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The Virulence in the Guinea-pig of Tubercle Bacilli Isolated before Treatment from South Indian Patients with Pulmonary Tuberculosis

3. Virulence related to Pretreatment Status of Disease and to Response to Chemotherapy

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This is the last of a series of three reports from the Tuberculosis Chemotherapy Centre Madras, on a study undertaken with the object of finding out whether differences in the virulence in the guinea-pig of tubercle bacilli isolated from South Indian tuberculous patients before the start of chemotherapy are related to the severity of the patients' disease on admission to treatment and to the subsequent response to chemotherapy. The 281 patients in this study were drawn from the patients admitted to a l-year comparison of four domiciliary chemotherapeutic regimens : (a) *3.9-5.5 mg/kg isoniazid plus 0.2-0.3 g/kg sodium PAS daily, divided into two doses (PH series) ;* (b) *7.8-9.6 mg/kg isoniazid alone daily in one dose (HI-I series) ; (c) 7.8-9.6 mg/kg isoniazid alone daily, divided into two doses (HI-2 series) ;* (d) *3.9-5.5 mg/kg isoniazid alone daily, divided into two doses (H series).*

No evidence was found of an association between the virulence of the organisms and any pretreatment condition of known prognostic importance. There was no association between pretreatment virulence and progress during treatment in the PH series (the most effective regimen). In the other series, however, the progress was more satisfactory in patients infected with organisms of low virulence than in those infected with organisms of high virulence, the association between virulence and progress attaining statistical significance in the combined HI-2 and H series (the least effective regimens) and only just failing to do so in the smaller HI-1 series.

Possible explanations are put forward both for the absence of an association between virulence and severity of disease on admission and for the presence of an association between virulence and response in the patients treated with isoniazid alone.

INTRODUCTION

During the course of a concurrent comparison of isoniazid plus p-aminosalicylic acid (PAS) with three regimens of isoniazid alone in the domiciliary treatment of pulmonary tuberculosis in South India (Tuberculosis Chemotherapy Centre, 1960), virulence tests in the guinea-pig were done on single cultures of tubercle bacilli obtained on admission (in one patient at three months) from 281 (89.2 %) of 315 patients in the main analysis of the comparison. In the second paper of the present series of three papers (Bhatia et al., 1961a [3]), these cultures were

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[3] See article on page 313.

shown to be, on the average, of lower virulence, and to have a wider range of virulence, than pretreatment cultures from British patients. Further, the virulence of multiple cultures obtained before treatment from the same Indian patient has been shown to be consistent (Bhatia et al., 1961b). It was therefore of interest to relate the virulence of the pretreatment cultures in the chemotherapy study to the extent and type of the disease found in the Indian patients on admission to the study, and to their progress under chemotherapy for one year.

The four regimens studied were :

PH (90 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.[1]

HI-1 (70 patients)

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

HI-2 (68 patients)

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

H (87 patients)

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

All of the 315 patients were aged 12 years or more, were resident in Madras City, had organisms sensitive to isoniazid on admission to the study and had not received more than two weeks' previous antituberculosis chemotherapy (the great majority had had no chemotherapy). All received the allocated regimen for one year unless death occurred or the treatment was changed because of clear-cut deterioration or serious drug toxicity.

During the course of the year, serious clinical or radiographic deterioration, necessitating a change of chemotherapy, occurred in 1% of the PH patients, 7% of the HI-1, 21% of the HI-2 and 17% of the H patients. Using very stringent criteria to assess the response to treatment, 8% of the PH, 27% of the HI-l, 41% of the HI-2 and 52% of the H patients were classified as having bacteriologically relapsed or active disease at the end of 12 months (including those whose chemotherapy had been changed because they had deteriorated); in addition 1 PH, 2 HI-2 and 3 H patients had died. Thus the PH regimen was the most effective, the HI-l was the next most effective and the HI-2 and the H regimens were the least effective.

In the present report the results of the virulence tests on the cultures obtained from the 281 patients are related *(a)* to their condition on admission to the study, and *(b)* to their progress during the year of chemotherapy.

METHODS

ASSESSMENTS OF PRETREATMENT DISEASE AND OF PROGRESS

A full account of the methods of assessing the extent and type of the patients' disease on admission to the study, and their progress during treatment is given elsewhere (Tuberculosis Chemotherapy Centre, 1960). All radiographic assessments were made on full-plate postero-anterior chest radiographs, taken on admission and at 6 and 12 months after the start of chemotherapy. On admission, the following assessments were made: *(a)* the extent of cavitation; *(b)* the number of lung zones involved in disease, the presence of any disease in each zone being recorded; *(c)* the total extent of the radiographic lesion, graded according to the total area occupied by the lesion; and *(d)* the type of disease in terms of the acuteness of the lesions. Changes in radiographic appearances were assessed over the 6-month and 12-month periods after the start of chemotherapy and the 12-month radiographs were examined for closure of cavities. All the radiographic assessments were made by an independent assessor (Dr Raj Narain) who was unaware of the treatment series of any patient.

The bacterial positivity of the sputum obtained on admission was graded on the results of the direct smear examination of a single specimen collected overnight (collection specimen). At the end of every month of treatment two collection specimens of sputum were obtained from each patient. At the end of three months, and monthly thereafter, a pair

[1] 1 lb. = 0.45 kg.

of laryngeal swabs was also taken. An assessment of the bacteriological response to treatment of the patient was based on the culture findings of these specimens obtained during treatment.

INCOME OF THE FAMILY

The total family income per month at the start of treatment was obtained by adding the incomes of the patient and of all the other family members. This total was divided by a figure representing the size and composition of the family, in which an adult male was counted as 1 standard unit, an adult female as 0.8 standard unit and a child under 15 years of age as 0.6 standard unit, to give the income per standard unit per month (Tuberculosis Chemotherapy Centre, 1960).

RATE OF INACTIVATION OF ISONIAZID

The rate of inactivation of isoniazid was determined for 268 (95.4%) of the 281 patients by the microbiological assay of isoniazid in the serum, four-and-a-half hours after a test dose of 3 mg of isoniazid per kg of body-weight given intramuscularly (Gangadharam et al., 1961). Patients with serum concentrations of 0.58 µg of isoniazid per ml, or more, were classified as slow inactivators, and those with concentrations of less than 0.58 µg of isoniazid per ml as rapid inactivators.

VIRULENCE TESTS

Virulence tests were carried out at the Microbiological Research Establishment, Porton, Wiltshire, England (Porton) and at the Tuberculosis Chemotherapy Centre (Madras). The measure of virulence used was based on the rate of progression of the disease in the guinea-pig, and has been described in detail by Mitchison et al. (1960, 1961 [1]). In brief, 1 mg (moist weight) of the culture was inoculated intramuscularly into each of two guinea-pigs, one of which was killed at 6 weeks and the other at 12 weeks. In 125 cultures tested at Porton, four guinea-pigs were inoculated with each culture, two being killed at 6 weeks and two at 12 weeks. At the post-mortem examination, the total extent of tuberculous disease was assessed as a score ranging from 0 to 100. The square root of the ratio of the score to the survival period in days was determined for each guinea-pig (whether sacrificed or dead from tuberculosis) and was termed the root-index. The root-index of virulence was defined as the mean of the root-indices for the two or four guinea-pigs inoculated with each culture, and has been used as the measure of virulence of the culture.

HOMOGENEITY OF THE VIRULENCE INVESTIGATION

In the first paper of the present series (Mitchison et al., 1961 [1]), a fuller account is given of the design of the investigation of virulence, together with a statistical evaluation of its homogeneity. There were certain features of the investigation which might have introduced heterogeneity into the results of the virulence tests, so that these results would have been influenced by factors other than the virulence of the cultures. The most important of these features were: *(a)* that the virulence tests were done partly on cultures soon after their isolation from the sputum of the patients and partly on cultures that had been stored, on the average, for 62 weeks at –20°C; *(b)* that the tests were carried out in 24 experiments over a period of two-and-a-half years; *(c)* that Duncan Hartley (DH) breed albino guinea-pigs were used at Porton and M-breed, mixed-colour guinea-pigs at Madras. However, it was established that storage at –20°C did not affect the virulence of the cultures and that inter-experimental variation was very small. The responses of the two breeds of guinea-pigs in the tests were found to be different, but a method for adjusting the results of the smaller Madras series to correspond to the results of the larger Porton series was successfully evolved; this adjustment has been used in the present report. Finally, antituberculosis chemotherapy may have been given for up to two weeks to 11 of the 281 patients before admission to the study. However, all of their cultures were sensitive to isoniazid and, in a paper to appear shortly (Subbaiah et al., 1961), it is shown that the virulence of cultures from the patients in the present study was not affected by three months of chemotherapy so long as the cultures were sensitive to isoniazid. In consequence, the values of the root-indices of virulence can be considered as giving a true measure of virulence, little influenced by these potential external sources of variation, and it is valid to study the associations between virulence and disease status in the total population of patients.

[1] See article on page 285.

TABLE 1
CONDITION OF PATIENTS ON ADMISSION TO TREATMENT RELATED TO VIRULENCE OF PRETREATMENT CULTURES

Condition on admission to treatment		Root-index of virulence						Mean
		0.0-		0.6-		0.9 or above		
		No. of patients	%	No. of patients	%	No. of patients	%	
Estimated age (years)	Under 25	27	30	38	31	21	31	0.74
	25-34	34	37	34	28	20	29	0.71
	35-44	15	16	31	25	16	24	0.76
	45 or above	15	16	19	16	11	16	0.70
Sex	Male	58	64	78	64	39	57	0.72
	Female	33	36	44	36	29	43	0.75
Total family income (in rupees [a]) per standard unit [b] per month	Less than 20	32	35	51	42	28	41	0.74
	20-	29	32	42	34	28	41	0.75
	30 or more	30	33	29	24	12	18	0.69
General clinical condition	Poor	13	14	35	29	11	16	0.75
	Fair	63	69	68	56	40	59	0.71
	Good	15	16	19	76	17	25	0.76
ESR (mm in 1 hour)	51 or more	70	77	90	74	43	63	0.71
	21-50	13	14	28	23	23	34	0.81
	0-20	8	9	4	3	2	3	0.65
Total patients		91	100	122	100	68	100	0.73

[a] Rs 4.76 = US $1.00.
[b] An adult male (15 years or over) was counted as 1 standard unit, an adult female (15 years or over) as 0.8 of a standard unit, and a child below 15 years as 0.6 of a standard unit.

RESULTS

The values of the root-index corresponded to the type of disease found at post-mortem in the guinea-pigs as follows: Root-indices of virulence of 0.00-0.59 were obtained with 91 (32.5%) of the 281 cultures. These cultures, of low virulence, were usually capable of producing in the guinea-pig lesions visible to the naked eye only at the site of inoculation or in its draining lymph-nodes. Root-indices of virulence of 0.60-0.89 were found with 122 (43.4%) of the cultures, which were of moderate virulence, producing only limited lesions in the visceral organs. Root-indices of virulence of 0.90 or above were obtained with 68 (24.2%) of the cultures; these were of high virulence, causing disease as extensive and rapidly progressive as that due to recently isolated drug-sensitive cultures from British patients (Bhatia et al., 1961a [1]).

VIRULENCE RELATED TO THE CONDITION OF THE PATIENT ON ADMISSION TO TREATMENT

Age and sex

No association is evident between the root-indices of virulence of the pretreatment cultures and the age or sex of the patients (Table 1).

[1] See article on page 313.

TABLE 2
RADIOGRAPHIC CONDITION OF PATIENTS ON ADMISSION TO TREATMENT RELATED TO VIRULENCE OF PRETREATMENT CULTURES

Radiographic condition on admission to treatment		Root-index of virulence						Mean
		0.0-		0.6-		0.9 or above		
		No. of patients	%	No. of patients	%	No. of patients	%	
Extent of cavitation	Extensive	9	10	17	14	9	13	0.75
	Moderate	46	51	66	54	31	46	0.72
	Slight	28	31	36	30	20	29	0.73
	Nil	8	9	3	2	8	12	0.76
Number of lung zones involved in disease	6 or 5	36	40	58	48	24	35	0.72
	4 or 3	38	42	48	39	26	38	0.72
	2 or 1	17	19	16	13	18	26	0.75
Extent of radiographic lesion	Gross or extensive	21	23	46	38	18	26	0.74
	Moderate or limited	63	69	70	57	39	57	0.71
	Slight or trivial	7	8	6	5	11	16	0.80
Total patients		91	100	122	100	68	100	0.73
Type of disease	Hyperacute	7	8	10	8	3	4	0.70
	Acute	34	40	42	34	28	41	0.73
	Mixed	38	44	59	48	30	44	0.74
	Chronic	7	8	11	9	7	10	0.76
Total patients		86 [a]	100	122	9 9	68	9 9	0.73

[a] Excluding five patients for whom the independent assessor reported the activity of the disease as unclassifiable.

Income of the family

The relationship between the family income and the virulence of the cultures was investigated because it was thought possible that cultures of low virulence might be less prevalent in those sections of the community with a relatively high income and consequently a higher resistance to tuberculosis. The findings however, indicate that cultures of low virulence occurred slightly more frequently in patients whose family income was high (Table 1), though even these patients were living under conditions of poor nutrition and overcrowding (Tuberculosis Chemotherapy Centre, 1959, 1960). The association does not attain statistical significance.

General clinical condition and erythrocyte sedimentation rate

The virulence of the pretreatment culture does not appear to be related to the general clinical condition of the patient nor was there an association with the erythrocyte sedimentation rate (ESR), determined by the Westergren method (Table 1).

Radiographic assessments

In Table 2, the root-indices of virulence of the 281 cultures are related to the extent of cavitation, the number of lung zones 'involved in disease and the total extent of the radiographic lesion. Cultures of slightly higher average virulence were obtained from

TABLE 3

BACTERIAL CONTENT OF SPUTUM ON ADMISSION TO TREATMENT AND RATE OF INACTIVATION OF ISONIAZID RELATED TO VIRULENCE OF PRETREATMENT CULTURES

Factor examined		Root-index of virulence: 0.0- No. of patients	0.0- %	0.6- No. of patients	0.6- %	0.9 or above No. of patients	0.9 or above %	Mean
Bacterial content of sputum (grade on smear of single collection specimen)	3-plus (heavy)	32	35	47	39	17	25	0.70
	2-plus(moderate)	26	29	40	33	22	32	0.75
	1-plus (scanty)	19	21	23	19	11	16	0.69
	Negative	14	15	12	10	18	26	0.78
	Total	91	100	122	101	68	99	0.73
Rate of inactivation of isoniazid	Rapid	38	43	45	39	29	44	0.74
	Slow	50	57	69	61	37	56	0.72
	Total	88 [a]	100	114 [b]	100	66 [c]	100	0.73

[a] Excluding three patients for whom the rate of inactivation of isoniazid was not determined.
[b] Excluding eight patients for whom the rate of inactivation of isoniazid was not determined.
[c] Excluding two patients for whom the rate of Inactivation of isoniazid was not determined,

patients with a smaller number of lung zones involved or a smaller extent of disease. Thus, the means of the root-indices were 0.75 for patients with l-2 zones and 0.72 for those with 3-6 zones involved in disease. Correspondingly, the means were 0.80 for patients with slight or trivial lesions, 0.71 for those with moderate or limited disease, and 0.74 for those with gross or extensive lesions. None of the associations is statistically significant.

The type of radiographic disease of 276 of the 281 patients was assessed in terms of the acuteness of the lesions (Table 2). There is a trend, which does not attain significance, suggesting that cultures of higher virulence came from patients with more chronic lesions.

Bacterial content of the sputum

No association is apparent between the bacterial content of a single collection specimen of sputum, as assessed by direct smear examination, and the virulence of the cultures (Table 3). The means of the root-indices of cultures from specimens graded as 3-plus, 2-plus, 1-plus and negative were 0.70, 0.75, 0.69 and 0.78, respectively.

Rate of inactivation of isoniazid

The cultures obtained from rapid and slow inactivators of isoniazid had similar degrees of virulence, the means of the root-indices being 0.74 and 0.72, respectively (Table 3).

In summary, there was no clear evidence of an association between virulence and any of the assessments of the condition of the patient on admission to the chemotherapy study. At most, there was a suggestion that strains of high virulence were obtained slightly more often from patients with less severe radiographic lesions, in particular, involvement of l-2 lung zones and a small total extent of their disease, but these trends did not attain statistical significance.

PRETREATMENT ASSOCIATIONS IN THE SEPARATE TREATMENT SERIES

In the preceding section it has been shown in the total of 281 patients that no clear associations exist between the virulence of the culture and any pretreatment factor which is known to influence the response to chemotherapy. However, it is necessary

TABLE 4

VIRULENCE OF PRETREATMENT CULTURES OBTAINED FROM PATIENTS IN THE FOUR TREATMENT SERIES RELATED TO IMPORTANT PROGNOSTIC ASSESSMENTS OF THEIR CONDITION ON ADMISSION TO TREATMENT

Condition on admission to treatment		Treatment series									
		PH		HI-1		HI-2		H		HI-2+ H	
		No. of patients	Mean root-index	No. of patients	Mean root-index	No. of patients	Mean root-index	No. of patients	Mean root-index	No. of patients	Mean root-index
Extent of cavitation	Extensive	15	0.75	7	0.73	5	0.86	8	0.70	13	0.76
	Moderate	38	0.72	33	0.73	34	0.65	38	0.76	72	0.71
	Slight	22	0.81	22	0.68	17	0.64	23	0.77	40	0.71
	Nil	7	0.73	1	1.03	4	0.54	7	0.87	11	0.75
Number of lung zones involved in disease	6 or 5	38	0.72	20	0.71	23	0.72	37	0.74	60	0.73
	4 or 3	30	0.76	32	0.69	26	0.64	24	0.80	50	0.72
	2 or 1	14	0.81	11	0.81	11	0.59	15	0.78	26	0.75
Bacterial content of sputum (smear examination)	3-plus	24	0.68	23	0.70	22	0.74	27	0.69	49	0.71
	2-plus	25	0.76	16	0.75	19	0.64	28	0.83	47	0.75
	1-plus	11	0.80	19	0.69	12	0.59	11	0.69	23	0.64
	Negative	22	0.79	5	0.80	7	0.59	10	0.91	17	0.78
Rate of inactivation of isoniazid	Rapid	27	0.74	29	0.74	23	0.73	33	0.73	56	0.73
	Slow	51	0.75	32	0.70	35	0.61	38	0.80	73	0.71

to establish that this holds true within each of the treatment series, since the association between virulence and response to chemotherapy will be studied separately in each of them. Accordingly, the results in each treatment series were examined to see if any associations existed between virulence and any of four assessments of the condition on admission which are of prognostic importance-namely, the extent of cavitation, the number of lung zones involved in disease, the bacterial content of the sputum (Tuberculosis Chemotherapy Centre, 1960) and the rate of inactivation of isoniazid (Selkon et al., 1961). The findings are summarized in Table 4. In the PH and HI-1 series there is little evidence of any association, except that the mean root-indices of virulence tended to be slightly lower with cultures from patients with a larger number of lung zones involved in disease and with more heavily positive sputum. However, in the HI-2 series, the patients with more extensive cavitation, a larger number of lung zones involved, and a higher bacterial content of the sputum, and who were rapid inactivators of isoniazid, tended to have, on the average, cultures of higher virulence. In the H series these associations are of about the same order, but in the opposite direction. Since the results of treatment in the HI-2 and the H series were very similar, it was possible, by a fortunate coincidence, to overcome the influence of these associations by considering the root-indices obtained in these two series together in the remaining sections of the report. It is evident (Table 4, last column) that there is little association between virulence and the various assessments of prognostic importance in the combined HI-2 and H series.

VIRULENCE RELATED TO PROGRESS OF THE PATIENTS UNDER CHEMOTHERAPY

All of the 281 patients were on the prescribed regimen for one year, with the following exceptions:

(1) 30 patients (1 PH, 5 HI-l, 13 HI-2, 11 H) had their treatment changed because of serious radiographic or clinical deterioration. (An independent

TABLE 5

CHANGES IN RADIOGRAPHIC APPEARANCES IN THE 12-MONTH PERIOD RELATED TO VIRULENCE OF PRETREATMENT CULTURES

Treatment series	Root-index of virulence	0-6 months								O-12 months							
		Total patients [a]		Moderate or greater improvement		Slight improvement or no change		Deterioration or death from tuberculosis		Total patients [b]		Moderate or greater improvement		Slight improvement or no change		Deterioration or death from tuberculosis	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
PH	0.0-	22	100	17	(77) [c]	4	(18)	1	(5)	22	100	19	(86)	1	(5)	2	(9)
	0.6-	36	101	29	81	6	17	1	3	36	100	31	86	4	11	1	3
	0.9 or above	21	100	16	(76)	5	(24)	0	(0)	21	100	18	(86)	3	(14)	0	(0)
	Total	79		62		15		2		79		68		8		3	
	Mean			0.73		0.77		0.70				0.74		0.80		0.61	
HI-1	0.0-	21	100	15	(71)	5	(24)	1	(5)	19	99	17	(89)	1	(5)	1	(5)
	0.6-	24	100	21	(88)	2	(8)	1	(4)	23	101	19	(83)	2	(9)	2	(9)
	0.9 or above	15	100	8	(53)	4	(27)	3	(20)	15	100	10	(67)	3	(20)	2	(13)
	Total	60		44		11		5		57		46		6		5	
	Mean			0.70		0.72		0.88				0.70		0.78		0.87	
HI-2 + H	**0.0-**	**46**	***100***	**30**	***65***	**13**	***28***	**3**	***7***	**46**	***100***	**35**	***76***	**2**	***4***	**9**	***20***
	0.6-	**58**	***100***	**39**	***67***	**11**	***19***	**8**	***14***	**58**	***101***	**37**	***64***	**5**	***9***	**16**	***28***
	0.9 or above	**29**	***100***	**17**	***59***	**9**	***31***	**3**	***10***	**29**	***100***	**15**	***52***	**4**	***14***	**10**	***34***
	Total	**133**		**86**		**33**		**14**		**133**		**87**		**11**		**35**	
	Mean			**0.71**		**0.72**		**0.79**				**0.68**		**0.83**		**0.79**	

[a] Excluding four patients (one in each series) who died of non-tuberculous conditions and five patients (2 PH, 2 HI-1, 1 HI-2) who had their chemotherapy changed on account of toxicity.

[b] Excluding four patients (one in each series) who died of non-tuberculous conditions and eight patients (2 PH, 5 HI-1, 1 HI-2) who had their chemotherapy changed on account of toxicity.

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

assessor (Dr K. S. Sanjivi) advised on the necessity for change of treatment due to radiographic deterioration.)

(2) five patients (1 PH, 1 HI-2, 3 H) died either from tuberculosis or with their tuberculosis contributing to the cause of death.

These 35 patients have been included, under the heading " deterioration or death from tuberculosis " from the month of change of treatment or death, in the subsequent analyses.

(3) four patients (one in each series) died from causes other than tuberculosis.

(4) eight patients (2 PH, 5 HI-1, 1 HI-2) had their treatment changed because of severe drug toxicity.

The 12 patients mentioned under (3) and (4) above have been excluded from the subsequent analyses from the month of death or change of treatment.

Changes in radiographic appearances

In Table 5 are set out the root-indices of virulence obtained with the pretreatment cultures from patients in the PH, the HI-1 and the combined HI-2 and H series, related to the over-all change in radiographic

appearances that occurred between 0 and 6 months and between 0 and 12 months after the start of chemotherapy. In the PH series there is no association between virulence and radiographic progress. However, in the other series patients with strains of low virulence tended to show more satisfactory progress, an association which was most evident in the O-12 month assessments. Thus, in the HI-1 series, 17 (89%) of 19 patients with cultures of low virulence had moderate or greater radiographic improvement over the 12 months, as compared with 29 (76 %) of 38 patients with cultures of moderate or high virulence. The corresponding results in the combined HI-2 and H series were 35 (76%) of 46 patients with cultures of low virulence and 52 (60%) of 87 patients with cultures of moderate or high virulence. Expressed in another way, the means of the root-indices of virulence in the HI-1 series were 0.70 for patients with moderate or greater improvement, 0.78 for those showing slight improvement or no change and 0.87 for those who deteriorated or died from tuberculosis. The corresponding means of the root-indices of virulence in the combined HI-2 and H series were 0.68, 0.83 and 0.79, respectively.

Correlation coefficients were calculated for the association between the root-indices of virulence and the changes in radiographic appearances (Table 6). In the PH series, the coefficients were small and statistically non-significant. They were relatively large and of about equal size for the HI-1 series during the 0-6 month and the 0-12 month periods, and for the combined HI-2 and H series during the 0-12 month period. The only coefficient to attain statistical significance was the one for the combined HI-2 and H series during the 0-12 month period ($r = +0.230$, $P = 0.008$), though the corresponding coefficient ($r = +0.242$) for the HI-1 series only just failed to do so ($P = 0.07$).

TABLE 6

CORRELATION COEFFICIENTS BETWEEN CHANGES IN RADIOGRAPHIC APPEARANCE AND ROOT-INDICES OF VIRULENCE

Treatment series	0-6 months		0-12 months	
	Correlation coefficient [a] (*r*)	P	Correlation coefficient [a] (*r*)	P
PH	+ 0.037	0.7-0.8	– 0.031	0.7-0.8
HI-1	+ 0.204	0.1	+ 0.242	0.07
HI-2 + H	+ 0.090	0.2-0.3	+ 0.230	0.008

[a] In calculating correlation coefficients a score of 0 was allotted for moderate or greater improvement, 1 for slight improvement or no change, and 2 for deterioration or death from tuberculosis.

Disappearance of cavitation

The root-indices of virulence are related to the disappearance of cavitation during the 12-month period in Table 7. Patients who showed no initial cavitation (7 PH, 1 HI-1, 11 HI-2+ H) were excluded from the analysis. No statistically significant association between pretreatment virulence and cavity closure is evident. In the PH series the means of the

TABLE 7

DISAPPEARANCE OF CAVITATION DURING THE 12-MONTH PERIOD RELATED TO VIRULENCE OF PRETREATMENT CULTURES

Treatment series	Root-index of virulence	Total patients [a]		Cavities remained		Cavities disappeared	
		No.	%	No.	%	No.	%
PH	0.0-	18	100	4	(22)[b]	14	(78)
	0.6-	36	100	15	42	21	58
	0.9 or above	18	100	5	(28)	13	(72)
	Total	72		24		48	
	Mean			0.75		0.73	
HI-1	0.0-	19	100	9	(47)	10	(53)
	0.6-	23	100	10	(43)	13	(57)
	0.9 or above	14	100	4	(29)	10	(71)
	Total	56		23		33	
	Mean			0.70		0.75	
HI-2 + H	0.0-	42	100	18	43	24	57
	0.6-	55	100	28	51	27	49
	0.9 or above	25	100	15	60	10	40
	Total	122		61		61	
	Mean			0.75		0.69	

[a] Excluding four patients (one in each series) who died of non-tuberculous conditions and eight patients (2 PH, 5 HI-1, 1 HI-2) who had their chemotherapy changed on account of toxicity.

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

root-indices of virulence were 0.75 for patients whose cavities remained apparent and 0.73 for those whose cavities disappeared. The corresponding means for the HI-1 series were 0.70 and 0.73 and, for the combined HI-2 and H series, 0.75 and 0.69, respectively.

Culture negativity

The results of culture of single collection sputum specimens obtained at monthly intervals from the patients in each of the series were related to the virulence of their pretreatment cultures. In the PH and HI-l series no associations were evident and the full results are not presented here. In the PH series at 6 months, negative cultures were obtained from 86 % of 21 patients with pretreatment cultures of low virulence and from 88 % of 56 patients with cultures of moderate or high virulence. The corresponding percentages at 12 months in this series were 90 % and 87%, respectively. In the HI-1 series negative cultures were obtained from 71% of 21 patients with pretreatment cultures of low virulence and from 76 % of 38 patients with cultures of moderate or high virulence at 6 months, and from 82% and 76%, respectively, at 12 months. The results of culture of the sputum specimens in the combined HI-2 and H series are shown in Fig. 1. There appears to be a suggestion of an association between low virulence and a high percentage of patients with negative cultures in this series from about the third month of treatment onwards. At 3 months, negative cultures were obtained from 55 % of 44 patients with pretreatment cultures of low virulence, from 52% of 56 patients with cultures of moderate virulence and from 29% of 28 patients with cultures of high virulence. The corresponding percentages at 6 months were 64 %, 47 % and 38 % and at 12 months 61%, 53 % and 41%, respectively. The differences at 3, 6 and 12 months between the percentages of patients with negative cultures do not attain statistical significance, though the 6-month findings only just fail to do so at the 5 % level.

Status at 12 months

In Table 8 a classification of the patients at 12 months, based primarily on the bacteriological response to treatment (Tuberculosis Chemotherapy Centre, 1960), is related to the virulence of the pretreatment cultures. A patient's disease has been termed bacteriologically quiescent if all the cultures (usually seven to nine) at 10, 11 and 12 months were negative. Those patients who, following at least three consecutive months of culture negativity, yielded an isolated positive culture at 10, 11 or 12 months have been considered as having doubtful bacteriological status. Patients whose cultures were all negative at three or more consecutive monthly

FIG. 1

PATIENTS WITH NEGATIVE SPUTUM CULTURES IN THE COMBINED HI-2 AND H SERIES RELATED TO VIRULENCE OF PRETREATMENT CULTURES

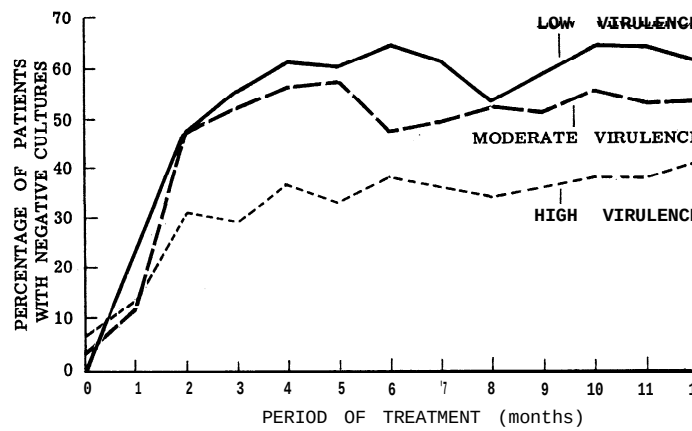


TABLE 8

CLASSIFICATION OF PATIENTS AT 12 MONTHS ACCORDING TO THEIR RESPONSE TO TREATMENT AND TO THE VIRULENCE OF THEIR PRETREATMENT CULTURES

Treatment series	Root-index of virulence	Total patients [a]		Bacteriologically quiescent or of doubtful status		Bacteriologically relapsed or active status		Change of chemotherapy due to deterioration or death from tuberculosis	
		No.	%	No.	%	No.	%	No.	%
PH	0.0-	22	*101*	20	*(91)* [b]	1	*(5)*	1	(5)
	0.6-	36	*100*	30	*83*	5	*14*	1	*3*
	0.9 or above	**21**	***100***	**21**	*(100)*	0	*(0)*	0	*(0)*
	Total	**79**		71		6		2	
	Mean			0.74		0.68		0.70	
HI-1	0.0-	19	*100*	15	*(79)*	3	*(16)*	1	(5)
	0.6-	23	*100*	17	*(74)*	4	*(17)*	2	(9)
	0.9 or above	15	*29*	11	*(73)*	2	*(13)*	2	(13)
	Total	57		43		9		5	
	Mean			0.71		0.74		0.87	
HI-2 + H	0.0-	46	*99*	25	*54*	13	*28*	8	*17*
	0.6-	58	*100*	29	*50*	16	*28*	13	*22*
	0.9 or above	29	*99*	10	*34*	12	*41*	7	*24*
	Total	133		64		41		28	
	Mean			0.68		0.75		0.77	

[a] Excluding four patients (one in each series) who died of non-tuberculous conditions and eight patients (2 PH, 5 HI-1, 1 HI-2) who had their chemotherapy changed on account of toxicity.

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

examinations, but who produced two or more positive cultures at 10, 11 or 12 months, have been classified as having bacteriologically relapsed disease. If the cultures were never negative at three consecutive monthly examinations the patients have been classified as having bacteriologically active disease. Finally, there were patients who had their chemotherapy changed because of radiographic or clinical deterioration in the presence of positive sputum, and those who died from tuberculosis.

In the PH series there was no evidence of an association between the status at 12 months and the virulence of the pretreatment cultures. However, in the other series, patients with a favourable result tended to have strains of low virulence. In the HI-l series, bacteriologically quiescent or doubtful status was attained by 15 (79 %) of 19 patients with cultures of low virulence and by 28 (74%) of 38 patients with cultures of moderate or high virulence. In the combined HI-2 and H series similar favourable results were obtained in 25 (54 %) of 46 patients with cultures of low virulence, as compared with 39 (45 %) of 87 patients with cultures of moderate or high virulence. The means of the root-indices of virulence in the HI-1 series were 0.71 for patients with quiescent or doubtful status, 0.74 for those whose disease

relapsed or remained active and 0.87 for those who deteriorated or died from tuberculosis. In the combined HI-2 and H series the corresponding means were 0.68, 0.75 and 0.77, respectively.

The correlation coefficients for the association between the status at 12 months and the root-indices of virulence are set out in Table 9. As with the coefficients for radiographic change, there was little correlation in the PH series. The correlation coefficient was largest (+0.209) in the HI-l series, but did not attain statistical significance. In the combined HI-2 and H series the coefficient was +0.168 and, the number of patients being larger, just attained significance (P = 0.05).

TABLE 9

CORRELATION COEFFICIENTS BETWEEN CLASSIFICATION OF PATIENTS AT 12 MONTHS ACCORDING TO THEIR RESPONSE TO TREATMENT AND ROOT-INDICES OF VIRULENCE

Treatment series	Correlation coefficient [a] *(r)*	P
PH	– 0.065	0.5-0.6
HI-1	+ 0.209	0.1
HI-2+ H	+ 0.168	0.05

[a] In calculating correlation coefficients a score of 0 was allotted for quiescent or doubtful status, 1 for relapsed or active status and 2 for deterioration or death from tuberculosis.

DISCUSSION

It has been found in this study that the virulence in the guinea-pig of cultures obtained from the patients immediately before the start of treatment was not related to the severity of their disease at that time. There are two possible explanations for this finding:

(1) Variation in virulence, as demonstrated in the guinea-pig, does not influence the natural history of tuberculosis in man.

(2) The influence of virulence has been obscured, partly because of the nature of the sample of patients studied and partly because the study was made at only one point in the course of the disease in each patient.

Reasons for considering that the second explanation may be correct merit further discussion.

There is reason to believe that the sample of patients in the study was not representative of all patients with pulmonary tuberculosis in the population from which they were selected-namely, residents of Madras City. The patients were admitted to the study because they had attended chest clinics with symptoms. Consequently, minor lesions would have been found among them less frequently than in a random sample of the population. Furthermore, it is probable that the age structure of the sample was also not representative. On the one hand, patients under 12 years of age were not admitted to the study. On the other hand, of the 279 patients aged 15 years or more, 16 % were at least 45 years old. In a sample survey of the prevalence of pulmonary tuberculosis in India (Indian Council of Medical Research, 1959), considering only those aged 15 years or more, with active or probably active radiographic disease, in six cities (Calcutta, Delhi, Hyderabad, Madanapalle, Patna and Trivandrum), the percentages of patients who were at least 45 years old ranged from 32% to 52 %; the corresponding percentages of patients aged 45 years or more among those who yielded a positive culture of tubercle bacilli from a single specimen ranged from 24% to 56%. Thus, the sample of patients presented here appears to have been deficient both in younger and in older patients.

A study of the severity of the disease in a sample taken on only a single occasion may obscure the true influence of virulence. As an example of one way in which this might occur, a hypothetical representation of the development of tuberculosis in patients infected with organisms of low and high virulence is shown in Fig. 2. In this system it is assumed that:

(1) The rate of development of the lesions in man is related to the virulence of the organism (in a manner analogous to the findings of the virulence tests in the guinea-pig), so that disease would develop more rapidly in those infected with highly virulent strains.

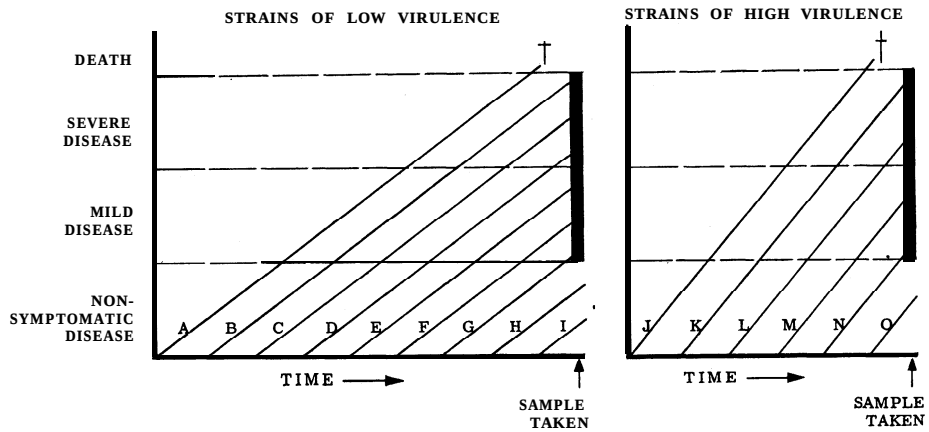
(2) Lesions start to develop at regularly spaced intervals of time.

(3) The majority of the lesions, whether caused by strains of high or of low virulence, show a steadily progressive course. (Patients with minor lesions tending to heal spontaneously would be unlikely to appear in the present sample at any point in the course of their disease and have, therefore, not been considered.)

Considering typical patients who developed tuberculosis, each of whom is represented by a diagonal line in Fig. 2, the results would be as follows.

FIG. 2

HYPOTHETICAL REPRESENTATION OF DEVELOPMENT OF LESIONS IN PATIENTS INFECTED WITH STRAINS OF LOW AND OF HIGH VIRULENCE



A, B . . . I: Patients with strains of low virulence. J, K . . . O: Patients with strains of high virulence.

Among patients infected with strains of low virulence, the patient infected earliest (Fig. 2, A) will have died by the time of the sample and will therefore not appear in it, three patients will have severe disease (Fig. 2, B, C, D), three will have mild disease (Fig. 2, E, F, G) and two will have disease which has not progressed sufficiently to cause the patient to attend at a clinic (Fig. 2, H. I). Correspondingly, in the patients infected with strains of high virulence, there will be one who had died before the sample was taken (Fig. 2, J), two with severe disease (Fig. 2, K, L), two with mild disease (Fig. 2, M, N) and one whose disease is asymptomatic (Fig. 2, O). Thus, of the five patients with severe disease, three will have been infected with strains of low virulence and two with strains of high virulence. Among those with mild disease there will also be three with strains of low virulence and two with strains of high virulence, so that no association between the severity of the disease and virulence would be apparent. From the geometry of the system it is apparent that there would also be no association if the spacing between the start of the disease was different for patients with strains of high and of low virulence. An extension of the concept to include variation among the patients within each virulence group in the speed at which their lesions develop, as a result of differences in host susceptibility or of the number of organisms with which they were infected, would not alter this conclusion. The system represented in Fig. 2 is one of several possible explanations for the absence of an association between virulence and the severity of the disease found in the present study. Evidence that it represents an approximation to the true state of affairs cannot be obtained from our findings, but is provided by the observations of Frimodt-Møller (1960). Frimodt-Møller carried out mass radiography surveys at approximately yearly intervals in a South Indian population. Cultures of tubercle bacilli from the cases in these surveys were tested for their virulence in the guinea-pig by methods similar to the method reported here. In a brief reference to unpublished results he has stated that untreated cases of tuberculosis with bacilli of low virulence had had radiographic lesions for a longer period of time than had cases from whom virulent bacilli were recovered (Frimodt-Møller, 1961). These findings are compatible with the model of Fig. 2, in which disease due to organisms of low virulence is represented as developing more slowly than disease due to highly virulent organisms.

Frimodt-Møller (1961) has also stated that there was an association between virulence and the age of the patient, strains of low virulence being more prevalent in the elderly. It is also evident that a higher proportion of attenuated bacilli in elderly patients would be expected from the hypothesis of Fig. 2, in which strains of low virulence are assumed to grow more slowly in the lesions than those of high virulence. In the present study, no association

was found between virulence and age, but, as has been commented on above, the age structure of the sample may not have been as representative as one obtained by mass radiography.

In considering the association between the virulence of the pretreatment cultures and the progress of the patients during their year of chemotherapy, it was necessary at first to establish that virulence was independent of other factors influencing progress. No association was found between virulence and any pretreatment condition of known prognostic importance, so that it is possible to consider directly its influence on progress. The two most important assessments of progress are the classification of the patients at 12 months according to their response to treatment and the change in radiographic appearances during the 12-month period. In the combined HI-2 and H series bacteriological quiescence (including doubtful bacteriological status) was attained by 54% of patients with cultures of low virulence and by 45 % of those with cultures of moderate and high virulence. Moderate or greater improvement over the 12 months was shown by 76% of patients with organisms of low virulence and by 60% of those with more virulent organisms. The two associations attained statistical significance at the 5% and 1% levels, respectively. Virulence did not appear to influence cavity closure. The associations between low virulence and satisfactory progress in the HI-1 series were of a similar order to those found in the combined HI-2 and H series. None of them attained statistical significance, although the association with radiographic progress only just failed to do so. In the PH series there was no evidence of an association between virulence and progress during treatment. Thus, in patients treated with isoniazid alone, the progress of the patients appeared to be more satisfactory in those infected with organisms of low virulence than in those infected with more virulent organisms.

Two reasons may be advanced to explain the association between pretreatment virulence and progress during chemotherapy. First, virulence may influence the extent of growth of sensitive bacilli in the lesions. In patients infected with organisms of high virulence, the greater multiplication of the sensitive bacilli might in itself have adverse clinical results and would also increase the probability of resistant strains emerging. Secondly, virulence might influence the growth of isoniazid-resistant organisms. For the latter mechanism to be responsible for the association between the virulence of pretreatment sensitive bacilli and progress, there would also have to be an association between the virulence of the resistant organisms and the virulence of the sensitive strains from which they had arisen. A comparison of the virulence of sensitive and resistant strains from the same patient will be reported elsewhere, but it can be stated here that they are, in fact, related. It is difficult to know which of these two mechanisms is the more important, particularly as there is no evidence on whether sensitive bacilli continue to grow in the lesions during chemotherapy. The early appearance of a higher proportion of negative sputum cultures from patients with cultures of low virulence (Fig. 1) suggests that virulence influences either the growth of sensitive bacilli or the early phase of growth of resistant bacilli.

If either of the two mechanisms considered above is responsible for the association between pretreatment virulence and progress during treatment, it would appear likely that they would operate to a smaller extent when combined chemotherapy with isoniazid and PAS was given. Thus, the presence of PAS in addition to isoniazid would tend to prevent any growth of sensitive organisms, and the growth of isoniazid-resistant organisms in the presence of PAS might be dependent on different causes from those operating in its absence. As an example of such causes, inadequate dosage of PAS or the presence of an exceptionally large bacterial population in the lesions might be of overriding importance in allowing resistant organisms to grow during treatment with both drugs. For these reasons it is not surprising that an association between virulence and progress during treatment appears to exist in those patients treated with isoniazid alone, whereas it is not apparent in those treated with isoniazid and PAS.

The associations that have been demonstrated between the virulence of the pretreatment cultures from the patients and the progress of their disease during treatment with isoniazid alone suggests that variation in virulence, as determined in the guinea-pig, has some influence on the course of human tuberculosis. However, the influence of virulence was not a large one. The associations only just reached conventional levels of statistical significance and were not evident at all in the PH series. Furthermore, the evidence is indirect, since it was obviously not possible to observe the course of the disease in untreated patients. The theoretical ideal would be to make observations at successive intervals on the same population in which the natural course of tuberculosis is not obscured by chemotherapy.

SUMMARY

1. Virulence tests in the guinea-pig were done on pretreatment, isoniazid-sensitive cultures of tubercle bacilli from 281 (89 %) of 315 patients participating in a comparison of four regimens of chemotherapy in the domiciliary treatment of pulmonary tuberculosis in South India. The regimens, and the dosages appropriate to patients weighing 100 lb. were: (1) PH-isoniazid 200 mg plus PAS (sodium) 10 g a day, divided into two doses; (2) HI-1–isoniazid 400 mg a day, in one dose; (3) HI-2–isoniazid 400 mg a day, divided into two doses; (4) H–isoniazid 200 mg a day, divided into two doses. The dosage of each regimen was graded according to the patient's weight. The regimens were given for 12 months.

2. The measure of virulence was based on the rate of progression of the lesions in the guinea-pig following the intramuscular injection of 1 mg of the culture, and was expressed as the root-index of virulence.

3. No associations were found between the root-indices of virulence of the cultures and any of the following assessments of the condition of the patients on admission to the study: age, sex, family income, general clinical condition, erythrocyte sedimentation rate, extent of cavitation, number of lung zones involved in disease in radiographs, total extent of the radiographic lesion, radiological acuteness of the lesions, bacterial content of the sputum, and rate of inactivation of isoniazid. Reasons are given for supposing that the absence of associations may have been due to the nature of the sample of the patients and to the study of each patient at only one point in the course of the disease.

4. In the combined HI-2 and H series-the regimens of lowest efficacy-good progress during treatment was associated with low virulence of the pretreatment cultures. Marked radiological improvement over the 12-month period was shown by 76% of the patients with cultures of low virulence, but by only 60% of those with cultures of moderate or high virulence. The corresponding proportions of patients achieving bacteriological quiescence by 12 months were 54% and 45 %. Both of these associations attained statistical significance. Pretreatment virulence did not appear to influence cavity closure.

5. In the HI-1 series the associations between the assessments of progress and the virulence of the pretreatment cultures were of similar magnitude to those found in the combined HI-2 and H series, but the number of patients being smaller, none attained statistical significance.

6. In the PH series-the regimen of greatest efficacy-no associations were found between any of the assessments of progress and the virulence of the pretreatment cultures.

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RÉSUMÉ

Des études de virulence du bacille tuberculeux, par inoculation au cobaye, ont été effectuées sur des souches sensibles à l'isoniazide, prélevées sur des malades avant traitement. Ces cultures provenaient de 281 des 315 malades engagés dans l'étude comparative de quatre régimes thérapeutiques administrés à domicile, dans le cadre des études entreprises à Madras (Inde). Ces régimes étaient les suivants pour les malades pesant 45 kg environ: 1) (PH) isoniazide 200 mg + PAS sodique, 10 g, dose quotidienne en deux fois; 2) (HI-1) isoniazide 400 mg, en une dose quotidienne; 3) (HI-2) isoniazide 400 mg en deux doses; 4) (H) isoniazide 200 mg en deux doses.

Les dosages étaient adaptés aux poids des malades. Le régime a été suivi pendant 12 mois.

La virulence des souches de bacilles a été évaluée d'après le taux de progression des lésions chez le cobaye, à la suite de l'injection intramusculaire d'1 mg de culture. Elle était exprimée en « racine de l'indice de virulence ».

On n'a pu établir aucun rapport entre cet indice et les facteurs suivants, relatifs aux malades admis à l'étude: âge, sexe, situation financière de la famille, état clinique général, vitesse de sédimentation des érythrocytes, étendue des lésions cavitaires, nombre de zones pulmonaires atteintes révélées par la radiographie, extension générale des lésions visibles à l'examen radiologique et leur degré d'activité, teneur en bacilles des crachats et taux d'inactivation de l'isoniazide. L'absence de relation entre ces divers facteurs peut s'expliquer par la nature de l'échantillonnage et par le fait que les malades n'ont été étudiés qu'à un moment de l'évolution de leur maladie.

Dans les groupes recevant les régimes H et HI-2 combinés (régimes de moindre efficacité), les progrès en cours de traitement ont été associés à la faible virulence des cultures avant traitement. Une amélioration visible à l'examen radiologique au cours de 12 mois a été observeé chez 76% des malades donnant des cultures de virulence faible ou moyenne, mais chez 60% seulement des malades ayant des bacilles modérément ou hautement virulents. Les proportions respectives de malades atteignant le stade de quiescence bactériologique après 12 mois étaient 54% et 45%. Dans les deux cas le rapport était statistiquement significatif. La virulence des souches avant traitement ne semble pas avoir affecté l'effacement des cavités.

Dans la série du régime HI-1, la relation entre les améliorations et le degré de virulence des cultures avant traitement était du même ordre de grandeur que celle des régimes H et HI-2 combinés, mais le nombre de malades étant faible, les chiffres n'étaient pas statistiquement significatifs.

Dans la série du régime PH, le plus efficace, on n'a trouvé aucun rapport entre les critères d'amélioration et la virulence des cultures avant traitement.

REFERENCES

Bhatia, A. L., Csillag, A., Mitchison, D. A., Selkon, J. B., Somasundaram, P. R. & Subbaiah, T. V. (1961a) *Bull. Wld Hlth Org.*, **25,** 313

Bhatia, A. L., Devadatta, S., Mitchison, D. A. Radhakrishna, S., Selkon, J. B. & Subbaiah, T. V. (1961b) *Tubercle (Lond.)* **,42,** 317

Frimodt-Møller, J. (1960) *Bull. Wld Hlth Org.*, **22,** 61

Frimodt-Møller, J. (1961) In: Tuberculosis Association of India, *Proceedings of the Seventeenth Tuberculosis Workers' Conference, held in Cuttack . . .*, New Delhi, p. 7

Gangadharam, P. R. J., Bhatia, A. L., Radhakrishna, S. & Selkon, J. B. (1961) *Bull. Wld Hlth Org.*, **25** (In press)

Indian Council of Medical Research (1959) *Tuberculosis in India. A sample survey 1955-1958,* New Delhi (*Special Report Series,* No. 34), p. 56

Mitchison, D. A., Bhatia, A. L., Radhakrishna, S., Selkon, J. B., Subbaiah, T. V. & Wallace, J. G. (1961) *Bull. Wld Hlth Org.*, **25,** 285

Mitchison, D. A., Wallace, J. G., Bhatia, A. L., Selkon, J. B., Subbaiah, T. V. & Lancaster, M. C. (1960) *Tubercle (Lond.),* **41,** 1

Selkon, J. B., Fox, W., Gangadharam, P. R. J., Ramachandran, K., Ramakrishnan, C. V. & Velu, S. (1961) *Bull. Wld Hlth Org.*, **25** (In press)

Subbaiah, T. V., Bhatia, A. L., Geraghty, E., Mitchison, D. A., Radhakrishna, S. & Selkon, J. B. (1961) *Bull Wld Hlth Org.*, **25** (In press)

Tuberculosis Chemotherapy Centre (1959) *Bull. Wld Hlth Org.*, **21,** 51

Tuberculosis Chemotherapy Centre (1960) *Bull. Wld Hlth Org.*, **23,** 535