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Maurice L. Cohn, … , Gardner Middlebrook, William F. Russell Jr.

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Research Article

COMBINED DRUG TREATMENT OF TUBERCULOSIS. I. PREVENTION OF EMERGENCE OF MUTANT POPULATIONS OF TUBERCLE BACILLI RESISTANT TO BOTH STREPTOMYCIN AND ISONIAZID *IN VITRO*

By MAURICE L. COHN, GARDNER MIDDLEBROOK and WILLIAM F. RUSSELL, Jr.

(From the Department of Research and Laboratories, National Jewish Hospital at Denver, and the Departments of Medicine and Microbiology, University of Colorado School of Medicine, Denver, Colo.)

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Treatment of tuberculosis with single drug regimens employing streptomycin, para-aminosalicylic acid, or isoniazid has proved to be clinically inferior to the use of combinations of these drugs (1–3). The superiority of combined drug regimens has been manifest both in the percentage of patients rendered bacteriologically negative and in the lower incidence of the emergence of drug resistance.

Ehrlich (4) first conceived the generalization that chemotherapy combining two or more effective agents with independent actions would prove superior to single drug treatment by tending to prevent the parasite from developing resistance to the action of either of the drugs. This principle was applied to antimicrobial attack *in vitro* on *Mycobacterium tuberculosis* by Middlebrook and Yegian (5), who also outlined the theoretical basis for prevention of the emergence of drug-resistant mutants of tubercle bacilli by combined drug treatment. Demerec (6) employed more refined genetic principles to elaborate upon this theory. Then Karlson and co-workers (7) demonstrated the suppressive effect of combined drugs (streptomycin, para-aminosalicylic acid and promin) upon the emergence of streptomycin-resistant tubercle bacilli during the chemotherapy of tuberculosis in man. Since that time many studies pertaining to combined drug therapy have been reported both from the laboratory and from clinical investigation.

Thus, although it has been impossible to provide clinical evidence for superiority of combined drug treatment in many other infectious diseases, there seems to be no doubt of its superiority over the use of single drugs in cavitary pulmonary or renal tuberculosis. Nevertheless, in spite of this established superiority of combined drug regimens, the sputa of 10 to 20 per cent of previously untreated patients fail to become bacteriologically negative, on conventional double-drug programs, without ancillary collapse procedures or surgical intervention (8–10). Many of these chemotherapeutic failures are the result of the emergence of mutant forms [1] of tubercle bacilli which are resistant to one or more of the antimicrobial agents employed (12, 13).

Clinical investigation and laboratory studies have established the fact that the action of a single antimicrobial agent in adequate concentration *in vitro* will usually select a drug-resistant population of tubercle bacilli if, initially, the number of organisms is sufficiently large, and if they multiply to yield a detectable colony. Such investigations depend upon laboratory determination of the prevalence of drug-resistant mutants in large populations of the parasites. Most attempts to estimate the prevalence of drug-resistant mutants have been performed with cultures containing relatively small numbers of organisms, in liquid media, and with techniques requiring repeated subculture.

[1] The advisability of employing the term *mutants* to describe typical tubercle bacilli which are found to be resistant to the antimicrobial action of a drug may be questioned. This usage has been justified on the following grounds: *1)* Such organisms immediately proliferate from previously unexposed populations of organisms just as though they were present in such populations prior to selection by the suppressive action of the drug on the susceptible organisms. *2)* All other peculiarities of their properties are just as immediately apparent. *3)* In the case of one strain of tubercle bacilli this has been proved by replica plating techniques which allow for precise estimation of the drug susceptibility of clones before any drug action (11).

Such techniques are not adequate for a real estimation of the prevalence of drug-resistant mutants; only a few studies (14, 15) have been performed which provide the quantitative information needed.

In order to provide a rational bacterial genetic basis for the superiority of combined drug regimens and, perhaps, also for the not uncommon failure of this approach to chemotherapy of tuberculosis with conventional drug dosages, quantitative information is required on the prevalence of mutants resistant to two drugs (double mutants) in wild, previously unexposed populations of tubercle bacilli. Whether or not, and, if so, how many mutant forms simultaneously resistant to both streptomycin and isoniazid can be selected *in vitro* from extremely large populations of tubercle bacilli exposed to both of these agents in combination obviously has important clinical implications. The purpose of this report (the first of a series) is to present the results of a study of this problem.

MATERIALS AND METHODS

1. Culture medium. The experiments were performed with base medium 7H-10,[2] the composition and preparation of which follows:

KH_2PO_4 (anhydrous)	1.5	Gm.
Na_2HPO_4 (anhydrous)	1.5	Gm.
L-glutamic acid	0.5	Gm.
Ammonium sulfate	0.5	Gm.
Glycerol (reagent grade)	5.0	ml.
Tri-sodium citrate $2H_2O$	0.4	Gm.
Ferric ammonium citrate	0.04	Gm.
$MgSO_4 \cdot 7H_2O$	0.05	Gm.
$CaCl_2 \cdot 2H_2O$	0.0005	Gm.
$ZnSO_4 \cdot 7H_2O$	0.001	Gm.
$CuSO_4 \cdot 5H_2O$	0.001	Gm.
Pyridoxine hydrochloride	0.001	Gm.
Biotin	0.005	Gm.

Dissolve the above in 1,000 ml. of distilled water, and adjust to pH 6.6. For growth on solid medium, add agar [3] in a final concentration of 1.5 per cent and add 0.25 mg. malachite green (oxalate salt) per ml. before autoclaving. After autoclaving, add oleic acid-albumin complex (16) in a final concentration of 0.5 per cent. For growth in liquid ST (standard transfer) medium, before autoclaving add Tween® 80 in a final concentration of 0.05 per cent, and add after autoclaving albumin instead of oleic acid-albumin complex, and omit agar. In either case, after autoclaving, add 0.2 per cent final concentration of glucose (from a concentrated solution previously autoclaved with 0.005 M citric acid), and catalase [4] in final concentration of 3 μg. per ml. from a stock solution, 1.0 mg. per ml. in 0.85 per cent saline, sterilized by passage through a sintered-glass filter.

2. Drugs. Sterile streptomycin sulfate powder in rubber-stopper bottles was dissolved aseptically in sterile distilled water to achieve a final concentration of 1,000 μg. of the base per ml. This stock solution was stored at −10° C. Isoniazid was dissolved in sterile distilled water to a final concentration of 1,000 μg. per ml. This solution was then autoclaved for 10 minutes at 15 pounds pressure (17), and stored at 4° C.

Petri dishes (100 mm. diameter) were prepared with the oleic acid-albumin-agar medium, designated 7H-10. The drugs were added aseptically to this sterile medium just prior to pouring. In the first experiment, four plates were prepared with 1.0 μg. of isoniazid per ml. of solid culture medium, four plates contained 2.0 μg. of streptomycin per ml., and 100 plates were poured with both 2.0 μg. of streptomycin and 1.0 μg. of isoniazid per ml. For the second experiment two plates were prepared containing 1.0 μg. of isoniazid per ml., two plates were prepared containing 1.0 μg. of streptomycin per ml., and 100 plates containing both 1.0 μg. of streptomycin and 1.0 μg. of isoniazid per ml. were poured. The third experiment was the same as the first except that 0.2 μg. of isoniazid instead of 1.0 μg. per ml. was used both in the plates with isoniazid alone and in the 100 plates testing the combined action of streptomycin and isoniazid. The fourth experiment was carried out in liquid medium (ST); it was designed to reveal the sterilizing rather than the bacteriostatic action of 2.0 μg. of streptomycin and 0.5 μg. of isoniazid per ml. of liquid culture medium. These concentrations of drugs were selected because such concentrations have been achieved in the serum of patients during treatment.

3. Cultures. Fully grown laboratory strains of tubercle bacilli (H37Rv and Pearson I), cultivated in Tween®-albumin liquid medium, 7H-4, were used (18). H37Rv was used in both the first and second experiments. However, the stock H37Rv strain has yielded catalase-positive,[5] isoniazid-resistant mutants in our laboratory only in very small numbers. This differs from our experience with most "wild" strains of tubercle bacilli freshly recovered from patients, such as the Pearson I strain. Studies *in vitro* on "wild" strains have demonstrated (19) that, by and large, critically low concentrations of isoniazid select many weakly isoniazid-resistant mutants which retain significant degrees of catalase activity (called catalase-positive) while higher concentrations of isoniazid result in the selection of only catalase-negative, highly isoniazid-resistant forms. Thus, an approximate estimate of the concentration of isoniazid in the free active state can be made after isoniazid-resistant

[2] A similar medium, 7H-9, is available from Difco Laboratories, Detroit, Mich.

[3] Baltimore Biological Laboratories, Inc., Baltimore, Md.

[4] Nutritional Biochemical Corp., Cleveland, Ohio (listed as catalase "crude").

[5] Catalase testing as performed in our laboratory is a crude test. A drop or two of a cold aqueous solution 15 per cent hydrogen peroxide and 5 per cent Tween® is placed on the colony of tubercle bacilli. Catalase-negative organisms give little or no foaming. Catalase-positive organisms yield foaming promptly.

mutants have been selected; low concentrations sterilize only the susceptible parent organisms while higher concentrations sterilize all but the highly isoniazid-resistant, catalase-negative mutants. Because the standard laboratory strain, H37Rv, fails to yield catalase-positive, isoniazid-resistant mutants, and, therefore, does not behave in the usual fashion toward high and low antimicrobially-active concentrations of isoniazid, the Pearson I strain which does yield both catalase-negative and catalase-positive mutants was employed in the third and fourth experiments.

4. Procedures. The first three experiments were performed in the same manner. Petri dishes containing only isoniazid were inoculated with 0.1 ml. of a 1×10^{-1} dilution of a seven to 10 day old dispersed culture of tubercle bacilli (neither filtered nor ground). These stock cultures in liquid medium have been shown to contain approximately 5×10^8 viable bacterial units per ml. (20). Distribution of the organisms on each plate was accomplished by means of a spreader while the plate was rotating on a turntable. Similarly, the plates containing streptomycin alone were inoculated with 0.1 ml. of the undiluted suspension, and the plates which contained both streptomycin and isoniazid were inoculated each with 0.2 ml. of the undiluted suspension. All of the plates were incubated at 36° C. under an atmosphere of 2 to 5 per cent carbon dioxide in air for at least 28 days. Drying out of the cultures was prevented by enclosing the plates in polyethylene plastic bags.

The fourth experiment was performed in duplicate. Two 250 ml. flasks containing 20 ml. of the liquid medium (ST) were inoculated with 0.2 ml. of an undiluted suspension of five day old vigorously growing Pearson I organisms. These flasks were incubated in the same way as the plates; however, two 0.1 ml. samples were removed from each duplicate flask at zero time, one, two and three weeks, and were plated on Petri dishes containing the 7H-10 solid medium without added drugs. These plates were incubated as indicated above.

RESULTS

Experiment No. 1

Four plates were used to test 1.0 μg. of isoniazid per ml. alone; four plates for 2.0 μg. of streptomycin alone; and 100 plates tested 2.0 μg. of streptomycin combined with 1.0 μg. of isoniazid (Table I). The 108 plates were incubated as described under Materials and Methods, and then were examined. The isoniazid plates yielded 99, 101, 106 and 107 colonies, an average of 103 colonies, consisting of isoniazid-resistant mutants. It is assumed that each of these colonies arose from a single drug-resistant mutant because of the relatively small numbers of organisms in each clump. Since there were roughly 5×10^6 viable bacterial units originally inoculated onto each isoniazid plate, it is evident that there was approximately one isoniazid-resistant mutant for every 5×10^4 drug-susceptible bacterial units. This is in agreement with previous estimates of the prevalence of isoniazid-resistant mutants in the H37Rv strain (15).

TABLE I

Results of Experiment 1

Number of plates	Inoculum* per plate	Drug conc.	Colonies per plate (actual)	Prevalence of resistance mutants
		μg./ml.		
4	5×10^6	INH† 1.0	99, 101, 106, 107	1 in 5×10^4
4	5×10^7	SM‡ 2.0	55, 59, 64, 70	1 in 1×10^6
100	1×10^8	INH 1.0 SM 2.0	No growth	<1 in 1×10^{10}

* Number of viable, bacterial units from a seven to 10 day old vigorously growing, well dispersed *H37Rv* culture in liquid medium (ST).
† Isoniazid.
‡ Streptomycin.

The streptomycin plates supported growth of 55, 59, 64 and 70 colonies, an average of 62 colonies, consisting of streptomycin-resistant mutants. This represents about one streptomycin-resistant mutant in 1×10^6.

The 100 plates testing the combined action of streptomycin and isoniazid yielded no growth whatsoever. In other words, no mutant which was simultaneously resistant to both streptomycin and isoniazid, the so-called double mutant, was present in 1×10^{10} viable bacterial units of the H37Rv strain. This population is about one-tenth of the number theoretically necessary to yield one such double mutant.

Only isoniazid-resistant mutants were recovered from the colonies present on those plates which contained isoniazid alone; and only streptomycin-resistant mutants were present in the colonies which grew on the plates containing 2.0 μg. of streptomycin per ml. of solid culture medium. The combination of 2.0 μg. of streptomycin and 1.0 μg. of isoniazid per ml. of culture medium completely prevented the appearance of any colonies.

Experiment No. 2

This experiment was the same as the first except that only 1.0 μg. of streptomycin per ml. was

TABLE II

Results of Experiment 2

Number of plates	Inoculum* per plate	Drug conc.	Colonies per plate (actual)	Prevalence of resistant mutants
		μg./ml.		
2	2.5×10^6	INH 1.0	23, 26	1 in 1×10^5
2	2.5×10^7	SM 1.0	30, 30	1 in 8×10^5
100	5×10^7	INH 1.0	10†	1 in 5×10^8
		SM 1.0		

* Number of viable bacterial units from a seven to 10 day old vigorously growing, well dispersed *H37Rv* culture in liquid medium (ST).

† Only 10 colonies appeared on a total of 100 plates; all susceptible to SM (streptomycin), 0.5 μg. per ml.; resistant to INH (isoniazid), catalase-negative.

employed (Table II). After the 104 plates had been incubated for 28 days, they were inspected for evidence of growth. The isoniazid plates revealed 23 and 26 large colonies, all of which proved to consist of isoniazid-resistant, catalase-negative organisms. There was one isoniazid-resistant mutant in 1×10^5 viable bacterial units. The streptomycin plates revealed a thin, diffuse spreading growth of streptomycin-susceptible organisms over the entire surface of each plate. These organisms had been prevented from proliferating during only part of the incubation period. A total of 60 large colonies were recognizable on these plates. These represented organisms which were streptomycin-resistant. Roughly speaking, this showed that among 8×10^5 drug-susceptible bacterial units there was one streptomycin-resistant mutant.

The 100 plates testing the combined action of streptomycin and isoniazid revealed 10 small colonies. These colonies proved on subculture to consist of pure populations of organisms which were susceptible to 0.5 μg. of streptomycin per ml. of culture medium and resistant to at least 0.5 μg. of isoniazid. Since approximately 5×10^9 viable bacterial units were seeded on these plates, only one isoniazid-resistant mutant in 5×10^8 drug-susceptible bacterial units was able to proliferate on medium containing the stated concentrations of both drugs together.

This experiment emphasized the fact that population size significantly modifies the antimicrobial action of streptomycin. A concentration of about 0.2 μg. of streptomycin per ml. of solid culture medium is usually inhibitory, if not bactericidal, for small numbers of tubercle bacilli of the H37Rv strain. These results show that when large numbers of tubercle bacilli are used, even a fivefold increase to 1.0 μg. of streptomycin per ml. is not completely inhibitory for all drug-susceptible organisms.

The usual minimum antibacterial concentration of isoniazid for small numbers of tubercle bacilli of the H37Rv strain is 0.05 to 0.075 μg. per ml. No significant antagonistic effect attributable to large inocula of tubercle bacilli has been observed in the case of isoniazid. The almost, but not quite, adequate concentration of streptomycin was certainly responsible for the marked reduction in numbers of isoniazid-resistant colonies which did grow. These experiments provide evidence for cross-antibacterial activity between streptomycin and isoniazid. However, when combined drug activity failed due to an inadequate concentration of Drug "A" (in this case, streptomycin), mutants grew which were resistant to Drug "B" (isoniazid) which was present in more effective concentration.

Experiment No. 3

The previous experiments demonstrated that a concentration of 2.0 μg. of streptomycin was the minimum totally inhibitory concentration for such a large population of tubercle bacilli. On the other hand, 1.0 μg. of isoniazid was greatly in excess of the minimum antibacterial concentration of isoniazid for small numbers of tubercle bacilli of this strain, H37Rv.

Catalase-positive, isoniazid-resistant mutants can be derived from the H37Rv strain by antimicrobially-active concentrations of isoniazid only with great difficulty, although catalase-negative, isoniazid-resistant mutants are readily selected. When low concentrations of isoniazid were under investigation, an organism which could produce both types of resistant mutants was necessary to establish the fact that the concentration of isoniazid was suboptimal, even though exceeding the minimum antibacterial concentration for the drug-susceptible parent stock. In such an experiment a strain which readily yields a significant number of catalase-positive, isoniazid-resistant mutants when subjected to the action of critically low concentrations of isoniazid might reveal significantly different responses to the antimicrobial activity of the combination of both drugs. Therefore, be-

cause it readily yields both types of mutants, the Pearson I strain was used.

The Pearson I strain (streptomycin-susceptible, isoniazid-susceptible, catalase-positive, pathogenic) was seeded on 108 plates using the same dilutions as in the preceding experiments (Table III). After incubation as before, these plates were read. The isoniazid plates revealed 34, 44, 45 and 54 colonies, respectively, an average of 44 colonies, composed of isoniazid-resistant mutants. In accordance with the previous assumption, this means that there was roughly one isoniazid-resistant mutant among 1×10^5 drug-susceptible bacterial units. There were 39, 39, 40 and 44 colonies on the streptomycin plates, an average of 40 colonies consisting of streptomycin-resistant mutants, or about one streptomycin-resistant mutant in 1×10^6 drug-susceptible bacterial units. The 100 plates containing both 2.0 μg. of streptomycin and 0.2 μg. of isoniazid per ml. of solid medium yielded no growth.

The colonies which grew on the plates containing only isoniazid were investigated for catalase activity. On one plate all 54 colonies were subjected to catalase testing: 41 colonies gave a negative reaction, and 13 colonies gave a positive reaction. Six colonies were selected at random from another of the isoniazid plates in order to investigate isoniazid resistance as well as catalase activity. One colony consisted of organisms resistant to 0.2 μg. of isoniazid, but susceptible to 5.0 μg. These organisms were catalase-positive. The five remaining colonies proved to consist of pure populations which were catalase-negative and resistant to both 0.2 and 5.0 μg. of isoniazid per ml. of medium. These same colonies were susceptible to the antimicrobial action of 2.0 μg. of streptomycin per ml.

TABLE III

Results of Experiment 3

Number of plates	Inoculum* per plate	Drug conc.	Colonies per plate (actual)	Prevalence of resistant mutants
		μg./ml.		
4	5×10^6	INH 0.2	34, 44,† 45, 54‡	1 in 1×10^5
4	5×10^7	SM 2.0	39, 39, 40, 44	1 in 1×10^6
100	1×10^8	INH 0.2 SM 2.0	No growth	<1 in 1×10^{10}

* Number of viable bacterial units from a seven to 10 day old vigorously growing, well-dispersed *Pearson I* culture in liquid medium (ST).

† Six colonies selected at random: one catalase-positive, R-0.2 INH (isoniazid), S-5.0 INH; five catalase-negative, R-0.2 and 5.0 μg. INH.

‡ Fifty-four colonies: 41 catalase-negative, 13 catalase-positive.

The results of this experiment confirmed our previous observations that exposure of large numbers of the Pearson I strain (total for the four isoniazid plates was 2×10^7 viable bacterial units) to 0.2 μg. of isoniazid per ml. selected both catalase-positive mutants which were resistant to 0.2 μg. of isoniazid and susceptible to 5.0 μg. of isoniazid per ml. of culture medium, and catalase-negative mutants which were resistant to both 0.2 and 5.0 μg. of isoniazid per ml.

Although this concentration of isoniazid was able to manifest effective antibacterial activity against all drug-susceptible parent bacilli, it was still low enough to select both types of resistant mutants. It seemed possible that a differential selection of the two types of resistant mutants might result under the influence of combined streptomycin and low isoniazid. No such differential selection occurred.

Experiment No. 4

The first three experiments demonstrated the antibacterial effect of combined drug action on very large populations of tubercle bacilli; this fourth experiment was designed to show whether this antibacterial activity was bacteriostatic or sterilizing. Viable colony counts were made by plating out from liquid culture medium (ST) containing drugs onto oleic acid-albumin-agar plates containing no drugs. Two duplicate flasks, each containing 0.5 μg. of isoniazid and 2.0 μg. of streptomycin per ml., were sampled in duplicate at zero time, and the samplings (each 0.1 ml.) plated on Petri dishes. These four control plates revealed confluent growth after 28 days' incubation. The flasks were sampled in duplicate after seven days of incubation. This time the plates revealed four, four, three and zero colonies. Sampling of the flasks at 14 and 21 days failed to yield any colonies, indicating a marked sterilizing effect upon the 2×10^8 viable bacterial units originally inoculated. This experiment confirms the observations of Singh and Mitchison (21) relating to the marked sterilizing activity of these two drugs for multiplying tubercle bacilli.

DISCUSSION

Replica plating techniques have established that isoniazid-resistant mutants of typical tubercle bacilli are present in large populations of isoniazid-susceptible organisms prior to any exposure to this drug (11). Presumably this is also true for streptomycin-resistant mutants. Therefore, whether or not these drugs also stimulate more rapid mutation to drug resistance is not a matter for further consideration in this report.

Analysis of the bacteriologic records of patients treated inadequately in the past with streptomycin and isoniazid revealed that the drug-resistant organisms which appeared during treatment were initially resistant to only one drug.[6] Theoretically, there is a definite but extremely low incidence of mutants simultaneously resistant to both of these drugs in enormous populations. Effective concentrations of both streptomycin and isoniazid should select such double mutants when they are present. It is evident that in our four experiments the populations tested were not sufficiently large to provide evidence on this point.

These experiments do establish by quantitative methods the phenomenon of cross-sterilization of multiplying single-drug-resistant mutants *in vitro* when streptomycin and isoniazid are employed, each in concentrations adequate to sterilize all multiplying parent drug-susceptible bacterial cells in "wild" populations of stock laboratory strains of H37Rv and Pearson I. Although the specific modes of action of these two drugs have not been biochemically defined, this evidence corroborates other observations that they have independent and unrelated mechanisms of action against drug-susceptible parasites.

These experiments also show that a nearly, but not quite, adequate concentration of streptomycin combined with relatively high concentrations of isoniazid reduces to a remarkable degree the survival of mutants resistant to isoniazid. Similarly, with an adequate concentration of streptomycin, a critically low concentration of isoniazid, alone unable to prevent the selection of weakly isoniazid-resistant, catalase-positive mutants, is quite sufficient to prevent the emergence of both isoniazid- and streptomycin-resistant mutants.

It should be emphasized that combined drug action, such as was tested here, actually *prevents* the emergence of drug resistance unless a spontaneous double mutant is encountered. These experiments failed to reveal even one double mutant from 2.5×10^{10} viable bacterial units.

Clinical experience indicated in the past that drug resistance would emerge provided only that the sputum remained persistently positive and combined drug therapy continued for a sufficient period of time. This led to the idea that combined drug treatment merely delayed the emergence of resistance. However, another interpretation of the emergence of drug resistance under these conditions (combined drug treatment and persistently positive sputum) is possible. This view is based on the results of Experiment No. 2, where an inadequate concentration of streptomycin allowed a few isoniazid-resistant mutants to be selected by fully adequate concentrations of isoniazid. According to this hypothesis, the patients who eventually excreted drug-resistant organisms had been unable to achieve or maintain for a sufficient period of time adequate concentrations of one of the two drugs in those sites where the tubercle bacilli were multiplying during the time that the other drug was being delivered in fully adequate concentration.

Finally, the results of these experiments provide a microbiologic explanation for the increased conversion rates in those clinical trials (8) which have employed lower dosage of isoniazid (3 to 5 mg. per Kg. per day) combined with higher dosage of streptomycin (7.0 Gm. or more per week). Five to 10 times higher concentrations of isoniazid (1.0 μg. per ml. as compared to 0.1 μg. per ml.) were required to prevent the *in vitro* emergence of catalase-positive, isoniazid-resistant mutants than were needed to exert a striking antibacterial effect against only the parent drug-susceptible population. Thus, in clinical practice, combined drug treatment employing lower dosages of isoniazid should be sufficient with adequate dos-

[6] It is possible that such drug-resistant organisms can be acquired from another patient by so-called *exogenous reinfection*. However, careful study of the records of the inadequately treated patients, referred to above, revealed not one case in which resistance appeared to a drug that the patient had not previously received. Therefore, these results are consistent with the generally accepted concept that the resistant forms of tubercle bacilli which emerge to predominance in the sputa of drug-treated patients are derived *in vivo* from spontaneous mutation in parent drug-susceptible populations.

age of streptomycin to prevent the multiplication of all mutants resistant to either drug (except, of course, double mutants).

SUMMARY

The antimicrobial activity of isoniazid combined with streptomycin was tested against large numbers of multiplying tubercle bacilli of the H37Rv and Pearson I strains in order to investigate the prevention of the selection of mutants simultaneously resistant to both drugs. Concentrations of streptomycin not quite sufficient alone to inhibit the growth of all streptomycin-susceptible bacterial cells were remarkably effective in combination with 20 times the minimum inhibitory concentration of isoniazid in reducing the emergence of drug-resistant mutants. Concentrations of isoniazid, whether only slightly or far in excess of the minimum inhibitory concentration, when combined with the minimum sterilizing concentration of streptomycin prevented the emergence of any drug-resistant mutants. Prevention of emergence of drug-resistant mutants was attributed to the cross-sterilizing action of the two drugs on individual mutants separately resistant to each one. The implications of these observations for clinical chemotherapy were discussed.

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