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Response of Patients Infected with Isoniazid-Resistant Tubercle Bacilli to Treatment with Isoniazid plus PAS Isoniazid Alone*

S.DEVADATTA, A.L. BHATIA, R.H. ANDREWS, WALLACE FOX, D. A. MITCHISON, S. RADHAKRISHNA, C.V. RAMAKRISHNAN, J.B. SELKON & S. VELU

This study from the Tuberculosis Chemotherapy Centre, Madras, compares patients infected with isoniazid-resistant tubercle bacilli ("R" patients) with those infected with isoniazid-sensitive tubercle bacilli ("S" patients) as regards, first, their pretreatment status and, secondly, their response to a year's chemotherapy, either with isoniazid plus p-aminosalicylic acid (PAS) or with isoniazid alone. With regard to the first comparison, there was, on admission to treatment, little difference between the R and S patients in terms of the extent of the radiographic lesion, the extent of cavitation or the bacterial content of the sputum, but there were major differences in the age and sex distributions, the R patients showing a greater preponderance of young males than the S patients. As to the second comparison, statistically significant differences in the bacteriological response to treatment of the S and the R patients were observed in both the isoniazid-plus-PAS and the isoniazid-alone series, the response of the S patients being much better than that of the R patients. When the response to treatment was assessed in terms of radiographic progress and weight changes, however, hardly any difference was observed between the progress of the S and the R patients. The reasons for the response in the R patients are discussed.

Information on the prevalence of infection with primarily isoniazid-resistant tubercle bacilli among tuberculous patients is available from a number of countries. Figures have been reported from several centres, including New York City (Chaves et al., 1955 1956), Great Britain (Fox et al., 1957), the Province of Quebec (Frappie, Desjardins & Panisset, 1957), Germany (Meissner, 1958), Austria (Dissmann & Iglauer, 1960), Madras City (Tuberculosis Chemotherapy Centre, 1959, 1960), Ashanti, Ghana (Bell & Brown, 1960) and Paris (Thibier et al., 1960). Rist & Crofton (1960) have recently published the results of a world survey.

Pepys, Mitchison & Kinsley (1960) have reported figures for patients with pulmonary tuberculosis presumed to have had no previous chemotherapy presenting for treatment in East Africa. Their figures, however, do not indicate the true prevalence of primary isoniazid-resistant infections as a number of patients subsequently confessed to having had previous chemotherapy.

There is, however, very little information on the effects of treatment with isoniazid, alone or in combination, in patients infected with isoniazid-resistant tubercle bacilli. A report from the Tuberculosis Chemotherapy Centre, Madras (Tuberculosis Chemotherapy Centre, 1959) has described the results of treatment with isoniazid plus p-aminosalicylic acid (PAS) of six of seven such patients and another report (Tuberculosis Chemotherapy Centre, 1960) summarized the disease status at one year for 20 patients, 14 treated with isoniazid alone and six with isoniazid plus PAS. The present paper studies the pretreatment clinical and bacteriological status of these 20 patients in the 1960 report and their progress during a year of treatment, and compares them with those of the 315 patients excreting isoniazid-sensitive organisms when they were admitted to the same study.

* From the Tuberculosis Chemotherapy Centre, Madras, India. The Centre is under the joint auspices of the India Council of Medical Research, the Madras State Government, the World Health Organization and the Medical Research Council of Great Britain.

MATERIAL AND METHODS

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

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

HI-1 (75 patients). Isoniazid alone, 7.8-9.6 mg/kg body-weight daily, divided into two doses, by mouth —i.e., 400 mg of isoniazid a day for a patient weighing 100 lb.

HI-2 (75 patients). Isoniaid alone, 7.8-9.6 mg/kg body-weight 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 body-weight daily, divided into two doses, by mouth —i.e., 200 mg of isoniazid a day for a patient weighing 100 lb.

Six patients (3 HI-1, 1 HI-2, 2H) have been excluded, three because they had received previous chemotherapy, which included isoniazid, for a period of more than two weeks and three of reasons given in the earlier report (Tuberculosis Chemotherapy Centre, 1960). There remain 315 patients (90 PH, 70 HI-1, 68 HI-2, 87 H) infected with isoniazid-sensitive tubercle bacilli, the "S" patients, and 20 (6 PH, 2 HI-1, 6 HI-2, 6 H) infected with isoniazid-resistant organisms, the "R" patients.

The patients were treated in their homes, the great majority attending the Centre weekly for a supply of medicine. The full details of the management have been given elsewhere (Tuberculosis Chemotherapy Centre, 1960).

The investigations before treatment included a full-plate chest radiograph, measurement of the weight, measurement of the erythrocyte sedimentation rate (ESR Westergren 200 mm, reading in mm at one hour), examination of four sputum specimens by smear and culture, tests of sensitivity to isoniazid on two cultures and qualitative estimation of the catalase activity of both of them and semi-quantitative estimation on all resistant cultures and on a sample of sensitive cultures. One of the cultures from each patient was tested for virulence in the guinea-pig and studied by a series of identification tests. Investigations during treatment included a monthly radiograph, two examinations of the sputum each month by smear and culture and, from the third month, a pair of laryngeal swabs by culture, also. A test of sensitivity to isoniazid and a qualitative catalase test were performed on one positive culture at the end of each month. If the culture was isoniazid-resistant, a semi-quantitative catalase test was undertaken as well. The patient's rate of inactivation of isoniazid was also determined at some time during the second six months of treatment.

Changes of chemotherapy

The reasons for which the chemotherapy was changed were drug toxicity, a clear-cut radiographic extension of the disease, confirmed by an independent assessor (Dr K. S.Sanjivi), or serious clinical deterioration.

BACTERIOLOGICAL PROCEDURES

Bacterial content of sputum

Sputum smears were examined by fluorescence microscopy and were graded as 3-plus (heavy), 2-plus (moderate), 1-plus (scanty) and negative. The specimens were treated with 4% sodium hydroxide, washed with distilled water and cultured on Lowenstein-Jensen medium without potato starch (Jensen, 1955). The cultures were examined weekly for eight to nine weeks (Tuberculosis Chemotherapy Centre, 1959).

Sensitivity tests

Tests of sensitivity of isoniazid were set up two or three days after the cultures became positive. If growth was insufficient, the cultures were reincubated to allow enough organisms to grow for preparation of the inoculum. The inoculum suspension was made by adding approximately 2 mg (moist weight) of bacilli, obtained a representative sample from all parts of the growth, to 1/4-ounce (approximately 10-ml) screw-capped bottles containing 0.5 ml of sterile distilled water and glass beads, and then shaking the bottle mechanically for one minute. Standard 3-mm loopfuls of this suspension were inoculated on to a series of slopes of Lowenstein-Jensen medium containing 0.2, 1, 5

and 50 isoniazid, as well as on to 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 slopes containing 0.025, 0.05, 0.1, 0.2 and 1 mg/ml isoniazid.

Definitions of isoniazid resistance

In the following definitions "growth" has been defined as 20 or more colonies. If the control (drug-free) slope yielded 100 or fewer colonies the test was repeated.

Before treatment, resistance to isoniazid was defined as:

(a) Growth on 1 mg/ml or a higher concentration, even if the result of a test on a second culture was sensitive.

(b)Growth on 0.2 mg/ml, followed by the same result on a repeat test on the same culture, even if the result of a test on a second culture was sensitive.

(c) Growth on 0.2 mg/ml, for two separate cultures, irrespective of the results of repeat tests.

During treatment, resistance to isoniazid was defined as:

Growth on 0.2 mg/ml or a higher concentration, irrespective of the results of repeat tests.

Catalase activity

Qualitative and semi-quantitative estimations of the catalase activity of cultures were carried out by the methods previously described (Tuberculosis Chemotherapy Centre, 1959). The catalase activity on the drug-free slope was estimated by both methods and that of the growth of 20 or more colonies on the isoniazid-containing slopes by the qualitative method only.

Identification tests

The cultures were examined by the following in vitro identification tests: (a) bacterial morphology; (b) colonial morphology on Lowenstein-Jensen slopes and on 7H-10 oleic-acid-albumin plates; (c) growth at 23^0C; (d) pigment production; (e) catalase activity on the drug-free slope and on the 50 mg/ml isoniazid slope; and (f) niacin production. The methods have been described by Thomas et al. (1961 [1]).

Virulence tests

Cultures were tested for virulence in the guinea-pig as described by Mitchison et al. (1960). In brief, 1 mg (moist weight) of a pretreatment culture of tubercle bacilli was inoculated intramuscularly into each of two guinea-pigs, one of which was killed at 6 weeks and the other at 12 weeks. At post-mortem examination the total extent of tuberculous disease in the spleen, liver, lungs and local glands was assessed as a score ranging from 0 to 100. The ratio of the score to the survival time in days was determined for each guinea-pig. The measure of virulence employed was the mean of the square root of the ratios for the two guinea-pigs which has been termed the root-index of virulence (Mitchison et al., 1961). Values of 0-0.59 were considered to indicate low virulence, 0.60-0.89 moderate virulence and 0.90 or above high virulence.

The majority of the virulence tests were done at the Microbiological Research Establishment, Porton, England; the results of the smaller series of tests done in Madras were adjusted to those obtained at Porton as described by Mitchison et al. (1961).

RESULTS

Prevalence of infection with isoniazid-resistant tubercle bacilli

All the 341 patients admitted to the study were interrogated on several occasions during the course of treatment, as their relationship of trust with the Centre's staff became more and more firmly established, to discover whether they had had previous chemotherapy and were concealing the fact. Relatives and, if necessary, friends were approached and dispensary cards from other hospitals and prescriptions from private doctors were scrutinized. One patient excreting isoniazid-sensitive and two excreting isoniazid-resistant tubercle bacilli admitted that they had had previous chemotherapy of more than two weeks' duration. Of the remaining 338 patients, 20, the "R" patients, had isoniazid-resistant organisms; and one had had a week of streptomycin plus isoniazid. It is considered that these 20 (5.9%) patients (6 PH, 2 HI-1, 6 HI-2, 6 H) out of the 338 who had had either no previous chemotherapy, or very little, had been infected with isoniazid-resistant organisms. The remaining patients had fully sensitive strains although seven of them had had up to two weeks' chemotherapy which included isoniazid.

[1] See article on page 747.

Among the patients classified as having sensitive organisms before treatment, there were seven with a culture which yielded growth of 20 or more colonies on 0.2 µg/ml but not on 1 µg/ml isoniazid, and with no growth on 0.2 µg/ml in a repeat test of this culture or in the test on the second culture from the patient. These seven patients responded to chemotherapy as favourably as the other patients who were classified as having initially sensitive organisms.

Levels of resistance of the pretreatment cultures

Table 1 gives the minimal inhibitory concentrations (MICs) of isoniaxid of the two cultures isolated before treatment from each of the 20 " R " patients. It will be seen that eight of these patients yielded two highly resistant cultures (MICs of 5 µg/ml or more) and that six patients yielded two cultures of low resistance (MICs of 1 µg/ml). There were three patients who yielded one resistant and one sensitive culture.

Identification tests

From 18 of the 20 patients an isoniazid-resistant culture isolated before the start of treatment was examined by the full series of identification tests (see page 809), with the exception, in five cases, of the test for growth at 23°C. All tests performed on these 18 cultures gave results typical of *Myco. tuberculosis* and, since they were niacin-positive, they have been classified as *Myco. tuberculosis* var. *hominis. The* remaining two cultures were not submitted to a full range of tests. One of them was tested for virulence in the guinea-pig and was found to be highly virulent (root-index of 1.12). This culture has, therefore, also been regarded as *Myco. tuberculosis. The* other pretreatment culture for the identification tests became contaminated after the sensitivity test had been performed and further investigations were not possible (the second pretreatment culture from the patient was no longer available). A culture from this patient at the end of 12 months' treatment was examined by the full series of tests and identified as *Myco. tuberculosis* var. *hominis.*

TABLE 1

MINIMAL INHIBITORY CONCENTRATION OF ISONIAZID OF TWO CULTURES ISOLATED BEFORE TREATMENT FROM PATIENTS INFECTED WITH ISONIAZID-RESISTANT TUBERCLE BACILLI

	Minimal inhibitory concentration of 2 cultures (µg/ml)	Number of patients
Both cultures resistant	1 and 1	6
	5 and 1	3
	5 and 5	5
	⩾50 and ⩾50	3
One culture resistant and the other sensitive	⩾5 and 0.2	1
	50 and 0.2	
	Total	20

PRETREATMENT COMPARISONS

Sex and age

There were major differences between the S and R patients in the age and sex distributions. Of the 315 S patients, 199 (63 %) were males, as compared with 18 (90%) of the 20 R patients. This difference attains statistical significance (P=0.01). Table 2 shows the age distribution of the male patients. Of the 199 S males 113 (57 %) were less than 35 years old, as compared with 15 (83%) of the 18 R males. This difference also attains statistical significance (P ≃ 0.02). Of the 116 S females, 85 (73%) were less than 35 years old; the R females were aged 13 and 23 years, respectively.

Clinical condition

Table 3 presents three pretreatment radiographic assessments made by an independent observer, Dr Raj Narain, who was unaware of the classification as S or R of any patient, and the pretreatment bacterial content of the sputum. Considering the

TABLE 2

AGE DISTRIBUTION OF THE MALE PATIENTS

Estimated age (years)	S patients		R patients	
Under 24	52	*26*	6	*(33)* [a]
25-34	61	*31*	9	*(50)*
35-44	43	*22*	2	*(11)*
45 or more	43	*22*	1	*(6)*
Total	199	*101*	18	*100*

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

TABLE 3
CONDITION ON ADMISSION TO TREATMENT

Condition	S patients		R patients	
	No.	%	No.	%
Extent of cavitation:				
Nil	22	*7*	2	*(10)*[a]
Slight	94	*30*	3	*(15)*
Moderate	182	*51*	13	*(65)*
Extensive	37	*12*	2	*(10)*
Total extent of disease :				
Trivial or slight	26	*8*	1	*(5)*
Limited or moderate	196	*62*	12	*(60)*
Extensive or gross	93	*30*	7	*(35)*
Number of lung zones involved in disease:				
1 or 2	61	*19*	4	*(20)*
3 or 4	126	*40*	10	*(50)*
5 or 6	128	*41*	6	*(30)*
Bacterial content of sputum : [b]				
Direct smear negative	50	*18*	2	*(10)*
Direct smear positive: 1-plus (scanty)	57	*18*	2	*(10)*
2-plus (moderate)	96	*30*	9	*(45)*
3-plus (heavy)	112	*36*	7	*(35)*
Total	315	*100*	20	*100*

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

[b] Based on a single collection specimen,

extent of cavitation, 12 % of the 315 S and 10 % of the 20 R patients had extensive cavitation and 51% and 65%, respectively, had moderate cavitation. There was little difference in terms of the total extent of the radiographic lesion, 30% of the S patients having extensive or gross disease, as compared with 35 % of the R patients, and, at the other extreme, 8 % of the S patients and 5% of the R patients having trivial or slight lesions. The distributions for the number of lung zones involved in disease were similar, although rather more of the S patients (41%) than the R patients (30 %) had 5 or 6 lung zones involved. The over-all distributions of bacterial content of the sputum were also similar, but there were fewer patients in the S series than in the R series with 2-plus and 3-plus direct smear findings (66%, as compared with 80%).

Rate of inactivation of isoniazid

Estimations of the rate of inactivation of isoniazid were carried out on 303 of the S patients and on 18 of the R patients by a method described by Gangadharam et al. (1961).[1] Of the S patients 181 (60%) were slow inactivators, as compared with 14 (78 %) of the R patients.

Virulence of the cultures in the guinea-pig

Table 4 gives the distribution of the root-indices of virulence (defined on page 809) of the strains from 281 S patients and 17 R patients. The mean root-index of virulence was 0.72 for both series.

TABLE 4
VIRULENCE IN THE GUINEA-PIG OF THE STRAINS OF TUBERCLE BACILLI FROM THE PATIENTS ON ADMISSION TO TREATMENT

Root-index of virulence [a]	S patients		R patients	
	No.	%	No.	%
0-0.59 (low virulence)	91	*32*	7	*(39)* [b]
0.60-0.89 (moderate virulence)	122	*43*	3	*(44)*
0.90 and above (high virulence)	68	*24*	3	(17)
Total [c]	231	99	18	*100*
Mean	0.73		0.71	

[a] The root-indices obtained in the Madras series were adjusted to those in the Porton series, as described by Mitchison et al. (1961).

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

[C] The virulence of the strain was not determined for 34 S patients and for 2 R patients.

Catalase activity of the cultures

Qualitative estimations of the catalase activity were performed on 618 isoniazid-sensitive pretreatment cultures from the 315 S patients and on 39 (36 resistant and three sensitive) of the 40 pretreatment cultures from the 20 R patients. One of the cultures from the S patients had 3-plus activity,

[1] See article on page 765.

615 had 2-plus and two had l-plus activity. Of the 36 resistant cultures tested from the R patients, 29 had 2-plus catalase activity and seven l-plus activity. The three sensitive cultures had 2-plus activity. The results of a semiquantitative estimation of the catalase activity were also available for 49 cultures obtained from a sample of the same number of S patients and for 33 resistant cultures obtained from 19 R patients. All the cultures from the S patients had 60%-100% activity, whereas of the 33 resistant cultures from the R patients, 23 had 60%-100% catalase activity and 10 (from six patients) 20%-50% activity (see Table 10, pages 818-819). There was thus some evidence of lesser catalase activity of the cultures from the R patients than of those from the S patients.

In summary, there was a much greater preponderance of young males among the R patients than among the S patients. There was little difference in terms of the extent of the radiographic lesion, the extent of cavitation or the bacterial content of the sputum. The catalase activity of the pretreatment cultures from the S patients was, on the average, greater than that of the cultures from the R patients, but there was no difference between them in respect of their virulence in the guinea-pig.

RESPONSE TO TREATMENT

Since 15 of the 20 R patients were males aged less *than 35* years, analyses (not tabulated here) were undertaken to compare the progress of these 15 patients and the 113 S males in the same agegroup. The findings were very similar to those of the comparison of the progress of the full populations of the 20 R and the 315 S patients. The comparisons of the full populations have therefore been presented in the subsequent sections, in which the patients receiving isoniazid plus PAS in the S group are compared with those receiving the same treatment in the R group, and the S and R patients have been compared for the three isoniazid regimens combined.

Weight changes

There were 80 S and five R patients who received isoniazid plus PAS for the full 12 months; 92% of the S and all the R patients gained weight, and the mean gain in weight was 10.3 lb. for the former and 9.8 lb. for the latter. Correspondingly, 92% of 167 S patients on isoniazid alone who received the prescribed chemotherapy for the 12 months gained weight, as compared with 91% of 11 R patients; the mean gain in weight was 11.0 lb. for the S and

TABLE 5

DISTRIBUTIONS OF THE ERYTHROCYTE SEDIMENTATION RATE ON ADMISSION TO TREATMENT AND AT 12 MONTHS [a]

ESR (mm in 1 hour)	Isoniazid plus PAS								Isoniazid alone							
	On admission				At 12 months				On admission				At 12 months			
	S patients		R patients		S patients		R patients		S patients		R patients		S patients		R patients	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0-10	0	0	0	(0)[b]	15	18	1	(17)	3	1	0	(0)	56	26	3	(21)
11-20	3	4	1	(17)	24	29	1	(17)	7	3	0	(0)	44	21	3	(27)
21-50	23	27	0	(0)	27	32	2	(33)	9	23	3	(21)	42	20	3	(21)
61 or more	53	69	5	(83)	16	19	1	(17)	153	72	11	(79)	32	15	2	(14)
Change of chemotherapy, tuberculous death deterioration, and	—	—	—	—	2	2	1	(17)	—	—	—	—	36	18	3	(21)
Total patients	34	100	6	100	34	100	6	101	212	99	14	loo	212	100	14	98

[a] Five patients who died of non-tuberculous conditions, eight patients who had their chemotherapy changed on account of toxicity and six patients for whom no results were available have been excluded.

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

9.7 lb. for the R patients. In the first six months, the mean weight gains were 9.7 lb. for the S and 9.2 lb. for the R patients who received isoniazid plus PAS, and 9.9 lb. for the S and 7.7 lb. for the R patients on isoniazid alone.

Erythrocyte sedimentation rate

The distributions of the ESRs at the start of treatment and at 12 months are set out in Table 5. The S and R series, whether on the combined chemotherapy or on isoniazid alone, showed an increase at 12 months in the proportions of patients with low ESRs (20 mm or less); the proportions of S and R patients with low ESRs were similar for the isoniazid-alone series.

Radiographic changes

The changes in the radiographic appearances were assessed by an independent observer, Dr Raj Narain, who was unaware of the treatment series or clinical details of any patient. In the first six months (Table 6) 97% of 87 S and 83 % of six R patients receiving isoniazid plus PAS showed radiographic improvement as did 84% of 219 S and 71% of 14 R patients receiving isoniazid alone. It will be noted that the proportions showing moderate or greater improvement in the isoniazid-alone series were 68 % for the S but only 29 % for the R patients. Over the 12-month period, 94% of 86 S and 67 % of six R patients receiving isoniazid plus PAS and 77 % of 216 S and 79% of 14 R patients on isoniazid alone showed radiographic improvement. The proportions showing moderate or greater improvement in the isoniazid-alone series were 69 % for the S and 64% for the R patients. Thus, by 12 months, the evidence of greater progress in the S patients than in the R patients receiving isoniazid alone, which was apparent at six months, had largely disappeared.

Changes in cavitation

Table 7 presents the independent assessment of the changes in cavitation for the 12-month period

TABLE 6

CHANGES IN RADIOGRAPHIC APPEARANCES IN THE FIRST SIX MONTHS AND IN THE 12-MONTH PERIOD [a]

Period		Isoniazid plus PAS				Isoniazid alone			
		S patients		R patients		S patients		R patients	
		No.	%	No.	%	No.	%	No.	%
0-6 months	Moderate or greater improvement	69	*79*	4	*(67)* [b]	149	*68*	4	*(29)*
	Slight improvement	15	*17*	1	*(17)*	35	*16*	6	*(43)*
	No change	1	*1*	0	*(0)*	12	*5*	1	*(7)*
	Deterioration with or without change of chemotherapy, or tuberculous death	2	*2*	1	*(17)*	23	*11*	3	*(21)*
	Total	87	*99*	6	*101*	219	*100*	14	*100*
0-12 months	Moderate or greater improvement	73	*85*	4	*(67)*	150	*69*	9	*(64)*
	Slight improvement	8	*9*	0	*(0)*	16	*7*	2	*(14)*
	No change	2	*2*	1	*(17)*	5	*2*	0	*(0)*
	Deterioration with or without change of chemotherapy, or tuberculous death	3	*3*	1	*(17)*	45	*21*	3	*(21)*
	Total	86	*99*	6	*101*	216	*99*	14	*99*

[a] Two separate assessments on standard radiographs. Patients who died of non-tuberculous conditions are excluded after their death and patients who had their chemotherapy changed or account of toxicity are excluded after the change.,

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

TABLE 7

CHANGES IN CAVITATION IN THE 12-MONTH PERIOD IN PATIENTS WITH CAVITATION ON ADMISSION TO TREATMENT[a]

	Isoniazid plus PAS				Isoniazid alone			
	S patients		R patients		S patients		R patients	
	No.	%	No.	%	No.	%	No.	%
Cavitation disappeared	50	*64*	2	*(33)*[a]	109	*54*	2	*(17)*
Cavitation less	20	*26*	3	*(50)*	46	*23*	6	*(50)*
Cavitation unchanged or more	6	*8*	0	*(0)*	9	*4*	1	*(8)*
Change of chemotherapy due to deterioration, and tuberculous death	2	*3*	1	*(17)*	38	*19*	3	*(25)*
Total patients with cavitation on admission	70	*101*	6	*100*	202	*100*	12	*100*

[a] Assessment on standard radiographs on admission to treatment and at 12 months. Patients who died of non-tuberculous conditions and patients who had their chemotherapy changed on account of toxicity have been excluded.

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

for the patients with cavitation on admission to treatment. Of the 78 S patients on isoniazid plus PAS, 64% had no cavitation at 12 months, as compared with 33 % of six R patients. The corresponding proportions for the 202 S and 12 R patients who received isoniazid alone for the whole year were 54 % and 17 %. Thus, the cavities disappeared in a greater proportion of S patients than R patients. However, there was little difference between these two groups of patients, whether on isoniazid alone or on the combined chemotherapy, if reduction and disappearance of cavitation were considered together.

TABLE 8

PATIENTS WITH NEGATIVE CULTURES OF SINGLE COLLECTION SPECIMENS OF SPUTUM TAKEN AT 3-MONTHLY INTERVALS

Months after start of chemotherapy	Isoniazid plus PAS				Isoniazid alone			
	S patients		R patients		S patients		R patients	
	Total patients[a]	Culture negative[b]	Total patients[a]	Culture negative[b]	Total patients[a]	Culture negative[b]	Total patients[a]	Culture negative[b]
0	90	8 *9*	6	0 *(0)*[c]	225	4 *2*	14	0 *(0)*
3	81	63 *78*	6	1 *(17)*	215	114 *53*	13	4 *(31)*
6	85	75 *88*	6	2 *(33)*	212	124 *58*	14	3 *(21)*
9	83	77 *93*	6	2 *(33)*	210	123 *59*	14	4 *(29)*
12	82	74 *90*	6	2 *(33)*	210	128 *61*	14	3 *(21)*

[a] Patients who died of non-tuberculous conditions are excluded after their death and patients who had their chemotherapy charmed en account of toxicity are excluded after the change; tuberculous deaths and patients who had their chemotherapy changed owing to deterioration remain-in the totals throughout.

[b] Even if the smear was positive.

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

Sputum culture results

Table 8 shows the number of patients with negative cultures on admission to treatment and at three, six, nine and 12 months after the start of chemotherapy based on *single* collection (overnight) specimens. At three months, 78% of 81 S patients and 17% of six R patients on isoniazid plus PAS yielded a negative culture. The corresponding proportions at six months were 88% and 33%, respectively, and there was little change at nine and 12 months. At three months, 53 % of 215 S patients and 31% of 13 R patients on isoniazid alone yielded a negative culture. The proportions at six months were 58 % and 21%, respectively, and there was little change subsequently.

Fig. 1 and Fig. 2 show, for the combined and single drug therapies respectively, the percentage of patients all of whose *multiple* cultures (an average of three) were negative at each monthly examination. The graphs demonstrate the superior response of the S patients, but also show that the bacteriological response among the R patients was apparent from the early months of treatment.

Response to treatment during the 12 months

Table 9 classifies the patients at the end of 12 months, mainly on the basis of their bacteriological response to treatment. *Favourable response* is *(a)* bacteriologically quiescent disease (all cultures, usually numbering 7-9, negative for at least the last three monthly examinations, that is, at 10, 11 and 12 months) or *(b)* disease of doubtful bacteriological status (one positive culture at one of the last three monthly examinations following at least three months of culture-negativity). *Unfavourable response* is *(a)* relapsed disease (bacteriologically positive on at least two occasions in the last three months following negative results for all the cultures at three or more consecutive monthly examinations), *(b)* bacteriologically active disease (no period of three consecutive months of culture-negativity, or change of chemotherapy due to radiographic or serious clinical deterioration) or *(c)* death from tuberculosis.

FIG. 1

PERCENTAGE OF PATIENTS ON ISONIAZID PLUS PAS WITH ALL CULTURES NEGATIVE AT EACH MONTH

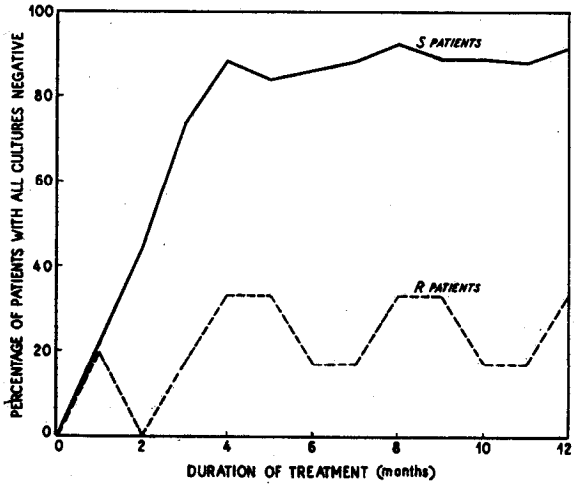
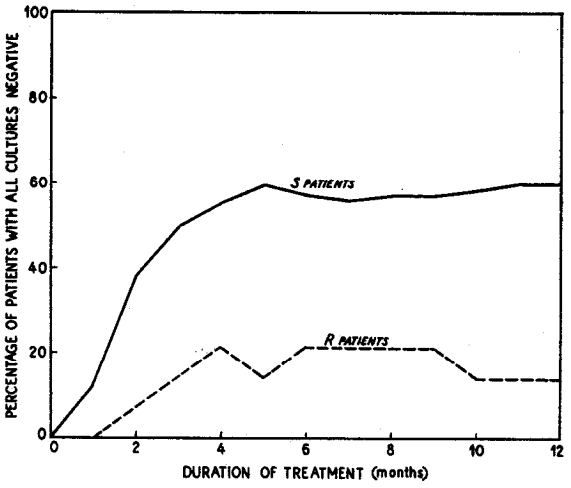


FIG. 2

PERCENTAGE OF PATIENTS ON ISONIAZID ALONE WITH ALL CULTURES NEGATIVE AT EACH MONTH



Only eight (9%) of 86 S patients on isoniazid plus PAS had an unfavourable response, as compared with five (83%) of six R patients, a statistically highly significant difference ($P < 0.001$); four of the R patients had bacteriologically active disease and the fifth had deteriorated radiographically and the chemotherapy had been changed. Of 216 S patients on isoniazid alone, 93 (43 %) had an unfavourable response, as compared with 12 (86%) of 14 R patients, also a highly significant difference ($P < 0.01$); eight of the 12 had active disease, one had relapsed disease, two had had their chemotherapy changed following a radiographic deterioration and one had died of tuberculosis.

The course of the disease in the R patients during the second year is considered on page 822.

TABLE 9
CLASSIFICATION OF THE PATIENTS AT THE END OF 12 MONTHS ACCORDING TO THEIR RESPONSE TO TREATMENT

Response	Isoniazid plus PAS				Isoniazid alone			
	S patients		R patients		S patients		R patients	
	No.	%	No.	%	No.	%	No.	%
Favourable [a]	78	*91*	1	*(17)*[b]	123	*57*	2	*(14)*
Unfavourable	8	*9*	5	*(83)*	93	*43*	12	*(86)*
Total	86[c]	*100*	6	*100*	216[d]	*100*	14	*100*

[a] Defined as bacteriologically quiescent disease or disease of doubtful status bacteriologically (page 815).
[b] The parentheses indicate percentages based on fewer than 25 observations.
[c] Excluding two patients who died of non-tuberculous conditions and two patients who had their chemotherapy changed on account of toxicity.
[d] Excluding three patients who died of non-tuberculous conditions and six patients who had their chemotherapy changed on account of toxicity.

OTHER FACTORS WHICH MIGHT INFLUENCE THE COMPARISON OF THE S AND THE R PATIENTS

Analyses were undertaken to. see whether the S and the R patients were similar in terms of their physical activity during the 12 months and in the regularity of their self-administration of the medicine. It will be appreciated that if the S patients had had much greater physical activity than the R patients or had been more irregular in the self-administration of their medicine, this might have given an advantage to the R patients unrelated to the effectiveness of the chemotherapy.

Physical activity

There were only minor differences in the physical activity of the S and the R patients during the 12 months of treatment. By the end of this period 80 (93%) of 86 S patients on isoniazid plus PAS had returned to part-time or full-time activity, as compared with five (83%) of six R patients. The corresponding proportions for the patients on isoniazid alone were 162 (75 %) of 216 S patients and nine (64%) of 14 R patients.

Regularity in self-administration of the medicine

One of the methods used to determine whether the patients were taking the medicament regularly was to test the urine for the presence of PAS in patients on isoniazid plus PAS (Simpson, 1956) and for isoniazid in patients on that drug alone (Gangadharam et al., 1958) at their weekly visit to the Centre. Of the 86 S patients on isoniazid plus PAS, there were 31% who yielded only positive results, as compared with 33 % of the six R patients on the combined chemotherapy. Of 214 S patients on isoniazid alone, 30 % yielded only positive results, as compared with 57% of the 14 R patients.

The 86 S patients on isoniazid plus PAS had a total of 4092 urine tests performed during the year at their weekly visits of which 4.8% were negative, as compared with 5.2% of 290 such tests in the six R patients. The corresponding proportions for the patients on isoniazid alone were 6.0% of 9643 teats in 214 S patients and 1.4% of 589 tests in the 14 R patients. These findings therefore suggest that the drugs were being taken with considerable regularity throughout the 12 months and that the R patients on isoniazid alone were more regular than the corresponding S patients.

RESPONSE OF THE R PATIENTS IN THE FIRST THREE MONTHS

It is possible that the clinical improvement observed in the R patients might conceivably represent a benign natural history of the disease and be unrelated to the chemotherapy. The progress of the R patients in the first three months of treatment has, therefore, been studied in detail in order to discover whether or not the responses coincided with the commencement of chemotherapy.

Weight changes

The mean weight gain of the six R patients on isoniazid plus PAS was 6.2 1b.by one month, 9.2 1b. by two months, 10.3 1b. by three months and 9.2 1b. by six months. For the R patients on isoniazid alone, the corresponding figures were 1.7 1b., 3.8 1b., 5.3 1b. and 7.7 1b. There was, thus, an association between gain in weight and the start of treatment, the patients having gained more weight in the first than in the second three months.

Erythrocyte sedimentation rate

All the six R patients on isoniazid plus PAS had elevated ESRs (more than 10 mm) at the commencement of treatment and all had lower values at three months. Similarly, of the corresponding 14 R patients on isoniazid alone, 12 had lower values at three months.

Radiographic changes

At an independent assessment, all six R patients on isoniazid plus PAS showed radiographic improvement in the first three months. In four the improvement was moderate and in the remaining two it was slight. Of the 14 R patients on isoniazid alone, 11 showed radiographic improvement in the first three months. In one of the improvement was considerable and in the other 10 it was slight. Of the remaining three patients, one showed no improvement, one moderate deterioration and one died of tuberculosis.

The assessor apportioned, between the first and the second three months, the over-all radiographic change observed in the first six months. Greater improvement occurred in the first three months in all the six patients on isoniazid plus PAS. Considering the 14 patients on isoniazid alone, greater improvement occurred in the first three months in five and in the second three months in two, and there was equal improvement in the two periods in five. One patient died in the first three months and one patient had his chemotherapy changed on account of radiographic deterioration in the second three months. Thus, there was evidence that the earlier period after the start of chemotherapy was the period of greater radiographic improvement for the patients on isoniazid plus PAS. For the patients on isoniazid alone it was usually the period of greater or equal improvement.

Changes in cavitation

At an independent assessment, one patient on isoniazid plus PAS had no residual cavitation, four had less and in one it was unchanged at the end of three months' treatment. Of the 12 patients with initial cavitation on isoniazid alone, the cavitation had disappeared in one by three months, was less in six, was unchanged in two and more in two, and one patient had died of tuberculosis.

The assessor apportioned the changes observed in the first six months between the first and the second three months. Five of the six patients on isoniazid plus PAS showed more improvement in the first three months and the sixth showed equal changes both periods. For the isoniazid-alone series, of 12 patients assessed, six showed greater improvement in the first three months, one showed equal improvement in both periods, one showed greater improvement in the second three months and four showed no change in either period. It may be concluded that both for the combined chemotherapy and for the isoniazid-alone series, there was evidence of greater improvement in cavitation in the first than in the second three months.

Sputum culture results

It may be seen from Fig.1 and 2 that there was a bacteriological response in the first three months both for the combined chemotherapy and for the isoniazid-alone series. All the four patients (one receiving isoniazid plus PAS and three isoniazid alone) who attained quiescence and six (four receiving isoniazid plus PAS and two isoniazid alone) who showed a fall in the bacterial content of the sputum, followed by a rise, demonstrated a clear benefit bacteriologically in the first three months.

In summary, the R patients demonstrated response by sever different criteria in the first three months of treatment and it seems likely that these were due to the effect of chemotherapy, as nearly all the patients had advanced disease, were clinically ill on admission to treatment and, in addition, ha little bed rest as they were all treated at home under ambulatory conditions.

RANKING OF THEIR PATIENTS

The 20 R patients were ranked according to their progress in the 12-month period (Table 10). In the ranking, primary consideration was given to bacteriological response. Among those who shoed a similar bacteriological response the ranking was based on the over-all radiographic progress and changes in the cavitation. If these were equal the higher rankings were give to patients with the

TABLE IO. RANKING OF THE R PATIENTS ACCORDING TO THEIR RESPONSE TO TREATMENT AND VARIOUS FACTORS ON

Patient's serial number	Ranking according to response in the 12-month period	Treatment [a]	Response In the 12-month period					
			Bacterio-logical	Radio-graphic [b]	Change in cavitation	Weight change (lb.[c])	Age (years)	Extent of cavitation
125	1	HI-2	Quiescent	4+	Disappeared	+14	25	Slight
247	2	PH	Quiescent	4+	Disappeared	+ 9	23	Slight
294	3	HI-2	Quiescent	3+	Less	+ 9	18	Moderate
212	4	H	Relapsed	3+	Disappeared	+ 9	26	Moderate
6	5	PH	Fall and rise	2+	Disappeared	+ 8	29	Slight
189	6	PH	Fall and rise	2+	Less	+13	20	Moderate
187	7	PH	Fall and rise	2+	Less	+ 5	29	Extensive
82	8	HI-2	Fall and rise	1+	None throughout	+ 5	24	Nil
104	S	HI-I	Fall and rise	1+	None throughout	+ 6	27	Nil
174	10	PH	Fall and rise	0	Less	+14	18	Moderate
145	11	HI-1	None	3+	Less	+12	33	Moderate
158	12	H	None	2+	Less	+19	33	Moderate
296	13	HI-2	None	2+	Less	+ 8	29	Moderate
12	14	H	None	2+	Unchanged	+17	37	Moderate
190	15	HI-2	None	2+	Less	+10	23	Moderate
2	16	HI-2	None	2+	Less	- 2	28	Moderate
244	17	PH	None	Deteriorated and chemotherapy changed			26	Moderate
225	18	H	None	Deteriorated and chemotherapy changed			47	Moderate
236	19	H	None	Deteriorated and chemotherapy changed			24	Moderate
84	20	H	None	Tuberculous death			13	Extensive

[a] See page 808.
[b] 4+, 3+, 2+, 1+ and 0 indicate exceptional, considerable, moderate, slight and no improvement, respectively.
[c] 1 lb. = 0.45 kg.
[d] See page 810.

more extensive cavitation and the larger total extent of disease on admission to treatment.

Table 10 shows the ranking at 12 months, in relation to the treatment, the progress in the 12-month period and various factors on admission to treatment. It will be seen that the first 10 patients showed a bacteriological response; four attained quiescence, though one subsequently relapsed, and six showed a clear fall in the bacterial content of the sputum, as judged by the smear and culture results, followed by a rise. The majority of the patients showed radiographic improvement, disappearance or a reduction in the size of cavitation and gains in weight.

Prognostic value of various factors on admission to treatment

It will be seen from Table 10 that there was no association between response to treatment and the age of the patients, the bacterial content of the

IN THE 12-MONTH PERIOD, WITH TABULATIONS OF SOME MEASUREMENTS OF PROGRESS
ADMISSION TO TREATMENT

Factors on admission to treatment								
Total extent of disease	Bacterial content of sputum [a]	MIC of isoniazid on first and second cultures (μg/ml) [d]	Highest isoniazid concentration allowing equal growth on first and second cultures (μg/ml) [e]		Catalase activity of first and second cultures [f]	Virulence in the guinea-pig		Rate of inactivation of isoniazid
						Culture injected	Root-index of virulence [g]	
Moderate	1-plus	1, 1	0.2	<0.2	80, 90	First	0.53	Slow
Moderate	2-plus	1, 5	<0.2	<0.2	70, 80	Second	1.12	Slow
Extensive	3-plus	1, 1	0.2	<0.2	80, 2+	Second	0.58	Slow
Limited	2-plus	≥ 5, 0.2	<0.2	<0.2	60, 2+	Second	0.57	Slow
Limited	2-plus	0.2, 50	<0.2	<0.2	2+, 80	Second	0.68	Rapid
Extensive	2-plus	1, 1	0.2	0.2	90, 2+	First	0.95	Rapid
Extensive	3-plus	50, 50	0.2	1	30, 40	—	—	Slow
Limited	P-PIUS	5, 1	<0.2	0.2	50, 50	Second	0.51	Slow
Trivial	Negative	0.2, 50	<0.2	<0.2	2+, –		—	Slow
Mod&ate	2-plus	1, 5	<0.2	<0.2	70, 80	First	0.70	Rapid
Moderate	3-plus	>50, >50	5	50	30, 20	First	0.64	Slow
Moderate	1-plus	5, 5	<0.2	1	30, 30	Second	0.55	Slow
Extensive	3-plus	5, 5	1	1	60, 80	Second	0.88	Slow
Moderate	2-plus	5, 5	0.2	0.2	20, 90	Second	1.24	Slow
Extensive	3-plus	1, 1	<0.2	0.2	80, 2+	First	0.74	Rapid
Moderate	2-plus	50, >50	0.2	0.2	90, 90	Second	0.54	Slow
Moderate	3-plus	5, 5	1	<0.2	90, 90	Second	0.52	Slow
Moderate	Negative	5, 5	1	1	80, 90	Second	0.56	Slow
Extensive	3-plus	1, 1	0.2	0.2	60, 60	First	0.56	
Gross	2-plus	1, 1	<0.2	0.2	20, 30	A third culture	0.59	

[e] Growth equal to that on the drug-free slope.
[f] Result of the semi-quantitative test expressed as a percentage and, when this was not available, that of the qualitative test: a dash indicates no result available.
[g] The root-indices obtained in the Madras series ware adjusted to those in the Porton series, as described by Mitchison et al. (1961).

sputum, the catalase activity or the virulence in the guinea-pig of the pretreatment cultures. Only four of 18 patients tested were rapid inactivators of isoniazid, but three of them demonstrated a bacteriological response. There was, however, some association between the extent of cavitation and the total extent of the disease initially and the response to treatment. Thus of the 10 R patients who showed some bacteriological response, only five had mode rate or extensive cavitation initially, as compared with all 10 patients who did not show such a response. Similarly, the total extent of the disease was classified as at least moderate in six of the former patients, as compared with all 10 of the latter.

Of the three patients who had one sensitive and one resistant culture before treatment, one attained bacteriological quiescence but then relapsed and the other two had a fall in the bacterial content of the sputum followed by a rise (these patients were ranked 4, 5 and 9, respectively). For two of the

three patients who had quiescent disease at 12 months both cultures had low levels of resistance (MIC of 1 µg/ml). On the other hand, the patient who died and one of the patients who deteriorated and had his chemotherapy changed (ranked 20 and 19, respectively) also had similar strains. Even so there is a suggestion that the patients with the highest ranking had lower MICs than the patients lower in the ranking. There seemed to be a strong& suggestion of an association between the response to treatment and the highest concentration of isoniazid which allowed growth equal to that on the drug-free control slope. Thus, only one of six patients who had one or more cultures which yielded equal growth on 1 µg/ml isoniazid or more showed a bacteriological response, as compared with nine of 14 patients with equal growth on 0.2 µg/ml isoniazid or less. However, all six patients whose cultures yielded equal growth on 1 µg/ml isoniazid or more had moderate or extensive cavitation initially, as compared with nine of the 14 patients with lower levels of resistance by this definition.

Response to treatment

Five of the six patients on isoniazid plus PAS, as compared with five of the 14 patients on isoniazid alone, showed a bacteriological response. Considering the patients on isoniazid alone further, four of eight patients on a moderate dosage of the drug (HI-1 and HI-2 regimens), as compared with only one of six patients on a low dosage (H regimen), showed a bacteriological response. However, the patients on the moderate dosage of isoniazid alone were at an advantage on admission to treatment in comparison with those on the low dosage of the drug. Thus, only five of the eight HI-1 and HI-2 patients had moderate or extensive cavitation, as compared with all six of the H patients.

Heterogeneity of the isoniazid-resistant cultures

Evidence of the presence of a component of isoniazid-sensitive organisms in the bacterial populations harboured by the R patients on admission to treatment was obtained from four methods: (1) by the finding that one of the two cultures before treatment was sensitive to isoniazid; (2) by the occurrence in the isoniazid-sensitivity test of greater growth on the drug-free control slope than on the slope containing 0.2 µg/ml isoniazid–a finding which suggests that sensitive organisms were being inhibited by isoniazid; (3) by the occurrence. in the isoniazid-sensitivity test of greater catalase activity on the drug-free control slope than on the slope containing 0.2 µg/ml isoniazid; and (4) by the recovery of isoniazid-sensitive cultures from the spleens of guinea-pigs infected with the isoniazid-resistant cultures. Table 11 shows the number of R patients who appeared to have harboured isoniazid-

TABLE 11

PRESENCE OF A COMPONENT OF ISONIAZID-SENSITIVE TUBERCLE BACILLI IN PATIENTS WITH ISONIAZID-RESISTANT CULTURES ON ADMISSION TO TREATMENT

Evidence for the presence of isoniazid-sensitive tubercle bacilli	Number of patients tested	Number of patients with a component of isoniazid sensitive-organisms	Evidence of a bacteriological response		Ranking according to response in the 12-month period (see Table 10)
			Quiescence at 12 months	Fall in the bacterial content of the sputum followed by a rise	
1. One culture sensitive and the other resistant to isoniazid	20	3	0	2	4, 5, 9
2. Less growth on 0.2 µg/ml slope than on drug-free slope	19	3	2	8	2,3,4,5,10,15,17,20
3. Less catalase activity on 0.2 µg/ml slope than on drug-free slope	19	7	1	3	2,5,6,8,11,12,16
4. Recovery of isoniazid-sensitive cultures from spleens of guinea-pigs	8	5	0	2	5, 10, 14, 11, 18

sensitive organisms according to these four methods of assessment and their response to treatment. According to method 1, three of the 20 patients were excreting isoniazid-sensitive organisms on admission treatment. The corresponding figures for method 2 were eight of 19 tested, for method 3, seven of 19 tested, and for method 4, five of eight tested. In all, 16 of the 20 patients gave evidence by one or more of the four criteria of having had an isoniazid-sensitive component on admission to treatment. It will be observed, however, that there was very little agreement between the methods (Table 11). Moreover, only about half the patients with evidence of isoniazid-sensitive tubercle bacilli obtained by any one of the four methods showed a bacteriological response. On the other hand, of the five patients (ranked 2,4,5,10 and 17) who were shown to have a component of sensitive organisms by at least two methods, four showed a bacteriological response.

LEVEL OF ISONIAZID-RESISTANCE OF THE CULTURES FROM THE R PATIENTS BEFORE AND DURING TREATMENT

The levels of isoniazid-resistance of the two cultures before the start of treatment and the levels for single cultures at the end of three, six, nine and 12 months of the allocated chemotherapy are presented in Table 12. In comparison with the first pretreatment culture, the level of resistance at 12 months was lower in three, the same in four and higher in six. In comparison with the second pretreatment culture, the level of resistance at 12 months was lower in four, the same in three and higher in six. Results were not available for three patients who were bacteriologically negative at 12 months, three more patients had had their chemotherapy changed and one had died. There was thus a slight tendency for an increase in the degree of isoniazid-resistance of the cultures isolated from patients at the end of 12 months of chemotherapy. A similar trend was also apparent when the cultures at three, six and nine months were compared with the pretreatment cultures.

CATALASE ACTIVITY OF THE CULTURES FROM THE R PATIENTS BEFORE AND DURING TREATMENT

Table 13 presents the catalase activity of the two cultures from the R patients before the start of treatment and those of single cultures at the end of three, six, nine and 12 months of the allocated chemotherapy. The results of the semi-quantitative test and, if these were not available, the results of the qualitative test, are given. In comparison with the results of the semi-quantitative tests on the first pretreatment culture, the catalase activity at 12 months was lower in seven and higher in four (results either before treatment or at 12 months were not available in nine case). Considering the second pretreatment culture, the catalase activity at 12 months was less in the eight, the same in one and more in none (results either before treatment or at 12 months were not available in 11 cases). There was, therefore, evidence of lower catalase activity of the cultures isolated from patients at the end of 12 months' chemotherapy in comparison with the pretreatment cultures. Further analysis revealed that this was also apparent in the cultures from the patients at six and at nine months, although not at three months.

In summary, there was a tendency for an increase in the degree of isoniazid-resistance of the cultures and a decrease in their catalase activity to occur during treatment.

CLASSIFICATION OF THE PATIENTS BY OTHER DEFINITIONS OF RESISTANCE

There are a number of definition of isoniazid-resistance in common use, some of which are quoted by Canetti (1957). Apart from the definition in use in the Tuberculosis Chemotherapy Centre, other main definitions include:

(a) Equal growth in the control tube and 1 ug/ml isoniazid tube, or any growth on 5 ug/ml isoniazid (Chaves et al., 1955, 1956; United States Veterans Administration, 1959).

(b) Growth on the 1 mg/ml tube equal to that of the control tube (quoted by Canetti, 1957). (This definition is still widely used on the Continent of Europe (Canetti, personal communication, 1960).)

None of the S patients in the present study would have been classified as harbouring resistant organisms by either of the above two definitions.

The 20 R patients have been reclassified according to these two definitions (Table 14). The United States Veterans Administration definition would have classified the cultures from eight of the 20 patients as isoniazid-sensitive. Five of these eight patients had an unfavourable response to treatment. The definition quoted by Canetti (1957) would have classified the cultures from 13 of the patients as isoniazid-sensitive. Of these 13, 10had an unfavourable response to treatment, However, all

TABLE 12

DEGREE OF ISONIAZID-RESISTANCE OF THE CULTURES ISOLATED FROM THE PATIENTS BEFORE TREATMENT AND AT THE END OF THREE, SIX, NINE AND 12 MONTHS OF CHEMOTHERAPY (MIC OF ISONIAZID IN µg/ml)

Regimen	Patient's serial number	Months after start of chemotherapy					
		0		3	6	9	12
		1st culture	2nd culture				
PH	6	0.2	50	–	>50	Culture negative	>50 [a]
	174	1	5	5	1	5	1
	187	50	50	5	>50	5	5
	189	1	1	1	50	50	50
	244	5	5	50	5	5	Change of chemotherapy
	247	1	5	Culture negative	Culture negative	Culture negative	Culture negative
HI-1	104	0.2	50	>50	>50	>50	50
	145	>50	>50	>50	>50	>50	5
HI-2	2	50	>50	5	5	5	5
	82	5	1	5	>50	>50	>50
	125	1	1	Culture negative	Culture negative	Culture negative	Culture negative
	190	1	1	>50	>50	50	5
	294	1	1	Culture negative	Culture negative	Culture negative	Culture negative
	296	5	5	5	5	5	5
H	12	5	5	1	5	5	50
	84	1	1	Died	–	–	–
	158	5	5	5	5	5	5
	212	≥ 5	0.2	5	5 [b]	Culture negative	5 [a]
	225	5	5	5	5	Change of chemotherapy	
	236	1	1	5	Change of chemotherapy		

[a] Result of a culture at 11 months.
[b] Result of a culture at five months.

three patients who had quiescent disease at 12 months would have been classified as having isoniazid-sensitive infections by both these definitions.

Another definition of isoniazid-resistance (Russell & Dye, 1957; Middlebrook et al., 1960) classifies cultures as resistant to isoniazid if 1% of the bacilli are resistant to 0.2 µg/ml isoniazid. By using a criterion which considers confluent growth as representing the growth of *approximately* 1% or more of the total organisms inoculated, both the cultures from each of five of the 20 R patients would be classified as isoniazid-sensitive. (Seven patients would have one culture classified as sensitive and the other as resistant and eight patients as both cultures resistant.) Of the five patients with both cultures classified as sensitive (ranked 2, 4, 5, 9 and 10), four had an unfavourable response.

PROGRESS OF THE R PATIENTS IN THE SECOND YEAR

Of the three patients (one on isoniazid plus PAS, two on isoniazid alone) who had quiescent disease

TABLE 13

CATALASE ACTIVITY OF THE CULTURES ISOLATED FROM PATIENTS BEFORE TREATMENT AND AT THE END OF THREE, SIX, NINE AND 12 MONTHS OF CHEMOTHERAPY [a]

Regimen	Patient's serial number	Months after start of chemotherapy					
		0		3	6	9	12
		1st culture	2nd culture				
PH	6	2+	80	–	10	Culture negative	0 [b]
	174	70	80	100	70	50	30
	187	30	40	10	30	50	10
	189	90	2+	90	50	70	100
	244	90	90	70	80	80	Change of chemotherapy
	247	70	80	Culture negative	Culture negative	Culture negative	Culture negative
HI-1	104	2+	–	0	10	10	0
	145	30	20	40	0	0	0
HI-2	2	90	90	40	70	50	70
	82	60	50	30	30	20	20
	125	80	90	Culture negative	Culture negative	Culture negative	Culture negative
	190	80	2+	80	40	80	90
	294	80	2+	Culture negative	Culture negative	Culture negative	Culture negative
	296	60	80	70	60	100	80
H	12	20	90	30	90	90	60
	84	20	30	Died	–	–	–
	158	30	30	50	10	20	10
	212	60	2+	80	80 [c]	Culture negative	50 [b]
	225	80	90	80	90	Change of chemotherapy	
	236	60	60	70	Change of chemotherapy		

[a] Result of the semi-quantitative test expressed as a percentage, but for a few of the pretreatment cultures, where this was not available, that of the qualitative test.

[b] Result of a culture at 11 months.

[c] Result of a culture at five months.

at 12 months, two received 200 mg of isoniazid daily and the third a placebo (calcium gluconate) in the second year. None of these three patients relapsed. All the 13 patients with active disease at 12 months who were still on the originally prescribed chemotherapy continued on it in the second year. One on isoniazid plus PAS (ranked 5) became bacteriologically quiescent from the fourteenth month. Of the remaining 12 patients two on isoniazid plus PAS (ranked 7 and 10) deteriorated radiographically in the sixteenth and seventeenth months, respectively, and three on isoniazid alone (ranked 14, 15 and 16) deteriorated in the thirteenth, eighteenth and nine teenth months, respectively, to an extent which necessitated a change of chemotherapy. In view of this high incidence of deterioration, the treatment of the remaining seven patients (one on isoniazid plus PAS and six on isoniazid alone), all of whom were excre-

TABLE 14
COMPARISON OF DIFFERENT DEFINITIONS OF ISONIAZID-RESISTANCE AND UNFAVOURABLE RESPONSE TO TREATMENT

Definition of isoniazid resistance	Results of 2 pretreatment isoniazid-sensitivity tests	Number of patients	Unfavourable response [a]	Ranking according to response in the 12-month period (see Table 10)
Growth on 1 µg/ml isoniazid slope equivalent to that on the control slope, or any growth on 5 µg/ml slope (Chaves et al., 1955, 1958; United States Veterans Administration, 1969)	Both sensitive	8	5	1, 2, 3, 6, 14, 15, 19, 20
	1 sensitive and 1 resistant	5	5	4, 5, 8, 9, 17
	Both resistant	7	7	7, 10, 11, 12, 13, 16, 18
Growth on 1 µg/ml isoniazid slops equivalent to that on the control slope (quoted by Canetti, 1957)	Both sensitive	13	10	1, 2, 3, 4, 5, 6, 8, 10, 14, 15, 16, 19, 20
	1 sensitive and 1 resistant	4	4	7, 9, 12, 17
	Both resistant	3	3	11, 13, 18
Definition used in the present study (see page 809)	1 sensitive and 1 resistant	3	3	4, 5, 9
	Both resistant	17	14	1, 2, 3, 6, 7, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20

[a] Defined as bacteriologically relapsed disease, bacteriologically active disease or death from tuberculosis (see gage 815).

ting tubercle bacilli, was changed in the thirteenth, sixteenth, seventeenth, nineteenth, nineteenth, twentieth and twenty-first months of treatment, respectively.

In summary, of the six patients on isoniazid plus PAS, two attained quiescence and three deteriorated radiographically necessitating a change of chemotherapy in the 2-year period. Of the 14 patients on isoniazid alone, two attained quiescence, five deteriorated radiographically necessitating a change of chemotherapy and one died of tuberculosis in the 2-year period. The remaining seven patients had active disease and had their chemotherapy changed in the second year. This observation is similar to the finding that the considerable majority of the S patients with bacteriologically active or relapsed disease at 12 months deteriorated radiographically or continued to excrete tubercle bacilli in the second year (Ramakrishnan et al., 1962).

DISCUSSION

There have been several reports on the prevalence of infection with isoniazid-resistant organisms amongst untreated tuberculosis patients in chest clinic practice. Chaves et al. (1955) reported a prevalence of 2.3% of " significant " isoniazid-resistance in New York City between 1953 and 1955, and these authors (1956) also reported, in a somewhat different population, a prevalence of 0.8% in New York City in 1955. Fox et al. (1957) reported a prevalence of 0.7% in Great Britain in 1955-56 and Bell & Brown (1960) a prevalence of 9.1% in Ashanti, Ghana, in 1958. A prevalence of 3.6% was found in 1956-57 in patients living in Madras City (Tuberculosis Chemotherapy Centre,

1959) and, in the present study, the prevalence in1957-58 was 5.9% (20 examples in 338 patients). The survey reported by Fox et al. (1957) was a national survey and is the only one based on a representative sample of chest clinics chosen by a random procedure. It is difficult to compare the above prevalence figures of drug-resistance because of differences in the methods of performing sensitivity tests and in interpreting the results (Canetti, 1957; Rist & Crofton, 1960). The two studies in Madras between 1956 and 1958 showed a considerably higher prevalence than that in Great Britain in 1955-56 (Fox et al., 1957), in which a very similar method of sensitivity testing and very similar criteria for isoniazid-resistance were used. There is little doubt that isoniazid alone has been more widely used in Madras than in Great Britain and this could explain the difference. There is no reason to think that the Madras figures include patients who had acquired drug-resistance as a result of chemotherapy for great care was taken to investigate whether the patients had ever received previous chemotherapy, and inquiries were made not only during the first year of patients' treatment, but also subsequently. Further, identification tests were undertaken in all 20 patients' (in detail in 18) and those showed that the infecting organism was Myco.tuberculosis var.hominis, so that the possibility of the infections being due to acid-fast organisms other than tubercle bacilli has also been excluded.

In the present study, a comparison was made of the disease status at the treatment in the 20 patients with isoniazid-resistant strains and the 315 patients with sensitive strains. There was hardly any difference in terms of the extent of the radiographic lesion, the presence, or the extent of cavitation or the bacterial content of the sputum. There were, however, major differences between them in the age and sex distributions. Thus, 18 of the 20 patients with primarily isoniazid-resistant strains were males, as compared with 199 (63%) of the 315 patients with isoniazid-sensitive strains, a statistically significant difference (P=0.01). Further, 17 (85%) of the 20 patients with resistant organisms were under 35 years of age, as compared with 198 (63%) of the 315 patients with sensitive organisms, a statistically significant difference (P-0.03). These findings may be compared with those of the earlier study (Tuberculosis Chemotherapy Centre, 1959) in which four of the seven patients who had primarily isoniazid-resistant infections were males, three of them being under the age of 35 years. Amalgamating the findings for the two studies, there were 22 (81%) males amongst 27 patients infected with isoniazid-resistant tubercle bacilli, as compared with 298 (62%) males amongst 487 patients infected with sensitive organisms. This difference attains statistical significance (P-0.03). Of the 27 patients infected with isoniazid-resistant organisms, 22 (81%) were under the age of 35 years, as compared with 304 (64%) of 478 patients infected with sensitive ones, a statistically significant differences (P-0.04).

A comparison was made between patients infected with organisms resistant to streptomycin or isoniazid or both with those infected with strains sensitive to both these drugs in the tow studies combined. It was found that of the 37 patients infected with resistant strains 29 (78%) were males, as compared with 304 (64%) of 477 patients with sensitive strains. Of the 37 patients infected with resistant strains 28 (76%) were under the age of 35 years, as compared with 302 (63%) of the 477 patients with sensitive strains. These differences do not attain statistical significance and are smaller than the corresponding differences encountered when isoniazid-sensitivity alone was considered, though they are in the same direction. Thus there was a suggestion that primarily isoniazid-and streptomycin-resistant organisms were more frequently found among male patients and among patients under the age of 35 years. These findings may be compared with those from Great Britain, where Fox et al. (1957) reported a higher prevalence of resistance to isoniazid, streptomycin or PAS in young women in comparison with the rest of their population of tuberculous patients.

In the present study, only three of the 20 patients with isoniazid-resistant infections had bacteriologically quiescent disease at one year-namely, one of six patients who received isoniazid plus PAS and two of 14 on isoniazid alone. The inferiority of the bacteriological response, both the patients on isoniazid plus PAS and of those on isoniazid alone, in comparison with the response of the patients with sensitive organisms receiving the same treatment attains statistical significance. Despite the clear-cut disadvantage in terms of the attainment of bacteriological quiescence, there was evidence of some bacteriological response in 10 patients, five of whom received isoniazid plus PAS and five isoniazid alone. Moreover, the majority of patients infected with isoniazid-resistant organisms showed radiographic improvement, including reduction in cavitation, had lower ESRs and gained weight.

Thus, over the 12-month period radiographic improvement was reported by an independent observer in 15 of the 20 patients. In five of them it was assessed as exceptional or considerable. The independent observer also reported that, of 18 patients with cavitated lesions initially, cavitation had disappeared in four and had become less in nine. Moreover, 15 of the 20 patients gained weight, four of them as much as 14 1b. or more. In all these assessments the progress approximated to that of the patients infected with sensitive organisms.

In the earlier study (Tuberculosis Chemotherapy Centre, 1959), of the six patients infected with isoniazid-resistant tubercle bacilli who were treated with isoniazid plus PAS whose response to therapy could be assessed, three attained bacteriological quiescence, four showed radiographic improvement which included the disappearance or reduction of the cavitation and five gained weight when assessed at the end of 12 months of chemotherapy. Amalgamating the findings for the two studies, of the 12 patients infected with isoniazid-resistant organisms who were treated with isoniazid plus PAS, four attained bacteriologically quiescent disease, as compared with 218 (87%) of 249 patients infected with sensitive organisms. This difference attains statistical significance ($P<0.001$).

Thibier et al. (1960) treated two patients, infected with isoniazid-resistant tubercle bacilli, with isoniazid plus PAS. One of them received this combination for over a year and the other for four-and-a-half months, after which period streptomycin was added. Both patients showed definite radiographic improvement by three months and both had negative cultures by the end of the first month of treatment. The authors, however, point out that these two patients authors, however, point out that these two patients had limited non-cavitated disease initially, with scanty expectoration of tubercle bacilli.

It is of interest to consider the various reasons why these patients infected with isoniazid-resistant tubercle bacilli in our study demonstrated response during the 12 months. They may have responded (a) because the natural history of untreated pulmonary tuberculosis in the Madras Patients, whether the tubercle bacilli are isoniazid-sensitive or isoniazid-resistant, follows a benign and improving course, or (b) because the infecting isoniazid-resistant organisms were attenuated to the patients and the disease in them was therefore spontaneously follo-wing an improving course, or (c)as a direct conse-quence of the chemotherapy, whether isoniazid plus PAS or isoniazid alone.

The first two are unlikely explanations as nearly all the patients had advanced disease and were clinically ill on admission to treatment, and all were treated at home under ambulatory conditions in an overcrowded environment. For example, 18 of the 20 patients had cavitated lesions on admission to treatment. Moreover, there was no difference in the virulence in the guinea-pig of the cultures from the patients infected with isoniazid-resistant strains in comparison with those from patients infected with sensitive strains, although the catalase activity of the former was found to be less on the average. Thibier et al. (1960) reported that seven of their 14 patients infected with isoniazid-resistant tubercle bacilli had cavitated lesions and thus found evidence that these organisms were producing major lesions in their patients.

There is some evidence in favour of the third explanation. First, the response was most marked in the early months, suggesting a direct association with the administration of the chemotherapy. Secondly, the type of chemotherapy appeared to influence the response. Thus, five of six patients on isoniazid plus PAS, as compared with five of 14 patients on isoniazid alone, demonstrated a bacteriological response. It is therefore likely that PAS made a contribution to the improvement in the patients on the combination.

The following further reasons may be put forward to explain why these patients infected with isoniazid-resistant organisms responded to treatment:

1. Three of the 20 patients were known, from the results of the sensitivity tests, to be excreting a mixture of isoniazid-sensitive and isoniazid-resistant organisms, and there was other evidence which suggested that 13 of the remaining 17 patients were also excreting a mixture of sensitive and resistant organisms. The sensitive organisms would be expected to respond to isoniazid.

2. There was a suggestion that patients excreting organisms with low levels of resistance to isoniazid before treatment responded better, on the average, than those excreting organisms with high levels of resistance. Also there was a tendency for the degree of isoniazid-resistance of the cultures to increase during treatment. Both these observations suggest that the strains with low levels of resistance were inhibited by the isoniazid.

3. The possibility must be considered that the isoniazid-sensitivity tests in the Centre are technically unreliable and that patients were classified as having resistant strains when the organisms were, in fact, sensitive. However, there is evidence that the isoniazid-sensitivity tests in the Centre are highly reproducible. A sample of 63 cultures (10 sensitive and 53 resistant to isoniazid), isolated from patients in the chemotherapy study before the start of treatment and at intervals up to the twenty-fourth month of treatment, was tested on two occasions. The pairs of tests were set up and read by separate workers. Of the 63 pairs, 56 had the same minimal inhibitory concentration in both tests. The other seven differed only one dilution step, and none of these yielded a sensitive result in one test and a resistant result in the other.

Caneti (1957) classified, according to eight different criteria of resistance (four of them in wide use at the time), 113 strains of tubercle bacilli isolated from pulmonary lesions after "various forms of treatment" with isoniazid. The proportion of resistant cultures ranged from 24% to 99%, the latter figure applying to the Medical Research Council's definition, which is very similar to that used in the present report. The 20 patients in the present study infected with isoniazid-resistant organisms (according to this definition) were also classified according to two other definitions of resistance—namely, the one in use by the United States Veterans Administration (1959) and a definition that is widely used on the Continent of Europe (quoted by Canetti, 1957). Although these two definitions classified the three patients who had quiescent disease at 12 months as having isoniazid-sensitive infections, which is in keeping with the satisfactory bacteriological response, both appeared to be less successful than the definition used in the present study in identifying patients infected with isoniazid-resistant strains who are likely to fail to attain bacteriological quiescence.

It may be concluded that this study has shown that the treatment of patients infected with isoniazid-resistant tubercle bacilli is unsatisfactory, whether isoniazid is used alone or in combination with PAS, and that only a small proportion of the patients will attain bacteriological quiescence. Although they frequently showed a good clinical and radiographic response in the first year of treatment, the longer course of the disease was towards serious radiographic deterioration.

SUMMARY

1. Twenty (5.9%) patients excreting isoniazid-resistant tubercle bacilli were encountered among 338 previously untreated patients with active pulmonary tuberculosis who were admitted to a chemotherapeutic study.

2. In this paper 315 of the patients infected with isoniazid-sensitive tubercle bacilli (the S patients) have been compared with the 20 patients infected with resistant organisms (the R patients). There was little difference in terms of the extent of the radiographic lesion, the extent of cavitation or the bacterial content of the sputum before the start of treatment. There were, however, major differences between them in the age and sex distributions, there being a great preponderance of young males among the R patients. Thus, of the 315 S patients, 199 (63%) were males, as compared with 18 (90%) of the 20 R patients, and of the 199 S males 113(57%) were less than 35 years old, as compared with 15 (83%) of the R males.

3. The catalse activity of the pretreatment cultures from the S patients was, on the average, grater than that of the cultures from the R patients, but there was, on the average, no difference between them in their virulence in the guinea-pig.

4. In all, 90 S patients and six R patients were treated with isoniazid plus PAS, while 225 S patients and 14 R patients received isoniazid alone in three different dosage schedules. The treatment was allocated for a year.

5. By the end of 12 months of treatment eight (9%) of 86 S patients on isoniazid plus PAS showed an unfavourable bacteriological response, as compared with five (83%) of the six R patients. Correspondingly, 93 (43%) of 216 S patients on isoniazid alone showed an infavourable response, as compared with 12 (86%) of the 14 R patients. The differences attain statistical significance in both the treatment series.

6. When over-all radiographic progress, as assessed by an independent observer, weight changes and changes in ESR were considered, the progress of the R patients in the first year approximated to that of the S patients, but radiographic deteriorations occurred subsequently.

7. Evidence has been adduced to show that the response of the R patients was due to the chemotherapy.

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RÉSUMÉ

Parmi les 338 malades, jusqu'alors non traités, présentant une tuberculose active, admis à participer à l'étude chimiothérapique, on en a trouvé 20 (5,9%) qui éliminaient des bacilles résistants à l'isoniazide.

Dans cet article, les auteurs comparent 315 des malades infectés par des bacilles sensibles à l'isoniazide (malades S) avec les 20 malades infectés par des bacilles resistants (malades R). Il y avait peu de differences entre eux, avant le début du traitement, quant à l'étendue des lesions révélées par la radiographie, à la grandeur des cavités ou à la teneur en bacilles des crachats. Par contre, il y avait entre eux de grandes différences dans la répartition par âge et sexe, car on trouvait une grande majorité de jeunes hommes parmi les malades R. Ainsi, sur les 315 malades S, 199 (63 %) étaient de sexe masculin, contre 18 sur 20 malades R; 113 des 199 males S (57%), contre 15 des 18 mâles R, avaient moins de 35 ans.

L'activité de la catalase des bacilles provenant des malades S avant traitement était, dans l'ensemble, plus élevée que celle des bacilles des malades R, mais il n'y avait pas de difference dans leur virulence pour le cobaye.

Quatre-vingt-dix malades S et 6 malades R ont été traités par l'isoniazide+PAS, 225 malades S et 14 malades R par l'isoniazide seul, en trois posologies différentes. Le traitement a été administré pendant une année.

A la fin des 12 mois de traitement, 8 des 86 malades S (isoniazide+PAS), soit 9%, contre 5 des 6 malades R (83%), n'avaient pas réagi favorablement, d'après le critère bactériologique. Parallèlement, 93 des 216 malades S (43%) traités à l'isoniazide seul, et 12 des 14 malades R soumis aux mêmes regimes, n'avaient pas réagi favorablement non plus, d'après le critère bactériologique. Ces différences, dans les deux séries, sont statistiquement significatives. (Les malades décédés à la suite d'autres maladies que la tuberculose et ceux pour qui la thérapeutique a été changée en raison de la toxicité du médicament, ont été exclus des statistiques.)

Si l'on considère les progrès que montre l'examen radiologique effectué par un observateur indépendant, les changements de poids et de vitesse de sedimentation des érythrocytes, l'amélioration des malades R, au cours de la premiere année, était à peu près la même que celle des malades S; mais les aggravations, révélée radiologiquement, survinrent plus tard.

Des faits militent en faveur de l'idée que la réponse des malades R était bien due à la chimiothérapie.

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