

Rate of Inactivation of Isoniazid in South Indian Patients with Pulmonary Tuberculosis *

2. Clinical Implications in the Treatment of Pulmonary Tuberculosis with Isoniazid either Alone or in Combination with PAS

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A series of studies on the rate of inactivation of isoniazid in Indian patients with pulmonary tuberculosis undergoing domiciliary chemotherapy with isoniazid, alone or in combination with p-aminosalicylic acid, has recently been undertaken by the Tuberculosis Chemotherapy Centre, Madras. In the first study, the serum isoniazid levels of the patients were determined four-and-a-half hours after intramuscular administration of a standard dose of 3 mg/kg body-weight of isoniazid and, according to whether the serum level was 0.58 µg/ml or above, or less than 0.58 µg/ml, the patient was classified as a slow or as a rapid inactivator. The present paper describes the second of these studies, in which the response to treatment of the slow and the rapid inactivators was compared. The results of this investigation suggested that there might be an association between response to treatment and rate of inactivation of isoniazid, since the slow inactivators were more often culture-negative during treatment and showed a higher proportion of individuals with bacteriologically quiescent disease at 12 months and a lower proportion with radiographic deterioration at six months than the rapid inactivators, while the slow inactivators who deteriorated radiographically or clinically to an extent warranting a change of treatment during the two years did so later than the corresponding rapid inactivators. There was slight evidence that the slow and the rapid inactivators differed in the speed of conversion to bacteriological negativity of those patients whose disease was bacteriologically quiescent at 12 months, but no evidence that they differed in the degree of positivity of sputum specimens that were positive on culture at six, nine or 12 months, or in the frequency with which the patients showed moderate or greater radiographic improvement at six months.

INTRODUCTION*

A previous report from the Tuberculosis Chemotherapy Centre, Madras, presented the results of a controlled comparison for a period of 12 months of three different regimens of isoniazid alone with a regimen of isoniazid plus p-aminosalicylic acid (PAS) in the domiciliary treatment of pulmonary tuberculosis (Tuberculosis Chemotherapy Centre, 1960). The patients in this chemotherapy study were classi-

ed as slow or rapid inactivators of isoniazid according to the serum concentration of isoniazid four-and-a-half hours after an intramuscular injection of 3 mg/kg body-weight of isoniazid (Gangadharam et al., 1961¹). Devadatta et al. (1960) have reported elsewhere that isoniazid-induced peripheral neuritis was encountered in 20 of the 341 patients admitted to the study, and that 18 of these 20 patients were slow inactivators of isoniazid. The analyses reported here were undertaken to discover whether an association existed between the rate of inactivation of isoniazid and the response to treatment with isoniazid alone or in combination

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¹ See article on page 765.

with PAS. The relationship between the rate of inactivation of isoniazid and the degree of resistance to isoniazid, the catalase activity and the virulence in the guinea-pig of cultures isolated from these patients during treatment will be reported later.

MATERIALS AND METHODS

Patients

In all, 341 patients with pulmonary tuberculosis were admitted to the controlled chemotherapy study. They were allocated at random to treatment with one of the following regimens for 12 months :

PH (96 patients). Isoniazid 3.9-5.5 mg/kg body-weight plus PAS (sodium) 0.2-0.3 g/kg daily, divided into two doses, by mouth—i.e., 200 mg of isoniazid plus 10 g of PAS (sodium) a day for a patient weighing 100 lb. (45.4 kg).

HZ-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.

HZ-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 of isoniazid a day for a patient weighing 100 lb.

At the end of 12 months' treatment patients whose disease was classified as bacteriologically quiescent or of doubtful status (see page 785) were allocated at random to treatment with calcium gluconate 0.5 g daily or isoniazid in a dosage of approximately 4.5 mg/kg daily for a further 12 months (Velu et al., 1961). Patients whose disease was classified as bacteriologically relapsed or active continued on the chemotherapy prescribed for their first year of treatment.

After excluding 26 patients for reasons given in the earlier report (Tuberculosis Chemotherapy Centre, 1960) there remained 315 patients (90 PH, 70 HI-1, 68 HI-2 and 87 H) who fulfilled the following criteria: they were aged 12 years or more; they had sputum positive for tubercle bacilli on culture examination with organisms sensitive to isoniazid; and they had not previously received more than two weeks of antituberculosis chemotherapy (96.5 % of the 315 patients had received none).

The isoniazid inactivation rates were not available for 14 of these 315 patients: in two patients the tests were contaminated; in one patient the blood was

collected half an hour too late; 11 patients (3 PH, 1 HI-1, 2 HI-2 and 5 H) had died before the earliest month selected for the test. Of the 11 patients who died, five did so from non-tuberculous causes and six from tuberculosis, two of the latter during the first month of treatment. In addition, two patients (one slow and one rapid inactivator) were excluded from the analysis as they had their treatment changed in the second month because of hypersensitivity to PAS. There remained 299 patients in this analysis—namely, 83 (92%) of the 90 PH, 68 (97%) of the 70 HI-1, 66 (97%) of the 68 HI-2 and 82 (94%) of the 87 H patients.

The clinical, radiographic and bacteriological methods used in this investigation have been described in detail elsewhere (Tuberculosis Chemotherapy Centre, 1960; Gangadharam et al., 1961¹). A brief description of the methods used is, however, given below.

Radiographic assessments

Radiographic assessments of the severity of the disease on admission to treatment and of the changes between 0 and 6 and between 0 and 12 months were made from full-plate postero-anterior radiographs by an independent assessor (Dr Raj Narain) who was unaware of the treatment regimen to which any patient had been allocated. The assessments of the severity of the disease on admission to treatment were: the extent of cavitation, graded as extensive, moderate, slight or nil; the extent of the radiographic lesions, classified as gross, extensive, moderate, limited, slight or trivial; and the number of lung zones involved in disease, the presence of disease in each zone being recorded no matter how limited in extent. For the radiographic changes between 0 and 6 and between 0 and 12 months, four grades of improvement were used—namely, exceptional, considerable, moderate and slight—and three of deterioration—namely, slight, moderate and considerable.

Bacterial content of the sputum

Before the start of the treatment a minimum of two overnight (collection) and two clinic (spot) specimens of sputum were obtained. At the end of every month of treatment two collection specimens, of sputum and, from the third month onwards, in addition, a pair of laryngeal swabs were obtained from each patient. Sputum specimens were examined

¹See article on page 765.

by fluorescence microscopy (Holst, Mitchison & Radhakrishna, 1959) and were cultured, after treatment with 4% NaOH, on Löwenstein-Jensen medium which did not contain potato starch (Jensen, 1955). Laryngeal swabs were cultured on the same medium (Tuberculosis Chemotherapy Centre, 1959).

Virulence in the guinea-pig

Pretreatment cultures from 266 of the 299 patients were tested for virulence in the guinea-pig. The degree of virulence was expressed as the root-index of virulence (Mitchison et al., 1961).

Rate of inactivation of isoniazid

The rate of inactivation of isoniazid was determined from the concentration of isoniazid in the serum four-and-a-half hours after an intramuscular injection of 3 mg/kg body-weight isoniazid. This concentration was measured by microbiological assay and patients with serum concentrations of 0.58 µg/ml or more were classified as slow inactivators and those with serum concentrations below 0.58 µg/ml as rapid inactivators (Gangadharam et al., 1961¹). The rate of inactivation of isoniazid was determined between the sixth and the twelfth month of treatment.

RESULTS

Number of slow and rapid inactivators

Of the 299 patients, 178 (60%) were slow inactivators of isoniazid and 121 (40%) were rapid inactivators. Although there were differences between the proportions of slow inactivators in the four treatment series—69 % in the PH, 53 % in the HI-1, 59% in the HI-2 and 56% in the H series—none of these differences was unusually large.

The results of this investigation are presented in two sections. The first examines whether the condition of the slow and rapid inactivators on admission to treatment was similar and the second whether there was any difference between the response to treatment of the slow and the rapid inactivators.

Condition on admission to treatment of slow and rapid inactivators of isoniazid

Age and sex. The age-distributions of the slow and the rapid inactivators were closely similar. Thus, 26 % of the slow and 33 % of the rapid inactivators

were under 25 years of age and 15 % of each group were aged 45 years or more.

Of the 178 slow inactivators 70% were males, as compared with 55% of the 121 rapid inactivators. This difference, which attains statistical significance ($P < 0.01$) and has been discussed elsewhere (Gangadharam et al., 1961¹), is not, however, of importance as there was no evidence from this study that response to treatment was related to the sex of the patients.

Disease condition on admission to treatment.

Table 1 presents, separately for the slow and for the rapid inactivators, three radiographic and two bacteriological assessments of the disease at the start of treatment. The distributions for the two groups were very similar. For example, the majority of both groups—namely, 63 % of the slow and 62 % of the rapid inactivators had moderate or extensive cavitation, the proportion with extensive cavitation being 10% and 12 %, respectively. In 62% of the slow and 64% of the rapid inactivators the total extent of disease was classified as limited or moderate; 28 % and 30 %, respectively, had extensive or gross disease. Considering the bacterial content of the sputum, 19 % of the slow and 15 % of the rapid inactivators had a negative result on smear examination and 37% and 36%, respectively, had a 3-plus (heavy) positive result. In 32 % of the slow and 34 % of the rapid inactivators, the pretreatment cultures were of low virulence in the guinea-pig and in 23 % of the slow and 25 % of the rapid inactivators they were of high virulence. The mean root-index of virulence for the slow inactivators was 0.72 and for the rapid inactivators 0.74.

Further analyses (not tabulated here) were undertaken separately for each of the four treatment series to see whether the distributions of slow and rapid inactivators *within each treatment series* differed in any of the above five factors. In the main the distributions were similar, but there were some differences which were as follows: In the PH series, 59 % of the slow inactivators, as compared with 74 % of the rapid inactivators, had moderate or extensive cavitation. In the HI-2 series, the slow inactivators were at an advantage in that 18% had extensive or gross disease, as compared with 30% of the rapid inactivators, and in respect of the guinea-pig virulence of their pretreatment cultures, the mean root-index of virulence being 0.61 for the slow inactivators and 0.73 for the rapid inactivators ($P = 0.02$). On the other hand, they were at a disadvantage in respect of the important prognostic factor, cavitation (Tuberculosis Chemotherapy Cen-

¹See article on page 765.

TABLE 1
CONDITION ON ADMISSION TO TREATMENT
OF SLOW AND RAPID INACTIVATORS OF ISONIAZID

Assessment on admission to treatment	Slow inactivators		Rapid inactivators		
	No.	%	No.	%	
<i>Extent of cavitation :</i>					
Nil	15	8	7	6	
Slight	51	29	39	32	
Moderate	94	53	61	50	
Extensive	18	10	14	12	
<i>Total extent of disease :</i>					
Trivial or slight	18	10	8	7	
Limited or moderate	110	62	77	64	
Extensive or gross	50	28	36	30	
<i>Number of lung zones involved in disease:</i>					
1 or 2	35	20	25	21	
3 or 4	77	43	44	36	
5 or 6	66	37	52	43	
<i>Bacterial content of sputum:</i>					
Direct smear negative	Culture negative	7	4	2	2
	Culture positive	25	14	16	13
Direct smear positive	Smear 1-plus (scanty)	34	19	16	13
	Smear 2-plus (moderate)	47	26	43	36
	Smear 3-plus (heavy)	65	37	44	36
Total	178	100	121	100	
<i>Root-index of virulence:</i>					
0.0 -	50	32	38	34	
0.6 -	69	45	45	41	
0.9 or above	36	23	28	25	
Total	155 ^a	100	111 ^b	100	

^a Results for 23 patients not available.

^b Results for 10 patients not available.

tre, 1960), 72 % having moderate or extensive cavitation, as compared with 56 % of the rapid inactivators. In the H series, 37 % of the slow inactivators had five or six lung zones involved in disease, as compared with 58% of the rapid inactivators, but on the other hand, 47% of the slow inactivators had 3-plus positive smears, as compared with 28% of the rapid inactivators. With the exception of the difference between the slow and the rapid inactivators in the HI-2 series in respect of the virulence of their pre-treatment cultures in the guinea-pig, none of these differences attains statistical significance.

In summary, the slow and the rapid inactivators were very similar in terms of the major pretreatment disease factors when all 299 patients were considered. When, however, the treatment series were examined separately, there was a suggestion that the slow inactivators were at an advantage in the PH series.

Comparison of response to treatment of slow and rapid inactivators of isoniazid

Changes of treatment during the first 12 months. Forty patients had their treatment changed during the 12 months. Of these changes, 32 were made on the recommendation of an independent assessor, Dr K. S. Sanjivi, on account of serious radiographic deterioration and two more were made on account of severe clinical deterioration. The procedure adopted for assessing radiographic deterioration that warranted a change of treatment has been presented in detail elsewhere (Tuberculosis Chemotherapy Centre, 1960). Since all these 34 patients yielded positive results, both on smear and on culture, at the time treatment was changed and since all had yielded three or more cultures which were resistant to isoniazid, they have been regarded as smear-and culture-positive for the remainder of the 12 months in the bacteriological analysis (see page 784).

The remaining six patients (all slow inactivators; 5 HI-1, 1 HI-2) had their treatment changed because of peripheral neuritis. Of these, four were positive on smear examination and five were positive on culture at the time of changing treatment (one in the fifth month, two in the sixth month, one in the tenth month and one in the eleventh month), and all of them had yielded two or more isoniazid-resistant cultures. Since most of the patients in this study who were still culture-positive at five months or later, and nearly all (86%) who yielded two or more cultures resistant to isoniazid during the first 12 months, failed to convert to bacteriological negativity during this period, these five patients have been regarded

as positive on smear and culture for the remainder of the 12 months. The sixth patient (HI-1) had treatment changed on account of peripheral neuritis in the seventh month, after having been culture-negative at three, four, five and six months. He has therefore been classified as bacteriologically quiescent at 12 months. While there may be an element of error involved in the above classification of the response of these six patients, it must be pointed out that their exclusion would have been a serious source of bias, because these six patients cannot be considered to be a random sample of the slow inactivators. Thus, whereas five of the six patients yielded two or more isoniazid-resistant cultures—a bad prognostic factor—only 20 (30%) of the remaining 69 slow inactivators in the HI-1 and HI-2 series did so, a significant difference ($P = 0.02$).

Radiographic response. The change in radiographic appearances of slow and rapid inactivators of isoniazid between 0 and 6 months is shown in Table 2. During the first six months of treatment, three slow inactivators (2 HI-1, 1 HI-2) had their treatment changed because of peripheral neuritis. Two of these (1 HI-1, 1 HI-2) had their treatment

changed after they had completed five months of treatment. These two patients have been included in Table 2 since it is unlikely that the radiographic appearances would have changed much during the sixth month. The proportion of slow and rapid inactivators who showed moderate or greater improvement at six months were 79% and 81%, respectively, in the PH series, 78% and 66% in the HI-1 series, 62% and 67% in the HI-2 series and 67% and 78% in the H series. On amalgamating the four series, the proportions were 72% and 73%, respectively. There was thus no difference between the rapid and the slow inactivators in this respect. The proportions of slow and rapid inactivators who showed radiographic deterioration were 0% and 4%, respectively, in the PH series, 0% and 15% in the HI-1 series, 3% and 11% in the HI-2 series and 9% and 14% in the H series. On amalgamating the four series, the proportions were 3% and 11%, respectively. In both the HI-1 and the amalgamated series the differences attain significance ($P = 0.02$ and $P < 0.01$, respectively). There was thus a clear difference between the proportions of slow and rapid inactivators who had deteriorated radiographically by six months.

TABLE 2
RADIOGRAPHIC RESPONSE IN THE FIRST SIX MONTHS IN SLOW AND RAPID INACTIVATORS OF ISONIAZID

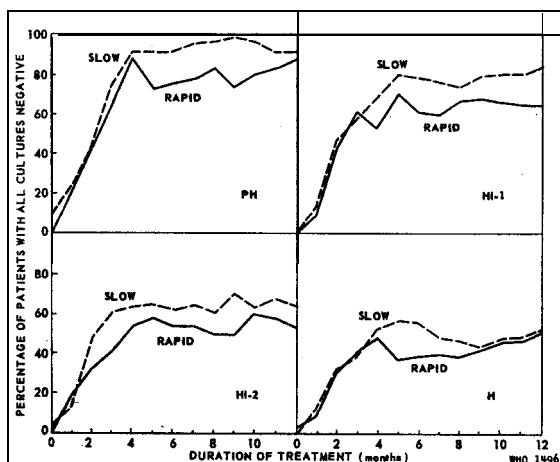
Treatment series	Rate of inactivation of isoniazid	Total patients		Radiographic response						Change of chemotherapy due to:			
				Moderate or greater improvement		Slight improvement or no change		Deterioration		Deterioration		Peripheral neuritis	
		No.	%	No.	%	No.	%	No.	%	No.	%		
PH	Slow	57	100	45	79	12	21	0	0	0	0	0	0
	Rapid	26	100	21	81	4	15	1	4	0	0	0	0
HI-1	Slow	36	100	28 ^a	78	7	19	0	0	0	0	1	3
	Rapid	32	100	21	66	6	19	4	12	1	3	0	0
HI-2	Slow	39	101	24 ^a	62	14	36	1	3	0	0	0	0
	Rapid	27	100	18	67	6	22	2	7	1	4	0	0
H	Slow	46	100	31	67	11	24	3	7	1	2	0	0
	Rapid	36	100	28	78	3	8	3	8	2	6	0	0
All series	Slow	178	101	128	72	44	25	4	2	1	1	1	1
	Rapid	121	100	88	73	19	16	10	8	4	3	0	0

^aIncluding one patient whose treatment was changed in the sixth month because of peripheral neuritis.

The analysis of the radiographic changes between 0 and 12 months (not tabulated here) was complicated by the fact that eight patients had had their treatment changed during the 12 months for reasons other than radiographic deterioration, six because of peripheral neuritis and two on account of severe clinical deterioration. An attempt was made to predict the probable radiographic progress of these eight patients between 0 and 12 months from their bacteriological and radiographic results up to the time they had their treatment changed. Bearing in mind that this may be inaccurate, the analysis of the 0 to 12-month radiographic changes yielded similar results to those obtained at six months, but the difference between the proportions of slow and rapid inactivators who had deteriorated was smaller.

Bacteriological response. The bacteriological response to treatment of the slow and the rapid inactivators of isoniazid was studied by comparing, at each month of treatment, the proportions of slow and rapid inactivators whose two collection specimens of sputum were negative on culture (see accompanying figure). All the 34 patients whose treatment was changed because of clinical or radiographic deterioration and five of the six patients whose treatment was changed because of peripheral neuritis have been regarded as culture-positive for the remainder of the 12 months after they changed treatment. The reasons for adopting this procedure are given on pages 782 and 783.

PERCENTAGE OF SLOW AND RAPID INACTIVATORS EACH MONTH WHOSE COLLECTION SPECIMENS OF SPUTUM WERE NEGATIVE ON CULTURE



In all the four treatment series, the proportion of patients whose sputum became culture-negative was larger in the slow than in the rapid inactivators. This difference became apparent at five months in the PH series, at four months in the HI-1 series, at two months in the HI-2 series, and at five months in the H series. The magnitude of the difference between the slow and the rapid inactivators remained fairly constant during the rest of the year in the HI-1 and HI-2 series, but became smaller at 11 and 12 months in the PH series, and extremely small between nine and 12 months in the H series. On amalgamating the four treatment series, the difference between the proportions for the slow and the rapid inactivators was 16% at five months, 15% at six and at seven months, 12% at eight months, 18% at nine months, 10% at 10 and at 11 months, and 11% at 12 months. The differences at five, six, seven, eight and nine months attain statistical significance.

Table 3 presents the month of bacteriological conversion (first of three or more consecutive months during which all the specimens were negative on culture) for the slow and the rapid inactivators who achieved bacteriological quiescence by 12 months. In all four series the majority of slow and rapid inactivators who converted did so within the first three months of treatment. Amalgamating the four series, 75% of the 119 slow inactivators converted bacteriologically in the first three months, 21% in the second three months and 4% between seven and 10 months. The corresponding proportions for the 70 rapid inactivators were 67%, 23% and 10%, respectively. None of these differences attains significance ($P > 0.1$). Thus, there was only a slight suggestion that the slow inactivators converted bacteriologically earlier than the rapid inactivators.

Analyses (not tabulated here) failed to demonstrate differences between the slow and the rapid inactivators in the degree of positivity, as assessed by smear examination, of the sputum specimens that were culture-positive at six, nine or 12 months.

In summary, there was a suggestion that the slow inactivators of isoniazid more frequently became negative on culture during treatment than the rapid inactivators. There was slight evidence of a difference between the slow and the rapid inactivators in the speed of conversion to bacteriological negativity among those patients who achieved quiescence by 12 months but not in the degree of positivity of the sputum specimens that were culture-positive at six, nine or 12 months.

TABLE 3
MONTH OF CONVERSION TO BACTERIOLOGICAL NEGATIVITY OF SLOW AND RAPID INACTIVATORS OF ISONIAZID

Treatment series	Rate of inactivation of isoniazid	Total patients		Month of conversion ^a					
				1-3		4-6		7-10	
		No.	%	No.	%	No.	%	No.	%
PH	Slow	51	101	38	75	12	24	1	2
	Rapid	21	100	12	(57) ^b	8	(38)	1	(5)
HI-1	Slow	24	100	18	(75)	5	(21)	1	(4)
	Rapid	18	101	14	(78)	3	(17)	1	(6)
HI-2	Slow	22	101	18	(82)	3	(14)	1	(5)
	Rapid	15	100	9	(60)	4	(27)	2	(13)
H	Slow	22	100	15	(68)	5	(23)	2	(9)
	Rapid	16	100	12	(75)	1	(6)	3	(19)
All series	Slow	119	100	89	75	25	21	5	4
	Rapid	70	100	47	67	16	23	7	10

^aFirst of three or more consecutive months during which all specimens were negative on culture.

^bThe parentheses indicate percentages based on fewer than 25 observations.

Classification of patients according to their response to treatment during the 12 months. The disease in the patients who completed 12 months of the prescribed chemotherapy was classified bacteriologically as "quiescent" if all the sputum specimens and laryngeal swabs examined during the last three months (that is, at 10, 11 and 12 months) were negative on culture; as of "doubtful status" if, following at least three consecutive months of culture negativity, the patient yielded an isolated positive culture during the last three months; as "relapsed" if, following at least three consecutive months of culture-negativity, two or more positive cultures were obtained during the last three months; and as "active" if the patient had never had a period of three consecutive months during which all the cultures were negative.

Table 4 presents the status of the slow and the rapid inactivators at 12 months according to the above classification of their response to treatment. In this table the nine patients (4 PH, 4 HI-1, 1 HI-2) whose disease was classified as of doubtful status have been amalgamated with the quiescent group. This procedure was adopted since they were relatively few in number and their subsequent progress was similar to that of the patients with quiescent disease (Velu

et al., 1961). In addition to the patients who completed 12 months of the prescribed chemotherapy there were 34 patients who had had their chemotherapy changed because of clinical or radiographic deterioration and six who had had their chemotherapy changed on account of peripheral neuritis. In the calculation of the percentages quoted below, five of the six patients whose treatment was changed because of peripheral neuritis have been regarded as having active disease at 12 months, and the sixth (HI-1) as having quiescent disease (see page 782). In all four treatment series, the proportion of slow inactivators whose disease was bacteriologically quiescent or of doubtful status was larger than that of the rapid inactivators, the figures being 95% and 85%, respectively, in the PH series,¹ 72% and 66%, respectively, in the HI-1 series, 59% and 56%, respectively, in the HI-2 series and 48% and 44%, respectively, in the H series. Amalgamating the four treatment series, 125 (70%) of the slow inactivators as compared with 74 (61%) of the rapid inactivators

¹When standardization was undertaken for the pretreatment differences in the extent of cavitation on admission to treatment (see page 781), these proportions were 95% and 87%, respectively.

TABLE 4
BACTERIOLOGICAL STATUS AT 12 MONTHS OF SLOW AND RAPID INACTIVATORS OF ISONIAZID

Treatment series	Rate of inactivation of isoniazid	Total patients		Bacteriological status at 12 months						Change of chemotherapy due to:			
				Quiescent or doubtful		Relapsed		Active		Deterioration		Peripheral neuritis	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
PH	Slow	57	101	54	95	2	4	1	2	0	0	0	0
	Rapid	26	101	22	85	2	8	1	4	1	4	0	0
HI-1	Slow	36	100	25	69	2	6	4	11	0	0	5	14
	Rapid	32	101	21	66	0	0	6	19	5	16	0	0
HI-2	Slow	39	101	23	59	3	8	4	10	8	21	1	3
	Rapid	27	100	15	56	2	7	4	15	6	22	0	0
H	Slow	46	101	22	48	4	9	16	35	4	9	0	0
	Rapid	36	100	16	44	1	3	9	25	10	28	0	0
All series	Slow	178	100	124	70	11	6	25	14	12	7	6 ^a	3
	Rapid	121	100	74	61	5	4	20	17	22	18	0	0

^a Five of these patients (4 HI-1, 1 HI-2) were classified as having bacteriologically active disease at 12 months and one (HI-1) as having quiescent disease.

had quiescent disease. None of these differences, however, attains significance, though that for the amalgamated series only just fails to do so ($P=0.07$).

Change of treatment due to deterioration occurred in 0% of the slow and 4% of the rapid inactivators in the PH series, 0% and 16% in the HI-1 series, 21% and 22% in the HI-2 series and 9% and 28% in the H series, respectively. On amalgamating the four treatment series, the proportions were 7% and 18%, respectively. The difference between the proportions of slow and rapid inactivators who deteriorated to an extent that warranted a change of treatment attains significance in the H series ($P \sim 0.05$) and in the amalgamated series ($P < 0.01$). It must be pointed out that five of the six slow inactivators whose treatment was changed because of peripheral neuritis have been classified as bacteriologically active but not as deteriorated at 12 months. However, if these five patients are classified as having deteriorated to an extent that warranted a change of treatment, the difference between the slow and rapid inactivators in the amalgamated series still attains significance ($P=0.05$). This association is discussed further on page 787.

Response during the second year. Of the 198 patients (124 slow and 74 rapid inactivators) whose

disease was classified bacteriologically as quiescent or of doubtful status at the end of the first 12 months, 186 (115 slow and 71 rapid inactivators) were allocated at random to treatment with either calcium gluconate 0.5 g daily or isoniazid in a dosage of approximately 4.5 mg/kg body-weight daily for a further 12 months. Of the 12 patients not allocated to treatment at random, six were misclassified at 12 months as having bacteriologically active disease and continued on their original chemotherapy, five were regarded as being unlikely to co-operate for a further year and were therefore given calcium gluconate and one was, in error, kept on his original treatment until 15 months before being changed to calcium gluconate.

There were no major differences between the patients randomly allocated to treatment with isoniazid or calcium, or between the slow and the rapid inactivators in each treatment series, in respect of the severity of the disease at the end of the first 12 months. In Table 5, the response during the second year of the slow and the rapid inactivators who were randomly allocated to treatment is related to the presence of cavitation at 12 months and to the second-year regimen. The disease in all the 59 slow inactivators who received isoniazid remained bacte-

TABLE 5
RESPONSE TO TREATMENT IN THE SECOND YEAR OF SLOW AND RAPID INACTIVATORS WHO HAD BACTERIOLOGICALLY QUIESCENT DISEASE OR DISEASE OF DOUBTFUL STATUS AT 12 MONTHS

Treatment in the second year	Cavitation status at 12 months	Total patients	Rate of inactivation of isoniazid					
			Slow			Rapid		
			Number of patients	Quiescent ^a throughout the second year		Number of patients	Quiescent ^a throughout the second year	
				No.	%		No.	%
Isoniazid	Non-cavitated	61	39	39	100	22	21	(95) ^b
	Cavitated	33	20	20	(100)	13	12	(92)
	Total	94 ^c	59 ^c	59	100	35	33	94
Calcium	Non-cavitated	68	38	36	95	30	26	87
	Cavitated	23	17	14	(82)	6	6	(100)
	Total	91	55	50	91	36	32	89

^aNot more than one positive culture in any 6-month period.

^bThe parentheses indicate percentages based on fewer than 25 observations.

^cExcluding one patient who died from a non-tuberculous cause in the 22nd month.

riologically quiescent (not more than one positive culture in any 6-month period) during the second year, as compared with 33 (94%) of the 35 rapid inactivators on isoniazid and 50 (91%) of the 55 slow inactivators and 32 (89%) of the 36 rapid inactivators on calcium gluconate. There was thus evidence that the slow inactivators who received isoniazid fared more favourably than the other patients.

Of the patients who continued on their initially prescribed chemotherapy for the whole of the first year, the disease in 16 was classified bacteriologically as relapsed and in 45 as active at 12 months. Of these 61 patients, 57 (33 slow, 24 rapid) continued on their initially prescribed treatment in the second year (Ramakrishnan et al., 1962). (Three patients (slow inactivators) did not continue on their initially prescribed chemotherapy because they were misclassified at 12 months as bacteriologically quiescent and consequently allocated at random to treatment with calcium gluconate or isoniazid for the second year, and one rapid inactivator had his treatment changed in the thirteenth month because of severe cor pulmonale. The one patient who was allocated to isoniazid had bacteriologically quiescent disease throughout the second year and the two who received calcium gluconate had bacteriologically active disease at 24 months. The patient with cor pulmonale

died in the fifteenth month.) At the end of the second year, 15 % of the 33 slow inactivators had bacteriologically quiescent disease, as compared with 17 % of the 24 rapid inactivators; 15 % of the slow inactivators had active disease, as compared with 42 % of the rapid inactivators, and 70% had deteriorated to an extent that warranted a change of treatment, as compared with 42% of the rapid inactivators. This difference between the proportions of the slow and the rapid inactivators who deteriorated in the second year does not attain significance. It should, however, be noted that it is in the opposite direction to that observed in the first year; this is discussed further below.

Occurrence during the two years of deterioration that warranted a change of treatment. Deterioration that was sufficiently serious to warrant a change of treatment occurred during the two years in 35 (20 %) of the 178 slow inactivators and 32 (27 %) of the 120 rapid inactivators whilst they were receiving their initially prescribed chemotherapy. The month at which the deteriorations occurred is given in Table 6. (These figures do not include one rapid inactivator (H series) who had bacteriologically active disease at 12 months and had his treatment changed in the thirteenth month because of cor

TABLE 6
MONTH IN WHICH SERIOUS RADIOGRAPHIC OR CLINICAL DETERIORATION OCCURRED DURING THE TWO YEARS
IN SLOW AND RAPID INACTIVATORS OF ISONIAZID

Month of treatment change	Treatment series											
	PH		HI-1		HI-2		H		All series			
	Slow inactivators	Rapid inactivators	Slow inactivators	Rapid inactivators	Slow inactivators	Rapid inactivators	Slow inactivators	Rapid inactivators	Slow inactivators No.	%	Rapid inactivators No.	%
0-4	0	0	0	0	0	0	0	0	0	0	0	0
5-8	0	1	0	1	3	3	2	4	5	14	9	28
9-12	0	0	0	4	5	3	2	6	7	20	13	41
13-16	0	1	0	2	0	1	7	1	7	20	5	16
17-20	0	0	4	1	1	0	6	2	11	31	3	9
21-24	1	1	0	0	1	1	3	0	5	14	2	6
Total	1	3	4	8	10	8	20	13	35	99	32	100

pulmonale but without evidence of radiographic deterioration, and one slow and one rapid inactivator whose disease relapsed bacteriologically with a radiographic deterioration in the second year, after having had their treatment changed to calcium gluconate at 12 months because they had bacteriologically quiescent disease.) Except for two patients who had their treatment changed in the first year on account of severe clinical deterioration, all these patients had their treatment changed on the recommendation of an independent assessor because of serious radiographic deterioration. All the patients had culture-positive results, with organisms resistant to isoniazid, at the time they changed treatment. Considering the PH series, the one slow inactivator who deteriorated and had his treatment changed did so in the second year, whereas one of three rapid inactivators did so in the first year. Considering the HI-1 series, none of four slow inactivators, as compared with five of eight rapid inactivators, who deteriorated and had their treatment changed did so in the first 12 months ($P = 0.07$). The corresponding proportions for the HI-2 series were eight of 10 and six of eight, respectively, and for the H series, four of 20 and 10 of 13, respectively ($P \sim 0.01$). Amalgamating the four treatment series, 34 % of the 35 slow inactivators who deteriorated and had their treatment changed did so in the first 12 months, as compared with 69% of the 32 rapid inactivators ($P \sim 0.02$). This association in the HI-1 and H series between the rate of inactivation and the

month in which deterioration that warranted a change of treatment occurred explains why at 12 months but not at 24 months there was a significant difference between the proportions of slow and rapid inactivators who had deteriorated to this extent.

Response to treatment of slow and rapid inactivators of isoniazid related to severity of disease on admission to treatment. Table 7 presents the response to treatment at 12 months of the slow and rapid inactivators of isoniazid related to three pretreatment assessments of the severity of disease that have been shown to be of prognostic value (Tuberculosis Chemotherapy Centre, 1960). These assessments were: the extent of cavitation; the degree of sputum positivity, as assessed by smear examination; and the virulence in the guinea-pig of pretreatment cultures of tubercle bacilli. (As there was little difference between the four treatment series, only the amalgamated figures are presented in Table 7.) Considering the extent of cavitation, the difference between the proportions of slow and rapid inactivators with slight or no cavitation who had bacteriologically quiescent disease at 12 months was 9 % and that for patients with moderate or extensive cavitation was also 9%. The difference for patients with smear-negative results was 3 % and for those with smear-positive results 9%. For patients with pretreatment cultures of low virulence (root-index of virulence of 0-0.59) the difference was 2% and for

TABLE 7
RESPONSE TO TREATMENT AT 12 MONTHS OF SLOW AND RAPID INACTIVATORS OF ISONIAZID RELATED TO SEVERITY OF DISEASE ON ADMISSION TO TREATMENT

Rate of inactivation of isoniazid	Assessment on admission to treatment											
	Extent of cavitation				Sputum positivity				Virulence in the guinea-pig (root-index of virulence)			
	Nil or slight		Moderate or extensive		Smear-negative		Smear-positive		0-0.59		0.60 or above	
	Total patients	Favourable response ^a No. %	Total patients	Favourable response ^a No. %	Total patients	Favourable response ^a No. %	Total patients	Favourable response ^a No. %	Total patients	Favourable response ^a No. %	Total patients	Favourable response ^a No. %
Slow	66	55 83	112	70 62	32	31 97	146	94 64	50	34 68	105	75 71
Rapid	46	34 74	75	40 53	18	17 (94) ^b	103	57 55	38	25 66	73	42 58

^a Bacteriologically quiescent disease or disease of doubtful status at 12 months.

^b This percentage is based on fewer than 25 observations.

patients with cultures of moderate or high virulence (root-index of virulence of 0.60 or above) 13 %. The only difference in response which attains significance was that for the patients with smear-positive results ($0.01 < P < 0.05$). There was thus a slight suggestion that the influence of the rate of inactivation of isoniazid on the response to treatment was greatest in patients who (a) had smear-positive specimens on admission to treatment and (b) were infected with tubercle bacilli of moderate or high virulence in the guinea-pig. There was, however, no evidence that the extent of cavitation on admission to treatment influenced the association between the rate of inactivation and the response to treatment.

Response to treatment related to the degree of rapid inactivation of isoniazid. Price Evans, Manley & McKusick (1960) have shown that homozygous rapid inactivators inactivate isoniazid more rapidly than heterozygous rapid inactivators. Analyses (not tabulated here) were therefore undertaken to compare the response to treatment of the 12 rapid inactivators who had very low serum concentrations of isoniazid (less than 0.2 µg/ml) four-and-a-half hours after the test dose of 3 mg/kg body-weight isoniazid with the remaining 109 rapid inactivators. There was no clear indication from these analyses that these 12 patients responded less favourably to treatment than the remaining rapid inactivators. It must be pointed out, however, that the numbers considered in this analysis were very small and that the genotype of the patients had not been determined.

DISCUSSION

Since Hughes, Schmidt & Biehl (1955) demonstrated that patients vary widely in their rate of inactivation of isoniazid, a number of investigations have been undertaken to determine whether these differences are associated with differences in response to treatment with isoniazid. Indirect evidence which suggests that such a relationship does exist is available from a study reported by Mandel et al. (1957) and more direct evidence has been presented by Mitchell & Bell (1957). Mandel et al. (1957) found, in a study on 56 patients in whom isoniazid-resistant cultures emerged during treatment, that catalase-negative strains occurred significantly more frequently amongst slow than amongst rapid inactivators. Cohn et al. (1954) had reported that catalase-negative isoniazid-resistant strains of tubercle bacilli were less virulent in the guinea-pig than catalase-positive isoniazid-resistant strains. Since this relationship between the catalase activity of tubercle bacilli and their virulence may also apply in man (Oestreicher et al., 1955), Mandel et al. (1957) suggested that slow inactivators could be expected to respond to treatment better than rapid inactivators. Mitchell & Bell (1957) studied 44 patients and related the frequency of sputum conversion to bacteriological negativity by the sixth month of treatment to the rate of inactivation of isoniazid. All the patients had received 12 mg/kg body-weight isoniazid daily, and in addition either PAS or streptomycin or both. At six months, specimens from 20 of the 22 slow inactivators were negative on culture, as compared with 14 of the 22 rapid inactivators. This study was

subsequently extended to include 123 patients, who were divided into four groups on the basis of the severity of the disease on admission to treatment and the chemotherapeutic regimens they received (Mitchell et al., 1958). In two of the four groups, the slow inactivators fared more favourably than the rapid inactivators in respect of the frequency of sputum conversion by six months, but in neither were the differences statistically significant. Also, the patients studied were a selected group, being the only patients, among a much larger number originally admitted to treatment, who received uninterrupted treatment with isoniazid for this period. Further, it was not reported whether the radiographic and bacteriological conditions of the slow and rapid inactivators at the start of treatment were similar.

On the other hand, two other studies have failed to demonstrate a relationship between response to treatment and the rate of inactivation of isoniazid (Biehl, 1957; Barclay, 1959). Biehl (1957) determined the rate of inactivation of isoniazid by the percentage of the daily dose of isoniazid excreted as free isoniazid in the urine in 24 hours and related this to the frequency of the emergence of isoniazid-resistant strains. The data from this investigation have been subjected to statistical analysis by Price Evans, Manley & McKusick (1960) and no significant association was found. Barclay (1959) stated that no relationship was observed in a study on 125 patients, but did not present the details of the investigation referred to.

The 299 patients considered in the present report were drawn from the 315 patients who were included in the main analysis of the chemotherapy study (Tuberculosis Chemotherapy Centre, 1960). On admission they had not received more than two weeks of previous antituberculosis chemotherapy (96.5% had received none) and all yielded cultures of tubercle bacilli which were sensitive to isoniazid. They were allocated at random to treatment for 12 months either with isoniazid plus PAS (PH regimen) or with isoniazid alone, either in a daily dosage, on the average, of 8.7 mg/kg given in one dose a day (HI-1 regimen) or in two doses a day (HI-2 regimen) or in a daily dosage, on the average, of 4.5 mg/kg given in two doses a day (H regimen). At the end of 12 months patients who had disease which was bacteriologically quiescent or of doubtful status were allocated at random to treatment for a further 12 months with 0.5 g of calcium gluconate daily or with isoniazid in a dosage of about 4.5 mg/kg daily. Patients who had disease which was bacteriologically

active or relapsed, but which had not deteriorated to an extent which warranted a change of treatment, continued on their initially prescribed chemotherapy for a second year. Of the 299 patients, 178 were classified as slow inactivators and 121 as rapid inactivators. The 178 slow and 121 rapid inactivators were very similar in terms of the major assessments of the condition of their disease on admission to treatment. However, when the disease condition of the slow and the rapid inactivators on admission to treatment was compared separately in the four treatment series, there was a suggestion that the slow inactivators in the PH series were possibly at an advantage in respect of the extent of cavitation. Since standardization for this pretreatment difference did not substantially alter the difference between the response to treatment of the slow and the rapid inactivators, the unstandardized percentages have been presented in this report. The results of this study have been presented for the four treatment series not only separately, but amalgamated also, because the associations encountered were in the same direction in all the series. Furthermore, the magnitudes of the differences between the responses of the slow and the rapid inactivators were of the same order.

Before considering the results, it is necessary to consider a source of possible bias in the analysis. Since the rate of inactivation was determined in the second six months of treatment, results are not available for the 11 patients who died before the determination was carried out. However, only six (1 PH, 1 HI-1, 4 H) of these 11 patients died from tuberculosis and two of them (both H) did so within the first month of treatment.

The results of the present investigation suggest that there probably was an association between response to treatment and the rate of inactivation of isoniazid. When the four treatment series were amalgamated the slow inactivators appeared to have responded more favourably to treatment than the rapid inactivators, in that:

- (a) They were more often culture-negative during the first 12 months of treatment.
- (b) A larger proportion of them had bacteriologically quiescent disease at 12 months ($P = 0.07$).
- (c) A smaller proportion of them showed radiographic deterioration at six months ($P < 0.01$).
- (d) those who deteriorated radiographically or clinically to an extent warranting a change of treat-

ment during the two years did so later than the rapid inactivators ($P < 0.02$).

There was slight evidence that the slow and the rapid inactivators differed in respect of the speed of conversion to bacteriological negativity of those patients who had bacteriologically quiescent disease at 12 months, but no evidence that they differed in the degree of positivity of sputum specimens which were positive on culture at six, nine or 12 months or in the frequency with which they showed moderate or greater radiographic improvement at six months. There was evidence amongst the patients with bacteriologically quiescent disease or disease of doubtful status at 12 months, and who received random isoniazid or calcium gluconate in the second year, that the slow inactivators who received isoniazid relapsed bacteriologically less frequently than the other patients.

It must be emphasized that the association suggested by this investigation between the rate of inactivation of isoniazid and the response to treatment was weak and that rapid inactivation could only have been responsible for the failure of chemotherapy in a small proportion, estimated to be approximately 4%, of the patients admitted to this study. Furthermore, the association has only been demonstrated in the amalgamated results of the four treatment series; in consequence, it cannot be said that it will necessarily hold true for each one of the regimens separately.

The rate of inactivation of isoniazid is genetically determined, slow inactivation being due to the presence of two recessive alleles (Knight, Selin St Harris, 1959; Price Evans, Manley & McKusick, 1960). As there were no major differences between the proportions of slow inactivators among healthy subjects and among patients with pulmonary tuberculosis in the American population studied by Price Evans, Manley & McKusick (1960), it seems reasonable to regard the preponderance of slow inactivators among the patients in this study as reflecting a preponderance of this genotype in the general South Indian population. Since the predominance of a recessive genotype in a population suggests that such a genotype confers a biological advantage, it could be argued that the slow inactivators responded more favourably to treatment than the rapid inactivators not because they inactivated isoniazid more slowly but because of this associated biological advantage. However, this seems unlikely, for there was little

difference between the slow and the rapid inactivators in the severity of their disease on admission to treatment and the relapse rates for the slow and the rapid inactivators who received calcium gluconate in the second year were similar.

SUMMARY

1. The rate of inactivation of isoniazid was determined in 299 of 315 patients in the main analysis of a controlled study of three regimens of isoniazid alone and one of isoniazid plus PAS in the domiciliary treatment of pulmonary tuberculosis. The patients were aged 12 years or more and all had yielded, on admission to treatment, cultures of tubercle bacilli which were sensitive to isoniazid.

2. The slow and the rapid inactivators were very similar in terms of the major assessments of the condition of their disease on admission to treatment when all 299 patients were considered. There were, however, minor differences between the slow and the rapid inactivators when the four treatment series were examined separately.

3. The differences between the response to treatment of the slow and the rapid inactivators in the four treatment series were fairly similar.

4. There was a suggestion that the slow inactivators responded slightly more favourably to treatment than the rapid inactivators, in four respects:

(a) They were more often culture-negative during treatment.

(b) A larger proportion of them had bacteriologically quiescent disease at 12 months.

(c) A smaller proportion of them showed radiographic deterioration at six months.

(d) Those who deteriorated radiographically or clinically to an extent warranting a change of treatment during the two years did so later than the rapid inactivators.

5. There was slight evidence that the slow and the rapid inactivators differed in the speed of conversion to bacteriological negativity of those patients who had bacteriologically quiescent disease at 12 months, but no difference between them in the degree of positivity of sputum specimens which were positive on culture at six, nine or 12 months or in the frequency with which they showed moderate or greater radiographic improvement at six months.

RÉSUMÉ

Le taux d'inactivation de l'isoniazide a été déterminé chez 299 parmi 315 malades, au cours de l'analyse d'une étude contrôlée portant sur trois régimes thérapeutiques d'isoniazide seul, et un régime d'isoniazide + PAS, dans le traitement à domicile de cas de tuberculose pulmonaire. Les malades avaient 12 ans ou plus, et les bacilles qu'ils hébergeaient lors du début du traitement donnaient des cultures sensibles à l'isoniazide. Les caractères principaux de la maladie étaient les mêmes chez les inactivateurs lents que chez les inactivateurs rapides, lors de l'examen des 299 malades dans leur ensemble. Il y avait cependant des différences mineures entre inactivateurs lents et inactivateurs rapides, si l'on considérait séparément les quatre séries de traitement.

Les différences dans la réponse au traitement des inactivateurs lents, et celle des inactivateurs rapides, dans les quatre séries à l'étude étaient analogues.

On pourrait penser que les inactivateurs lents répon-

dent légèrement mieux au traitement, d'après les indices suivants: *a)* ils étaient plus souvent négatifs à la culture, au cours du traitement; *b)* une plus forte proportion d'entre eux avaient atteint la forme quiescente de la maladie, à l'examen bactériologique, au bout de 12 mois; *c)* une plus faible proportion d'entre eux présentèrent une détérioration de leur état à 6 mois, révélée par la radiographie; *d)* ceux qui présentaient une telle détérioration, visible cliniquement ou par radiographie, le faisaient plus tardivement que les inactivateurs rapides.

On n'a pas réuni de preuves permettant d'affirmer que les inactivateurs rapides différaient des inactivateurs lents *a)* par la rapidité de la conversion à la négativité bacillaire des malades ayant atteint l'état de quiescence après 12 mois; *b)* par le degré de positivité des crachats positifs à la culture à 6, 9, ou 12 mois; *c)* par la fréquence des améliorations plus ou moins importantes, visibles à la radiographie, à 6 mois.

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