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VOLUNTARY ORGANISATIONS AND TUBERCULOSIS CONTROL PROGRAMMES

In many countries of the world voluntary organisations were pioneers in the anti-tuberculosis movement. Some doubts have been expressed of late, as to whether it was necessary for such organisations to take active part in the movement especially because the idea of Welfare State is catching the imagination of people, and National Governments and inter-Governmental International Organisations such as the W.H.O. are also now taking increasing part in tuberculosis control work.

At the International Congress on Tuberculosis held last September in Canada, one of the subjects discussed was the place of voluntary organisations in the campaign against tuberculosis especially in the changing pattern of social life in different countries. Speaker after speaker, particularly from the newly independent countries of Africa, emphasised the need for voluntary Tuberculosis Associations taking an active part in stimulating public opinion in favour of the anti-tuberculosis movement. Even representatives from countries where tuberculosis has now been more or less controlled emphasised the view that voluntary organisations in those parts too have an important part, especially in getting the public to know that tuberculosis requires continuous vigilance to avoid a sense of complacency and slackening of effort. These countries want the community to be educated to realise that it will be a long time before tuberculosis can be eradicated. In fact, the emphasis during the discussions was on redoubling of efforts by voluntary organisations everywhere.

These organisations to function effectively and on voluntary basis should have wide representation drawn from different sections of the community—persons who have real interest in the tuberculosis problem and abiding faith

in community effort to solve it. It would be necessary also to have associated with them medical men to give guidance on technical matters. Further, these voluntary associations should have practical service programmes which they can undertake and which will appeal to the people. Such programmes should include health education, case-finding, social services, rehabilitation, etc. etc. The ultimate control of tuberculosis in a country will depend not only upon the treatment of patients, but also on effective preventive methods in which education always plays an important part.

The International Conference holds the view that voluntary associations can serve best if they are independent of Government control. This, however, should not prevent them from working in very close co-operation with Government agencies both in the planning and execution of tuberculosis control programmes. It was their concensus of opinion that voluntary tuberculosis associations can demonstrate in many fields the effectiveness of different types of programmes and these after a time can be handed over, if found necessary, to Government agencies. It is also important to note that the international conferences provide a very useful forum for various voluntary organisations throughout the world to help them in formulating their plans and programmes. It is essential to emphasise that these Associations should take the community along with them in implementing their plans. What Sir William Osier said years ago, that 'The battle against tuberculosis is not a doctor's affair. It belongs to the entire public'—still holds good.

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 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-Moller, Mathew and 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 chemotherapeutic 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*

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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 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 will be reported subsequently (Ramakrishnan *et al.*, 1962).

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 has been given (Mitchison *et al.*, 1961) 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 has been given by Mitchison *et al.* (1961). 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. Subbaiah *et al.* (1961), have 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 provided the sputum specimens from these patients are listed in the acknowledgements in the paper by Mitchison *et al.* (1961). 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; 1961). In brief, the sputum culture was subcultured on to a Lowenstein-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 postmortem 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 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 Lowenstein-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 and Russel (1959), as modified by Subbaiah, Mitchison and Selkon (1960)], solidified with silica gel according to the method of Selkon and 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) 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 per cent) of the 65 British cultures as compared with 99 (79 per cent) of the 125 Indian cultures did not kill any of the guinea-pigs. Furthermore, two or more guinea-pigs per culture were killed by 24 (37 per cent) of the British cultures and by only 12 (10 per cent) 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 per cent) of the 28 British cultures and by 13 (24 per cent) of the 55 Indian cultures. The difference

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	79
	1	25	38	14	11
	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	76
	1	14	50	8	15
	2	7	25	5	9
	Total	28	100	55	100

between the British and the Indian cultures in their ability to kill guinea-pigs attains statistical significance at the 0.1 per cent 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

^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).

^b 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).

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		Total	
	No.	%	No.	%	No.	%		
0.0-	0	0	0	0.0	0	0.0	0	0.0
0.2-	0	0	1	0.8	11	8.5	12	4.7
0.4-	0	0	34	27.2	33	25.6	67	26.4
0.6-	0	0	39	31.2	44	34.1	83	32.0
0.8-	18	28	32	25.6	23	17.8	55	21.5
1.0-	43	66	16	12.8	16	12.4	32	12.6
1.2-	4	6	3	2.4	2	1.6	5	2.6
Total	65	100	125	100.0	129	100.0	254	100.1
Mean	1.05		0.75		0.71		0.73	
Standard deviation ^a	0.05		0.21		0.18		0.20	

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 ^b	0.12		0.30	

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 per cent) of the 125 Indian cultures in the Porton series and with 22 (40 per cent) of the 55 Indian cultures in the Madras series. Among the Indian cultures, 39 (31.2 per cent) in the Porton series and 12 (22 per cent) in the Madras series were moderately attenuated, with root-indices of virulence of 0.60-0.79, while 35 (28.0 per cent) cultures in the Porton series and 21 (38 per cent) 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 draining 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), 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 per cent 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.

SPLEEN CULTURES

The spleens of the guinea-pigs in the Madras series were cultured either by rubbing the cut surface on Lowenstein-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 per cent) of 28 animals infected with British tubercle bacilli and from 16 (31 per cent) of 52 animals infected with Indian tubercle bacilli. The corresponding results for 12-week guinea-pigs were 20 (71 per cent) and 13 (25 per cent) of the animals. Both of these differences attain significance at the 0.1 per cent 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 per cent) of 30 animals whose root-index was less than 1.3, as compared with 24 (92 per cent) 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 per cent) of 65 guinea-pigs with root-indices of less than 0.9 as compared with 25 (64 per cent) 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 per cent level.

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

^a 3-plus: innumerable discrete colonies; 2-plus: 20-100 colonies; 1-plus: 1-19 colonies.

^b Excluding 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 ⁰	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.	%
3-plus ...	7	25	3	6
2-plus ...	15	54	13	25
1-plus ...	5	18	8	29
Negative	1	4	15	29
Total ...	28	101	52 ^b	100

^a 3-plus: innumerable discrete colonies; 2-plus: 20-100 colonies; 1-plus: 1-19 colonies.

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

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	0	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

^a 3-plus: innumerable discrete colonies; 2-plus: 20-100 colonies; 1-plus: 1-19 colonies.

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

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 ^a	4	2	4
23	0	0	0	0
24	0	0	1	2
Total	28	101	55	102
Mean	17.9		17.6	

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

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

VIAL 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)]. The mean of the counts of 58 suspensions from British cultures was $7.38 \log_{10}$ viable units per ml (range, 6.62-8.75 \log_{10} viable units per ml), and on 24 suspensions from Indian cultures was $7.47 \log_{10}$ viable units per ml (range, 6.74-8.18 \log_{10} 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) 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.

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 ¹	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 ²	44	6.9700	2.31	<0.005
d	Duplicate tests	24 ³	5.0625				54 ⁴	3.0185		

¹ Against the term indicated in the 'British cultures' column.

² NS indicates that the variance ratio is less than 1.0.

³ Four missing observations were estimated by standard statistical techniques.

⁴ One missing observation was estimated by standard statistical techniques.

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-pigs 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.*, 1961), 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 will be presented in a further paper (Ramakrishnan *et al.*, 1962), which is concerned with the progress of the patients during treatment.

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 and Selkon, 1960) and to thiacetazone (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.

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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ALLERGY PRODUCING CAPACITY OF THE MADRAS AND DANISH BCG VACCINES AS SEEN AMONG SCHOOL CHILDREN IN BANGALORE

By RAJ NARAIN, KUL BHUSHAN AND M. SUBRAMANIAN¹

Introduction

The allergy producing capacity of the Madras and Danish Vaccines was compared in 1950. On the basis of allergy produced by the two vaccines it was estimated that the Indian vaccine corresponded to 1/12th strength of Danish Vaccine (Edward, L. B. *et al.*, 1953). The strength of the Danish Vaccine at that time was one and a half times that of Madras Vaccine in terms of moist weight, the Madras Vaccine containing 0.050 mgm. per dose as against 0.075 mgm. of Danish Vaccine.

The strength of the Indian Vaccine was equalised with that of the Danish Vaccine (i.e. 0.075 mgm. per dose) with effect from 8.10.1951 and this continues to be the strength of the Indian vaccine since then (D'Silva, C. B. 1960, Director, BCG Laboratory, Guindy—Personal Communication). A later comparison of both the vaccines carried out by the WHO (TRO) in 1955 showed that the mean tuberculin reaction produced after vaccination with the Danish vaccine was about 2 mm. larger than that produced by the Indian Vaccine (*Bull. Wld. Hlth. Org.* 1955).

Ranganathan (1951) quoting Roelsgaard then working in Pakistan using the Indian vaccine stated that there was no significant difference in conversion rates between Danish and Madras vaccines in spite of the fact that the Danish vaccine was 50 per cent stronger than the Indian vaccine. In the same report, Ranganathan also stated that the conversion rates for mass vaccination in India had shown marked variation from batch to batch. Frimodt Möller (1953) has also stated that his investigations had shown quite unmistakably that (i) the vaccine produced in India is highly potent and (ii) that Indian children can produce very satisfactory allergy after BCG vaccination, which compares well with that of European children.

WHO (TRO) 1957 stated that Mass Campaign Vaccination produced a uniform and reasonably high level of allergy in Indian school children.

Dr. Frimodt Moller (1960) has however stated that at the Madanapalle Research Centre, the Madras vaccine did not produce a satisfactory level of allergy. This finding is contradictory to what has been noted by other workers including the earlier report by Dr. Frimodt Moller (1953). It may be noted that the first round of BCG vaccination was completed at Madanapalle Research Centre in August 1951 and therefore the strength of the Madras vaccine used could have been only 0.050 mgm. per dose.

Kul Bhushan (1960) showed that the post-vaccination allergy attained with the vaccine from Madras was higher than that reported by Frimodt Moller (1960) and that retests had shown very little change from December 1952 to April 1958 in different batches of Madras vaccine.

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A further comparison of the allergy produced by the Danish and Madras vaccines was therefore carried out in Bangalore during 1959-60 by the National Tuberculosis Institute, Bangalore, South India.

Method and Material

Students from 3 Boys' High Schools in Bangalore formed the study population. The total included in the study was 1259 (Table I). Their ages varied between 11 and 19 years, only 2 per cent being above 19 years of age. These children were tested with 1 TU RT 23 containing 0.05 per thousand Tween, on the dorsal aspect of the upper third of one of the forearms. The reactions were read 3-4 days after testing. Some of these boys had been vaccinated during the Mass Campaign earlier, but no attempt was made to ascertain the exact time when these vaccinations were done. The presence of the

TABLE I
*Extent of work done among High School Boys of Bangalore
at Prevaccination stage*

Number tested with 1 TU	1259
Number Read	1168
Number of reactors (14 mm. or more)	389
Number of reactors (13 mm. or less) ...	779
No. available for allocation to vaccination or control group	*678
No. vaccinated with either vaccine	447
Controls, i.e., No vaccination	231
* All were also given 20 TU at the time of vaccination.	

vaccination scar was the only criterion used for judging whether an individual had been vaccinated during the Mass Campaign earlier or not. 1168 boys only were available for the reading. 779 boys, reacting with 13 mm. or less, were considered eligible for vaccination. Though it was the intention to vaccinate all these and at the same time to do a tuberculin test with 20 TU RT 23 containing 0.05 per thousand Tween, only 678 accepted both vaccination and the-20 TU test. The boys who accepted vaccination were allocated to one of the following three categories:

Vaccinated with {	(i) Madras Vaccine	(G): 211
	(ii) Danish Vaccine	(S): 236
	(iii) Controls (No vaccination)	(O): 231

These groups were given code symbols G, S and O respectively. Randomisation was done making use of random number tables. The strength of the two vaccines used was the same, viz., 0.075 mgm., per dose. The code of treatment for each eligible individual was read out to the vaccinator by another person. All vaccinations were given intracutaneously in the middle of the left deltoid region. The 20 TU test was given on the mid-volar surface of the right forearm at the time of vaccination.

Three months later, 575 boys out of 678 from the vaccinated and control group, were available for retesting with 1 TU RT 23 with Tween (Table I A). In addition, a group of 221 other boys was also tested for the sake of avoiding bias at the time of reading of the tuberculin test. This group comprised persons who were either absent from

TABLE I-A.
Number of boys tuberculin tested (with 1 TU RT 23) and read at the post-vaccination stage—3 months after vaccination.

Eligible for follow-up	678
Number tested	575
Number read	524

testing or those tested but not available for reading or those with reactions of more than 13 mm. at the initial examination and thus not eligible for vaccination. This retesting was done on the mid-volar surface of the left forearm. At the time of retesting the left shoulders of all the available boys were examined and vaccination lesions, when present, were measured and recorded. As some of the boys vaccinated in the group had already scars from previous vaccinations (177), a careful examination was made in each case to decide which of the scars was due to recent vaccination. The judgment of the reader on the basis of the appearance of the scar was the final criterion for deciding whether the scar was recent or old. Only the recent scars were measured. However this group has been analysed separately in subsequent tables. Of the 575 boys who were retested only 524 were available for the reading of reactions. A follow-up was done at one year in which both 1 TU and 20 TU tests were given simultaneously. The number of boys tested was 381 of which 328 only were available for reading (Table I B). The bias prevention group was also included in this retesting at one year. This was selected from among those who were absent at the reading of pre-vaccination test, and those who refused vaccination, but those who had previous positive reactions of 14 mm. or more were not included in this group.

TABLE I-B.
Number of boys tuberculin tested (with 1 TU and 20 TU RT 23) at the Post-vaccination stage—1 Year after vaccination.

Eligible for follow-up	495
Number tested	381
Number read	328

The Danish vaccine from State Serum Institut was air-shipped under refrigeration from Copenhagen to Bangalore. During transit the ice in the containers was replenished at Delhi. The Madras vaccine was sent to Bangalore from Madras by air under refrigeration. Only one batch of either vaccine was used. The tuberculin dilutions 1 TU and 20 TU used in this study were also obtained from the State Serum Institut, Copenhagen.

All tuberculin reactions in this study, both of 1 TU and 20 TU were read by the same WHO TRO trained nurse (Miss J. McLary). All vaccinations were given by another similarly trained nurse (Miss I. Mundt). The post-vaccination lesions were read by a third WHO TRO trained reader (Miss D. Randgaard). Throughout the study only the transverse diameters of tuberculin indurations and the vaccination lesions were measured and recorded. The readers of tuberculin reactions and BCG lesions had no access to the cards at the time of reading.

Results

Table II gives the number of boys vaccinated with the two vaccines and the controls and those who completed the retesting at three months, separately for those with previous BCG scars and those without such scars.

TABLE II

Number of children who were allocated to the two (Danish and Madras) vaccines or the Controls and the number of those who completed the post-vaccination test at 3 months.

Total allocated for each category	Total completing the post-vaccination tuberculin test	
	With no previous BCG Scars	With previous BCG or doubtful scars
Madras (G) 211	115	42
Danish (S) 236	130	54
Controls (O) 231	136	47
Total 678	381	143

Figure I A shows the distribution of tuberculin reactions to 1 TU for those who had no previous BCG scars at the pre-vaccination test and Figure I B, for those with BCG scars-definite or doubtful. The distribution of reactions for those with no BCG scars is bimodal in shape with fairly good separation at the level of 10-14 mm. For those with previous BCG scars (Figure I B) the distribution of reactions is scattered; more than half of the reactions are below 8 mm.

Figure II shows the distribution of reactions to 1 TU at pre-vaccination and at three months after vaccination for all those who were given Madras or Danish vaccine and also for the controls. Distribution of reactions among the vaccinated at the pre-vaccination stage is almost the same for the three groups except for the absence of 12-14 mm. reactions in the Controls. The measure of scatter namely standard deviation of the post-vaccination tuberculin reactions is 3.8 mm. in the case of Madras Vaccine and 4.5 mm. in the case of Danish vaccine. However, this difference is not statistically significant ($F = 1.4 < F_{.05}$ $V_1 = 129, V_2 = 114$).

Table III shows the average size of reaction to 1 TU at the pre-vaccination stage and at three months after vaccination for those vaccinated for the first time and for those with evidence of previous vaccination. Mean size of BCG vaccination lesions for those vaccinated for the first time (who had no scars) is also given. The average size of pre-vaccination tuberculin reactions was 3.2 mm. for the groups vaccinated with either of the two vaccines, and 3.1 mm. for the control group. The average size of post-vaccination tuberculin reactions for the two vaccinated groups showed no difference, viz., 11.8 mm. and 11.9 mm. for the Madras and the Danish Vaccines respectively. The mean size of vaccination

TABLE III

Number of boys vaccinated with Danish and Madras Vaccines, the Controls and mean Indurations of Pre-vaccination and Post-vaccination Tuberculin reactions to 1 TU RT 23, in the unvaccinated and previously vaccinated groups.

		Vaccinated for the first time		Revaccinated (with previous BCG Scars)		Size of Vaccination lesion (mm)
		No.	Average Size (mm)	No.	Average size (mm)	
Pre- Vaccination	G	115	3.2	42	3.9	
	S	130	3.2	54	4.6	
	O	136	3.1	47	5.7	
Post- Vaccination	G	115	11.8	42	14.1	6.9
	S	130	11.9	54	13.7	6.8
	O	136	3.8	47	10.3	

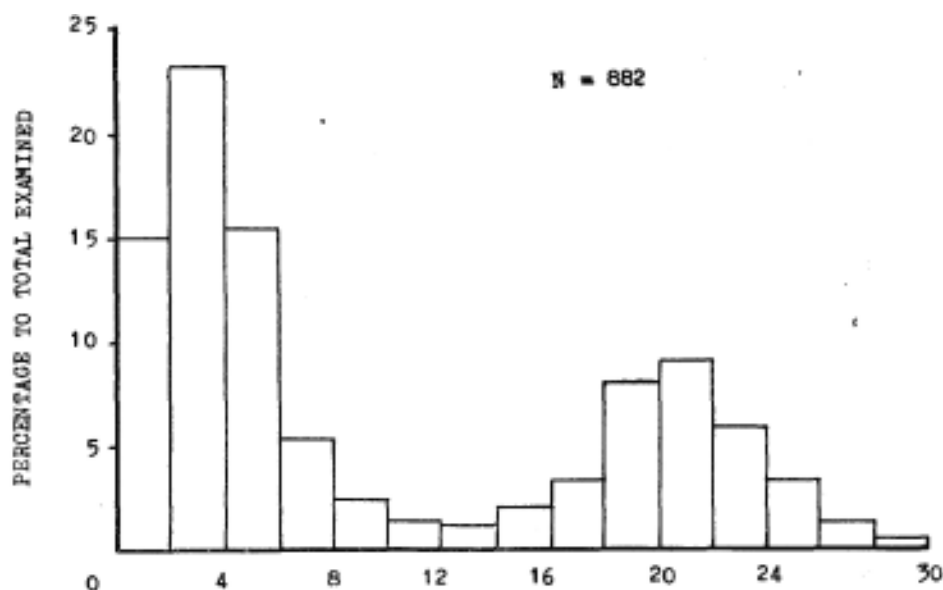


FIG. 1A.— Distribution of reactions to 1 TU RT 23 with Tween among the persons who had no evidence of B.C.G. or doubtful scars at the pre-vaccination stage

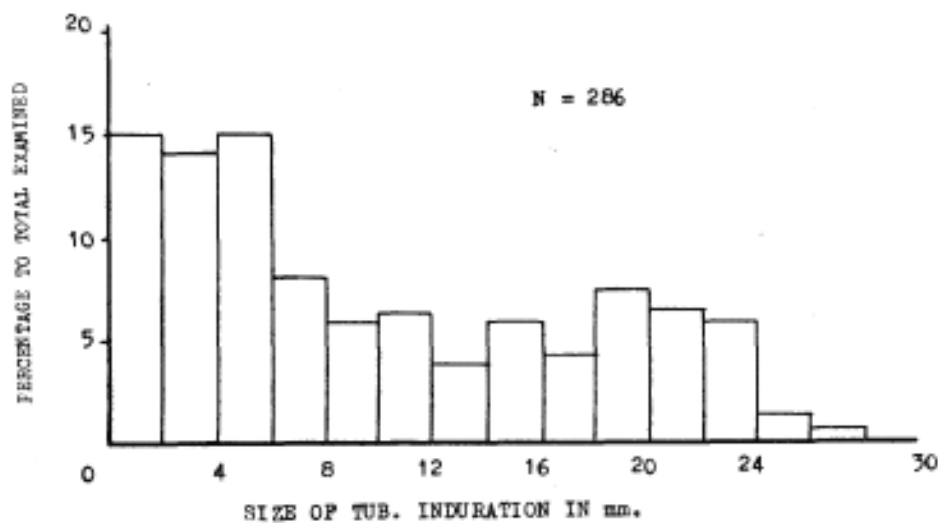


FIG. 1B.—Distribution of reactions to 1 TU RT 23 with Tween among the persons who had B.C.G. or doubtful scar at the pre-vaccination stage

lesions was also not different viz., 6.9 and 6.8 mm. for the Madras and the Danish vaccine respectively. In both the groups the difference in the mean size of induration between the pre- and post-vaccination tests was about 9 mm. as against 0.7 mm. for the Control Group. The number and percentage of reactors showing at least an average increase of

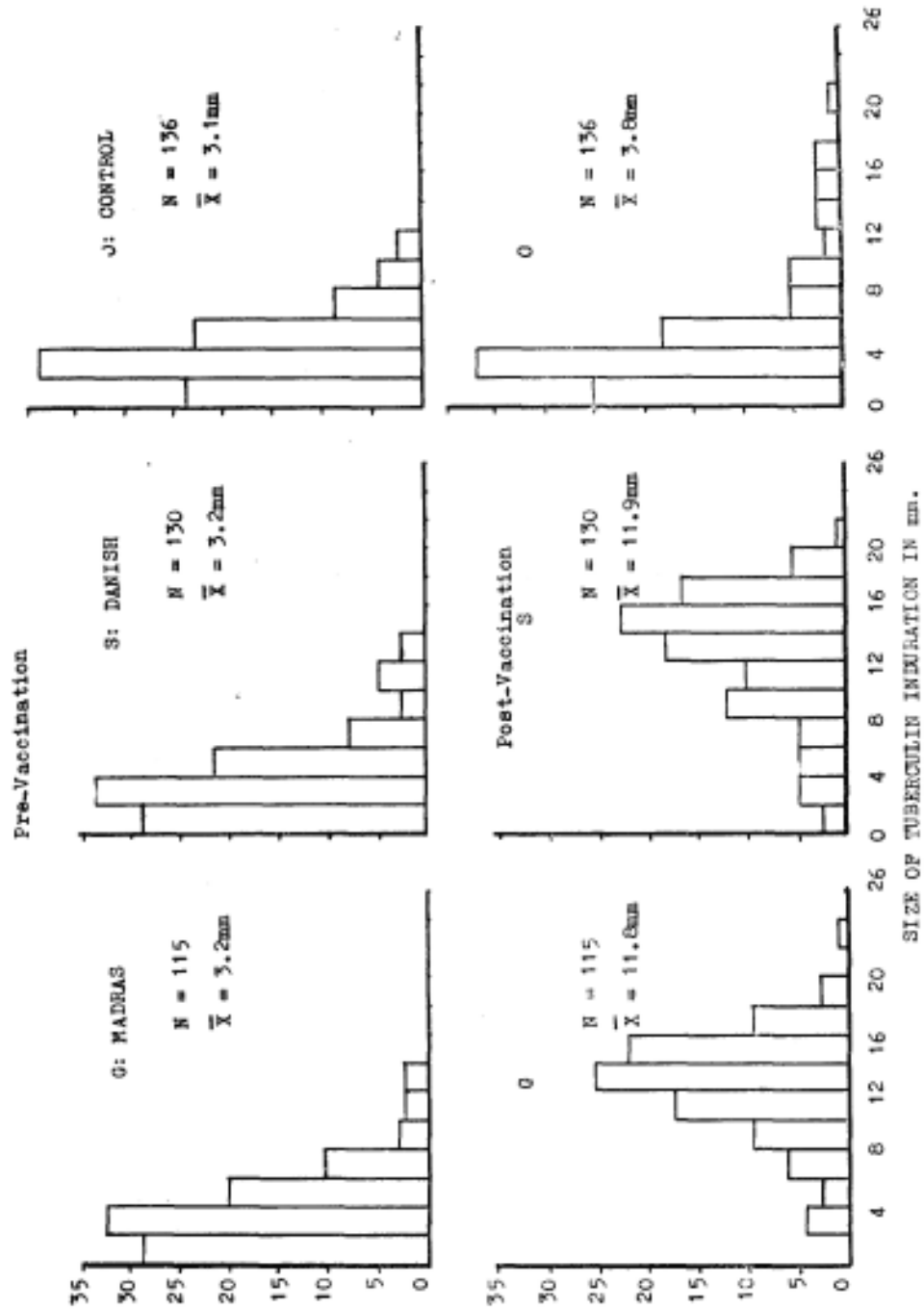


FIG. II. Distribution of size of Pre- and Post-vaccination (3 months) Tuberculin indurations to 1 TU RT 23 (with Tween) in the groups vaccinated with Madras and Danish vaccines and the control group.

9 mm. from their pre-vaccination level of allergy is presented in Table IV in relation to each mm. size of pre-vaccination induration. Percentage of those showing this average increase varies from 69 to 25 for Madras vaccine and 67 to 33 for Danish vaccine. These percentages vary inversely with the pre-vaccination induration up to 7 mm. Among all those showing a pre-vaccination induration of 8 mm. or more, not one has shown this increase of 9 mm. which is the average noted for the group.

While Table IV shows percentage of reactors having an increase of at least 9 mm. over the pre-vaccination allergy, Table V gives the mean size of post-vaccination reactions in relation to different groups of pre-vaccination induration size. For the pre-vaccination induration of 0-2 mm. the average increase due to vaccination for either vaccine is a little over 10 mm.; for the group 3-5 mm. average increase is 8 mm., and for the group 6-8 mm. it is 6.6 mm. for the Madras vaccine and 7.2 mm. for the Danish vaccine. For the group with 9-13 mm., this average increase is about 4 mm. for both the vaccines. There is no difference between the two vaccines in this respect. The small average increase of 4 mm. only in the group with 9-13 mm. pre-vaccination reactions may be noted.

TABLE IV
Number and percentage of persons whose post-vaccination reactions at 3 months are at least 9 mm. greater than their pre-vaccination tuberculin induration for Madras and Danish vaccines and the Controls

Pre-vacc. Tub. size (mm)	G		% to Total	S		% to Total	O		% to Total
	No.	group		No.	group		No.	group	
0	23	33	69.7	25	37	67.6	1	32	3.1
2	15	21	71.4	14	22	63.6	1	27	3.8
3	10	16	52.5	14	21	66.7	2	25	8.0
4	8	15	53.3	8	18	44.4	1	22	4.6
5	2	8	25.0	6	10	60.0	—	9	0
6	3	8	37.5	3	7	42.9	—	5	0
7	1	4	25.0	1	3	33.3	—	7	0
8	—	2	0	—	2	0	—	4	0
9	—	2	0	—	3	0	—	2	0
10	—	1	0	—	3	0	—	1	0
11	—	2	0	—	3	0	—	2	0
12	—	2	0	—	1	0	—	—	0
13	—	1	0	—	0	0	—	—	0
Total	62	115	53.9	71	130	54.6	5	136	3.7

TABLE V
Average size of post-vaccination allergy (3 months after vaccination) in relation to different sizes of pre-vaccination sensitivity for the two vaccines

Pre-vaccination size groups for 1 TU (mm)	No.	G		No.	S	
		Pre-vaccn. Average (mm)	Post-vaccn. average (mm)		Pre-vaccn. average (mm)	Post-vaccn. average (mm)
0-2	54	0.8	11.0	59	0.7	11.0
3-5	39	3.8	11.8	49	3.8	11.9
6-8	14	6.6	13.2	12	6.6	13.8
9-13	8	10.9	14.8	10	11.0	15.2

Table VI gives the size of post-vaccination induration in the re-vaccinated group (those who had BCG scars before vaccination in the present study) in relation to indurations

as seen at the pre-vaccination tuberculin test. Here also there is no significant difference ($t=53$, $V=94$) in the average size of post-vaccination allergy for the two vaccines (14.1 mm. for the Madras vaccine, 13.7 mm. for the Danish vaccine). But the post-vaccination allergy in the re-vaccinated group is more than that among those vaccinated for the first time (11.8 and 11.9 mm.). Of the total of 96 persons (Table VI) showing a vaccination scar as many as 67 showed reactions below 6 mm. to 1 TU at the pre-vaccination test and nearly half (49) gave reactions of only 3 mm. or less. It was not determined how long ago, these people had been vaccinated; either the earlier vaccination was with a weak vaccine or the allergy had waned because of a long interval.

TABLE VI

Size of post-vaccination allergy on the basis of corresponding pre-vaccination size for the re-vaccinated group

Pre-vaccination tuberculin size (mm)	Post-vaccination size ITU			
	No.	G Average (mm)	No.	S Average (mm)
0	13	13.8	13	9.9
2	9	12.0	5	13.8
3	2	15.5	7	14.1
4	5	15.8	5	15.4
5	1	16.0	7	13.0
6	1	10.0	2	17.5
7	1	15.0	1	19.0
8	3	14.0	1	14.0
9	2	15.0	2	16.0
10	—	—	6	15.3
11	1	13.0	3	17.0
12	3	17.7	1	16.0
13	1	15.0	1	18.0
Total	42	14.1	54	13.7

Table VII shows the control group with regard to the pre- and post-vaccination allergy. It is seen that although there is no significant rise in the post-vaccination allergy amongst

TABLE VII

Distribution of Tuberculin reactions (and their percentages) to 1 TURT23 with Tween at pre- and post-vaccination (3 months) stages for the unvaccinated control groups with and without evidence of previous vaccination scar.

Induration size in mms.	Group without previous scar				Group with previous scar			
	Pre-Vaccn.		Post-Vaccn.		Pre-Vaccn.		Post-Vaccn.	
	No.	% to total	No.	% to total	No.	% to total	No.	% to total
0- 2	32	23.5	34	25.0	7	14.9	2	4.3
2- 4	52	38.2	50	36.8	6	12.8	4	8.5
4- 6	31	22.8	25	18.4	14	29.8	5	10.6
6- 8	12	8.8	7	5.1	4	8.5	2	4.3
8-10	6	4.4	7	5.1	6	12.8	5	10.6
10-12	3	2.2	2	1.5	7	14.9	4	8.5
12-14	—	—	3	2.2	3	6.4	11	23.4
14-16	—	—	3	2.2	—	—	10	21.3
16-18	—	—	3	2.2	—	—	1	2.1
18-20	—	—	1	0.7	—	—	1	2.1
20-22	—	—	1	0.7	—	—	2	4.3
Total	136		136		47		47	
Mean Size	3.1		3.8		5.7		10.5	

those who had not been vaccinated before (from mean size 3.1 mm. to 3.8 mm.) yet in persons who had BCG scars of earlier vaccination, the retest showed an average rise of about 5 mm. induration (from mean size of 5.7 to 10.3 mm.). The cause of this rise in this group on a retest after 3 months is not clear; it may partly be due to the boosting effect of the tuberculin test on previously vaccinated persons (Magnus, K.—1957; Kul Bhushan—1958-59).

Non-specific Allergy

It may be recalled that all those reacting up to 13 mm. were given 20 TU RT 23 with 0.05 per thousand Tween irrespective of their allocation to either vaccine or to the control group. The reactions to 20 TU were read in 645 persons of which 495 had no previous BCG scar. Distribution of reactions to 20 TU amongst these 495 persons is given in Figure III. It can be seen that the distribution of reactions is more or less flat, being almost parallel to the baseline up to 20 mm. Comparing Figure I A and Figure III,

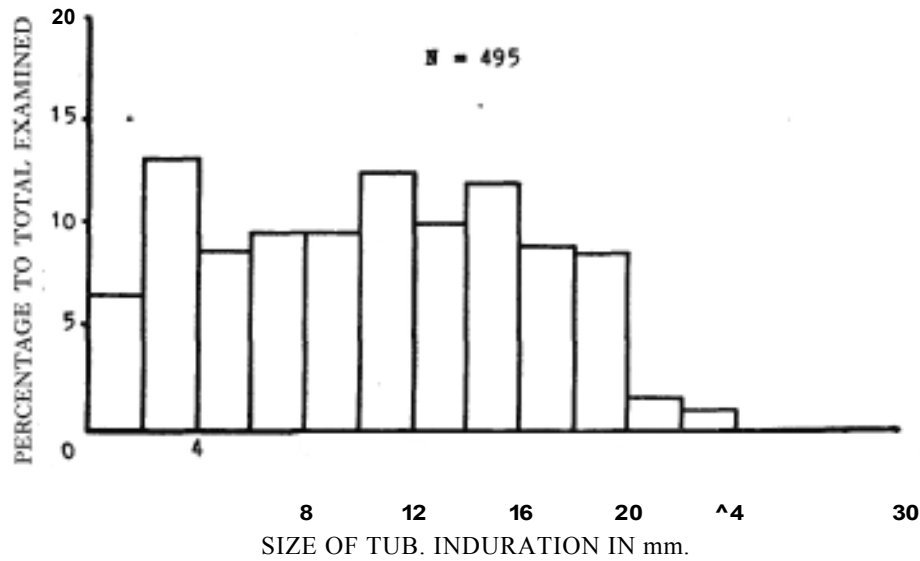


FIG. III- Distribution of reactions to 20 TU RT 23 with Tween among those with 13 mm. or less reactions to 1 TU RT 23 and with no evidence of B.C.G. or doubtful scar at the pre-vaccination stage

it would be noted that most of the persons giving smaller reactions to 1 TU have given a larger reaction to the higher dose. These reactions to 20 TU have been correlated with reactions to 1 TU in Table VIII from which it can be seen that out of the 495, 442 or 89.3 per cent reacted to 1 TU with an induration of 6 mm. or less. Of these 165 or 37.5 per cent reacted with 6 mm. or less to 20 TU also. Among 135 'O' reactors to 1 TU, 28 (21 per cent) gave reactions above 14 mm. and 75 (56 per cent) above 6 mm. to 20 TU.

At the three month follow-up 20 TU test was not given. The size of reactions as elicited by 1 TU at three months has been correlated with the 1 TU and 20 TU reactions at the pre-vaccination stage. For this purpose Table VIII has been divided into six sections with 6 mm. and 13 mm. as the points of division. Each of these six sections has been analysed separately in Table IX which shows that all those with pre-vaccination induration

TABLE VIII

Correlation between the size of pre-vaccination reactions to 1 TU and 20 TU RT 23 with Tween

		Size of Indurations to 1 TU RT 23 with Tween (mm)														Total
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	
Size of Indurations to 20 TU RT 23 with Tween (mm)	0	14	—	12	1	3	1	1								32
	1	—	—	—	—	—	—	—								—
	2	13	—	11	6	3	1	1								35
	3	9	—	11	3	3	2	1								29
	4	10	—	7	4	3										24
	5	6	—	3	4	2		3								18
	6	8	—	4	8	6		1								27
	7	4	—	5	4	3	2						1			19
	8	2	—	5	8	4	1		1	1						22
	9	9	—	1	5	4	2	2		1	1					24
	10	15	—	3	6	4	5	2								35
	11	6	—	3	3	5	3		3	3						26
	12	6	—	5	4	9	1		1					1		27
	13	5	—	3	5	1	4	3		1						21
	14	9	—	9	3	7	3	1		1						33
	15	3	—	2	5	3	5	1		1	3			2		25
	16	5	—	3	—	7	5	2		1	1	1			1	27
	17	2	—	2	1	1	2	1		1	2	2	1		1	16
	18	7	—	2	3	2	2	3		4	2	4	1	1		31
	19	—	—	3	—	1	1			2		4				11
	20	—	—	—	1	1	—	1							2	5
	21	—	—	—	2	1										3
	22	2	—	—	—	—	—	—		—				1	1	4
23	—	—	—	—	1	—	—		—						1	
Total	135	—	94	76	74	40	23	15	11	7	4	7	5	4	495	

TABLE IX

Correlation between the size of Pre- and Post-Vaccination (3 month) reactions to 1 TU RT 23 (with Tween) for the six zones of Table VIII based on the pre-vaccination reaction to 1 TU and 20 TU.

Pre-Vaccination Reaction to 20 TU ↓	Pre-Vaccination Reactions to 1 TU →	0-6 mm.		7-13 mm.	
		Pre-Vaccin.	Mean Reaction to 1 TU		Post-Vaccin.
			Post-Vaccin.	Pre-Vaccin.	
0-6 mm.	Madras	1.7	10.7	—	—
	Denish	1.7	10.7	—	—
7-13 mm.	Madras	2.7	12.0	8.3	9.5
	Denish	2.2	11.0	8.5	11.0
14 mm. or more	Madras	2.8	12.2	9.8	14.6
	Denish	3.2	13.4	9.8	15.2

of 0-6 mm. to 1 TU (irrespective of their reaction to 20 TU) showed an increase of 9 mm. or more induration, at post-vaccination test. But those showing a pre-vaccination induration of 7-13 mm. to 1 TU regardless of their reaction to 20 TU showed an increase of about 1-5 mm. in their post-vaccination allergy. It may also be noted that the rise in post-vaccination allergy as elicited by 1 TU does not seem to have any relation with the pre-vaccination allergy to 20 TU.

Follow-up after one year

Though 328 boys completed the 1 TU and 20 TU tests at one year, only 200 who had no BCG scar at the pre-vaccination stage had completed the tuberculin tests at all the 3 stages, namely, pre-vaccination, three-month and one year. These have been analysed separately in Table X which gives the average size of reactions to 1 TU at 0 day,

TABLE X

Mean size of reactions and their standard errors to 1 TU RT 23 with Tween at pre-vaccination and post-vaccination stages at 3 months and one year; and to 20 TU RT 23 with Tween (at pre-vaccination and post-vaccination stage at 1 year) in those completing all the examinations in the groups vaccinated with Madras and Danish vaccines and the Controls.

Dose and time	Madras Vaccine		Danish Vaccine		Controls	
	Mean (mm)	S.E. of mean	Mean (mm)	S.E. of mean	Mean (mm)	S.E. of mean
No.	60		63		77	
1TU(0day)	3.0	.39	3.0	.35	3.0	.26
1TU (3-month)	12.0	.53	11.6	.58	3.2	.43
1TU(1Year)	10.5	.63	10.8	.62	4.7	.42
20TU(Oday)	9.3	.78	9.8	.71	9.7	.66
20TU(1year)	18.4	.36	18.2	.39	9.8	.63

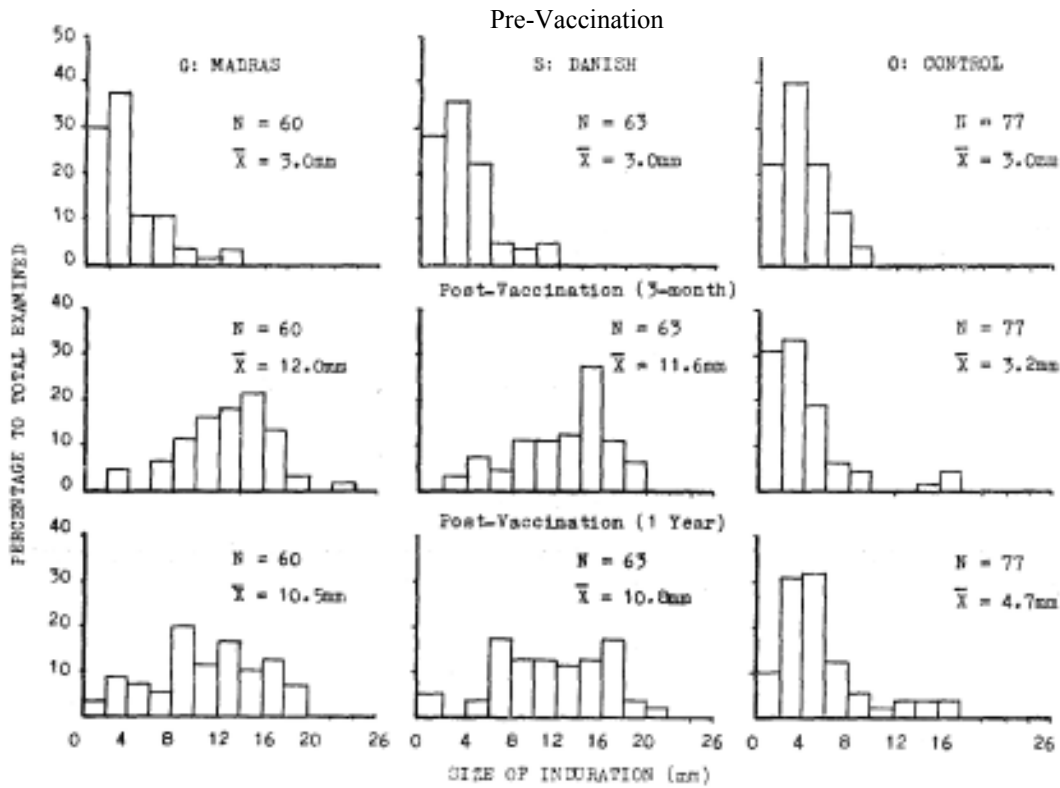


FIG. IV A—Distribution of size of pre- and post-vaccination (at 3 Months and 1 Year) Reactions to 1 TU RT 23 (with Tween) among those completing all the tests in the groups vaccinated with Madras and Danish Vaccines and the Control Group

3-month and at 1 year and to 20 TU at 0-day and at one year. The mean size of post-vaccination reactions for Madras and Danish vaccines at 1 year to 1 TU was 10.5 mm. and 10.8 mm. respectively while to 20 TU it was 18.4 mm. and 18.2 mm. The average increase in allergy from pre-vaccination mean size of reactions for either vaccine at 1 year was about 8 mm. for both the tests.

The distribution of reactions to 1 TU at 0-day, 3 months and at 1 year and to 20 TU at 0-day and at 1 year are also given in Figures IV A and IV B respectively. The slight

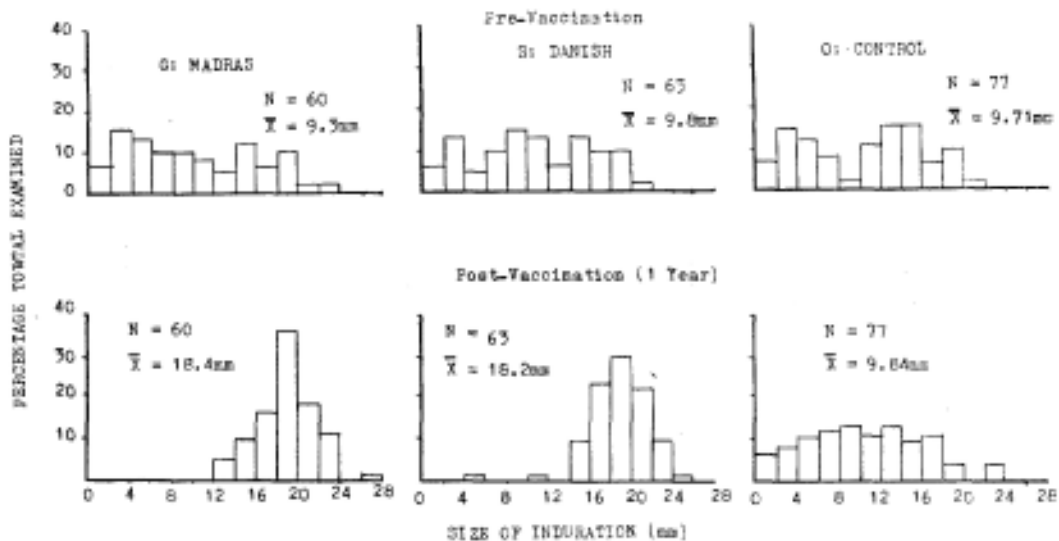


FIG. IV B—Distribution of size of pre- and post-vaccination (at 1 Year) tuberculin Reactions to 20 TU RT 23 (with Tween) in the Groups vaccinated with Madras and Danish Vaccines and the Control Groups

scatter at 3 months in the reactions with the Danish vaccine as shown in Figure II is also seen at 1 year in Fig. IV A. No such scatter is seen in Fig. IV B with the 20 TU test at one year. The scatter seen at 3 months and at 1 year with 1 TU does not seem to have any significance.

Discussion

No difference in the allergy producing capacity between the Madras and Danish vaccines has been found in this study among high school boys in Bangalore (South India). The analysis according to:

- (i) average increase of allergy from the mean pre-vaccination level;
- (ii) rise of allergy from each mm. size of pre-vaccination reaction to 1 TU at 3 months;
- (iii) the mean size of induration, to 20 TU at 1 year among those vaccinated for the first time; and
- (iv) size of vaccination lesion produced by the vaccines at three months, also did not show any difference.

These remarkably identical results in the allergy producing capacity of the Madras and the Danish vaccines should dispel doubts regarding the allergy producing capacity of the Madras vaccine raised in some earlier studies (Edwards, L. B., *et al.*, 1953, WHO TRO 1955 and Frimodt Moller 1960). The better results obtained with the Madras vaccine in this study as compared with the previous studies quoted, may be due not only to the increase in the dose of vaccine, (from 0.050 mgm. moist weight to 0.075 mgm.) but also to the improvements introduced during 1955 and 1957 in the preparation of the Madras vaccine, namely higher compression during nitration (Ranganathan 1956) and cooling before filtration (Ranganathan and Srinivasan 1958).

The protective value of the Danish vaccine has been well shown in the M.R.C. Trial (1956) (1959). As the allergy produced by both Danish and Madras vaccines are practically the same in the present study, it is reasonable to deduce that the protective value of the Madras vaccine is not likely to be different from that of the Danish vaccine. However, the present study was confined to a single batch of each of the two vaccines in the standard strength only and no dilutions or other strengths of the two vaccines were compared. It may be mentioned that other studies from NTI (unpublished) (1960-61) using other batches of the Danish and Madras vaccines, with standard and double doses of each also showed similar results.

The post-vaccination level of allergy achieved at 3 months and at 1 year with 1 TU was however less than that reported in some other studies (Frimodt Moller 1953, Kul Bhushan 1960). The relatively lower level of post-vaccination allergy in the present study may be due to differences in the tuberculin used. In the studies quoted above (Frimodt Möller 1953 and Kul Bhushan 1960), 5 TU of RT 22 without Tween was used. In the present study 1 TU RT 23 with 0.05 per thousand Tween was used. NTI (unpublished 1960) and Kul Bhushan (1960) have shown that, on an average, 1 TU RT 23 with Tween elicits about 3-4 mm. less induration than 5 TU RT 22 without Tween at 3 months after vaccination.

Allergy in the Re-vaccinated

Although the post-vaccination level of allergy at three months amongst those vaccinated for the first time was 11.8 and 11.9mm., amongst the revaccinated it was 14.1 and 13.7 mm. (Table II). This higher allergy in the revaccinated group may be due to either revaccination itself or due to boosting effect of the tuberculin tests (WHO TRO 1957, Magnus K. 1957 and Kul Bhushan 1958-59) or both. The boosting effect of tuberculin test only amongst the controls with previous BCG or doubtful lesions (from 5.7 mm. to 10.3 mm.) (Table II B) has also been observed.

Non-specific allergy

It has been suggested that non-specific reactions to tuberculins are common in some parts of India (Edwards, L. B. *et al.*, 1955). Intermediate tuberculin reaction between 5 and 8 mm. or even more to 1 TU in this area may not be due to infection with *Mycobacterium Tuberculosis*, but may probably be due to non-tuberculous infections or unknown factors. Such non-specific sensitivity is represented by intermediate size of tuberculin reactions to 1 TU with Tween, or 5 TU without Tween, or larger reactions to a higher dose.

It has already been observed (Table VIII) that in a large number of persons even zero reactions to 1 TU RT 23 with Tween show large reactions to 20 TU. The limitations of 1 TU RT 23 with Tween in bringing out the post-vaccination allergy has already been pointed out. Thus this 1 TU is probably not quite suitable for eliciting either non-specific allergy or allergy induced by BCG.

Post-vaccination allergy amongst intermediate reactors

From Tables III and IV it has been shown earlier that there is a progressive fall in the increase in the post-vaccination allergy with a rise in pre-vaccination allergy. For the group showing a pre-vaccination allergy of 9-13 mm. (Table IV) the post-vaccination rise in allergy was about 4 mm. If a rise in post-vaccination allergy can be taken as a criterion for the protection offered by BCG, then vaccination of the group with pre-vaccination reactions of 9-13 mm. may not materially contribute to the protective value of vaccination in a control programme, but it will need further investigations.

Conclusion

1. No difference in the allergy producing capacity was elicited between the Danish and the Madras BCG vaccines amongst school children in Bangalore either at 3 months or at one year. This also applies to the size of the scars produced by both vaccines.
2. The allergy in persons, who show a pre-vaccination induration of 9 mm. or more to 1 TU RT 23 with Tween is not increased by more than 4 mm. after vaccination. What effect vaccination may have in such groups is an open question.
3. A large number of persons with zero reactions to 1 TU give large reactions to 20 TU. Thus 1 TU RT 23 with Tween does not elicit non-specific allergy. It may not be adequate for eliciting post-vaccination allergy also.

Acknowledgments

Many persons have helped to carry out this study. Special mention must be made of the field teams who carried out this work. The authors are grateful to Dr. P. V. Benjamin, Adviser in Tuberculosis, Government of India, for his encouragement and advice in the preparation of this paper. They are also grateful to the members of the Technical Co-ordinating Committee of the National Tuberculosis Institute, especially to Dr. Bordia, Mr. Waaler, Mr. Andersen and Prof. Jambunathan for their comments and helpful criticism.

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CORTICO-STEROIDS IN CLINICALLY RESISTANT CASES OF PULMONARY TUBERCULOSIS

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Clinically resistant cases represent the failure of chemotherapy. Recently some reports have appeared indicating beneficial effects when corticosteroids are used as an adjunct to chemo-antibiotics in such cases.

Barre *et al.*, (1955) treated 25 patients with steroids for 1-3 months. Radiologically eight patients showed a marked and nine slight improvement, two of them improved but relapsed later on again and six did not show any results. Improvement in general condition was noticed in 22 cases.

Shubin and Heiken (1956) in a controlled trial noted the response of prednisolone in chronic cases (who had taken more than one course of antimicrobial therapy without any effect). Out of the 30 patients he noted early radiological improvement, sputum conversion and decrease in resistance to streptomycin in 12 patients. In remaining 18, one death occurred due to carcinoma pancreas and seventeen showed little change and still had positive sputum but demonstrated a feeling of well-being. Only 2 showed progression of disease. No side effects were seen.

Beneficial results have also been reported by Hart (1954), Turiaf and Marland (1955), Cockran and Clayson (1955). Hockert *et al.*, (1956) and Climie *et al.*, (1956),

According to Cannetti (1954) the various reasons that could be attributed to the failure of chemotherapy are:

1. Development of resistant bacilli. (At present we are not dealing with such a status of bacilli).
2. Resting state of bacilli.
It is true that bacilli in cavities have the best oxygen supply the body can provide but oxygen is not everything, as nutritive medium (caseous substance) may become exhausted or the bacilli may become older and thus cease to grow.
3. Intracellular situation of the bacilli e.g., streptomycin acts only on the extra-cellular bacilli.
4. It is true that chemotherapy acts on bacilli which are multiplying but it is the state of active metabolism which matters and not the multiplication alone.

The effect of chemotherapeutic agents is to interfere with metabolic reactions which are essential to synthesis of vital substances in the bacillus and the facts known so far show that the metabolic reactions interfered with are different for every antibacterial agent. Thus it becomes quite conceivable that if the bacillus has no metabolic reactions of the kind in which streptomycin intervenes it will behave as resting in relation to it, but it may not

be in the same state in relation to I.N.H., or the metabolic reactions may be depressed to a state that the bacillus seems to be resting in relation to many antibacterial agents at the same time.

Shubin *et al.*, (1958) pointed out the role of poor host resistance and possible chronic adrenal insufficiency, the definite role of which is still not properly understood. In such cases therefore after the use of all the available antibacterial agents a stalemate is reached.

Since it was expected that the corticosteroids might bring the resting bacilli back to the state of active metabolism and also help in removing chronic adrenal insufficiency, it was proposed to use the corticosteroids as adjuvants with antitubercular treatment in clinically resistant cases.

Material and Methods

The material for study was selected from cases admitted to K.T.B. Clinic and Hospital. The cases taken up for present study showed clinical and radiological evidence of Pulmonary Tuberculosis with demonstrable *mycobacterium tuberculosis* in sputum. They had already received one or more courses of treatment and showed no further improvement with triple drug therapy in the hospital. In the meantime the report of culture and sensitivity was received (depicted in Table 1).

TABLE I
Number of Cases

Antibiotic	Sensitive	Partially resistant	Resistant
SM	5	8	9
INK	4	12	7
PAS	15	7	—

None of the cases showed total resistance to the two anti-tubercular drugs together.

Prednisolone was then added to the triple drug regimen which these patients were already getting. The therapeutic regimen employed was as follows:

SM	1 Gm O.D. intramuscularly	} for 3 months
INH	10 mg/K.G. body weight orally	
PAS	12 Gm/day in two divided doses orally.	

Prednisolone 5 mg. Q.I.D. for 1 month
5mg. T.D.S. for 20 days
5mg. B.D. for 20 days
5 mg. O.D. for 20 days
and then withdrawn.

Radiologically these cases were classified according to the recommendations of the Sub-Committee on Classification of the Indian Tuberculosis Association (1940).

The effect of therapy was noted on the parenchymal lesion and the specific changes were classified as improved/stationary/deteriorated. The cases showing improvement were sub-divided into those with marked, moderate or slight improvement.

The criteria for specific changes were as follows:

Marked: More or less complete clearing of infiltration or clearing to the extent of at least two zones and/or clearing of atelectasis and/or cavity closure.

Moderate: Diminution in size of cavity and/or radiological clearing of infiltration of at least one zone but less than two zones. Slight:

Radiological clearing of infiltration less than one zone.

In all 25 such cases have been studied. These cases themselves served as their control as they were not improving on chemotherapy alone before the institution of prednisolone to their regimen.

They belonged to age group 16-52 years. 18 cases were males and 7 were females. They have been ailing for long period, average duration being 3- years and 3 months (range 2-5 years).

7 cases (28 per cent) had temperature ranging from normal up to 99° F, 9 cases (36 per cent) had temperature ranging from 99° F to 100° F and another 9 cases (36 per cent) had temperature above 100° F. The average weight was 99 lb. (Range 54-103 lb.)

E.S.R. was as shown in following Table 2.

TABLE 2

Range of E.S.R.	No. of cases and percentage
Normal (0-15 mm)	Nil.
Slight increase (15-40 mm)	4 (16 per cent)
Moderate increase (40-50 mm)	15 (60 per cent)
Marked rise (above 80 mm)	6 (24 per cent)

All the cases had a positive sputum. Results of Gaffky count (Keers and Ridgen 1945) of the number of bacilli done was as depicted in Table 3.

TABLE 3

Gaffky count	No. of cases	Percentage
I	4	16 per cent
II	9	36 per cent
III	2	8 per cent
IV	8	32 per cent
V	0	—
Above V	2	8 per cent

The radiological status of the cases was as shown in Table 4.

TABLE 4

Extent of disease		Stage of disease				
Unilateral	Bilateral	I	II	III	Exudative	Productive
9 cases	16 cases	1 case	3 cases	21 cases	16 cases	9 cases
(36%)	(64%)	(4%)	(12%)	(84%)	(64%)	(36%)

The cavitory disease was present in 20 cases only and the total number of cavities was 38.

In every case a routine laryngeal and bronchoscopic examination was done. Six cases (24 per cent) had laryngitis and 10 cases (40 per cent) had endobronchial lesion.

The range of B.P. was Systolic 92-104/Diastolic-60-88 mm. Hg., average being 96/68 mm. Hg.

The range of fasting blood sugar was 60-106 mg per cent average being 89 nag. per cent. The glucose tolerance test was normal in all cases and thus none of the cases included in the present trial were diabetic.

The 17 Ketosteroid values ranged from 3.1 to 11.4 mg/hours, average being 6.1 mg/24 hours.

Results and Discussion

The improvement in general condition was noticed in 15 cases (60 per cent) the remaining were stationary. None of the cases deteriorated. The improvement started as early as the first week after starting the corticosteroids.

Appetite

Gain in appetite occurred in all the patients to a variable extent, however, marked improvement was seen in 23 cases (92 per cent) and moderate improvement in 2 cases (8 per cent).

Cough

Improvement in cough was noticed in all cases but marked improvement was seen in 15 cases (60 per cent), moderate in 8 cases (32 per cent) and slight in 2 cases (8 per cent).

Temperature

Before the start of prednisolone therapy all cases were febrile (range being 99°F to 104°F). In majority of cases (88 per cent) the temperature came within normal limits at the end of three months. In one case (4 per cent) the temperature ranged between 99°F to 100°F and in the remaining two cases (8 per cent) it remained above 100°F.

Weight changes

The weight gain after 3 months' therapy occurred in 24 cases (96 per cent) while only one case (4 per cent) remained stationary. By the end of 3 months there was an average gain of 11.21b.

E.S.R.

Before treatment all cases had raised E.S.R. with 84 per cent' showing moderate to marked rise. With treatment a normal E.S.R. was noticed in 10 cases (40 per cent) slightly increased in 14 cases (56 per cent). Only one case had a moderately raised E.S.R. at the end of 3 months treatment. ,

With the addition of corticosteroids a rapid fall of E.S.R. towards the normal levels was noticed in the first month of treatment so much so that 5. cases (20 per cent) reached normal level, and slightly raised E.S.R. was found in 5 cases (20 per cent) and 15 cases (60 per cent) only still had a moderately raised E.S.R.

Sputum conversion

All patients were positive for A.F.B. on smear and culture examination before the start of prednisolone. At the end of 3 months eight cases (32 per cent) became sputum negative as confirmed by a culture examination. In the remaining 17 cases (69 per cent) a reduction in Gaffky count was noticed (in 14 cases smear showed G. I count and the remaining 3 showed G. II count).

The sputum conversion reported by Shubin (1956) was 40 per cent. Other workers like Hockart *et al.*, (1956), and Harris (1956) have also reported rapid sputum conversion in such cases with the use of corticosteroids.

Radiological improvement

The results are depicted in the following Table 5:

TABLE 5

Improvement	Exudative cases (16 cases)			Productive cases (9 cases)			Results at the end of 3 months
	1st month	2nd month	3rd month	1st month	2nd month	3rd month	
Marked	—	2 cases	3 cases	—	—	—	3 cases (12 per cent)
Moderate	5 cases	5 cases	8 cases	—	1 case	1 case	9 cases (36 per cent)
Slight	9 cases	9 cases	5 cases	1 case	—	1 case	6 cases (24 per cent)
Stationary	2 cases	—	—	8 cases	8 cases	7 cases	7 cases (28 per cent)
Deterioration	—	—	—	—	—	—	—

Thus in the present series there were three cases (12 per cent) who showed marked radiological clearing and 9 cases (36 per cent) who showed moderate clearing. These results are highly commendable in view of the fact that these cases previously failed to respond to the routine antituberculous treatment and is a pointer for the definite place of the use of corticosteroid in clinically resistant cases.

All cases with predominantly exudative lesion showed improvement. Out of 9 predominantly productive cases, seven cases remained stationary while moderate and slight improvement was seen in one case each at the end of 3 months treatment. In these two cases the improvement seen was due to the disappearance of existing exudative element.

Thus the present study reveals that as far as possible the corticosteroids may be used in predominantly exudative lesions.

Cavity Closure

There were 38 cavities present before treatment, out of which, without the addition of temporary collapse measure or surgical intervention, 6 cavities (15.8 per cent) completely closed and 12 cavities (31.6 per cent) showed reduction in size. The remaining 20 (52.6 per cent) did not show any change but out of them 2 cases achieved an open negative status.

Laryngitis

Out of the 6 cases with a laryngeal lesion two cases (33.3 per cent) showed complete healing while another two cases (33.3 per cent) showed slight to moderate improvement and in the remaining two cases (33.3 per cent) the lesion remained stationary.

Endobronchial lesion

In 10 cases with endobronchial lesion, complete healing was seen in 4 cases (40 per cent), moderate improvement in 3 cases (30 per cent) and in the remaining 3 cases (30 per cent), the lesion remained stationary.

B. P. and Corticosteroids

Before start of therapy there was hypotension in most of the cases. With start of prednisolone an increase in both diastolic and systolic pressures occurred. After treatment the range of B.P. was 104-166/78-110 mm. Hg., average being 124.4/89.6 mm. Hg.

In only two cases (8 per cent) the diastolic pressure registered a rise above 100 mm. Hg. in the last week of 3rd month which came down to normal with the withdrawal of prednisolone.

Blood Sugar and Corticosteroid

In none of the cases was hyperglycaemia found. The average fasting blood sugar of every month was as shown in the following Table 6:

TABLE 6

Fasting blood sugar level before starting prednisolone	Fasting blood sugar		after treatment
	1st month	2nd month	3rd month
94mg%	95mg%	98 mg%	104 mg%

Excretion of 17 Ketosteroids

Before the start of therapy in nearly all cases hypofunction of adrenal was found, but with the administration of prednisolone an increase in excretion was noticed which was as depicted in the following Table 7:

TABLE 7

17 Ketosteroids	Before (mg/24 hours)	After 3 months (mg/24 hours)
Average	6.1	9.7
Range	3.1—11.4	6.2—14.0

Toxicity

No untoward reaction with the present dosages employed was seen in present series.

Summary

The literature on the use of Corticosteroids in clinically resistant cases of pulmonary Tuberculosis has been reviewed and the results of the use of prednisolone in 25 such

cases presented. The present study indicates that addition of corticosteroids in such cases can be done with benefit.

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NEWS AND NOTES

XVIth International Tuberculosis Conference, Toronto

The Sixteenth International Tuberculosis Conference was held in Toronto from September 6th to 15th, 1961. Dr G. J. Wherrett, Executive Secretary of the Canadian Tuberculosis Association and President of the IUAT presided. About 2,000 delegates from over seventy different countries attended. The following were present from India:

Dr P. V. Benjamin	Dr Hafizullah
Dr B. K. Sikand	Dr A. G. Patel
Dr T. J. Joseph	Dr Nanasaheb Gokhale
Shri B. M. Cariappa	Dr P. R. J. Gangadharan
Dr B. B. Yodh	Dr D. R. Nagpaul
Dr J. Frimodt-Moller	Dr M. K. Vaidya

XVIIth International Tuberculosis Conference, Rome

The International Union elected Prof. Omodei Zorini of Italy as President of the Union. The next Conference of the Union will be held in Rome, Italy, in October 1963 under the auspices of the 'Federazione Italiana contra la Tuberculosis'.

Informal meeting of the Eastern Regional Committee

An informal meeting of the Eastern Regional Committee of the International Union Against Tuberculosis was held in Toronto, Canada in September last at the time of the XVIth International TB Conference. Delegates from Australia, Japan, Malaya, Singapore and Thailand attended the meeting. Shri B. M. Cariappa, Secretary and Treasurer of the Eastern Regional Committee presented a report on the work of the Committee since it met last in Sydney in May, 1960 up to August, 1961.

The next regular meeting of the Committee will be held in Bangkok, Thailand during October-November, 1962.

Indian team in Canada

The following persons visited Canada to study and observe anti-tuberculosis measures there under the auspices of the Colombo Plan Fellowship:

Dr B. K. Sikand, Director, New Delhi TB Centre.
Shri B. M. Cariappa, Secretary, Tuberculosis Association of India, New Delhi.
Dr Hafizullah, Superintendent, C. D. Hospital, Srinagar.
Dr A. G. Patel, Chief Medical Officer, D.G.D. Anti-TB Clinic, Anand, Gujarat State.
Dr M. K. Vaidya, Medical Superintendent, Karnatak Health Institute, Ghataprabha Mysore State.

XVIIIth Tuberculosis Workers' Conference, Bangalore

The Eighteenth Tuberculosis and Chest Diseases Workers' Conference will be held in Bangalore from 16th to 19th January 1962 under the auspices of the Tuberculosis Association of India. Dr R. N. Tandon, formerly Professor of Tuberculosis, King George Medical College, Lucknow, will preside over the Conference,

Arrangements for holding the Conference in Bangalore are being made by Dr U. Sundarraya Shetty, Honorary Secretary, Mysore State TB Association. Delegates who require accommodation in Bangalore during the conference period should contact Dr Shetty. Delegates may also inform him whether they wish to join excursions to TB institutions and other places of general interest so that adequate transport and other arrangements may be made.

Anti-tuberculosis Campaign in Maharashtra

On the suggestion of the Maharashtra State Advisory Board on Tuberculosis, the Government of Maharashtra agreed to gear-up all activities connected with the prevention and treatment of tuberculosis by launching an anti-tuberculosis campaign throughout the State of Maharashtra for a period of one month starting on 10th October, 1961.

Beit Memorial Fellowship for Medical Research

The next election of the Junior Fellows for the award of the Beit Memorial Fellowship will take place in May 1962. Junior fellowships have successive values of £900, £950 and £1,000 for three years.

Forms of applications and detailed information may be obtained from the Secretary to the Government of India, Ministry of Health, New Delhi 2.

ANTI-TUBERCULOUS DRUGS

By J. BALTRUSAITIS, North Bay, Ontario, Canada

Tuberculosis is notoriously slow to heal. No treatment is known which will kill tubercle bacilli promptly. Specific drugs prevent only bacilli from multiplying for many months, reproductive capacities of tubercle bacilli are impaired and the result is equivalent to bacteriocidal action. Thus, emphasis has to be placed not only on very prolonged but, also upon uninterrupted drug therapy. Interruption of treatment permits a restoration of vitality on the part of bacilli, which may be difficult to overcome.

Specific treatment should be continued as long as any evidence of improvement is seen and for considerable time thereafter. A rule frequently stated is that treatment should be continued for at least 12 months after the last positive culture, the last evidence of roentgenographic improvement or the last indication that cavity was present. The total duration of drug administration usually at least 18 to 24 months, if the above rule is followed.

1. *First line anti-tuberculous drugs*: Drugs, which are used most commonly in the treatment of tuberculosis, because of their effectiveness and not too serious side effects, such as allergic reactions, gastrointestinal upsets etc. They are:

1. **Streptomycin.** An antibiotic, given IM.

It is used often in combination with other two drugs, isoniazid and para-amino salicylic acid, as long as 3 to 4 months, until the sputa becomes negative. Because of its toxicity to the hearing nerve, it is seldom used beyond a four month period.

2. **Isoniazid.** Given by mouth (Tablets) is of low toxicity and comparatively of high effectiveness. Can be given for prolonged period of time, up to 2 years or more, provided that patient tolerates it well. Occasionally, it may cause symptoms of nervous system, such as polyneuritis and psychosis as well as unusual nervousness, insomnia and headache. Pyridoxine (vit. B 6) if given concurrently, helps to prevent the toxic symptoms of nervous system.

3. **Paramino-salicylic-acid (PAS).** If used as acid dosage of 12 gms. If as sodium salt, dosage of 15 gms. daily. It is of lowest effectiveness against tubercle bacilli but

it is very important to prevent or postpone resistance to streptomycin and isoniazid. One drawback is that it has to be taken in comparatively large amounts (12 to 15 gms. daily) and also sometimes causes unpleasant gastro-intestinal symptoms, such as indigestion, heartburn, nausea and even vomiting and diarrhea. PAS should always be used in combination with another drug, and is never used alone.

Most often used therapeutic regimens are:

Streptomycin plus Isoniazid plus PAS.

Isoniazid plus PAS. Streptomycin plus

PAS. Streptomycin plus Isoniazid. For advanced disease, Isoniazid and PAS may be superior to Isoniazid and intermittent streptomycin.

2. *Second line anti-tuberculous drugs*: These drugs are used when a patient cannot tolerate the above mentioned first line drugs or when resistance of tubercle bacilli develops while using the above drugs. These drugs are newer, there is less clinical experience with them, many of them are quite toxic. In view of this, they are used for shorter periods and under strict clinical and laboratory supervision. Most often these drugs are administered while patient is still at sanatorium. The following drugs are used:

1. **Viomycin.** Antibiotic drug, given IM. in doses of 2 gms. twice a week. Most often it is used for patients being prepared for surgical treatment, for 2 to 3 weeks before surgery and 8 to 10 weeks after operation.

2. **Cycloserine (Sero-mycin 'lilly').** Given orally in doses of 0.25 gm. twice daily. Higher doses are toxic to nervous system, blurred vision, convulsions, jitteriness, psychosis etc.

3. **Pyrazinamide.** Given orally in dosage of 20 to 40 mg./kg. per day. It is used for short periods up to 30 to 60 days and only under strict hospital supervision because of its harmful effects on liver.

4. **Kanamycin.** Recently introduced antibiotic in treatment of tuberculosis, given IM in dosage of 0.5 gm. twice daily. It may,

however, lead to deafness in a short period of time, which limits its therapeutic value in any long term programme of treatment of tuberculosis.

It should, however, be emphasized that the above mentioned second line anti-tuberculous drugs are of short term effectiveness, of approximately 3 to 6 months duration and, therefore, not good for long treatment lasting 2 years or more. Any plan of treatment with these drugs should include a programme of thoracic surgical intervention at the earliest appropriate time.

3. *Steroid drugs (cortizone etc.):* These drugs are sometimes used concurrently with anti-tuberculous drugs for symptomatic improvement of severely ill patients with adrenocortical hypofunction and in-patients who are hypersensitive to tuberculo-protein.

4. *New drugs:* Which are still being experimented with on animals or being tried on humans.

1. **Isoxyl.** This preparation is being experimented with on animals in Germany. If good results of the animal experiments are clinically confirmed, the well tolerated preparation could possibly replace PAS.
2. **Thioamide** (Alpha-ethyl, thio isonicotinamide) being tried on humans in U.S.A. in whom tubercle germs showed resistance to INH and streptomycin. Dose 0.5 to 1.0 gm. daily in divided doses. Optimum duration of treatment is unknown.
3. **Ethambutol.** Supposedly as active as streptomycin in mice, not experimented with on humans in U.S.A. as yet.

ABSTRACTS

Toxic and Allergic Drug Reactions during the Treatment of Tuberculosis

In 628 new cases of tuberculosis, allergic reactions to Streptomycin occurred in 44 patients (8 per cent) and to P.A.S. in 54 (or 8.7 per cent). Toxic reaction to Streptomycin in 44 (or 8 per cent) patients and to P.A.S. in 3 (or 0.5 per cent) patients.

Allergic reaction to both Streptomycin and P.A.S. was seen in 23 (50 per cent), of 472 patients who had both drugs for at least six weeks.

The principle causes of failure in treatment are:

1. Tubercle bacilli initially resistant to one or more of the drugs.
2. Failure of the patients to take the treatment advised leading to resistant bacilli.
3. Interference with treatment because of the development of toxic or allergic drug reaction.

Children are free from both toxic and allergic reactions.

Increasing age had no effect on allergic reaction although it gave increasing liability to toxic reaction from Streptomycin.

The majority of allergic reactions occurred in the first five weeks.

Allergic and toxic reactions occurred twice as commonly in women as in men.

Isoniazid gave rise to no reactions of any kind.

[John Morrison Smith and Maia H. Zirk; *Tubercle, London*, (1961), 92, 287.]

An investigation of the value of Ethionamide with Fyrazinamide or Cycloserine in the treatment of Chronic Pulmonary Tuberculosis

Ethionamide with Pyrazinamide or Cycloserine was given to 62 chronic patients of pulmonary tuberculosis with positive sputum in whom treatment with other drugs had failed.

Half the patients in both the groups had negative sputum culture at 3 and 6 months.

The majority of patients with positive cultures after 3 months treatment showed bacterial resistance to either ethionamide or the companion drug.

Resistance to ethionamide and pyrazinamide developed within less than 3 months of treatment, but ethionamide resistance seldom developed before 6 months when this drug was given with Cycloserine.

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Resistance to Cycloserine occasionally developed after 9 months treatment.

After one year of treatment 18 out of 62 patients continued to receive treatment. The treatment was stopped in the others because of side effects.

[A report from the Research Committee of the British Tuberculosis Association; *Tubercle, London*, (1961), 42, 269.]

Pulmonary Calcification and Histoplasmosis

In 71 (63.9 per cent) of the 111 autopsies of cases with pulmonary and nodal calcification organism resembling *Histoplasma Capsulatum* were found in the Calcific foci stained by methanamine silver method.

35 patients had splenic calcification and 20 per cent of these revealed organisms resembling *Histoplasma Capsulatum*.

There was no co-relationship between the incidence of positive nodules and the occupation of individual complement fixation and collodion tests or blood obtained at autopsy were negative for antibodies against *Histoplasma Capsulatum*. In one the culture was positive for *Histoplasma Capsulatum*.

(Mashburn, J. D.; Dazson, D. F. and Young, J. H.; *Amer. Rev. Resp. Dis.*, Vol. 84, No. 2, August, 1961.)

Pulmonary adaptation to altered Thyroid Metabolism

Pulmonary function in 7 hyperthyroid and 5 hypothyroid patients with evidence of pulmonary disease showed that both total ventilation and alveolar ventilation were significantly greater in hyperthyroid group than in the hypothyroid group. Patients with pulmonary disease should be protected as much as possible against any treatment or situation that will increase metabolic demands.

(Ellison Lois, T. and Ellison Robert, T.; *Amer. Rev. Resp. Dis.*, Vol. 84, No. 2, August, 1961.)

The surgical management of large Pulmonary Blebs and Bullae

Among 14- patients, 7 patients had bilateral bullous disease (Group I) and 7 had unilateral disease (Group II) associated with greater or lesser degree of Generalized pulmonary emphysema.

All had unilateral surgery.

In Group I there had been post-operative complications such as retention of secretions, atelectasis, empyema, bronchopleural fistula, enlargement and rupture of the contralateral cyst.

One had a late death in a follow up of nine years.

In Group II there were no complications and no deaths in a follow up of ten years. Careful judgement is to be exercised in selecting cases for surgical treatment.

Though the improvement by surgical interference may not be permanent especially in cases with diffuse pulmonary emphysema but it has a definite palliative benefit.

(*Spear, Harol, C.; Daughtry, DeWitt, C.; Chesney John, C. and As her Marks; Amer. Rev. Resp. Dis., Vol. 84, No. 2, August, 1961.*)

Ileocaecal Tuberculosis with particular reference to isolation of Myco-Bactrium Tuberculosis

Of 67 cases of abdominal tuberculosis 47 were having hyperplastic ileocaecal tuberculosis diagnosed clinically, histopathologically and bacteriologically.

A culture positive for *M. Tuberculosis* or guinea pig inoculation or presence of caseation. Necrosis may or may not be present. Their absence does not negate the diagnosis of Ileocaecal Tuberculosis and suggestive of Crohn's disease.

Hyperplastic form of Ileocaecal tuberculosis is not so uncommon as this form probably accounts for a large number of cases of chronic Ileocaecal granuloma of Crohn's type. The tubercle bacilli isolated in all of the cases has been classified as of human type.

(*Wig, K. L.; Chitkara, N. L.; Gupta, S. P., Kishore, K.; and Manchanda, R. L.; Amer. Rev. Resp. Dis., Vol. 84, No. 2, August, 1961.*)

The occurrence of Intra-Thoracic Calcification in Sarcoidosis

The Incidence of Thoracic Calcification was about 10.1 per cent in 256 patients of sarcoidosis and in those in whom diagnosis of sarcoidosis was not established.

A majority of patients with sarcoidosis with calcification reacted to neither tuberculin nor histoplasmin.

Calcifications were as often related to histoplasmosis as to tuberculosis. Patients with sarcoidosis reacted less often to tuberculin (30.1 per cent) and histoplasmin (85 per cent) than did patients in whom this diagnosis was not established.

A transient elevation of serum calcium concentration was noted in 29 per cent of patients with

sarcoidosis and sustained elevation in less than 3 per cent.

No relation to thoracic calcification was apparent.

(*Harold L. Israel, Maurice Sones, Robert L. Roy and George N. Stein.; Amer. Rev. Resp. Dis., Vol. 84, No. 1, July, 1961.*)

The Supra-renal Function in Allergic Asthma

Determination of the Plasma and Urinary 17-OH-Corticoids and of the Urinary 17-Ketosteroids before and after ACTHZN

Adrenal Function was studied by means of 24 hours test with ACTHZN given intramuscularly in 22 patients of definite origin who had never received corticosteroids treatment.

The urinary 17-Ketosteroids, the 17-Hydroxycorticoids and the plasma 17-Hydroxycorticoids were studied.

Elimination of 17-Ketosteroids was diminished before the administration of ACTH. After the ACTH, the increase of 17-Ketosteroids was¹ practically non-existent (+ 6 per cent).

The elimination of 17-Hydroxycorticoids was within normal limits. The increase after stimulation was less (+145 per cent) while in normal it was (4-300 per cent). The proportion of plasma 17-Hydroxycorticoids was higher. This manifested in inverse ratio to the intensity of symptoms.

The increase of Plasma Corticoids eight hours after stimulation with ACTH was found to be diminished.

In Allergic Astmas there is disturbance of such clinical adrenal insufficiency.

(*Jorge Raul Vaccarezza; Dis. Chest. Vol. 40, No. 2, August, 1961.*)

Sarcoidosis

Review of 111 patients of sarcoidosis showed that 25 per cent were asymptomatic and remained asymptomatic.

The diagnosis depends upon clinical, histological and immunological findings. Recurrences and exacerbations are noteworthy only because of their absence.

Recovery with apparent inactivity of disease was seen in 65 per cent.

Corticosteroids afforded no benefit except temporary amelioration of acute symptoms.

(*Theodore Bacharact; Amer. Rev. Resp. Dis., Vol. 84, No. 1, July, 1961.*)