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The Emergence of Isoniazid-Resistant Cultures in Patients with Pulmonary Tuberculosis during Treatment with Isoniazid alone or Isoniazid plus PAS*

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Previous reports from the Tuberculosis Chemotherapy Centre, Madras, have described a comparison of four regimens (three of isoniazid alone and one of isoniazid plus PAS) in the treatment of pulmonary tuberculosis and an investigation of the serum isoniazid levels in the patients concerned. The present report studies the emergence of isoniazid-resistant organisms in these patients during treatment. All patients with an unsatisfactory response to treatment yielded resistant cultures, showing that the isoniazid dosage was never too low to inhibit sensitive organisms. From the degree of resistance of the first resistant cultures and of the six-month cultures from the patients treated with isoniazid alone it was concluded that resistance emerged in two stages. In the first stage, very early in treatment, highly resistant mutant bacilli grew freely whatever the isoniazid dosage, but mutants of lower resistance were prevented from growing to an extent dependent on the peak isoniazid concentration in the serum. Consequently, when the isoniazid dosage was increased the proportion of patients with resistant organisms decreased, since fewer low-resistance strains were able to develop. In the second stage, organisms with relatively low resistance continued to multiply, though still partially inhibited by isoniazid, and became more resistant, particularly in slow inactivators. The first-stage events determined the results of treatment since, once resistance had emerged, its extent was unrelated to the patient's eventual progress. These findings emphasize the importance of early intensive chemotherapy and adjustment of the isoniazid dosage according to peak serum concentrations rather than concentrations measured three or six hours after the dose. Concomitant administration of PAS prevented emergence of isoniazid resistance in many patients and in others delayed its emergence and reduced its degree, possibly because growth in the secondstage was slow.

INTRODUCTION

A previous report from the Centre (Tuberculosis Chemotherapy Centre, 1960) presented the results of a controlled comparison of isoniazid plus p-aminosalicylic acid (PAS) with three regimens of isoniazid alone in the domiciliary treatment of pulmonary tuberculosis in South India. In terms of the attainment of bacteriologically quiescent disease by 12 months, the regimen of isoniazid plus PAS was the most effective, a moderate dosage of isoniazid (approximately, 8.7 mg/kg body-weight) given alone in one dose a day was less effective and the same moderate daily dosage and a low daily dosage (on the average, 4.5 mg/kg) both given alone in two doses a day were least effective. There was also a suggestion in all four treatment series that the slow inactivators of isoniazid responded to

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treatment slightly better than the rapid inactivators (Selkon et al., 1961).

With the exception of a few patients who died or had their chemotherapy changed in the early months, failure to respond to treatment was always accompanied by the emergence of isoniazid-resistant tubercle bacilli. An attempt has therefore been made in the present report to investigate whether differences in the progress of the patients receiving the various regimens of treatment could be explained in terms of the *degree of resistance* of the resistant organisms. In the patients who were treated with isoniazid alone, an increase in the dosage has been shown to lead to the attainment of higher serum concentrations of isoniazid (Gangadharam et al., 1961b), and it is possible that these higher serum concentrations might have prevented the growth of organisms with low degrees of resistance, thus decreasing the chance of resistant bacilli appearing in the sputum. Further, the concomitant administration of PAS might have either delayed or prevented the emergence of isoniazid-resistance. These possibilities have been examined by considering the degree of resistance and the month of emergence of isoniazid-resistant strains in the slow and rapid inactivators in the four treatment series. Further papers will describe certain other characteristics of the resistant strains, particularly their virulence in the guinea-pig, and the elimination of sensitive organisms during treatment, since these factors might also have influenced the response of the patients to treatment.

MATERIALS AND METHODS

PATIENTS

In all, 341 patients aged 12 years or more with newly diagnosed, culture-positive, pulmonary tuberculosis were allocated at random to treatment with one of four regimens of chemotherapy for 12 months (Tuberculosis Chemotherapy Centre, 1960). The four regimens studied were:

PH (96 patients)

Isoniazid 3.9-5.5 mg/kg body-weight plus sodium PAS 0.2-0.3 g/kg daily, divided into two doses, by mouth–i.e., 200 mg of isoniazid plus 10 g of sodium PAS a day for a patient weighing 100 lb *(45.4* kg).

HI-1 (75 patients)

Isoniazid alone, 7.8-9.6 mg/kg daily in one dose, by mouth–i.e., 400 mg of isoniazid a day for a patient weighing 100 lb.

HI-2 (75 patients)

Isoniazid alone, 7.8-9.6 mg/kg daily, divided into two doses, by mouth,–i.e., 400 mg of isoniazid a day for a patient weighing 100 lb.

H (95 patients)

Isoniazid alone, 3.9-5.5 mg/kg daily divided into two doses, by mouth–i.e., 200 mg a day for a patient weighing 100 lb.

The size of the dose prescribed was related to the patient's weight. It will be appreciated that the size of the average single dose of the HI-1 regimen was twice that of the HI-2 regimen which was, in turn, twice that of the H regimen.

The patients received the allocated chemotherapy for 12 months (and in some instances for 24 months) unless it was changed on account of clear-cut radiographic extension of the disease confirmed by an independent assessor, serious clinical deterioration or peripheral neuropathy. The management of the patients has been described in detail elsewhere (Tuberculosis Chemotherapy Centre, 1960).

For reasons given in the earlier report (Tuberculosis Chemotherapy Centre, 1960) 26 of the 341 patients were excluded from the main analysis, 22 because they had isoniazid-resistant cultures on admission. A further 10 patients (five PH, one HI-1, one HI-2, three H) are excluded from the present analysis. Three patients died from tuberculosis and two more had their treatment changed on account of drug-toxicity, all during the first three months while they were still excreting isoniazid-sensitive organisms. The other five died from non-tuberculous causes during the year. There remained 305 patients (85 PH, 69 HI-1, 67 HI-2, 84 H) all of whom had isoniazid-sensitive cultures on admission to treatment and had previously received, at the most, two weeks of antituberculosis chemotherapy (the great majority had received none).

ASSESSMENTS ON THE PATIENTS

Full details of the assessments made on the patients on admission and during their treatment

have been given in the main report on the study (Tuberculosis Chemotherapy Centre, 1960). The more important of these, which have been used in the present report, are as follows.

On the patient's admission to the study, the severity of the disease was assessed in terms of the extent of cavitation and the total extent of disease read from a full-plate postero-anterior radiograph by an independent observer (Dr Raj Narain). In addition, a smear prepared from a sputum specimen that had been collected overnight was graded for its bacterial content, using fluorescence microscopy.

The progress of the patients during treatment was classified according to the bacteriological status of their disease at 12 months. At the end of each month of treatment, two specimens of sputum and, from the third month onwards, a pair of laryngeal swabs from each patient were cultured. A patient was considered to have quiescent disease (and a favourable response) at 12 months if all of the specimens taken at 10, 11 and 12 months were negative on culture (seven to nine culture results were usually available during this period). Included in this category, in the present report, are also those few patients who, following at least three months of culture negativity, yielded a single positive culture at 10, 11 or 12 months, since their subsequent progress was similar (Velu et al., 1961). The remaining patients with an unfavourable response were bacteriologically active at 12 months, or at the time that their treatment was changed because of radiographic or clinical deterioration, or when they died. Of the six patients who had their treatment changed because of the occurrence of peripheral neuritis, one has been regarded as having quiescent disease and five as having active disease at 12 months, for reasons given by Selkon et al. (1961).

CULTURE AND ISONIAZID SENSITIVITY TESTS

Sputum specimens, after treatment with 4% sodium hydroxide, and laryngeal swabs, after treatment with 4% sulfuric acid, were cultured on Löwenstein-Jensen medium without potato starch by methods previously described (Tuberculosis Chemotherapy Centre, 1959). Usually within three days of the culture becoming positive, sensitivity tests to isoniazid were set up on two pretreatment cultures and on one culture obtained from each patient at the end of each month of treatment. The inoculum suspension was made by adding, as judged by eye, approximately 2 mg (moist weight) of bacilli, obtained as a representative sample of the growth on the Löwenstein-Jensen medium slope, to ¼-ounce (7-ml) screw-capped bottles containing 0.5 ml of sterile distilled water and six glass beads. After the bottle had been shaken mechanically for one minute, a 3-mm loopful of this suspension (containing about 10^5 viable units) was inoculated on to each of a series of slopes of Löwenstein-Jensen medium containing 0.2, 1, 5 and 50 µg/ml isoniazid, and on a drug-free slope as a control. The standard sensitive strain of *Mycobacterium tuberculosis*, H37Rv, was also set up with each series of tests, on a drug-free slope and on slopes containing 0.025, 0.05, 0.1, 0.2 and 1 µg/ml isoniazid.

The tests were read after four weeks incubation at 37°C; they were repeated on the rare occasions when the drug-free slope yielded 100 or fewer colonies. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of isoniazid on which less than 20 colonies grew. Cultures isolated during treatment which had MICs of 0.2 µg/ml were regarded as sensitive, and those with MICs of 1 µg/ml or more as resistant. An MIC of 1 µg/ml was regarded as a low degree of resistance, 5 µg/ml as moderate resistance, 50 µg/ml as high resistance and greater than 50 µg/ml as very high resistance to isoniazid.

RATE OF INACTIVATION OF ISONIAZID

The rate of inactivation of isoniazid was determined by microbiological assay of the isoniazid present in the serum four-and-a-half hours after a test dose of 3 mg of isoniazid per kg body-weight, given intramuscularly (Gangadharam et al., 1961a). Patients with serum concentrations of 0.58 µg/ml or more isoniazid were classified as slow inactivators and those with concentrations of less than 0.58 µg/ml isoniazid as rapid inactivators. The rate of inactivation of isoniazid was determined in 299 of the 305 patients; in two patients (one PH, one HI-1) the tests were contaminated; in one patient (PH) the blood was collected half-an-hour too late, and three patients (one HI-2, two H) died before the test was done.

RESULTS

The results are presented in four sections. In the first the variation encountered in the isoniazid-sensitivity tests is considered. The second presents the pattern of emergence of resistance and studies the influence of two background factors, the speed of emergence of resistance and the condition of the patient's disease on admission to treatment, on the degree of resistance. The third section describes the proportions of patients in the four treatment series, considered separately as slow or rapid inactivators, who yielded resistant cultures and the degree of resistance of these cultures. In the fourth section the response to treatment of those patients who yielded isoniazid-resistant cultures during the year was studied in relation to the degree of the resistance of their cultures.

VARIATION IN RESULTS OF ISONIAZID-SENSITIVITY TESTS

Variation between duplicate tests on the same culture

The reproducibility of the isoniazid-sensitivity test was studied by comparing the results of duplicate tests set up on 63 cultures, five obtained from patients in the study before the start of treatment and the remainder between five months and 24 months, while the patients were still receiving their initially allocated chemotherapy. The duplicate tests were set up from the same culture within a few days of each other by separate workers, who were unaware of the purpose of the investigation, and were read independently.

The results of the duplicate tests are summarized in the first line of Table 1. Of the 63 cultures, 10 were sensitive, having an MIC of 0.2 µg/ml or less in the first test, 10 had an MIC of 1 µg/ml, 15 had an MIC of 5 µg/ml, 13 had an MIC of 50 µg/ml and 15 had an MIC of more than 50 µg/ml. In the second test, 56 had the same MIC and the remaining seven had MICs which were only one dilution step different; four were less resistant and three were more resistant. None of the cultures yielded a resistant result (MIC of 1 µg/ml or more) in one test and a sensitive result (MIC of 0.2 µg/ml) in the other test.

Variation in degree of resistance of six- and seven-month cultures isolated from the same patient

The variation inherent in the sampling of the bacterial populations in the lesions of patients was investigated by comparing the results on different sputum specimens from the same patient. The tests on cultures isolated at six and seven months were chosen for this comparison since only one positive culture isolated at each month was tested for its sensitivity and since the results on six-month cultures have been used in the following sections of the report. The results obtained on the six-month and seven-month cultures that were isolated from each of 66 patients who had sensitivity test results on cultures obtained at both of these months are summarized in the second line of Table 1. All of the 132 cultures tested were resistant to isoniazid. In 58 of the 66 patients the six- and seven-month

TABLE 1

COMPARISON OF THE PO-COLONY AND EQUAL-GROWTH END-POINTS IN DUPLICATE ISONIAZID SENSITIVITY TESTS AND IN SENSITIVITY TESTS ON SIX-MONTH AND SEVEN-MONTH CULTURES ISOLATED FROM THE SAME PATIENT

Comparison	Total cultures	Comparison of the results in the 2 sensitivity tests							
		MIC in first test or in test on six-month culture *higher* (No. of dilution steps [a])				The *same* MIC in both tests	MIC in first test or in test on six-month culture lower (No. of dilution steps [a])		
		4	3	2	1		1	2	3
Between duplicate sensitivity on tests on same culture	63	0	0	0	4	56	3	0	0
Between six-month and seven-month cultures from same patient	66	0	1	0	0	58	6	0	1

[a] The isoniazid concentrations in the tests were 0.2, 1, 5 and 50 µg/ml.

cultures had the same MIC. The seven-month culture had an MIC which was one dilution step higher than the six-month culture in six patients, and an MIC three dilution steps higher in one patient. In one patient the seven-month MIC was three dilution steps lower than the six-month MIC.

Consistency of resistance in multiple cultures from the same patient

The consistency with which cultures, once resistant, were succeeded by further resistant cultures from the same patients was also examined. Of the 305 patients in this study, 120, after yielding a resistant culture, had an isoniazid-sensitivity test result on at least one subsequent culture. From these 120 patients, 703 cultures, which were tested for their sensitivity to isoniazid, were isolated after the emergence of the first isoniazid-resistant culture until the end of the year of treatment or until the month in which their treatment was changed. Of the 703 cultures, only 11 (1.6 %) were sensitive to isoniazid.

EMERGENCE OF ISONIAZID-RESISTANT CULTURES DURING TREATMENT

Response of the patients to treatment related to the emergence of resistance

Of the 305 patients in the study, 136 yielded one or more isoniazid-resistant cultures during the first year while receiving their allocated treatment. These 136 patients included all of the 103 patients whose disease failed to become bacteriologically quiescent at 12 months. The proportion of cultures that were resistant to isoniazid at each month of treatment increased during the first five months and, at six months, 97 % of the 90 cultures were resistant. In the second six months, only nine of the 415 cultures tested were sensitive; these were obtained from eight patients (one H, three HI-2, two HI-1, two PH). Of the eight patients, seven (two slow inactivators, five rapid inactivators) attained quiescent disease; one (slow inactivator) yielded a resistant culture at 11 months and, since two positive cultures had been obtained in the last three months of treatment, was classified as having active disease. Thus, an unfavourable response was invariably accompanied by the emergence of resistance and there was no evidence of the persistent excretion of isoniazid-sensitive organisms, even in rapid inactivators receiving a low dosage of isoniazid. The isolation on culture of isoniazid-sensitive organisms in the second six months was a rare phenomenon and did not presage a failure to attain quiescent disease at 12 months.

Proportions of patients with resistant cultures

One or more isoniazid resistant cultures were obtained from 57 (68%) of the 84 H patients, 33 (49%) of the 67 HI-2 patients, 30 (43%) of the 69 HI-1 patients and from 16 (19 %) of the 85 PH patients. Thus, in the isoniazid-alone series, the proportion of patients with resistant cultures was highest in the H series and lowest in the HI-1 series. Considerably fewer of the PH patients yielded resistant cultures than did the patients treated with isoniazid alone.

Speed of emergence of resistant cultures

The month of treatment at which the first (or only) resistant culture was isolated from the slow and rapid inactivators of isoniazid in the four treatment series is set out in Table 2. The first resistant culture was obtained at one to four months from 75 % of the 57 H patients who yielded resistant cultures, from 85% of the 33 HI-2 patients, from 83 % of the 30 HI-1 patients and from only 25 % of the 16 PH patients. Thus, the majority of the resistant cultures were obtained during the first four months in the patients treated with isoniazid alone, but the emergence of resistance was delayed in the PH series. The speed of emergence of resistance was similar in the three isoniazid-alone series, the proportions of resistant cultures obtained for the first time at one or two months being 56% in the H series, 55 % in the HI-2 series and 60 % in the HI-1 series. First resistant cultures emerged at about the same time in the slow and rapid inactivators in each of the treatment series.

Degree of resistance of the first resistant cultures

The degree of resistance of the first resistant cultures, and the month of treatment in which they were obtained, are set out in Table 3. First resistant cultures with MICs of 1 μg/ml were obtained from 21% of 100 patients in the first four months of treatment and from 31% of 36 patients in the remaining eight months. Thus, the degree of resistance of the first resistant cultures was not related to the duration of treatment before their emergence.

Effect of continued treatment on the degree of resistance

The effect of continued treatment on the degree of resistance to isoniazid is shown in the accompa-

TABLE 2
MONTH OF EMERGENCE OF THE FIRST ISONIAZID-RESISTANT CULTURE IN SLOW AND RAPID INACTIVATORS OF ISONIAZID IN THE FOUR TREATMENT SERIES

Treatment series	Rate of Inactivation of isoniazid	Total patients who yielded resistant cultures	Month of emergence of the first resistant culture							
			1-2		3-4		5-6		7-12	
			No. of patients	%	No. of patients	%	No. of patients	%	No. of patients	%
PH	Slow	8	0	(0) [a]	1	(12)	4	(50)	3	(38)
	Rapid	8	1	(12)	2	(25)	4	(50)	1	(12)
	All patients	16	1	(6)	3	(19)	8	(50)	4	(25)
HI-1	Slow	16	8	(50)	5	(31)	1	(6)	2	(12)
	Rapid	14	10	(71)	2	(14)	1	(7)	1	(7)
	All patients	30	18	60	7	23	2	7	3	10
HI-2	Slow	18	6	(33)	8	(44)	1	(6)	3	(17)
	Rapid	14	11	(79)	2	(14)	0	(0)	1	(7)
	All patients	33 [b]	18 [b]	55	10	30	1	3	4	12
H	Slow	30	18	60	5	17	4	13	3	10
	Rapid	25	12	48	6	24	6	24	1	4
	All patients	57 [c]	32 [c]	56	11	19	10	18	4	7

[a] The parentheses Indicate percentages based on fewer than 25 observations.
[b] Indicates the inclusion of one patient whose rate of inactivation was not determined.
[c] Indicates the inclusion of two patients whose rate of inactivation was not determined.

nying figure. The proportion of cultures with MICs of 1 µg/ml decreased from 24% of 25 resistant cultures at one month, to 16% of 87 at six months, and to 7% of 72 at nine months. Thereafter, it remained fairly constant and was 8% of 52 at 12 months. The proportion of cultures with MICs of 5 µg/ml and 50 µg/ml remained fairly constant over the year. The proportion of cultures with MICs of greater than 50 µg/ml increased from 20 % of 25 at one month to 26 % of 87 at six months, to 39% of 72 at nine months and to 40% of 52 at 12 months. This increase in the degree of resistance during treatment was shown by both the slow and the rapid inactivators of isoniazid.

The degree of resistance of successive resistant cultures from the same patient was also studied by amalgamating all first resistant cultures, and, correspondingly, all resistant cultures obtained thereafter at each month subsequent to the isolation of the first resistant culture. MICs of 1 µg/ml were obtained with 23 % of the 136 first resistant cultures, with 16% of the 94 resistant cultures at the first subsequent month, with 11 % of the 101 resistant cultures at the second subsequent month, with 6 % of the 64 resistant cultures at the sixth subsequent month and with 3% of the 38 resistant cultures at the tenth and eleventh subsequent months. Thus, the tendency for the degree of resistance to increase during treatment was at least as evident in successive cultures from the same patient as in successive months of treatment. The increase in the degree of resistance was also more evident in the earlier than in the later resistant cultures.

In order to avoid bias in the above analysis, the data were examined further (but are not tabulated here) to see whether changes in the degree of resistance were similar in patients who had few or many resistant cultures during the year of treatment

TABLE 3

DEGREE OF RESISTANCE TO ISONIAZID OF FIRST RESISTANT CULTURE RELATED TO THE MONTH OF EMERGENCE

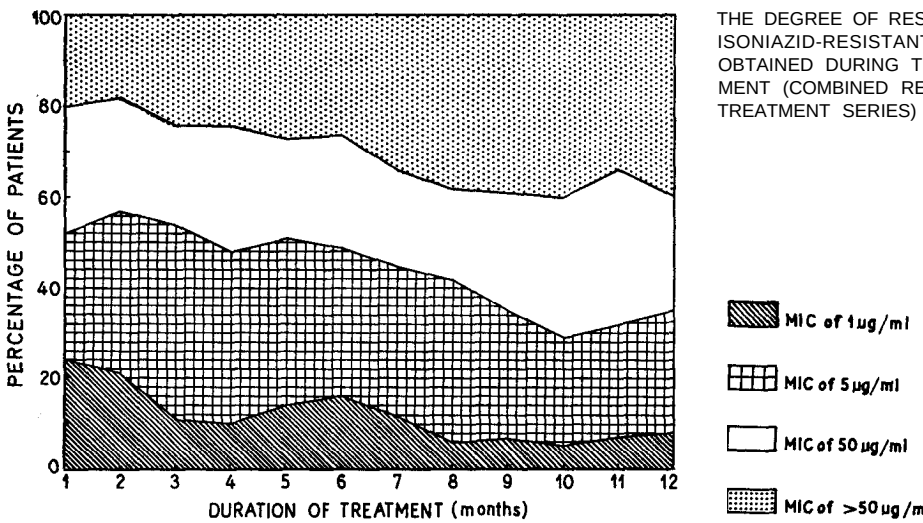
Month of treatment	Total cultures		Minimal inhibitory concentration of isoniazid (μg/ml)							
			1		5		50		>50	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
1-4	100	*100*	21	*21*	35	*35*	25	*25*	19	*19*
5-8	**30**	*100*	8	*27*	7	*23*	5	*17*	10	*33*
9-12	**6**	100	3	*(50)*[a]	2	*(33)*	0	*(0)*	1	*(17)*
Total	136	*100*	32	*24*	44	*32*	30	*22*	30	*22*

[a] The parentheses indicate percentages based on fewer than 25 observations.

and whether the patients who became bacteriologically negative or who changed treatment, and were therefore lost from the population studied, differed from the remaining patients in the degree of resistance of their cultures. No reason was found to alter the conclusion that the degree of resistance in the individual patient increased as treatment was continued.

Influence of the pretreatment condition of the disease on the emergence of resistance

The relationship between the pretreatment condition of the disease and the emergence of resistance has been studied in the first isoniazid-resistant culture obtained from each patient and in a population of resistant cultures termed the " supplemented six-month " cultures. The first isoniazid-resistant cultures were chosen for study since they were the cultures that most closely represented the types of resistant mutant that originally emerged from the patient's population of sensitive bacilli. The supplemented six-month cultures consisted of a single culture obtained from each patient at six months, provided that this culture was available and resistant. If the six-month culture was negative or contaminated, the resistant culture isolated at seven or at five months was included, in this order of preference. The five-month resistant culture from four patients who had their treatment changed during the sixth month was also included. Cultures from seven



THE DEGREE OF RESISTANCE OF. ISONIAZID-RESISTANT CULTURES OBTAINED DURING THE YEAR OF TREATMENT (COMBINED RESULTS IN ALL FOUR TREATMENT SERIES)

patients (three H, two HI-2, two HI-1) who had their treatment changed or died before completing five months of treatment were excluded. The supplemented six-month cultures were chosen for study because an appreciable number of patients had their treatment changed during the following few months.

Table 4 relates the pretreatment condition of the disease to the proportion of patients who yielded resistant cultures and to the degree of resistance both for the first and for the supplemented six-month resistant cultures. The four treatment series are not presented separately since analyses (not tabulated here) showed that they were similar in respect of the associations considered below.

Proportion of patients with resistant cultures. As can be seen from the first two columns of Table 4, one or more resistant cultures were obtained from 99 (52%) of 191 patients with extensive or moderate cavitation on admission, but from only 37 (32 %) of 114 patients with slight or no cavitation, a highly significant difference ($P<0.001$). Patients with gross or extensive disease also yielded resistant cultures more often than those with less extensive disease. Considering the bacterial content of single pretreatment collection specimens, resistant cultures were obtained from 52% of 201 patients with 3-plus or 2-plus smear gradings and from 31% of 104 patients with I-plus or negative smears; this difference is highly significant ($P<0.001$). In a similar manner a higher proportion of patients with initially more severe disease yielded supplemented six-month resistant cultures.

Degree of resistance. Table 4 also relates the degree of resistance of the first resistant cultures to the condition of the patients on admission to treatment. Cultures with MICs of 1 µg/ml were obtained from 20% of the 99 patients with resistant cultures who had moderate or extensive cavitation and from 30% of 37 with slight or no cavitation. The corresponding proportions were 25% for the 48 patients who had gross or extensive disease on admission, as compared with 22 % for the 88 patients who had moderate or less disease, and 23% for the 104 patients who had 3-plus or 2-plus positive smears as compared with 22% for the 32 patients who had l-plus positive or negative smears. An absence of association between the degree of resistance and the pretreatment severity of the disease was also noted with the supplemented six-month cultures (Table 4).

In summary, there was an association between the proportion of patients who yielded one or more isoniazid-resistant cultures during treatment and the extent of cavitation, the total extent of disease and the degree of sputum positivity on admission to treatment. On the other hand there was no evidence that, in the patients who yielded resistant cultures, the degree of resistance of either the first or the supplemented six-month cultures was related to the initial condition of the patients on admission to treatment.

COMPARISON OF THE DEGREE OF RESISTANCE OF ISONIAZID-RESISTANT CULTURES IN THE FOUR TREATMENT SERIES

This section compares the emergence of isoniazid-resistance in the four treatment series and in the slow and rapid inactivators of isoniazid. The purpose of this comparison was to determine whether the dosage of isoniazid and the differences in the serum levels of isoniazid attained by slow and rapid inactivators (Gangadharam et al., 1961b) were related to the frequency of emergence of resistant cultures and to the degree of their resistance. For simplicity of presentation, only the first and the supplemented six-month resistant cultures (for definition see page 279) are considered here.

First resistant cultures

In Table 5 are set out the numbers of patients who yielded one or more isoniazid-resistant cultures during the year, the degree of resistance of the first resistant cultures and the bacteriological status at one year of the patients in the four treatment series. The eight subgroups, formed by the slow and rapid inactivators in the four treatment series, are arranged in order of the response to treatment that was obtained (Table 5, last column). Thus, the H regimen in rapid inactivators was the least effective and the PH regimen in slow inactivators was the most effective. The proportion of patients who yielded isoniazid-resistant cultures in each of the subgroups decreases in the same order, from 69 % of the 36 H rapid inactivators to 14% of the 57 PH slow inactivators.

Among the patients who received isoniazid alone, cultures with MICs of 1 or 5 µg/ml were obtained from 44% of the 36 rapid inactivators in the H series, from 35% of the 46 slow inactivators in the H series, from 26% of the 27 rapid inactivators

TABLE 4

DEGREE OF RESISTANCE TO ISONIAZID OF THE RESISTANT CULTURES ISOLATED DURING TREATMENT RELATED TO THE CONDITION OF THE DISEASE ON ADMISSION TO TREATMENT

Pretreatment assessments		First isoniazid-resistant culture										Supplemented six-month isoniazid-resistant culture									
		Total patients examined (a)	Patients with resistant cultures (b)		Minimal inhibitory concentration of isoniazid (μg/ml)							Total patients examined (c)	Patients with resistant cultures (d)		Minimal inhibitory concentration of isoniazid (μg/ml)						
					1		5		50		>50				1		5		50		>50	
			No.	% of (a)	No.	% of (b)	No.	% of (b)	No.	% of (b)	No.	% of (b)		No.	% of (c)	No.	% of (d)	No.	% of (d)	No.	% of (d)	No.	% of (d)
Extent of cavitation	Extensive or moderate	191	99	*52*	20	20	34	34	24	24	21	27	185	80	43	12	*15*	28	35	19	24	21	26
	Slight	92	30	*33*	8	27	IO	33	4	13	8	27	91	23	25	4	(17) [a]	6	(26)	6	(26)	7	(30)
	Nil	22	7	(32)	3	(43)	2	(29)	1	(14)	1	(14)	22	6	(27)	2	(33)	1	(17)	1	(17)	2	(33)
Total extent of disease	Gross or extensive	88	48	55	12	*25*	18	33	10	21	8	17	84	38	45	7	18	11	29	II	29	9	24
	Moderate or limited	191	83	*43*	17	20	25	*30*	19	23	22	27	189	67	35	9	73	22	33	15	22	21	*31*
	Slight or trivial	26	5	19	2	(43)	3	(60)	0	(0)	0	(0)	25	4	*16*	2	(50)	2	(50)	0	(0)	0	(0)
sputum positivity on direct smear	3-plus	111	66	59	16	24	24	*36*	12	*18*	14	*21*	108	53	49	9	*17*	20	38	II	*21*	13	*25*
	2-plus	90	38	*42*	8	27	IO	26	II	29	9	24	86	32	36	6	*19*	8	25	9	28	9	*28*
	1-plus	55	19	*35*	2	(11)	7	(37)	4	(27)	6	(32)	53	17	32	1	(6)	4	(24)	4	(24)	8	(47)
	Negative	49	13	*27*	5	(38)	5	(38)	2	(15)	1	(8)	49	7	14	2	(29)	3	(43)	2	(29)	0	(0)
Total patients		305(136		45	31	23	46	34	29	21	30	21	298 [b]	109	37	18	*17*	35	32	26	24	30	28

[a] The parentheses indicate percentages based on fewer than 25 observations.

[b] Excluding seven patients who had died or had their treatment changed before completing five months of treatment,

TABLE 5

DEGREE OF RESISTANCE TO ISONIAZID OF THE FIRST RESISTANT CULTURES FROM PATIENTS IN THE FOUR TREATMENT SERIES

Treatment series	Rate of inactivation of isoniazid	Total patients (a)	Total patients with first isoniazid-resistant cultures		Minimal inhibitory concentration of isoniazid (μg/ml)								Patients with bacteriologically quiescent disease at one year	
					1		5		50		>50			
			No.	% of (a)	No.	% of (a)	No.	% of (a)	No.	% of (a)	No.	% of (a)	No.	% of (a)
PH	Slow	57	8	14	3	5	3	5	1	2	1	2	54	95
	Rapid	26	8	31	5	19	3	12	0	0	0	0	22	85
	All patients	85[a]	16	19	8	9	6	7	1	1	1	1	78[a]	92
HI-1	Slow	36	16	44	1	3	9	25	4	11	2	6	26	72
	Rapid	32	14	44	2	6	4	12	2	6	6	19	21	66
	All patients	69[b]	30	43	3	4	13	19	6	9	8	12	48[b]	70
HI-2	Slow	39	18	46	1	3	5	13	6	15	6	15	23	59
	Rapid	27	14	52	4	15	3	11	3	11	4	15	15	56
	All patients	67[b]	33[b]	49	5	7	9[b]	13	9	13	10	15	38	57
H	Slow	46	30	65	4	9	12	26	7	15	7	15	22	48
	Rapid	36	25	69	10	28	6	17	5	14	4	11	16	44
	All patients	84[a]	57[a]	68	15[b]	18	18	21	13[b]	15	11	13	38	46
All isoniazid-alone series	Slow	121	64	53	6	5	26	21	17	14	15	12	71	59
	Rapid	95	53	56	16	16	13	14	10	10	14	15	52	55
	All patients	220	120	54	23	10	40	18	28	13	29	13	124	56

[a] Indicates the inclusion of two patients whose rate of inactivation of isoniazid was not determined.
[b] Indicates the inclusion of one patient whose rate of inactivation of isoniazid was not determined.

in the HI-2 series and from 20% of the remaining 108 patients in the HI-2 and HI-1 series. On the other hand, the proportions of patients with cultures that had MICs of 50 µg/ml or more are similar in each of the subgroups. Thus, it appears that the decrease in the proportion of patients with resistant strains, from the rapid inactivators of the H series to the slow inactivators of the HI-1 series, is mainly due to a deficiency of patients with cultures of low or moderate degrees of resistance. This conclusion, though uncertain for the data for the HI-1 series and for the HI-2 slow inactivators, is supported by the tidings on the supplemented six-month cultures presented below.

Among the cultures with low or moderate degrees of resistance, a further association is evident between the degree of resistance and the rate of inactivation of isoniazid, such that the proportion of cultures with low degrees of resistance is higher in the rapid inactivators than in the slow inactivators. Thus, among those patients in the H series who had cultures with MICs of 1 or 5 µg/ml, only four of 16 cultures from slow inactivators had cultures with an MIC of 1 µg/ml, as compared with 10 of the 16 corresponding cultures from rapid inactivators. Similar findings are also evident in the remaining two series. Amalgamating the results in the three isoniazid-alone series, an MIC of 1 µg/ml was obtained with six (19%) of the 32 cultures with MICs of 1 or 5 µg/ml from slow inactivators and from 16 (55 %) of the 29 corresponding cultures from rapid inactivators, a significant difference ($P<0.01$).

A comparison of the degree of resistance of cultures from patients in the PH and the HI-2 series is of interest, since the patients on these two regimens had similar serum concentrations of isoniazid (Gangadharam et al., 1961b). Cultures with MICs of 50 µg/ml or more were obtained from 2% of the 85 PH patients, but from 28% of the 67 HI-2 patients, a highly significant difference ($P<0.001$). The proportions of cultures with MICs of 1 or 5 µg/ml were, however, similar in the PH series (16 % of 85 patients) and in the HI-2 series (21% of 67 patients). Thus, the concomitant administration of PAS with isoniazid resulted in a considerable decrease in the proportion of patients who yielded cultures with high or very high degrees of resistance.

Supplemented six-month resistant cultures

The degree of resistance of the supplemented six-month resistant cultures from the slow and rapid inactivators in the four treatment series is set out in Table 6, also arranged in order of efficacy of the regimens. These cultures were usually obtained after the first resistant cultures when the degree of resistance had increased slightly. The associations found with the first resistant cultures were also evident in the supplemented six-month cultures, but the proportions of cultures with MICs of 50 µg/ml, as well as those with MICs of 1 and 5 µg/ml, varied according to the treatment regimen and the rate of inactivation. In the three series treated with isoniazid alone, cultures with MICs of 1, 5 or 50 µg/ml were obtained from 56% of the 36 H rapid inactivators, from 38 % of the 45 H slow inactivators, from 37% of the 27 HI-2 rapid inactivators and from 21 % of the remaining 105 patients in the HI-2 and HI-1 series, again demonstrating a decrease in the proportions of cultures with low, moderate or high degrees of resistance as the dose of isoniazid was increased. Cultures with MICs of more than 50 µg/ml were obtained from 11 % to 19 % of the H patients, the HI-2 patients and the HI-1 rapid inactivators, but from only 6% of the HI-1 slow inactivators. Thus, the differences between the six subgroups of the isoniazid-alone series in the proportion of patients who had resistant strains is mainly accounted for by the patients who yielded cultures of low, moderate or high degrees of resistance, and not by patients with cultures of very high resistance.

As with the first resistant culture, the relative proportion of resistant cultures with different degrees of resistance within each treatment series was related to the rate of inactivation of isoniazid. However, the effect of the rate of inactivation also occurs in cultures with MICs of 50 µg/ml. Thus, of the cultures with MICs of 50 µg/ml or less in the H series, the proportions of cultures with MICs of 1, 5 and 50 µg/ml were 6 %, 29 % and 65 %, respectively, of the 17 cultures from slow inactivators, as compared with 50 %, 35 % and 15 % respectively of the 20 cultures from rapid inactivators. A similar trend is evident in the HI-2 series and in the HI-1 series. Amalgamating the results on cultures with MICs of 50 µg/ml or less in the three isoniazid-alone series, cultures with MICs of 1, 5 or 50 µg/ml were obtained from 6 %, 34% and 59%, respectively, of the 32 slow inactivators and from 35 %, 49% and 16%, respectively, of the 37 rapid inactivators. These differences are highly significant ($P<0.001$).

TABLE 6

DEGREE OF RESISTANCE TO ISONIAZID OF THE SUPPLEMENTED SIX-MONTH [a] CULTURES FROM PATIENTS IN THE FOUR TREATMENT SERIES

Treatment series	Rate of inactivation of isoniazid	Total patients (a)	Total patients with supplemented six-month isoniazid-resistant cultures		Minimal inhibitory concentration of isoniazid (µg/ml)							
					1		5		50		>50	
			No.	% *of* (a)	No.	% of (a)	No.	% of (a)	No.	% of (a)	No.	% of (a)
PH	Slow	57	5	*9*	0	*0*	3	*5*	1	*2*	1	*2*
	Rapid	26	6	*23*	3	*12*	3	*12*	0	*0*	0	*0*
	All patients	85[b]	11	*13*	3	*4*	6	*7*	1	*1*	1	*1*
HI-1	Slow	35	9	*26*	0	*0*	3	*9*	4	*11*	2	*6*
	Rapid	31	13	*42*	1	*3*	4	*13*	2	*6*	6	*19*
	All patients	67[c]	22	*33*	1	*2*	7	*10*	6	*9*	8	*12*
HI-2	Slow	38	15	*40*	1	*3*	3	*8*	4	*10*	7	*18*
	Rapid	27	13	*48*	2	*7*	7	*26*	1	*4*	3	*11*
	All patients	65	28	*43*	3	*5*	10	*15*	5	*8*	10	*15*
H	Slow	45	23	*51*	1	*2*	5	*11*	11	*24*	6	*13*
	Rapid	36	25	*69*	10	*28*	7	19	3	*8*	5	*14*
	All patients	61	46	*60*	11	*14*	12	*15*	14	*17*	11	*14*
All isoniazid-alone series	Slow	118	47	*40*	2	*2*	11	*9*	19	*16*		
	Rapid	94	51	*54*	13	*14*	18	*19*	6	*6*	14	*15*
	All patients	213	98	*46*	15	*7*	29	*14*	25	*12*	29	*14*

[a] Cultures isolated at either six, seven or five months in this order of preference.
[b] Indicates the inclusion of two patients whose rate of inactivation of isoniazid was not determined.
[c] Indicates the inclusion of one patient whose rate of inactivation of isoniazid was not determined.

INFLUENCE OF THE DEGREE OF RESISTANCE TO ISONIAZID ON THE SUBSEQUENT RESPONSE TO TREATMENT

Of the 136 patients who yielded one or more isoniazid-resistant cultures, 33 (24 %) attained bacteriologically quiescent disease at 12 months (for definition see page 275). Seventeen of these 33 patients had yielded one resistant culture, six had two resistant cultures, three had three resistant cultures and seven had four or more resistant cultures. Since, as shown above, there was a tendency for the degree of isoniazid-resistance to increase with continued treatment, it was of particular interest to determine whether the subsequent response of the patients to treatment was related to the degree of resistance of their first resistant culture or to that of their supplemented six-month resistant culture.

The degree of resistance of the first resistant culture and the proportion of patients who yielded one or more resistant cultures during the year but nevertheless had bacteriologically quiescent disease at 12 months are set out in Table 7. Bacteriologically quiescent disease was attained by 19% of 57 H patients who had one or more resistant cultures, by 12 % of 33 HI-2 patients, by 30 % of 30 HI-1 patients and by 56% of 16 PH patients. The difference between the PH series and the combined isoniazid-alone series in the proportion of patients with resistant cultures who had a favourable response is highly significant ($P<0.01$). A favourable response was obtained by 19 (26%) of 72 slow inactivators and by 14 (23%) of 61 rapid inactivators with resistant cultures.

Amalgamating the three isoniazid-alone series, bacteriologically quiescent disease was attained by 22% of the 23 patients with first resistant cultures that had an MIC of 1 µg/ml, by 20% of 40 with MICs of 5 µg/ml, by 14% of 28 with MICs of 50 µg/ml and by 24% of 29 with MICs of greater than 50 µg/ml. Of the 32 slow inactivators who had first resistant cultures with MICs of 1 or 5 µg/ml, nine (28 %) attained bacteriologically quiescent disease as compared with four (14%) of the 29 corresponding rapid inactivators. These differences do not attain statistical significance. Thus, there was no clear evidence of an association between the degree of resistance of the first resistant culture and the subsequent response of the patients to treatment.

The degree of resistance of the supplemented six-month resistant cultures and the response to treatment of the patients who yielded these cultures is shown in Table 8. There was a suggestion that patients with isoniazid-resistant cultures more often had quiescent disease at 12 months in the PH series (36% of 11 patients) than in the combined isoniazid-alone series (15 % of 98 patients). With regard to the degree of resistance of the cultures from patients treated with isoniazid alone, quiescent disease was attained by 27 % of 15 patients with cultures having MICs of 1 µg/ml and by 13 % of 83 patients with cultures having MICs of 5 µg/ml or more. A favourable response was obtained by 31% of 13 slow inactivators and by 13 % of 31 rapid inactivators who had cultures with MICs of 1 or 5 µg/ml. However, none of these differences attains statistical significance.

In summary, a favourable response to the allocated regimen of treatment was obtained among patients who yielded resistant cultures more often in the PH series than in the series treated with isoniazid alone. However, no definite association existed between the degree of resistance and the further response of the patients treated with isoniazid alone. There was a slight suggestion that the response in patients with supplemented six-month cultures of low degrees of resistance and in slow inactivators with cultures of low or moderate degrees of resistance was better than in the remaining patients.

DISCUSSION

Before relating the degrees of resistance of cultures from the patients treated with isoniazid alone to the dosage of isoniazid that they received and to their inactivation status, it was necessary to examine two other factors which might have influenced the degree of resistance. The first of these was the duration of treatment before resistance emerged. There were no appreciable differences between the times taken for the first resistant cultures to appear in the three isoniazid-alone series. Furthermore, the degree of resistance of the first resistant cultures was not associated with the month of their emergence. Thus, any differences between the four treatment series or between the

TABLE 7

RESPONSE TO TREATMENT RELATED TO THE DEGREE OF RESISTANCE TO ISONIAZID OF THE FIRST RESISTANT CULTURE

Treatment series	Minimal inhibitory con centration of isoniazid (μg/ml)								All patients with resistant cultures	
	1				50		>50			
	No. of patients	Favourable response [a] No.	%	No. of patients	Favourable response [a] No.	%	No. of patients	Favourable response [a] No.	%	No. of patients	Favourable response [a] No.	%
PH	8	6		6	2		1	0		1	1		16	9	(56)
HI-I	3	0		13	4		6	2		8	3		30	9	30
HI-2	5	3		9	1		9	0		10	0		33	4	12
H	15	2		18	3		I3	2		11	4		57	11	19
All isoniazid-alone series:															
Slow inactivators	6	2	(33) [b]	26	7	27	I7	2	(12)	15	3	(20)	64	14	22
Rapid inactivators	16	3	(19)	13	1	(8)	10	2	(20)	I4	4	(29)	63	10	19
Total patients treated with isoniazid alone	23 [c]	6	(22)	40 [c]	8	20	28"	4	14	29	7	24	120	24	20

[a] Bacteriologically quiescent disease at I2 months.

[b] The parentheses indicate percentages based on fewer than 25 observations.

[c] including one patient whose rate of inactivation of isoniazid was not determined.

TABLE 8

RESPONSE TO TREATMENT RELATED TO THE DEGREE OF RESISTANCE TO ISONIAZID OF THE SUPPLEMENTED SIX-MONTH RESISTANT CULTURES

Treatment series	Minimal inhibitory concentration of isoniazid (µg/ml)								All patients with resistant cultures	
	1		5		50		>50			
	No. of patients	Favourable response [a]	No. of patients	Favourable response [a]	No. of patients	Favourable response [a]	No. of patients	Favourable response [a]	No. of patients	Favourable response [a]
		No. %		No. %		No. %		No. %		No. %
PH	**3**	**2**	**6**	**1**	**1**	**0**	**1**	**1**	**11**	**4 (36)**
HI-1	**1**	**0**	**7**	1	6	0	8	3	22	4 *(18)*
HI-2	**3**	**3**	**10**	1	5	0	10	0	28	4 *14*
H	**11**	**1**	**12**	2	14	3	11	1	48	7 *15*
All isoniazid-alone series :										
Slow inactivators	**2**	**1 (50) [b]**	**11**	3 *(27)*	19	1 *(5)*	15	0 *(0)*	47	5 *11*
Rapid inactivators	13	3 *(23)*	18	1 *(6)*	6	2 *(33)*	14	4 *(29)*	51	10 *20*
Total patients treated with isoniazid alone	1 5	4 *(27)*	29	4 *14*	25	3 *12*	29	4 *14*	98	15 *15*

[a] Bacteriologically quiescent disease at 12 months.

[b] The parentheses indicate percentages based on fewer than 25 observations.

slow and rapid inactivators in the degree of resistance of the first resistant cultures could not have been due to the period of treatment preceding their emergence. The second factor which might have influenced the degree of resistance was the severity of the patient's disease on admission to treatment. However, although patients whose initial disease was more severe yielded resistant cultures with greater frequency, *the degree of resistance* of their cultures was similar to those obtained from patients with less severe disease. Similar observations were made by Fox and Sutherland (1955) on patients treated with isoniazid alone. Furthermore, as can be seen from Table 15 of the main report of the present study (Tuberculosis Chemotherapy Centre, 1960) and from further analyses (not tabulated here), the initial severity of the disease in patients with resistant cultures was similar in the three treatment series and in slow and rapid inactivators of isoniazid. It therefore seems reasonable to regard the differences between the' treatment series and between the slow and rapid inactivators in the degree of resistance of the first and the supplemented six-month resistant cultures (cultures at six months, or, when unobtainable, at five or seven months, see page 219 as having been due only to the dosage of isoniazid received by the patients and to the rate at which it was inactivated.

The size of a single dose of isoniazid given in the three series treated with isoniazid alone ranged from about 2.2 mg/kg (given twice a day) in the H series, to 4.4 mg/kg (given twice a day) in the HI-2 series, and to 8.7 mg/kg (given once a day) in the HI-1 series. Gangadharam et al. (1961 b) measured the concentrations of isoniazid in the serum of a sample of the patients in the study and their findings are summarized in Table 9. The six subgroups, formed by dividing the patients in each series into rapid or slow inactivators of isoniazid, could be arranged in order of increasing peak concentrations, which ranged from 0.7 µg/ml in the H rapid inactivators to 6.6 µg/ml in the HI-1 slow inactivators. The same order was obtained if the six subgroups were arranged according to the therapeutic response, as measured by the proportion of patients who had attained bacteriologically quiescent disease at 12 months. On the other hand, the therapeutic response in the subgroups was not related to other measures of the time-serum concentration curves in the patients and, in particular, it was not related to the period during a day that inhibitory concentrations of isoniazid were present in the serum. It was therefore concluded that the response to treatment in the six subgroups was determined by the peak concentration of isoniazid attained.

TABLE 9

PEAK SERUM CONCENTRATIONS AND BACTERIOLOGICAL CLASSIFICATION AT 12 MONTHS OF THE PATIENTS IN THE THREE ISONIAZID-ALONE TREATMENT SERIES[a]

Treatment series	Single dose of isoniazid (mg/kg)	Rate of inactivation of isoniazid	Peak serum concentration of isoniazid (µg/ml)	Percentage of patients with quiescent disease at 12 months
HI-1	8.7	Slow	6.6	*72*
		Rapid	4.5	*66*
HI-2	4.4	Slow	2.6	*59*
		Rapid	1.9	*56*
H	2.2	Slow	1.2	*48*
		Rapid	0.7	*44*

[a] From Gangadharam et al. (1961b).

The present study has shown that the proportion of patients in the six subgroups who yielded one or more resistant cultures during the year of treatment decreased as the therapeutic response (and the peak concentrations) increased. The percentages were 69% for the H rapid inactivators, 65% for the H slow inactivators, 52% for the HI-2 rapid inactivators, 46% for the HI-2 slow inactivators and 44% for both the rapid and slow inactivators in the HI-1 series. This progressive decrease in the proportion of patients with resistant cultures was accounted for by a corresponding deficit of patients with first resistant cultures of low or moderate degrees of resistance; the proportion of patients whose first resistant cultures were of high or very high degrees of resistance were similar in the six subgroups. With the supplemented six-month resistant cultures the deficits of patients occurred among those with cultures of low, moderate or high degrees of resistance, but not in those with cultures of very high degrees of resistance, presumably because the supplemented six-month cultures were usually isolated after the first resistant cultures and there was evidence that the degree of resistance increased during treatment. Middlebrook (1952) and Canetti & Grosset (1961) have shown that sensitive strains of tubercle bacilli contain isoniazid-resistant mutants of different degrees of resistance.

The findings of the present study suggest that in patients with low peak serum concentrations the growth of mutants of low or moderate degrees of resistance occurred more frequently than in those with higher peak concentrations and that the differences between the subgroups in the proportion of patients with resistant cultures was determined by the ability of these mutants to multiply. Furthermore, the ultimate response of the patients, that is, whether they attained bacteriologically quiescent disease, appeared also to be largely dependent on the initial inhibition of growth of these mutants, since there was little evidence that the attainment of quiescent disease *after* the emergence of resistance was associated with the degree of resistance of the first or supplemented six-month resistant cultures.

No evidence was found that a low dosage of isoniazid, even in rapid inactivators, resulted in persistent excretion of sensitive organisms, as has been reported by Russell and Middlebrook (1956) and by Mitchell and Bell (1957). Indeed, all of the 103 patients whose disease failed to become quiescent yielded one or more resistant cultures, the excretion of sensitive cultures in the last six months of treatment occurred rarely and was not associated with the regimen or the rate of inactivation of isoniazid, and resistant cultures emerged at similar periods after the start of treatment in the rapid and slow inactivators of the three isoniazid-alone series. Thus, while variation in peak serum concentrations was the factor that seemed to determine the emergence of bacilli with low degrees of resistance, there was no reason to believe that the serum concentrations attained by any patient in the present study were too low to inhibit sensitive organisms.

In addition to the association between the *emergence* of cultures with low and moderate degrees of resistance and the peak serum concentrations of isoniazid, a further association was evident, among the patients from whom a resistant culture *had been obtained,* between the degree of resistance and the rate of inactivation of isoniazid in each of the treatment series. After amalgamating the results in the three isoniazid-alone series, first resistant cultures with low degrees of resistance were obtained from 19% of the 32 slow inactivators and from 55% of the 29 rapid inactivators who had yielded cultures of low or moderate degrees of resistance. A similar tendency for cultures from rapid inactivators to be less resistant than cultures from slow inactivators was also evident in the supplemented six-month resistant cultures, but, in keeping with the findings on the emergence of resistant cultures, the association existed in cultures with high degrees of resistance as well as in those with low and moderate degrees.

A possible explanation for the existence of the two associations considered above is as follows. Barclay, Koch-Weser & Ebert (1954), Youatt (1958) and Tsukamura, Tsukamura & Nakano (1963) have shown that labelled isoniazid is rapidly bound by isoniazid-sensitive tubercle bacilli, but not by highly resistant bacilli. On the assumption, supported by the observations of Tsukamura, Tsukamura & Nakano (1963), that bacilli with fairly low degrees of resistance also bind isoniazid, it is suggested that many of these organisms, including those that are dormant, bind isoniazid and are inhibited in their growth immediately or fairly soon after the start of treatment. The extent of binding would tend towards a maximal value determined by the peak serum concentration during successive doses of isoniazid. If the peak concentrations were low, many of the bacilli with low degrees of resistance would bind insufficient isoniazid to prevent growth, but if the peak concentrations were high, these bacilli would be inhibited and only the more resistant bacilli could grow, thus accounting for the negative association between the emergence of cultures of low or moderate degrees of resistance and the peak serum concentration. However, among those resistant bacilli that were able to continue multiplying, there would be some with relatively low degrees of resistance whose growth would be impeded by isoniazid. These organisms would therefore tend to become more resistant; in the present study the degree of resistance of resistant cultures was found to increase slightly during treatment. In these multiplying organisms there would be a race between the binding of isoniazid at available sites and the creation of new sites in the daughter cells at which binding might occur. In slow inactivators isoniazid would be present for longer periods in the lesions and the bacilli would therefore bind more isoniazid than in rapid inactivators. Thus, higher degrees of resistance would be expected in cultures from slow inactivators than in those from rapid inactivators as long as the organisms were only partially inhibited by isoniazid.

In essence, the hypothesis is that there are two stages in the emergence of isoniazid resistance. In the first stage, organisms which are sensitive or

have low degrees of resistance absorb enough isoniazid to prevent their growth throughout treatment, while more resistant organisms continue to grow. The critical degree of resistance separating organisms that are inhibited and those that grow is related to the peak serum concentrations of isoniazid and not to the length of time that isoniazid is present. In the second stage there is an increase in the degree of resistance of some of the organisms that continue to grow. Those with relatively low, " border-line " degrees of resistance become more resistant (rapidly at first and then more slowly) as a result of multiple step selection during growth. Selection for an increase in resistance is more intense the longer isoniazid is present in the serum (and in the lesions), so that resistant organisms within the border-line group become more resistant in slow inactivators than in rapid inactivators. The remaining organisms with higher degrees of resistance grow freely and are unaffected by treatment. The events in the first stage, but not those in the second stage, determine the outcome of treatment.

The association between the degree of resistance in border-line cultures (MICs of 1 and 5 µg/ml) and the inactivation rate of the patient was evident in the first resistant cultures (Table 5). This finding implies that the second stage in the emergence of resistance must have started before these cultures were obtained. The majority of the first resistant cultures were obtained during the first four months of treatment, so that the critical first-stage events–determining whether or not resistant organisms multiply at all-must have occurred even earlier. Thus the eventual response of the patients was probably decided at a very early stage, perhaps within a few days or weeks of the start of treatment. This suggests that in planning chemotherapeutic regimens particular emphasis must be placed on giving intensive chemotherapy both with isoniazid, in at least a moderate dose (400 mg in a single daily dose), and with other drugs during the early weeks of treatment to prevent the growth of resistant mutants. The importance of the early phase of intensive treatment is supported by the finding of the Medical Research Council (1962) that the administration of streptomycin for the first six weeks increased the efficacy of a regimen of isoniazid plus PAS in the treatment of pulmonary tuberculosis with cavitation.

It is evident from the hypothesis that studies relating the degree of resistance of well-established resistant cultures to the concentration of isoniazid in the serum can be misleading in the formulation of the proper dosage of isoniazid for individual patients. The second-stage events (which are irrelevant to therapeutic response) will have already occurred in these cultures, so that strains from slow inactivators will have considerably higher degrees of resistance than those from rapid inactivators. Since the difference between serum isoniazid concentrations in slow and rapid inactivators increases during the period after the dose has been taken, the best correlation may be found between the degree of resistance and the serum concentrations measured later than at the peak, for instance at three or six hours after the dose. Yet it is the peak isoniazid concentration (which determines the first-stage events) that is best related to the outcome of treatment with isoniazid and should be used in preference to the three-hour or six-hour concentration in deciding on dosage.

The influence of the addition of PAS to treatment with isoniazid can be seen by comparing the emergence of resistance in the PH and HI-2 patients whose serum isoniazid concentrations were found to be similar (Gangadharam et al., 1961b). First, only 19% of the 85 PH patients yielded a resistant culture, as compared with 49% of the 67 HI-2 patients. Secondly, resistant cultures were obtained during the first four months of treatment from 25 % of the PH patients and from 85 % of the HI-2 patients who yielded resistant cultures at any time during the 12 months of treatment. Thus, the concomitant administration of PAS prevented the emergence of resistance in a considerable proportion of the patients and, in those who yielded resistant cultures, their emergence was delayed. A further difference between the series was that low or moderate degrees of resistance were found in 88 % of the first resistant cultures in the PH series, but in only 42% of the corresponding cultures in the HI-2 series. The supplemented six-month cultures also had lower degrees of resistance in the PH series than in the HI-2 series. An explanation for these differences is that PAS either inhibited the growth of isoniazid-resistant mutants completely or, if growth occurred, slowed it down considerably, thus accounting for the delay in the emergence of resistance. If it is accepted that growth of border-line resistant organisms during treatment resulted in an increase of the degree of their resistance, then slowing down of their growth by PAS would also lead to a lower degree of resistance than if isoniazid were used alone. The preponderance of cultures with low or moderate

degrees of resistance to isoniazid in patients treated with isoniazid plus PAS is consistent with the finding of Nassau & Hamilton (1955) that cultures resistant to isoniazid and PAS were more frequently cat&se-positive than were cultures resistant only to isoniazid.

From 6% to 13 % of the patients in the four treatment series yielded one or more isoniazid-resistant cultures but nevertheless attained bacteriologically quiescent disease by 12 months. It must be noted that although the percentage of patients who yielded resistant cultures but subsequently attained bacteriological quiescence was low, this percentage nevertheless represented more than half of the patients in the PH series who excreted one or more isoniazid-resistant cultures. Thus, the prognostic significance of the emergence of an isoniazid-resistant culture in patients treated with isoniazid plus PAS is not as serious as in patients treated with isoniazid alone.

SUMMARY

1. The results are reported of isoniazid sensitivity tests on cultures obtained at monthly intervals from 305 South Indian patients with pulmonary tuberculosis who participated in a comparison of four regimens of domiciliary chemotherapy. The regimens (and the dosages appropriate to patients weighing 100 lb. (45.4 kg)) were: *(a)* PH–200 mg of isoniazid plus 10 g of PAS (sodium) a day, divided into two doses; *(b)* HI-1–400 mg of isoniazid a day, in one dose; (c) HI-2–400 mg of isoniazid a day, divided into two doses; *(d)* H–200 mg of isoniazid a day, divided into two doses. The dosage of each regimen was graded according to the patient's weight. None of the patients had had more than two weeks of previous chemotherapy, and all had isoniazid-sensitive organisms on admission to treatment. They were classified as slow or rapid inactivors of isoniazid.

2. The degree of resistance was measured as the minimal concentration of isoniazid that prevented the growth of 20 or more colonies after inoculation of approximately 10^5 viable units. The degree of resistance was reasonably reproducible in duplicate tests on the same culture and in tests on cultures obtained at six months and at seven months from the same patient.

3. All of the 103 patients who failed to attain bacteriologically quiescent disease at 12 months yielded resistant cultures. In the second six months of treatment, only nine of 415 cultures tested were isoniazid-sensitive and the finding of a sensitive culture was not associated with the dosage of isoniazid or with the rate of its inactivation. The serum concentrations of isoniazid attained by any patient did not, therefore, appear to be too low to inhibit sensitive organisms.

4. The three isoniazid-alone series were similar in the month in which the first resistant culture emerged; 80% of the resistant cultures emerged during the first four months of treatment. The degree of resistance of the first resistant cultures was not associated with the month in which it emerged.

5. There was a slight increase in the degree of resistance of resistant cultures as treatment was continued.

6. The degrees of resistance of the first resistant cultures and of the " supplemented six-month " resistant cultures (comprising a culture obtained from each patient at six months, or, if not available or negative, at seven or five months) were not associated with the extent of cavitation, the total extent of disease or the bacterial content of the sputum of the patients on admission to treatment.

7. The proportion of patients with resistant cultures in the six sub-groups, formed by dividing each of the isoniazid-alone series into rapid and slow inactivators, ranged from 69% in the H rapid inactivators, to 65% in the H slow inactivators, to 52% in the HI-2 rapid inactivators, to 46 % in the HI-2 slow inactivators and to 44% in the HI-1 rapid and slow inactivators. The corresponding percentages of those who yielded supplemented six-month resistant cultures were 69 %, 51%, 48 %, 40%, 42% and 26%, respectively. The progressive decrease in the proportion of patients with resistant cultures was mainly due to corresponding deficiencies of patients who yielded first resistant cultures with low or moderate degrees of resistance or supplemented six-month cultures with low, moderate or high degrees of resistance; the proportion of patients with cultures of higher degrees of resistance

remained fairly constant in each subgroup. Since the order of these subgroups arranged according to the proportion of patients yielding resistant cultures is the same as the order arranged according to the peak concentrations of isoniazid obtained in the serum during treatment, it seems that the early growth of resistant mutants with relatively low degrees of resistance and, therefore, the proportion of patients who yielded resistant cultures were dependent on the peak serum concentrations attained.

8. In the eight subgroups of rapid and slow inactivators in the four treatment series, failure to respond to treatment, as assessed by the proportion of patients who did not attain bacteriologically quiescent disease at 12 months, was closely associated with the proportion of patients who yielded first or supplemented six-month resistant cultures. However, there was no definite association between the degree of resistance of these resistant cultures and the subsequent response to treatment. Thus, the ultimate response of the patients appeared to depend on the ability of resistant mutants to multiply in the early stages of treatment.

9. A further association existed between the degree of resistance of resistant cultures that had emerged and the rate of inactivation of isoniazid. Of first resistant cultures with low or moderate degrees of resistance, cultures with low degrees of resistance were obtained from 19% of 32 slow inactivators and from 55 % of 29 rapid inactivators. A similar association existed among six-month supplemented cultures. It is suggested that the higher degree of resistance in slow inactivators is due to partial inhibition of multiplying resistant bacilli by the prolonged presence of low concentrations of isoniazid and consequent selection of more highly resistant organisms.

10. Resistant cultures were obtained from 19 % of the 85 PH patients and from 49% of the 67 HI-2 patients (similar serum isoniazid concentrations were found in the two series) during treatment. The first resistant culture was obtained during the first four months in 25 % of the 16 PH patients and in 85 % of the 33 HI-2 patients who yielded resistant cultures; low or moderate degrees of resistance were found in 88% of the PH cultures and in 42% of the HI-2 cultures. Thus, the concomitant administration of PAS frequently prevented the emergence of resistance, and, in those who yielded resistant cultures, its emergence was delayed and the degree of resistance was low.

11. It is concluded that the response to 12 months' treatment depends largely on whether mutants with low degrees of resistance are able to multiply in the very early stages of treatment. Since the growth of these mutants is prevented by high peak concentrations of isoniazid, it is suggested that intensive chemotherapy should be given at the start of treatment.

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RÉSUMÉ

Les auteurs exposent les résultats du test de sensibilite à l'isoniazide, appliqué tous les mois aux bacilles tuberculeux provenant de 305 sujets de 1'Inde méridionale, atteints de tuberculose pulmonaire, et soumis, par groupes, à quatre regimes thérapeutiques à domicile, qui, pour des malades pesant environ 45 kg étaient les suivants: série PH: 200 mg d'isoniazide + 10 g par jour de PAS (sel sodique), en deux doses; série HI-1 : 400 mg d'isoniazide par jour, en deux doses; série H-2: 400 mg d'isoniazide par jour, en deux doses; série H: 200 mg d'isoniazide par jour, en deux doses. Les doses étaient adaptées au poids de chaque malade. Aucun des sujets n'avait eu, auparavant, plus de deux semaines de chimiothérapie, et au commencement du traitement, tous les bacilles étaient sensibles à l'isoniazide. Les malades furent classés en inactivateurs rapides et inactivateurs lents.

Le degré de resistance a été évalué d'après la concentration minimum d'isoniazide inhibant le développement au point de ne permettre la croissance que de 20 colonies

au maximum, à partir d'un inoculum de 10^5 bacilles. Le degré de résistance était le même, à une approximation satisfaisante, dans les tests répétés avec la même culture ou les tests effectués 6 ou 7 mois après sur les mêmes malades.

Les 103 malades qui n'atteignirent pas le stade de quiescence bactériologique après 12 mois, présentaient des bacilles résistants. Dans les 6 derniers mois du traitement, 9 seulement des 415 cultures soumises au test étaient sensibles à l'isoniazide, et aucune relation n'a pu être établie entre la résistance et la quantité d'isoniazide du régime thérapeutique ou la vitesse d'inactivation. La concentration du sérum en isoniazide, atteinte par chaque malade, ne paraissait donc pas trop faible pour inhiber les bacilles sensibles.

Le mois où apparurent les premieres cultures r&istantes, les trois séries comportant de l'isoniazide seulement se comportaient de façon analogue; 80% des cultures résistantes apparurent durant les 4 premiers mois de traitement. Le degré de résistance des premieres cultures résistantes n'était pas en relation avec le mois de leur apparition.

On constata une légère augmentation de la résistance chez les cultures résistantes au fur et à mesure que le traitement progressait.

Le degré de résistance des premieres cultures résistantes et des cultures supplémentaires (une culture provenant de chaque malade après 6 mois de traitement – ou éventuellement 5 ou 7 mois) n'était en relation ni avec l'étendue des cavités, ni avec celle de la maladie en général, ni avec la teneur en bacilles des crachats, au moment de l'admission des malades.

La proportion de malades présentant des cultures résistantes, dans les six sous-groupes formés en répartissant chacun des groupes ne recevant que de l'isoniazide en inactivateurs rapides et inactivateurs lents, s'échelonnait entre les limites suivantes: série H, 69% chez les rapides à 65 % chez les lents ; série HI-2, 52 % chez les rapides à 46 % chez les lents; série HI-1, 44 % dans les deux groupes. Les proportions correspondantes des malades des groupes ci-dessus donnant des cultures résistantes à 6 mois étaient 69 %, 51%, 48 %, 42 %, et 26 % respectivement. Cette proportion décroissante de malades présentant des cultures résistantes étant en rapport avec le maximum de la teneur du sérum en isoniazide, il semble que la proportion de malades donnant des cultures résistantes dépende de ce facteur.

Dans les 8 sous-groupes (inactivateurs rapides et lents) des 4 séries de traitement, l'échec du traitement – évalué par la proportion de malades n'ayant pas atteint le stade de quiescence bactériologique en 12 mois –, était en rapport étroit avec la proportion de malades présentant des cultures résistantes. Cependant, il n'y avait pas de rapport net entre le degré de résistance des cultures et la réponse subséquente au traitement. Il semble que, finalement, la réponse des malades dépende de la faculté des mutants résistants de se multiplier au cours des premiers stades du traitement.

On a constaté une relation entre le degré de résistance des cultures et le taux d'inactivation de l'isoniazide. On a obtenu, parmi les premières cultures résistantes, des cultures présentant un faible degré de résistance chez 19 % de 32 inactivateurs lents, et 55 % de 29 inactivateurs rapides. Il en était de même pour les cultures prélevées à 6 mois. On peut penser que le degré plus élevé de résistance chez les inactivateurs lents est dû à l'inhibition partielle de la multiplication des bacilles résistants par la présence, durant des périodes prolongées, de faibles concentrations sériques d'isoniazide, et la sélection d'organismes plus résistants qui s'ensuivit.

L'expérience a montré également que l'administration de PAS avec l'isoniazide permettait d'éviter ou de retarder l'apparition de la résistance, et d'atténuer le degré de la résistance manifestée par les bacilles.

Les auteurs concluent que le résultat d'une chimiothérapie de 12 mois dépend en grande partie de la possibilité pour les mutants résistants de se multiplier durant les premiers stades du traitement. Puisque l'expérience montre que leur croissance est inhibée par des taux élevés d'isoniazide sérique, ils estiment qu'une chimiothérapie intensive devrait être administrée au début du traitement.

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