Activity of Cycloserine and Structurally Related Compounds Against *M. leprae*-infected Mice

TO THE EDITOR:

Previously, Shepard and Chang ([10]) demonstrated that cycloserine, when incorporated in mouse chow and fed continuously at a concentration of 0.5% for up to 11 months, delayed and partially suppressed the growth of *Mycobacterium leprae* in the mouse foot pad. However, in that study, following inoculation of 5×10^3 *M. leprae*, counts of *M. leprae* at 13 months were as follows:

Controls	1.4×10^6
DDS 0.1%	3×10^4
INH 0.1%	3×10^4
PAS 0.6%	3×10^4
Cycloserine 0.5%	4.1×10^5

Thus cycloserine was judged to be only minimally active. In studies of the Leonard Wood Memorial ([2]), in which patients were assigned to treatment on a random basis, cycloserine was found about as effective as dapsone in terms of the clearance of bacilli from the skin and nasal secretions. However, after 48 weeks only 4 of 14 patients treated with cycloserine showed clinical improvement, while 14 of 18 patients responded to dapsone. A number of other reports ([1, 6, 7, 8]) have documented the clinical and/or bacteriologic efficacy of cycloserine in leprosy. Because of the recent synthesis by Hynes of two structurally related compounds, glycyl hydroxamic acid and beta alanyl hydroxamic acid, their demonstrated activity against saprophytic and pathogenic mycobacteria *in vitro* ([3, 4]), and the ability of beta alanyl hydroxamic acid, but not of glycyl hydroxamic acid, to reduce the number of viable tubercle bacilli in the lungs and spleen of mice infected with H37Rv strain of *M. tuberculosis* to a comparable degree to that obtained with streptomycin ([3]), we decided to evaluate the activity of these three agents against *M. leprae*-infected mice.

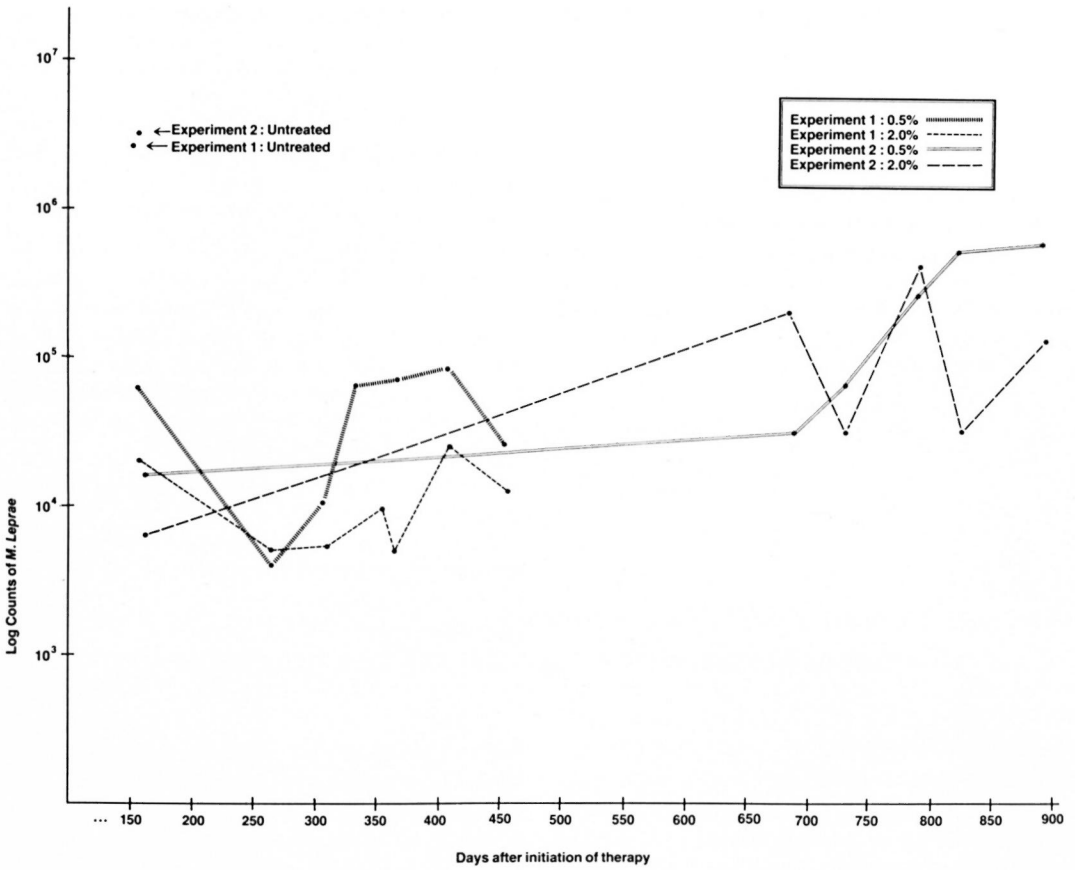
In the initial study (Experiment 1), 240 female BALB/c weanling mice were inoculated in both hind foot pads with 5×10^3 *M. leprae* of an extensively studied strain. Groups of 15 mice were fed a diet from day 60 to day 150 (kinetic technique of Shepard[9]) containing no drug or cycloserine, glycyl hydroxamic acid, or beta alanyl hydroxamic acid in concentrations of 0.0025%, 0.025%, 0.1%, 0.5%, and 2%. Diets were prepared fresh every two weeks by dissolving drugs in water. These diets were stored refrigerated and placed in the mouse feeders twice weekly. On approximately day 150, foot pad harvests from two mice (four feet) from each group were performed and *M. leprae* enumerated using standard techniques. Subsequently, at approximately bimonthly intervals, enumeration of *M. leprae* was performed in a similar manner in those groups of animals in which antimicrobial activity appeared to be present at the time of discontinuation of therapy.

All dietary concentrations of glycyl hydroxamic acid and beta alanyl hydroxamic acid studied were inactive: At the completion of therapy, the number of *M. leprae*/foot pad in untreated mice was 2.5×10^6 and $>6 \times 10^5$ in all treated groups. Lower dietary concentrations of cycloserine (0.0025%, 0.025%, 0.1%) also had no effect on the multiplication of *M. leprae*; the numbers of *M. leprae*/foot pad at the completion of therapy were $>10^6$. However, 0.5% and 2% dietary cycloserine prevented multiplication of *M. leprae* for over one year following the cessation of therapy (The Figure).

Because of the unexpected and significant activity of cycloserine at 0.5% and 2%, these groups of animals were restudied in the manner previously described (Experiment 2). Unfortunately, mouse harvests from these studies were interrupted while the laboratory was being relocated.

Results of Experiment 2 are presented in The Figure. Again, 0.5% dietary cycloserine appeared active and resulted in a delay of growth of *M. leprae* of 570 days (linear correlation coefficient is 0.98 when the results of day 161 are eliminated). Two percent cycloserine appeared to prevent sustained multiplication of *M. leprae* for over two years.

It is not clear why the activity of cycloserine found in these studies was superior



THE FIGURE. Results of active cycloserine therapy in Experiments 1 and 2.

to that reported by Shepard. Possible explanations include a difference in the susceptibility of the strains of *M. leprae* studied or in the preparation and storage of the drug-containing diet.

The utility of cycloserine for clinical application in the treatment of leprosy is limited by its irritant effects on the central nervous system. Doull, *et al.* [2] in the treatment of leprosy found that with initial doses of 250 mg daily, which were slowly increased to 1 g daily by seven weeks, cycloserine was well tolerated for the 48 weeks it was given. The usual dose of cycloserine for adults is about 750 mg per day; this is associated with a small risk of toxic reactions. Holmes found in 60 tuberculosis patients that if cycloserine serum levels are monitored and maintained between 20 μg and 40 μg per ml, bacteriologic and radiographic improvement occurred, uncomplicated by cycloserine toxicity [5].

For other bacterial diseases there is a well known advantage and often synergistic effect when utilizing combinations of antibiotics with different loci of action. Cycloserine inhibits cell-wall synthesis, a site thus far not exploited in the chemotherapy of leprosy.

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Mycobacterium X Identified as *Mycobacterium avium intracellulare* (Probably Mixed with *M. leprae* in Early Subcultures)

TO THE EDITOR:

Several strains of mycobacteria were cultivable from *Mycobacterium leprae*-infected human and armadillo tissues in media containing straight-chain dimethylated *n*-alkanes as the sole source of carbon and energy. The primary cultures and early subcultures did not grow on Löwenstein or in Dubos media, but in the foot pads of mice produced a disease similar to that obtained following injection of host-grown *M. leprae* ([7, 8]). The later subcultures, however, maintained in our laboratory on tetradecane–agar media, were also cultivable on Löwenstein media. These results suggest that early subcultures might be mixed isolates of *M. leprae* and a cultivable strain of mycobacteria. Today, out of the 18 cultures of *Mycobacterium X* that we possess, there is only one in our collection (AD-92) which still does not grow on Löwenstein medium. The isolates, identified as *M. avium intracellulare* by standard biochemical tests, have identical characteristics. They appear as rough, nonpigmented, unbilicated colonies on tetradecane media and have the following characteristics on which classification can be based. Slow, nonpigmented growth develops on Löwenstein medium at 30°C and 37°C; 0 ± growth at 20°C; no growth was registered at 42°C. NO_3 reduction, thiophen-2-carboxylic acid hydrazide (TCH), β-glucosidase, catalase S.Q., Tween 80 hydrolysis, acid phosphatase, 5% NaCl tolerance, arylsulfatase (3–14 days), and urease reactions were negative. NAP, catalase 68°C, tellurite reduction, nicotinamidase, pyrazinamidase reactions were positive. Cultures were resistant to isoniazid (INH) (0.2 μg), p-aminosalicylic acid (PAS) (0.5 μg), ethionamide (20 μg), and sensitive to streptomycin (4 μg), rifampin (40 μg), ethambutol (2 μg), capriomycin (50 μg), and cycloserine (30 μg) (per ml, respectively).

Two cultures of *Mycobacterium X* were confirmed as *M. intracellulare* (Serotype 19) by an independent laboratory (Dr. A. Laszlo, Health and Welfare Canada, National Research Centre for Tuberculosis).

The frequent isolation of cultivable mycobacteria from *M. leprae*-infected tissues has constantly been the subject of discussion and controversy, and has often been blamed on experimental error or technical incompetence. Claims of cultivation of *M. leprae* and reports on cultivation of mycobacteria from leprosy-derived tissues have appeared frequently in the literature every year during the past century. The pertinent literature has been reviewed in the past ([17]),