ECs was found to be 50–100% [4]. In the present *in vitro* study, it was 27.75% after 24 hr, and it was reduced to 12.66% on the third day. It seems that the microenvironment supports the phagocytic action of the ECs which was lost in culture.

It is reported that the phagocytic index of Schwann cells is 15.9% after 24 hr and reaches 67.2% by the third day [9]. This suggests a high affinity of bacilli toward Schwann cells (SC). It seems that Schwann cells *in vivo* in leprous neuropathy influence phagocytic activity of ECs and both of these cells (EC and SC) seem to be complementary.

The majority of live bacilli were seen intracellularly (Fig. 3), while irradiated ones were mainly seen extracellularly. It appears that viablity of the bacillus is essential for its recognition by ECs.

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Clofazimine-resistant *M. leprae*

To the Editor:

Several years ago, Dr. T. Warndorff-van Diepen reported in the pages of the Journal a case of clofazimine-resistant leprosy [7]. Approximately eight months earlier, Dr. Warndorff had sent me two suspensions of *Mycobacterium leprae,* requesting that I test the susceptibility of the organisms to clo-

TABLE 1. *History of specimens received from Addis Ababa.*

Date	Inoculum		Harvest		Remarks
	Source (No. of AFB per specimen)	Number ($\times 10^3$)	Time (days)	Results ($\times 10^5$)	
26 Oct. 1981	Biopsy specimen	5.00	185	0.362	
	(2.16×10^8)		246	0.359	Lost
	Mouse harvest	1.90	185	0.308	
	(1.26×10^5)		246	0.738	
			373	1.17	Passaged
5 Nov. 1982	Mouse harvest	1.17	121	0.510	
	(1.17×10^5)		170	0.540	Passaged
26 April 1983	Mouse harvest	3.80	120	0.074	
	(5.40×10^4)		169	4.35	
			215	38.2	Passaged for study of drug susceptibility (Table 2)

fazimine in my laboratory. The importance of confirming her results was self-evident.

The suspensions were brought by a traveler from Addis Ababa to Jerusalem on wet ice in an insulated container. The trip required several days, and no ice remained in the container at the time it was received in Jerusalem. According to the information supplied, one of the suspensions had been prepared from a fresh skin biopsy specimen; the second specimen represented the results of a harvest from mouse food pads that had previously been inoculated with *M. leprae* obtained from a skin biopsy specimen from the same patient. On receipt of the suspensions, the *M. leprae* were counted, with the results shown in Table 1. The suspension prepared from the biopsy specimen was diluted so as to contain 5×10^3 acid-fast bacilli (AFB) per 0.03 ml, and inoculated into both hind foot pads of one group of CBA mice. The second suspension without dilution was inoculated into both hind foot pads of a second group of CBA mice.

TABLE 2. *Test of susceptibility to dapsone and clofazimine. Inoculum* = 5×10^3 M. leprae *per foot pad.*

Drug	Time from inoculation to harvest (days)	No. of *M. leprae* per foot pad ($\times 10^5$)
None	93	2.61
	100	5.28
	104	14.9
	107	10.9
Dapsone		
0.0001 g %	106	0.106
	118	0.320
0.001 g %	105	0.373
	115	<0.053
0.01 g %	105	0.053
	115	<0.053
Clofazimine		
0.0001 g %	106	4.05
0.001 g %	106	0.053
	118	0.373

As shown in Table 1, no evidence of multiplication of the organisms obtained from the skin biopsy specimen was detected as late as 8 months after receipt of the specimens in Jerusalem, at which time the mice were lost. A harvest from mice performed 246 days after inoculation with the organisms stated to have been obtained by mouse harvest suggested that the organisms were multiplying, a harvest performed 373 days after inoculation yielded 1.17×10^5 AFB, and the organisms were subinoculated into new CBA mice. This passage coincided with a zoonosis that inhibited multiplication of *M. leprae* in our mice. Immediately, new animal quarters were prepared, and new specific-pathogen-free (SPF) CBA breeding stock was obtained from the National Institute of Medical Research in London. At the first opportunity thereafter, approximately 6 months after inoculation of the passage mice, a harvest was performed and the organisms were subinoculated into SPF CBA mice. During this second passage, the *M. leprae* multiplied as expected, so that

approximately 7 months later it was possible to carry out a third passage, this time inoculating enough mice to permit testing of the susceptibility of the organisms to both dapsone and clofazimine.

Passage mice were inoculated into both hind foot pads with 5×10^3 *M. leprae* per foot pad, and groups of 15 mice were administered drug-free diet(s), diets containing clofazimine in a concentration of 0.0001 g or 0.001 g per 100 g diet, or dapsone in a concentration of 0.0001, 0.001, or 0.01 g per 100 g mouse diet, beginning on the day of passage. The results of the tests of drug susceptibility are shown in Table 2. That the inoculum included a large proportion of viable *M. leprae* is demonstrated by the multiplication to $>10^6$ organisms per foot pad within 107 days after passage. Harvests performed at this same time from drug-treated mice demonstrated multiplication only in those administered clofazimine in the smallest concentration; multiplication of the *M. leprae* appears to have been inhibited by clofazimine in a concentration of 0.001 g per 100 g diet, and by dapsone in the smallest concentration administered.

Thus, the organisms have been shown to be fully susceptible to dapsone and "resistant" only to clofazimine administered in a concentration of 0.0001 g per 100 g diet. Whether the failure of clofazimine in this concentration to inhibit multiplication of *M. leprae* represents evidence of the emergence of a drug-resistant mutant, or merely reflects a variation of the minimal effective dose of clofazimine among "wild" strains, cannot be stated at this time; the susceptibility to clofazimine of too few strains of *M. leprae* has been tested to permit establishment of criteria of susceptibility and resistance to clofazimine. The available data, representing five strains ([1, 3, 4, 6, 7]), demonstrate that, except for Dr. Warndorff's strain, all of the strains tested thus far, for which the data have been published, are inhibited from multiplication by clofazimine administered to mice in a concentration of 0.0001 g per 100 g.

The discrepancy between our results and those reported earlier ([7]) requires explanation. [Unfortunately, attempts to isolate *M. leprae* from the suspensions sent to Antwerp ([7]) were unsuccessful (S. R. Pattyn, personal communication).] The study carried out in Addis Ababa involved drug administration beginning only 60 days after the inoculation of the mice, and the first harvests of *M. leprae* were carried out only 9 months after inoculation. In fact, the criterion for susceptibility of *M. leprae* to dapsone depends upon drug administration from the day of inoculation, and harvesting from drug-treated mice immediately after harvests have yielded unmistakable evidence that the organisms have multiplied in untreated control mice. In the case of Dr. Warndorff's strain, without the results of simultaneous harvests from control and treated mice performed at the time that multiplication was near maximal in the control mice, one cannot be certain that the multiplication in treated mice presented in Dr. Warndorff's table ([7]) had not occurred before drug administration was begun. Finally, the published data are insufficient to exclude the possibility that even fully susceptible *M. leprae* continue to multiply, albeit slowly, during continued administration of clofazimine; such a phenomenon has been described for both cycloserine ([5]) and methimazole ([2]).

Alternatively, the possibility of "back-mutation" of a clofazimine-resistant mutant to a susceptible one cannot be ruled out. Although back-mutation to dapsone susceptibility has not been encountered (R. J. W. Rees and C. C. Shepard, personal communications; unpublished data from this laboratory), the failure of back-mutation to have been observed with respect to dapsone resistance does not exclude the possibility of this phenomenon with respect to clofazimine resistance. That no other clofazimine-resistant mutant *M. leprae* have been isolated has prevented the study of this phenomenon. Certainly, the data given in this present report would have been more convincing had they been obtained in the course of the initial isolation, or on first passage. On the other hand, the organisms may be seen to have multiplied through only about one-million-fold from the original inoculum to final passage to drug-treated mice; a very high mutation frequency would be necessary to explain the current findings in terms of a back-mutation.

Finally, the possibility of a laboratory error, in which the mice inoculated with *M. leprae* received from Addis Ababa were re-

placed with mice that had been inoculated with organisms of another strain, must be considered. It is unfortunate that Dr. Warndorff had not tested the susceptibility of her isolate to dapsone; this might have provided another marker. Militating against such an error, however, is the fact that the strain of *M. leprae* with which, at a given time, almost all of the mice in our animal quarters are infected has previously been shown to be susceptible to clofazimine in a concentration of 0.0001 g per 100 g ([1]).

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