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Combinations of Drugs Against *Mycobacterium leprae* Studied in Mice[1]

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Although work on the action of single drugs against *M. leprae* in mice is now extensive (reviewed in ([12])), not much has been done on combinations of drugs. One report ([8]) concerned combinations of DDS with DPT, INH, PAS, or SM [3] ; when given alone, the latter four drugs were found to have limited activity, i.e., they were inactive in part of the experiments or they possessed only bacteriostatic effect. When given with DDS, none had any potentiating effect; PAS was partially inhibitory, INH seemed to be slightly inhibitory, and DPT and SM were without effect. In another experiment ([9]) the effect of PAB on DDS was studied; weak inhibition was observed when a low dosage of DDS and a high dosage of PAB were given.

Further work on combinations of antileprosy drugs is related here. The combinations studied include several drugs that have been found to be bactericidal for *M. leprae*.

MATERIALS AND METHODS

The methods have been described earlier ([6, 10]). In brief, the drugs were mixed in the diet and administered for a limited period during the logarithmic phase of the multiplication of *M. leprae* in the foot pads of mice. The bacillary growth curve in the treated mice was compared to that in the control mice, and the amount of growth delay was estimated from the graphed results. By this kinetic method of drug administration, drugs that are merely bacteriostatic prevent bacterial multiplication only while they are present in the body, whereas bactericidal drugs, because they eliminate part or all of the bacterial population, delay the appearance of bacterial growth for periods that persist after the drug has disappeared from the body. Moreover, since the drugs are present for only a limited period, they are put to a more severe test. The strains of *M. leprae* were ones in continuous passage in mouse foot pads.

RESULTS

TMP and DDS. TMP strongly potentiates the effect of sulfonamides against certain bacteria and malaria parasites through "sequential blockade", i.e., TMP and sulfonamides both interfere with folate synthesis—sulfonamide (and DDS) in an early step by competing with PAB, and TMP in a later step by inhibiting dihydrofolate reductase ([1]). In effect, the combination of TMP and sulfonamide also has a broader anti-bacterial spectrum since the potentiating effect extends to bacteria that have borderline sensitivities to the individual drugs. However, TMP did not potentiate DDS against *M. leprae* (Table 1 and Figure 1); in fact, the results suggest a weak antagonistic effect, since the growth delay was somewhat shortened when TMP was given with DDS. TMP was tested in high dosage (0.1% or approximately 100 mg/kg/day), and 0.01%, and it was inactive by itself. DDS was tested at 0.01% (the intake that produces approximately the same blood and tissue levels as standard dosages in man ([13])), at 0.0001% (the usual minimal effective dosage ([14])), and at the next lower dosage, 0.00001%. The 33 days of delay observed with 0.00001% DDS and 0.1% TMP was probably not significant.

INH, PAS, and PAB with DDS. INH, PAS, and PAB have been found to antagonize partially the effect of DDS against *M. leprae* ([8, 9]). As before, they were tested at their maximum tolerated dose; in addition PAS and PAB were tested at the same dosage as INH against the minimal effec-

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[3] Abbreviations used throughout: B663, clofazimine; DDS, dapsone; DPT, thiambutosine; Eth, ethionamide; INH, isoniazid; PAB, para-aminobenzoic acid; PAS, aminosalicylic acid; SM, streptomycin; TMP, trimethoprim.

TABLE 1. *Effect of combinations of TMP and DDS against* M. leprae.

Drug[a,b]	Growth delay (days)
0.1% TMP	0
0.01% TMP	0
0.01% DDS	143
0.01% DDS + 0.1% TMP	119
0.01% DDS + 0.01% TMP	107
0.0001% DDS	83
0.0001% DDS + 0.1% TMP	41
0.0001% DDS + 0.01% TMP	81
0.00001% DDS	0
0.00001% DDS + 0.1% TMP	33
0.00001% DDS + 0.01% TMP	0

[a] The drugs were administered from the 75th to the 135th day after infection.
[b] Abbreviation: TMP, trimethoprim; DDS, dapsone.

tive dosage of DDS. When given alone, only PAS (and DDS) was anti-bacterial in this experiment (Table 2, Figure 2). Against 0.01% DDS, only PAB appeared to have an antagonistic effect. Against 0.0001% DDS, none of the compounds had distinct effect.

B663, Eth, and DDS. When tested singly in higher dosages, DDS, B663, and Eth have been found to be bactericidal against *M. leprae* [11] and these results were confirmed in the present experiment (Tables 3 and 4, and Figure 3). For the studies of combinations, two dosages were tested with each drug—a high level corresponding to acceptable dosages in man, and the minimal effective dosage. The results in Tables 3 and 4 are arranged in order of the growth delay produced by the single drugs, so that inspection down the columns of results can reveal any additive effect produced by the combinations. Of the eight combinations, only two, 0.0001% B663 plus 0.0001% DDS and 0.1% Eth plus 0.01% DDS, failed to manifest additive effect.

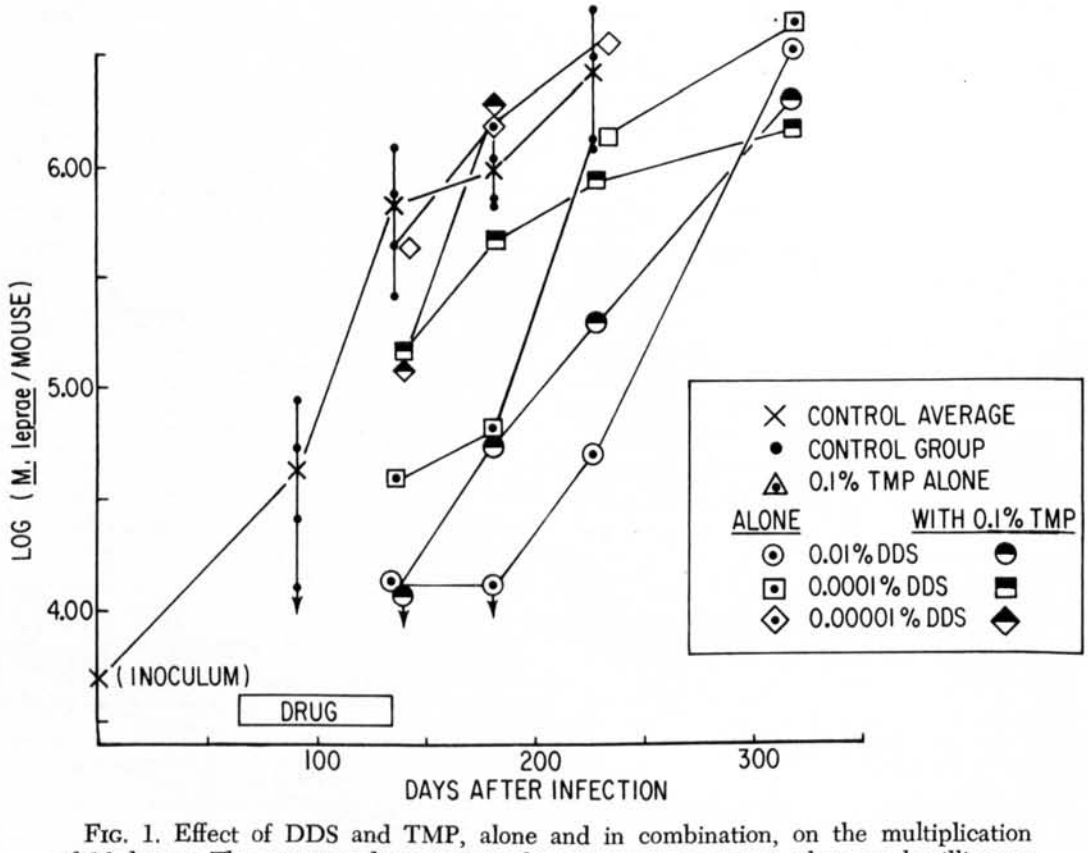

FIG. 1. Effect of DDS and TMP, alone and in combination, on the multiplication of *M. leprae*. The arrows indicate maximal estimates in instances where no bacilli were found during the counting procedure.

Combinations of these drugs at the other dosage level gave additive results.

DISCUSSION

The results with drugs tested singly against *M. leprae* in this and other laboratories have recently been reviewed ([12]). Singly, the drugs of the first two experiments (Tables 1 and 2) have had limited activity or no activity. TMP (0.1% in the diet) was inactive alone. DPT (0.1%) has not been active in all experiments when administered continuously, and in one kinetic experiment it was bacteriostatic. INH (0.01%) has been active with continuous administration, but not in kinetic experiments. PAS (0.6%) has been active with continuous administration, but inactive or only bacteriostatic in kinetic experiments. None of these drugs (TMP, DPT, INH, PAS, and SM) have been found to add to the activity of DDS, and PAS was partially antagonistic in an earlier experiment ([8]).

The last three compounds, B663, DDS, and Eth when tested singly, have given bactericidal-type results in kinetic experiments and combinations of these three were usually mutually additive. Rifampin, a drug more rapidly bactericidal than B663, DDS, or Eth is also being tested in

TABLE 2. *Effect of combinations of INH, PAS, or PAB with DDS.*

Drug[a,b]	Growth delay (days)
0.01% INH	0
0.6% PAS	94
1.0% PAB	0
0.1% DDS	213[c]
0.01% DDS + 0.01% INH	169
0.01% DDS + 0.6% PAS	178
0.01% DDS + 1.0% PAB	77
0.0001% DDS	80
0.0001% DDS + 0.01% INH	102
0.0001% DDS + 0.6% PAS	89
0.0001% DDS + 0.01% PAS	105
0.0001% DDS + 1.0% PAB	82
0.0001% DDS + 0.01% PAB	107

[a] Drugs were administered from the 73rd day to the 145th day after infection.

[b] Abbreviations: INH, isoniazid; PAS, para-aminosalicylic acid; PAB, para-aminobenzoic acid; DDS, dapsone.

[c] Estimated from last bacillary count which was $10^{5.09}$.

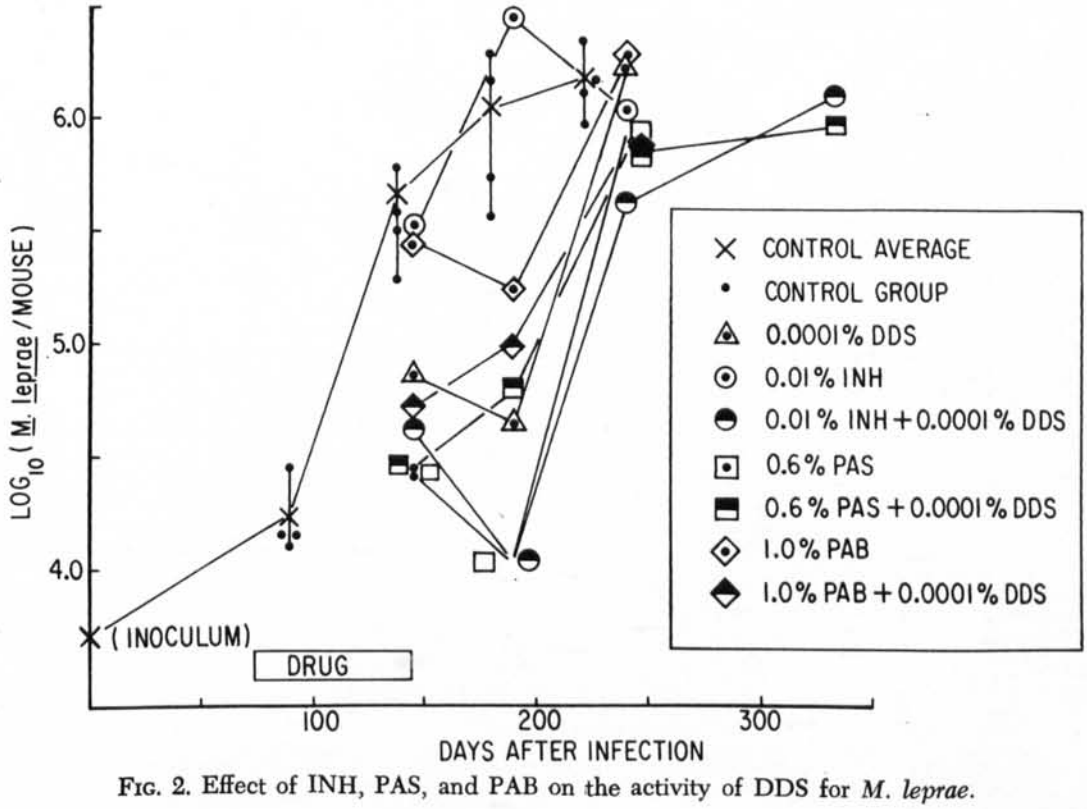

FIG. 2. Effect of INH, PAS, and PAB on the activity of DDS for *M. leprae*.

TABLE 3. *Effect of combinations of B663, DDS, and Eth in high dosage.*

Drug[a,b]	Growth delay (days) B663 0.004%	Eth 0.1%	DDS 0.01%
0.004% B663	(342)[c]		
0.1% Eth	>405	(235)[c]	
0.01% DDS	>407	>408	(165)[c]
0.01% DDS + 0.1% Eth	>408		

[a] Abbreviations: B663, clofazimine; Eth, ethionamide; DDS, dapsone.
[b] Drugs were administered from the 70th to the 133rd day after infection.
[c] Parentheses indicate results with single drugs.

TABLE 4. *Effect of combinations of B663, DDS, and Eth in low dosage.*

Drug[a,b]	Growth delay (days) B663 0.0001%	DDS 0.0001%	Eth 0.01%
0.0001% B663	(103)[c]		
0.0001% DDS	95	(102)[c]	
0.01% Eth	162	157	(86)[c]
0.0001% DDS + 0.01% Eth	194		

[a] Abbreviations: B663, clofazimine; Eth, ethionamide; DDS, dapsone.
[b] Drugs were administered from the 70th to the 133rd day after infection.
[c] Parentheses indicate results with single drugs.

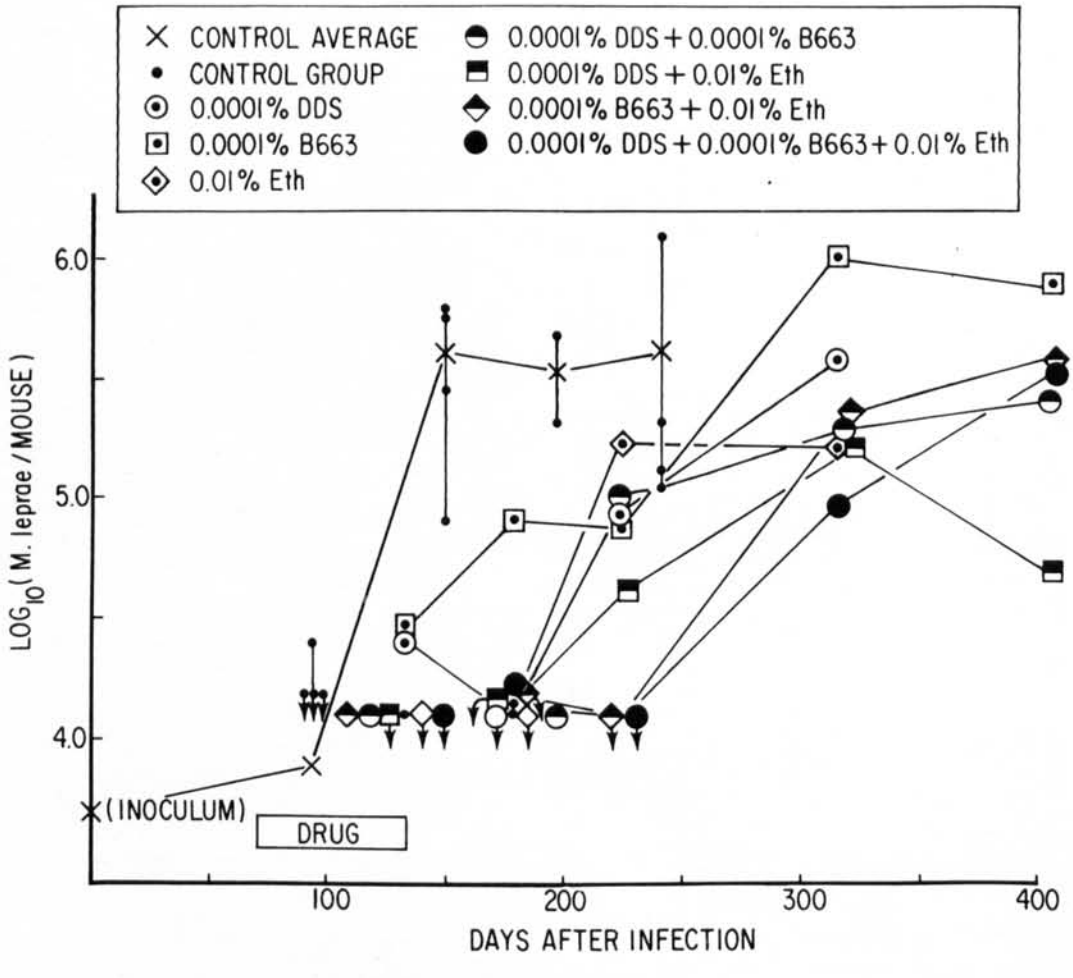

FIG. 3. Activity for *M. leprae* of DDS, B663, and Eth alone and in combination.

combination with DDS, and preliminary results indicate an additive effect here also. Thus the results to date indicate that tests of combinations should concentrate on drugs that are bactericidal when given alone. Combinations should probably be tested in mice before they are tried in humans in order to detect antagonisms between drugs, a possibility that would increase the risk of development of resistance to the drugs in the combination.

The mechanism of action of DDS is thought to be a competition with PAB in the synthesis of folate compounds, so PAB was expected to antagonize the action of DDS, as it does the action of sulfonamides on other bacteria. However, the antagonism of PAB was weak; its antagonism had also been weak in the earlier experiment ([9]). A possible explanation, which is consistent with its low minimal effective dosage, is that DDS is better able to penetrate *M. leprae*, so that the relative concentrations of DDS and PAB at the site of the condensing enzyme favors DDS.

The use of drug combinations in therapy has inherent disadvantages because of the added risk of drug toxicity and the greater difficulty in determining which drug is responsible for a toxic reaction. Nevertheless, drug combinations need to be considered in the treatment of leprosy for two reasons. One is that the combination might be more rapidly effective than the more active member of the combination. The therapy of lepromatous leprosy frequently requires five years or more, and many patients lapse treatment, especially when their disease appears to them to improve. The present results indicate that combinations of B663, DDS, and Eth may be more rapidly effective than the single drugs. Mouse experiments with the design used here correspond, however, only to the initial part of the bactericidal process in the patient. At the time the drug administration was begun in the experiments, the live bacterial population was probably not more than 10^4 per mouse, whereas lepromatous patients probably average about 10^{11} *M. leprae* in their tissues ([7]). Other experimental models that develop larger populations might make possible the study of a greater portion of the killing curve. Nevertheless, the only way to determine the last and critical part of the curve is apparently to study relapse rates in patients who have stopped therapy at various times, especially since the histologic location of the bacilli, their metabolic state, and the disposition of active drug may be involved.

The other reason for considering drug combinations is that they may prevent the emergence of drug-resistant bacteria, as they do in tuberculosis. Resistance to DDS has been demonstrated ([4, 11]). The rate of eventual appearance of drug resistance in lepromatous patients entering treatment with DDS or sulfetrone was first estimated to be very low—perhaps 1 in 1000 in patients beginning therapy with DDS or sulfetrone ([4]) and perhaps 1 in 300 in those starting with glucosulfone (Promin) ([14]). However, the peak time of appearance appears to be 12-18 years after the initiation of therapy so cases are continuing to appear; the eventual rate in patients with lepromatous leprosy starting on dapsone or sulfetrone is not yet determined but will be considerably higher than the previous estimates of less than 1% ([3]).

The reason that drug combinations are more effective than single drugs in preventing the appearance of drug-resistant bacilli is, of course, that the resistance ratio (proportion of drug-resistant bacilli) to a combination of unrelated drugs is the product of the resistance ratios to each member of the combination. The present mouse model is not suited for the determination of resistance ratios of *M. leprae*, again because the number of *M. leprae* in the mouse is inadequate. Nevertheless, the emergence of significant amounts of drug-resistance appears by itself to be indication for the introduction of effective drug combinations in the primary treatment of leprosy.

In patients whose bacilli have already developed DDS-resistance, combinations of anti-leprosy drugs would have even more indication. Not enough experience has accumulated to indicate the rate of resistance that will eventually develop to B663, Eth, and rifampin when they are given alone. One might urge that, since these patients are no longer able to benefit from DDS, they ought henceforth to be treated with

combinations of at least two of these three drugs or of other drugs that may be found to be as effective.

Leprosy patients may have clinical tuberculosis and the problem of antagonisms between anti-bacterial drugs then appears. The results to date would suggest that PAS should not be used in a patient receiving DDS. Other anti-tuberculosis drugs are now available whose actions clearly do not relate to PAB metabolism. These include rifampin, the only anti-mycobacterial drug that appears distinctly effective in both diseases ([2, 5]).

SUMMARY

Combinations of drugs were tested in infections of *Mycobacterium leprae* in mice. Trimethoprim, a drug that potentiates the activity of sulfonamides against many bacteria and parasites, was not active by itself against *M. leprae* and appeared to antagonize dapsone slightly. Para-aminobenzoic acid, the theoretical antagonist of dapsone, was only partially antagonistic in a particular combination. Aminosalicylic acid, isoniazid, and thiambutosine when tested singly have irregular or only bacteriostatic activity against *M. leprae*; they did not potentiate the activity of dapsone. Clofazimine, ethionamide, and dapsone when tested singly have bactericidal activity for *M. leprae*; in most of the various possible combinations they potentiated each other's action.

RESUMEN

Se probaron combinaciones de drogas en infecciones por *Mycobacterium leprae* en ratones. La trimetoprima, uno droga que potencia la actividad de las sulfonamidas contra una gran cantidad de bacterias y parásitos, no fué activa por si misma contra el *M. leprae* y apareció como antagonizando ligeramente a la dapsona. El ácido para-aminobenzoico, que es el antagonista teórico de la dapsona, fué sólo parcialmente antagónico en una combinación determinada. El ácido aminosalicílico, la isoniazida y la tiambutosina cuando se probaron solas tuvieron una actividad irregular o solamente bacteriostática contra el *M. leprae* no potenciaron la actividad de la dapsona. La clofazimina, la etionamida y la dapsona cuando se probaron aisladas tuvieron actividad bactericida contra el *M. leprae;* en la mayor parte de las combinaciones posibles entre ellas, cada una potenció la acción de las otras.

RÉSUMÉ

Des associations médicamenteuses ont été étudiées dans des infections par *Mycobacterium leprae* chez la souris. La trimethoprime, qui est un médicament qui renforce l'activité des sulfamides contre beaucoup de bactéries et de parasites n'était pas active par elle-même contre *M. leprae* el semblait dotée d'une légère activité de compétition avec la dapsone. L'acide para-aminobenzoïque, antagoniste théorique de la dapsone, ne révélait qu'une capacité d'antagonisme partiel à l'égard de cette combinaison particulière. L'acide aminosalicylique, l'isoniazide, et la thiambutosine, quand on les essaie isolément, présentaient une activité irrégulière, ou seulement bactériostatique, contre *M. leprae;* ils ne renforçaient pas l'activité de la dapsone. Etudiées isolément, la clofazimine, l'éthionamide et la dapsone présentaient une activité bactéricide pour *M. leprae;* avec la plupart des diverses combinaisons possibles, ils renforçaient leur action mutuelle.

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Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.

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