lates HCl secretion by the isolated gastric mucosa of the bullfrog. On a molar basis, human gastrin is about ½ as potent as pentagastrin and about 1/200 as potent as porcine gastrin. Maximal secretory response to human gastrin, pentagastrin, and porcine gastrin occurred at 2.5×10^{-6}, 2.5×10^{-6} or 2.5×10^{-5}M, and 2.5×10^{-8} *M*, respectively.

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Retardation of the Emergence of Isoniazid-Resistant Mycobacteria by Phenothiazines and Quinacrine (33522)

ROBERT E. MANION, S. GAYLEN BRADLEY, AND WENDELL H. HALL

Medical Service, Veterans Administration Hospital, Minneapolis, Minnesota 55417; and The Department of Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota 55455

Sevag and his co-workers (1–5) have reported that Atabrine (quinacrine hydrochloride) in combination with an antibiotic prevents the emergence of antibiotic-resistant strains of bacteria from a normal population. They have described the use of such combinations in the prevention of emergence of antibiotic-resistant populations of *Staphylococcus aureus* and *Escherichia coli*. When they tested an antibiotic-resistant organism, they found the quinacrine–antibiotic combination to be ineffective in delaying the onset of growth.

Warren *et al.* (5) have found that with isoniazid-sensitive strains of *Mycobacterium tuberculosis* and *M. bovis* resistance to isoniazid (INH) is much less apparent in the course of six subcultures if sublethal concentrations of quinacrine are added. They have also found that quinacrine in combination with dihydrostreptomycin sulfate or para-aminosalicylic acid (PAS) eliminated the emergence of resistance to these antituberculous drugs.

In the light of these investigations, it was thought that the measurement of the effect of various heteroanthracenes on the growth rates of various mycobacteria in the presence of marginally or minimally inhibitory amounts of INH would be worthwhile. Comparison of the effects of various heteroanthracenes to those of PAS, ethionamide, and ethambutol on development of resistance to INH was made. The following report compares the various systems as measured photometrically.

Materials and Methods. M. tuberculosis strain $H_{37}Ra$ and *M. intracellulare* strain P-55 ("Battey" mycobacterium, Runyon Group III) were grown in Middlebrook 7H9 broth (Difco) enriched with oleate–dextrose–albumin complex (Difco) and with 0.5% Tween 80 (Atlas Chemical Co.) for dispersed growth. Test organisms were grown on slants of Lowenstein-Jensen medium and were then inoculated into flasks of 7H9 broth. Each tube of broth (9.5 ml) used in the test was then inoculated with 0.5 ml of a 72–96 hr

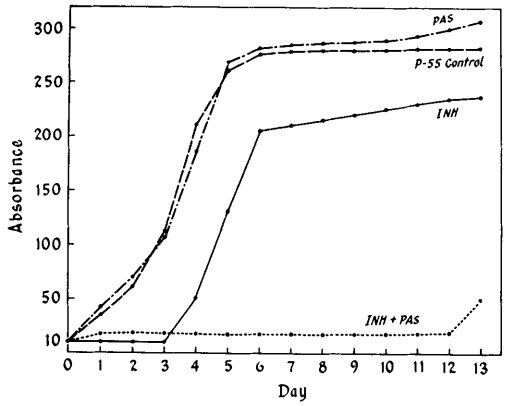

FIG. 1. Retardant effect of *p*-aminosalicylic acid (PAS) on resistance of the Battey organism (strain P-55) to isoniazid (INH). Each drug concentration was 10 μg/ml.

broth culture. Each tube contained 5, 10, or 50 μg/ml of the antibiotic, 5, 10, or 50 μg/ml of the resistance retarding compound or one of the possible combinations of these concentrations. Incubation was at 37° in a tissue culture rotating drum.

Turbidities were measured (after shaking in a Vortex mixer) in a Coleman Jr. spectrophotometer at 600 mμ. Daily readings were made for a period of 3 weeks.

The sources of the antituberculous drugs were as follows: isoniazid and sodium para-aminosalicylic acid, Panray Division, Ormont Drug and Chemical Co., Englewood, N.J.; streptomycin sulfate, Pfizer Laboratories, Brooklyn, N.Y.; ethionamide, Ives Laboratories, New York, N.Y. and ethambutol, Lederle Laboratories, Pearl River, N.Y.

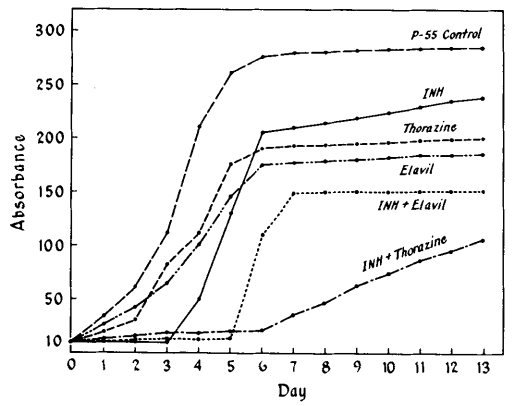

FIG. 2. Delay of resistance of the Battey culture (strain P-55) to INH by a phenothiazine (Thorazine) and by a dibenzazepine (Elavil). Each drug concentration was 10 μg/ml.

The agents used to retard resistance to antituberculous drugs were obtained as follows: chlorpromazine hydrochloride, Smith, Kline, & French Labs., Philadelphia, Pa.; promethazine hydrochloride, Wyeth Labs., Philadelphia, Pa.; hydroxyzine hydrochloride, J. B. Roering Co., New York, N.Y.; amitryptyline hydrochloride, Merck, Sharp, & Dohme, West Point, Pa.; acriflavine hydrochloride, National Analine Co., New York, N.Y.; and quinacrine hydrochloride, Winthrop Labs., New York, N.Y.

Results. The system employing the "Battey" strain was found to be the most reliable for detecting compounds that retard the emergence of drug-refractory populations.

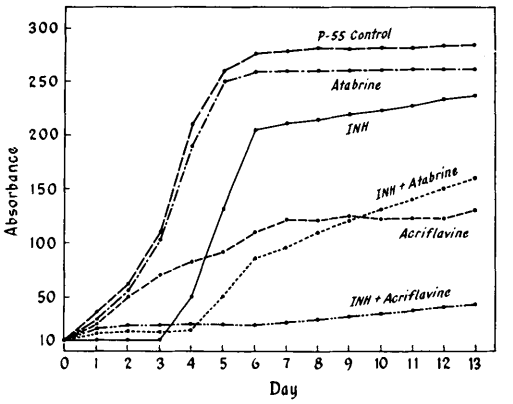

FIG. 3. Retardant effect of quinacrine HCl (Atabrine) and of acriflavine on resistance of the Battey organism of INH. Each drug concentration was 10 μg/ml.

This strain (P-55) was moderately resistant to 10 μg of INH/ml. This drug was selected for testing the effects of resistance retardants because outgrowth reproducibly occurred after a lag of 3–4 days. The data from which the curves in the three figures were drawn represent averages of 3 to 7 experiments.

Figure 1 indicates the ability of PAS to retard the resistance of the P-55 strain to INH at a combination of 10 μg/ml each. It will be noted that the strain of mycobacteria used is resistant to PAS and that the "Battey" organism manifests substantial resistance to INH after the third day. Of the

compounds tested that were effective in retarding resistance development (Figs. 2 and 3) PAS was the most efficacious. PAS delayed the growth in the presence of INH until day 13 of incubation.

Chlorpromazine (Thorazine) at 10 μg/ml, as well as other common phenothiazines tested, such as promethazine (Phenergan) and hydroxyzine (Atarax), when combined with INH at 10 μg/ml served to retard growth until day 6 and manifested the effect through day 13, by which time turbidities had reached only one third those of the bacterial control cultures without any drug additions (Fig. 2).

A combination of quinacrine hydrochloride (Atabrine) and INH each at a concentration of 10 μg/ml gave results with the Battey organism similar to those obtained with amitriptyline and INH, with only a slightly earlier escape and higher final turbidity (Fig. 3). Quinacrine did not substantially extend the lag in growth imposed by INH but it slowed the rate of outgrowth of the drug refractory population. Acriflavine alone depressed growth, and with INH (each at 10 μg/ml) acriflavine was more effective than quinacrine in retarding outgrowth; however, this probably reflects the additive toxicity of each drug.

Amitriptyline (Elavil), a representative of the dibenzazepine antidepressants, was found by Sevag to be effective in retarding the streptomycin resistance of *S. aureus* at a concentration of 75 μg/ml. With the P-55 strain of "atypical" mycobacteria it was found to be less effective in preventing outgrowth with INH than was chlorpromazine (Thorazine) at a concentration of 10 μg/ml (Fig. 2). With the $H_{37}Ra$ strain of *M. tuberculosis* a definite stimulation of growth was evident with amitriptyline alone at 5 μg/ml and a slight retardation of growth at 10 μg/ml.

With streptomycin (in place of INH) at 5 μg/ml or more, no growth was evident until day 8 when the P-55 strain was used, and the resistance-retardant compounds tested (quinacrine, chlorpromazine and promazine) did not prolong this time. Although the P-55 strain is highly resistant to PAS (over 50 μg/ml), the addition of minimal amounts (5 μg/ml) of this drug to INH or streptomycin at 5 μg/ml markedly reduced the viable population of the cultures.

Ethionamide, when used in combination with INH in cultures of P-55, did not delay outgrowth, and the growth curves of the combinations closely paralleled that of the ethionamide alone. Similarly, ethambutol with INH gave a growth response by strain P-55 almost identical to that with ethambutol alone.

Discussion. While it has been shown that the outgrowth of drug-refractory organisms of certain strains of mycobacteria can be delayed or prevented by quinacrine and some of the major phenothiazine tranquillizers, it was observed that their efficacy did not approach that of PAS when combined with INH *in vitro*. They do seem to be more potent than ethambutol when tested in a similar system.

Since INH is known to be a powerful chelating agent with ability to combine with certain divalent metallic ions, this drug may affect nucleic acid biosynthesis and metabolism. LePecq (6) has reported that quinacrine combined with DNA interfers with the action of deoxyribonuclease. Consequently, it would appear that the quinacrine–DNA complex may be of such a nature as to alter the action of INH. Kurnick (7) found quinacrine combines with DNA in the proportion of 1 molecule of quinacrine per 4 nucleotides. Similarly Lerman (8) concluded that the acridine molecules are inserted firmly between 2 sequential base pairs in the central core of the DNA requiring some local untwisting and extension of the double helix. Hele (9) showed that phenothiazine drugs also form insoluble complexes with nucleic acids, indicating a possible similar mode of action with these compounds.

It is apparent that there is a diverse species sensitivity to the "anti-DNA" drugs tested, as well as variations when combined with antituberculous drugs. The effects of the retardants were less consistent when combined with streptomycin than with INH, but this may indicate a variation in the original

resistance patterns of the mycobacteria tested.

Summary. Growth of *Mycobacterium intracellulare* strain P-55 ("Battey" organism) was most strikingly retarded by a combination of isoniazid (INH) and para-aminosalicylic acid (PAS), though the organism was resistant to PAS and only marginally sensitive to INH. Among the phenothiazines, chlorpromazine, promethazine, and hydroxyzine also delayed outgrowth in the presence of INH by the "Battey" organism. Quinacrine hydrochloride also prolonged growth inhibition of strain P-55 in the presence of INH. The dibenzazepine, amitriptyline, was less effective than the phenothiazines in delaying INH resistance in strain P-55. The P-55 strain was quite sensitive to streptomycin but none of the resistance-retardants delayed outgrowth of drug-refractory organisms. Though strain P-55 was resistant to PAS at 50 μg/ml, only 5 μg PAS/ml with either INH or streptomycin was able to vastly reduce the viable count. Neither ethionamide nor ethambutol prolonged the lag in growth of the "Battey" organism caused by INH.

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