given weekly *per os* prevented this MCV decrease. Initiated 6 months after the inoculation, this treatment improved the MCV decrease on the inoculated side up to the value of the other side.

TABLE 2. *Sciatic nerve motor conduction velocities in 8-week-old normal, 6 month-inoculated and then 2 months rifampin-treated mice.*

Groups	Right sciatic MCV (m/s)	Left sciatic MCV (m/s)
Normal mice 8 weeks old	59.9 ± 17.0 (n = 16)	56.4 ± 16.9 (n = 14)
6 months inoculated mice	43.3 ± 10.0[a] (n = 8)	30.1 ± 4.9[b,c] (n = 8)
8 months inoculated and 2 months rifampin treated mice	48.5 ± 10.4[d] (n = 8)	43.9 ± 19.2[d] (n = 8)

[a] Significantly less than right sciatic MCV of normal mice, $p < 0.05$, Student's *t* test.
[b] Significantly less than left sciatic MCV of normal mice, $p < 0.001$, Student's *t* test.
[c] Significantly less than right sciatic MCV of 6 months inoculated mice, $p < 0.01$, Student's *t* test.
[d] Not significantly different from normal mice or 6 months inoculated mice, $p > 0.05$, Student's *t* test.

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NADH-Methemoglobin Reductase and Reticulocytosis

TO THE EDITOR:

Magna and Beiguelman ([5]) have shown that the activity of NADH-methemoglobin reductase varies widely among leprosy patients under dapsone treatment and is negatively correlated to their hemoglobin level. Since dapsone has a hemolytic effect ([1]), while NADH-methemoglobin reductase is more active in younger than in older erythrocytes ([3, 4]), it was supposed that this correlation might be due to an increase in the reticulocyte rate in leprosy patients.

This hypothesis was tested by studying venous blood samples of leprosy patients (30 males and 30 females) submitted to chronic sulfone therapy. The activity of NADH-methemoglobin reductase was determined according to Scott's method ([6]) slightly modified, and the hemoglobin level was determined following Benesch, *et al.*'s method ([2]). The reticulocyte rate was obtained as usual. The mean and standard deviation of the variables studied are given in The Table.

THE TABLE. *Mean values ($\bar{x}$) and standard deviations (s) of NADH-methemoglobin reductase activity ($10^4 \cdot A_{600}$/min), hemoglobin level (g%) and reticulocyte rate (%) of 60 adult leprosy patients under sulfone therapy.*

Variable	Sex	$\bar{x}$	s
NADH-methemoglobin reductase	M	43.54	10.76
	F	61.73	14.14
	M + F	52.63	15.48
Hemoglobin	M	13.16	1.52
	F	11.05	1.95
Reticulocytes rate	M	2.34	1.41
	F	2.41	1.63
	M + F	2.38	1.51

In the present study, no significant correlation was found between the NADH-methemoglobin reductase activity and the hemoglobin level ($r = -0.05$ in males and $r = 0.20$ in females) or between the former and the reticulocyte rate ($r = 0.04$ in males and $r = -0.23$ in females).

It also seems important to point out that in the samples analyzed in the present study the NADH-methemoglobin reductase activity among females was significantly higher as compared to males.

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