

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

2. Comparison with Virulence of Tubercle Bacilli from British Patients

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A series of studies has been undertaken by the Tuberculosis Chemotherapy Centre Madras, with the ultimate object of finding out whether differences in the virulence of the tubercle bacilli isolated from Indian tuberculous patients before the start of chemotherapy are related to the severity of the patients' disease and to the subsequent response to treatment. This paper—the second in the series—presents the results of a comparative investigation of the virulence in the guinea-pig of tubercle bacilli obtained from Indian and from British tuberculous patients before treatment. In this investigation, which was carried out at the Centre and at the Microbiological Research Establishment, Porton, England, the virulence of the Indian and the British cultures was assessed by guinea-pig mortality, by the "root-index of virulence" (based on the post-mortem tuberculous disease score and the survival period of the animal), and by the results of spleen culture. The Indian cultures were found, on the average, to be less virulent and to show a wider range of virulence than the British cultures, both in the Porton and in the Madras series of experiments. A further indication of the heterogeneity of the Indian tubercle bacilli was provided by the results of tuberculin tests: whereas the British cultures appeared to be homogeneous in their ability to induce tuberculin allergy in the guinea-pig, the Indian cultures showed considerable variation in this respect.

INTRODUCTION

'After Frimodt-Møller, Mathew & Barton (1956) had reported that a considerable proportion of cultures of tubercle bacilli from untreated South Indian patients was attenuated in the guinea-pig, it was planned to investigate the virulence of cultures from

Indian patients participating in controlled chemotherapy studies at the Tuberculosis Chemotherapy Centre, Madras. In the first stage of this investigation (Mitchison et al., 1960), isoniazid- and streptomycin-sensitive cultures, shown to be tubercle bacilli by *in vitro* tests, and obtained before treatment from the Indian patients were compared with corresponding British cultures for their virulence in three breeds of guinea-pig. The Indian cultures were found to be, on the average, less virulent and to have a wider range of virulence than the British cultures.

The second stage of the investigation, which overlapped in part with the first stage, was planned to

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relate the virulence of the cultures obtained before treatment from Indian patients participating in a comparison of various domiciliary regimens of chemotherapy (Tuberculosis Chemotherapy Centre, 1960) to the degree and type of the patients' pulmonary disease on admission to treatment and to the patients' progress during treatment with the prescribed chemotherapy. The results of this study are reported in the third paper of the present series (Ramakrishnan et al., 1961¹).

During the course of the second stage, cultures from untreated British patients were also tested for their virulence, partly to serve as sensitive indicators

of inter-experimental variation in the series, partly to provide evidence on the differences between Indian and British cultures in a larger number of tests, and partly to establish that the Indian cultures were as attenuated, relative to British cultures, as they had been in the first stage of the investigation. An account is given in the first paper of the present series (Mitchison et al., 1961²) of the manner in which the cultures from British patients were used to estimate inter-experimental variation. We report here the comparison of the virulence of the cultures from Indian and British patients.

METHODS

A full account of the methods employed in the investigation of virulence is given in the accompanying paper by Mitchison et al. (1961).² In brief, these are as follows:

CULTURES OF TUBERCLE BACILLI

Indian

A total of 281 cultures of tubercle bacilli were obtained from the sputum of 281 Indian patients participating in a controlled comparison of various regimens of chemotherapy (Tuberculosis Chemotherapy Centre, 1960). All of these cultures were sensitive to isoniazid. Of the 281 patients, 269 had received no previous chemotherapy as far as could be discovered, 11 had probably received up to two weeks of chemotherapy, and one had received three months of the prescribed regimen in the controlled comparison. In a paper to appear shortly (Subbaiah et al., 1961), it is shown that the virulence of cultures from Indian patients was not affected by three months of chemotherapy, provided that the cultures remained sensitive to isoniazid. The results of the virulence tests on the cultures from the 12 patients who had received some chemotherapy have therefore been included.

British

Of the 93 British cultures, 92 were obtained from the same number of British patients with newly diagnosed and previously untreated pulmonary tuberculosis. The clinics and hospitals which pro-

vided the sputum specimens from these patients are listed in the acknowledgements in the paper by Mitchison et al. (1961).² The remaining culture was obtained by mistake from a patient who had received previous chemotherapy, but the results for this culture have been included for the reason given above, since the culture was sensitive to isoniazid, streptomycin and PAS.

VIRULENCE TESTS

The majority of the virulence tests were carried out at the Microbiological Research Establishment, Porton, Wiltshire, England, on albino Duncan Hartley (DH-breed) guinea-pigs. The remainder were done at the Tuberculosis Chemotherapy Centre, Madras, India, on mixed-colour (M-breed) guinea-pigs.

Root-index of virulence

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, 1961³). In brief, the sputum culture was subcultured on to a Löwenstein-Jensen medium slope and, after three weeks' incubation, 1 mg (moist weight) of the growth on the subculture 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 of the tests at Porton, each culture was inoculated into four guinea-pigs, two being killed at 6 weeks and two at 12 weeks. At the post-mortem examination of the animals, the total extent of tuberculous disease was assessed as a score ranging from 0 to 100. The square root of the ratio of the

¹ See article on page 323.

* See article on page 285.

score to the survival time in days was determined for each guinea-pig (whether sacrificed or dead from tuberculosis) and was termed the " root-index ". The mean of the root-indices for all of the guinea-pigs infected with a culture was termed the " root-index of virulence " for that culture.

Spleen cultures and Mantoux tests

Spleen cultures and Mantoux tests were done only at Madras. The spleens of the guinea-pigs were cultured either by rubbing the cut surface of the spleen on a Löwenstein-Jensen medium slope, or by homogenizing part of the spleen in about 1 ml of water and inoculating a loopful of the suspension on to the same medium. Mantoux tests were done with 0.1 ml of 1 : 100 Old Tuberculin intracutaneously four weeks after infection of the guinea-pigs. The diameters of the areas of erythema were read 48 hours later, in two directions at right angles to each other, and the mean of the two readings was taken as the measure of tuberculin sensitivity for each guinea-pig.

Viable counts on the infecting suspensions

Counts of the numbers of viable units in the suspensions used for infecting the guinea-pigs were done in a sample of the tests at Porton. Counts were set up in 7H-10 medium (Cohn, Middlebrook & Russell (1959), as modified by Subbaiah, Mitchison & Selkon (1960)), solidified with silica gel according to the method of Selkon & Mitchison (1957).

ARRANGEMENT OF EXPERIMENTS

Porton series

Virulence tests were done on 254 Indian and 65 British cultures at Porton. The tests were set up in 13 experiments and in a total of 1018 guinea-pigs. Of the Indian cultures, 125 were each injected into four guinea-pigs and the remaining 129 were each injected into two guinea-pigs. In each experiment, tests were done on five British cultures, each inoculated into four guinea-pigs.

Madras series

In all, 55 Indian and 28 British cultures, each injected into two guinea-pigs, were tested in 11 experiments at Madras, on a total of 166 guinea-pigs. Cultures from 28 of the 55 Indian patients were also tested in the Porton series.

There are two main comparisons of virulence available between the Indian and the British cultures, namely :

(a) Between the 254 Indian and the 65 British cultures in the Porton series. (For certain comparisons, such as the mortalities among the guinea-pigs, it is appropriate to compare only the 125 Indian cultures that were each tested in four animals with the 65 British cultures, all of which were tested in four animals.)

(b) Between the 55 Indian and the 28 British cultures, each tested in two guinea-pigs in the Madras series.

It will be appreciated that these two comparisons are not independent, since cultures from 28 of the Indian patients were tested in both.

Homogeneity of the investigation

Since facilities for large-scale experiments on guinea-pigs were not available at Porton until towards the end of the investigation, 143 of the 254 Indian cultures in the Porton series were stored for an average of 62 weeks at -20°C before being subcultured in the virulence test. The remaining 111 Indian cultures in the Porton series, all of the Indian cultures in the Madras series, and all of the British cultures in both series were subcultured within 10 weeks of their becoming positive. Further, the tests were set up in 24 experiments over a period of two-and-a-half years. However, Mitchison et al. (1961) ¹ have shown that storage at -20°C did not affect the virulence of the cultures. Also, inter-experimental variation in virulence was found to be very small in both series and has been ignored in the majority of the analyses in the present report.

RESULTS

MORTALITY OF GUINEA-PIGS

The frequencies with which the British and Indian cultures caused death of the guinea-pigs from tuberculosis have been compared separately for the Porton and the Madras series (Table 1). In the

Porton series, considering only those cultures each of which was tested in four guinea-pigs, 16 (25 %) of the 65 British cultures as compared with 99 (79 %) of the 125 Indian cultures did not kill any of the

¹See article on page 285.

TABLE 1
ABILITY OF INDIAN AND BRITISH CULTURES OF TUBERCLE BACILLI TO CAUSE DEATHS FROM TUBERCULOSIS IN GUINEA-PIGS

Series	Number of guinea-pigs per culture which died from tuberculosis	British cultures		Indian cultures	
		No.	%	No.	%
Porton (4 guinea-pigs per culture)	0	16	25	99	7 9
	1	25	38	14	1 1
	2	21	32	10	8
	3	2	3	2	2
	4	1	2	0	0
	Total	65	100	125	100
Madras (2 guinea-pigs per culture)	0	7	25	42	7 6
	1	14	5 0	8	15
	2	7	25	5	9
	Total	28	100	5 5	100

TABLE 2
VIRULENCE IN THE GUINEA-PIG OF BRITISH AND INDIAN CULTURES OF TUBERCLE BACILLI TESTED IN THE PORTON SERIES

Root-index of virulence	British cultures		Indian cultures			
	4 guinea-pigs per culture		4 guinea-pigs per culture		2 guinea-pigs per culture	
	No.	%	No.	%	No.	
0.0-	0	0	0	0.0	0	0.0
0.2-	0	0	1	0.8	11	8.5
0.4-	0	0	34	27.2	33	25.6
0.6-	0	0	39	31.2	44	34.1
0.8-	18	2 8	32	25.6	23	17.8
1.0-	43	6 6	16	12.8	16	12.4
1.4-	4	6	3	2.4	2	1.6
Total	65	100	125	100.0	129	100.0
Mean	1.05		0.75		0.71	
Standard deviation ^a	0.06		0.21		0.18	

^a Square root of the component of variance for cultures in the same experiment, estimated from the analyses of variance presented in Tables 5 and 6 of Mitchison et al. (1961) (see article on page 265).

TABLE 3
VIRULENCE IN THE GUINEA-PIG OF BRITISH AND INDIAN CULTURES OF TUBERCLE BACILLI TESTED IN THE MADRAS SERIES

Root-index of virulence	British cultures		Indian cultures	
	No.	%	No.	%
0.0-	0	0	0	0
0.2-	0	0	4	7
0.4-	0	0	17	31
0.6-	0	0	12	22
0.8-	3	11	5	9
1.0-	8	29	7	13
1.2-	11	39	7	13
1.4-	6	21	3	5
Total	28	100	55	100
Mean	1.25		0.79	
Standard deviation ^a	0.12		0.30	

^a Square root of the component of variance for cultures in the same experiment, estimated from the analysis of variance presented in Table 6 of Mitchison et al. (1961) (see article on page 265).

guinea-pigs. Furthermore, two or more guinea-pigs per culture were killed by 24 (37 %) of the British cultures and by only 12 (10 %) of the Indian cultures. The greater ability of the British cultures to cause deaths from tuberculosis is also seen in the results obtained in the Madras series. At least one animal per culture was killed by 21 (75 %) of the 28 British cultures and by 13 (24 %) of the 55 Indian cultures. The difference between the British and the Indian cultures in their ability to kill guinea-pigs attains statistical significance at the 0.1% level in both series.

ROOT-INDICES OF VIRULENCE

The root-index of virulence combines the results of mortality and post-mortem score of the guinea-pigs inoculated with a culture. The values of the root-index of virulence obtained in the Porton series are set out in Table 2, and those in the Madras series are set out in Table 3.

In examining the ranges of the values of the root-indices of virulence in the Porton series, it is appropriate to compare the results on the British cultures,

each of which was tested in four guinea-pigs, with the results on the 125 Indian cultures that were also tested in four animals. Root-indices of virulence of 0.80 or more, indicating extensive disease in the visceral organs, were obtained with all of the 93 British cultures in the Porton and Madras series, but with only 51 (40.8 %) of the 125 Indian cultures

TABLE 4

COMPARISON OF TWO METHODS OF SPLEEN CULTURE

Result of spleen culture ^a	Method 1		Method 2	
	Number of guinea-Pigs	%	Number of guinea-Pigs	%
3-plus	12	14	5	7
2-plus	29	35	25	33
1 -plus	25	30	26	34
Negative	18	21	20	26
Total	84 ^b	100	76	100

^a3-plus: innumerable discrete colonies; 2-plus: 20-100 colonies; 1-plus: 1-19 colonies.

^bExcluding four guinea-pigs which yielded a contaminated result on spleen culture, and two guinea-pigs which died of non-tuberculous conditions.

TABLE 5

RESULTS OF SPLEEN CULTURES FROM GUINEA-PIGS INFECTED WITH BRITISH OR INDIAN CULTURES OF TUBERCLE BACILLI

Result of spleen culture	6-week guinea-pigs infected with:				12-week guinea-pigs infected with:			
	British tubercle bacilli		Indian tubercle bacilli		British tubercle bacilli		Indian tubercle bacilli	
	No.	%	No.	%	No.	%	No.	%
3-plus	7	25	3	6	4	14	3	6
2-plus	15	54	13	25	16	57	10	79
1 -plus	5	18	21	40	8	29	17	33
Negative	1	4	15	29	0	0	22	42
Total	28	101	52 ^b	100	28	100	52 ^b	100

^a3-plus: innumerable discrete colonies; 2-plus: 20-100 colonies; 1-plus: 1-19 colonies;

^bExcluding two guinea-pigs which yielded a contaminated result on spleen culture, and one guinea-pig which died of a non-tuberculous condition.

in the Porton series and with 22 (40%) of the 55 Indian cultures in the Madras series. Among the Indian cultures, 39 (31.2 %) in the Porton series and 12 (22 %) in the Madras series were moderately attenuated, with root-indices of virulence of 0.60-0.79, while 35 (28.0%) cultures in the Porton series and 21 (38%) cultures in the Madras series had values of less than 0.60, indicating a high degree of attenuation with disease usually confined to caseation at the site of inoculation and in its pining lymph-node.

It is probable that, in the Porton series (Table 2), the variation in the values of the root-indices of virulence was slightly greater in the Indian cultures tested in two guinea-pigs than in those tested in four guinea-pigs, owing to the greater influence in the former group of the natural variation in response of the guinea-pigs. True variation in virulence from culture to culture, after the elimination of this natural variation among the guinea pigs and inter-experimental variation (if any), was estimated in both the Porton and the Madras series as a standard deviation (last line of Tables 2 and 3). The estimates were 0.06 for the 65 British cultures and 0.20 for the 254 Indian cultures in the Porton series. In the Madras series the corresponding estimates were 0.12 and 0.30. The estimates for the Indian cultures have been shown to be significantly greater than zero in both series (Mitchison et al. (1961),¹ Tables 5 and 6, P <0.001) However, although the estimate for the British cultures was significantly greater than zero in the Porton series (P=0.001), it did not differ significantly from zero in the smaller Madras series (P=0.1).

In the Porton series the means. of the root-indices of virulence obtained with the 65 British cultures and with the 254 Indian cultures were 1.05 and 0.73, respectively. In the Madras series the means were 1.25 with the 28 British cultures and 0.79 with the 55 Indian cultures. In view of the greater variation among the Indian cultures (see above), the statistical significance of the difference between the means in each series was tested by Cochran's modified *t* test; both the differences were significant at the 0.1% level.

In summary, the average of the root-indices of virulence with the Indian cultures was lower than with the British cultures. Furthermore, the true variation in the values with the Indian cultures, expressed as a standard deviation, was about three times as great as that for the British cultures.

¹See article on page 285.

TABLE 6
RESULTS OF SPLEEN CULTURES FROM GUINEA-PIGS.
IN RELATION TO VIRULENCE

Result of spleen culture ^a	Guinea-pigs infected with British tubercle bacilli		Guinea-pigs infected with Indian tubercle bacilli ^b		
	Root-index		Root-index		
	Less than 1.30	1.30 or above	Less than 0.60	0.60-0.89	0.90 or above
3-plus	3	8	0	Q	6
2-plus	15	16	1	3	19
1-plus	11	2	9	20	9
Negative	1	0	23	9	5
Total	30	26	33	32	39

^a3-plus: innumerable discrete colonies; 2-plus: 20-100 colonies; 1-plus: 1-19 colonies.

^bExcluding four guinea-pigs which yielded a contaminated result on spleen culture, and two guinea-pigs which died of non-tuberculous conditions.

SPLEEN CULTURES

The spleens of the guinea-pigs in the Madras series were cultured either by rubbing the cut surface on Löwenstein-Jensen medium (method 1) or by inoculating the medium with an organ suspension (method 2). The results obtained by the two methods (Table 4) have been pooled, since there was no apparent difference in the positivity of the cultures obtained with them ($P = 0.1-0.2$), and since the ratio of British to Indian cultures tested in the guinea-pigs was approximately the same for the two methods.

The results of the cultures from the spleens of guinea-pigs infected with British or Indian tubercle bacilli are set out in Table 5. In 6-week guinea-pigs, heavily positive (3-plus or 2-plus) spleen cultures were obtained from 22 (79 %) of 28 animals infected with British tubercle bacilli and from 16 (31%) of 52 animals infected with Indian tubercle bacilli. The corresponding results for 12-week guinea-pigs were 20 (71%) and 13 (25 %) of the animals. Both of these differences attain significance at the 0.1% level. Thus, as judged by the results of spleen cultures, British tubercle bacilli were more virulent than Indian tubercle bacilli.

The results of the spleen cultures are related to the values of the root-indices obtained in the guinea-pigs in Table 6. Among the guinea-pigs infected with

British tubercle bacilli, spleen cultures of high (3-plus or 2-plus) positivity were obtained from 18 (60 %) of 30 animals whose root-index was less than 1.3, as compared with 24 (92%) of 26 animals with root-indices of 1.3 or more. A stronger association between heavy positivity of spleen cultures and high root-indices is evident in the results in the animals infected with Indian cultures. Heavily positive spleen cultures were obtained from four (6%) of 65 guinea-pigs with root-indices of less than 0.9, as compared with 25 (64 %) of 39 guinea-pigs with root-indices of 0.9 or more. The correlation between the positivity of the spleen cultures and the root-indices of virulence was calculated separately for the British and the Indian cultures, using scores of 0, 1, 2 and 3, respectively, for cultures in the negative, 1-plus, 2-plus and 3-plus categories. The correlation coefficients were 0.431 and 0.698 for the two series, respectively; both attain statistical significance at the 0.1% level.

TABLE 7
RESULTS OF MANTOUX REACTIONS IN GUINEA-PIGS
INFECTED WITH BRITISH OR INDIAN CULTURES
OF TUBERCLE BACILLI

Mean diameter of reaction (mm)	British cultures		Indian cultures	
	No.	%	No.	%
14	2	7	2	4
15	0	0	2	4
16	2	7	14 ^a	25
17	8	29	7	13
18	8 ^b	29	19	35
19	4	14	1	2
20	2	7	5	9
21	1	4	2	4
22	1	4	2	4
23	0	0	0	0
24	0	0	1	2
Total	28	101	55	102
Mean	17.9		17.6	

^aIncluding one culture for which the result was based on one guinea-pig only, the other having died before 30 days.

^bIncluding two cultures for each of which the result was based on one guinea-pig only, the other having died before 30 days.

TABLE 8
RESULTS OF MANTOUX TESTS IN GUINEA-PIGS INFECTED WITH BRITISH
OR INDIAN CULTURES OF TUBERCLE BACILLI
ANALYSIS OF VARIANCE

Term	Source of variation	British cultures					Indian cultures			
		DF	Mean square	Term tested against	F	P	DF	Mean square	F ^a	P
a	Cultures (C)	27	6 1130				54	7.8972		
b	Experiments (E)	10	11.6170	c	4.04	0.005	10	11.9770	1.72	0.1
c	Cultures in same experiment C(E)	17	2.8753	d	-	NS ^b	44	6.9700	2.31	<0.005
d	Duplicate tests	24 ^c	5.9625				54 ^d	3.0185		

^aAgainst the term indicated in the "British cultures" column.

^bNS indicates that the variance ratio is less than 1.0.

^cFour missing observations were estimated by standard statistical techniques.

^dOne missing observation was estimated by standard statistical techniques.

VIABLE COUNTS ON THE INFECTING SUSPENSIONS

During the course of the experiments at Porton, viable counts were done on a sample of the suspensions used for inoculating the guinea-pigs in the virulence tests (see Fig. 1 and 2 of Mitchison et al. (1961)¹). The mean of the counts on 58 suspensions from British cultures was 7.38 log₁₀ viable units per ml (range, 6.62-8.75 log₁₀ viable units per ml), and on 24 suspensions from Indian cultures was 7.47 log₁₀ viable units per ml (range, 6.74-8.18 log₁₀ viable units per ml). This difference between the means does not attain statistical significance. Thus it is improbable that the lower virulence of the Indian cultures is due to a difference in the mean viable units of the British and the Indian organisms in the infecting suspensions.

Mitchison et al. (1961)¹ have shown that there was no evidence of variation in the values of the root-index of virulence due to the preparation of the infecting dose, nor was there any association between the values of the counts reported here and the root-indices of virulence obtained with the guinea-pigs inoculated with the suspensions. Consequently, the variation in virulence from culture to culture is unlikely to be caused by differences in the size of the infecting dose.

MANTOUX REACTIONS

The distributions of the mean diameters of the Mantoux reactions are set out in Table 7 for the 28 British cultures and the 55 Indian cultures in the Madras series. The means of the two distributions were closely similar—namely, 17.9 mm and 17.6 mm, respectively.

The diameters of the reactions were examined by analysis of variance. Since the tests were done at four weeks, the readings on the 6-week and 12-week guinea-pigs were considered as duplicate observations. Variation in the diameters of the reactions between duplicate guinea-pigs appeared similar for the animals infected with British or Indian cultures (Table 8, term d, P > 0.1). When the variation in reaction size from culture to culture in the same experiment was compared with the variation between duplicate guinea-pigs (Table 8, terms c and d), the results with British cultures appeared homogeneous, whereas those with Indian cultures were heterogeneous (P < 0.005). These findings indicate that the Indian cultures varied in their ability to produce allergy in the guinea-pig. The variation, expressed as a standard deviation (the square root of the component of variance due to this source), was, however, only 1.4 mm.

¹See article on page 285.

DISCUSSION

In a previous report (Mitchison et al., 1960), drug-sensitive tubercle bacilli obtained before treatment from South Indian patients with pulmonary tuberculosis were found to be less virulent in the guinea-pig, on the average, than corresponding cultures from British patients. Evidence of the difference was obtained from four different assessments of virulence in the guinea-pig. These assessments were: (a) the amount of disease in the organs at post-mortem examination, assessed as a score; (b) the mortality from tuberculosis; (c) the positivity of cultures from the spleen; and (d) the "index", that is, the ratio of the post-mortem score to the period of survival (in days) of the guinea-pig. Of these assessments, the index was considered the best single measure of virulence since it measured the rate of progression of the lesions and since comparable values were obtained whether the guinea-pig had been killed or had died from tuberculosis. Statistically convincing evidence of the lower mean virulence of the Indian cultures was obtained from the scores and from the indices. However, the data on mortality and spleen cultures, though suggesting a large difference between the Indian and the British cultures, could not form the basis of firm conclusions because of the small numbers of cultures tested. In addition to these findings on the *mean* virulence of the Indian and the British cultures, the *range* of virulence of the Indian cultures, as measured in terms of the index, appeared to have been greater than was found among the British cultures.

The findings in the present report, based on a larger number of cultures and obtained by the same method of testing for virulence, confirm and strengthen these conclusions. The difference between the Indian and the British cultures in their ability to kill guinea-pigs and in the degree of positivity of the spleen cultures were substantial and attained a high degree of statistical significance. The index has been replaced, on statistical grounds, by the root-index of virulence (Mitchison et al., 1961¹), but the root-index retains all the advantages of the index. The root-indices of virulence obtained with the Indian cultures were not only lower, on the average, than

those obtained with the British cultures, but the range of their values was wider. The true variation in virulence from culture to culture, in terms of standard deviations of the root-index of virulence, was estimated to be about three times as large for the Indian as for the British cultures. The estimates, based on 254 Indian and 65 British cultures tested in England in the Porton series, and on 55 Indian and 28 British cultures tested in India in the Madras series, can be considered reasonably precise. Approximately one-third of the Indian cultures were similar in virulence to the British cultures, and about one-third were very attenuated, usually failing to produce visible disease in the visceral organs of the guinea-pig.

In a previous publication (Mitchison et al., 1960) a hypothesis was proposed to explain the lowered mean virulence and the wider range of virulence of cultures of tubercle bacilli from Indian patients. Evidence bearing on this hypothesis is presented in the third paper in this series (Ramakrishnan et al., 1961²), which is concerned with the progress of the patients during treatment, and will also be discussed in a further publication.

Apart from any considerations of the mechanisms by which attenuated tubercle bacilli arise and are maintained in India, it is clear that the variation in virulence found among the Indian cultures is an indication of their heterogeneity. Further evidence that Indian cultures are more heterogeneous than British cultures is provided by our findings on the Mantoux reactions in the guinea-pigs and by the wider range of susceptibility to hydrogen peroxide (Subbaiah, Mitchison & Selkon, 1960) and to thi-acetazone (Thomas et al., 1961) that has been demonstrated in Indian cultures. Whatever factors are responsible for the maintenance of tubercle bacilli of a fairly uniform high virulence in Great Britain may also be responsible for maintaining uniformity in other characters, even though an association between virulence and any one of the characters is very small. Equally, there may be factors other than selection pressures for virulence that induce more variation in Indian than in British cultures.

¹See article on page 285.

²See article on page 323.

SUMMARY

1. A culture of tubercle bacilli was obtained from the sputum of each of 281 South Indian and 93 British patients with pulmonary tuberculosis, of whom all except two had not had more than two weeks of antituberculosis chemotherapy. The virulence in the guinea-pig of these cultures was examined in two series of experiments, one at Porton in which 254 Indian and 65 British cultures were tested, and the other at Madras in which tests were done on 55 Indian and 28 British cultures. Cultures from 28 Indian patients were examined in both series.

2. The guinea-pigs were each injected with 1 mg of bacilli by the intramuscular route, and were killed 6 or 12 weeks later. The amount of visible disease at post-mortem examination was given a score. The best measure of virulence was considered to be the root-index, defined as the square root of the ratio of the score to the survival period of the guinea-pig.

3. As assessed by the mortality from tuberculosis

in the guinea-pig, by the root-indices of virulence and by the results of culture of the guinea-pig spleens, the Indian cultures were found to be less virulent, on the average, than the British cultures in both the Porton and the Madras series. The Indian cultures also had a wider range of virulence; about one-third were as virulent as the British cultures and about one-third were attenuated to the extent that visible disease was usually confined to the site of inoculation and its draining lymph-nodes. The true variation in the root-indices of virulence was three times larger with the Indian than with the British cultures.

4. The diameter of the Mantoux reactions of guinea-pigs tested with 100 TU of Old Tuberculin four weeks after infection with the Indian cultures was found to be, on the average, the same as in those infected with the British cultures. The British cultures appeared homogeneous in their ability to cause tuberculin allergy, but the Indian cultures were heterogeneous.

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RÉSUMÉ

Une forte proportion des cultures de bacilles tuberculeux isolées de malades non traités, de l'Inde méridionale, ayant présenté une virulence atténuée pour le cobaye, les auteurs les ont comparées à des cultures isolées en Grande-Bretagne. Ils ont utilisé comme critère la racine carrée de l'indice de virulence (défini dans un autre article), le résultat de la culture des rates des cobayes, et la mortalité des cobayes. Les résultats ont confirmé que les souches indiennes de bacilles tuberculeux étaient nettement moins virulentes et avaient une gamme

de virulence plus étendue que les souches britanniques: un tiers environ des souches indiennes étaient aussi virulentes que les souches britanniques, mais un tiers environ étaient si fortement atténuées que seul le point d'inoculation et les ganglions avoisinants trahissaient l'infection. La variation de la virulence (selon la racine carrée de l'indice) était trois fois plus élevée pour les souches indiennes que pour les souches britanniques. Ces dernières présentaient une homogénéité du pouvoir allergène que les souches indiennes n'avaient pas.

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