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## CHEMOTHERAPY TRIALS IN INDIA

This issue is a Special number incorporating some of the papers received from the Tuberculosis Chemotherapy Centre, Madras, giving results of the investigations made there. These papers have already been published in the *Bulletin of the World Health Organization*. We are reproducing them in this Journal so that those who may not have access to the *Bulletin* can have the benefit of reading them. We have also an arrangement by which certain articles published in the Indian Journal of Tuberculosis will be reproduced by the *Tubercle*, London and some of their articles will be published in this Journal also.

The articles in this issue give the results of investigations done on Indian patients. Though some of these may appear to be of academic interest only, they are important because they contribute to the knowledge of the Epidemiology of Tuberculosis in this country and also indicate the factors that influence the successful treatment of tuberculous patients with modern anti-bacterial drugs. For example, the virulence of tubercle bacilli isolated from Indian patients as well as the role of diet in their treatment are of epidemiological interest. The rate of inactivation of isoniazid in Indian patients is of practical significance in the treatment of this disease.

It has been noted already that the virulence of tubercle bacilli isolated from patients in India varies a good deal from that noted in bacilli isolated from patients in England. However, it may be mentioned that there are bacilli in Indian patients as virulent as those found in patients in western countries also. Further, when once the disease develops there does not seem to be significant difference in the type of the disease between those developed with varying degrees of virulence. Further investigation to find out why this is so seems necessary.

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It is evident that the work done in this Chemotherapy Centre is contributing substantially to our knowledge of the various aspects connected with the practical application of tuberculosis control methods under Indian conditions, with special reference to domiciliary treatment. Research work of this type takes considerable time to complete as also to get valid results,

involving at the same time comparatively heavy costs. The Madras Centre has made a good start and the results so far achieved have been acclaimed as extremely useful. Tuberculosis workers in India earnestly hope that full scope and opportunities will be provided for this Centre to pursue its investigations un-interrupted.

# THE VIRULENCE IN THE GUINEA-PIG OF ISONIAZID-SENSITIVE TUBERCLE BACILLI ISOLATED FROM SOUTH INDIAN PATIENTS BEFORE TREATMENT AND AFTER THREE MONTHS OF CHEMOTHERAPY\*

T. V. SUBBAIAH,<sup>1</sup> A. L. BHATIA,<sup>1</sup> ELLEN GERAGHTY,<sup>2</sup> D. A. MITCHISON,<sup>2</sup>  
S. RADHAKRISHNA<sup>1</sup> & J. B. SELKON<sup>1</sup>

In order to find out whether chemotherapy with isoniazid affects the virulence in the guinea-pig of tubercle bacilli that do not develop resistance to the drug, virulence tests were carried out on isoniazid-sensitive cultures obtained from 20 South Indian tuberculous patients before treatment and after three months of chemotherapy with isoniazid. No significant difference in virulence was observed between the cultures obtained on admission to treatment and those obtained after three months of chemotherapy. This is a finding with important implications for large-scale surveys of the distribution of attenuated strains of tubercle bacilli from untreated patients in India and other countries. Detailed and repeated inquiries as to previous chemotherapy are not important in such surveys, provided that sensitivity tests are done on all the cultures.

## INTRODUCTION

In confirmation of the findings of Frimodt-Moller, Mathew and Barton (1956) and Frimodt-Moller (1957), Mitchison *et al.* (1960) and Bhatia *et al.* (1961a) have shown that cultures of tubercle bacilli isolated from South Indian patients, resident in Madras City, were, on the average, less virulent in the guinea-pig and had a wider range of virulence than cultures from British patients. All of the Indian cultures studied were fully sensitive to isoniazid and almost all were obtained from patients before the start of antituberculosis chemotherapy. However, a small proportion of them were from patients who had received short periods of treatment, probably with isoniazid. It is well established that the emergence of isoniazid-resistance is associated with loss of virulence in the guinea-pig (Middlebrook, 1957), but there appears to be no evidence as to whether chemotherapy with isoniazid affects virulence in the absence of the emergence of resistance. In consequence, it was thought desirable to compare the virulence of *isoniazid-sensitive*

cultures obtained from patients before treatment and after three months of chemotherapy with isoniazid.

An opportunity arose to make the comparison during the course of a concurrent study of four chemotherapeutic regimens in the treatment of pulmonary tuberculosis in South Indian patients (Tuberculosis Chemotherapy Centre, 1960). In brief, the regimens employed were:

*PH.* Isoniazid 3.9-5.5 mg/kg body-weight plus *p*-aminosalicylic acid (sodium salt) 0.2-0.3 g/kg daily, divided into two doses, by mouth.

*HI-1.* Isoniazid alone, 7.8-9.6 mg/kg daily, in one dose by mouth.

*HI-2.* Isoniazid alone, 7.8-9.6 mg/kg daily, divided into two doses, by mouth.

*H.* Isoniazid alone, 3.9-5.5 mg/kg daily, divided into two doses, by mouth.

Sensitive cultures were obtained from 20 patients participating in the study, both before treatment and after three months of the allocated regimen. Twelve of the patients received

\* This paper is also published in the *Bulletin of the World Health Organization*.

<sup>1</sup> Tuberculosis Chemotherapy Centre, Madras, India. The Centre is under the joint auspices of the Indian Council of Medical Research, the Madras State Government, the World Health Organization and the Medical Research Council of Great Britain.

<sup>2</sup> Unit for Research on Drug Sensitivity in Tuberculosis (Medical Research Council of Great Britain), Postgraduate Medical School of London, London, England.

TABLE I  
*Root-indices of isoniazid-sensitive cultures obtained on admission to treatment  
 and after three months of chemotherapy*

Patient number	0-month culture (on admission to treatment)				3-month culture*			Mean root-index of virulence	
	Experiment number		6-week root-index	12-week root-index	Root-index of virulence	6-week root-index	12-week root-index		Root-index of virulence
1	Por.	If	0.89	0.61	0.75	0.81	1.00	0.90	0.83
2	..	2†	1.09	0.87	0.95	0.99	1.02	1.00	0.99
3	..	3†	0.77	0.32	0.54	0.45	0.56	0.50	0.52
4	..	3†	0.96	0.84	0.90	1.00	1.16	1.08	0.99
5	..	4†	0.81	0.47	0.64	0.71	0.32	0.52	0.58
6	..	4	0.88	0.32	0.60	0.66	0.32	0.49	0.54
7	..	5‡	0.44	0.90	0.67	0.32	0.45	0.38	0.53
8	..	5	1.08J	1.00	1.04	0.65	0.35	0.50	0.77
9	..	5	1.14	0.96	1.05	1.38	0.93	1.16	1.10
10	..	5	0.37	0.32	0.34	0.66	0.46	0.56	0.45
11	..	6	1.01	0.56	0.78	0.89	0.81	0.85	0.82
12	..	6	0.66	0.88	0.77	0.75	0.26	0.50	0.64
13	..	7	0.79	0.82	0.80	0.66	0.46	0.56	0.68
14	..	8	0.93	0.86	0.90	0.96	0.49	0.72	0.81
15	..	8	1.12	1.37	1.24	0.82	0.44	0.63	0.94
16	..	10	1.10	0.87	0.98	0.71	0.94	0.82	0.90
17	..	10	0.42	0.30	0.36	0.45	0.35	0.40	0.38
18	..	12	1.12	1.10	1.11	1.10	0.96	1.03	1.07
19	..	12	0.93	1.05	1.00	1.06	0.84	0.95	0.98
20	..	12	1.01	0.62	0.82	0.81	0.89	0.85	0.83
Mean			0.88	0.75	0.81	0.79	0.65	0.72	0.77

\* All the 3-month cultures were stored in the deep-freeze and all were tested in Por. 13.

† Deep-freeze stored culture.

‡ Non-tuberculous death; missing observation estimated by statistical techniques (see article by Mitchison *et al.* (1962) on page 71).

the PH regimen, three the HI-1, two the HI-2 and three the H. We report here on the virulence of these cultures in the guinea-pig.

#### METH

##### ODS Cultures

Sputum specimens were cultured on Lowenstein-Jensen medium and sensitivity tests were done on the cultures by the methods described previously (Tuberculosis Chemotherapy Centre 1959). The identification test procedures, which included the niacin test, were those of Thomas *et al.* (1962).<sup>1</sup> All except one of the cultures, which became contaminated, were identified as *Mycobacterium tuberculosis* var. *hominis*, and all were sensitive to isoniazid and streptomycin. Cultures were flown to England and were either tested for their virulence within

eight weeks of having become positive or stored at  $-20^{\circ}\text{C}$  for periods of up to 60 weeks until required. Storage at  $-20^{\circ}\text{C}$  has been shown not to affect virulence (Mitchison *et al.*, 1962<sup>2</sup>).

##### Virulence tests

The measure of virulence 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, 1962<sup>2</sup>). In brief, 1 mg. (moist weight) of a 3-week-old culture was injected into each of two guinea-pigs of the Duncan Hartley breed (mean weight, 435g; range, 300-520 g.) at the Microbiological Research Establishment, Porton, Wiltshire, England. One of these animals was killed at 6 weeks and the other at 12 weeks. At post-mortem examination, the total extent of tuberculous

<sup>1</sup> See article on page 99,

<sup>2</sup> See article on page 71.

disease in the spleen, liver, lungs and local glands was assessed as a score ranging from 0 to 100. For reasons given elsewhere (Mitchison *et al.*, 1962<sup>1</sup>), the square root of the ratio of the score to the survival time in days (whether the guinea-pig died or was sacrificed) was determined for each animal, and was called the 6-week root-index or the 12-week root-index. The mean of the 6-week and 12-week root-indices of the two guinea-pigs was employed as the measure of virulence, and was termed the 'root-index of virulence'.

Some of the cultures were tested for virulence in four guinea-pigs (two killed at 6 weeks and two at 12 weeks). On grounds of simplicity, the analysis is based on the results on only two of these animals (one killed at 6 weeks and one at 12 weeks), selected at random.

#### *Arrangement of experiments*

The virulence tests were done in a series of experiments (which included tests on other cultures) at Porton and, in conformity with the nomenclature adopted by Mitchison *et al.* (1962),<sup>1</sup> they are referred to as For. 1, 2 .... The virulence of strain H37Rv and of five recently isolated British cultures was tested in each of these experiments, principally to detect inter-experimental variation (for a fuller account, see Mitchison *et al.*, 1962<sup>1</sup>). The cultures obtained from the patients before the start of the prescribed regimen were tested either fresh or after storage at  $-20^{\circ}\text{C}$  in 10 of these experiments (Table 1), but those obtained after three months' treatment were all stored at  $-20^{\circ}\text{C}$  and were tested together in experiment For. 13. Of the 80 guinea-pigs used, one died from a non-tuberculous cause in experiment For. 8, and the value of its root-index was estimated from the results on the remaining animals in the experiment, as described by Mitchison *et al.* (1962).<sup>1</sup>

#### RESULTS

The root-indices obtained in the virulence tests on the 40 cultures from 20 patients are set out in Table 1. Inter-experimental variation in the series of experiments Por. 1 to Por. 13 was found to be very small (Mitchison *et al.*, 1962<sup>1</sup>), so that the results obtained in the different experiments have been considered as a unit and examined by analysis of variance (Table 2).

#### *Variation between patients*

The means of the root-indices of virulence for the two cultures from each patient are shown in the last column of Table 1. The variation between these means from patient to patient was significantly greater than the estimate of variation between the root-indices of virulence of duplicate cultures from the same patient (Table 2, terms a and c,  $P < 0.005$ ). Thus, there were consistent differences between patients in the virulence of their cultures.

#### *Difference between 0-month and 3-month cultures*

The means of the root-indices of virulence on the 0-month and 3-month cultures from the 20 patients were 0.81 and 0.72 respectively (Table 1). The difference does not attain statistical significance (Table 2, term b,  $P = 0.07$ ). Furthermore, the mean of the root-indices of virulence obtained with strain H37Rv in experiments Por. 4-12 was 0.85 (strain H37Rv was not set up in For. 1-3), and in Por. 13 it was 0.82. The corresponding means with the British cultures were 1.05 in Por. 1-12 and 1.03 in Por. 13. These results suggest that the root-indices obtained in Por. 13, which included all the tests on 3-month cultures, were, on the average, very slightly lower than in the earlier experiments, among which the 0-month cultures were distributed. Thus, the difference between the means of the root-indices on 0-month and 3-month cultures, as well as being small and not statistically significant, may also have resulted in part from slight systematic under-scoring in experiment Por. 13.

#### *Variation between cultures from the same patient*

An estimate of variation between duplicate cultures from the same patient was provided by the interaction of the difference between months and the differences between patients. From this estimate, the variation in virulence between pairs of cultures from the same patient did not appear to be greater than the residual variation in response of the guinea-pigs (Table 2, terms c and h,  $P = 0.1$ ).

#### *Remaining terms in the analysis of variance*

The difference between the means of the 6-week and 12-week root-indices is statistically significant (Table 2, term d,  $P < 0.005$ ). This finding has been a consistent feature of all

<sup>1</sup> See article on page 71.

TABLE 2

*Root-indices of isoniazid-sensitive cultures obtained on admission to treatment and after three months of chemotherapy*  
analysis of variance

*Design of the investigation*—20 patients; two cultures from each patient; each culture inoculated into two guinea-pigs; total of 80 guinea-pigs.

Term	Source of variation	Sum of squares	DF	Mean square	Term tested against	F	P
a	Patients (P)	3.5295	19	0.1858	c	3.83	<0.005
b	0 and 3 months (M)	0.1739	1	0.1739	c	3.59	0.07
c	Interaction M X P	0.9214	19	0.0485	h	1.61	0.1
d	6 and 12 weeks (W)	0.3551	1	0.3551	h	11.80	<0.005
e	Interaction W x P	0.4778	19	0.0251	g	...	NS <sup>1</sup>
f	Interaction W x M	0.0013	1	0.0013	g	...	NS
g	Interaction W x P X M	0.6937	19	0.0365			
h	Residual (e + f + g)	1.1728	39	0.0301			
	Total ...	6.1527	79	0.0779			

<sup>1</sup> NS indicates that the variance ratio is less than 1.0.

analyses of virulence tests done by the method reported here. It arises from a non-linear relationship between the post-mortem score and the period of survival of the guinea-pigs. As both the first-order interactions with this difference between the 6-week and 12-week results were small and did not attain statistical significance, they were pooled with the second-order interaction. The resultant residual mean square (Table 2, term h, 0.0301) was similar to the corresponding mean square (0.0264) in a larger sample of virulence tests on Indian cultures done by the same method (see Table 6 in Mitchison *et al.*, 1962<sup>1</sup>).

#### DISCUSSION

The main findings of the present study of *isoniazid-sensitive* cultures of tubercle bacilli are as follows: (a) there was considerable variation between patients in the virulence of their cultures in the guinea-pig; (b) sensitive cultures obtained from the patients before treatment and after three months of chemotherapy, including isoniazid, did not appear to differ in their virulence. However, the probability that a difference existed was close to the 5 per cent level; (c) the variation between duplicate cultures from the same patient was no greater than the natural variation in response

<sup>1</sup> See article on page 71.

of the guinea-pigs. Thus, in the present study, cultures from a given Indian patient had a characteristic and consistent degree of virulence, which was unaffected by a 3-month period of chemotherapy, so long as the cultures remained sensitive to isoniazid. These findings confirm and extend the observations of Bhatia *et al.* (1961 b), who tested the virulence of three cultures obtained over a 6-week period before the start of chemotherapy from each of 12 patients, and who also found that the differences in virulence between patients were consistent.

In accompanying papers the virulence in the guinea-pig of 281 cultures obtained before the start of chemotherapy from the same number of South Indian patients in Madras is related to the extent and type of their disease before treatment (Ramakrishnan *et al.*, 1962<sup>2</sup>), and is compared with the virulence of cultures from British patients (Bhatia *et al.*, 1961a). These cultures were all sensitive to isoniazid and were all regarded as being pretreatment cultures, although, at the time they were obtained, anti-tuberculosis chemotherapy had probably been given to 11 of the 281 patients for up to two weeks and certainly to one patient for three months; the remaining 269 patients had almost certainly had no previous chemotherapy (Tuberculosis Chemotherapy Centre, 1960). Our finding that three months of chemotherapy

<sup>2</sup> See article on page 124.

did not appear to influence the virulence of isoniazid-sensitive cultures provides good justification for considering all the 281 cultures as either genuinely pretreatment or having the same virulence as pretreatment cultures.

This finding is likely to be of general importance for epidemiological investigations in the future. Studies of the virulence of cultures from patients in other parts of India and in other countries would be of value in determining the geographical area in which attenuated cultures exist. Such studies might provide information on the spread of tubercle bacilli within India and from one country to another. In these investigations it will be a great advantage to be able to assume, from the evidence reported here, that the virulence of any isoniazid-sensitive culture is the same, or nearly the same, as that of a pretreatment (isoniazid-sensitive) culture from the same patient and that, even if the patient has had some chemotherapy, this has not altered the virulence of his strain. It is easy to do sensitivity tests, but difficult to get reliable histories of chemotherapy, since these require detailed and repeated inquiries over a period of many months, during which increasing confidence can be established with the patient and his family (Tuberculosis Chemotherapy Centre, 1959, 1960). It is most unlikely that circumstances which would allow of such exhaustive inquiries would be available in wide-spread epidemiological surveys.

## SUMMARY

1. Cultures of tubercle bacilli sensitive to isoniazid and streptomycin were obtained from 20 South Indian patients before and at three months after the start of chemotherapy with isoniazid plus PAS or with isoniazid alone. These cultures were tested for their virulence in the guinea-pig.

2. Statistically significant variation was found between patients in the mean virulence of their cultures.

3. No significant difference in virulence was found between the pretreatment cultures and the cultures obtained at the end of three months' chemotherapy.

4. The variation in virulence between duplicate cultures from the same patient was no greater than the natural variation in response of the guinea-pigs.

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## THE ROLE OF DIET IN THE TREATMENT OF PULMONARY TUBERCULOSIS\*†

### AN EVALUATION IN A CONTROLLED CHEMOTHERAPY STUDY IN HOME AND SANATORIUM PATIENTS IN SOUTH INDIA

C. V. RAMAKRISHNAN, KANTHI RAJENDRAN, P. GEORGE JACOB, WALLACE FOX AND  
S. RADHAKRISHNA

Before the advent of antituberculosis chemotherapy, a diet rich in calories, proteins, fats, minerals and vitamins was generally considered to be an important, if not essential, factor in the treatment of tuberculosis. The introduction of specific antituberculosis drugs, however, has so radically altered the management of the disease that the role of diet has to be reconsidered in the light of the recent advances in treatment. An evaluation of the influence of diet in the treatment of pulmonary tuberculosis with isoniazid plus *p*-aminosalicylic acid was recently undertaken by the Tuberculosis Chemotherapy Centre, Madras, in the course of a controlled comparison of home and sanatorium chemotherapy for tuberculous patients from a poverty-stricken community in Madras City. Despite the fact that during the year of treatment the home patients subsisted on a markedly poorer diet, were physically more active and, on the average, gained less weight than the sanatorium patients, the overall response to treatment in the home series closely approached that in the sanatorium series, although there was a tendency for tubercle bacilli to disappear earlier in the latter. Direct evidence has been presented that none of the dietary factors studied (calories, carbohydrates, total and animal proteins, fats, minerals and vitamins) appears to influence the attainment of quiescent disease among tuberculous patients treated for one year with an effective combination of antimicrobial drugs, and that initial chemotherapy of patients at home can be successful even if the dietary intake is low throughout the period of treatment.

The role of diet in the treatment of tuberculosis, both in human beings and in experimental animals, has been the subject of a large number of papers, and many standard works recommend a diet rich in calories, proteins, fats, minerals and vitamins in therapy (McLester and Darby, 1952; Pagel, Simmonds and Macdonald, 1953; Hudson, 1957; Davidson, Meikle-John and Passmore, 1959). The opportunity has been taken during a controlled chemotherapy study of tuberculous patients conducted by the Tuberculosis Chemotherapy Centre, Madras, to re-evaluate the role of diet in treatment when an *effective* combination of antimicrobial drugs is being administered.

A dietary study was undertaken among patients with newly diagnosed pulmonary tuberculosis who were admitted to a comparison of home and sanatorium treatment for 12 months with a standard oral combination of anti-

tuberculosis drugs, isoniazid plus the sodium salt of *p*-aminosalicylic acid (sodium PAS) (Tuberculosis Chemotherapy Centre, 1959).

The patients in this study were drawn from the lower income groups or from the unemployed in Madras City, which is the largest urban community in South India. Their living conditions were, with few exceptions, poor. The patients were allocated at random to treatment at home or in sanatorium and all were prescribed the same chemotherapy. The diet of the patients in the home series depended on their economic status and their individual tastes. On the other hand, the sanatorium patients had a diet which conformed to standards laid down by the Madras Government for tuberculous patients and was occasionally supplemented by food obtained from outside the sanatorium.

There were, therefore, grounds for expecting a difference in the diet of the home and the sana-

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

† This paper is also published in the *Bulletin of the World Health Organization*.

torium series of patients during the year of treatment. The present report is an evaluation of the magnitude of this difference in the diet and its influence, in the presence of standard combined chemotherapy, on the course of the disease in the home and the sanatorium patients, as judged by the radiographic response and the attainment of bacteriological quiescence.

The Medical Research Council of Great Britain, through its Tuberculosis Research Unit, is responsible for the scientific direction of the research in accordance with the plans prepared by a Project Committee consisting of representatives of the co-operating agencies and the Senior Medical Officer of the Centre.

Dr C. Gopalan and Dr V. N. Patwardhan of the Nutrition Research Laboratories of the Indian Council of Medical Research gave valuable advice at every stage of the study; Miss Swaran Pasricha, their unit's nutritionist, assisted in the initiation of the dietary assessments. Dr P. V. George, Nutrition Officer in the Public Health Department, Madras Government, furnished data on the composition of a number of South Indian cooked dishes (personal communication, 1958). Professor B. S. Platt gave valuable advice during the planning stage of the study and with the manuscript. Dr Ian Sutherland of the Medical Research Council's Statistical Research Unit made many helpful suggestions throughout the study.

## I. General Plan and Conduct of the study

### PATIENTS IN THE PRESENT ANALYSIS

Between September 1956 and September 1957, 193 patients were allocated at random to treatment at home or in sanatorium; all of them were aged 12 years or more and had newly diagnosed disease with tubercle bacilli in their sputum, and the majority had advanced cavitated lesions and were clinically ill on admission to treatment (Tuberculosis Chemotherapy Centre, 1959). Of these, 30 were excluded from the main analysis for reasons detailed in the 1959 report, leaving 163 patients (82 home, 81 sanatorium) with initially isoniazid-sensitive organisms, in the main comparison. Of these 163 patients, six (three home, three sanatorium) were excluded from the dietary study because complete data were not available. Three (one home, two sanatorium) died before any dietary assessments could be made. A fourth (home)

was electrocuted at work before an assessment during treatment could be undertaken, and one patient (home) had dysphagia due to carcinoma of the oesophagus, restricting his dietary intake. Another patient (sanatorium) was discharged before the assessment of his diet in sanatorium could be made. There remain 157 patients for the dietary study, of whom 79 (46 males, 33 females) were treated at home and 78 (48 males, 30 females) in sanatorium. Of the 157 patients, seven (all home) were strict vegetarians, who, quite apart from not eating meat or fish, did not even have eggs in their diet, although they drank milk.

### THE USUAL DIETARY

The usual diet of the income group from which the patients were drawn consists of a very light breakfast (often the water in which the rice is cooked, with perhaps a small quantity of rice) and two fuller meals (sometimes only one), one at noon and the other in the evening. The main meals consist largely of cooked rice, one of them, or sometimes both, having a small amount of green vegetables and pulses; on some occasions very small quantities of flesh foods (usually fish), fats and fruits are also eaten. Beverages, usually tea and occasionally coffee, are drunk two to four times a day, but with very little milk.

### METHODS AND PROCEDURES

Two methods of diet survey were employed:

- (a) The oral questionnaire method.
- (b) Weighment of uncooked and cooked foods.

The oral questionnaire was the standard procedure for the great majority of the assessments in this report. The weighment method was used in parallel with the oral questionnaire in a comparatively small number of the patients to confirm the reliability of the oral questionnaire results.

#### *Oral questionnaire*

The assessment of dietary intake by the oral questionnaire method was conducted as follows. The dietitian carefully interrogated the individual patients and, sometimes, other members of the family. The aim was to elicit precise information on the amounts of different articles of food consumed by the patient and the various members of the family in the course

of the day. Standard vessels and containers and measures of graded sizes were shown to the patients to help them express the amounts of various articles of diet consumed. This method was the same as the one used by the Nutrition Research Laboratories of the Indian Council of Medical Research (Pasricha, 1958, 1959) except that there was a slight modification in respect of the use of the adult equivalent adopted by Aykroyd, Patwardhan and Ranganathan, (1951). It is usually possible to make a fairly accurate assessment of an individual's dietary intake by assessing the total quantity of food consumed by the whole family and, on the basis of adult equivalents, calculating the proportion of food consumed by the individual. In the present study, however, most of the patients were seriously ill when they started treatment (Tuberculosis Chemotherapy Centre, 1959) and had little or no appetite, so that they were consuming very much less than their normal intake of food. In such circumstances, the adult equivalent gives misleadingly high values and the patients were therefore asked to express, with the aid of standard vessels, the actual quantities of food they ate. The adult equivalent was applied only for items like oil, dhal (pulses) and sugar, where it is difficult to measure the actual quantity consumed. As reported elsewhere (Tuberculosis Chemotherapy Centre, 1959), the Centre distributed small quantities of milk powder to the families of patients under treatment for consumption by the whole family. The quantity of milk powder consumed by the patient was calculated, on the basis of adult equivalents, from the total quantity distributed to the family for the month. Special articles of diet consumed by the patient alone—for example, eggs or fruits—were also taken into consideration in calculating the dietary intake of the patient. In order to make valid comparisons of the diet before and during treatment, the above procedure was adopted for all the dietary assessments during the year.

The dietary surveys were performed at the Centre, in the patients' homes or in the sanatorium. Because of the severely limited family budgets and the consequently almost unvarying diet, the patients in both series were able to state with confidence the quantity of food consumed by them when at home; it was only occasionally necessary to consult other family members. There is no doubt that the good relationship established between the patients'

families and the Centre's staff prior to the commencement of the dietary study greatly facilitated the dietitian's assessments. In sanatorium also, the diet was a standard one, and again the patients had no difficulty in describing it.

#### *Weighment*

The weighment method of dietary assessment involved the actual weighing of foods, both in their raw, uncooked state and again after cooking. This technique was used for an assessment at the end of three months of treatment in a group of patients studied intensively (see page 53 *et seq.*). The weighment technique had to be applied differently in the home and the sanatorium series.

*Weighment at home.* The weighment assessment was made on three consecutive days, domestic scales being used. The dietitian visited the home by appointment twice a day, once in the morning to weigh the raw foods and the second time to weigh the total food cooked for the family and to assess the patient's share of it. The following instructions were given to the patients and their families prior to the visits:

(a) All purchases for the day were to be made early in the morning or on the previous evening and shown to the dietitian at her morning visit.

(b) No food was to be included in the diet if it had not been shown to the dietitian in the morning when the raw food was weighed.

(c) The family was not to eat the cooked food before it was weighed at the second visit.

(d) For convenience in weighment, the family was asked to use the same vessels on all the three days.

(e) The family was instructed not to make any extra or unusual purchases of food.

Raw foods, such as rice, dhal, vegetables, meat, sugar and jaggery (coarse brown sugar), were weighed. (Tea leaves, condiments and the like were not taken into account). The mean dietary intake for the family over the three days was first calculated from the raw foodstuffs. Next, the amount of cooked food consumed by the patient during this period was expressed as a proportion of the total food consumed by the family. By applying this proportion to the mean dietary intake for the family based on the raw foodstuffs, the patient's

dietary intake was calculated. It was necessary to adopt this procedure because food values were available only for raw foodstuffs.

There were 22 patients in the home series whose diets were assessed by the weighment technique (see below). The wage-earners were casual labourers receiving daily wages in 14 of the 22 families and were paid monthly in eight. Four of these eight monthly wage-earners were patients and therefore not in employment when the weighment assessment was made. Thus, the majority of patients were in families who did not have a lump sum available once a month to alter the standard of their diet in the days immediately after its receipt. In the remaining four patients the weighment assessment was commenced six, 11, 14 and 29 days, respectively, after the payment of the monthly wage.

*Weighment in sanatorium.* In sanatorium, the cooked foods actually consumed by the patients were weighed, the equivalents in terms of raw food were determined, and the dietary values were calculated. For items such as oil, the mean quantity consumed per sanatorium patient was taken. As the diet was a standard one, a 1-day assessment was considered adequate. Food supplements, such as coffee and snacks from the sanatorium canteen, or food brought by relatives from home, were also taken into account.

COMPARISON OF ORAL QUESTIONNAIRE AND WEIGHMENT TECHNIQUES

In order to verify whether the oral questionnaires were yielding reliable data for the patients under study, a comparison was made of the oral questionnaire and weighment techniques in the last 45 patients (22 home, 23 sanatorium) in the main analysis of the chemotherapy study. The comparison was made three months after admission to treatment, the oral questionnaire being conducted on the day before the weighment survey was started. The results are presented in Table 1. The distributions of calories, total proteins, animal protein, fats and carbohydrates by the two methods were closely similar both for the home and for the sanatorium series. Thus, 13 patients in the home series had a daily intake of 2000 calories or more when assessed by the oral questionnaire method, as compared with 12 patients by the weighment technique; the corresponding figures for the sanatorium patients were 21 and 22, respectively.

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TABLE I  
Comparison of oral questionnaire and weighment methods in the assessment of dietary intake at three months

		Home		Sanatorium	
		Oral	Weigh-ment	Oral	Weigh-ment
Calories	Less than 1,000	1	1	0	0
	1,000-	1	0	0	0
	1,200-	3	4	0	0
	1,400-	2	3	1	0
	1,600-	1	1	0	0
	1,800-	1	1	1	1
	2,000-	2	1	2	0
	2,200-	6	6	17	20
	2,600-	3	2	2	2
	3,000 or more	2	3	0	0
Total proteins (g)	0-	0	0	0	0
	10-	1	0	0	0
	20-	0	2	0	0
	30-	6	6	0	0
	40-	2	2	1	0
	50-	8	7	0	1
	60-	2	1	14	9
	70-	1	0	8	12
80 or more	2	4	0	1	
Animal proteins (g)	0-	9	10	0	0
	10-	10	8	0	0
	20-	1	2	1	0
	30-	0	0	18	20
	40-	2	2	4	3
50 or more	0	0	0	0	
Carbo-hydrates (g)	Less than 100	0	0	0	0
	100-	1	1	0	0
	200-	4	6	2	0
	300-	3	3	14	12
	400-	8	8	7	11
	500-	5	2	0	0
600 or more	1	2	0	0	
Fats (g)	0-	4	6	0	0
	10-	8	7	0	0
	20-	7	4	0	0
	30-	1	2	1	1
	40-	0	1	5	4
	50-	0	0	5	9
60 or more	2	2	12	9	
Total patients		22	22	23	23

Considering patients with an intake of at least 50 g. of total proteins, the numbers in the home series were 13 by the oral questionnaire and 12 by the weighment method, and the corresponding figures in the sanatorium series were 22 and 23, respectively. There was also a general similarity in the findings in respect of the animal protein, carbohydrates and fats. It may be concluded that the oral questionnaire method, which was simple and not so time-consuming as the weighment technique, was just as reliable. This observation is in general agreement with the findings reported by Venkatachalam, Srikantiah and Gopalan (1954) and Pasricha (1959). It was decided, in the light of the comparison, that there was no need to undertake further weighment assessments in this study.

#### THE TWO GROUPS FOR DIETARY ASSESSMENT

For the purpose of the dietary assessments, the 157 patients in this report were studied in two main groups:

- (a) the less intensively investigated group of 112 patients (57 home, 55 sanatorium);
- (b) the intensively investigated group of 45 patients (22 home, 23 sanatorium).

The patients in the less intensively investigated group were assessed by the oral questionnaire method at some time during the second six months of treatment. On that occasion, two assessments were undertaken for each patient, the first being a retrospective assessment of the dietary intake immediately before admission to treatment, and the second an assessment of the patient's current diet. The pretreatment assessments, being retrospective and, hence, dependent on the patient's memory, are probably less reliable than the current assessments. Nevertheless, they permit valid comparisons between the home and the sanatorium series.

In the intensively investigated group the oral questionnaire was undertaken at six *set* dates—namely, on admission to treatment, and at six weeks, three months, six months, nine months and one year after the start of treatment; *none* of the assessments in this group was retrospective. In addition, a weighment assessment was undertaken at three months.

In cases of gross exaggeration or under-estimation of the dietary intake which were not compatible with the family's spending capacity, the diet survey was repeated. If the patients

were suffering from an illness other than tuberculosis that interfered with their usual intake of food, the dietary assessments were postponed.

## II. Results of Oral Questionnaire Survey

This section presents the distributions of dietary intake (*a*) both before treatment and during the second six months of treatment, for all the patients—that is, for the 112 (57 home, 55 sanatorium) patients in the less intensively investigated group and the 45 (22 home, 23 sanatorium) in the intensively investigated group combined (for the latter group, the results included in the tabulations are those of the pretreatment assessment and the assessment at nine months)—and (*b*) at the six *set* examinations, for the intensively investigated group.

#### RESULTS IN THE TWO GROUPS COMBINED

##### *Total calories*

Before the start of treatment the daily calorie intake was low in the majority of patients in both series (Table 2). Thus, 71 per cent of the 79 home and 68 per cent of the 78 sanatorium patients had a total daily intake of less than 1800 calories, 22 per cent in each series having less than 1000 calories. At the assessment during treatment the intake had increased in both series, but to a greater extent in the sanatorium patients, 58 per cent of the home as compared with 99 per cent of the sanatorium patients having an intake of more than 1800 calories a day. However, 11 (14 per cent) patients at home and only one in sanatorium claimed a daily intake of 3000 calories or more.

##### *Proteins*

Before the start of treatment the total daily protein intake was less than 50 g. in 80 per cent of the home and 79 per cent of the sanatorium patients (Table 3). There were seven patients (five home, two sanatorium) who had an intake of less than 10 g., the intake being 5-9 g. in six and 1.5 g. in the seventh. These patients all had severe toxæmia. (Six had an intake of less than 500 calories and the seventh 514 calories a day.) At the assessment during treatment 47 per cent of the home patients and all (100 per cent) of the sanatorium patients had a total protein intake of more than 50 g. The animal-protein data have not been tabulated

TABLE 2  
Total calorie intake: Assessments before treatment and during treatment\*

Calories	Before treatment		During treatment	
	Home	Sana- torium	Home	Sana- torium
	No. %	No. %	No. %	No. %
Less than 1,000	17 22	17 22	4 5	0 0
1,000-	10 13	5 6	3 4	0 0
1,200-	18 23	13 77	4 5	0 0
1,400-	9 11	5 6	13 16	0 0
1,600-	2 3	13 77	9 77	1 1
1,800-	5 6	11 74	6 8	2 5
2,000-	4 5	3 4	6 5	14 75
2,200-	10 13	7 9	13 79	49 63
2,600-	2 3	0 0	8 10	11 74
3,000 or more	2 3	4 5	11 74	1 7
Total patients	79 102	78 700	79 700	78 700

\* Assessed in the second six months of treatment.

here, but during treatment six (8 per cent) of the home and all of the sanatorium patients had a daily intake of 30 g. or more of animal protein; the majority of the home series—namely, 61 (77 per cent)—had an intake of less than 20 g. a day, and 27 (34 per cent) had an intake of less than 10 g.

*Fats*

The great majority of patients—namely, 72 (91 per cent) of the home and 65 (83 per cent) of the sanatorium series—had an intake of less than 40 g. of fat daily before treatment, the intake being less than 20 g. in 59 per cent of the home and in 41 per cent of the sanatorium patients. During treatment the intake of fat in the home series remained practically unaltered, but in sanatorium it increased to a marked degree. Thus, only nine (11 per cent) of the home as compared with all (100 per cent) of the sanatorium patients had an intake of more than 40 g. at the assessment during treatment, 59 per cent of the latter having an intake of 60 g. or more of fat per day. The results have not been tabulated here.

*Carbohydrates*

The pretreatment daily intake of carbohydrates (not tabulated here) in both series was

similar and in the majority of patients—namely, 81 per cent of the home and 83 per cent of the sanatorium series—it was less than 400 g., 27 per cent and 26 per cent, respectively, having an intake of less than 200 g. During treatment a greater proportion of the home patients (48 per cent) than the sanatorium patients (28 per cent) had an intake of 400 g. or more. Whereas 12 patients in the home series consumed over 600 g. of carbohydrates a day, none of the sanatorium patients had so large an intake. On the other hand, 18 per cent of the home patients and only 1 per cent of the sanatorium patients had a daily intake of less than 300 g. In summary, though the mean intake of carbohydrates was the same in both series, there was greater variation among the home patients, for whom carbohydrates, in the form of rice, were the major article of diet.

*Minerals and vitamins*

In view of the similarity in the pretreatment diet of the two series of patients in terms of total calories, proteins, fats and carbohydrates, the mineral and vitamin contents of the diet before the start of treatment were not calculated. However, the intakes of minerals—namely,

TABLE 3  
Total protein intake: Assessments before treatment and during treatment\*

Proteins (g)	Before treatment		During treatment	
	Home	Sana- torium	Home	Sana- torium
	No. %	No. %	No. %	No. %
0-	0 0	1 7	0 0	0 0
5-	5 6	1 1	0 0	0 0
10-	2 3	1 7	0 0	0 0
15-	8 10	4 5	1 1	0 0
20-	8 70	7 9	2 3	0 0
25-	10 13	9 72	3 4	0 0
30-	19 24	21 27	18 23	0 0
40-	11 14	18 23	18 23	0 0
50-	12 15	6 8	13 16	3 4
60-	1 1	3 4	13 16	23 20
70-	2 3	3 4	4 5	32 41
80 or more	1 1	4 5	7 9	20 26
Total patients	79 100	78 100	79 100	78 100.

\* Assessed in the second six months of treatment.

TABLE 4  
Total mineral intake: Assessment during treatment\*

		Home		Sanatorium	
		No.	%	No.	%
Calcium (g)	Less than 0.5	22	28	0	0
	0.5-1.0	39	49	1	1
	1.0-1.5	14	18	54	69
	1.5-2.0	4	5	22	28
	2.0 to 2.5	0	0	1	1
Phosphorus (g)	Less than 0.5	4	5	0	0
	0.5-1.0	22	28	0	0
	1.0-1.5	38	48	10	13
	1.5-2.0	14	18	53	68
	2.0 to 2.5	1	1	15	19
Iron (mg)	Less than 5	1	1	0	0
	5-10	2	3	0	0
	10-15	21	27	3	4
	15-20	15	19	36	46
	20-25	11	14	38	49
	25-30	16	20	1	1
	30-35	10	13	0	0
	35-40	0	0	0	0
	40-45 or more	0	0	0	0
Total patients ...	79	100	78	100	

\* Assessed in the second six months of treatment.

calcium, phosphorus and iron (Table 4)—and of vitamin A, carotene, thiamine, riboflavine, nicotinic acid and ascorbic acid during treatment were calculated to see whether differences existed between the two series. The calculations are based on the mineral and vitamin contents of uncooked foodstuffs and, as such, the results do not take into account losses in cooking.

**Calcium.** Among the home patients, 77 per cent had a daily dietary intake of less than 1 g. of calcium, as compared with 1 per cent of the sanatorium patients, 28 per cent of the home patients receiving less than 0.5 g. of calcium a day (Table 4).

In South India, betel leaves, to which slaked lime has been added, are commonly chewed, and this habit adds to the daily intake of calcium. Before their admission to treatment 63 (79 per cent) of the patients in the home series

and 48 (62 per cent) of those in the sanatorium series chewed betel leaves. After admission to treatment it was easier for the patients in the home series to continue the habit, which was forbidden in sanatorium although the restriction was not rigidly enforced. This additional source of calcium has not been taken into account in the calculations.

**Phosphorus.** In the home series only 67 per cent of the patients had an intake of 1 g. or more of phosphorus (Table 4) daily, as compared with all (100 per cent) of those in the sanatorium series.

**Iron.** Considering the intake of iron (Table 4), the means were the same though the distributions were widely different. Thus, 30 per cent of the home series received less than 15 mg. daily, as compared with 4 per cent in the sanatorium series, and at the other extreme, 37 per cent of the home patients and 1 per cent of the sanatorium patients had an intake of 25 mg. or more. The great majority of the sanatorium patients—namely, 95 per cent—had a daily intake of 15-24 mg. of iron in their diet.

**Vitamin A.** While 96 per cent of the 79 home patients had less than 1000 International Units (IU) of vitamin A in their diet daily, all (100 per cent) of the sanatorium patients had an intake of more than 1000 IU and 68 per cent had an intake of 2000 IU or more.

**Carotene.** Of the home patients, 37 (47 per cent) were receiving less than 600 IU of carotene daily, as compared with one (1 per cent) of the sanatorium patients. At the other end of the scale, four (5 per cent) of the home and 10 (13 per cent) of the sanatorium patients had a daily intake of 2000 IU or more.

**Thiamine.** Of the home patients, 38 (48 per cent) had an intake of less than 400 IU of thiamine, as compared with none (0 per cent) of the sanatorium patients. In all, 13 (17 per cent) of the home and 54 (69 per cent) of the sanatorium patients had intakes of 600 IU or more.

**Riboflavine.** Whereas 69 (87 per cent) of the home patients received less than 500 µg. of riboflavine daily, none of the sanatorium patients had such a low intake, 76 (97 per cent) having 1500 µg. or more daily.

**Nicotinic acid.** Although the mean intakes were very similar for the two series, 22 (28

TABLE 5

Serial assessments of the total calorie intake during the 12 months

Calories	Home						Sanatorium					
	Before treatment	6 weeks	3 months	6 months	9 months	12 months	Before treatment	6 weeks	3 months	6 months	9 months	12 months
Less than 1,000	4	0	1	0	0	0	6	0	0	0	0	0
1,000-	3	1	1	1	2	1	2	0	0	0	0	0
1,200-	5	3	3	6	1	0	3	0	0	0	0	0
1,400-	4	2	2	2	6	5	4	0	1	0	0	0
1,600-	0	4	1	1	0	1	4	1	0	0	0	0
1,800-	0	3	1	0	1	1	3	2	1	0	0	0
2,000-	0	2	2	3	0	3	1	7	2	1	2	3
2,200-	6	1	6	6	5	5	0	11	17	17	18	14
2,600-	0	3	3	2	4	2	0	2	2	4	3	6
3,000 or more	0	3	2	1	3	4	0	0	0	1	0	0
Total patients ...	22	22	22	22	22	22	23	23	23	23	23	23

per cent) of the patients in the home series had an intake of less than 15 mg. of nicotinic acid a day, as compared with five (6 per cent) of those in the sanatorium series. At the other extreme, 14 (18 per cent) of the home but none (0 per cent) of the sanatorium patients had an intake of 25 mg. or more daily.

*Ascorbic acid.* At home, 20 (25 per cent) of the patients had an intake of less than 25 mg. of ascorbic acid a day, as compared with four (5 per cent) in sanatorium. The majority of patients in both series—namely, 67 (85 per cent) of the home and 67 (86 per cent) of the sanatorium patients—had intakes of less than 100 mg. a day.

It may be concluded, on the basis of the above findings, that the dietary intakes were similar in the two series before the start of treatment. Once treatment had begun, however, patients admitted to sanatorium received a clearly superior diet. The differences in the mean intake of calories, total proteins, animal proteins, fats, calcium, phosphorus, vitamin A, thiamine and riboflavin all attained statistical significance at the 0.1 per cent level. While there was little difference in the mean intake of carbohydrates between the two series, there was a greater variation among the home patients.

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RESULTS IN THE INTENSIVELY INVESTIGATED GROUP

A more detailed study of the trend of dietary intake over the 12-month period was possible in the intensively investigated group of 45 patients (22 home, 23 sanatorium). The findings for calories, total proteins, animal proteins, fats and carbohydrates are set out below.

*Total calories*

Considering the calorie intake (Table 5), the pretreatment distributions for the two series were broadly similar, 16 patients in the home and 15 in the sanatorium series receiving less than 1,600 calories daily. At six weeks the calorie intake had increased in both series, but to a greater extent in the sanatorium series; six home patients but no sanatorium patient still had an intake of less than 1,600 calories. At three months there was evidence of a further, though slight, increase in calories in both series. The assessments at six, nine and 12 months showed little further change, and the greater calorie intake in the sanatorium patients was maintained.

*Total proteins*

The six assessments of total protein intake made in the 12-month period are set out in

TABLE 6  
Serial assessments of the total protein intake during the 12 months

Proteins (g)	Home						Sanatorium					
	Before treatment	6 weeks	3 months	6 months	9 months	12 months	Before treatment	6 weeks	3 months	6 months	9 months	12 months
0-	1	0	0	0	0	0	0	0	0	0	0	0
10-	0	0	1	0	0	0	1	0	0	0	0	0
20-	6	0	0	3	2	0	5	0	0	0	0	0
30-	7	9	6	4	7	6	9	0	0	0	0	0
40-	2	4	2	4	2	4	6	0	1	0	0	0
50-	4	4	8	5	2	6	1	1	0	0	0	0
60-	1	2	2	4	4	1	1	17	14	1	1	2
70-	1	0	1	1	3	1	0	5	8	6	12	10
80 or more	0	3	2	1	2	4	0	0	0	16	10	11
Total patients	22	22	22	22	22	22	23	23	23	23	23	23

Table 6. In both series there was an increase in the intake at six weeks, as compared with the pretreatment findings, the increase being more marked in the sanatorium series. Thus, the total protein intake was 40 g. or more in eight of the home patients before treatment, as compared with 13 at six weeks. In the sanatorium series the corresponding figures were eight before treatment and 23 at six weeks. There was little change in the distribution in the home series at the rest of the assessments, but in the sanatorium series at six, nine and 12

months there were 22, 22 and 21 patients, respectively, with an intake of more than 70 g., as compared with five at six weeks and eight at three months.

#### Animal protein

The animal protein in the diet was low in both series before the start of treatment, 19 of the home and 17 of the sanatorium patients having an intake of less than 20 g. The intake remained low at all the assessments during treatment for the home series, 20 patients at six

TABLE 7  
Serial assessments of the total fat intake during the 12 months

Fats (g)	Home						Sanatorium					
	Before treatment	6 weeks	3 months	6 months	9 months	12 months	Before treatment	6 weeks	3 months	6 months	9 months	12 months
0-	4	5	4	3	6	6	2	0	0	0	0	0
10-	6	9	8	9	7	7	7	0	0	0	0	0
20-	9	4	7	6	4	2	5	0	0	0	0	0
30-	0	2	1	3	1	5	7	0	1	0	0	0
40-	2	0	0	0	2	0	1	5	5	1	0	0
50-	1	0	0	0	1	1	1	7	5	4	4	1
60 or more	0	2	2	1	1	1	0	11	12	18	19	22
Total patients	22	22	22	22	22	22	23	23	23	23	23	23

Weeks, 19 at three months, 18 at six months, 18 at nine months and 17 at 12 months still having an intake of less than 20 g. In contrast, no patient in the sanatorium series had so low an intake during treatment and all at six weeks and 22 at three months had an intake of 30 g. or more a day and the considerable majority—namely, 18, 19 and 19—received 50 g. or more at the assessments made at six, nine and 12 months, respectively. The data have not been tabulated here.

*Fats*

There was little change in the intake of fats among patients in the home series at any time in the year (Table 7). In the sanatorium series, however, there was a marked increase in intake at six weeks and a further increase in the last three assessments. Thus, no sanatorium patient had an intake of 60 g. or more before admission to treatment, as compared with 11 at six weeks, 18 at six months and 22 at 12 months.

*Carbohydrates*

An analysis of the carbohydrate intake (not tabulated here) showed an increase in both series in the first six weeks. Thus, 10 home patients had an intake of 300 g. or more a day before treatment, as compared with 17 at six weeks.

In the sanatorium series the corresponding figures were eight and 21. There was little change in the sanatorium series for the rest of the year, but in the home series rather more patients had large carbohydrate intakes (400 g. or more) at three months and subsequently than at six weeks. The analysis confirmed the finding in the less intensively investigated group that the home series had a wider range of carbohydrate intake than the sanatorium series.

In summary, the findings in the intensively investigated group of patients showed that the patients had very stable diets from three to 12 months and that the sanatorium series were at a clear-cut advantage throughout the year.

**III. Rest and Physical Activity**

The home patients were advised to rest for the first month or two and were then allowed, if their disease showed signs of improvement, to undertake light activity. The majority of the patients were ambulant much of the time and were quite often not at home when visits were paid by the staff. In sanatorium, very ill patients had absolute bed rest with bed-pan facilities. The less ill patients had their activity restricted, and throughout the 12 months the maximum time that the patients were officially

TABLE 8

*Assessment of the physical activity of the patients during the 12 months*

Physical activity	Home				Physical activity	Sanatorium			
	6 months		12 months			6 months		12 months	
	No.	%	No.	%		No.	%	No.	%
Resting ...	5	6	1		Complete bed rest	1	1	0	0
Slight ...	42	53	10	13	Up to toilet only	6	8	0	0
Part-time ...	29	37	34	44	Up 2 hours	62	81	66	85
Full-time ...	3	4	32	42	Up 4 hours		10	12	15
All patients	79	100	77 <sup>1</sup>	100	All patients	77 <sup>2</sup>	100	78	100

<sup>1</sup> Excluding, at 12 months only, two patients in whom chemotherapy was changed on account of serious radiographic deterioration at the 10th and 12th months respectively.

<sup>2</sup> Excluding, at six months only, one patient who was discharged from sanatorium at the time of assessment.

allowed to be out of bed was four hours a day. A fuller description of the management is given in an earlier report (Tuberculosis Chemotherapy Centre, 1959).

The physical activity of the patients at six and 12 months is set out in Table 8. While 41 per cent of the home patients were engaged in at least part-time activity at six months, only 10 per cent of the sanatorium patients were up for four hours a day. At 12 months the proportions were 86 per cent and 15 per cent, respectively, 42 per cent of the home patients being on full-time activity. It is difficult to compare the activity of the home and sanatorium patients, but it is considered that part-time activity in a home patient clearly represented more than the maximum permitted activity of a sanatorium patient. It may be concluded that the home patients were physically much more active during the 12 months than the patients in sanatorium. This greater physical activity in the home patients increases their dietary disadvantage.

#### IV. Income of the Home Families during the Year

Considering an adult male (15 years or over) as being equivalent to one standard unit, an adult female (15 years or over) as 0.8 standard unit, and a child under the age of 15 years as 0.6 standard unit (India, Ministry of Commerce, 1949), the average monthly income per standard unit over the 12 months was calculated for the families of home patients. Thus expressed, 87 per cent of the families had less than Rs 30<sup>1</sup> per standard unit per month, 68 per cent having less than Rs 20. The figure varied from Rs 2.54 to Rs 40.50, the average for all home families being Rs 17.20.

An indication of the purchasing power of the income per standard unit is given by the sum that was spent by the Madras Government on food for the patients in the sanatorium series. Rs 1.84 daily was spent for the non-vegetarian patients and Rs 1.66 daily for the vegetarian patients—that is, Rs 55.20 for a 30-day month for the non-vegetarians and Rs 49.80 for the vegetarians. These sums are based on bulk purchases of food for an institution with over 600 patients, so that it is very unlikely that a patient treated at home would be able to pur-

chase the same with an identical daily expenditure. Also, Chaudhuri (1959), when referring to the current cost of living in India, wrote: 'At present price levels, a balanced diet for one adult costs at least Rs 1.50 a day (Rs 45.00 a month); and the minimum total requirement, including food and clothing, is about Rs 60-70 per month.' It may therefore be concluded that the economic status of the home families was poor during the year.

#### V. Response to Treatment of the Home and the Sanatorium Series

A detailed analysis in an earlier report (Tuberculosis Chemotherapy Centre, 1959) showed that the response to treatment of the patients at home closely approached that of the patients in sanatorium. Because the patients under treatment at home had, on the average, more extensive disease on admission to the study, statistical standardization of the results was undertaken, to allow for the pretreatment differences. For the full details the reader is referred to the earlier (1959) report. In brief, measures of the radiographic and the bacteriological response were standardized for pretreatment differences in (a) the extent of cavitation, (b) the number of lung zones involved in disease and (c) the extent of cavitation and the lung-zone involvement combined; these factors were selected because they were found to be of major prognostic importance (Tuberculosis Chemotherapy Centre, 1959), and because the two series differed in both respects at the start of treatment. Whereas in the earlier report the statistical standardization was based on 163 patients, the percentages in Table 9, referred to below, are based on the 157 patients in the present analysis.

##### RADIOGRAPHIC RESPONSE

Considering the radiographic response first, the important findings are set out in Table 9 (first part). For the period 0-6 months, the unstandardized percentages for patients showing considerable or exceptional radiographic improvement were 30.8 for the home series and 38.5 for the sanatorium series, the former being 80 per cent of the latter. When allowance was made for the pretreatment differences in

<sup>1</sup>Rs 4.80 = US\$ 1.00.

TABLE 9

Percentages of patients showing favourable radiographic and bacteriological response, standardised for pretreatment differences in extent of cavitation and lung-gone involvement

Radiographic response					Bacteriological response					
Nature of response		Percentages standardized for pretreatment differences in:		Home as % of san.	Nature of response		Percentage standardized for pretreatment differences in:		Home as % of san.	
		(Unstandardized)	Home	San.			(Unstandardized)	Home	San.	
Percentage of patients with considerable or exceptional radiographic improvement	0-6 months	Extent of cavitation	30.8	38.5	Percentage of patients with negative culture on a single collection specimen	0-6 months	Extent of cavitation	87.2	97.3	90
		Lung-zone involvement	32.8	36.2			91	91		
	Cavitation and lung zones	31.5	38.7	82		91				
		31.8	35.9	89		93				
0-12 months	(Unstandardized)	Extent of cavitation	41.8	52.6	0-12 months	(Unstandardized)	Extent of cavitation	87.0	93.4	93
		Lung-zone involvement	42.5	51.0			83	96		
	Cavitation and lung zones	43.3	53.1	82		95				
		44.0	49.6	89		97				
Percentage of patients with before treatment in whom it disappeared	At 12 months	(Unstandardized) Extent of cavitation	37.1	54.9	Percentage of patients no longer with active disease bacteriologically	At 12 months	(Unstandardized) Extent of cavitation	83.5	92.3	90
		Lung-zone involvement	42.6	50.6			84	93		
	Cavitation and lung zones	41.5	53.2	78		93				
		43.3	49.8	87		94				

the extent of cavitation between the series, the standardized percentages were 32.8 and 36.2, respectively, the former being 91 per cent of the latter. Standardization for lung-zone involvement also slightly reduced the difference between the series, the response in patients at home becoming 82 per cent of that in the sanatorium patients. When allowance was made simultaneously for both the pretreatment differences, the standardized percentages were 31.8 for the home patients and 35.9 for the sanatorium patients, the former being 89 per cent of the latter. Considering the period 0-12 months, the percentages of patients showing considerable or exceptional radiographic improvement were 41.8 for the home series and 52.6 for the sanatorium series, the former being 79 per cent of the latter. The contrast between these percentages was reduced by standardization for both cavitation and lung-zone involve-

ment; thus, the standardized percentages were 44.0 for the home and 49.6 for the sanatorium patients, the former being 89 per cent of the latter. Standardization for both pretreatment factors considerably reduced the difference between the home and the sanatorium series in respect of cavity closure for the 12-month period, the standardized percentages being 43.3 for the home series and 49.8 for the sanatorium series, the former being 87 per cent of the latter.

**BACTERIOLOGICAL RESPONSE**

The bacteriological response is considered in Table 9 (second part). The effect of allowing for the pretreatment differences in extent of cavitation and lung-zone involvement was a slight reduction in the contrast between the results for the home and the sanatorium patients. This applies to all three measures of response—

TABLE 10  
*Distribution of first month of persisting culture negativity in patients with bacteriologically quiescent disease at one year*

First month of persisting culture negativity	Home patients		Sanatorium patients	
	No.	%	No.	%
1	9	15	12	17
2	9	15	19	27
3	15	25	19	27
4	17	29	16	23
5	2	75	1	6
6	1		2	
7	2		0	
8	1		1	
9	2		0	
10	1		0	
Total ...	59	99	70	100

namely, the percentage of patients with a negative culture on a single collection specimen at six months and at 12 months and the percentage of patients no longer having bacteriologically active disease at 12 months. Thus, for example, the unstandardized percentage of patients with a negative culture at six months was 87.2 for the home and 97.3 for the sanatorium patients, whereas after standardization for both extent of cavitation and lung-zone involvement, the percentages were 90.4 and 97.0, the former being 93 per cent of the latter. At 12 months, the same percentage, standardized both for cavitation and lung-zone involvement, was 89.4 for the home and 92.2 for the sanatorium patients, the former being 97 per cent of the latter. Considering patients who no longer had bacteriologically active disease at 12 months, the standardized percentage (86.1) for the home patients was 94 per cent of that for the sanatorium patients (92.0).

A corresponding analysis (not tabulated here), undertaken to standardize both for extent of cavitation and for bacterial content of sputum, led to very similar conclusions.

Thus, when allowance was made, so far as was possible, for some of the important pre-treatment differences, the difference in the disease status of the home and the sanatorium patients at the end of one year was 'small. However, there was evidence that the patients

in the sanatorium series attained bacteriological quiescence more rapidly than those in the home series (Table 10). Thus, 50 (71 per cent) of the 70 sanatorium patients who attained bacteriological quiescence had shown sputum conversion by three months, as compared with 33 (56 per cent) of 59 home patients ( $P=0.1$ ). This difference was practically unchanged when standardization was undertaken for the pre-treatment differences in the extent of cavitation and the number of lung zones involved in disease.

## VI. Weight changes in the 12-month period

Gain in weight is a time-honoured method of assessing clinical improvement in patients with active tuberculosis. It is, therefore, of particular interest to compare the gains in weight in the two treatment series. (The mean weights on admission to treatment were 88, 88, 70 and 75 lb., respectively, for the home males, sanatorium males, home females and sanatorium females.) The findings are set out in Table 11 and in the accompanying figure. It will be seen that the males and females in both series gained, on the average, a considerable amount of weight, the gains being consistently greater in the sanatorium patients. A clear difference was apparent between the home and the sanatorium series from the second month onwards in respect of the males, and from the first month onwards in respect of the females. By six months, the home males had gained, on the average, 9.7 lb. while those in sanatorium had gained 15.4 lb.; the corresponding gains for the females were 11.3 lb. and 17.3 lb., respectively. The differences, both for the males and for the females, attain statistical significance at the 1 per cent level. There was little change in the average weight of the home patients in the second six months, but the sanatorium patients continued to gain weight, especially the females, who gained a further 6.9 lb. on the average. This continuing gain in weight can be clearly seen in the figure. It may be concluded that the sanatorium patients, both male and female, gained considerably more weight than the home patients. However, as already demonstrated in the previous section, this did not result in over-all superior clinical results at the end of the year in the sanatorium series.

TABLE 11

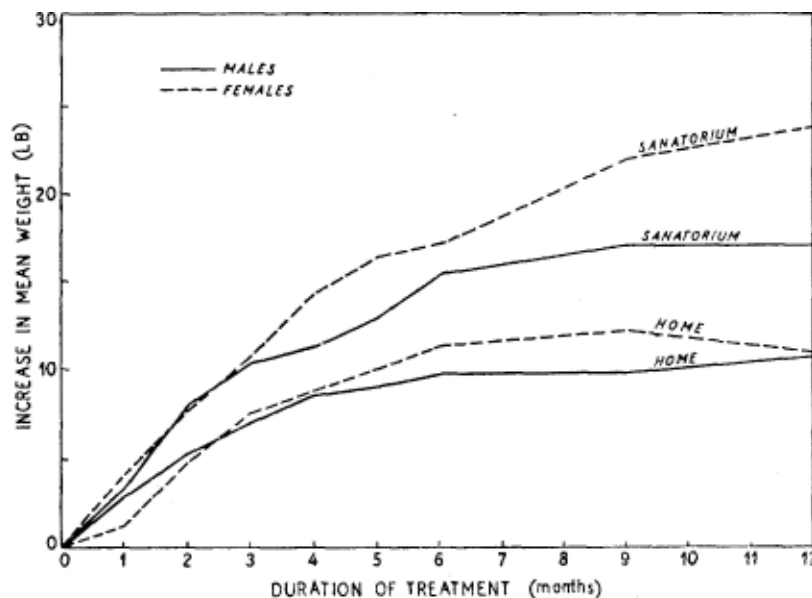
Weight changes in the 12-month period, according to sex of patients and place of treatment

Period	Males				Females			
	Home		Sanatorium		Home <sup>1</sup>		Sanatorium	
	Total patients weighed	Increase in mean weight (lb. <sup>2</sup> )	Total patients weighed	Increase in mean weight (lb.)	Total patients weighed	Increase in mean weight (lb.)	Total patients weighed	Increase in mean weight (lb.)
0-1 month	46	2.9	48	3.2	26	1.2	30	4.0
0-2 months	46	5.2	48	8.0	26	4.7	30	7.7
0-3 months	46	7.0	48	10.3	26	7.5	30	10.7
0-4 months	46	8.5	48	11.2	26	8.7	30	14.4
0-5 months	46	9.0	48	13.0	26	10.0	31	16.4
0-6 months	46	9.7	48	15.4	26	11.3	30	17.3
0-9 months	46	9.7	48	17.1	26	12.2	30	22.1
0-12 months	46	10.7	43	17.1	26	11.0	30	24.2

<sup>1</sup> Excluding seven patients who became pregnant during the 12-month period.

<sup>2</sup> 1 lb. = 0.45 kg.

Weight changes in the 12-month period, according to sex of patients and place of treatment



Further analyses were undertaken to investigate whether the patients who gained considerable amounts of weight had also responded better than the patients who gained less weight or who failed to gain weight. The findings are set out in Tables 12 and 13.

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WEIGHT GAIN IN RELATION TO RADIOGRAPHIC RESPONSE

Considering favourable radiographic response (Table 12), defined as moderate, considerable or exceptional improvement (assessed by

TABLE 12  
Favourable radiographic response at 12 months, related to weight change over the 12 months<sup>1</sup>

Weight change (0-12 months)	Home			Sanatorium		
	Number of patients	Patients with favourable radiographic response		Number of patients	Patients with favourable radiographic response	
		No.	%		No.	%
Loss or no change	4	0	(0) <sup>2</sup>	0	...	...
<i>Gain of:</i>						
less than 7 lb. <sup>3</sup> ...	22	15	(68)	5	5	(83)
7-13 lb. ...	20	16	(80)	6	16	(100)
14-20 lb. ...	18	14	(78)	22	19	(86)
21 lb. or more ...	8	6	(75)	34	30	88
Total patients ...	72	51	71	78	70	90

<sup>1</sup> Excluding seven home patients who became pregnant during the 12-month period.

<sup>2</sup> The parentheses indicate percentages based on fewer than 25 observations.

<sup>3</sup> 1 lb. = 0.45kg.

TABLE 13  
Bacteriologically active disease at 12 months, related to weight change over the 12 months<sup>1</sup>

Weight change (0-12 months)	Home			Sanatorium		
	Number of patients	Patients with bacteriologically active disease		Number of patients	Patients with bacteriologically active disease	
		No.	%		No.	%
Loss or no change	4	3	(75) <sup>2</sup>	0	...	...
<i>Gain of:</i>						
less than 7 lb. <sup>3</sup> ...	22	1	(5)	6	1	(17)
7-13 lb. ...	20	5	(25)	16	0	(0)
14-20 lb. ...	18	3	(17)	22	4	(18)
21 lb. or more ...	8	0	(0)	34	2	6
Total patients ...	72	12	17	78	7	9

<sup>1</sup> Excluding seven home patients who became pregnant during the 12-month period.

<sup>2</sup> The parentheses indicate percentages based on fewer than 25 observations

<sup>3</sup> 1 lb. = 0.45 kg.

an independent observer unaware of the treatment of any patient), 20 (77 per cent) of 26 patients, who gained at least 14 lb. during the year, showed a favourable radiographic response at 12 months, as compared with 80 per cent of 20 who gained 7-13 lb. and 68 per cent of 22 who gained less than 7 lb. There was, thus, no evidence of an association between gain in weight and favourable radiographic response. In the sanatorium series there was also no evidence of an association; 88 per cent of 34 patients who gained 21 lb. or more had a favourable radiographic response during the year, as compared with 86 per cent of 22 patients who gained 14-20 lb., 100 per cent of 16 who gained 7-13 lb. and 83 per cent of six who gained less than 7 lb. There were four patients in the home series who failed to gain weight, and none had had a favourable radiographic response, but this finding may well be due to a failure of some of the patients to gain weight *because* their disease was responding unfavourably. It cannot be assumed that unfavourable radiographic response is necessarily a direct consequence of a failure to gain weight.

#### WEIGHT GAIN IN RELATION TO BACTERIOLOGICAL RESPONSE

Considering bacteriologically active disease at twelve months (Table 13), three (12 per cent) of 26 patients in the home series who gained at least 14 lb. during the year had active disease, as compared with six (14 per cent) of 42 who gained 13 lb. or less. In the sanatorium series, the corresponding proportions were six (11 per cent) of 56 and one (5 per cent) of 22 patients. These figures provide no evidence that the larger gains in weight were associated with a favourable bacteriological response in either series. There were four patients in the home series who had failed to gain weight and three of these had bacteriologically active disease at 12 months, so that there was some evidence that failure to gain weight and a poor bacteriological response were associated. As with the radiographic findings, however, it cannot be assumed that the poor response was a consequence of the failure to gain weight.

#### VII. Response to Treatment in Relation to the Dietary Intake during the Year

The object of this section is to relate directly the level of the dietary intake during the year to two important measures of the response to

treatment—namely, radiographic improvement at 12 months, and the bacteriological assessment of the disease status at 12 months.

#### RADIOGRAPHIC RESPONSE AT 12 MONTHS

Table 14 relates various factors of the diet to favourable radiographic response at 12 months (moderate, considerable or exceptional improvement at an independent assessment). The findings for the home and the sanatorium patients are presented separately. Considering total calories first, there was no association between the dietary intake and the radiographic response. In the home series, 25 (76 per cent) of 33 patients with an intake of less than 1800 calories showed at least moderate improvement, as compared with 24 (71 per cent) of 34 patients with an intake of 2200 or more calories. In the sanatorium series, also, there was no difference between the responses of patients with low and high total calorie intakes. Considering total proteins, 75 per cent of 24 home patients with an intake of less than 40 g. a day responded well, as compared with 67 per cent of 24 receiving 60 g. or more a day. In the sanatorium patients, also, there was no association between protein intake and response; thus, 95 per cent of 58 patients receiving less than 80 g. a day showed at least moderate improvement, as compared with 75 per cent of 20 receiving 80 g. or more. Considering animal proteins, 83 per cent of 30 home patients receiving less than 10 g. a day responded well, as compared with 76 per cent of 17 patients receiving 20 g. or more. In the sanatorium series, too, there was no evidence of a beneficial effect of a large intake of animal protein, 93 per cent of 27 patients receiving between 30 and 40 g. a day responding well, as compared with 85 per cent of 34 patients receiving 50 g. or more a day. Both at home and in sanatorium, the response in relation to total intake of fats was very similar for the different levels of intake. Considering total carbohydrates, 78 per cent of 32 patients in the home series with an intake of less than 350 g. a day responded well, as compared with 71 per cent of 31 with an intake of 450 g. or more a day. In the sanatorium series, the response was also, if anything, less satisfactory in the patients with a higher intake, for all of 24 patients consuming less than 350 g. fared well, as compared with 85 per cent of 54 with higher intakes.

A number of analyses (not tabulated here) were undertaken to investigate possible asso-

TABLE 14  
Favourable radiographic response at 12 months, related to the dietary intake during treatment

	Home			Sanatorium		
	Total patients	Favourable radiographic response <sup>1</sup>		Total patients	Favourable radiographic response	
		No.	%		No.	%
<i>Calories:</i>						
Less than 1,400	11	7	(64) <sup>2</sup>	0	...	...
1,400-1,800-	22	18	(82)	1	1	(100)
1,800-2,200-	12	9	(75)	16	15	(94)
2,200-2,600 or more	15	11	(73)	49	46	(94)
	19	13	(68)	12	8	(67)
<i>Total proteins</i>						
(g):						
Less than 30	6	4	(67)	0	---	---
30-	18	14	(78)	0	---	---
40-	18	14	(78)	0	---	---
50-	13	10	(77)	3	2	(67)
60-	13	9	(65)	23	22	(96)
70-	4	2	(50)	32	31	(97)
80— or more	7	5	(71)	20	15	(75)
<i>Animal proteins</i>						
(g):						
0-	30	25	83	0	---	---
10-	32	20	63	0	---	---
20-	11	9	(82)	0	---	---
30-	3	2	(67)	27	25	(93)
40-	3	2	(67)	17	16	(94)
50 or more	0	0	---	34	29	(85)
<i>Fats (g):</i>						
Less than 10	18	13	(72)	0	---	---
10-	30	22	73	0	---	---
20-	22	16	(73)	0	---	---
40-	8	6	(75)	32	29	(91)
60-	0	0	---	31	30	(97)
70 or more	1	1	(100)	15	11	(73)
<i>Carbohydrates</i>						
(g):						
Less than 300	14	10	(77)	1	1	(100)
300-	18	15	(83)	23	23	(100)
350-	9	6	(67)	32	30	(94)
400-	7	5	(77)	16	11	(96)
450-	6	5	(83)	5	4	(80)
500 or more	25	17	65	1	1	(100)
Total patients	79	58	73	78	70	90

<sup>1</sup> Defined as moderate, considerable or exceptional improvement.

<sup>2</sup> The parentheses indicate percentages based on fewer than 25 observations.

TABLE 15  
Bacteriologically active disease at 12 months related to the dietary intake during treatment

	Home			Sanatorium		
	Total patients	Patients with bacteriologically active disease <sup>1</sup>		Total patients	Patients with bacteriologically active disease	
		No.	%		No.	%
<i>Calories:</i>						
Less than 1,400	11	2	(18)	0	...	...
1,400-1,800-	22	4	(18) <sup>2</sup>	1	0	(0)
1,800-2,200-	12	3	(25)	16	0	(0)
2,200-2,600 or more	15	4	(27)	49	6	12
	19	1	(5)	12	1	(8)
<i>Total proteins</i>						
(g):						
Less than 30	6	0	(0)	0	---	---
30-	18	3	(77)	0	---	---
40-	18	6	(33)	0	---	---
50-	13	3	(23)	3	0	(0)
60-	13	2	(75)	23	2	(9)
70-	4	0	(0)	32	3	9
80 or more	7	0	(0)	20	2	(70)
<i>Animal proteins</i>						
(g):						
0-	30	4	73	0	---	---
10-	32	8	25	0	---	---
20-	11	1	(9)	0	---	---
30-	3	1	(33)	27	2	7
40-	3	0	(0)	17	3	(78)
50 or more	0	0	---	34	2	6
<i>Fats (g):</i>						
Less than 10	18	1	(6)	0	---	---
10-	30	8	27	0	---	---
20-	22	5	(23)	0	---	---
40-	8	0	(0)	32	4	73
60-	0	0	---	31	1	3
70 or more	1	0	(0)	15	2	(73)
<i>Carbohydrates</i>						
(g):						
Less than 300	14	3	(27)	1	0	(0)
300-	18	2	(77)	23	1	(4)
350-	9	3	(33)	32	4	73
400-	7	2	(29)	16	2	(13)
450-	6	1	(77)	5	0	(0)
500 or more	25	3	72	1	0	(0)
Total patients	79	14	18	78	7	9

<sup>1</sup> For definition, see page 67.

<sup>2</sup> The parentheses indicate percentages based on fewer than 25 observations.

ciations of certain vitamin content of the diet—namely, vitamin A, carotene, thiamine, riboflavine, nicotinic acid and ascorbic acid—and the mineral content of the diet—namely, calcium, phosphorus and iron—with the radiographic and bacteriological response to treatment; none showed any evidence of association.

It may be concluded that there was no association between radiographic improvement and the intake of any of the dietary factors studied.

#### BACTERIOLOGICAL RESPONSE AT 12 MONTHS

Table 15 relates various dietary factors to unfavourable bacteriological response—that, is bacteriologically active disease at 12 months. (A patient was considered to have active disease if he never had three consecutive months of culture negativity or if two or more cultures were positive among an average of seven to nine examined in the last three months of the year, i.e., at 10, 11 and 12 months. Two patients who produced an insolated positive culture at 12 months and were classified as having disease of bacteriologically doubtful status in an earlier report (Tuberculosis Chemotherapy Centre, 1959) have been regarded in the present paper as having bacteriologically active disease, since they produced a positive culture at 13 months also).

Considering the total calories, 18 per cent of 33 home patients with an intake of less than 1800 calories had bacteriologically active disease at 12 months, as compared with 15 per cent of 34 with an intake of 2200 calories or more. In the sanatorium series, seven patients had active disease and all had an intake of 2200 or more calories. Considering total protein, 12 per cent of 24 patients with an intake of less than 40 g. in the home series had active disease, as compared with 8 per cent of 24 with an intake of 60 g. or more. The percentage of sanatorium patients with active disease was very similar for patients receiving between 60 and 80 g. a day (9 per cent) and for those receiving 80 g. or more a day (10 per cent). The amount of animal protein appeared unimportant, for 13 per cent of 30 home patients receiving less than 10 g. a day had bacteriologically active disease at 12 months as compared with 12 per cent of 17 patients with an intake of 20 g. or more. In the sanatorium series, too, there was no evidence

of an association between intake of animal protein and bacteriological response. Examination of the findings for fats and carbohydrates likewise revealed no evidence of an association between intake and bacteriologically active disease. Further analyses (not tabulated here) showed no evidence of an association between the vitamin and mineral content of the diet and the likelihood of bacteriologically active disease at 12 months.

In summary, an examination of the response to treatment as measured by two major assessments—namely, favourable radiographic response at 12 months and unfavourable bacteriological response during the year—yielded no evidence that the diet played an important role either for the patients at home or for those in sanatorium.

#### VIII. Discussion

Many authorities still consider that a rich diet makes a definite contribution to the treatment of pulmonary tuberculosis (McLester and Darby, 1952; Pagel, Simmonds and Macdonald, 1953; Hudson, 1957; Davidson, Meiklejohn and Passmore, 1959). A recent correspondence in the *Transactions of the Royal Society of Tropical Medicine and Hygiene* illustrates how debatable the question is (Barlovatz, 1959; Haddock, 1959). A search of the literature, however, has not revealed any evaluation of the role of diet in patients undergoing treatment for tuberculosis with standard combined chemotherapy. A controlled comparison has now been made of the response to isoniazid plus sodium PAS of patients with pulmonary tuberculosis treated at home for a year with that of patients treated with the same combination of drugs in sanatorium (Tuberculosis Chemotherapy Centre, 1959), with concomitant observations on the diet. The patients were drawn from a poverty-stricken, malnourished and over-crowded section of the community in Madras City. On admission to treatment, the great majority had far advanced cavitated disease and all had tubercle bacilli in the sputum, a large proportion on smear examination, the organisms being sensitive to isoniazid. Whereas the patients admitted to sanatorium were treated under favourable conditions of accommodation, rest and nursing and received the sanatorium diet, the poverty-stricken patients treated at home remained in their over-

crowded conditions and had much less rest, a poor diet, little nursing and could not be depended upon to take their medicament regularly (Tuberculosis Chemotherapy Centre, 1959). The present report is based on 157 patients who had dietary assessments.

The diet before treatment and during treatment was assessed by the oral questionnaire technique (Pasricha, 1958, 1959); the reliability of this method had already been shown by other workers (Venkatachalam, Srikantiah and Gopalan, 1954; Padmavati, Lakhanpal and Gupta, 1958; Pasricha, 1959) and has been confirmed in the present study by a comparison with the weighment technique. In the present study, closer agreement was obtained for protein intake as assessed by the two methods than that reported by Pasricha (1959). The oral questionnaire method was selected for the present investigation because the technique is particularly suited to assessments of the 'poor South Indian' diet which is so commonly encountered in the community under investigation (Patwardhan, 1952; Dakshinamurti and Devadatta, 1956) because the number of assessments to be undertaken was large and because the assessments of the diet before treatment for 112 of the patients had to be made retrospectively.

Before the start of treatment the patients in both the home and the sanatorium series had similar diets, the total calories, total and animal proteins, fats and carbohydrates all being low. The patients were, at that time, clinically ill, many seriously so, and their appetites were poor. During treatment the patients in sanatorium received a superior diet in terms of total calories, total fats and total and animal proteins, as well as in terms of phosphorus and several vitamins. The main food factor in the diet of the home patients during treatment was carbohydrate, for the staple article of their diet was rice. As an example of the inferiority of the diets in the home series during treatment, 30 per cent of the patients treated at home, as compared with 96 per cent of the sanatorium patients, had an intake of 60 g. or more of protein, and only 8 per cent of the patients treated at home, as compared with *all* the patients treated in sanatorium, had a daily intake of 30 g. or more of animal protein. The difference in the diet is magnified by the fact that the home patients had much less rest, for the majority had returned to part-time or full-time activity and many were in their normal occupations

before the end of the year. In contrast, in sanatorium only a small proportion of the patients were permitted to be up four hours a day, a degree of activity which was clearly less than the part-time activity in the home series.

The dietary intake in both series increased in the first three months of treatment, especially in the first six weeks, and from six months onwards the dietary intake was essentially stabilized. The increase in dietary intake in the sanatorium series was, however, much more marked. It is of interest to consider why these increases in diet occurred. At the start of treatment the patients in both series were ill and suffering from toxæmia. There was a rapid reduction in toxæmia after the start of treatment and it is likely that the increase in food intake was due to the consequent increase in appetite. The sanatorium patients had, in addition, a well-balanced diet provided regularly. The domiciliary patients bought as rich a diet as they were able to afford, but because their purchasing capacity was low (Tuberculosis Chemotherapy Centre, 1959) this rarely gave them the same intake as was routinely available for the patients in sanatorium.

The response of the patients in the home series to a year's chemotherapy closely approached that of the sanatorium patients to the same combination of drugs, even though the diet of the home series was much inferior. Thus, it is evident that none of the factors in the diet could have been important in the attainment of quiescence. This was investigated further by studying whether, within each series of patients, the intake of any of the main dietary factors was associated with the radiographic or bacteriological response. No associations were found. Thus, for example, considering total protein, 75 per cent of 24 home patients who had less than 40 g. a day had a favourable radiographic response, as compared with 67 per cent of 24 home patients with an intake of 60 g. or more a day. Similarly, 72 per cent of 18 home patients with an intake of less than 10 g. of fats had a favourable response, as compared with 74 per cent of 31 home patients with an intake of 20 g. or more a day.

There was, however, evidence that the sanatorium patients whose disease attained quiescence responded more rapidly bacteriologically than the corresponding home patients. This may have been due to the superior diet in sanatorium. Other factors which may have

contributed are the greater rest which the sanatorium patients had and the supervised medicine administration in sanatorium, which ensured that the patients had their medicament regularly; there was evidence that a number of the home patients did not take the medicine regularly (Tuberculosis Chemotherapy Centre, 1959).

The weight changes in the 12-month period are of considerable interest. Both series gained weight, the average gain being considerably larger in the sanatorium patients than in the home patients. Thus, the average weight gain of home males was 10.7 lb. and of sanatorium males 17.1 lb., and the corresponding gains in the females were 11.0 lb. and 24.2 lb., respectively. The greater increase in weight in the sanatorium patients was not indicative of a more satisfactory therapeutic response. It presumably resulted from the larger dietary intake and the lesser degree of physical activity.

It has been shown in this study that the diet plays little, if any, part in the attainment of bacteriological quiescence *at the end of a year in patients receiving standard combined chemotherapy*. Since overcrowding has also been shown to be unimportant (Tuberculosis Chemotherapy Centre, 1959), it may be concluded that the successful treatment of patients in their homes in developing countries need not await an increase in the standard of living. Successful treatment of patients on a mass scale can begin as soon as adequate supplies of medicaments are available, and as soon as the necessary supervision of the patients can be organized.

The study is continuing and the dietary intake is being assessed in a period of follow-up in order to evaluate the role of diet in relation to the maintenance of bacteriological quiescence and in the occurrence of relapse. The findings will be the subject of a separate report.

### IX. Summary

1. A study was undertaken of the diet of 157 patients with pulmonary tuberculosis admitted to a controlled comparison of treatment with isoniazid plus PAS for a year at home with the same treatment in sanatorium.

2. The patients were drawn from a poverty-stricken section of the community living in overcrowded conditions in Madras City.

3. A comparison has been made of the dietary status of the home and the sanatorium patients before and during treatment, and the role of the diet in the attainment of bacteriological quiescence of the tuberculous disease has been evaluated.

4. The assessments of the dietary intake were made by the oral questionnaire method. In an analysis based on 45 patients it gave results similar to a dietary weighing technique.

5. The dietary intake before the start of treatment was assessed for all the 157 patients, and 112 of them had another assessment in the second six months of treatment; the remaining 45 patients were investigated at five set dates during treatment—namely, at six weeks, three months, six months, nine months and one year.

6. Before treatment the patients in both series had poor and similar diets.

7. During the early months of treatment, the dietary intake of the patients in both series increased. However, the sanatorium patients received a clearly superior diet throughout the year in terms of total calories, fats, total and animal proteins, phosphorus and several of the vitamins.

8. The home patients were physically more active during treatment than the sanatorium patients, further accentuating the dietary disadvantage of the home series.

9. The home patients gained on the average 10.8 lb. in weight over the 12-month period, as compared with 19.8 lb. for the sanatorium patients. This greater weight gain among the sanatorium patients was not, however, indicative of superior clinical results.

10. The response to treatment (as measured by the radiographic and bacteriological progress) was not directly associated with the level of dietary intake of any of the food factors, either in the patients treated at home or in those treated in sanatorium.

11. It may be concluded that none of the dietary factors studied appears to have influenced the attainment of quiescent disease among tuberculous patients treated with an effective combination of antimicrobial drugs for a period of one year. The successful initial treatment of patients at home is therefore possible even if the levels of dietary intake are low.

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**THE VIRULENCE IN THE GUINEA-PIG OF TUBERCLE BACILLI ISOLATED  
BEFORE TREATMENT FROM SOUTH INDIAN PATIENTS  
WITH PULMONARY TUBERCULOSIS\***

1. HOMOGENEITY OF THE INVESTIGATION AND A CRITIQUE OF THE  
VIRULENCE TEST

D. A. MITCHISON,<sup>1</sup> A. L. BHATIA,<sup>2</sup> S. RADHAKRISHNA,<sup>2</sup> J. B. SELKON,<sup>2</sup>  
T. V. SUBBAIAH<sup>2</sup> AND J. G. WALLACE<sup>1</sup>

A series of studies on the virulence in the guinea-pig of tubercle bacilli isolated before treatment from Indian tuberculous patients admitted to a controlled comparison of different regimens of domiciliary chemotherapy has recently been undertaken by the Tuberculosis Chemotherapy Centre, Madras. The main object of these studies was to determine whether the differences in virulence of the tubercle bacilli obtained from Indian patients before the start of chemotherapy were related to the severity or type of the patients' disease at that time and to the subsequent response to treatment. Before these relationships could be investigated, however, it was necessary to find out whether the results of the virulence tests, which were carried out over a period of two-and-a-half years at the Centre and at the Microbiological Research Establishment, Porton, England, could be considered as a unified whole—that is, as if they had all been done on the same day in the same laboratory.

A proportion of the cultures was stored at  $-20^{\circ}\text{C}$  for 44-78 weeks, but this did not affect their virulence. Inter-experimental variation was found to be small in the Porton series of tests and undetectable in the Madras series, and the results in the latter series could be successfully adjusted to those in the former by allowing for differences in the means and standard deviations of the distributions for the two series. The measure of virulence used was found to be reasonably acceptable for the analysis of variance technique. Suggestions are made as to ways of improving the efficiency of the experimental design in future studies.

### Introduction

Cultures of tubercle bacilli obtained from untreated South Indian and British patients with pulmonary tuberculosis were compared by Mitchison *et al.* (1960) for their virulence in the guinea-pig. These cultures were sensitive to isoniazid and streptomycin and had been identified as *Mycobacterium tuberculosis* by a number of *in vitro* tests. In agreement with the earlier work of Frimodt-Møller, Mathew and Barton (1956) and Frimodt-Møller (1957), the average virulence of the cultures from the Indian patients was found to be lower than that of the cultures from the British patients and also the range of virulence was wider, only about

30 per cent of the Indian cultures being as

virulent as those from British patients. These findings have been confirmed and extended in the second of the present series of three papers (Bhatia *et al.*, 1961a). Further, Bhatia *et al.* (1961b) have shown that the variation in virulence among cultures obtained over a 6-week period from the same untreated Indian patient was no greater than the natural variation in the response of the guinea-pigs to the virulence test and was considerably less than the variation in virulence from patient to patient. Thus, individual Indian patients yielded strains of a consistent degree of virulence. As a result of these findings, it became meaningful to

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<sup>1</sup> Unit for Research on Drug Sensitivity in Tuberculosis (Medical Research Council of Great Britain), Postgraduate Medical School of London, London, England.

<sup>2</sup> Tuberculosis Chemotherapy Centre, Madras, India. The Centre is under the joint auspices of the Indian Council of Medical Research, the Madras State Government, the World Health Organization and the Medical Research Council of Great Britain (MRC).

investigate whether the differences in virulence of the tubercle bacilli obtained from Indian patients before the start of chemotherapy were related to the severity or type of the patients' disease at that time, and to the subsequent response to treatment with antituberculosis drugs. Such an investigation has now been made, and is reported in the third of the present series of papers (Ramakrishnan *et al.*, 1961<sup>1</sup>).

From October 1957 to December 1958 a total of 341 South Indian patients were admitted to a controlled comparison of four regimens of chemotherapy in the domiciliary treatment of tuberculosis (Tuberculosis Chemotherapy Centre, 1960). After the exclusion of 22 patients because they yielded isoniazid-resistant cultures on admission, and four for other reasons, there remained 315 patients in the main analysis of the results of this comparison. Virulence tests in the guinea-pig were carried out on single cultures obtained before the start of the prescribed regimen (except in one patient, whose culture was obtained three months after the start of treatment) from 281 (89.2 per cent) of the 315 patients.

The results of the virulence tests on these 281 patients are related to assessments of the extent and type of their disease on admission to the chemotherapy trial, and to their progress during one year's treatment with the prescribed regimen, in the third paper in this series (Ramakrishnan *et al.*, 1962<sup>1</sup>). However, for any associations found to be meaningful, it was essential to be able to consider the results of the virulence tests as a unified whole, that is, as if they had all been done on the same day in the same laboratory. There were certain features of the investigation which might be expected to introduce serious heterogeneity into the results of these tests. We therefore report here, as a preliminary to the third paper, the findings on experiments which were incorporated in the investigation to measure the degree of homogeneity, and also the measures taken to standardize the results where a source of heterogeneity was found.

The more important possible sources of heterogeneity and the measures taken to estimate them, or to adjust for them, were as follows:

1. Facilities for large-scale experimental work on animals were not available until the latter part of the period of intake of patients

to the chemotherapy trial, so that the majority of the cultures from patients admitted at the beginning of the period were stored at  $-20^{\circ}\text{C}$  in a deep-freeze cabinet. The effect of storage in the deep-freeze was examined by comparing the virulence of pairs of cultures from the same Indian patient, one culture in each pair having been tested shortly after isolation and the other after storage in the deep-freeze.

2. The virulence tests were carried out in two series of experiments, the large series at the Microbiological Research Establishment, Porton, Wiltshire, England (Porton) and the other at the Tuberculosis Chemotherapy Centre, Madras, India (Madras). These experiments were done over a period of two-and-a-half years. The extent of the variation in the results from experiment to experiment was examined in several ways:

(a) In both series of experiments, the results of the tests on the cultures from the Indian patients themselves provided evidence on inter-experimental variation.

(b) A sample of cultures of tubercle bacilli was obtained from untreated British patients, and these cultures were included in both series of experiments. Since the virulence of British cultures was known to vary less than the virulence of Indian cultures (Mitchison *et al.*, 1960), the results of the tests on the British cultures would be a particularly sensitive indicator of inter-experimental variation.

(c) In the majority of the experiments at Porton, a standard strain, H37Rv, of moderate virulence, was included among the cultures tested.

3. The breed of guinea-pig used at Porton was different from that used at Madras. Although the same method of virulence testing was used, the responses in the two breeds differed substantially. The results of the smaller Madras series were therefore adjusted to make them comparable with those obtained at Porton.

These findings are followed in the present paper by a statistical critique of the virulence test, concerned principally with (a) the measure of virulence and the validity of its use in analysis of variance, (b) the advantages of various possible arrangements of guinea-pigs in the tests, and (c) the relative efficiencies of the two breeds of guinea-pig in measuring virulence.

See article on page 124.

## Methods

### NOMENCLATURE

The Tuberculosis Chemotherapy Centre will be referred to as Madras and the experiments carried out there as Mad. 1, 2 . . . , the Microbiological Research Establishment as Porton, with experiments For. 1, 2 . . . , and the MRC's Unit for Research on Drug Sensitivity in Tuberculosis as London. Some of the results of the virulence tests described here—namely, those on cultures from 73 of the Indian and 22 of the British patients—have already been reported by Mitchison *et al.* (1960), using the same nomenclature.

### PATIENTS

#### *Indian patients*

Virulence tests were carried out on single cultures of tubercle bacilli from 281 South Indian patients. All of these patients had been admitted to a chemotherapeutic study at Madras (Tuberculosis Chemotherapy Centre, 1960) and had contributed to the main analysis on 315 patients. Virulence tests on cultures from the remaining 34 patients were not done, since 23 cultures were contaminated during storage or transport from Madras to England, eight were mislaid, and three failed to grow on sub-culture after storage in the deep-freeze. The 281 patients with virulence-test results conformed to the following important criteria:

(a) The patients were aged 12 years or more and were living in Madras City.

(b) All the patients had pretreatment cultures that were sensitive to isoniazid and all except three had organisms sensitive to streptomycin. The emergence of streptomycin-resistance is not associated with loss of virulence (Feldman, Karlson and Hinshaw, 1948; Steenken and Wolinsky, 1948; Karlson and Gainer, 1951). No attempt was made to exclude cultures resistant to *p*-aminosalicylic acid (PAS) for reasons reported elsewhere (Selkon *et al.*, 1960; Tuberculosis Chemotherapy Centre, 1960). Sensitivity tests were carried out on Löwenstein-Jensen medium (Tuberculosis Chemotherapy Centre, 1959). [The Löwenstein-Jensen medium referred to here, and throughout the text, did not contain potato starch (Jensen, 1955).]

(c) The great majority of the patients had not had any previous antituberculosis chemotherapy so far as was known. Up to two weeks of such chemotherapy had been received by 11 patients. In one patient an isoniazid-sensitive culture, obtained three months after the start of the prescribed chemotherapy, was tested for virulence, because a pretreatment result was not available. The results of virulence tests on cultures from these 12 patients have been included, since Subbaiah *et al.* (1962<sup>1</sup>) have shown that the virulence of cultures from Indian patients is unaffected by three months of chemotherapy provided that the organisms remain sensitive to isoniazid.

(d) The patients had subsequently followed the initially prescribed regimen of treatment in the trial for 12 months with, at most, minor variations, unless chemotherapy was stopped or changed owing to death, deterioration or major toxicity.

#### *British patients*

Virulence tests were done on single cultures from 93 patients of British extraction, aged 12 years or more and with newly diagnosed, untreated, and moderately or far advanced pulmonary tuberculosis. A test was done, by mistake, on one culture from a patient who had received previous antituberculosis chemotherapy, but the organisms were sensitive to isoniazid, streptomycin and PAS. The results of this virulence test have been included for the reason given above.

### CULTURES OF TUBERCLE BACILLI

The sputum specimens obtained from Indian patients were cultured at Madras, and those from British patients at London, the same method (Tuberculosis Chemotherapy Centre, 1959) being used—namely, homogenization with 4 per cent sodium hydroxide, followed by inoculation on to slopes of Löwenstein-Jensen medium.

#### *Cultures from Indian patients*

As soon as the cultures from the Indian patients had become positive they were stored in Madras, either for a short period at 37°C (138 'fresh cultures') or for 44-78 weeks (average, 62 weeks) at -20°C (143 'deep-freeze-stored

<sup>1</sup> See article on page 45.

cultures'). After storage, the cultures for testing at Porton were sent by air to London for subcultivation in the virulence test, in parallel with the cultures from British patients. Of the 138 fresh Indian cultures, 132 were stored for up to eight weeks (usually less than six weeks), three for nine weeks, and three for 10 weeks. One of the 143 deep-freeze-stored cultures had to be decontaminated by treatment with sulfuric acid at London.

#### *Cultures from British patients*

All of the cultures from the British patients were stored at 37°C for periods of up to eight weeks. Those to be tested for virulence at Madras were sent by air, to be set up as fresh cultures in parallel with the cultures from Indian patients.

#### *Strain H37Rv*

A standard strain of *Myc. tuberculosis* var. *hominis*, H37 Rv, was obtained from Dr R. J. W. Rees, National Institute for Medical Research, London. This strain, which had recently been passaged through mice, was inoculated on to a number of slopes of Lowenstein-Jensen medium, which were then stored at -20° C in a deep-freeze. In each experiment one of these slopes was removed from the deep-freeze and subcultivated in the virulence test.

#### IDENTIFICATION TESTS

The wide range of identification tests employed for 73 of the cultures from Indian patients included in this study, and already described by Mitchison *et al.* (1960), was slightly restricted for the remaining cultures to: (a) a smear from the growth on Lowenstein-Jensen medium stained by the Ziehl-Neelsen method; (b) colonial morphology on 7H-10 oleic-acid-albumin agar medium and on Lowenstein-Jensen medium; (c) examination for ability to grow at 23°C; (d) abnormal pigmentation on Lowenstein-Jensen medium in the dark and after exposure to light; (e) the niacin test; (f) a qualitative catalase test; and (g) sensitivity to *p*-acetamido-benzaldehyde thiosemicarbazone (thiacetazone). The methods employed are described fully elsewhere (Thomas *et al.*, 1962<sup>1</sup>). In addition, Mantoux tests with 0.1 ml of 1:100 Old Tuberculin were done four weeks after infection on all animals tested at Madras.

Identification tests were not done systematically on the cultures from British patients, since they were fairly uniformly of high virulence in the guinea-pig.

#### VIRULENCE TESTS

The origin and diet of the guinea-pigs and the procedures for the virulence test have been described in detail by Mitchison *et al.* (1960) so that the methods will only be presented briefly here.

#### *Guinea-pigs*

At Porton, a total of 1138 Duncan Hartley (DH) breed albino animals (99 per cent males) was used, of average weight 426 g. (range, 300-580 g.). At Madras a total of 166 M-breed, mixed-colour guinea-pigs (69 per cent males) was used, of average weight 370 g. (range, 258-608 g.).

#### *Infecting dose*

The initial diagnostic culture, after storage as described earlier, was subcultivated on Löwenstein-Jensen medium. After three weeks' incubation at 37°C, growth from the subculture was weighed out into screw-capped bottles containing glass beads. Sterile distilled water (at Madras), shown not to be bactericidal to tubercle bacilli, or 0.1 per cent bovine albumin in water (at London) was added and the bottles were shaken to prepare a suspension of the bacilli. Either two or four guinea-pigs (depending on the particular virulence test) were each injected in the right thigh muscle with 0.5 ml. of the suspension, which contained 1 mg. (moist weight) of bacilli. From some of the suspensions, a viable count was set up in 7H-10 medium [Cohn, Middlebrook and Russell (1959), as modified by Subbaiah, Mitchison and Selkon (1960)], solidified with silica gel according to the method of Selkon and Mitchison (1957).

#### *Root-index of virulence*

When two guinea-pigs were infected, one was killed at 6 weeks and the other at 12 weeks; when four animals were infected, two were killed at each time. If, when two guinea-pigs were infected, one of them died before six weeks, then it was counted as a 6-week guinea-pig and the surviving guinea-pig was killed at 12 weeks. If both guinea-pigs died, then the

<sup>1</sup> See article on page 99.

one that died first was counted as the 6-week guinea-pig and the other was counted as the 12-week guinea-pig. Similar rules applied when four guinea-pigs were infected. At post-mortem examination the amount of visible disease was assessed as a score ranging from 0 to 100. The maximum score for the spleen was 40, for the liver 30, for the lungs 20, and for the site of inoculation, and its draining lymphnodes, 10. Animals that died, either of tuberculosis or from natural causes, were scored in the same way. The total score for each animal (whether dying or killed) was divided by its survival time in days to give an index. This index is a measure of the rate at which lesions develop in the organs and, by inference, it also measures the approximate rate at which tubercle bacilli multiply in the animal body. Further, it combines the results of score and mortality. For reasons given later in the present report (see page 85), the square roots of the 6-week and the 12-week indices were calculated and termed the '6-week root-index' and the '12-week root-index', respectively. The mean of the root-indices for all the animals infected with a culture was termed the 'root-index of virulence' and has been employed as the measure of virulence in the present report.

#### *Non-tuberculous deaths*

Non-tuberculous deaths were defined as those occurring in animals with a total score of less than 40, and a lung score of less than 20 (the maximum for this organ). Defined in this way 16 non-tuberculous deaths (1.4 per cent of 1138 animals) occurred in the experiments at Porton and two (1.2 per cent of 166 animals) in the experiments at Madras. If such a death occurred 30 or more days after infection, and if there was no evidence of an infection or other cause of death which could be confused with tuberculosis, then a root-index was calculated as described earlier, and the result was included. If the guinea-pig died less than 30 days after infection, or if the nature of the lesions was obscure, then the root-index was estimated as follows. When the guinea-pig concerned was one of four injected with the culture, the value of the root-index of the paired 6-week or 12-week guinea-pig was taken as the estimate. When the missing value was from, say, the single-6-week-guinea-pig-injected with a culture,

then its root-index was estimated by adding to the root-index of the corresponding 12-week guinea-pig the mean difference between the 6-week and 12-week root-indices of all the remaining guinea-pigs in the particular experiments, whether their results are reported here or not (see below). The results on 10 animals at Porton and on two animals at Madras had to be estimated in this manner.

#### ARRANGEMENT OF EXPERIMENTS

Both at Porton and at Madras a series of experiments was set up, the interval between successive experiments being six weeks. Consequently, in all except the first and the last experiment of each series, the 6-week guinea-pigs from one experiment and the 12-week guinea-pigs from the previous experiment were killed and scored together. In both series, virulence tests on cultures not reported here were also carried out (by the same method) in nearly all of the experiments. The order of preparing the infecting suspensions, of injecting the doses, and of killing the guinea-pigs was randomized, and the identity of the infecting organisms was not known to the observer who assessed the score.

#### *Porton series*

The Porton series (Table 1) consisted of 13 experiments, in which 274 Indian cultures were tested. These 274 cultures were from 254 of the 281 patients, two cultures from each of 20 patients being tested twice, for the comparison of fresh and deep-freeze-stored cultures (see below). In experiments Por. 1 to Por. 3 all the Indian cultures were fresh, and each culture was injected into four guinea-pigs. Some of the cultures in experiments Por. 4 and Por. 5, and all of them in Por. 6 and later experiments, had been stored in the deep-freeze. In Por. 4 and subsequent experiments, only a sample averaging 28 per cent of the cultures was set up in four guinea-pigs per culture. The purposes of these samples were to obtain estimates of the duplicate error of the test for 6-week and 12-week guinea-pigs and to facilitate certain comparisons, reported in the accompanying paper by Bhatia *et al.* (1961 a), with the British culture which were also injected into four guinea-pigs each (see below). For each of the remaining

TABLE 1  
Arrangement of experiments in the Porton series (DH guinea-pigs)

Experiment No.	Results in the present report								Total number of guinea-pigs in experiment (including results not in the present report)
	Indian cultures							Number of guinea-pigs	
	Tested in 2 guinea-pigs		Tested in 4 guinea-pigs		Total	British cultures (tested in 4 guinea-pigs)	Strain H 37 Rv (8 guinea-pigs in each experiment)		
	Fresh	Deep-freeze-stored	Fresh	Deep-freeze-stored					
For. 1	0	0	21	0	21	5	0	104	104
2	0	0	27	0	27	5	0	128	136
3	0	0	20	0	20	5	0	100	108
4	31	3		1	43	5	1	152	164
5	3	15	1	9	28	5	1	104	164
6	0	15	0	8	23	5	1	90	164
7	0	21	0	8	29	5	1	102	168
8	0	3	0	4	7	5	1	50	158
9	0	2	0	0	2	5	1	32	84
10	0	15	0	4	19	5	1	74	174
11	0	11	0	7	18	5	1	78	152
12	0	8	0	7	15	5	1	72	166
13	0	22 <sup>1</sup>	0	0	22	5	1	72	190
Total	34	115	77	48	274	65	—	1138	1932

<sup>1</sup> Including the 20 cultures in the comparison of the virulence of fresh and deep-freeze-stored cultures.

cultures, two guinea-pigs were infected per culture. In all, 125 Indian cultures were each injected into four guinea-pigs and 129 were each injected into two guinea-pigs.

The effect of storage in the deep-freeze was investigated in pairs of cultures from 20 Indian patients. One of the two cultures from each patient was tested fresh, in experiment Por. 4, and is included among the other Indian cultures. The second culture of the pair was stored in the deep-freeze and was then tested in experiment Por. 13, each culture being injected into two guinea-pigs. The results of the tests on the second cultures are excluded from all analyses other than that of this comparison.

In each experiment at Porton, five British cultures were tested, totalling 65 cultures in the series. Each British culture was injected into four animals. As a further control on inter-experimental variation, strain H37Rv was tested in eight animals in each experiment from Por. 4 onwards. In addition to serving as a test for inter-experimental variation, these animals were also used for a further study, described later (page 82), of the variations in virulence

due to preparation of the infecting suspension, and of the effect of a 10-fold decrease in the size of the dose.

#### Madras series

The Madras series (Table 2) consisted of 12 experiments, in which 55 Indian cultures were tested. Of these 55 cultures, 27 were tested only at Madras and, together with the 254 cultures in the Porton series, make up the total of 281 cultures from the same number of Indian patients in the investigation. Of the remaining 28 cultures, 23 were also tested in the Porton series; the other five were from patients from whom alternative pretreatment sputum cultures were tested in the Porton series. These 28 cultures do not, therefore, contribute to the total of 281 patients. They provide a check on the adjustment of the results obtained with cultures tested only at Madras. All of the 55 Indian cultures were fresh. A total of 28 British cultures was tested in the series. Each 'Culture in the series, whether Indian or British, was injected into two guinea-pigs.

TABLE 2  
Arrangement of experiments in the Madras series (*M guinea-pigs*)

Experiment No.	Results in the present report					Total number of guinea-pigs in experiment (including result not in the present report)
	Indian cultures			British cultures	Number of guinea-pigs	
	Tested only at	Tested at Madras and Porton	Total			
Mad. 1	4	0	4	2	12	12
2	3	0	3	1	8	24
3	2	0	2	1	6	18
4	4	0	4	2	12	20
5	4	0	4	2	12	14
6	7	0	7	3	20	24
7	0	3	3	2	10	17
8	0	7	7	3	20	36
9	2	9	11	5	32	50
10	0	5	5	3	16	44
11	0	0	0	0	0	20
12	1	4	5	4	18	41
Total	27	28	55	28	166	320

**Results**

The results of the virulence tests are described in two sections. Section A deals with the homogeneity of the investigation, principally with the effect on the root-index of virulence of such factors as conditions of storage of the culture, inter-experimental variation, variations in the preparation of the infecting suspension, and differences in the responses of the two breeds of guinea-pig. Section B consists of a critical analysis of the virulence test itself, together with suggestions for its modifications in future work. The results of the tests were examined by analysis of variance. This statistical method is based on certain assumptions—namely, additivity of effects and homogeneity of variance in a normally distributed population. The extent to which the results of virulence tests reported here satisfied these conditions is described in section B.

**A. HOMOGENEITY OF THE INVESTIGATION**

*Identification tests*

Of the total of 306 cultures from the 281 Indian patients (duplicate cultures being obtained from

20 patients in the comparison of fresh and deep-freeze-stored cultures and from five patients in the comparison between virulence tests in the Porton and the Madras series), 287 (93.8 per cent) were examined for their identity. The results on 262 cultures were among those reported fully by Thomas *et al.* (1962<sup>1</sup>), and the findings on the remaining 25 cultures were similar. In brief, all 287 were found to be *Myc. tuberculosis* and, of 279 examined, all were of the human type, as indicated by a positive niacin test.

*Comparison of fresh and deep-freeze-stored cultures*

The effect of storage in the deep-freeze on the virulence of cultures was investigated in pairs of cultures from 20 Indian patients. The pairs of sputum specimens which yielded the cultures were obtained at intervals, on the average, of two days from each other. One of the cultures from each patient was tested as a fresh culture, in experiment Por. 4, and the other was stored in the deep-freeze for, on the average, 57 weeks (range, 51-59 weeks) before being tested in experiment Por. 13. This period of storage is similar to the mean period for all deep-freeze-stored cultures in the investigation (62 weeks; range, 44-78 weeks). The results are set out in full, as a representative sample of virulence-test data, in Table 3. A few of the cultures had been tested in four animals in experiment Por. 4, and for these one 6-week and one 12-week root-index were selected at random for simplicity in analysis. The means of the root-indices of virulence were 0.64 for the fresh cultures in Por. 4 and 0.71 for the deep-freeze-stored cultures in Por. 13. This difference does not attain statistical significance (Table 3, term b, P = 0.1). However the difference measures not only any tendency for deep-freeze-stored cultures to differ in mean virulence from fresh cultures, but also any difference in the average virulence of cultures tested in Por. 4 and Por. 13. Separate analyses were therefore done of the tests on British cultures and on "strain H37Rv included as controls in Por. 4 and Por. 13, and these showed no evidence of a significant difference between the experiments. Nevertheless, examination of the means of the tests on the British cultures and on strain H37Rv in For. 4 and Por. 13 (Table 3) suggests that, if these

<sup>1</sup> See article on page 99.

TABLE 3

*Comparison of fresh and deep-freeze-stored Indian cultures in experiments Por. 4 and Por. 13*

Patient No.	Indian		Fresh cultures (For. 4) Deep-freeze-stored cultures (Por. 13)			
	6-week root-index	12-week root-index	Root-index of virulence	6-week root-index	12-week root-index	Root-index of virulence
1	0.84	1.08	0.96	!	0.93	1.02
2	0.45	0.32	0.38	0	0.49	0.60
3	0.45	0.32	0.38	0	0.55	0.58
4	0.81	0.62	0.72	0	0.46	0.46
5	0.45		0.40	0.42	0.67	0.73
6	0.84	1.02		0.93	1.02	0.90
7	0.81	0.94	0.88	0.96	0.92	0.94
8	0.67	0.32	0.50	0.89	0.73	0.81
9	0.81	0.89	0.85	0.94	0.88	0.91
10	1.15	0.57	0.86	0.66	0.66	0.66
11	0.84	0.45	0.64	0.89	0.62	0.76
12	0.63	0.35	0.49	0	0.32	0.38
13	0.45	0.26	0.36	0.78	0.60	0.69
14	1.01	0.62	0.82	0.89	0.62	0.76
13	0.45	0.72	0.58	0.45	0.55	0.50
16	0.71	0.63	0.67	0.82	0.61	0.72
17	0.87	0.56	0.72	0.66	0.62	0.64
18	0.63	0.32	0.48	0.75	0.55	0.65
19	0.45	0.77	0.61	0.87	0.75	0.81
20	0.84	0.32	0.58	0.62	0.77	0.70
Mean	0.71	0.57	0.64	0.75	0.67	0.71
Mean for British cultures	1.11	1.11	1.11	1.11	0.96	1.03
Mean for H37Rv	1.02	0.82	0.92	0.94	0.71	0.82

*Analysis of variance*

Term	Source of variation	Sum of squares	DF	Mean square	Term tested against	F	P
a	Patients (P)	1.8950	19	0.0997	c	3.52	0.005
b	Storage — fresh and deep freeze - (S)	0.0952	1	0.0952	c	3.36	0.1
c	Interaction S X P	0.5379	19	0.0283	g	1.51	0.2
d	6 and 12 weeks (W)	0.2464	1	0.2464	e	9.51	0.005
e	Interaction W X P	0.4915	19	0.0259	g	1.38	>0.2
f	Interaction W X S	0.0106	1	0.0106	g	—	NS <sup>1</sup>
g	Interaction W X P X S ...	0.3572	19	0.0188			
	Total ...	3.6338	79	0.0460			

<sup>1</sup> NS indicates that the variance ratio is less than 1.0.

TABLE 4  
Virulence of fresh and deep-freeze-stored cultures from Indian patients in the Porton series

Experiment	Fresh cultures		Deep-freeze-stored cultures	
	Number	Mean root-index of virulence	Number	Mean root-index of virulence
Por. 1-Por. 3 ...	68	0.74	0	—
For. 4 ...	39	0.71	4	0.60
For. 5 ...	4	0.71	24	0.74
Por. 6-Por. 13 ...	0	—	115	0.74
Total ...	111	0.73	143	0.74

means indicate any systematic difference between the two experiments, it is in a direction that would imply a greater mean virulence in the deep-freeze-stored than in the fresh cultures. Further evidence that storage in the deepfreeze did not alter the virulence of Indian cultures is provided by the close similarity of the mean of the root-indices of virulence with fresh cultures (0.73) to the mean with deepfreeze-stored cultures (0.74) in the entire Porton series (Table 4). In summary, evidence was obtained that deep-freeze-stored cultures did not differ from fresh cultures in virulence.

*Inter-experimental variation*

*Indian cultures.* The results of virulence tests on single cultures from Indian patients were divided into three groups, which were analysed separately: (a) Porton series, four guinea-pigs per culture; (b) Porton series, two guinea-pigs per culture; (c) Madras series, all

TABLE 5  
Indian and British cultures tested in four guinea-pigs in the Porton series: Analysis of variance  
Design of the investigation: (1) Indian cultures: 125 cultures in 11 experiments; each culture injected into four guinea-pigs; total of 500 guinea-pigs. (2) British, cultures : 65 cultures ; five cultures in each of 13 experiments; each culture injected into four guinea-pigs; total of 260 guinea-pigs.

Term	Source of variation	Indian cultures				British cultures				
		DF	Mean square	Term tested against	F	P	DF	Mean square	F <sup>1</sup>	P
a	Cultures (C) ...	124	0.1978				64	0.0388		
b	Experiments (E) ...	10	0.1625	c	—	NS <sup>2</sup>	12	0.0723	2.33	0.01-0.02
c	Cultures in same experiment C (E) ...	114	0.2009	h	8.85	<0.001	52	0.0310	1.95	0.001
d	6 and 12 weeks (W) ...	1	3.6006	f	183.70	<0.001	1	1.1246	34.50	<0.001
e	Interaction W X C ...	124	0.0310	h	1.37	0.02	64	0.0201	1.26	0.1-0.2
f	Interaction W x E ...	10	0.0196	g	—	NS	12	0.0326	1.90	0.05
g	Interaction W X C (E) ...	114	0.0320	h	1.41	0.01	52	0.0172	1.08	>0.2
h	Duplicate guinea-pigs ...	245 <sup>3</sup>	0.0227				126 <sup>4</sup>	0.0159		
i	6 weeks ...	120	0.0215	i	1.11	0.5 <sup>5</sup>	62	0.0110		
j	12 weeks ...	125	0.0238	I			64	0.0207	1.88	0.02 <sup>5</sup>

<sup>1</sup> Against the term indicated in the "Indian cultures" column.

<sup>2</sup> NS indicates that the variance ratio is less than 1.0.

<sup>3</sup> For five non-tuberculous deaths (all in 6-week guinea-pigs) missing observations were estimated as described in the text (page 75).

<sup>4</sup> For four non-tuberculous deaths (three in 6-week guinea-pigs) missing observations were estimated as described in the text (page 75).

<sup>5</sup> Two-tail probability.

TABLE 6

*Indian and British cultures tested in two guinea-pigs: Analysis of variance*

Design of the investigation : (1) Indian cultures: (a) Porton series: 129 cultures in 10 experiments; total of 258 guinea-pigs, (b) Madras series: 55 cultures in 11 experiments; total of 110 guinea-pigs  
(2) British cultures: Madras series: 28 cultures in 11 experiments; total of 56 guinea-pigs

Term	Source of variation	Indian cultures								British cultures				
		Porton series				Madras series				Madras series				
		DF	Mean square	Term tested against	F	P	DF	Mean square	F <sup>1</sup>	P	DF	Mean square	F <sup>1</sup>	P
a	Cultures (C)		0.0988				54	0.2158			27	0.0576		
b	Experiments (E)	9	0.1943	c	2.12	0.04	10	0.2449	1.17	>0.2	10	0.0426	—	NS <sup>2</sup>
c	Cultures in same experiment C (E)	119	0.0915	e	3.43	<0.001	44	0.2092	8.47	<0.001	17	0.0664	1.69	0.1
d	6 and 12 weeks (W)	1	1.9745	e	73.95	<0.001	1	1.8331	74.21	<0.001	1	0.4866	12.41	<0.00
e	Interaction W x C	128	0.0267				54	0.0247			27	0.0392		
f	Interaction W X E	9	0.0317	g	1.20	>0.2	10	0.0115	—	NS	10	0.0275	—	NS
g	Interaction W x C (E)	119	0.0264				44	0.0270			17	0.0460		

<sup>1</sup> Against the term indicated in the "Porton series" column.

<sup>2</sup> NS indicates that the variance ratio is less than 1.0.

with two guinea-pigs per culture. The analyses of variance for these three groups are set out in Tables 5 and 6. In the Porton series where each culture was injected into four animals (Table 5), 125 cultures were tested in 11 of the 13 experiments. There is no evidence that the variation in mean virulence from experiment to experiment (inter-experimental variation) was greater than the variation from culture to culture in the same experiment (Table 5, terms b and c). In the Porton series where each culture was injected into two animals (Table 6), a further 129 cultures were tested in 10 of the experiments. Statistically significant evidence of inter-experimental variation is apparent here (Table 6, term b,  $P=0.04$ ). Finally, in the Madras series, 55 cultures were tested in 11 experiments; no inter-experimental variation is apparent (Table 6, term b).

*British cultures.* In each of the 13 experiments in the Porton series, five cultures from different British patients were tested, each in four guinea-pigs. The analysis of variance is set out in Table 5. The variation in mean virulence from experiment to experiment is significantly greater than the variation from

culture to culture in the same experiment (Table 5, terms b and c,  $P=0.01-0.02$ ). Since the variation from culture to culture in the same experiment is smaller for the British cultures (mean square, 0.0310) than for the Indian cultures (mean square, 0.2009), the results on the British cultures are likely to provide a more sensitive test for inter-experimental variation, even though the total number of British cultures tested was fewer.

The analysis of variance of the virulence-test results on the 28 British cultures tested in 11 experiments at Madras is set out in Table 6. No significant inter-experimental variation was found (Table 6, term b).

*Strain H37Rv.* In the Porton series, strain H37Rv was tested in Per. 4 and in all subsequent experiments. The culture to be tested in each experiment was taken from the deep-freeze and inoculated on to two Lowenstein-Jensen medium slopes. After the usual 3-week incubation period, two separate infecting suspensions were prepared from each slope. From each suspension, two guinea-pigs were infected with 1.0 mg. of bacilli and a further two with 0.1 mg. of bacilli. Thus eight animals were used in

TABLE 7

*Strain H37Rv in the Porton series: Analysis of variance*

*Design of the investigation:* 10 experiments; in each experiment two infecting suspensions, each in two doses, each injected into two guinea-pigs; total of 80 guinea-pigs

Term	Source of variation	Sum of squares	DF	Mean square	Term tested against	F	P
a	Infecting suspensions (I)	0.6908	19	0.0364			
b	Experiments (E)	0.5375	9	0.0597	k	2.83	0.01
c	Infecting suspensions in same experiment I (E)	0.1533	10	0.0153	i	—	NS <sup>1</sup>
d	Doses of 1.0 and 0.1 mg (D)	0.5136	1	0.5136	f	12.62	<0.005
e	6 and 12 weeks (W)	0.2453	1	0.2453	i	10.85	<0.005
f	Interaction D x I	0.7726	19	0.0407	i	1.81	0.1
g	Interaction W X I	0.4305	19	0.0227	i	1.01	>0.2
h	Interaction W X D	0.0256	1	0.0256	i	1.14	>0.2
i	Interaction W X D X I	0.4267	19	0.0225			
j	(g + h + i)	0.8828	39	0.0226			
k	(c + g + h + i)	1.0361	49	0.0211			
	Total	3.1051	79	0.0393			i

<sup>1</sup> NS indicates that the variance ratio is less than 1.0.

TABLE 8

*Estimates of inter-experimental variation*

Series	Type of culture	Number of guinea-pigs per culture	Degrees of freedom		Inter-experimental variation	
			Experiment s	Cultures in same experiment	P	Square root of component of variance (standard deviation)
Porton	Indian	4	10	114	NS <sup>1</sup>	0.00
	Indian	2	9	119	0.04	0.07
	British	4	12	52	0.01-0.02	0.05
	H37Rv	8	9	492	0.01	0.07
Madras	Indian	2	10	44	>0.2	0.06
	British	2	10	17	NS	0.00

<sup>1</sup> NS indicates that the variance ratio is less than 1.0.

<sup>2</sup> Term k in Table 7.

each experiment. The full analysis of variance of the results of the virulence tests is set out in Table 7. Statistically significant evidence of inter-experimental variation exists (Table 7, term b,  $P=0.01$ ).

*Estimates of inter-experimental variation.* Estimates of the extent of the variation from experiment to experiment, expressed as a standard deviation (the square root of the component of variance due to this source in the analyses of variance in Tables 5, 6 and 7), are set out in Table 8. In addition, the probability that these estimates differ from 0.00 is shown. The estimates range from 0.00 to 0.07.

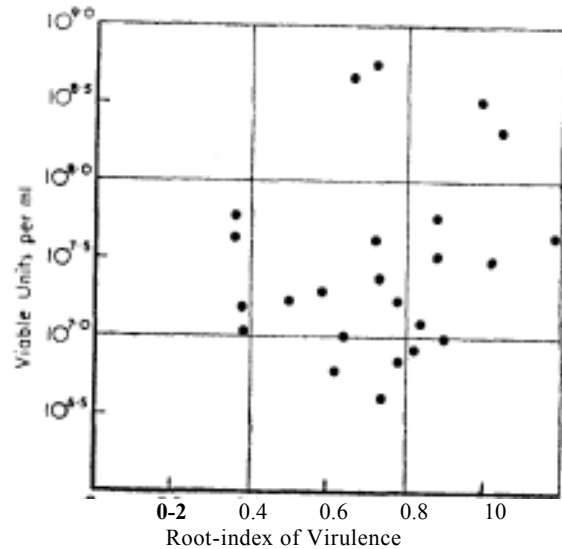
In summary, the presence of inter-experimental variation has been sought for in six analyses and demonstrated in only three. The estimated magnitude of the variation is very small, and probably contributed less than 0.07 to the standard deviation of the root-index of an individual guinea-pig.

#### *Variation between infecting suspensions*

As mentioned above, two separate infecting suspensions of strain H37Rv were used in each experiment. No evidence was obtained of differences between the root-indices of the animals infected with these suspensions in the same experiment (Table 7, term c). In each experiment the infecting suspension was injected in two doses, 1.0 and 0.1 mg. The mean of the root-indices was 0.93 for animals receiving 1.0 mg. and 0.77 for those receiving 0.1 mg. The difference attains significance at the 0.5 per cent level (Table 7, term d). Thus, the variation in dose resulting from miscellaneous errors in the preparation of the infecting suspension was inconsiderable, whereas a known 10-fold decrease in dose decreased to a definite (though small) extent the values of the root-indices of virulence.

Further evidence on the effect of variation in the infecting suspensions is provided by relating the viable count on the infecting suspension to the root-index of virulence obtained with it, separately for tests on 24 Indian cultures (Fig. 1) and on 58 British cultures (Fig. 2). No association is evident (Fig. 1:  $r=0.20$ ,  $P>0.3$ ; Fig. 2:  $r=0.10$ ,  $P>0.4$ ). Thus, variation in the numbers of bacilli in the infecting suspensions, as measured by the viable counts, did not influence the values of the root-indices of virulence. Clumps of bacilli

FIG. 1  
*Viable counts on infecting suspensions from 24 Indian cultures related to root-indices of virulence*



were sometimes visible in the infecting suspension, and it will be appreciated that the viable counts cannot, therefore, be considered as accurate measures of either the number or the weight of bacilli present. In summary, no

FIG. 2  
*Viable counts on infecting suspensions from 58 British cultures related to root-indices of virulence*

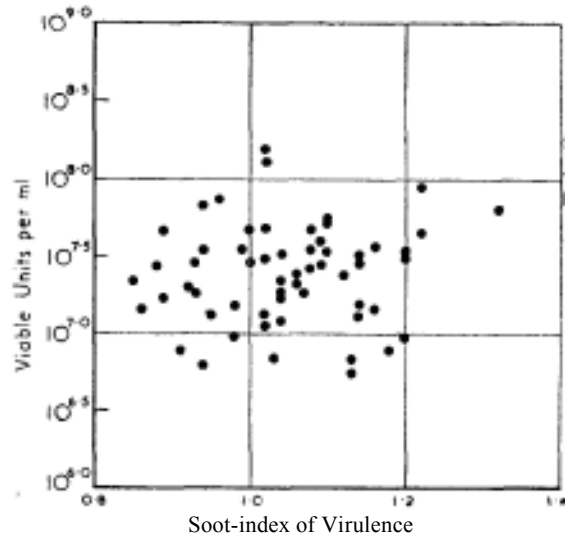
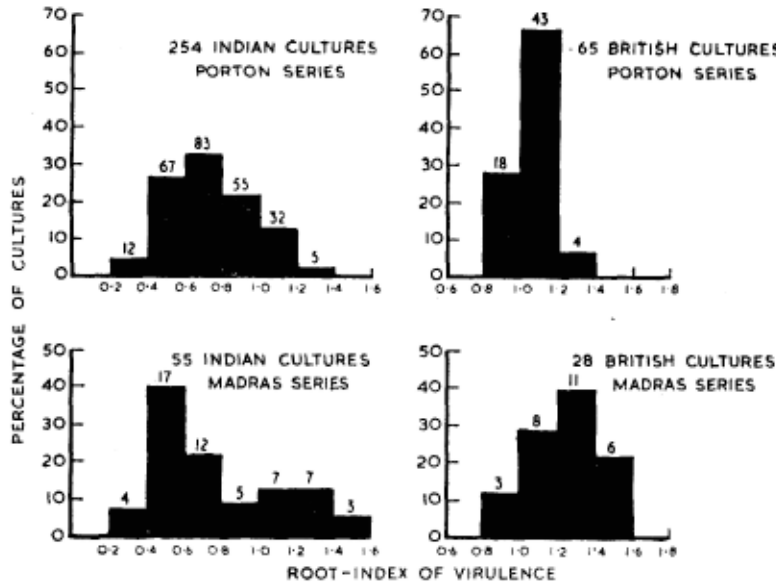


FIG. 3

Distributions of root-indices of virulence obtained with Indian and British cultures in the Porton and the Madras series



evidence of the existence of variation between infecting suspensions was obtained, and, if such variation existed, it was apparently unrelated to the root-index of virulence.

*Further terms in the analyses of variance*

The further terms in the analyses of variance set out in Tables 5, 6 and 7 will be considered in section B.

*Adjustment of the Madras to the Porton Series*

Histograms of the root-indices of virulence on single cultures from the 254 Indian patients in the Porton series and from the 55 Indian patients in the Madras series are set out on the left in Fig. 3 (the data are also given in Table 2 of Bhatia *et al.* (1961a)). It is apparent, particularly in the Porton series, that the distributions approximate to normality, but that they differ both in their means and in their standard deviations, which are:

	Mean	Standard deviation
Indian cultures, Porton series	0.7285	0.223
Indian cultures, Madras series	0.7886	0.336

Since cultures from 27 of the Indian patients were tested only in the Madras series, their

root-indices were adjusted to correspond to the Porton series by allowing for the differences both in the means and in the standard deviations of the two distributions, using the following equation:

$$\frac{Y-0.7285}{0.223} = \frac{X-0.7886}{0.336} \dots\dots\dots (1)$$

where X is the root-index of virulence before adjustment and Y is the adjusted value.

$$\text{From (1) } Y = 0.66 X + 0.21 \dots\dots\dots (2)$$

Cultures from 28 of the 55 patients in the Madras series were also tested in the Porton series, and in 23 of these patients the same culture was tested in both series. For these 28 patients, the values of the root-indices of virulence obtained in the Madras series both before (X) and after adjustment (Y) are compared in Table 9 with the root-indices obtained in the Porton series. The success of the adjustment was evaluated by comparing the variation between the adjusted root-index in the Madras series and the root-index obtained on the (usually) same culture in the Porton series, with the natural variation in response from guinea-pig to guinea-pig estimated from the 6-week

TABLE 9  
Effect of adjustment of root-indices of virulence on cultures from 28 Indian patients tested in both the Madras and the Porton series

Patient No.	Madras series		Porton series
	Observed root-index of virulence (X)	Adjusted root-index of virulence (Y)	Observed root-index of virulence
1	0.44	0.50	0.58
2	0.46	0.51	0.83
3	0.50	0.54	0.48
4	0.54	0.57	0.52
5	0.58	0.59	0.36
6	0.58 <sup>1</sup>	0.59	0.97
7	0.59	0.60	0.47
8	0.60	0.61	0.64
9	0.63	0.63	0.66
10	0.64	0.63	0.43
11 <sup>1</sup>	0.68	0.66	0.52
12	0.68	0.66	0.47
13	0.70	0.67	0.41
14	0.76	0.71	0.54
15	0.80	0.74	0.58
16	0.90	0.80	0.65
17 <sup>1</sup>	0.93	0.82	0.48
18	0.98	0.86	0.82
19	1.06	0.92	1.00
20	1.08	0.92	0.89
21	1.10	0.94	0.83
22	1.14	0.96	0.93
23 <sup>1</sup>	1.21	1.01	0.99
24	1.22	1.02	1.08
25	1.28	1.05	1.08
26 <sup>1</sup>	1.38	1.12	1.18
27	1.41	1.14	0.90
28 <sup>1</sup>	1.46	1.17	1.10

Source of variation	DF	Mean square	F	P
Between adjusted Madras				
root-index and observed Porton root-index for same patient ...	28	0.0140	1.01	>0.9
Residual <sup>2</sup> ...	27	0.0138		

<sup>1</sup> Different cultures from the same patient tested in the two series.

<sup>2</sup> Interaction weeks X patients obtained from the results at Porton for the 28 patients.

and 12-week root-indices of the same 28 cultures in the Porton series. The adjustment appears to have been successful, since the former variation was practically the same as the latter.

In a previous publication (Tuberculosis Chemotherapy Centre, 1960), which presented

the main findings of a study of three different regimens of isoniazid alone compared with isoniazid plus PAS, the results of the virulence tests reported here were used to assess more precisely the response to the four different regimens. In this connexion, the results of the tests on the cultures from the 27 patients tested only in the Madras series were adjusted to correspond to the Porton series, but the procedure used for adjustment differed slightly from the method described above. The adjustment equation, corresponding to equation 2, was derived in the same manner, but from the distributions of the *square roots of the means* of the 6-week and 12-week indices (score divided by survival period) and not, as above, from the distributions of the *means of the square roots* of these indices. The differences found between the adjusted values obtained by these two methods were small.

#### B. CRITIQUE OF THE VIRULENCE TEST

In the following section the root-indices of Indian cultures tested in the Madras series are the observations unadjusted by the procedure described above.

#### Variation between cultures

Among the cultures obtained from Indian patients the variation in the root-indices of virulence from culture to culture was greater than the natural variation in the response of the guinea-pigs in the tests. This difference attains significance at the 0.1 per cent level in both the Porton and the Madras series (Table 5, terms c and h, and Table 6, terms c and e). Among the cultures from British patients, significant variation from culture to culture was found in the Porton series Table 5, term c,  $P=0.001$ ), but not in the smaller Madras series (Table 6, term c,  $P=0.1$ ). The implications of these findings are considered further in the paper by Bhatia *et al.* (1961a).

#### Precision of the estimates of virulence of Indian cultures

The 95 per cent confidence limits for root-indices of virulence in tests done on Indian cultures in four guinea-pigs in the Porton series and on two guinea-pigs in the Porton and <sup>1</sup> the Madras series have been calculated from the residual mean squares in the analyses

of variance (Table 5, term h, and Table 6, term e). They are as follows:

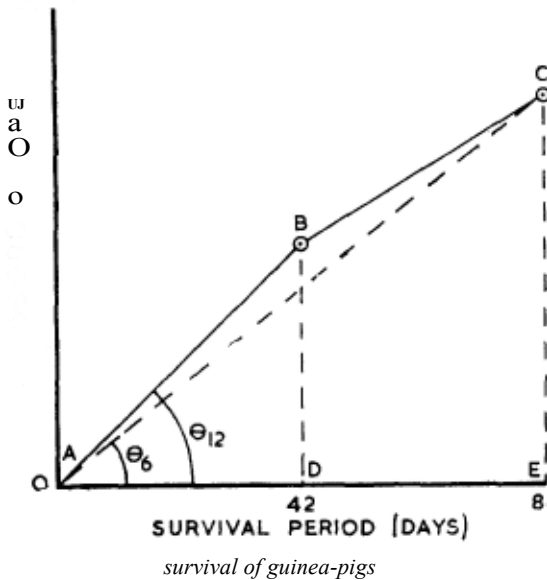
Series	Number of guinea-pigs per culture	Residual	95% confidence limits of root-index of virulence
Porton	4	Duplicate guinea-pigs (Table 5)	$\pm 0.151$
Porton	2	Interaction: weeks X cultures (Table 6)	$\pm 0.231$
Madras	2	Interaction: weeks X cultures (Table 6)	$\pm 0.222$

*Relationship between post-mortem score and survival period*

The relationship between the post-mortem score and the survival period of the guinea-pigs in a virulence test is represented diagrammatically in Fig. 4, where B and C represent the findings on 6-week and 12-week animals, respectively. The index of the 6-week guinea-pigs is the score, BD, divided by the survival period, AD, or  $\tan \theta_6$ . Similarly, the index of the 12-week guinea-pigs is  $\tan \theta_{12}$ . If a linear relationship between score and survival period exists, then  $\theta_6 = \theta_{12}$ , and the 6-week and 12-week indices would be equal. However, the 6-week indices were found to be greater,

FIG. 4

*Relationship between post-mortem score and period of*



on the average, than the 12-week indices (and similarly for the root-indices), indicating a non-linear relationship between score and survival period (A—B—C in Fig. 4). The difference between weeks attains a high degree of statistical significance in all of the analyses both of the indices (not tabulated here) and of the root-indices (term d of Tables 5 and 6 and term e of Table 7).

*The square root transformation*

In an earlier publication (Mitchison *et al.*, 1960) the post-mortem score divided by the survival period in days was defined as the index and the mean of the 6-week and 12-week indices (termed here the *index* of virulence) was employed as a measure of virulence. In the present report the mean of the square roots of the 6-week and 12-week indices has been used instead, and is called the *root-index* of virulence. The extent to which the square root transformation resulted in data showing additivity of effects together with normal and independent distribution of the errors with constant variance, conditions necessary for the valid application of the analysis of variance technique, is considered below.

*Homogeneity of error variance.* The residual mean squares in the analyses of variance are set out in Tables 10 and 11, related to values of the indices of virulence in the former and to values of the root-indices of virulence in the latter. High mean squares were associated with high values of the indices of virulence, especially in the Madras series, in the Porton series with two guinea-pigs per culture and in the 12-week duplicate guinea-pigs on British cultures in the Porton series. Bartlett's tests applied to the data tabulated yielded significant evidence of heterogeneity ( $P < 0.001$ ) in all the three series. After application of the square root transformation there appeared to be little association between the values of the residual mean squares and the root-indices of virulence. Bartlett's tests yielded evidence of heterogeneity only in the Porton series with four guinea-pigs per culture, and even here the chance probability ( $P = 0.01-0.02$ ) was larger than the corresponding probability ( $P < 0.001$ ) based on the indices of virulence.

The variation among residual mean squares obtained in the 13 experiments of the Porton series was also studied, and the findings are presented in Table 12. The mean squares

TABLE 10  
Residual mean squares related to indices of virulence

Race of patient	Index of virulence	Porton series						Madras series	
		4 guinea-pigs per culture				2 guinea-pigs per culture		2 guinea-pigs per culture	
		Between duplicate 6-week indices		Between duplicate 12-week indices		Weeks X cultures interaction		Weeks x cultures interaction	
		DF	Mean square	DF	Mean square	DF	Mean square	DF	Mean square
Indian	0.0-0.4-	36	0.0368	37	0.0178	47	0.0315	19	0.0110
	0.4-	48	0.0615	49	0.0573	53	0.0504	14	0.0474
	0.8 or above	36	0.0817	39	0.0652	26	0.1138	19	0.1221
	Total	120	0.0601	125	0.0481	126	0.0564	52	0.0614
British	0.7-	28	0.0495	29	0.0455		— <sup>1</sup>		— <sup>2</sup>
	1.1 or above	34	0.0581	35	0.1153				
	Total	62	0.0542	64	0.0837			27	0.2370
Bartlett's test	Corrected $\chi^2$	35.573				14.911		39.729	
	DF	9				2		3	
	P	<0.001				<0.001		<0.001	

<sup>1</sup> Estimates of mean squares have not been tabulated since there were no cultures.

<sup>2</sup> Estimates of mean squares have not been tabulated since 25 of the 28 cultures had indices of virulence of 1.1 or above.

tabulated were calculated from the results with Indian and British cultures on duplicate 6-week and duplicate 12-week guinea-pigs. There was greater variation among the residual mean squares based on indices of virulence than among the corresponding mean squares based on root-indices of virulence. Bartlett's test yielded evidence of heterogeneity among the former ( $P=0.001-0.01$ ), but no evidence of heterogeneity among the latter ( $P=0.1-0.2$ ).

*Additivity of effects.* Additivity of the two main effects of the analysis of variance—namely, between cultures and between weeks—would correspond to a similar relationship between the score and the survival period at different levels of virulence. The mean differences between the 6-week and the 12-week results, related both to the index and to the root-index

of virulence, are set out in Table 13. In general, there is a trend, not easy to see, indicating that the mean difference was low for low values of the *index* and for high values of the *root-index*. This trend implies that the condition of additivity was not satisfied completely either for the index or for the root-index.

In the Porton series with four guinea-pigs per culture, the constancy of the difference between weeks was tested by comparing the interaction between cultures and weeks with the residual mean square between duplicate guinea-pigs; in the Porton series with two guinea-pigs per culture and in the Madras series, it was studied by a test developed by Tukey (1949). The results of these tests of additivity, considered separately for the Indian and the British cultures, are set out in Table 14. Among the five

TABLE 11  
Residual mean squares related to root-indices of virulence

Race of patient	Root-index of virulence	Porton series						Madras series	
		4 guinea-pigs per culture				2 guinea-pigs per culture		2 guinea-pigs per culture	
		Between duplicate 6-week root-indices		Between duplicate 12-week root-indices		Weeks x cultures interaction		Weeks X cultures interaction	
		DF	Mean square	DF	Mean square	DF	Mean square	DF	Mean square
Indian	0.0-	34	0.0261	35	0.0199	43	0.0265	20	0.0160
	0.6-0.9 or above	54	0.0224	55	0.0311	59	0.0268	13	0.0201
		32	0.0152	35	0.0162	24	0.0251	19	0.0263
	Total	120	0.0215	125	0.0238	126	0.0263	52	0.0208
British	0.8-	32	0.0105	33	0.0166		— <sup>1</sup>		— <sup>2</sup>
	1.05 or above	30	0.0115	31	0.0250				
	Total	62	0.0110	64	0.0207			27	0.0392
Bartlett's test	Corrected x <sup>2</sup>	20.469				0.030		4.915	
	DF	9				2		3	
	P	0.01-0.02				0.98		0.1-0.2	

<sup>1</sup> Estimates of mean squares have not been tabulated since there were no cultures.

<sup>2</sup> Estimates of mean squares have not been tabulated since 25 of the 28 cultures had root-indices of virulence of 1.05 or above.

sets of tests on indices, significant non-additivity was found in two—namely, in the results in the Porton series on Indian cultures set up on four guinea-pigs each (P=0.001) and in the Madras series on British cultures (P=0.03). In the corresponding tests on root-indices, there was also evidence of non-additivity in two of the sets of tests, again in the Porton series on Indian cultures (P=0.02), and in the Madras series on Indian cultures (P=0.01-0.02).

The reasons for non-additivity with the transformed data were examined further in the Madras series on Indian cultures, by plotting the products obtained in Tukey's test for individual cultures against the values of the root-index, and it seems probable that it was mainly

due to tests in which the 12-week guinea-pig had died from tuberculosis. The proportion of 12-week guinea-pigs which died from tuberculosis was high in the Madras series, there being 12 (21.8 per cent) deaths among 55 guinea-pigs infected with Indian cultures (Table 15). In the tests where the 12-week guinea-pig died before 12 weeks, the 12-week root-index was not very different from the 6-week root-index, and consequently the difference between weeks was smaller than usual. A further consequence of tuberculous deaths is that the indices or root-indices of virulence obtained in those tests in which they occurred are slightly biased in an upward direction, owing to the non-linear relationship between

TABLE 12  
Residual mean squares for experiments in the Porton series

Experiment No.	Degrees of freedom	Between duplicate guinea-pigs mean square	
		Index	Root-index
Por. 1	52	0.0410	0.0163
2	64	0.0928	0.0307
3	50	0.0421	0.0163
4	27	0.0604	0.0230
5	27	0.0488	0.0223
6	23	0.0504	0.0161
7	25	0.0591	0.0199
8	17	0.1366	0.0291
9	18	0.0525	0.0223
10	24	0.0240	0.0091
11	24	0.0421	0.0157
12	10	0.0667	0.0181
13	10	0.0666	0.0166
Por. 1-13	371	0.0592	0.0204
Bartlett's test	Corrected		
	$\chi^2$	30.477	17.810
	DF	12	12
	P	0.001-0.01	0.1-0.2

score and survival period. Thus, if the 12-week guinea-pig died early, its root-index would be similar to the 6-week root-index, which is, on the average, higher than the root-index of virulence.

Although additivity between main effects is considered to be the most essential characteristic of the population for the valid application of the analysis of variance technique (Snedecor, 1956), the root-index of virulence, employed as a measure of virulence, is an average of the responses in 6-week and 12-week guinea-pigs and is meaningful despite minor variations in the size of the difference between weeks at different levels of virulence.

*Normality of distributions.* The distributions of the indices of virulence obtained with Indian and British cultures in the Porton and the Madras series are shown in Fig. 5. The corresponding distributions of the root-indices of virulence are illustrated in Fig. 3. It is apparent that the square root transformation has resulted in distributions that are more nearly symmetrical. This is particularly true of the large series of Indian cultures tested at Porton; the distribution of the indices of virulence showed positive skewness ( $g_1=0.81$ ,  $P<0.001$ ) and negative kurtosis ( $g_2 = -0.09$ , non-significant), whereas the distribution of the root-

FIG. 5

Distributions of indices of virulence obtained with Indian and British cultures in the Porton and the Madras series

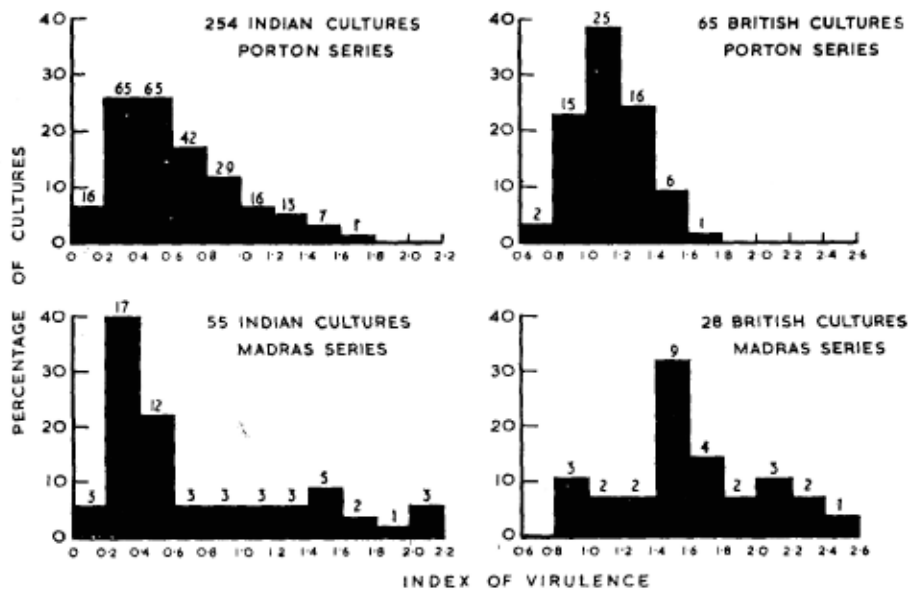


TABLE 13  
Differences between 6-week and 12-week indices and root-indices, related to the virulence of cultures

Series	Race of patient	Index of virulence	Difference between 6-week and 12-week indices		Root-index of virulence	Difference between 6-week and 12-week root-indices	
			Number of cultures	Mean		Number of cultures	Mean
Porton	Indian	0.0-0.4-0.8 or above	85 103 66	0.167 0.279 0.283	0.0-0.6-0.9 or above	79 115 60	0.185 0.188 0.126
		Total	254	0.243	Total	254	0.172
		British	29 36	0.306 0.229	0.8-1.05 or above	33 32	0.171 0.091
	Total	65	0.263	Total	65	0.132	
Madras	Indian	0.0-0.4-0.8 or above	20 15 20	0.268 0.551 0.324	0.0-0.6-0.9 or above	21 14 20	0.312 0.356 0.133
		Total	55	0.365	Total	55	0.258
	British	0.8 or above	28 <sup>1</sup>	0.489	0.9 or above	28 <sup>2</sup>	0.186

<sup>1</sup> Including 25 cultures with an index of 1.1 or above.

<sup>2</sup> Including 25 cultures with a root-index of 1.05 or above.

indices of virulence was less skewed ( $g_1=0.31$ ,  $P=0.05$ ) but had more kurtosis ( $g_2= -0.65$ ,  $P=0.04$ ).

In summary, the results of applying the square root transformation were:

(a) Heterogeneity of error variance was substantially decreased, especially in the Madras series and in the Porton series with two guinea-pigs per culture.

(b) Non-additivity was decreased slightly in the Porton series and increased slightly in the Madras series. The Porton series contributed the major portion of the virulence tests, so that, on balance, a slight gain may have been obtained by the transformation.

(c) A more symmetrical distribution of the results of the virulence tests was obtained.

Thus, more reliance can be placed on the results of the analysis of variance using the root-indices in place of the indices. However, when all the analyses of variance considered in section A were done on the indices, none of the conclusions that could be drawn from them differed from those reported here, nor were the probability levels altered appreciably.

*The arrangement of guinea-pigs in the test*

The main purpose in killing guinea-pigs at two set dates (separated by six weeks) after infection was to obtain evidence on the progress of the disease. Thus, it has been reported that Indian cultures of low virulence produce disease which tends to regress between 6 and 12 weeks (Mitchison *et al.*, 1960). Having

TABLE 14  
Results of tests of additivity in the Porton and the Madras series

Series	Race of patient	Number of guinea-pigs per culture	Source of variation	DF	Index			Root-index		
					Mean square	F	p	Mean square	F	p
Porton	Indian	4	Interaction : week X cultures	124	0.0841	1.56	0.001	0.0310	1.37	0.02
			Between duplicate guinea-pigs	245	0.0540			0.0227		
	2	Interaction: week X cultures	128	0.0586			0.0267			
		Non-additivity	1	0.0291	—	NS <sup>1</sup>	0.0482	1.81	0.2	
	Residual	127	0.0588			0.0266				
	British	4	Interaction : weeks X cultures	64	0.0844	1.22	0.2	0.0201	1.26	0.1-0.2
Between duplicate guinea-pigs			126	0.0692			0.0159			
Madras	Indian	2	Interaction : week x cultures	54	0.0660			0.0247		
			Non-additivity	1	0.0203	—	NS	0.1407	6.25	0.01-0.02
	Residual	53	0.0668			0.0225				
	British	2	Interaction : weeks x cultures	27	0.2370			0.0392		
			Non-additivity	1	1.0830	5.30	0.03	0.0820	2.19	0.1-0.2
	Residual	26	0.2044			0.0375				

<sup>1</sup> NS indicates that the variance ratio is less than 1.0.

TABLE 15  
Deaths from tuberculosis in guinea-pigs in the Porton and the Madras series

Series	Guinea-pig group	Indian cultures			British cultures		
		Number of guinea-pigs	Deaths from tuberculosis		Number of guinea-pigs	Deaths from tuberculosis	
			No.	%		No.	%
Porton	6-week	379	8	2.7	130	8	6.2
	12-week	379	49	12.9	130	69	5.31
	Total	758	57	7.5	260	11	29.6
Madras	6-week	55	6	70.9	28	9	32.1
	12-week	55	12	21.8	28	19	67.9
	Total	110	18	16.4	56	28	50.0

obtained this evidence, it is pertinent to inquire whether any further advantage was gained by this procedure and, if not, whether killing all the animals at either 6 weeks or 12 weeks would be an improvement in future virulence tests.

*Inter-experimental variation.* A subsidiary reason for killing the guinea-pigs at 6 weeks and at 12 weeks was to reduce inter-experimental variation due to systematic changes in scoring from one day of post-mortem examination to another, by taking the average of the two test results for the same culture obtained on successive days of examination. Estimates of inter-experimental variation, expressed as standard deviations, were derived separately for 6-week root-indices, 12-week root-indices and root-indices of virulence, and are set out in Table 16. These estimates range from 0.00 to 0.07 for the 6-week root-indices, from 0.00 to 0.08 for the 12-week root-indices and from 0.00 to 0.07 for the root-indices of virulence. No apparent reduction in inter-experimental variation has resulted from the use of 6-week and 12-week guinea-pigs in the tests, nor is there any clear evidence that it is smaller with either 6-week or 12-week animals.

*Efficiencies of various arrangements.* The efficiencies of various possible arrangements of guinea-pigs were studied, using the root-indices obtained in the Porton series. By efficiency is meant the ability of an arrangement to

TABLE 16  
*Estimates of inter-experimental variation with 6-week root-indices, 12-week root-indices and root-indices of virulence*

Series	Type of culture	Number of guinea-pigs per culture	Inter-experimental variation (square root of component of variance)		
			6-week		Root-indices of virulence
			root-indices	root-indices	
Porton	Indian	4	0.00	0.00	0.00
	Indian	2	0.04	0.08	0.07
	British	4	0.03	0.07	0.05
	H37Rv	8	0.07	0.08	0.07
Madras	Indian	2	0.07	0.00	0.06
	British	2	0.00	0.00	0.00

distinguish between the virulence of different cultures. It has been expressed (Table 17) as the relative numbers of guinea-pigs required to equalize the variance ratio (the ratio of the mean square for cultures in the same experiment to the residual mean square), that is, to equalize the relative efficiencies of the various arrangements. The arrangements considered were:

- (a) All guinea-pigs killed at 6 weeks.

TABLE 17  
*Efficiencies of various arrangements of guinea-pigs in the virulence tests*  
(Derived from root-indices of virulence in the Porton series)

Relative number of guinea-pigs required to equalize the variance ratios						
Race of patient	2 guinea-pigs per culture			4 guinea-pigs per culture		
	Both killed at 6 weeks	Both killed at 12 weeks	1 killed at 6 weeks and 1 at 12 weeks	All killed at 6 weeks	All killed at 12 weeks	2 killed at 6 weeks and 2 at 12 weeks
Indian	100*	97*	136*	100†	97†	104†
British	100*	128*	309*	100†	128†	129†

\* Derived from mean squares in Table 5 and analysis of variance done on one 6-week guinea-pig and one 12-week guinea-pig, selected at random from two at 6 weeks and two at 12 weeks.

† Derived from mean squares in Table 5.

TABLE 18  
*Tests on residual mean squares*

Race of patient		Index		Root-index	
		6-weeks results	12-week results	6-week results	12-week results
Indian	Corrected				
	$\chi^2$ *	5.437	16.167	2.263	4.958
	DF	2	2	2	2
	P	0.07	<0.001	0.3	0.09
British	F	1.17	2.53	1.10	1.51
	DF	34,28	35,29	30,32	31,33
	P	>0.2	0.005-0.01	>0.2	0.1-0.2

\* Bartlett's test.

- (b) All guinea-pigs killed at 12 weeks.  
(c) Half the guinea-pigs killed at 6 weeks and the other half at 12 weeks.

In a design with two guinea-pigs per culture, the relative numbers of guinea-pigs required for equal efficiency with Indian cultures are 100, 97 and 136, respectively, for experimental arrangements *a*, *b* and *c*. The corresponding figures with British cultures are 100, 128 and 309, respectively. In a design with four guinea pigs per culture, the relative numbers are 100, 97 and 104 for Indian cultures, and 100, 128 and 129 for British cultures. Thus, it appears that arrangement *c*, employed in the Porton series (two animals per culture) and in the Madras series, is the least efficient, and that the most efficient design is probably to kill all the guinea-pigs at six weeks, whether two or four guinea-pigs are used in the test.

*Homogeneity of error variance, and normality of distributions in the separate 6-week and 12-week results.* The residual mean squares (between duplicate guinea-pigs) in the Porton series were related (separately for 6-week and 12-week results and for Indian and British cultures) to values of indices of virulence and root-indices of virulence in Tables 10 and 11. The results of Bartlett's tests on these residuals for Indian cultures are set out in the upper half of Table

18. The mean squares based on 6-week root-indices show least evidence of heterogeneity ( $P=0.3$ ). For British cultures, the mean squares based on 6-week indices and 6-week root-indices were least heterogeneous (lower half of Table 18,  $P>0.2$ ).

The distributions of the indices and root-indices obtained with Indian and British cultures at 6 and 12 weeks are set out in Table 19. For Indian cultures, the distribution of the 6-week root-indices was more nearly normal than the other distributions, with neither the skewness nor kurtosis attaining statistical significance. For the British cultures, none of the distributions showed significant evidence of skewness or kurtosis. However, the estimates of  $g_1$  and  $g_2$  were closer to zero for the distribution based on the 6-week root-indices than for that based on the 6-week indices.

In summary, considering both homogeneity of variance and normality of the distributions, the 6-week root-indices appear most amenable to analysis of variance, both for Indian and for British cultures. This suggests that it would be an advantage in the design of future virulence tests to kill all guinea-pigs at six weeks.

#### *The breeds of guinea-pig*

A comparison of the results obtained from Indian cultures with the DH-breed guinea-pigs (two animals per culture) at Porton and the M-breed guinea-pigs at Madras showed that the variation from culture to culture among the root-indices obtained with the DH-breed animals was less than that which occurred with the M-breed animals (Fig. 3; Table 6, term *c*). Furthermore, the natural variation in the response of individual guinea-pigs of the two breeds was similar (Table 6, term *e*) and the average of the root-indices of virulence with the DH breed (0.728) was not very different from the average with the M breed (0.794). These findings indicate that, with cultures of high virulence, the root-indices of the DH-breed guinea-pigs were smaller than those of the M-breed guinea-pigs (this tendency was also apparent in the root-indices of virulence, shown in Fig. 3, and in the deaths from tuberculosis, shown in Table 15, for the highly virulent cultures from British patients carried out, in both series), whereas, with cultures of low virulence, the root-indices of the DH-breed guinea-pigs were larger than those of the M-

TABLE 19

Distributions of indices and root-indices for 6-week and 12-week guinea-pigs in the Porton series

Race of patient	Index	6-week guinea-pigs		12-week guinea-pigs		Root-index	6-week guinea-pigs		12-week guinea-pigs	
		No.	%	No.	%		No.	%	No.	%
		Indian	0.0-	12	4.7		59	23.2	0.0-	0
0.2-	39		75.4	71	28.0	0.2-	6	2.4	49	19.3
0.4-	53		20.9	42	76.5	0.4-	38	75.0	66	26.0
0.6-	56		22.0	31	72.2	0.6-	74	29.7	64	25.2
0.8-	32		72.6	23	9.1	0.8-	75	29.5	46	18.1
1.0-	25		98	16	6.3	1.0-	46	75.7	22	5.7
1.2-	20		79	5	2.0	1.2-	13	5.1	5	2.0
1.4-	8		3.1	5	2.0	1.4 or above	1	0.8	0	0.0
1.6-	5		7.0	2	0.8					
1.8 or above	4		1.6	0	0.0					
Total ...	254	100.0	254	100.1	Total ...	254	100.0	254	100.1	
$g_1$	0.80		0.99		$g_1$	0.7		0.36		
P	<0.001		<0.001		P	>0.2		0.01-0.02		
$g_2$	0.46		0.45		$g_2$	-0.44		-0.68		
P	0.1-0.2		0.1-0.2		P	0.1-0.2		0-0.3		
British	0.2-	0	0	1	2	0.6-	0	0	4	6
	0.4-	0	0	2	3	0.8-	8	72	31	48
	0.6-	1	2	12	18	1.0-	45	69	25	38
	0.8-	7	77	20	57	1.2-	12	18	5	8
	1.0-	18	28	15	23	1.4 or above	0	0	0	0
	1.2-	24	37	8	72					
	1.4-	11	77	6	9					
	1.6-	2	3	1	2					
	1.8 or above	2	3	0	0					
	Total ...	65	101	65	100	Total ...	65	99	65	100
$g_1$	0.38		0.23		$g_1$	0.10		0.06		
P	0.2-0.3		0.4-0.5		P	0.7-0.8		0.8-0.9		
$g_2$	0.38		-0.30		$g_2$	-0.08		-0.19		
P	0.5-0.6		-0.6		P	0.8-0.9		0.7-0.8		

breed guinea-pigs. Thus, the tests on DH-breed guinea-pigs were less effective in distinguishing differences of virulence than were those on M-breed guinea-pigs.

The efficiency with which the two breeds distinguished differences in virulence was again

expressed as the relative numbers of guinea-pigs of the two breeds required for equal efficiency (Table 20). For every 100 M-breed animals the number of DH-breed guinea-pigs which would result in equal efficiency was estimated as 308 for Indian cultures and 347

TABLE 20  
*Efficiencies of DH-breed and M-breed guinea-pigs  
 in the virulence tests*  
 (Derived from root-indices of virulence)

Race of patient	Relative number of guinea-pigs required to equalize the variance ratios	
	DH-breed	M-breed
Indian	308*	100*
British	347†	100†

\* Derived from mean squares in Table 6.

† Derived from mean squares in Table 6 and analysis of variance done on one 6-week guinea-pig and one 12-week guinea-pig, selected at random from two at 6 weeks and two at 12 weeks.

for British cultures. In making this comparison of the relative efficiencies of the DH-breed and the M-breed guinea-pigs, it has been assumed that the scoring procedure was uniform at Porton and Madras, even though it was necessarily done at the two centres by different observers. This assumption is supported by evidence presented previously (Mitchison *et al.*, 1960).

### Discussion

The conditions under which the present investigation of virulence was carried out might have introduced factors, in addition to the virulence of the cultures, affecting the results of the tests. The initial plan was to do all tests in M-breed guinea-pigs. Difficulties in breeding these animals under tropical conditions made this impossible, and large-scale facilities at Porton only became available towards the end of the study. In consequence, the tests were done in two breeds of guinea-pig, the M breed at Madras and the DH breed at Porton, and about half of the Indian cultures were tested after storage at  $-20^{\circ}\text{C}$ . Furthermore, they were tested in 25 experiments, extending over a period of two-and-a-half years. However, the results of the Madras series were successfully adjusted, to permit amalgamation with those of

the much larger Porton series, storage at  $-20^{\circ}\text{C}$  was found not to have affected the virulence of the cultures, and interexperimental variation was shown to have been very small. Thus, the results of the tests appear to give a true measure of virulence, little influenced by these potential external sources of variation.

In an earlier publication (Mitchison *et al.*, 1960) the index—that is, the post-mortem score divided by the survival time in days—was adopted as the measure of virulence for four reasons. First, it indicated the rate of development of the lesions and was thus related to a more fundamental measure, the rate of multiplication of the bacilli in the organs of the guinea-pig. Secondly, it had an advantage over the use of the score alone, since it allowed comparison of the results of different tests, irrespective of whether deaths had occurred before the appointed day for the sacrifice of the animals. Thirdly, the results obtained with 6-week and 12-week guinea-pigs could be combined to give a single measure of virulence, thus gaining information on the progress of the disease with little loss in precision of the estimates of virulence. Finally, the indices obtained in the virulence tests, which were less numerous than those reported here, appeared acceptable for the standard statistical technique of analysis of variance. In the present report, on a much larger number of tests, the conditions underlying the use of analysis of variance with the indices did not seem to be entirely satisfied. In consequence, the square root of the index—that is, the root-index—was adopted as the measure of virulence. The results of the analyses of variance of the root-indices can be accepted with greater confidence since the square root transformation largely eliminated heterogeneity of error variance, and the root-indices were more nearly symmetrical in distribution than the indices. Nevertheless, additivity of the difference between weeks and the differences between cultures was not entirely satisfied by the root-indices of virulence in the tests on Indian cultures. However, the degree of non-additivity appeared to be small, so that the conclusions in the present report and in the accompanying papers (Bhatia *et al.*, 1961a; Ramakrishnan *et al.*, 1962<sup>1</sup>; Subbaiah *et al.*, 1962<sup>2</sup>), based on analyses of variance applied

<sup>1</sup> See article on page 124.

<sup>2</sup> See article on page 45.

to root-indices, can be considered as reliable. It will be appreciated that the other three reasons listed above for using the index also apply to the root-index.

The effect of the square root transformation is to expand the lower range of values of the index and contract the higher range. Equally spaced indices of 0.0, 0.5 and 1.0 correspond approximately to root-indices of 0, 0.7 and 1.0, in which the interval 0.0-0.7 is more than twice the interval 0.7-1.0. In a given series of tests, low values of the root-index will affect the mean *more* than low values of the index, and high values of the root-index will have *less* influence on it than high values of the index. Virulence in a system of the type used here is difficult to define in quantitative terms, and there is no definite evidence that either of these alternative measures is preferable in a biological sense. With markedly attenuated cultures, the disease produced is confined mainly to the site of inoculation and its draining lymph-nodes. The lesions in these sites, with the scoring system used in the present paper, are allocated only a possible total of 10 out of a maximum score of 100 for the whole guinea-pig. Thus, variation in virulence among such cultures results in only small changes in the score and therefore in the index, but causes relatively larger changes in the root-index. The square root transformation can therefore be considered as a simple alternative to the more cumbersome (but equally empirical) procedure of re-allocating the arbitrary score values in different proportions among the possible sites of the lesions to give more weight to variation among attenuated cultures.

Scores have frequently been used in experimental tuberculosis of guinea-pigs to assess the extent of the lesions visible to the naked eye in the organs (Table 21). The allocation of the scores among the organs has always been empirical and has varied from one investigation to another. The proportion of the total score which could be allocated to the site of inoculation and the lymph glands varied from 0 per cent to 62 per cent. Equal weights were usually given to the spleen, liver and lungs. The scoring system used in the present report resembles the system used by Feldman (1943), except for minor differences in the relative weights of the lesions in the spleen, liver and lungs. So far as can be discovered, no investigation of whether scores are acceptable

TABLE 21  
*Scoring systems in experimental tuberculosis of guinea-pigs*

Reference	Maximum score (expressed as a percentage of the total score) for extent of tuberculosis in:			
	Spleen	Liver	Lungs	Site of inoculation and draining lymph-nodes
Feldman (1943)	35	25	30	10
Steenken and Wolinsky (1947)	25	25	25	25
Bloch <i>et al.</i> (1949)	33	33	33	0
Dessau, Yeager and Kulish (1949)	12	12	12	62
Marshak and Kuschner (1950)	33	33	33	0
Mitchison <i>et al.</i> (1962)*	40	30	20	10

\* Present report.

for the analysis of variance technique has previously been described.

The main purpose of the investigation described here and in the companion papers was to relate the virulence in the guinea-pig of the Indian cultures to assessments of the patients' condition. Under these circumstances, and with limited numbers of guinea-pigs, it is more efficient to test a large number of cultures, each in a small group of guinea-pigs, than to make accurate estimates of virulence with large groups on a few cultures. In the basic test, two guinea-pigs were therefore infected. Of these two guinea-pigs one was killed at 6 weeks and one at 12 weeks, the latter in order to gain additional information on the progress of the disease. A reason for seeking this information was to make possible a comparison between the biological characteristics of attenuation in these isoniazid-sensitive cultures and attenuation in their isoniazid-resistant variants (the latter were tested in the same set of experiments and the results will be reported elsewhere). The arrangement with two guinea-pigs, one killed at 6 weeks and the other at 12 weeks, was found to be less efficient in detecting differences in virulence between cultures than the other possible arrangements, in which the two animals are both killed either at 6 weeks or at 12 weeks;

the loss in efficiency was small in the tests on Indian cultures, but larger in those on British cultures. A further advantage in killing both animals at the same time in future tests would be that non-additivity of the two main effects would cease to be a cause for lack of confidence in the analyses of the results. As to the choice between 6 and 12 weeks as the time for killing both animals, the 6-week period is preferable since the 6-week root-indices were found to be more homogeneous and their distribution did not depart significantly from normality. In addition, the proportion of deaths from tuberculosis, which is here shown to be a source of minor bias, would be lowered and, finally, the results of the tests would be available earlier. In the future design of virulence tests on cultures comparable to those reported here, it might well not be necessary to gain additional information on the progress of the disease during more than one interval after infection. Under such circumstances the procedure which would be most efficient and also most amenable to analysis of variance would be to kill two or more animals, all at six weeks.

The differences in efficiency found between the DH and the M breeds of guinea-pig is of interest. Cultures of high virulence produced lower root-indices and fewer deaths from tuberculosis in the DH-breed than in the M-breed animals, whereas cultures of low virulence produced higher root-indices in the DH-breed animals. Consequently, with similar arrangements and numbers of guinea-pigs in the tests, about three of the DH-breed animals were required for every one of the M-breed animals to achieve equal ability in discriminating between the virulence of cultures. In the interests of economy in guinea-pigs, it would clearly be an advantage to use only the M breed. In this breed, to kill all animals at six weeks would be of particular importance, since the heterogeneity and bias introduced by the high proportion of deaths from tuberculosis in 12-week animals was higher in the Madras than in the Porton series.

### Summary

1. Virulence tests in the guinea-pig were done on 281 isoniazid-sensitive cultures obtained from the same number of Indian patients on admission to a study of various regimens of domiciliary chemotherapy in the treatment of pulmonary tuberculosis, and on 93 cultures

from newly diagnosed, untreated British patients. The tests on 254 of the Indian cultures and on 65 of the British cultures were in DH-breed guinea-pigs at Porton, and the remaining 27 Indian cultures and 28 British cultures were tested in M-breed guinea-pigs at Madras.

2. In the test, 1 mg. of each culture was injected by the intramuscular route into two guinea-pigs or, for 125 Indian cultures and all 65 British cultures in the Porton series, into four guinea-pigs. Half the animals were sacrificed at 6 weeks and the other half at 12 weeks; the extent of tuberculosis in the organs was scored at the post-mortem examination, the maximum score per guinea-pig being 100. Animals dying before the appointed day were similarly scored. The score on each animal was divided by its survival period to give an index. The mean of the square roots of the 6-week index and the 12-week index (the root-index of virulence) was taken as the measure of virulence since it was found to be more acceptable for the analysis of variance technique than the index of virulence used by Mitchison *et al.* (1960).

3. Of the 254 Indian cultures in the Porton series, 143 were stored at  $-20^{\circ}\text{C}$  for 44-78 weeks (average, 62 weeks) before being tested. A comparison carried out on pairs of cultures, one stored at  $-20^{\circ}\text{C}$  and the other tested fresh, from 20 Indian patients showed no clear evidence of alteration in virulence.

4. The tests were done in 13 experiments at Porton and in 12 experiments at Madras over a period of two-and-a-half years. The results on the Indian and British cultures in both series, and on strain H37Rv, set up as a control in the majority of the experiments at Porton, indicated that inter-experimental variation was small in the Porton series and could not be detected in the Madras series.

5. In the tests on strain H37Rv, variation in the preparation of the infecting suspension did not appear to influence the root-indices of virulence, nor were the viable counts on the suspensions of Indian and British cultures associated with the values of the root-index. However, a known 10-fold decrease in the dose of bacilli lowered the root-index to a small extent.

6. To obtain comparable results throughout the study, the root-indices of virulence in the Madras series were adjusted to those in the Porton series by allowing for differences in the

means and standard deviations of the distributions for the two series. The adjustment appeared to be successful, since the adjusted root-indices in the Madras series were the same, within the limits of error of the test, as the root-indices in the Porton series obtained in tests done in both laboratories on cultures from the same 28 Indian patients.

7. The results of the tests in the Porton series indicate that to kill all guinea-pigs six weeks after infection would have the advantages of greater efficiency in detecting differences in the virulence of the cultures, of yielding results more acceptable for the analysis of variance technique, and of rapidity.

8. Cultures of high virulence produced fewer deaths from tuberculosis and lower root-indices in the DH-breed than in the M-breed guinea-pigs, whereas cultures of low virulence produced higher root-indices in the DH-breed guinea-pigs. In consequence, differences in virulence were shown less efficiently with the DH-breed guinea-pigs, about three DH animals being of equal efficiency to one M-breed animal.

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# IDENTIFICATION OF TUBERCLE BACILLI FROM INDIAN PATIENTS WITH PULMONARY TUBERCULOSIS\*†

K. L. THOMAS, S. JOSEPH, T. V. SUBBAIAH AND J. B. SELKON

Pretreatment cultures of bacilli from Indian patients with active pulmonary tuberculosis admitted to a controlled domiciliary chemotherapy study by the Tuberculosis Chemotherapy Centre, Madras, were subjected to a series of *in vitro* tests designed to identify the bacilli as human or bovine tubercle bacilli, or as anonymous mycobacteria. For the purposes of comparison, pretreatment cultures from British patients with pulmonary tuberculosis were examined by the same series of identification tests.

Cultures identifiable as mammalian tubercle bacilli were obtained from all the 341 Indian patients admitted to the chemotherapy study. Tests for niacin production were carried out on the cultures from 277 of these patients; all gave positive results, indicating that the bacilli in question were *Mycobacterium tuberculosis* var. *hominis*. The cultures from the Indian patients yielded results similar to those of the cultures from the British patients in all the *in vitro* tests except the thiacetazone-sensitivity test. In this test the Indian cultures differed from the British cultures, being on the average less sensitive and showing greater variation in sensitivity among themselves.

## Introduction

A search of the literature has not revealed any previous reports of taxonomic studies of tubercle bacilli obtained from Indian patients. The objects of this paper are to report on the *in vitro* characteristics of cultures of tubercle bacilli obtained from Indian patients, to compare these characteristics with those of cultures from patients of British origin and to report on the anonymous mycobacteria encountered in this study. The cultures which are the subject of this paper were obtained from Indian patients participating in a controlled study of four different regimens of domiciliary chemotherapy (Tuberculosis Chemotherapy Centre, 1960) and from British patients attending chest clinics in England.

It was particularly important to identify whether the cultures obtained from Indian patients were *Mycobacterium tuberculosis* var. *hominis* or *Myco. tuberculosis* var. *bovis*, for anonymous mycobacteria can cause pulmonary disease, clinically and anatomically indistinguishable from tuberculosis (Timpe and Runyon, 1954; Wood, Buhler and Pollak, 1956; Nassau and Hamilton, 1957; Runyon, 1959). It has also been suggested (Palmer, 1953) that

the high prevalence of low-grade tuberculin sensitivity encountered in some countries, including India (World Health Organization Tuberculosis Research Office, 1955), may be the result of infection with anonymous mycobacteria. Since Indian cultures of tubercle bacilli vary widely in their virulence in the guinea-pig, many strains being attenuated (Frimodt-Moller, Mathew and Barton, 1956; Mitchison *et al.*, 1960), guinea-pig inoculation is not a reliable identification test for Indian strains of tubercle bacilli. For the same reason animal virulence studies are not reliable as a means of differentiating between human and bovine strains of *Myco. tuberculosis* isolated from Indian patients. A series of *in vitro* tests was therefore selected which would differentiate mammalian strains of tubercle bacilli from the anonymous myco-bacteria and identify strains of tubercle bacilli as either human or bovine.

## Methods

### MYCOBACTERIAL CULTURES

#### *Indian cultures*

Cultures were obtained before treatment was started from each of the 341 patients, all of

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whom had active pulmonary tuberculosis, who were admitted to a controlled study of four different chemotherapeutic regimens (Tuberculosis Chemotherapy Centre, 1960). The patients admitted to this study (*a*) had sputum which either contained acid-alcohol-fast bacilli on smear examination (93.0 per cent of the 341 patients) or yielded cultures that morphologically resembled *Myco. tuberculosis* (no patients failed to be admitted to the study because they yielded cultures of mycobacteria without this resemblance), (*b*) were aged 12 years or more, (*c*) were resident in Madras City and (*d*) had not, so far as was known, previously received more than two weeks of antituberculosis chemotherapy (95.3 per cent had received none). Three patients were, however, subsequently found to have had more than two weeks of antituberculosis chemotherapy before admission to the study.

It was the intention to examine by the series of identification tests, one pretreatment culture from each of the 341 patients. Since all the cultures could not be studied within six weeks of their becoming positive, a proportion (S3 per cent) were stored at  $-20^{\circ}\text{C}$  until examined. The pretreatment cultures from 54 of these 341 patients could not be examined by the series of identification tests because the culture selected for examination was either contaminated (33), failed to grow on subculture (12) or was mislaid (nine). Twenty-eight of the 33 cultures which were contaminated, and all the 12 cultures which failed to grow on subculture had been stored at  $-20^{\circ}\text{C}$ . None of the contaminants was an anonymous mycobacterium. There thus remained cultures from 287 patients (including the three who had received more than two weeks of previous chemotherapy) which were studied by the series of identification tests. No culture was excluded from this investigation because of resistance to streptomycin, *p*-aminosalicylic acid (PAS) or isoniazid.

#### *British cultures*

Cultures were obtained from 77 newly diagnosed and previously untreated British patients with pulmonary tuberculosis attending a number of chest clinics in England. Specimens of sputum obtained from these patients were sent to the Unit for Research on Drug Sensitivity in Tuberculosis (Medical Research Council of Great Britain), Postgraduate Medical School of London, for culture. Posi-

tive cultures were dispatched by air to Madras. Cultures from British patients were selected for this study if they were sensitive to streptomycin, PAS and isoniazid. They were included in all the batches of *in vitro* identification tests set up, roughly in the proportion of one British to four Indian cultures.

#### SPUTUM CULTURE

The sputum from both the Indian and the British patients was cultured on Lowenstein-Jensen medium after treatment with 4 per cent NaOH and washing with distilled water (Tuberculosis Chemotherapy Centre, 1959).

#### CONTROL STRAINS

Three control strains—*Myco. tuberculosis* strains H37Rv and BCG and the photochromogenic anonymous mycobacterium strain 0735 (Selkon and Mitchison, 1959)—were set up with each batch of tests. These strains were obtained from Dr D. A. Mitchison of the Postgraduate Medical School of London and were maintained by serial subculture on Lowenstein-Jensen medium.

#### MEDIA

The Lowenstein-Jensen medium referred to throughout the text did not contain potato starch (Jensen, 1955). The 7H-10 oleic-acid-albumin agar medium and 7H-10 Tween-albumin liquid medium were prepared as described by Cohn, Middlebrook and Russell (1959).

#### IDENTIFICATION TESTS

Cultures were examined for the following characteristics:

##### *In vitro tests*

*Bacterial morphology.* Bacterial morphology and acid-alcohol fastness were studied by examining, with a magnification of X 700, smears prepared from 4-week-old cultures grown on Lowenstein-Jensen medium and stained by the Ziehl-Neelsen method (Mackie and McCartney, 1959). The organisms were regarded as typical mammalian tubercle bacilli if they were strongly acid-alcohol fast, slender rods without excessive beading or barring, averaging 2-6  $\mu$ . in length and without branching.

*Colonial morphology.* Cultures incubated at 37°C for four weeks on Lowenstein-Jensen medium slopes and on 7H-10 oleic-acid-albumin agar plates were examined for colonial morphology, the former macroscopically and the latter with a plate microscope (magnification X8.75). Colonies on Lowenstein-Jensen medium were regarded as typical tubercle bacilli if they had a moderate or coarsely granular mat surface and were buff-coloured. The typical characteristics of the colonies of tubercle bacilli on 7H-10 oleic-acid-albumin agar plates were a granular mat surface, an irregular edge, a grey periphery with a central umbo and heaping of growth at the edges of adjoining colonies.

*Growth at 23°C.* Two slopes of Lowenstein-Jensen medium were inoculated with a suspension containing either about 0.01 mg. or about 1 mg. (moist weight) of culture; one was incubated at 37°C and the other at 23°C. The slopes were examined for growth at four and five weeks. Cultures were regarded as typical of tubercle bacilli if they did not yield growth at 23°C.

*Pigmentation.* Two Lowenstein-Jensen medium slopes were inoculated and then incubated at 37°C, one exposed to the electric light of the incubator room and the other in a tightly closed light-proof box. After four weeks' incubation, the cultures were examined for pigmentation and then kept on the laboratory bench at room temperature (approximately 30° C) for two weeks, one culture being exposed to daylight and the other remaining in the dark. The degree of pigmentation was then re-examined. If the colonies were buff-coloured, and if no increase in pigmentation had occurred after exposure to daylight, the culture was reported as typical.

*Catalase activity.* Catalase activity was determined by a qualitative method (Tuberculosis Chemotherapy Centre, 1959). Cultures were tested for catalase activity after four weeks' incubation on Löwenstein-Jensen drug-free medium and on Löwenstein-Jensen medium containing 50 µg/ml isoniazid. In order to obtain growth on the medium containing 50 µg/ml isoniazid, the slopes were heavily inoculated with approximately 2 mg. (moist weight) of the parent culture. Strains whose growth on the drug-free medium did not yield excessive catalase activity or whose growth on 50 µg/ml isoniazid had no catalase activity were regarded as typical tubercle bacilli.

*Niacin test.* The production of niacin was studied, using the method of Gilani and Selkon (1958). Two slopes of 7H-10 oleic-acid-albumin agar medium were heavily inoculated and incubated at 37° C. Growth on one slope was tested for niacin production after four weeks. If it was negative, the other slope was tested after six weeks' incubation. Cultures which gave positive results were regarded as *Myco. tuberculosis* var. *hominis* and those which were negative at the end of six weeks as either bovine or anonymous strains.

*Cord formation.* Tubercle bacilli, but not usually anonymous mycobacteria, have the ability to grow in tightly bound cords of parallel bacilli (Middlebrook, Dubos and Pierce, 1947; Selkon and Mitchison, 1959); this ability was examined in slide culture after 7-10 days' growth by the method of Sievers (1949).

*Arylsulfatase activity.* Arylsulfatase activity was examined in 2-week-old cultures in 7H-10 Tween-albumin liquid medium containing M/100 phenolphthalein disulfate by the method described by Whitehead, Wildy and Engback (1953). Human strains of tubercle bacilli do not possess detectable arylsulfatase activity (Whitehead, Wildy and Engback, 1953; Selkon and Mitchison, 1959).

*Drug-sensitivity.* The sensitivity of cultures to isoniazid and to *p*-acetamidobenzaldehyde thiosemicarbazone (thiacetazone) was determined by the method described by the East African/British Medical Research Council Thiacetazone/Diphenylthiourea Investigation (1960). For the thiacetazone-sensitivity tests, the slopes contained 0.25, 0.5, 1, 2, 4, 8, 16 and 32 µg/ml thiacetazone and 1.0 per cent triethylene glycol (the solvent for thiacetazone). The results of the sensitivity tests have been reported as the minimal inhibitory concentration (MIC) of the drug or as the resistance ratio (RR)—namely, the MIC for the test strain divided by the MIC for the control strain, H37Rv.

#### *In vivo test*

*Virulence in the guinea-pig.* Cultures were tested for virulence in the guinea-pig by the method described by Mitchison *et al.* (1960). In brief, 1 mg. (moist weight) of a 3-week-old subculture was inoculated intramuscularly into each of two guinea-pigs, one of which was killed at six 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 roots of the ratios for the two guinea-pigs and has been termed the root-index of virulence (Mitchison *et al.*, 1962<sup>1</sup>). Root-indices of virulence of 0-0.59 were considered as indicating a low degree of virulence, those of 0.60-0.89 as indicating a moderate degree of virulence and those of 0.90 or more as indicating a high degree of virulence. Guinea-pigs were Mantoux-tested four weeks after infection, using 0.1 ml. of 1:100 Old Tuberculin. The Mantoux tests were read after 48 hours and the results were expressed as the two diameters of the area of erythema taken at right angles to each other.

### Results

#### CULTURES EXAMINED BY THE SERIES OF IDENTIFICATION TESTS

The results obtained with the 287 Indian cultures that were examined by the series of *in vitro* identification tests will be presented in two sections, the first comparing the results obtained with 285 cultures which were regarded as tubercle bacilli with those obtained with the 77 British cultures of tubercle bacilli and the second dealing with the results of the two cultures which were regarded as anonymous mycobacteria.

#### *The 285 Indian and 77 British cultures identified as tubercle bacilli*

The results obtained with the series of identification tests on the 285 Indian cultures and the 77 British cultures regarded as tubercle bacilli are summarized in Table 1. The results of the tests for cord formation and arylsulfatase activity are not included in this table as these tests were performed on only a small proportion of the cultures at the start of this study. The great majority of the cultures were examined by all the tests, except the test for catalase activity of the growth on medium containing 50 µg/ml isoniazid and the test for growth at 23°C. Indeed, only 14 Indian and 12 British cultures were not examined by all the other tests. With the exception of one British culture (A420), which was not examined for colonial morphology on 7H-10 medium or for niacin production,

none of the cultures missed more than one of the identification tests. The tests of colonial morphology and pigmentation were not done because of either contamination or poor growth of the culture. The niacin test was not performed on 14 cultures (eight Indian, six British) because they were examined at the beginning of the study, before this test was introduced.

The Indian cultures behaved similarly to the British cultures in all the tests and all the cultures yielded results typical of mammalian tubercle bacilli, with the exception of 12 cultures (eight Indian, four British) which yielded growth at 23°C. The results of the full series of identification tests for these 12 cultures are shown in Table 2. Seven of the eight Indian cultures and three of the four British cultures yielded growth of less than 20 colonies at 23°C. The remaining cultures (one Indian, one British) yielded between 20 and 100 colonies. None of the 12 cultures yielded results atypical of mammalian tubercle bacilli in any other test, none was resistant to isoniazid and the guinea-pigs infected with 11 of them were strongly Mantoux-positive (diameters of erythema, 15 mm X 15 mm). The root-indices of five of the eight Indian cultures were, however, indicative of moderate to low virulence in the guinea-pig, but this is in keeping with previous findings on the virulence in the guinea-pig of tubercle bacilli isolated from Indian patients (Mitchison *et al.*, 1960). These 12 cultures have, therefore, been regarded as mammalian tubercle bacilli.

The 12 cultures of tubercle bacilli that yielded growth at 23°C were examined early in the study, when the test was performed by inoculating Lowenstein-Jensen medium slopes directly from a culture, using a wire loop. This method inoculated approximately 1 mg. (moist weight) of bacillary mass on to each slope. Eight of the nine Indian and four of the five British cultures tested by this method yielded growth at 23°C. Subsequently, the method was changed and the slopes were inoculated with a loopful of an aqueous suspension containing 4 mg. (moist weight) of bacilli per ml. None of the 163 Indian and 24 British cultures tested by this method of inoculating the slopes yielded growth at 23°C. The growth encountered at 23°C with the 12 cultures by the earlier method was thus presumably due to the very heavy inoculum used. The ability of tubercle bacilli

<sup>1</sup> See article on page 71.

TABLE I  
Results of identification tests on Indian and British pretreatment cultures

Source Of culture	Bacterial morphology	Colonial morphology		Growth at :		Pigmentation		Catalase activity		Niacin test
		On L-J slope	On 7H-10 plate	37°C	23°C	In light	In dark	On Drug-Free Slope	On 50 µ/ml isoniazid slope	
	Number of cultures with results typical of <i>Myc. tuberculosis</i>	285	285 281	285 167	283 283	285 41	277			
Indian patients	Number of cultures with atypical results	0	0 0	0 8	0 0	0 0	0			
	Number of cultures examined by the test*	285 100%	285 281 100% 98.6%	285 175 100% 61.4%	283 283 99.2% 99.3%	285 41 100% 14.4%	277 97.2%			
	Number of cultures with results typical of <i>Myc. tuberculosis</i>	77	77 70	77 25	77 77	69 17	71			
British patients	Number of cultures with atypical results	0	0 0	0 4	0 0	0 0	0			
	Number of cultures examined by the test†	77 100%	77 70 100% 90.9%	77 29 100% 37.7%	77 77 100% 100%	69 17 89.6% 22.1%	71 92.2%			

\* The percentages shown are based on a total of 285 cultures,  
† The percentages shown are based on a total of 77 cultures.

to grow at 23°C when heavily inoculated has been reported elsewhere (Csillag, 1961).

In summary, all the 285 Indian and 77 British mammalian tubercle bacilli. All the 277 Indian and 77 British cultures first tested for acid production were niacin-positive, and were therefore sensibility to human strains.

*Cultures identified as anonymous mycobacteria*

Two cultures (13986 and 15361) gave results which identified them as anonymous mycobacteria (Table 3). Culture 13986 yielded a deep-orange colour even on incubation in the dark and was thus a scotochromogen (Runyon, 1959—Group II). Culture 15361 yielded increased pigmentation only after exposure to light and was thus a photochromogen (Runyon, 1959—Group I). These two cultures have not been

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regarded as being the etiological agent of the patient's pulmonary disease for the following reasons. Culture 13986 was characteristic of mammalian tubercle bacilli, was sensitive to isoniazid and not abnormally pigmented. The culture was first tested for acid production and was niacin-positive, and was therefore sensibility to human strains. A further pretreatment culture from this patient was also sensitive to isoniazid and not abnormally pigmented. The isoniazid-resistance and abnormal pigmentation of culture 13986 only appeared on subculture after it had been stored for many months at—20°C. This culture was eventually shown to be a mixture of two different strains which were obtained in pure culture. One of these strains had the *in vitro* characteristics described in Table 3; the other had the characteristics typical of *Myc. tuberculosis* var. *hominis*. Culture 15361 was obtained from a patient whose other pretreatment culture was isoniazid-sensitive and not photochromogenic. From

TABLE 2  
Results of the full series of identification tests for the eight Indian and four British cultures which yielded growth at 23°C

Source of culture	Culture No.	Identification test										Guinea-pig test results			
		Bacterial morphology	Colonial morphology L-J 7H-10 slope	Degree of growth <sup>1</sup> at: 37°C	Degree of growth <sup>1</sup> at: 23°C	Pigmentation In light	Pigmentation In dark	Urease activity	Niacin test	Cord formation	Arylsulphatase activity	Sensitivity to penicillin (A.C.) (µg/ml)	Mantoux (1:100 OT) (mm)	Root-index of virulence	
Indian patients	11385	T <sup>2</sup>	T	T	2+	1	T	T	2+	Not done	Positive	Negative	<0.2	18×20 17×19	0.53
	11514	T	T	T	2+	1+	T	T	2+	Not done	Positive	Negative	<0.2	22×26 17×24	0.62
	11731	T	T	T	3+	5	T	T	2+	Not done	Positive	Negative	<0.2	13×16 15×20	1.03
	13613	T	T	T	3+	3	T	T	2+	Positive	Positive	Negative	<0.2	17×19 18×18	0.55
	13906	T	T	T	3+	3	T	T	2+	Not done	Positive	Negative	<0.2	16×15 Not done	0.47
	14601	T	T	T	3+	16	T	T	2+	Positive	Positive	Negative	<0.2	Not done	0.96
	15129	T	T	T	3+	6	T	T	2+	Not done	Positive	Negative	<0.2	20×20 23×21	0.51
	15920	T	T	T	3+	4	T	T	2+	Not done	Positive	Negative	<0.2	20×15 21×17	1.07
British patients	A 420	T	T	C <sup>3</sup>	3+	6	T	T	2+	Not done	Positive	Negative	<0.2	16×20 20×17	1.15
	A 424	T	T	T	3+	8	T	T	2+	Not done	Positive	Negative	<0.2	17×17 16×22	1.52
	A 457	T	T	T	3+	2	T	T	2+	Not done	Positive	Negative	<0.2	18×18 21×20	1.33
	I 530	T	T	T	3+	1+	T	T	2+	Not done	Positive	Negative	<0.2	20×23 Not done	1.49

<sup>1</sup> Degree of growth: 3+ = confluent growth; 2+ = innumerable discrete colonies; 1+ = 100-20 colonies; figures below 20 without the plus sign = number of colonies. <sup>2</sup> T = typical of mammalian tubercle bacilli. <sup>3</sup> C = contaminated.

TABLE 3

Identification test results for the two cultures regarded as anonymous mycobacteria

Identification test	Result			
	Culture No. 13986		Culture No. 15361	
<i>Bacterial morphology :</i>	Typical acid-alcohol-fast bacilli		Acid-alcohol-fast cocco-bacilli	
<i>Colonial morphology:</i>				
Lowenstein-Jensen medium	Smooth glistening surface, deep yellow in colour		Smooth mat surface, buff-coloured	
7H-10 medium	Dome-shaped, orange colonies with smooth glistening surface and entire edge		Fine granular, mat surface, grey colour, irregular edge	
<i>Degree of growth* at:</i>				
37°C	3+		34+	
23°C	2+		24+	
<i>Pigmentation:</i>				
In light	Orange		Light yellow Typical	
In dark	Orange		buff-coloured	
<i>Catalase activity:</i>				
On drug-free slope	24+		2+	
On 50 Mg/ml isoniazid slope	1+		2+	
<i>Niacin test:</i>	Negative		Negative	
<i>Drug sensitivity:</i>	<i>First test</i>	<i>Second test</i>	<i>First test</i>	<i>Second test</i>
Streptomycin RR	I†	1	16†	16
Isoniazid MIC (µg/ml)	0.2†	1	>50†	>50
Thiacetazone MIC (µg/ml)	>32	>32	8	
<i>Drug sensitivity of another pretreatment culture from the same patient:</i>				
Streptomycin RR	0.5†		0.5†	
Isoniazid MIC (µg/ml)	0.2†		0.2†	

\* For explanation, see Table 2.

† Tests set up when the culture was first isolated.

the first month of treatment and for more than 12 months, this patient consistently yielded isoniazid-resistant, catalase-negative cultures which were not abnormally pigmented or photochromogenic. A culture obtained after 12 months of treatment was examined by the full series of identification tests and classified as *Myc. tuberculosis* var. *hominis*.

#### CULTURES NOT EXAMINED WITH THE SERIES OF IDENTIFICATION TESTS

Cultures obtained before the start of treatment from 54 of the 341 patients admitted to

the chemotherapy study were not studied with the series of identification tests for the reasons given earlier (see page 100). Two cultures from each of 47 of these 54 patients and one culture from each of the remaining seven patients obtained before the start of treatment were, however, tested when first isolated for their sensitivity to isoniazid. After the sensitivity tests had been read, qualitative catalase tests were carried out on the growth on the drug-free slope and on any isoniazid-containing slopes which yielded growth of 20 or more colonies. The tubes were then left on the

TABLE 4  
Sensitivity of Indian and British cultures of tubercle bacilli to thiacetazone

Minimal inhibitory concentration (µg/ml)	Number of cultures								Resistance ratio	Number of cultures			
	20-colony MIC				100-colony MIC					20 colony RR			
	Indian cultures		British cultures		Indian cultures		British cultures			Indian cultures		British cultures	
	No.	%	No.	%	No.	%	No.	%		No.	%	No.	%
<0.25	6	2.7	7	70.6	21	9.3	15	22.7	<0.25	16	7.7	2	3.0
0.5	38	16.9	25	37.9	41	18.2	28	42.4	0.5	34	15.1	22	33.3
1	37	16.4	23	34.8	56	24.9	21	31.8	1	53	23.6	25	37.9
2	100	44.4	11	76.7	85	37.8	2	3.0	2	77	34.2	17	25.8
4	34	75.7	0	0.0	21	9.3	0	0.0	4	33	74.7	0	0.0
8	3	1.3	0	0.0	0	0.0	0	0.0	8	5	2.2	0	0.0
16	2	0.9	0	0.0	0	0.0	0	0.0	16	1	0.4	0	0.0
32	0	0.0	0	0.0	0	0.0	0	0.0	32	1	0.4	0	0.0
>32	5	2.2	0	0.0	1	0.4	0	0.0	>32	5	2.2	0	0.0
Total ...	225	99.9	66	100.0	225	99.9	66	99.9	Total ...	225	99.9	66	100.0

laboratory bench exposed to daylight at room temperature (approximately 30°C) for two to four days and re-examined for the development of abnormal pigmentation.

Of the 54 patients, 52 yielded cultures which were sensitive to isoniazid (MIC < 0.2 µg/ml). As none of the cultures from these 52 patients showed excessive catalase activity or was abnormally pigmented or photochromogenic, and all were sensitive to isoniazid, they were regarded as *Myco. tuberculosis*. (Confirmation was obtained for 17 of these 52 patients from cultures obtained during treatment, which were examined by the series of identification tests and classified as *Myco. tuberculosis* var. *hominis*.) One patient yielded one culture sensitive to isoniazid and another culture resistant to isoniazid (growth on 5 µg/ml, but not on 50 µg/ml). Neither of these cultures was abnormally pigmented and the growth of the isoniazid-resistant culture on the 5 µg/ml. slope was catalase-negative. Both cultures were therefore probably tubercle bacilli. A further culture obtained from this patient after 12 months' treatment was examined by the full series of identification tests and identified as *Myco. tuberculosis* var. *hominis*. The remaining patient yielded two cultures which had a low degree of resistance to isoniazid (MIC of 1 µg/ml). One of these two cultures was tested

for guinea-pig virulence and proved to be highly virulent (root-index of virulence, 1.12). It was therefore regarded as a culture of mammalian tubercle bacilli.

In summary, all the 54 patients yielded cultures which have been considered to be mammalian tubercle bacilli.

#### NOTES ON IDENTIFICATION TESTS

##### *Catalase activity of growth on medium containing 50 µg/ml isoniazid*

Only 40 of 188 Indian and 17 of 54 British cultures of tubercle bacilli yielded any growth on the 50 µg/ml slope, even though a heavy inoculum of approximately 2 mg. (moist weight) of culture was used. The failure to obtain growth with such a large proportion of the cultures was thus a serious limitation of this test. It is possible, however, that a larger inoculum may prove more satisfactory in this respect.

##### *Niacin production*

Niacin production was tested for on 271 Indian and 67 British cultures of tubercle bacilli after four weeks' incubation at 37°C. Positive results were obtained with 263 (97.0 per cent) of the Indian cultures and 65 (97.0 per cent) of the British cultures. The remaining eight Indian and two British cultures were,

TABLE 5

Results of repeat thiacetazone-sensitivity tests on cultures with resistance ratios of 4 or more in the first test

	20-colony MIC at first test				20-colony RR at first test		
	4 µg/ml	8 µg/ml or more	Total		4	8 or more	Total
Number of cultures	34	10	44		33	12	45
Number of cultures retested	25	7	32		30	9	39
20-colony MIC on retest (µg/ml)	No. %	No. %	No. %	20-colony RR on retest	No. %	No. %	No. %
<1	5 20	1 (14)*	6 79	<1	6 20	3 (33)	9 23
2	12 48	2 (28)	14 44	2	9 30	2 (22)	11 28
4	5 20	2 (28)	7 22	4	10 33	2 (22)	12 37
8	1 4	1 (14)	2 6	8	3 10	1 (77)	4 10
16	0 0	1 (14)	1 3	16	0 0	1 (77)	1 3
32	1 4	0 (0)	1 5	32	0 0	0 (0)	0 0
>32	1 4	0 (0)	1 3	>32	2 7	0 (0)	2 5

\* Percentages based on fewer than 25 observations are enclosed in parentheses, as an indication of the small totals.

however, positive when tested after six weeks' incubation. The control strain BCG was tested in 49 of the 51 batches of niacin tests and strains H37Rv and 0735 in all 51 batches of tests. Strains BCG and 0735 were niacin-negative in all tests and strain H37Rv was always niacin-positive. This method of testing for the presence of niacin can thus be relied on to differentiate human strains of tubercle bacilli from bovine strains and anonymous mycobacteria, provided that the cultures are incubated at 37°C for at least six weeks.

*Thiacetazone-sensitivity test*

The thiacetazone-sensitivity test proved unsatisfactory as an identification test for Indian cultures of tubercle bacilli, as these showed considerable variation in their sensitivity to this drug. In this respect, the Indian cultures differed from the British cultures. The distribution of the sensitivity to thiacetazone of 225 of the 285 Indian and 66 of the 77 British cultures identified as tubercle bacilli by the series of identification tests is set out in Table 4. Three distributions are presented: (a) the minimal inhibitory concentrations (MICs) inhibiting the growth of 20 or more colonies; (b) the MICs inhibiting the growth of 100 or more colonies; and (c) the resistance ratios

(RRs) for the 20-colony end-point. With the 20-colony definition of growth, the mean MIC for the Indian cultures was between 1.0 and 2.0 µg/ml thiacetazone, whereas for the British cultures it was between 0.5 and 1.0 µg/ml thiacetazone. The Indian cultures also had a wider range of sensitivity to thiacetazone than the British cultures; the MIC for Indian cultures ranged from <0.25 to >32 µg/ml thiacetazone (19.5 per cent had MICs of >2 µg/ml) as compared with from <0.25 to 2.0 µg/ml thiacetazone for the British cultures. The difference between the Indian and the British cultures was also shown by the 100-colony definition of growth, though the range of sensitivity of the Indian cultures to thiacetazone was somewhat reduced. The results of the sensitivity tests, when expressed as resistance ratios, were similar to those expressed as MICs. In order to determine whether the Indian cultures, which on the 20-colony definition of growth yielded growth on 2.0 µg/ml thiacetazone or had RRs of 4 or more, really were resistant to these concentrations of thiacetazone, cultures which yielded such results were retested. The results of the repeat tests are shown in Table 5. Considering the results expressed as MICs, 12 (38 per cent) of the 32 cultures which grew on 2 µg/ml or more thiaceta-

zone in the first test, and were retested, yielded growth on 2 µg/ml or more in the second test. This is an appreciably higher proportion than the 20 per cent of the 225 cultures which yielded growth on 2 µg/ml or more in the first test. The results expressed as RRs were similar; of the 39 cultures which had RRs of 4 or more in the first test and were retested, 19 (42 per cent) yielded similar results in the second test, as compared with 20 per cent of the 225 cultures which yielded RRs of 4 or more in the first test. These findings suggest that with Indian cultures, although there was considerable technical variation in the test, there were also genuine differences between cultures in respect of their sensitivity to thiacetazone.

### Discussion

Of the 287 cultures of Indian patients that were studied by the full series of identification tests, 285 yielded cultures which were classified as mammalian tubercle bacilli. The remaining two cultures, which were classified as anonymous mycobacteria, were not regarded as the etiological agents of the patients' pulmonary disease, because (a) they were isolated from each patient on only one occasion and (b) both patients yielded other cultures, which were regarded as typical tubercle bacilli.

The 285 Indian cultures identified as mammalian tubercle bacilli behaved similarly to the British cultures in the series of *in vitro* identification tests, except in respect of their sensitivity to thiacetazone. The Indian cultures were on the average less sensitive to thiacetazone than the British cultures and also showed greater variation in their sensitivity. Part of the variation in thiacetazone sensitivity of the different Indian cultures was due to technical reasons, but part was due to genuine differences between the cultures from different Indian patients, a small proportion of cultures being resistant to 2 µg/ml thiacetazone. The difference between the Indian and the British cultures was demonstrated by both the 20-colony and 100-colony definitions of growth. It is of interest that a difference has previously been demonstrated between Indian and British cultures of tubercle bacilli in respect of their sensitivity to PAS (Selkon *et al.*, 1960). In the case of PAS, however, the difference was present only when growth was defined as 20 or more colonies, and not when it was defined as 100 or more colonies.

All the 277 Indian cultures of tubercle bacilli tested for niacin production were positive and were therefore regarded as human strains. The absence of infection with bovine tubercle bacilli among our patients is a little surprising in view of the report that 1.8 per cent of the cattle and 2.8 per cent of the buffaloes in the Madras urban and adjacent rural areas are tuberculin reactors (Indian Council of Agricultural Research, personal communication). It is possible, however, that an appreciable proportion of the positive reactions was due to infections with mycobacteria other than the tubercle bacillus, or that transmission of infection to humans occurs infrequently in India because of the local custom of drinking milk only if boiled and the mild nature of the disease in Indian cattle (Mallick, Aggarwal and Dua, 1942; Iyer, 1944).

For 54 further patients, less complete information was available. Cultures from 53 of these patients were classified as tubercle bacilli on the findings that their growth was inhibited by 0.2 µg/ml isoniazid, that they were not photochromogenic and that they did not show abnormal pigmentation. Sensitivity to 0.2 µg/ml isoniazid is not, however, a completely reliable criterion on which to classify cultures as *Myc. tuberculosis*, for a small proportion of anonymous mycobacteria are inhibited by this concentration of isoniazid (Lester, Botkin and Colton, 1958; Marks and Trollope, 1960). The anonymous mycobacteria which have, however, been reported by other authors as sensitive to 0.2 µg/ml isoniazid were photochromogens (Lester, Botkin and Colton, 1958) and scotochromogens (Marks and Trollope, 1960) and not strains which showed pigmentation similar to tubercle bacilli (Runyon, 1959—Group III) which are invariably resistant to 0.2 µg/ml isoniazid (Jenkins, 1959; Marks and Trollope, 1960). Since the 53 cultures were not photochromogenic or abnormally pigmented, it seems reasonable, therefore, to regard them as tubercle bacilli on the basis of their sensitivity to 0.2 µg/ml isoniazid. The culture from the remaining patient was isoniazid-resistant, but was regarded as *Myc. tuberculosis* because of its high degree of virulence in the guinea-pig (Wilson and Miles, 1955).

The failure to detect any cases of pulmonary disease simulating tuberculosis in which anonymous mycobacteria could be incriminated as the etiological agent suggest that the prevalence

of infections due to these organisms may be lower in Madras City than in some other areas—for example, Georgia, USA (Crowed *al.*, 1957) Thinsdale, 111., USA (Lester, Botkin and Colton, 1958) and Great Britain (Selkon and Mitchison, 1959). It must be emphasized, however, that the 341 patients studied in this report were a selected group. All the patients presented with symptoms and a high proportion had extensive disease. They were admitted to treatment only if they yielded at least one sputum specimen that was positive on smear or culture examination. Furthermore, the prevalence of low-grade tuberculin sensitivity may be lower in Madras City (Andrews *et al.*, 1960) than in other areas in India (World Health Organization Tuberculosis Research Office, 1957).

### Summary

Cultures from 287 of 341 South Indian patients admitted to a controlled chemotherapy study were examined by a series of *in vitro* identification tests and compared with cultures from 77 British patients.

The cultures from 285 of the 287 Indian patients were identified as mammalian tubercle bacilli. Of the remaining two cultures, one was a mixture of a scotochromogenic anonymous mycobacterium and typical tubercle bacilli and the other was a photochromogenic anonymous mycobacterium. Neither of these two anonymous mycobacteria was regarded as the etiological agent of the patient's pulmonary disease because neither was isolated on more than one occasion and both patients yielded other cultures which were regarded as typical tubercle bacilli.

Cultures from the remaining 54 of the 341 patients were also regarded as mammalian tubercle bacilli, but on the basis of a limited number of tests. Cultures regarded as tubercle

bacilli were thus obtained from all the 341 Indian patients admitted to the chemotherapy study.

The cultures from 277 Indian patients were tested for niacin production; all yielded positive results and were therefore classified as *Mycobacterium tuberculosis* var. *hominis*.

The Indian cultures of tubercle bacilli yielded results similar to those of the British cultures in all the *in vitro* identification tests except the test for sensitivity to thiacetazone. The Indian cultures differed from the British cultures in that they were on the average less sensitive and showed greater variation among themselves in their sensitivity to thiacetazone.

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## RATE OF INACTIVATION OF ISONIAZID IN SOUTH INDIAN PATIENTS WITH PULMONARY TUBERCULOSIS \* †

### 1. MICROBIOLOGICAL ASSAY OF ISONIAZID IN SERUM FOLLOWING A STANDARD INTRAMUSCULAR DOSE

P. R. J. GANGADHARAM, A. L. BHATIA, S. RADHAKRISHNA AND J. B. SELKON

Since isoniazid is metabolized in man to several derivatives with little or no specific activity against the tubercle bacillus, its rate of inactivation in the body may have an important bearing on its efficacy as an antituberculosis drug. The inactivation rate, though constant in any one person, is known to vary from individual to individual and from race to race. A series of studies on the rate of inactivation of isoniazid in Indian patients with pulmonary tuberculosis has recently been undertaken at the Tuberculosis Chemotherapy Centre, Madras. The present paper describes the first of these studies, in which the concentration of isoniazid in the serum of patients admitted to a controlled comparison of four domiciliary chemotherapeutic regimens was determined by microbiological assay four-and-a-half hours after administration of a standard dose of isoniazid (3 mg/kg body-weight). Patients with serum levels of 0.58 µg/ml or more were classified as slow inactivators of isoniazid and those with levels below 0.58 µg/ml as rapid inactivators. By this definition, 195 (61 per cent) of the 321 patients studied were found to be slow inactivators and 126 (39 per cent) rapid inactivators. A relationship was shown between sex and the rate of inactivation, there being a significantly higher proportion of rapid inactivators among the females than among the males. The observed estimates of the error of the microbiological assay procedure are discussed and possible ways of reducing the error suggested.

#### INTRODUCTION

Isoniazid is metabolized in man to several derivatives of little or no antimicrobial activity against the tubercle bacillus (Bernstein *et al.*, 1952; Hughes, 1953; Cuthbertson, Ireland and Wolff, 1953). The rate of inactivation of isoniazid varies widely from subject to subject (Hughes, Schmidt and Biehl, 1955; Middlebrook and Dressier, 1956), but is constant for a given individual (Hughes, Schmidt and Biehl, 1955; Bell and Riemensnider, 1957b). The catalase activity and the degree of isoniazid-resistance of strains of tubercle bacilli isolated from patients during treatment with isoniazid have been shown to be related to the rate of inactivation of isoniazid (Mandel *et al.*, 1957; Canetti and Grosset, 1958); the isoniazid-resistant strains which emerged during treatment in slow inactivators had a higher degree of resistance and were more frequently catalase-negative than the corresponding strains from rapid inactivators. Mitchell and Bell (1957)

have provided suggestive evidence that the rate of inactivation of isoniazid influences the speed with which sputum conversion occurs,

The relationship between the rate of inactivation of isoniazid and the response to treatment has, therefore, been investigated in 321 of the 341 patients admitted to a controlled study of four different chemotherapeutic regimens administered at home, undertaken by the Tuberculosis Chemotherapy Centre (1960). In three of the regimens isoniazid was the sole antimicrobial agent, and in the fourth it was given with PAS. The serum isoniazid concentrations were determined after a standard test-dose of 3 mg/kg isoniazid. The test-dose of isoniazid was given by intramuscular injection instead of by mouth to avoid irregularities in absorption from the intestinal tract, as the patients, being treated at home, were not amenable to the dietary restrictions recommended by Bell and Riemensnider (1957b). The serum isoniazid concentrations were determined by the

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

† This paper is also being published in the *Bulletin of the World Health Organization*.

microbiological assay method of Mandel *et al.* (1956) in preference to chemical methods (Kelly and Poet, 1952; Cuthbertson *et al.*, 1954; Short, 1954; Poole and Meyer, 1958; Berte *et al.*, 1959) as the latter are either too cumbersome, not sufficiently sensitive or not specific for the antimicrobially active free isoniazid and hydrazones.

We report here investigations on the microbiological assay technique and the results of determinations of isoniazid inactivation rates of the patients in the controlled study. Other articles to be reprinted in this journal are concerned with the serum isoniazid concentrations in these patients when they were receiving their prescribed chemotherapy (Gangadharam *et al.*, 1961) and with the relationship between the rate of inactivation of isoniazid and the response to treatment (Selkon *et al.*, 1961).

#### MATERIALS AND METHODS

The 341 patients admitted to the controlled comparison of four regimens of domiciliary chemotherapy (Tuberculosis Chemotherapy Centre, 1960) had newly diagnosed pulmonary tuberculosis, and were aged 12 years or more.

The four prescribed regimens were:

*PH.* Isoniazid 3.9-5.5 mg/kg body-weight plus PAS (sodium salt) 0.2-0.3 g/kg daily, in two divided doses by mouth.

*HI-1.* Isoniazid alone, 7.8-9.6 mg/kg daily, in one dose by mouth.

*HI-2.* Isoniazid alone, 7.8-9.6 mg/kg daily, in two divided doses by mouth.

*H.* Isoniazid alone, 3.9-5.5 mg/kg daily, in two divided doses by mouth.

Of the 341 patients, three are not considered in this report since they had received more than two weeks of antituberculosis chemotherapy prior to admission to the study; the vast majority (96.2 per cent) of the remaining 338 patients had received none.

The results of the isoniazid inactivation rates of 17 patients were not available for the following reasons: 12 patients had died before the sixth month, the earliest month selected for the test; two had taken their discharge against medical advice; in two patients the tests were

contaminated and in one patient the venipuncture was performed after five instead of four-and-a-half hours. The isoniazid inactivation rates of the remaining 321 patients were determined between their sixth and twelfth months of treatment. Additional investigations (see pages 116 and 117) were carried out on 16 newly diagnosed patients who fulfilled the criteria required for admission to the controlled study (Tuberculosis Chemotherapy Centre, 1960).

#### Procedure

The patient was taken off all drugs for two days before the test. In order to confirm that the treatment had, in fact, been discontinued, a specimen of urine was collected immediately before the injection of isoniazid and examined for the presence of isoniazid by the combined naphthoquinone-mercuric chloride test (Gangadharam *et al.*, 1958). A test dose of 3 mg/kg body-weight isoniazid<sup>1</sup> was given by intramuscular injection and a specimen of venous blood was collected four-and-a-half hours later. No dietary restrictions were imposed on the patient either before or during the test.

#### Culture medium

Liquid 7H-10 medium (Cohn, Middlebrook and Russell, 1959), without glycerol, was used in this investigation, with the following modifications:

(a) For growth of the inoculum, the medium contained, in addition to the basic ingredients, 0.5 per cent bovine albumin fraction V (Armour Laboratories), 0.2 per cent glucose, 0.05 per cent Tween 80 and 50 Mg/ml streptomycin (final concentrations).

(b) For the assay procedure, the medium contained, in addition to the basic ingredients, 0.5 per cent bovine albumin fraction V, 0.2 per cent glucose, 10 µg/ml *p*-aminobenzoic acid and 50 µg/ml streptomycin (final concentrations).

#### Assay organism

A culture of *Mycobacterium tuberculosis* strain H37RvSR, resistant to streptomycin, obtained by *in vitro* selection from strain H37Rv, was used as the assay organism. It was main-

<sup>1</sup> The preparations used were Neoteben (Bayer A. G., Leverkusen, Germany) and Isonic (Chemidica S. A., Montreux, Switzerland), supplied in ampoules containing 100 mg/ml isoniazid.

tained by monthly subculture on Lowenstein-Jensen medium containing 1024 µg/ml streptomycin.

#### *Test*

A 1/5 dilution of the serum was prepared by adding 2.0 ml of the serum to 8.0 ml of the 7H-10 test medium. Serial twofold dilutions, from 1/10 to 1/80, were then prepared by adding 1.0, 0.5, 0.25 and 0.125 ml of the 1/5 dilution of serum to 7H-10 test medium, to make up the final volume to 2.0 ml. Whenever sufficient serum was available (95 per cent of sera), tests were set up in duplicate from the same 1/5 dilution of the serum. The 2.0-ml volumes of the 1/5 to 1/80 dilutions of serum were inoculated with 0.1 ml of an 8-day-old culture of H37RvSR grown in the Tween-containing 7H-10 liquid medium, and then incubated at 37°C for five days. Smears were prepared by pipetting the unshaken deposit of growth on to slides, with the visual assistance of a concave mirror. The slides were stained in Coplin jars by the Ziehl-Neelsen technique and counterstained with Loeffler's methylene blue.

With each batch of tests, a control series of tubes was set up in duplicate containing 0.00, 0.02, 0.04 and 0.08 µg/ml isoniazid in water, prepared from a stock solution (100 µg/ml isoniazid) sterilized by filtration through sintered glass.

#### *Reading and recording of the results*

At least six fields of each smear were examined under x 700 magnification and the proportion of acid-fast bacilli was estimated as 0 per cent, 25 per cent, 50 per cent, 75 per cent or 100 per cent. The dilution end-point was defined as the dilution of serum (the mean dilution if tests were set up in duplicate) producing 50 per cent loss of acid-fastness and was determined, where necessary, by interpolation. For example, if none of the bacilli in the 1:10 dilution, and all the bacilli in the 1:20 dilution were acid-fast, then the dilution which could have produced 50 per cent loss of acid-fastness was estimated as 1:15. The isoniazid concentration of each test serum was obtained by dividing the geometric mean of the isoniazid concentrations producing 50 per cent loss of acid-fastness in the controls set up in the study by the dilution end-point of the serum.

## RESULTS

The results of the microbiological assays of isoniazid were analysed after transformation to a logarithmic scale in which a twofold decrease in the serum dilution producing 50 per cent loss of acid-fastness, or a two fold increase in isoniazid concentration, was given a value of one working unit. One working unit is, therefore, equivalent to one dilution step in the assay. In consequence, *all* mean isoniazid concentrations quoted are geometric means. Where appropriate, the transformed values were examined by analysis of variance.

#### *Investigation of batch differences*

The occurrence of variation between different batches of assays was studied on 399 sera, 321 from the same number of patients (page 112), 30 from further tests carried out on six of these 321 patients (page 116) and 48 from the eight additional patients studied in the investigation reported on page 116. The isoniazid concentrations of the 399 sera were assayed in 29 batches of tests, the number of sera tested in each batch ranging from four to 33. The control dilutions were contaminated in seven batches, leaving 22 batches in which the results on the controls were available. In calculating the isoniazid concentration of a test serum, it is usual (Mandel *et al.*, 1956) to correct for possible variation from batch to batch by dividing the concentration of isoniazid which produces 50 per cent loss of acid-fastness in the control in the particular batch by the dilution of the test serum which produces a similar loss of acid-fastness (the dilution end-point). Such a procedure assumes that factors affecting the results in any batch alter the end-point of the test serum and the control in a similar direction. This assumption was examined in the following way.

First, the isoniazid concentrations of the test sera were calculated by employing the controls set up in the same batch as the test serum. The averages of the isoniazid concentrations of the test sera in each batch are set out under method I in Table 1. The variation in these averages from batch to batch was found to be significantly greater than the variation in isoniazid concentration from serum to serum within the same batch ( $P < 0.005$ ). The magnitude of this batch variation, expressed as a standard deviation (the squareroot of the com-

TABLE 1  
Distribution of the mean concentrations of isoniazid in the sera in different batches of tests, calculated by two methods

Mean concentration of isoniazid ( $\mu\text{g/ml}$ )	Test batches			
	Calculated from control in each batch (method I)		Calculated from the mean of controls in all batches (method II)	
	No.	%	No.	%
<0.35	0	(0)*	1	3
0.35-	3	(14)	1	3
0.44-	6	(27)	14	48
0.56-	6	(27)	3	10
0.70-	5	(27)	5	17
0.88-	1	(5)	5	17
>1.11	1	(5)	0	0
Total	22	707	29	98

\* The parentheses indicate percentages based on fewer than 25 observations.

ponent of variance due to this source in the analysis of variance), was 0.32 dilution step.

Secondly, the isoniazid concentrations of the test sera were calculated by dividing the mean of the isoniazid concentrations which produced 50 per cent loss of acid-fastness in the controls in the 22 batches (0.0344  $\mu\text{g/ml}$  isoniazid) by the dilution end-point of the serum. The averages of the isoniazid concentrations in the 29 batches expressed in this way are set out under method II in Table 1. Again, the variation of these averages from batch to batch was significantly greater than the variation from serum to serum within the same batch ( $P=0.01$ ). The magnitude of the batch variation, also expressed as a standard deviation, was 0.26 dilution step, an estimate lower than the corresponding estimate of 0.32 obtained by the first method. Thus, there is no evidence from our results that the factors responsible for batch variation altered the end-points of the test serum and the control in the same direction in each batch. In calculating the isoniazid concentrations of the test sera the second method was therefore employed.

The concentrations of isoniazid which produced 50 per cent loss of acid-fastness in the

controls set up in the 22 batches are presented in Table 2. The distribution of these concentrations has a mean of 0.0344  $\mu\text{g/ml}$  and a range of 0.030-0.060  $\mu\text{g/ml}$ . The skewness of this distribution suggests that a single factor, such as the ability of the test organisms to grow in the medium, may have been the principal cause of variation in the control end-points. It will be appreciated that the dilutions of the test sera but not those of the controls, contained human serum and that the presence of this human serum might have promoted the growth of the test organism. Thus, the failure of the controls to diminish batch variation might be due to this difference.

#### Effect of streptomycin

In the early stages of the use of the microbiological assay procedure in this laboratory, 39 (7.5 per cent) of 522 tests set up were contaminated. Streptomycin 50  $\mu\text{g/ml}$  (final concentration) was therefore added to both the medium used for growing the test organism and that used for microbiological assay. This alteration in the technique was followed by a reduction of the contamination rate to 0.5 per cent of the 1005 subsequent tests. In order to be certain, however, that the addition of streptomycin did not influence the micro-

TABLE 2  
Distribution of the control concentrations of isoniazid which produced 50% loss of acid-fastness in different batches of tests

Concentration of isoniazid which produced 50 % loss of acid-fastness* ( $\mu\text{g/ml}$ )	Control batches	
	No.	%
0.030	13	(59)†
0.035	2	(9)
0.037	2	(9)
0.040	0	(0)
0.046	1	(5)
0.053	1	(5)
0.060	1	(5)
Total	22	101

\* Mean concentration which produced 50% loss of acid-fastness = 0.0344  $\mu\text{g/ml}$ .

† The parentheses indicate percentages based on fewer than 25 observations.

biological assay of isoniazid, the following investigation was conducted. The isoniazid concentrations of seven sera were determined by three different methods. In method A, a medium containing 50 µg/ml streptomycin was inoculated with a culture of H37RvSR grown in a medium containing the same concentration of streptomycin. In method B, a medium not containing streptomycin was inoculated with the culture of H37RvSR grown in a medium containing 50 µg/ml streptomycin. In method C, neither the medium used for the assay nor that used for growing the inoculum contained streptomycin. The smears prepared from these tests were examined in a random order. The mean concentration of isoniazid for the seven sera was estimated as 0.32 µg/ml with method A, 0.46 µg/ml with method B, and 0.36 µg/ml with method C. As the differences are neither in the same direction nor statistically significant, it can be concluded that the addition of streptomycin did not interfere with the assay of isoniazid.

*Stability of isoniazid in serum during storage in the deep-freeze*

As sera were generally stored in the deep-freeze at -20°C, sometimes for many weeks, before their isoniazid concentrations were estimated, the stability of isoniazid in serum during storage was investigated. The isoniazid concentrations of 10 sera were assayed within 12 hours of collection of the blood from the patients and then again at the end of two, four,

TABLE 3  
*Stability of isoniazid in serum during storage at -20°C*

Serum number	Serum concentration of isoniazid (µg/ml)				
	Weeks of storage at -20°C				
	0	2	4	6	8
1	0.29	0.29	0.29	0.26	0.26
2	1.04	1.04	1.04	0.93	0.69
3	0.29	0.29	0.29	0.26	0.23*
4	1.04	1.14	1.04	1.04	1.04
5	1.04	1.04	1.04	1.04	0.69
6	1.04	1.14	1.04	0.93	0.69
7	0.26	0.26	0.23	0.17	0.17
8	2.07	1.37	1.14	1.04	1.04
9	1.04	1.04	1.04	0.92	1.04
10	1.04	1.04	1.04	0.69	0.92
Mean (µg/ml)	0.75	0.74	0.70	0.61	0.57

\* One missing value has been estimated using standard statistical techniques.

six and eight weeks of storage in the deep-freeze. The smears prepared from these tests were heat-fixed and then kept for staining until all the smears were available. They were stained in one batch and the slides were read in a random order. The results of this experiment are presented in Tables 3 and 4. The concentration of isoniazid decreased from week to week and there was a linear relationship

TABLE 4  
*Analysis of variance of data in table 3*

Term	Source of variation	Sum of squares	Degrees of freedom	Mean square	F	P
a	Sera	43.6575	9	4.8508		
b	Weeks of storage	1.2536	4	0.3134	9.44	<0.001
c	Linear regression	1.1653	1	1.1653	35.09	<0.001
d	Deviation from regression	0.0883	3	0.0294	...	NS*
e	Residual	1.1631	35†	0.0332		
Total ...		46.0742	48†			

\* NS indicates that the variance ratio is less than 1.0.

† One missing value has been estimated using standard statistical techniques.

TABLE 5  
*Consistency of individual isoniazid inactivation rates*

Treatment regimen	Number of patients	Mean serum concentration of isoniazid ( $\mu\text{g/ml}$ )					
		Before treatment	Duration of treatment				
			7 days	3 months	6 months	9 months	12 months
PH	6	0.58	0.53	0.56	0.62	0.65	0.54
HI-1	3	0.31	0.36	0.25	0.43	0.41	0.29
HI-2	2	0.84	0.84	0.84	1.04	1.09	1.14
H	3	0.26	0.31	0.43	0.42	0.50	0.44
All regimens	14	0.45	0.46	0.47	0.57	0.60	0.54

between the log concentration and the duration of storage (Table 4, term c,  $P < 0.001$ ). The fall in the isoniazid concentration during storage is estimated as 3.7 per cent per week. This estimate has been used to correct the results obtained with sera which had been stored for more than seven days before being assayed. In contrast to these findings with serum, a solution of 100  $\mu\text{g/ml}$  isoniazid in water, stored at  $4^\circ\text{C}$  and tested concurrently with the sera, showed no alteration in the concentration of isoniazid during six weeks of storage.

#### *Consistency of individual isoniazid inactivation rates*

The consistency of individual rates of inactivation of isoniazid was investigated in 14 patients (6 PH, 3 HI-1, 2 HI-2, 3H), six of whom were part of the main population of 321 patients under study (page 112). Serum concentrations of isoniazid were determined four-and-a-half hours after an intramuscular test-dose of 3 mg/kg isoniazid, just before treatment was started, and then again after seven days and after three, six, nine and 12 months of treatment. The results are set out in Table 5. Considering all treatment groups, there was a very slight suggestion of an increase in the mean concentration of isoniazid with time. The trend (linear regression), however, is not statistically significant ( $0.1 < P < 0.2$ ). This applied to all four regimens.

The mean weight of the 14 patients was 82.6 lb. (range, 59-109 lb.) on admission to

treatment, 88.4 lb. at six months and 89.8 lb. at 12 months.

#### *Accuracy of the test*

The error of the test procedure for determining the isoniazid inactivation rate on different occasions in a patient is composed of the error of the microbiological assay and of other procedures in the test, such as inadvertent variation in the test-dose of isoniazid injected and variation in its rate of absorption. During the course of the study, a number of estimates were made of the variation from different sources that contributed to the error of the test procedure. These are set out in Table 6.

*Error of the assay procedure.* Estimates of the variation from assay to assay, carried out on the same test serum in the same batch of assays, were obtained from (a) the experiment on the stability of isoniazid in serum during storage in the deep-freeze (Table 6, term a), and (b) the untabulated results of duplicate tests on 65 sera, which were set up and read in a random order (Table 6, term b). Combining these estimates, the standard deviation was 0.15 dilution step (Table 6, term c). Since this variation was between assays in the same batch, and since differences from batch to batch were found to be significant (page 113), an estimate of the total error of the assay is provided by adding to the former, the variation from batch to batch (Table 6, term d, 0.26 dilution step). Thus calculated, the total error was 0.30 dilution step

TABLE 6  
*Estimates of error due to different sources in the determination of serum concentration of isoniazid in patients four-and-a-half hours after an intramuscular test-dose of 3 mg/kg isoniazid*

Source of error	Estimate of error			
	Term	Source of estimate	Degrees of freedom	Standard deviation (dilution steps)
<i>Assay procedure:</i>				
Between assays on same serum in same batch	a	Table 4, term e <sup>1</sup>	35	0.18
	b	Page 116 <sup>2</sup>	65	0.14
	c	Combined estimate (a and b)	100	0.15
	d	Page 116; Table I <sup>3</sup>	28	0.26
	e	c+d		0.30
<i>Test procedure:</i>				
Between tests on same patient	f	Table 5, Page 117 <sup>5</sup>	67	0.60
	g	Combined estimate (f and g)	42	0.44
	h		109	0.54
Test procedures other than in the assay	i	h-e		0.45

<sup>1</sup> Residual from experiment on the effect of storage of sera at -20°C.

<sup>2</sup> Between duplicate tests on the same serum.

<sup>3</sup> Between batches of tests.

<sup>4</sup> Between tests on the same patient from experiment on the stability of individual isoniazid inactivation rates.

<sup>5</sup> Between patients with test results at six and 12 months.

when expressed as a standard deviation (Table 6, term e). As an example of the interpretation of this estimate in terms of isoniazid concentrations, if 20 assays, randomly distributed in different batches, were carried out on the same serum with an isoniazid concentration of 1.0 µg/ml, then in 19 of these assays the estimated concentration would be expected to be between 1.50 and 0.67 µg/ml.

*Error of the test procedure.* The error of the test procedure—that is, the variation in the determinations of the serum isoniazid concentration on different occasions on the same patient—was estimated from (a) the results of multiple tests on the same patient in the experiment on the consistency of individual isoniazid inactivation rates (Table 6, term f), and (b) the results of duplicate tests (not tabulated here) carried out on 43 patients, at six and 12 months (Table 6, term g). The combined estimate

(Table 6, term h) of this variation, expressed as a standard deviation, was 0.54 dilution step. If 20 test procedures were carried out on different occasions on the same patient, and the mean of the serum isoniazid concentrations at four-and-a-half hours was 1.0 µg/ml, then in 19 of these tests the isoniazid concentration would be expected to be between 2.08 and 0.48 µg/ml. *Error of the test procedures apart from the assay.* The error of the test procedures apart from the assay was calculated by subtracting the error of the microbiological assay from the total error of the test procedures. Expressed as a standard deviation, this error was equal to 0.45 dilution step (Table 6, term i).

*Serial serum concentrations from half an hour to five-and-a-half hours*

Serial isoniazid serum concentrations were determined in eight newly diagnosed, previously

TABLE 7

*Serum concentrations of isoniazid at different intervals after intramuscular injection of 3 mg/kg isoniazid*

Rate of inactivation of isoniazid	Patient number	Serum concentration of isoniazid ( $\mu\text{g/ml}$ )				
		Hours after administration of test-dose				
		1	1	2	4 1	5 1
Slow	1	1.82	2.75	2.06	1.03	1.03
	2	3.68	2.75	2.06	1.17	1.03
	3	2.06	2.06	2.06	1.03	1.03
	4	2.75	2.75	1.82	1.38	1.17
	5	2.30	2.75	2.06	1.17	1.03
	Mean	2.44	2.60	2.01	1.15	1.06
Rapid	1	3.68	2.06	1.03	0.34	...
	2	2.75	1.38	1.38	0.34	0.17
	3	1.03	2.06	1.03	0.52	0.34
	Mean	2.18	1.80	1.14	0.39	0.24

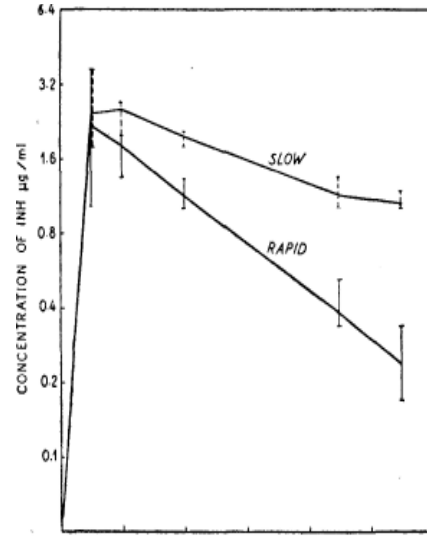
untreated patients half an hour, one hour, and two, four-and-a-half and five-and-a-half hours after an intramuscular injection of 3 mg/kg isoniazid. The results are presented in Table 7 and in Fig. 1. At four-and-a-half hours, five of the eight patients had serum concentrations above 0.58  $\mu\text{g/ml}$  and three had serum concentrations below 0.58  $\mu\text{g/ml}$ . For reasons given in the next section, the former have been classified as slow inactivators and the latter as rapid inactivators of isoniazid.

The highest mean serum concentration observed for the five slow inactivators was 2.60  $\mu\text{g/ml}$  and that for the three rapid inactivators was 2.18  $\mu\text{g/ml}$ .

The serum concentrations at half an hour varied considerably between patients and there was no clear difference between rapid and slow inactivators. At one hour, a difference was

FIG. 1

*Serial mean serum concentrations of isoniazid (INH) in five slow and three rapid inactivators after an intramuscular dose of 3 mg/kg body-weight*



The dotted and solid vertical lines indicate the range of the observations for the slow and rapid inactivators, respectively.

discernible, but there was still considerable variation between patients in the same inactivation group and slight overlapping between the groups. The variation between patients in the same inactivation group was markedly less at two, four-and-a-half and five-and-a-half hours and there was a clear distinction between the mean concentrations of the slow and rapid inactivators. The mean concentration of the slow inactivators was 1.8 times that of the rapid inactivators at two hours, 2.9 times at four-and-a-half hours and 4.4 times at five-and-a-half hours.

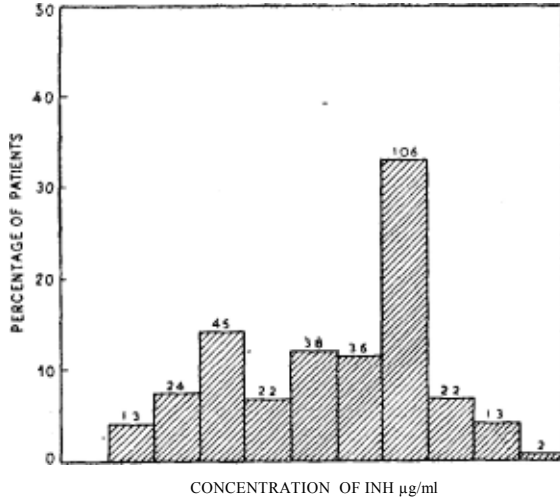
#### *Distribution of serum concentrations of isoniazid of South Indian patients*

The distribution of the serum concentrations of isoniazid of the 321 South Indian patients (page 112) four-and-a-half hours after an intramuscular injection of 3 mg/kg of iso-

FIG. 2

Distribution of serum concentrations of isoniazid (INH) four-and-a-half hours after an intramuscular dose of 3 mg/kg body-weight

0.17 0.24 0.34 0.49 0.69 0.97 1.37 1.93 2.73



The number above each block indicates the number of patients on whom determinations of serum concentration of isoniazid were made.

niazid is set out in Fig. 2. The distribution is bimodal and appears to consist of two normal distributions which overlap, particularly in the 0.49-0.69 µg/ml class interval. The means of the two distributions are approximately 0.30 and 1.10 µg/ml. In order to determine whether the 38 patients in the 0.49-0.69 µg/ml class interval really did belong to one or other of these distributions and were not a group with an intermediate rate of inactivation, the rate of inactivation of isoniazid of 37 of them was retested. Of the 37 patients who were retested, 17 (46 per cent) had serum concentrations above 0.69 µg/ml and 9 (24 per cent) below 0.49 µg/ml. Although this evidence does not exclude the possibility of there being an intermediate group, it does suggest that at least the majority of patients in this class interval do belong to one or other of the main distributions. It was therefore decided to separate the two distributions arbitrarily at the mid-point (0.58 µg/ml) of this class interval and to classify those with serum concentrations at or above 0.58 µg/ml as slow inactivators of isoniazid and those with serum concentrations below 0.58 µg/ml as rapid inacti-

vators. According to this classification, 195 (61 per cent) of the 321 patients were slow inactivators and 126 (39 per cent) were rapid inactivators.

The standard deviation of the serum concentrations of isoniazid for the slow inactivators was estimated to be 0.54 dilution step. It was shown on page 117 that the error of the test procedure was also estimated as 0.54 dilution step. Thus, all of the variation among the slow inactivators is likely to be due to the error of the test procedure. The corresponding standard deviation for the rapid inactivators could not be estimated as a relatively large proportion of the patients had serum concentrations of isoniazid below the level of sensitivity of the assay.

*Relationship between sex, body-weight, age and rate of inactivation of isoniazid*

There was no evidence of an association between the serum concentration of isoniazid and body-weight either among the 195 slow inactivators (correlation co-efficient (r) = 0.11, P=0.1-0.2) or among the 126 rapid inactivators (r=0.17, P=0.07). The distribution of weights for the slow and rapid inactivators is presented, separately for males and females, in Table 8. Considering the males, the weight distributions for the slow and rapid inactivators are very similar, the means being 98.3 and 100.4 lb., respectively. Among females, there was a slight suggestion that the rapid inactivators

TABLE 8

*Distribution of weights according to rate of inactivation of isoniazid and sex of patients*

Weight (lb. *)	Males		Females	
	Slow inactivators	Rapid inactivators	Slow inactivators	Rapid inactivators
	No.	%	No.	%
60-69	2	1	4	7
70-79	4	3	14	26
80-89	26	18	17	31
90-99	56	40	13	24
100-109	26	18	3	6
110-119	18	13	3	6
120 or above	9	6	8	11
Total	141	99	71	100
Mean	98.3		85.9	

\* 1 lb. = 0.45 kg.

TABLE 9  
Proportion of slow inactivators among male and female patients according to body-weight

Weight	Males		Females	
	Total	Percentage of slow inactivators	Total	Percentage of slow inactivators
60-79	11	(55)†	44	41
80-89	35	74	33	52
90-99	77	73	20	(65)
100 or above	89	60	12	(50)
Total ...	212	67	109	50

\* 1 lb. = 0.45 kg.

† The perenthesis indicate percentages based on fewer than 25 observations.

weighed less than the slow inactivators; thus, 10 (18 per cent) of the 55 rapid inactivators weighed 60-69 lb. as compared with four (7 per cent) of the 54 slow inactivators ( $P \approx 0.2$ ). The mean weight for the rapid inactivators was 81.2 lb. as compared with 85.9 lb. for the slow inactivators ( $P \approx 0.06$ ).

Of 212 male patients, 141 (67 per cent) were slow inactivators as compared with 54 (50 per cent) of 109 females, the difference being highly significant ( $P < 0.01$ ). A sub-analysis was undertaken to see if this difference was evident in the various weight-groups and the findings are presented in Table 9. It can be seen that the proportion of slow inactivators among the males is uniformly higher in all the weight-groups than the corresponding proportion for the females. It may therefore be concluded that the difference between the sexes in respect of the rate of inactivation of isoniazid cannot be attributed to differences in body-weight and is presumably a genuine sex difference. An analysis (not tabulated here) revealed that there was no association between the rate of inactivation of isoniazid and the age of the patient.

#### DISCUSSION

The results of determinations of the rate of inactivation of isoniazid reported here have demonstrated that South Indian patients can

be divided into two groups—namely, the rapid and the slow inactivators — as has been previously reported for North American patients (Middlebrook and Dressier, 1956; Price Evans, 1959). The success of a method for determining the rate of inactivation of isoniazid can best be judged by its ability to distinguish these two groups of patients. In this study, 38 (12 per cent) of the 321 patients had serum concentrations between 0.49 and 0.69 *fig/ml*, the class interval in which the distributions of the slow and rapid inactivators overlapped, and thus could not be assigned with certainty to either of these two groups. This percentage of indeterminate results may be compared broadly with the estimates of other workers (Mandel *et al.*, 1959; Price Evans, 1959), although there were differences between the three studies, not only in the procedures employed, but also in the class intervals used for grouping the isoniazid assay results. The results of the above-mentioned workers are rather more satisfactory than those reported here; thus, 1.5 per cent of the 254 patients studied by Mandel *et al.* (1959) and 8 per cent of the 123 patients reported by Price Evans (1959) had indeterminate serum concentrations of isoniazid. It is therefore necessary to consider modifications of the technique used at the Tuberculosis Chemotherapy Centre which could eliminate or decrease the proportion of patients with indeterminate results.

Increased precision in classifying patients as rapid or slow inactivators can be obtained in two ways: (a) by increasing the difference between the means of the distributions for the slow and rapid inactivators, and (b) by decreasing the variation within each of these distributions. Considering the first approach, the results of serial assays on patients indicate that the longer the period between the peak serum concentration and the collection of blood for assay, the greater is the difference in the mean serum concentrations of isoniazid between the rapid and the slow inactivators. Middlebrook and Dressier (1956) recommended six hours as the optimum period after oral administration of a 4 mg/kg test-dose. In this study, an interval of four-and-a-half hours between the administration of the test-dose and the collection of blood was chosen, because it was thought that the difference between the times when peak serum concentrations are attained following intramuscular and oral administration would be

about one-and-a-half hours. Peak serum isoniazid concentrations were, however, achieved after about 30 minutes following the intramuscular injection of the test-dose, and after about 70 minutes following oral administration of a dose of about the same size (Gangadharam *et al.*, 1961), a difference of only 40 minutes. The original estimate of one-and-a-half hours was thus 50 minutes too long. However, if the period between administering the test-dose and collecting the blood had been prolonged beyond four-and-a-half hours, a larger proportion of sera would have contained concentrations of isoniazid that were below the limit of sensitivity of the assay method, and this would have impaired the statistical analysis of the results. A longer interval than four-and-a-half hours could, however, be employed if a larger test-dose of isoniazid than 3 mg/kg were used. In future studies at the Tuberculosis Chemotherapy Centre it is proposed to administer an intramuscular test-dose of 6 mg/kg isoniazid and to collect blood six hours later.

Considering the second approach, a decrease in the variation from patient to patient within each of the two inactivation groups would also result in greater precision in the classification of the patients. Owing to the presence of the patients who could not be classified as slow or rapid inactivators with certainty, and because a proportion of assay results lay below or above the range of the method, exact estimates of the standard deviations of the distributions of rapid and slow inactivators could not be calculated. Nevertheless, it appears probable that the error of the test procedure—that is, the variation from test to test on the same patient—accounted for the variation from patient to patient in the group of slow inactivators, which is the better defined of the two distributions (Fig. 2).

In considering the error of the test procedure, it is important to realize that it is derived from two sources. The source yielding the greater part of the error (standard deviation of 0.45 dilution step) consists of the effects of factors other than the microbiological assay, such as possible inaccuracies in the measurement of the test-dose or variations in its rate of absorption. These two sources of error might be controlled by injecting a carefully measured dose in a large volume by the intravenous route.

Jenne (1960), however, using the intravenous route, obtained a similar proportion of indeterminate results to that encountered in our study. The second source is the error of the assay itself, which has a slightly smaller standard deviation (0.30 dilution step). A considerable portion of this error was due to batch variation, and it is a disturbing comment on the present method that the controls set up to eliminate this variation entirely failed to do so. While it has not been possible to define and estimate all the causes of error in the test procedure, it is believed that the estimates obtained of certain variations which contributed to it have been helpful in indicating which aspects of the tests should receive most attention in future attempts to reduce the error. In the meantime, until a procedure is available which completely separates the distributions of slow and rapid inactivators, it would seem advisable to carry out repeat tests on those patients whose first results are indeterminate, and classify them on the basis of the mean of the two tests.

Mandel *et al.* (1956) had previously reported that the microbiological assay of isoniazid was unaffected by the addition of 100 µg/ml streptomycin to undiluted human serum. However, these authors did not report the concentration of streptomycin present in the serum dilution which produced 50 per cent loss of acid-fastness. The present investigation has shown that 50 µg/ml streptomycin added to the assay medium probably did not interfere with the assay, and was responsible for a marked reduction in contamination rates.

The finding in the present study that isoniazid in serum was destroyed at a rate of 3.7 per cent per week for eight weeks during storage at  $-20^{\circ}$  C is at variance with the findings of Bell and Riemensnider (1957a), who reported that there was no loss of isoniazid on up to 21 weeks' storage in the deep-freeze.

The rate of inactivation of isoniazid has been shown to be determined genetically, slow inactivation being a simple Mendelian recessive trait (Knight, Selin and Harris, 1959; Price Evans, 1959) which occurs with different frequencies in different racial groups (Harris, Knight and Selin, 1958; Armstrong and Peart, 1959). Harris, Knight and Selin (1958) found that 44 per cent of Americans of Caucasian ancestry were slow inactivators, as compared

with 12 per cent of Americans of Japanese descent, and Armstrong and Peart (1959) found that only 5 per cent of Eskimos were slow inactivators as compared with 56 per cent of non-Eskimos. Price Evans (1959) found that 52 per cent of Americans of Caucasian ancestry were slow inactivators and the remainder rapid inactivators. Our finding that the proportion of slow inactivators in Indian patients was 61 per cent is thus similar to the proportion of this group in Americans of Caucasian descent.

The relationship shown in this study between the rate of inactivation of isoniazid and sex is different from the findings of Price Evans (1959). In our investigation, the proportion of rapid inactivators among female patients was significantly higher than that among male patients and this difference could not be accounted for by differences in body-weight. Price Evans (1959), on the other hand, demonstrated a relationship between the rate of inactivation of isoniazid and body-weight, but not between the rate of inactivation and sex. It is difficult to explain this discrepancy. It may be due to the fact that his population contained a larger proportion of children.

We have no definite information on the effect of malnutrition on the rate of inactivation of isoniazid. Suggestive evidence is, however, afforded by the serial determinations of the inactivation rates of 14 patients that were done on admission to treatment and after three, six, nine and 12 months of treatment. Although the patients were malnourished on admission to treatment and gained, on the average, 7 lb. in weight during the 12 months of treatment, there was no corresponding alteration in their rates of inactivation of isoniazid.

#### SUMMARY

1. The serum isoniazid concentrations were determined four-and-a-half hours after an intra-muscular injection of 3 mg/kg isoniazid in 321 of 341 patients participating in a controlled chemotherapy study.

2. The addition of streptomycin in a final concentration of 50 µg/ml was found to have no effect on the assay.

3. Isoniazid in serum was destroyed at a rate of 3.7 per cent per week for eight weeks during storage at -20°C.

4. Estimates were obtained of the error of the procedure for determining the rate of inactivation of isoniazid (0.54 dilution step) and for the method of microbiological assay of isoniazid in serum (0.30 dilution step). The error due to factors other than that of the microbiological assay method was estimated as 0.45 dilution step.

5. A defect of the microbiological assay method was the presence of batch variation, which was not corrected for by the use of isoniazid controls. The error introduced by batch variation was estimated as 0.26 dilution step.

6. Patients with serum isoniazid concentrations of 0.58 µg/ml or more were classified as slow inactivators and those with less than 0.58 µg/ml as rapid inactivators. Of the 321 patients studied, 195 (61 per cent) were classified as slow inactivators and 126 (39 per cent) as rapid inactivators.

7. The rate of inactivation of isoniazid in individual patients was found to remain constant during 12 months of treatment.

8. A relationship was shown between sex and the rate of inactivation of isoniazid, there being a significantly higher proportion of rapid inactivators among the females as compared with the males.

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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**

C. V. RAMAKRISHNAN,<sup>1</sup> A. L. BIATIA,<sup>1</sup> WALLACE FOX,<sup>1</sup> D. A. MITCHISON,<sup>2</sup> S. RADHAKRISHNA,<sup>1</sup>  
J. B. SELKON,<sup>1</sup> T. V. SUBBAIAH,<sup>1</sup> S. VELU<sup>1</sup> AND J. G. WALLACE<sup>2</sup>

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 1-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-1 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 per cent) 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), these cultures were 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.

<sup>1</sup> Tuberculosis Chemotherapy Centre, Madras, India. The Centre is under the joint auspices of the Indian Council of Medical Research, the Madras State Government, the World Health Organization and the Medical Research Council of Great Britain.

<sup>2</sup> Unit for Research on Drug Sensitivity in Tuberculosis (Medical Research Council of Great Britain), Postgraduate Medical School of London, London, England.

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.<sup>1</sup>

*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 per cent of the PH patients, 7 per cent of the HI-1, 21 per cent of the HI-2 and 17 per cent of the H patients. Using very stringent criteria to assess the response to treatment, 8 per cent of the PH, 27 per cent of the HI-1, 41 per cent of the HI-2 and 52 per cent 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-1 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 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

<sup>1</sup> 1 lb. = 0.45 kg.

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 per cent) 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.*, 1962<sup>1</sup>). 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, 1962<sup>2</sup>). 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 squareroot 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.*, 1962<sup>2</sup>), 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  $-20^{\circ}\text{C}$ ; (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  $-20^{\circ}\text{C}$  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 an accompanying paper (Subbaiah *et al.*, 1962<sup>3</sup>), 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.

#### Results

The values of the root-index corresponded to the type of disease found at post-mortem

<sup>1</sup> See article on page 111.

<sup>2</sup> See article on page 71.

<sup>3</sup> See article on page 45.

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*) per standard Unit† per month	Less than 20	32	35	51	42	28	41	0.74
	20-30 or more	29	32	42	34	28	41	0.75
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	16	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

\* Rs 4.76 = US \$ 1.00.

† 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.

in the guinea-pigs as follows: Root-indices of virulence of 0.00-0.59 were obtained with 91 (32.5 per cent) 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 per cent) 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 per cent) 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).

**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).

*Income of the family*

The relationship between the family income and the virulence of the cultures was investigated

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 patient	%	No. of patients	%	No. of patients	%	
Extent of cavitation	Extensive	9	70	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	72	0.76
Number of lung zones involved in disease	6 or 5 4	36	40	58	48	24	55	
	or 3	38	42	48	39	26	38	
	2 or 1	17	19	16	13	18	26	
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	76	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*	100	122	99	68	99	0.73

\* Excluding five patients for whom the independent assessor reported the activity of the disease as unclassifiable.

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

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						Mean	
	0.0-		0.6-		0.9 or above			
	No. of patients	%	No. of patients	%	No. of patients	%		
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	79	11	16	0.69
	Negative	14	75	12	70	18	26	0.78
	Total	91	700	122	707	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*	100	114†	700	66‡	100	0.73

\* Excluding three patients for whom the rate of inactivation of isoniazid was not determined.

† Excluding eight patients for whom the rate of inactivation of isoniazid was not determined.

‡ Excluding two patients for whom the rate of inactivation of isoniazid was not determined-

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 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 1-2 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

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

of high virulence were obtained slightly more often from patients with less severe radiographic lesions, in particular, involvement of 1-2 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 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

Treatment series	Root-index of virulence	0-6 months				0-12 months					
		Total patients <sup>1</sup>		Moderate or greater improvement	Slight improvement or no change	Deterioration or death from tuberculosis	Total patients <sup>2</sup>		Moderate or greater improvement	Slight improvement or no change	Deterioration or death from tuberculosis
		No.	%				No.	%			
PH	0.0-	22	100	17 (77) <sup>3</sup>	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

<sup>1</sup> 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.

<sup>2</sup> 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.

<sup>3</sup> The parentheses indicate percentages based on fewer than 25 observations.

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 associa-

tion 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-1, 13 HI-2, 11 H) had their treatment changed because of serious radiographic or clinical deterioration. (An independent 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 0-12 month assessments. Thus, in the HI-1 series, 17 (89 per cent) of 19 patients with cultures of low virulence had moderate or greater radiographic improvement over the 12 months, as compared with 29 (76 per cent) of 38 patients with cultures of moderate or high virulence. The correspond-

ing results in the combined HI-2 and H series were 35 (76 per cent) of 46 patients with cultures of low virulence and 52 (60 per cent) 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 ( $P = 0.07$ ).

#### Disappearance of cavitation

The root-indices of virulence are related to the disappearance of cavitation during the

TABLE 6

Correlation coefficients between changes in radiographic appearance and root-indices of virulence

Treatment series	0-6 months		0-12 months	
	Correlation* coefficient (r)	P	Correlation* coefficient (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

\* 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.

TABLE 7  
Disappearance of cavitation during the 12-month period related to virulence of pretreatment cultures

Treatment series	Root-index of virulence	Total patients*		Cavities remained		Cavities disappeared	
		No.	%	No.	%	No.	%
	0.0-0.6-0.9 or above	18	700	4 (22)†		14	(75)
		36	700	15	42	21	58
		18	700	5	(28)	13	(72)
PH	Total	72		24		48	
	Mean			0.75		0.73	
	0.0-0.6-0.9 or above	19	100	9 (47)		10	(53)
		23	100	10	(43)	13	(57)
		14	100	4	(29)	10	(77)
HI-1	Total	56		23		33	
	Mean			0.70		0.75	
HI-2	0.0-0.6-0.9 or above	42	100	18	43	24	57
+H		55	100	28	51	27	49
		25	100	15	60	10	40
	Total	122		61		61	
	Mean			0.75		0.69	

\* 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.

† The parentheses indicate percentages based on fewer than 25 observations.

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

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*

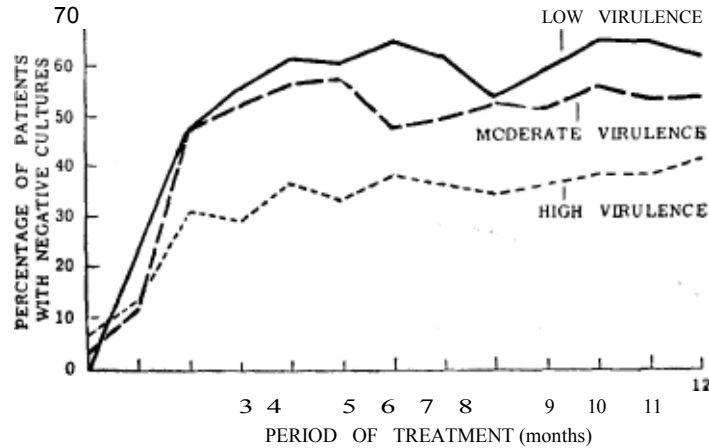
The results of culture of single collection sputum specimens obtained at monthly intervals from the patients in each of the series were related to the virulence of their pretreatment cultures. In the PH and HI-1 series no associations were evident and the full results are not presented here. In the PH series at 6 months, negative cultures were obtained from 86 per cent of 21 patients with pretreatment cultures of low virulence and from 88 per cent of 56 patients with cultures of moderate or high virulence. The corresponding percentages at 12 months in this series were 90 per cent and 87 per cent, respectively. In the HI-1 series negative cultures were obtained from 71 per cent of 21 patients with pretreatment cultures of low virulence and from 76 per cent of 38 patients with cultures of moderate or high virulence at 6 months, and from 82 per cent and 76 per cent, 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 per cent of 44 patients with pretreatment cultures of low virulence, from 52 per cent of 56 patients with cultures of moderate virulence and from 29 per cent of 28 patients with cultures of high virulence. The corresponding percentages at 6 months were 64 per cent, 47 per cent and 38 per cent and at 12 months 61 per cent, 53 per cent and 41 per cent, 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 per cent 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 Che-

FIG. 1

*Patients with negative sputum cultures in the combined HI-2 and H series related to virulence of pretreatment cultures*



motherapy Centre, 1960), is related to the virulence of the pre-treatment 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 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-1 series, bacteriologically quiescent or doubtful status was attained by 15 (79 per cent) of 19 patients with cultures

of low virulence and by 28 (74 per cent) 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 per cent) of 46 patients with cultures of low virulence, as compared with 39 (45 per cent) 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-1 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 ( $P=0.05$ ).

### 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

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 <sup>1</sup>		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	(9.7) <sup>2</sup>	1	(5)	1	(5)
	0.6-	36	100	30	83	5	17	1	3
	0.9 or above	21	100	21	(700)	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	(76)	1	(5)
	0.6-	23	100	17	(74)	4	(77)	2	(9)
	0.9 or above	15	99	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	77
	0.6-	58	700	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	

<sup>1</sup> 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.

<sup>2</sup> The parentheses indicate percentages based on fewer than 25 observations.

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

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* ( <i>r</i> )	P
PH	- 0.065	0.5-0.6
HI-1	+ 0.209	0.1
HI-2 + H ...	+ 0.168	0.05

\* 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.

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

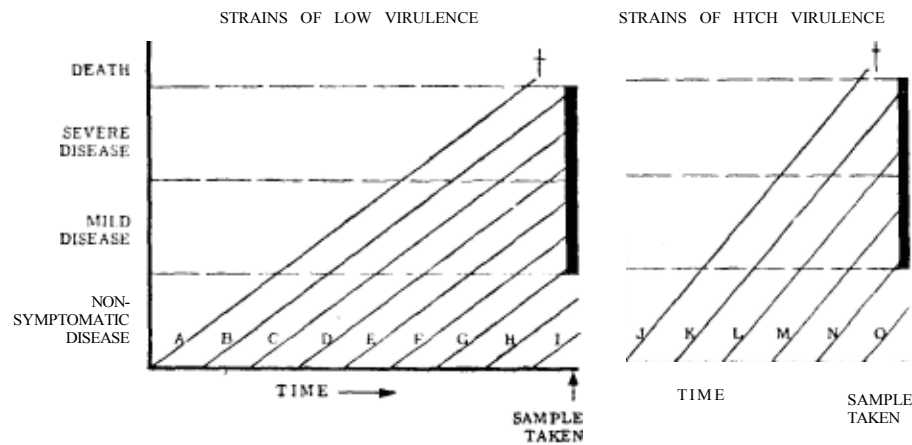
the sample was also not representative. On the one hand, patients under 12 years of age were not admitted to the study. On the other hand, of the 279 patients aged 15 years or more, 16 per cent were at least 45 years old. In a sample survey of the prevalence of pulmonary tuberculosis in India (Indian 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 per cent to 52 per cent; 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 per cent to 56 per cent. 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

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.

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. 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-Moller (1960). Frimodt-Moller 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-Moller, 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-Möller (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 per cent of patients with cultures of low virulence and by 45 per cent of those with cultures of moderate and high virulence. Moderate or

greater improvement over the 12 months was shown by 76 per cent of patients with organisms of low virulence and by 60 per cent of those with more virulent organisms. The two associations attained statistical significance at the 5 per cent and 1 per cent 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 over riding 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 per cent) 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 100lb. 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 per cent of the patients with cultures of low virulence, but by only 60 per cent of those with cultures of moderate or high virulence. The corresponding proportions of patients achieving bacteriological

quiescence by 12 months were 54 per cent and 45 per cent. 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.

### Acknowledgements

Our thanks are due to the nursing staff of the Tuberculosis Chemotherapy Centre who arranged for the collection of sputum specimens from the patients. Dr P. R. J. Gangadharam (Madras), Mr C. Narayanan Nair (Madras), Mr K. L. Thomas (Madras), Dr A. Csillag (London), Dr E. Geraghty (London), Miss J. Lloyd (London), Dr M. C. Lancaster (Porton) and Mr J. Randles (Porton) assisted with the animal experiments. Dr Ian Sutherland (Statistical Research Unit, Medical Research Council of Great Britain) gave advice on the statistical methods, and some of the computations were done by Mr K. Ramachandran (Madras) and Mr P. R. Somasundaram (Madras). The facilities at Porton were very kindly provided by Dr D. W. Henderson.

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## NEWS AND NOTES

### **XXII Annual Meeting**

The XXII Annual General Meeting of the Tuberculosis Association of India will be held in New Delhi on Thursday, the 26th April, 1962 at the Conference hall of the Association, 3 Red Cross Road, New Delhi.

The Central Committee of the Association will also meet on the same day in the same place.

A meeting of the Standing Technical Committee will be held on Friday the 27th April in the Committee room of the Tuberculosis Association of India, New Delhi.

### **1962 Health Visitors' Course**

The 1962 Tuberculosis Health Visitors Course commenced in the New Delhi TB Centre on 2nd January, 1962. Thirteen candidates deputed by various states are taking the training.

### **Postgraduate Refresher Course, Hyderabad**

A short-term postgraduate refresher course was held at Hyderabad from 2nd to 27th February, 1962 under the auspices of the Andhra Pradesh TB Association.

### **Next Meeting of the Eastern Regional Committee**

The next meeting of the Eastern Regional Committee of IUAT will be held in Bangkok, Thailand during the later half of November

this year. The meeting will be held for three days out of which one day will be given to administrative and official matters. Two days will be devoted to the discussion on scientific subjects. This meeting is sponsored under the joint auspices of the Eastern Regional Committee of the IUAT and the Anti-Tuberculosis Association of Thailand.

### **Chest and Heart Association—India Scholarship**

The Chest and Heart Association, London has awarded a scholarship to the value of £350 to a medical graduate from India for study in Tuberculosis in the United Kingdom in 1962. The candidate will be selected by the Tuberculosis Association of India. Details of Scholarship and other particulars are available with the Secretary, Tuberculosis Association of India, 3 Red Cross Road, New Delhi.

### **New Drugs and their efficacy**

The Director General of Health Services (Drugs Controller), Government of India, Ministry of Health, New Delhi requests Tuberculosis Institutions to report to him any toxic or undesirable or hitherto unnoticed effects of drugs which they may come across in the course of professional practice. It will be appreciated if details regarding the name of the drug involved, name of the manufacturer, batch number, and if possible, the name of the dealer from whom the drug was purchased could be furnished to the Directorate.

# The Indian Journal of Tuberculosis

## ABSTRACTS

Vol. IX

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Abst No. 2

### **Pulmonary Tuberculosis in Contacts: A Ten Year Survey**

Over a period of ten years 81 (12 per cent) of the 664 contacts of 155 active pulmonary tuberculous cases developed active tuberculosis. Of these 81 contacts had 8 (1.2 per cent) non-respiratory tuberculosis, 12 (1.8 per cent) had childhood type primary pulmonary tuberculosis necessitating hospital treatment and 61 (9.2 per cent) had adult type pulmonary tuberculosis.

Of the 61 cases of adult Pulmonary Tuberculosis, about one third (22) were discovered in the first year, about one half (32) in the first two years and about three quarters (46) in the first five years.

Difficulties of Long term supervision have been discussed.

*A. W. Lees; G. W. Allan; J. Smith and W. F. Tyrrell. Dis. Chest, Vol. 40, No. 5, Nov., 61.*

### **The Streptomycin Concentration in Tuberculous Cavities**

Streptomycin activity inside the Cavity was investigated in 31 cases.

Daily Streptomycin gave superior results compared with administration upon alternate days.

Thick walled cavities did not contain a high streptomycin concentration as often as thin walled cavities.

*K. D. Arosenius, V. O. Bjork and G. Laurell. Thorax (1961), 16,361.*

### **Pulmonary Resection in Patients Harboring Drug Resistant Tubercle Bacilli**

265 Pulmonary Resections done on 220 patients harbouring tubercle bacilli resistant to Streptomycin, P.A.S. and/or Isoniazid showed tuberculous complications in 23.4 per cent and mortality rate of 9.1 per cent.

Drug Therapy, extent of Disease, presence of culturable tubercle bacilli, type of surgery and patients' co-operation had a great influence on the ultimate results.

*David Precora: Amr. Rev. Resp. Dis., Vol. 84, No. 4, Oct., 61.*

### **A Trial of Corticotrophin and Prednisone with chemotherapy in Pulmonary Tuberculosis**

Three hundred and forty-six patients with acute pulmonary tuberculosis were allocated at random to treatment.

(a) 119 patients with chemotherapeutic drugs (Streptomycin 1 gm., Soda PAS 16 gms. and Isoniazid 300 mgms. daily) control series.

(b) 111 patients were given Corticotrophin 30 I.U. for 3 months daily in addition to chemotherapy in the control group.

(c) 116 patients were given Prednisone 30 mgm. daily for 3 months along with Chemotherapy as in (a)

All the three groups had chemotherapy for six months.

In (b) (A.C.T.H. group) and (c) (Prednisone group) the Clinical improvement was very rapid as shown by increase in weight, fall in the Erythrocyte sedimentation rate and average temperature and the improvement was maintained after sloping the hormones. The progress of the (a) (Control group) was slower, but by twelve months the improvement was similar to that of the two hormone series.

The (b) (A.C.T.H.) and (c) (Prednisone) series had significantly less residual radiographical shadowing at three months and prednisone series at six and twelve months, than the Control series.

Transient radiographic deterioration between three and four months was observed in 47 patients (5 Control, 12 A.C.T.H. and 30 Prednisone).

Clinical rebound phenomenon occurred in 6 A.C.T.H. and 34 Prednisone patients.

There was little difference between three series in terms of Cavitation changes and sputum conversion at any time. Almost all the patients in the three series had negative cultures at six months and all but 1 Control patients at nine and 1 at twelve months.

Severe hypersensitive reactions to Streptomycin, PAS or isoniazid, Streptomycin toxicity or PAS intolerance which necessitated a change

of chemotherapy occurred in 31 patients (6 treated with 20 needles for the 100 mgm/ml vaccine. Control, 7 A.C.T.H. and 9 Prednisone).

In 22 patients (15 A.C.T.H. and 7 Prednisone) the needles of the guns to obtain adequate side effects caused the hormone therapy to be penetration may partially account for the difference changed or stopped before the end of the courses between the groups in both tuberculin sensitivity and lesions produced after vaccination.

It is concluded that prednisone has a place The pressure required was greater for the with Chemotherapy, in the treatment of selected 20 needles guns used in Lancashire than for the cases of acute extensive pulmonary tuberculosis. others.

*A Report from the Research Committee of the British Tuberculosis Association, Tubercle, London, (1961), 42, 391.*

It is concluded that 6 needles were not sufficient for satisfactory vaccination, 20 needles probably provide the most satisfactory vaccination and a 40 needles gun may be impracticable.

### **B.C.G. Vaccination by Multiple Puncture: Preliminary Report**

The pressure at which the needles are released must be sufficient to produce their full penetration into the skin.

The results of a study in Lancashire and Salford on B.C.G. vaccination by multiple puncture have been reviewed.

*A Report from the Research Committee of the British Tuberculosis Association, Tubercle, London, (1961), 42, 413.*

Six groups of children were vaccinated with 70 or 100 mgm/ml strength of vaccine and given 6, 20 or 40 punctures. All the 898 vaccinated children tested in Lancashire and almost all (90 per cent or more) of the 866 vaccinated children in Salford were positive to the Heaf-test 10 to 13 weeks after vaccination.

### **The Significance of Radiographic Calcification in the lungs of Coal Workers**

In Lancashire those vaccinated with 20 needles and given 70 mgm/ml vaccine had higher tuberculin sensitivity than those vaccinated with either 60 or 40 needles.

Lesions responsible for the radiographic changes in six coal workers on clinical and Necropsy findings proved entirely Pneumococciotic, there being no evidence of Pulmonary Tuberculosis.

There was no significant difference in sensitivity according to the number of needles used with 100 mgm/ml vaccine.

It is concluded that radiographic appearance of extensive nodular pulmonary calcification is more in Pneumoconiosis than in Pulmonary Tuberculosis.

*In Salford:* There was no significant difference in sensitivity according to number of needles used with 70 mgm/ml strength, but those vaccinated with 20 needles and the 100 mgm/ml vaccine, there was a higher conversion rate than those given 6 needles.

*J. P. Lyons and A. J. Watson: Tuberc., Land., (1961), 42, 457.*

### **Liquid or Freeze Dried B.C.G. Vaccine: Persistence of Tuberculin Sensitivity in School children after vaccination**

The majority of the minor complications reported in both areas were in those vaccinated with 20 needles. At one year after vaccination the proportion of tuberculin positive were lower than between 10 and 13 weeks in Salford and very considerably lower in Lancashire.

258 children were vaccinated in 1957 with Danish Liquid B.C.G. vaccine and 216 with British Freeze dried B.C.G. vaccine. Both groups did not show change in sensitivity between 3 months and one year after vaccination in either group.

In Lancashire, at one year those vaccinated with 20 needles and given 70 mgm/ml vaccine had a higher sensitivity than those given either 6 or 40 punctures. There were no significant difference according to the number of needles used with 100 mgm/ml vaccine.

Between one and four years after there was significant decline in sensitivity to 3 T.U.

In Salford at one year the children receiving 6 puncture had a significantly lower positivity than those vaccinated with 40 needles for both strengths of vaccine and also than those vaccina-

In Danish Vaccine group decline in sensitivity was seen from 98 to 81 per cent and in the British Vaccine group from 93 to 71 per cent.

The changes were a result of reversion to negativity and not of weakening of sensitivity. Significance of decline of sensitivity in relation to protection against tuberculin is not clearly understood.

*Christine L. Miller and Barbara J. Kinsley: British Med. Jour., 1322, Nov., 18, 61.*

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Ind. J. Tub., Vol. IX, No. 2

**Cavitated Pulmonary Tuberculosis: A Long Term Follow up of 283 patients with special Reference to the Significance of the Persistent Cavity**

283 cases with Cavitory Pulmonary Tuberculosis who received no major surgical treatment were followed for 5—14 years.

156 of the 283 patients were alive at the end of the survey. There were 34 relapses. 9 of the patients were unco-operative, 25 were co-operative but had had insufficiently prolonged Chemotherapy and all responded satisfactorily to further treatment.

P. G. Arblaster, K. W. Cross, I. R. McWhinney and Janet Yates: *Tubercle*, London, (1961), i2, 428.

**The Treatment of Chronic Cavitating Pulmonary Tuberculosis by Long Term Chemotherapy**

46 out of the 50 patients with chronic Cavitating Pulmonary Tuberculosis not amenable to surgical treatment but sensitive to 2 or more of the 3 standard drugs treated with long term chemotherapy were negative.

40 out of 45 followed for 3 years were still negative.

22 out of 24 followed for 5 years were still negative.

D. H. Blake, *Tubercle*, Lond., (1961), 42, 438.

*LETTER TO THE EDITOR*

**FOLLOW UP OF BCG VACCINATION PROGRAMME**

SIR,

The article by ‘ Billimoria’ on the above subject in September 1960 issue of your journal is rather misleading. A comparison was attempted to be made between two groups standing on altogether different bases. To find the efficacy of vaccination programmes by following up two groups of persons varying to a maximum extent in the age, sex and epidemiological characteristic is unscientific.

The comparison has been made between the prevalence of cases in the unvaccinated and vaccinated groups. The former consists of ONLY mantoux positives (presumable infected) at the time of vaccination and the latter of mantoux negatives (presumable uninfected). Even without vaccination it will be possible to prove that the prevalence of cases and the emergence of new cases is greater among mantoux positives compared with that among mantoux negatives where it will be significantly small. In such uncomparable groups the introduction of the effect of BCG Vaccination will be only to mislead readers. If, at all, any comparison is to be made it should be on two groups identical in every respect (age, sex and tuberculin status) but with one group having been vaccinated while the other acts as controls. An analysis of data from such studies only will help those interested in finding the efficacy of vaccination. Unfortunately there is yet no short cut to this method.

*National TB Institute,  
Bangalore-3*

Sincerely yours,  
A. M. SUBRAMANIAM

**WANTED**

Doctor experienced in tuberculosis work for surveying the incidence and conducting a pilot treatment scheme in the Dandakaranya Project, S. Orissa. Salary based on Christian Medical Association of India scales, with rural living allowance. Christian preferred.

*Apply:*

**Director, Bengal Refugee Service, 5 Russell Street, Calcutta-16**