Minimal Effective Dosages in Mice of Clofazimine (B.663) and of Ethionamide against *Mycobacterium leprae*[1] (34162)

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Clofazimine (B.663) is one of the most active of a series of phenazine compounds with antituberculosis activity synthesized by Barry and his colleagues (1). It is very slowly excreted and, on continuous administration, accumulates in the tissues and may even form crystals there (2). It is reported to have good therapeutic effect in leprosy in dosages of 100–300 mg/day (3, 4), and it is the drug of choice in the treatment of infections with sulfone-resistant *Mycobacterium leprae* (5). Its chief disadvantage is that it pigments the skin strongly in currently used dosages (6). It is active against *M. leprae* in mice when administered continuously in dosages in the range of 0.01% in the diet (7–9).

Ethionamide is a "second-line" antituberculosis drug. The hepatotoxicity that it causes in some patients is reversible when the drug is stopped (10). Its clinical activity in previously untreated leprosy is not clear (11). In mice, when tested by the kinetic method (12) in a dosage of 0.2% in the diet for 75 days, it had the residual effect characteristic of bactericidal activity (unpublished result).

Materials and Methods. The mice were inoculated in the right hind foot pad with 5000 *M. leprae* of a mouse passage strain. The drugs were administered as mixtures in the unpelleted diet according to the schedule indicated in Figs. 1 and 2. With the highest dosages (0.1% ethionamide and 0.01% B.663), the dry drugs were mixed directly into the diet; with the lower dosages, ethanolic solutions of the drugs were added. The B.663 was received through the courtesy of Dr. W. A. Vischer, J. R. Geigy, Basel, and ethionamide through the courtesy of Dr. John N. Williams, Ives Laboratories, New York City. All drug diets were mixed in a twin-shell liquids–solids blender. The controls received unpelleted diet without drug. The counts of *M. leprae* were carried out on pools of the foot pad tissues of 4 mice in nearly all instances, except that the pools of the groups treated from 76 to 167 days represented 2 mice from 295 days onward in the B.663 mice and from 204 days onward in the ethionamide mice. Earlier publications describe details of the methods for working with *M. leprae* in mice (13, 14) and for counting *M. leprae* microscopically (15). Two drug schedules were used. By the kinetic method (12), the drugs were administered only for the 89-day period beginning 76 days after infection. By the other, called the continuous method, the drugs were administered continuously from the day of infection until bacterial growth in the controls had reached the plateau phase. The kinetic method makes it possible to distinguish drug treatments that are merely bacteriostatic while drug is present from those that produce bactericidal effects or bacteriostatic effects that persist for a time after disappearance of drug. The amount of bacterial growth delay was estimated by graphical comparison with the curve for the average of the controls.

Results. The results with B.663 are given in Fig. 1. A dosage as low as 0.0001% was active. This dosage delayed bacterial growth a total of 170 days, or 95 days longer than the period of drug administration. The dosage of 0.001% delayed growth somewhat

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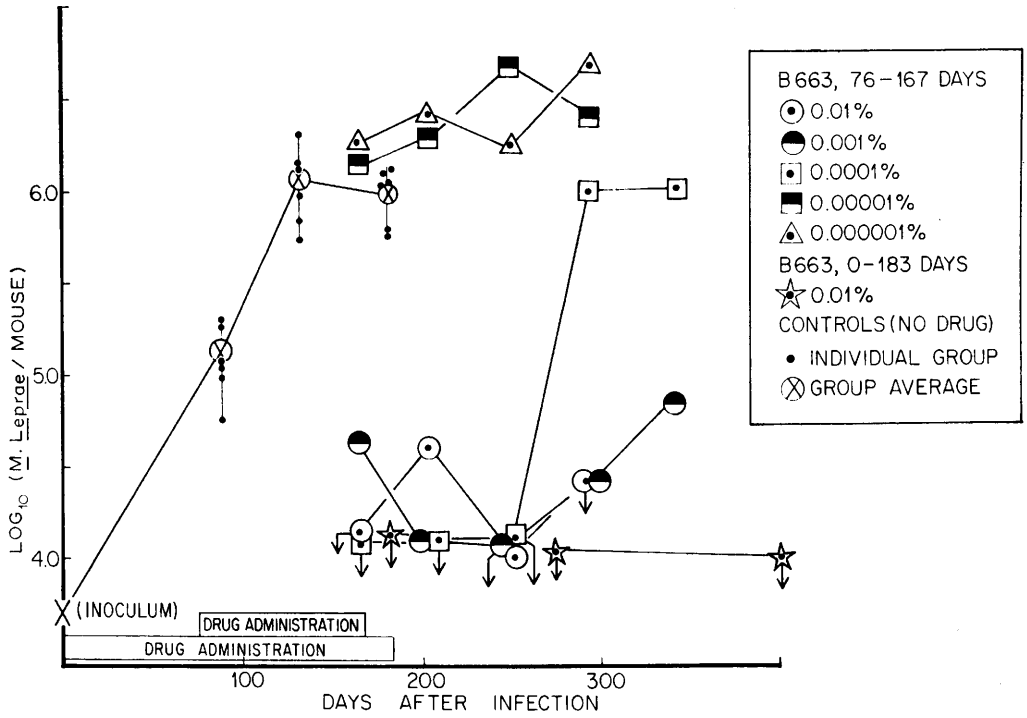

FIG. 1. Effect of B.663 on multiplication of *M. leprae*. The drug was administered by mixture into the diet in the concentration shown. The values marked by arrows indicate maximal estimates in cases where no bacilli were found during the counting procedure.

longer but apparently allowed some late growth to appear eventually. With 0.01% on either schedule there was no evidence of subsequent bacterial growth.

The B.663 appears to have stopped bacterial growth shortly after its administration was begun, even in the lowest active dosage, 0.0001%. Because of its tendency to accumulate in the tissues one might have expected that some time must elapse before the concentration in the environment of the organisms could reach effective levels. Similarly, with the next lower dosage, 0.00001%, there was no suggestion that it accumulated to effective local concentrations during the period it was given.

The results with ethionamide are given in Fig. 2. The minimal active dosage was 0.01%; at this dosage bacterial growth appeared as soon as the drug was stopped. When the next higher dosage, 0.1%, was administered from 76 to 167 days, bacterial growth was delayed at least 267 days; the last harvest in this group was so low that it was not clear whether it actually represented new growth. When 0.1% was given from 0 to 183 days, no bacterial growth was detected and the growth delay was estimated as at least 374 days. In the previous unpublished experiment 0.2% ethionamide was administered from 73 to 164 days; no evidence of growth was seen, and this finding was estimated to be a growth delay of greater than 299 days.

Discussion. The interpretation of the results, in terms of antibacterial effects, is simpler for ethionamide because it is a rapidly excreted drug (10). In its minimal effective dosage, 0.01% in the diet, ethionamide delayed growth only for the period of its administration. Its effect was pure bacteriostasis acting only during the period of drug administration. In a dosage of 0.1% from 76 to 167 days ethioamide produced a delay of at least 267 days, or 176 days longer than the period of its administration. Such delay can,

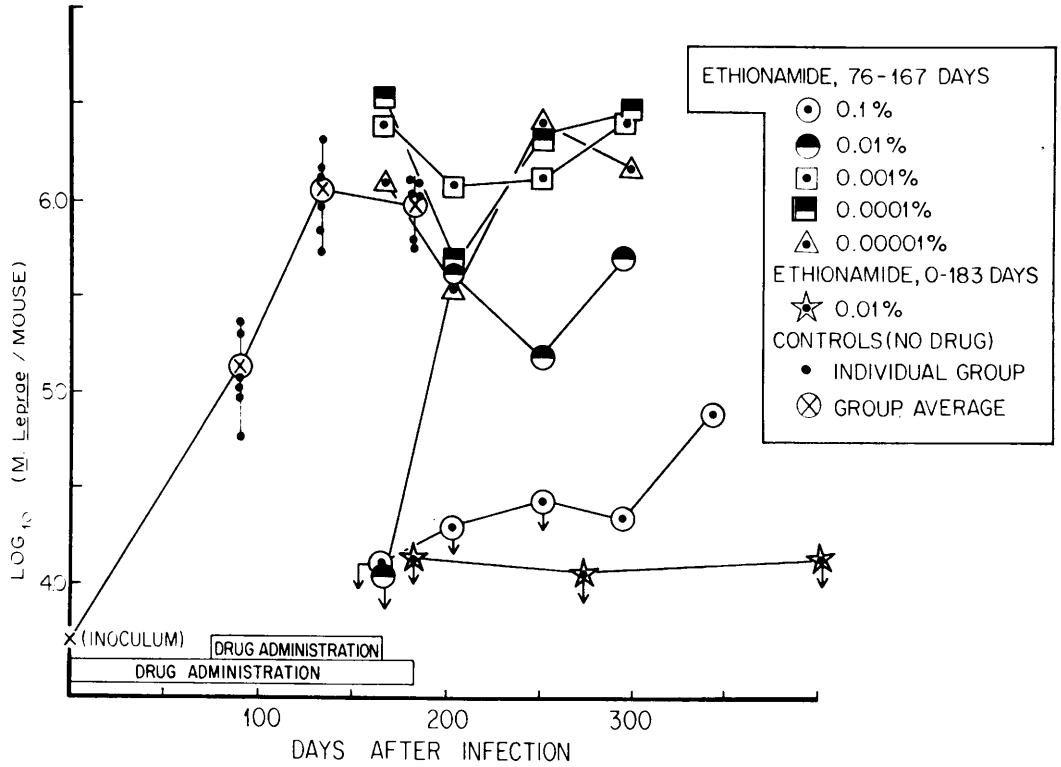

FIG. 2. Effect of ethionamide on multiplication of *M. leprae*. The drug was administered by mixture into the diet in the concentration shown. The values marked by arrows indicate maximal estimates in cases where no bacilli were found during the counting procedure.

in theory, arise from the time required for elimination of drug, from persisting bacteriostasis, or from bactericidal effect. The concentration of ethionamide in the environment of the bacilli probably fell below the minimal inhibitory concentration in a day. There is no evidence in the present experiment to discriminate between bactericidal effect and bacteriostasis that persists temporarily after disappearance of drug. If the delay is ascribed to killing of most of the bacteria, with subsequent replacement of killed bacteria by multiplication of the surviving fraction at a normal logarithmic growth rate of 12.5 days/generation (16), the 175 days of delay lasting beyond the period of drug administration and drug elimination, would amount to a delay of 14.0 generation times and would be equivalent to a 1.6 $\times$ 10^4-fold decrease. If the bacterial population that was present when the drug was started had been decreased to this extent, a few bacilli might have remained to initiate the possible late growth. In a dosage of 0.1% from 0 to 183 days, ethionamide produced at least 190 days of growth delay beyond the period of drug administration and drug elimination. If this delay is ascribed to bactericide, one could estimate similarly that none of the population present when drug was started survived, and that even longer observation would not have revealed growth.

In contrast, B.663 is a slowly excreted drug. Present knowledge of drug elimination at the low concentrations involved is too meager to allow one to rule out the possibility that drug persistence accounts for all the growth delay that was observed. The mice receiving 0.01% B.663 had obviously pigmented tissues and the pigment remained in the foot pad tissues, and especially in the foot bones, of the mice killed at 402 days, or 219 days after the drug was discontinued. Evidence that B.663 does kill *M. leprae* in

humans receiving 100–200 mg a day has, however, been obtained in studies in which bacterial viability was judged by changes in bacterial morphology (3, 4) and in a study in which bacterial viability was tested more rigorously by mouse foot pad inoculation (C. C. Shepard, L. Levy and P. Fasal, unpublished). (L. Levy, U. S. Public Health Service Hospital, San Francisco, has also obtained evidence that 0.0001% B.663 in the diet exerts residual activity against *M. leprae* in mice, personal communication.)

Against *M. tuberculosis* infections in mice, the minimal effective dosage of B.663 is about 2 mg/kg/day, as judged by the lengthening of survival time (2). A course of treatment concluded 4 weeks before infection increased survival time (2), as did a single days's treatment with 100 mg/kg on the day of infection (17). It is interesting that, in the curative treatment of experimental tuberculosis in the mouse, the combination of B.663 and ethionamide produced more rapid and more extensive bacterial killing than either drug alone (18).

The present results with B.663 against *M. leprae* suggest that lower dosages than those currently employed might be effective, and that pigmentation may not be a necessary accompaniment of activity against *M. leprae*. To estimate the minimal effective dosage in man from the minimal effective dosage in mice is difficult, however, because of the unequal distribution of B.663 in different tissues (2) and presumably even in different cells in the same tissue. The minimal effective dosage in mice, 0.0001% in the diet, amounts to about 0.1 mg/kg/day; this intake in man would be about 7 mg/day, or 1/15 to 1/45 the current dosages.

The same minimal effective dosage, 0.0001% in the diet, was found in mice for 4,4′-diaminodiphenylsulfone (DDS) (19), the standard drug in treatment of leprosy. The usually accepted dosages of DDS in man produce about 100 times the apparent minimal inhibitory concentration, and a repository form, 4,-4′-diacetyldiaminodiphenylsulfone, has since been found active in human leprosy in first trial (20). In the case of B.663, spaced ingestion or injection of the unmodified compound would be suggested, since its elimination from the tissues is very slow. Simplification of drug administration, for therapy or chemoprophylaxis, has special value in leprosy because many of the important endemic areas have limited medical resources.

Summary. The activity of B.663 and ethionamide against *Mycobacterium leprae* in mice was determined at dosages of drug that varied by factors of 10. The minimal effective dosage of B.663 was 0.0001% in the diet: that of ethionamide was 0.01% in the diet. The very low minimal effective dosage of B.663 suggests that spaced ingestion or injection might be effective against leprosy in man.

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