The
Indian Journal of Tuberculosis

Vol. XIII New Delhi, March 1966 No. 2

THE 21st CONFERENCE

The twenty-first Conference of Tuberculosis and Chest Diseases Workers was held in Calcutta from the llth to the 14th February, 1966. West Bengal had the honour of being the host State. About 600 delegates, and others interested in the subject attended the Conference. The Conference is drawing larger number of delegates year by year proving that its contributions are becoming more and more useful. It has reached the significant age of maturity this year. It is hoped that its deliberations will be more effective from now on incorporating both youthful vigour and maturity of judgement.

The deliberations of the Conference covered very wide fields. There were Panel Discussions and Symposia on the 8th WHO Report, District Tuberculosis Control Programme, Relapse in Tuberculosis, Sensitivity Tests for Second-line and TB drugs, Surgery in Tuberculosis, Public Health Nursing and Social Work in Tuberculosis, Chronic Bronchitis and Childhood Tuberculosis. Besides, there were special sessions with original contributions on Epidemiology, Toxicity of Thiacetazone, in addition to many assorted papers of great value. It is not possible in short space to summarise even very briefly the contributions or the conclusions arrived at. The Transaction which will be published later by the Tuberculosis Association of India will incorporate all the contributions and will be of great value to all workers. It may, however, be mentioned that the deliberations avoided the beaten tracks in many areas and were specifically engaged in finding simpler and more profitable measures for the control of Tuberculosis according to the culture, capacity and conditions of the country. By doing so the Conference contributed many new thoughts, new approaches and new types of field and other services for trials.

It is felt that many important details may be missed by the workers unless they are serious readers of the Transactions. This loss may be mitigated if the Editorial Board of this Journal is assigned the responsibility of collecting important informations and recommendations and publish them in this journal. The same Committee may also be given the responsibility to ear-mark important Papers read at the Conference for publication in this Journal.

The Tuberculosis Association of India is encouraging States to hold their own Conferences and a few States are already doing so. It is time now to decide how often these should be held to avoid overlapping of Conferences. The All India Conference should continue as usual its annual sessions. The

States may be requested to have their own at two years' interval or so specially if the annual conference is being held in the neighbouring states.

It is said that man's mastery over others depends largely on the discovery of the "Wheel" and the "Conference", the former for tapping immense power from the Nature and the latter for harnessing common wisdom and will for unified action. To conquer this great scourge of Tuberculosis we need both. Science will give us power. Conferences will confer wisdom to apply this power judicially.

P.K. Sen

AWARD OF T.A.I. GOLD MEDAL

The first Award of the Tuberculosis Association of India's Gold Medal for outstanding work in the Tuberculosis field was conferred on Dr. A.C. Ukil of Calcutta by Shrimati Padmja Naidu, Governor of West Bengal, at the time of the XXI TB and Chest Diseases Workers Conference held in Calcutta in February, 1966.

Born on 14th November, 1888 Dr. Ukil graduated from the Calcutta Medical College in 1914 after a brilliant academic career. In 1922, he proceeded to Paris for post-graduate work in Pathology and Bacteriology at the Pasteur Institute. He was associated with Professor Weinberg and Professor Calmette. On return from Paris after two years he started practice in Calcutta in Clinical Pathology, Bacteriology and Phthisiology. In 1927, he was appointed the Director of Tuberculosis Enquiry, Indian Research Fund Association. In 1928-29, he was awarded the Ghosh Travelling Fellowship of the Calcutta University and returned to the Pasteur Institute in Paris for further research work. He was appointed in 1931 the Physician-in-charge of the Chest Department, Medical College Hospitals, Calcutta, which he developed into an important centre of teaching and research in Tuberculosis in the country. He also continued as the Director of the I.R.F A. Enquiry in tuberculosis at the All-India Institute of Hygiene and Public Health, Calcutta, till 1943, in which capacity he made outstanding contributions in the epidemiology of Tuberculosis.

On the appointment of Dr. P.K. Sen as the Physician-in-charge, Dr. Ukil was appointed in 1948 the Consulting Physician to the Chest Department of the Medical College Hospital and continued to serve as such till 1963, when his illness forced him to suspend his activities.

He was a member of the first Medical and Publicity Sub-Committee of one of the earliest Anti-Tuberculosis Organizations in India, the Tuberculosis Association of Bengal, which was re-named the Bengal Tuberculosis Association in 1939. After serving the Bengal Association in various capacities, he took over the office of the Chairman from the late Dr. B.C. Roy in 1948 and held it for the next fifteen years before relinquishing it for reasons of health in 1963. He was elected an Emeritus Fellow of the American College of Chest Physicians. He also served as Councillor Member of the Council of International Union Against Tuberculosis. He became a corresponding member of the French Pathological Society and a corresponding editor of the Danish Journal Mycopathologiaet Mycologia Applicata.

Dr. A. C. Ukil

Dr. Ukil was a member of the Standing Technical Committee of the Tuberculosis Association of India and he was also a member of the Central Committee. He was President of the All-India Tuberculosis Workers' Conference in 1949. He was a Honorary Member of the Calcutta Branch of the Indian Medical Association and was elected President of the All-India body in 1955-56. He was also an Honorary Member of the Indian Association for Chest Diseases and served as its President for the year 1960. He was President of the Medical College Re-Union in 1961.

He was the Consulting Physician for Chest Diseases of the Institute of Post-graduate Medical Education and Research since its creation. He was appointed Principal and Superintendent of the Medical College Hospitals but resigned after serving for a period of nearly one and half years.

He was elected President of the Calcutta Rotary Club in 1935 and became the Districts Governor of Rotary International in 1942-43. He was awarded the Kaiser-I-Hind Gold Medal in 1941. He had been for a long time associated with the Masonic Movement in India and has held some of its highest offices. In 1955-56, he was nominated the Sheriff of Calcutta.

He served the National Institute of Science of India of which he is a Fellow and later became its President in 1955-56. He also served as an editor of "Science and Culture" from 1938 to 1957. He was elected President of the Medical and Veterinary Section of the Indian Science Congress in 1941. He was Sir J.C. Bose Memorial lecturer in 1956. He was also the President of the Indian Science News Association in 1961-62.

He was associated with the Asiatic Society —(formerly the Royal Asiatic Society) for a

long time. He was awarded the Barclay Memorial Medal of the Society in 1951 and in 1960-61 was elected its President. He was responsible for acquiring land and the construction of the imposing building in which the Society is now housed.

In the field of medical education he has left his mark. He was senator of the Calcutta University for a number of years and for a period, the Dean of the Faculty of Medicine of the University. He was Honorary Fellow of the State Medical Faculty of Bengal Consulting Physician for Chest Diseases, Medical College Hospitals, Calcutta, and the Institute of Post-Graduate Medical Education and Research. In his capacity as the Principal of the Calcutta Medical Colleges he took considerable interest in shaping the medical education of the State of West Bengal. He was awarded the Coates Memorial Medal of the Calcutta University in 1925 for researches in medicine. He was appointed the Basanta Memorial Lecturer in Public Health of the University in 1954-55.

He was invited abroad on a number of occasions to participate in international conferences and seminars. In 1948 he travelled to Washington as a participant in the 4th International Congress on Tropical Medicine and Malaria. In 1951, he visited the U S.S.R. as a member of a team of Indian scientists and academicians led by Prof. P.C. Mahalanobis and travelled widely in the various states of the country in order to acquaint himself with the existing medical and public health facilities as well as the state of medical education and research activities there. In April, 1955 he was invited by the World Health Organization to participate in a conference in Geneva and in September of the same year he visited Paris to take part in meetings, of the International Union Against Tuberculosis. In 1962 he was invited by the Cornell University at Ithaca, U.S.A., to participate in the History of Medicine Symposium held there.

Dr. Ukil's indefatigable energy led him to take up leadership in such diverse fields as safety education, nutrition and dietetics, history of medicine and science in India and many others.

In the early part of his career, during the first World War, he was arrested and interned in his home town for a period by the British rulers because of his association with the revolutionaries in Bengal.

In appreciation of such a remarkable career and contribution towards tuberculosis control, the Tuberculosis Association of India honours him by the award of its "Gold Medal" for 1966.

ENHANCING OF TUBERCULIN ALLERGY BY PREVIOUS TUBERCULIN TEST(S)

RAJ NARAIN, S.S. NAIR, G. RAMANATHA RAO, P. CHANDRASEKHAR AND PYARE LAL
(National Tuberculosis Institute. Bangalore)

Tuberculin tests repeated after an interval of time, at a different site, have been reported to elicit larger reactions than the first test. Magnus and Edwards (1955) suggested that intradermal testing in school children every year may prevent waning of BCG induced allergy. Magnus (1957) showed that waning allergy in case of BCG vaccinated guinea-pigs can be substantially enhanced by an intradermal injection of PPD in a dose of 5 TU or more and by old tuberculin in a dose of 1 TU. Kul Bhushan (1958-59) studied the phenomenon among the vaccinated and unvaccinated tuberculin positive and negative school children, and reported enhancing of allergy among the negative vaccinated. Ferebee and Mount (1963) demonstrated boosting on serial tuberculin testing among mental hospital patients. They found very little boosting in 4 schools for the mentally retarded and thought that this lack of boosting in the school group was due to annual testing with tuberculin. Whatever boosting had to take place, occurred with the first test or tests and subsequent tests added very little.

The National Tuberculosis Institute (NTI) is carrying out repeated surveys with tuberculin testing, X-ray and bacteriological examinations in a previously untested and unvaccinated rural population (Longitudinal Survey). In this survey reactors of 13 mm or less to 1 TU have been tested with 20 units for a study of low-grade reactions. The present study of a possible enhancing effect of tuberculin test was undertaken and so planned that it may help in a better interpretation of the data collected in the Longitudinal Survey.

Objectives :

To study the possible enhancing of tuberculin allergy by previous test(s) with 1 TU and with both 1 and 20 units.

Study population

Due to lack of knowledge of the factors which niay influence the enhancing effect, the most suitable size of study population could not be estimated. Somewhat arbitrarily, in the same area as the Longitudinal Survey, eight villages with an estimated population of about 4,000 in which no previous BCG vaccination or tuberculin testing had been done, were selected.

Methods

All houses in each village were numbered. A card showing name of the village, serial number, the household number, name, age, sex and relationship with the head of the household etc., was prepared for each individual by a house-to-house visit. All *de jure* residents, even though temporarily away and thus not likely to be available for testing, and all visitors who had slept the previous night in the village, even though not likely to be available at later stages of the study, were registered.

A map of the village showing the number and location of each house was drawn for convenience in the location of households in subsequent visits.

Before visiting a village, cards to be used were serially numbered (individual number), and by random selection three fourths of the cards were coded for a tuberculin test. Persons registered on these cards were each given an intradermal test on the upper third of the volar surface of the left forearm with 1 unit, PPD RT 23 containing 0 05 per thousand Tween 80 in 0.1 cc of the buffered diluent. The remaining one-fourth, coded for the control group, were given 0.1 cc of the diluent containing Tween 80 and chinosol (1 TU controls). At the time of testing, both shoulders of each individual were examined and the presence or absence of a BCG scar (definite or doubtful) recorded, primarily to exclude the vaccinated immigrants.

Three-fourths of the individual cards coded for the 1 TU test were also coded for a further test with 20 TU. The remaining one-fourth were coded for a test with the diluent only (20 TU controls). The 20 TU tests, in 0.1 cc of the diluent with Tween 80, or the placebo were given at the time of 1 TU test reading, only to those who had 13 mm or smaller longitudinal diameter of induration, the site being middle of the dorsal surface of the left forearm.

The two tuberculins and the placebos used were labelled with codes only. None of the field workers had any knowledge of the codes.

At two months, half of the registered households in each village were selected as a simple random sample in order to administer a 1 TU test to half the population. This was done irrespective of whether the particular individual

in these households was tested at 0 month or not. It was considered operationally more convenient to test all persons in half the households rather than half the persons in each household. The site for retesting was the middle third of the dorsal surface of the right forearm.

All tuberculin reactions were read after 3-4 days and longitudinal diameter of induration in millimetres dictated to a secretary. The knowledge whether a person had been tested before or not was guarded from the reader. Reader and tester for the 1 TU tests were the same in the two rounds. During the periodical tests for reading of tuberculin reactions carried out by the Institute the reader always had good internal consistency.

The initial 1 TU and 20 TU tests were carried out during August and September, 1963 ; the repeat 1 TU test, after 2 months, during October and November, 1963. Entire population is planned to be retested with 1 TU at 18 months. The present report is based on the results of the first and second rounds only.

Population Surveyed

Population eligible for various tests and attendances at various stages of the study are given in Table 1. Fig. 1 is a schematic representation of the accomplished design of the study. The population under report comprises of those tested and read both at 0 and at 2 months and those tested with placebo at 0 month but with 1 TU at 2 months. Age and sex distributions among the test-read at Round I, the re-rested and read at Round II and also among the con-

TABLE 1

Eligible, tested and read at 0 month and at two months

At 0 month	Number of persons	At *2* months*		
		Eligible for 1 TU	Tested	Read
For 1 TU test:				
Eligibles	3217	1346	1096	1025
Tested	2600	1240	1008	949
Read	2357	1208	976	920
For 1 TU control;				
Eligibles	1023	424	356	333
Tested	806	391	327	307
Read	759	383	319	300
For 20 TU test:				
Eligibles	1379	698	559	529
Tested	1359	689	554	525
Read	1275	638	513	487
For 20 TU control:				
Eligibles	439	217	177	170
Tested	431	214	175	168
Read	406	201	165	158
Reaction ≥ 14 mm) to 1 TU	539	293	240	221

* Excluding those registered for the first time at 2 months only.

trols tested with tuberculin for the first time at Round II were not much different.

Results

I. Increase in allergy seen at retest :

Fig. 2 shows the distribution of 1 TU indurations for those test-read at both 0 and 2 months, and for the controls tested only at 2 months. Only two age groups (0-14) years and 'all ages' have been shown.

In these histograms and in those for younger age groups (not shown) it is not possible to draw a clear line of demarcation between the "negative" and "positive" reactors. This lack of clear separation between the two groups had also been reported earlier (Raj Narain et al. 1963). The area is one with high prevalence of non-specific allergy.

The greater mean allergy of 2.2 mm in the retested group in comparison with the 1 TU results at 0 month (10.9 mm compared to 8.7 mm) can be due to many factors : new infection during the intervening two months, the effect of time interval (including any possible reader bias) and enhancing effect, if any, of the earlier tuberculin tests. But the greater mean allergy of 1.7 mm seen for retests at II round, compared with the 1 TU mean reaction for the controls also tested at 2 months, (10.9 mm against 9.2 mm) can only be due to the enhancing of allergy as a result of the tests at first round. All other factors are likely to have affected the two groups equally. (Also see discussion). The difference is statistically significant at 99% level of confidence.

2. Some factors associated with boosting: The effect of time interval of 2 months and

TABLE 2

Increase in mean allergy at 2 months in 1 TV reactions at 0 month
by 2 mm belts

Induration to 1 TU at 0 month	Number	Mean induration to 1 TU at 2 months	Increase in allergy at 2 months
1	2	3	4
0—1	139	4.6	4.6
2—3	207	4.5	2.1
4—5	150	6.9	2.4
6—7	111	8.4	1.9
8—9	42	13.0	4.6
10—11	28	14.5	4.1
12—13	22	17.6	5.4
14—15	25	16.4	1.7
16-17	26	16.9	0.2
18—19	15	21.5	2,9
20—21	27	22.1	1.7
22—23	19	23.7	1.3
24-25	20	25.2	0.8
26—27	30	26.3	—0.3
28—29	22	26.9	—1.6
30 +	37	27.2	—5.7
Total	920	10.9	2.2

factors other than boosting (including reading and other errors) can be estimated by comparing the mean allergy in '1 TU controls' at 2 months (9.2 mm) with that for those tested at 0 month (8.7 mm). The age and sex distribution of the two groups was similar, and the difference 0.5mm is statistically not significant ($0.40<P<0.50$). Therefore, one may assume that in the retested group the difference between the reactions at 0 month and those at 2 months is mainly due to boosting. A comparison of these two sets of reactions has shown that the factors associated with boosting are :

(a) Initial allergy to 1 TU :

Increase in mean allergy at 2 months for each 2 mm belt of induration to 1 TU at 0 month, among those tested at both rounds, is shown in Table 2. The greatest increase in mean allergy is seen for those with (0-1) and (8-13) mm reactions at 0 months. When persons who have 0-1 mm initial reactions are retested, a decrease in allergy at the second test is not possible and the induration can either remain the same or increase. Similarly those with 26 to 30 mm or bigger reactions are much more likely to show only a decrease in allergy on a retest. That such a phenomenon occurs is shown in Table 3, in which only extreme ends of the distribution of indurations in 2 mm belts at 2 months have been compared with their mean indurations at 0 month. "Change" in allergy from 2 months to 0 month is shown in the last column. The Tuberculin reactions of 0-1 mm at 2 months show an "increase" in allergy at 0 month ! Tuberculin reactions of 2-3 mm at 2 months do so but to a very small degree. Similarly those with 26 mm or larger reactions at 2 months show a much greater "decrease" of allergy at 0 month than similar large reactions at 0 month show at 2 months in Table 2.

Thus the extreme upper and lower limits of Table 2 may be disregarded. The increase in allergy is then seen to be most marked in those with 1 TU reactions of 8-13 mm at 0 month and, to a smaller extent, continues to be seen more or less upto initial induration of 23 mm,

A table similar to Table 2 was prepared by using Chauvenet's criterion to exclude extreme values (Geigy, J.R., 1956). The greater degree of increase in allergy among those with intial indurations of 8-13 mm was well marked. Further, the increase in allergy at round IT could be seen even for the large reactions beyond 23 mm.

(b) Initial allergy to 20 TU:

A 20 TU test was given only to those with 13 mm or smaller reactions to 1 TU. The choice of this level was, more or less, arbitrary. The

TABLE 3

*'Change" in mean Allergy at 0 month in 1 TU reactions at 2 months (Reverse of Table 2)**

Induration at 2 months	Number	Mean Induration at 0 month	"Change" in allergy at 0 month
1	2	3	4
0—1	165	3.0	3.0
2—3	109	2.7	0.1
4—5	101	3.7	—0.9
—	—	—	—
—	—	—	—
24—25	42	18.3	—6.3
26-27	55	21.8	—4.7
28—29	21	22.9	—5.5
30+	42	25.2	—7.5

* Only extreme ends of the distribution are shown.

intention was to avoid large reactions to 20 TU and at the same time to test all, except the definitely positives, with 20 TU. Reactions of 14 mm and above to 1 TU were regarded as fairly certain evidence of infection with Myco. tuberculosis.

1 TU results at 2 months away the 20 TU tested and read are available for 487 (Table 1). Increase in 1 TU indurations at two months correlated with the 20 TU reactions at 0 month is shown in tabular form :

Size of 20 TU Indurations	Number	Mean Induration to 1 TU at 0 month	Increase in mean induration at 2 months
0-4 mm	102	1.7	0.6
5-9 mm	92	3.2	0.2
10-14 mm	83	3.1	2.3
15 mm and above	210	5.6	6.0
All Indurations	487	3.9	3.1

The increase in allergy is seen only among those showing an initial induration of 10 mm or more to 20 TU*. New infection is not likely to be more frequent among this group than among those with 0-9 mm reactions to 20 TU. This greater increase of allergy among the 20 TU reactions of 10 mm and more can also be seen from Fig. 3, which shows the lines of regression of reactions at 2 months on initial 1 TU reactions seperately for (0-9) and > 10 mm reactions to 20 TU. The difference in the rate of increase of allergy from the first to second round in the two groups is statistically significant at the 99.9% level of confidence. Further, correlation analysis shows that the reactions at 2 months are more highly correlated with 20 TU indurations than with the 1 TU reactions at 0 month**.

(c) The addition of the 20 TU test at 0 month:

Table 4 compares the increase in allergy by size of 1 TU reactions, among the 20 TU tested and the 20 TU controls. The mean increase in allergy among those given both tests is 3.0 mm, while among those given only 1 TU at 0 month, it is 2.6 mm. The difference is not statistically significant. Thus, for the group as a whole, addition of the 20 TU test does not seem to add materially to the increase in allergy elicited by the retest. But increase in allergy due to the addition of 20 TU becomes marked, if initial allergy of 8 mm or higher is considered:

Induration at 0 months to 1 TU	Increase in mean induration at 2 months	
	20 TU tested	20 TU controls
0-7mm	2.7mm(1.4)mm	2.5 mm(1.3)mm
8-13mm	5.4mm (5.4)mm	2.9mm(2.9)mm

Figures in brackets represent increase in mean indurations, after excluding by Chauvenet's criterion persons whose reations have undergone extreme changes (Geigy, J. R., 1956).

Number showing initial allergy of 8-13 mm are small especially among the controls (Table 4) The addition of the 20 TU test seems to influence the enhancing of allergy only among those with 0 month indurations to 1 TU of 8-13 mm. The difference does not attain statistical significance ($0.10<P<0.20$).

To further examine the slightly greater increase in allergy among the 20 TU tested, regression lines of the tuberculin indurations at two months on the initial 1 TU reactions among the 20 TU tested and 20 TU controls are shown in Fig. 4. Reactions below 4 mm to 1 TU at 0 month have been excluded because they are subject to one sided errors as shown in Table 3. The regression lines indicate that the two groups may behave differently. The overall difference is not demonstrable in Table 4, because the two lines cross each other and the divergence between the two groups becomes apparent only for large initial reactions.

(*d) Influence of age:*

Increase in allergy in the retested group s

* Almost identical results were obtained when those with (10-13) mm reactions to 1 TU. who could possibly include some infected with Myco. Tuberculosis, were excluded from the table.

** The reactions at 2 months are more highly correlated with the initial reactions to 20 TU than similar reactions to 1 TU, the respective correlation coefficients of 0.59 and 0.45 being significantly different at 99% level of confidence. If 20 TU reactions are kept constant the partial correlation coefficient between 1 TU reactions at 0 and at 2 months is only 0.22. Keeping 1 TU reactions at 0 month constant the partial correlation coefficient between 20 TU reactions at 0 month and 1 TU reactions at 2 months has a significantly higher value (0.48). This confirms that the reactions at 2 months are more closely correlated with the 20 TU reactions at 0 month than with the 1 TU reactions.

TABLE 4

Increase in mean Induration to 1 TU at 2 month for the 20 TU tested and the 20 TU controls by 1 TU reactions at 0 month in 2mm Belts

Induration (mm) to 1 TU at 0 month	20 TU tested		20 TU controls	
	Number	Mean increase in induration at 2 months	Number	Mean increase in induration at 2 months
1	2	3	4	5
0-1	118	4.3	19	6.0
2-3	149	2.3	57	1.1
4-5	117	2.0	32	3.7
6-7	77	1.9	33	1.8
8-9	33	6.2	9	—1.1
10-11	15	2.9	12	6.1
12-13	16	6.6	6	2.3
All indurations (< 13mm.)	525*	3.0	168*	2.6

*4 persons who refused the 20 TU test and 2 who refused the placebo have been excluded

more for 'all ages' than in the younger age group (0-14) years, whether the comparison is made with the controls tested at 2 months or with the 0 month reactions (Fig. 2). This suggests that boosting may be more in older age groups.

Influence of age in detail has been studied for the combined group of the 20 TU tested and the 20 TU controls (Table 5). The enhancing of allergy at the second test is less for age groups below 10 years, but increases with rise in age. Beyond 45 years of age enhancing of allergy is observed but is less than that in age group (25-44) years.

The 20 TU tested and the 20 TU controls studied separately by age groups (not tabulated) showed a similar general trend of greater increase of allergy with rise in age.

The rise in increase of allergy with age may, wholly or partly, be due to the associated increase in initial tuberculin allergy (including non-specific allergy) and new infections. The latter two also increase upto a certain age and add to the increase in allergy at the second test. It may, however, be possible to eliminate the effect of initial allergy, if (0-4) mm reactors to 20 TU are considered to have no initial

TABLE 5

Mean increase in TU reactions by age at 2 months for those with 13 mm. or smaller initial reactions

Age-Group	Number	Increase in mean allergy at 2 months
1	2	3
0-4	139	0.7
5-9	137	1.0
10-14,	151	2.2
15-24	81	4.5
25-44	116	6.6
45 +	69	4.9
All ages	693*	2.9

* Excluding the six who refused the 20 TU test or the placebo.

allergy. As no difference in the increase of allergy by age was found between (0-4) and (5-9) mm reactors to 20 TU, the two have been combined in Table 6, which shows the rise in mean allergy to 1 TU at 2 months by age groups, among the (0-9) mm and 10 mm or bigger reactions to 20 TLJ. Among those with (0-9) mm reaction to 20 TU, there is no difference in the mean increase in allergy in different age groups. Thus, age, by itself, does not seem to influence the increase in tuberculin allergy among these ‘ negative” reactors to 20 TU. It is only among those with initial 20 TU reactions of 10 mm or more, (the number of children below 5 years is only 5) that the increase in allergy rises definitely with rise in age.

Increase in allergy among those with 14 mm or larger initial 1 TU reactions was also studied by age (Table not presented). No rise in allergy in successive age groups was seen.

(e) Influence of sex:

In section 1, the average increase in mean induration at 2 months due to boosting was 1.7 mm. This average increase was greater in the female (2.5 mm) than in the male (0.9 mm). The ‘20 TU tested’ showed an increase in mean induration of 3.4 mm for females and 2.7 mm for males. The ‘20 TU controls’ showed a similar increase of 3.0 mm for females, and 2.1 mm for males. The differences in the mean increase of allergy between the sexes are not statistically significant. Those with 14 mm or larger reactions to 1 TU at 0 month showed a decrease in mean induration of 1.2 mm for males, but for females there was an increase of 0.5 mm. This difference nearly attains significance at 95% level (P=0.055). When all the 920 tested and retested are considered together, the increase in allergy at 2 months for males is 1.6 mm and for females is 2.7 mm, and the difference between the sexes is statistically significant at 95% level.

Tables similar to 5 and 6 were also drawn separately for the two sexes. The increase in mean allergy was somewhat greater in the female but not consistently so in all age groups.

It is likely that there is greater boosting of allergy in females than in males.

3. Supporting evidence in favour of some of the factors in Section 2:

The effect of retesting at 2 months only has been studied. In order to further examine some of the relationships observed or the hypothesis formulated in Section 2 it may be relevant to examine the data from the first 50 villages in the Longitudinal Survey (Raj Narain et al, 1965). 14,412 persons in the same area were tuberculin tested with 1 TU and a further

TABLE 6

Increase in mean allergy at 2 months by age among those with (0—9) and 10 mm. and bigger indurations to 20 TU at 0 month

Age Group	20 TU indurations of (0—9) mm		20 TU indurations of ≥ 10 mm.	
	Mean Number	Increase	Number 4	Mean Increase
1	2	3		5
0-4	95	0.4	5	2.0
5-9	59	0.4	40	2.8
10-14	24	0.5	82	3.2
15-24			46	5.4
25-44	17	0.4	74	8.2
45+			45	4.9
All ages	195	0.4	292	5.0

test with 20 TU was given only to those with 13 mm or smaller reactions to 1 TU. After 18 months all were retested with 1 TU. The increase in tuberculin allergy at 18 months by age and by size of 20 TU reactions is shown in Table 7 and by size of initial 1 TU reactions in Table 8. In order to reduce the error due to new infection, all those showing a rise of 20 mm or more have been excluded (Raj Narain et al, 1965).

Table 7 shows that:

(i) Increase in allergy at round II has risen with age among those with (0-4), (5-9) and 10 mm and bigger reactions to 20 TU. In Section 2(d) it did not rise at two months for those with initial 20 TU reactions of (0-9) mm (Table 6).

(ii) Increase in allergy has risen, almost in each age group, with the rise in size of initial reaction to 20 TU, the increase being especially marked in the group with 10 mm or larger reactions to 20 TU. Findings at 18 months may not be strictly comparable with those at 2 months, but, broadly speaking, seem to confirm the finding of the present report that increase in tuberculin allergy is correlated with size of initial reaction to 20 TU and with age.

Table 8 shows that the increase in allergy at round II does not seem to be correlated with size of initial 1 TU reactions. This is contrary to the findings in Section 2(a). Perhaps it is due to the fact that 1 TU reactious do not accurately separate the "negatives" from those showing non-specific allergy. Nearly 60% of those with 0-4 mm reactions to 1 TU gave 10 mm or bigger reactions to 20 TU.

Table 8 drawn separately for the two sexes (not presented) did not show any difference in the boosting of allergy in the two sexes. In view of the contrary finding in Section 2(e), the

TABLE 7

(Longitudinal Survey—First 50 villages)

Increase in allergy to 1 TU after 18 months by age and by size of initial reaction to 20 TU

Age Group	Reactions to 20 TU					
	(0-4) mm		(5-9) mm		(10+) mm	
	Number	Increase in mean induration to ITU	Number	Increase in mean induration to ITU	Number	Increase in mean induration to 1 TU
1	2	3	4	5	6	7
0—4	1733	1.1	218	1.1	199	1.5
5—9	838	1.7	340	2.1	850	2.4
10—14	248	2.4	180	1.8	1241	2.9
15—24	46	2.6	69	2.7	1182	4.2
25—34	43	3.5	37	3.2	1111	5.5
35-44	28	3.0	17	3.2	674	5.7
45+	73	3.8	46	3.4	928	6.8
All ages	3009	1.5	907	2.6	6185	3.8
Numbers excluded (those showing a rise of 20 mm or more)	48		14		233	

possibly greater boosting of allergy in the female may, at present, be regarded an open question.

4. Not all show evidence of Boosting:

So far, mean indurations have been compared to study enhancing of allergy in the group as a whole. But boosting is not uniformly distributed in the entire group and therefore comparison of mean indurations has certain limitations This is well illustrated in Fig 5, which shows the 'scatter' of 1 TU reactions for the 20 TU tested separately for individuals with (0-9) and 10mm or bigger reactions to 20 TU at 0 month. Among the former, about 80% show indurations of 5 mm or less to 1 TU at each round. There are hardly any (5%) who show a bigger increase or decrease than 5 mm from their 0 month reactions. (Appendix Table 1 A). It is only among those with 10 mm or bigger reactions to 20 TU that more than a third (40%) show an increase in allergy of more than 5 mm at 2 months, while hardly any (4%) show such a decrease in allergy (Appendix Table 1 B). The mean increase of 2.2 mm seen in the retested group at 2 months (Fig. 2) is mainly due to the large increase in allergy among the 40%. Thus, boosting is mainly confined only to a part of the group with 10 mm or larger reactions to 20 TU or to an estimated 20% or less of the total population. Among the 10 mm and larger reactors to 20 TU the increase in mean allergy in the two sexes was almost similar.

TABLE 8

(Longitudinal Survey—First SO villages) Increase in allergy to 1 TU at 18 months by size of initial induration to 1 TU*

Reaction to 1 TU in T round (mm)	Number	Increase in mean induration 11 round (mm)
1	2	3
0—1	5774	3.3
2—3	2732	2.1
4—5	1054	2.3
6—7	763	2.5
8—9	437	2.5
10—11	353	3.1
12—13	326	2.4
14—15	375	1.2
16—17	401	1.9
18—19	285	2.7
20—21	287	2.2
22—23	351	0.2
24—25	330	0.3
26—27	292	—1.3
18—29	147	—2.8
30+	176	—3.3
All indurations	14083	2.5

* Excluding 319 with an increase of 20 mm or more in their I round reactions.

Discussion

Present study has been carried out in a previously untested and unvaccinated area where only about 25% of the population show 14mm or bigger indurations to 1 TU and of the remaining about 60% show 10 mm or larger reactions to 20 TU. Findings from the Longitudinal Survey also confirm these results and the high prevalence of non-specific allergy in the area.

The crucial consideration is whether the enhancing of allergy seen in Section 1 is really due to the tuberculin tests at Round I. A comparison of the retested and the controls tested at the same time eliminates any reader bias and the influence of other factors associated with the interval. Therefore, the difference in mean allergy of 1.7 mm between these two groups can be due only to the effect of previous tests. It may be stressed that for any kind of reader bias or any other factor to produce the highly specific differences correlated with such factors as initial 1 TU and 20 TU allergy, one or two tuberculin tests, age and sex (Section 2), to say the least, would be extremely difficult**. It is, therefore, concluded that previous tests do boost the allergy at retests.

The most significant finding of this paper would appear to be that boosting caused by

** In three of the eight villages both at 0 and at 2 months the traverse diameters of induration of 1 TU reactions were also read by another reader for a different purpose. These readings, among the 350 persons available for analysis, also show increase in allergy at 2 months and its similar correlation with size of initial 1 TU and 20 TU reactions. Other findings are also similar. For a reader bias of any kind to have operated in a similar fashion in such great detail with two different readers is extremely unlikely.

previous tuberculin test(s) whether with 1 TU or with both 1 TU and 20 TU is not uniform for the entire group and is associated with certain factors. When the allergy initial test is low, the increase at retest is small or negligible, the boosting is most marked among those with intermediate grades of tuberculin allergy, namely, initial reactions of 8-13 mm to 1 TU and 10 mm or more to 20 TU. Almost all those with (8-13) mm reactions to 1 TU gave 10 mm or bigger reactions to 20 TU and are therefore included in the latter group. No precise definition of the term "non-specific" allergy is available but 10 mm or bigger reactions to 20 TU (from among those with (0-13) mm or with (0-9) mm reactions to 1 TU may be regarded, by and large, as evidence of such allergy. Then this finding would suggest that boosting effect of a tuberculin test may be more marked in areas, such as the present one, where prevalence of 'non-specific' allergy is high. A definite conclusion on this point may be possible by conducting a similar study in an area with low prevalence of non-specific allergy.

Influence of age presents some difficulty in interpreting the results. Table 6 shows that, among those with less than 10 mm reactions to 20 TU, the different age groups show almost no increase of allergy at 2 months, while among those with 10 mm or bigger initial reactions to 20 TU, there is a definite rise with age in the increase of mean indurations. This would seem to suggest that age may not be a factor by itself but data from an altogether different study presented in Table 7 (Section 3) suggests that age by itself may be a factor. After an interval of 18 months, even among those with initial reactions of (0-4) mm to 20 TU, the increase in allergy rises with age. Whether age is an independent factor affecting boosting or not, the fact remains that increase in allergy rises with age and this rise may be more in communities with high prevalence of non-specific allergy.

The enhancement of allergy by a tuberculin test may be of the nature of anamnestic phenomenon (Rich, 1951). The wonder is that it occurs in man with as low a dose as 1 TU PPD. Magnus (1957) found boosting with 5 TU but not with 1 TU of RT 19-20-21 in vaccinated guinea-pigs. Probably, the addition of Tween 80 has made this difference, as very little less due to absorption of tuberculin on the surface of glass takes place. A tuberculin test cannot create or cause sensitivity in man. It can only increase an existing allergy, especially if it is of an intermediate grade. The enhancement of such allergy among the BCG vaccinated is well marked and was observed first by Magnus (1955). In an earlier report (Raj Narain et al, 1961) it was found that mean increase in allergy with a 1 TU retest after 3 months in previously vaccinated school children was 4.6 mm compared with 0.7 mm in the unvaccinated. Non-specific allergy and perhaps "waned" allergy due to infection with Mycobacterium tuberculosis also show similar intermediate grades of allergy and, if so, greater boosting among these may not be surprising.

In passing, the report by Ferebee and Mount (1963) that there was little or no increase in allergy in school children may be commented upon. They suggest that this lack of enhancing effect was due to previous annual tuberculin tests. Although we have no experience with tuberculin tests at more than two points of time, yet the possibility may be suggested that the children in Massachusetts schools did not have much initial allergy and were possibly non-reactors to 20 TU and thus did not show enhancing of allergy on a retest.

The boosting effect of a previous tuberculin test has several implications :

(i) A far reaching implication would be on the design of future epidemiological surveys. In all surveys where follow-up studies are planned, tuberculin testing of the entire study population may not be advisable. Only a randomly selected portion of the survey population may be tested in each round. Something similar to the design of the present study would appear to be suitable. In this way a better understanding of the changes in tuberculin status in later rounds would be possible.

(ii) Conversion rates, i.e., proportion of tuberculin negatives who become positive, must not be used for estimates of incidence of new infection as has often been done. This aspect has been discussed in detail elsewhere (Raj Narain et al, 1965).

A further implication, which is known, but, far too often not followed (Annual Report, BCG vaccine Laboratory, 1962-63 ; D'Arcy Hart, et al, 1964), is that conversion rates for the measurement of post-vaccination allergy cannot be relied upon. The number of reports of the adequancy of the Indian BCG vaccine in the early stages based upon "conversion rate" (Ranganathan, 1951) were misleading. Only measurement of increase in mean allergy after vaccination, by the WHO teams, later showed the vaccine to be a weak one and improvements leading to an equal allergenic potency with the Danish Vaccine became possible (Raj Narain et al, 1961).

(iii) In clinical practice, caution in interpreting a repeat tuberculin test is needed. For example, for diagnostic purposes a reaction of 6 to 8 mm to 1 TU may not usually be consi-

dered as definite evidence of infection with Myco. tuberculosis. In such cases, when, some time later, the test is repeated an enhanced reaction of 10 or 12 mm or even a larger reaction, may also not be taken as evidence of infection.

(iv) Studies on the influence of steroids, chemotherapy, and chemoprophylaxis on tuberculin allergy which require serial tuberculin testing of the same population could also be influenced by the enhancing effect.

Another rather interesting effect of 'boosting' may be recorded. It was reported earlier (Raj Narain et al, 1963) that the percentage of reactors at the 18 mm level in different age groups was more or less equal in the two sexes. Above this level the percentage of reactors was more in the female and below this level it was more in the male. For the first 50 villages of the Longitudinal Survey this phenomenon is illustrated in Figs. 6 and 7 for the two rounds respectively. In Fig. 6 the percentage reactors in the two sexes is equal at the 20 mm level. In Fig. 7 (as a result of 'boosting' !) this has risen to the 22 24 mm level. The significance of this observation is not known.

Summary

Enhancing of tuberculin allergy as a result of previous tests with I TU and with both 1 and 20 units has been studied by a random allocation of these tests and placebo to an untested and unvaccinated population of 8 villages. There was high prevalence of 'non-specific allergy' in the study area. It has been found that a tuberculin test does enhance the allergy elicited by a subsequent test. The enhancing effect is associated with initial allergy to tuberculin, especially that elicited by a 20 TU test, increase being almost confined to those with 10 mrn and larger reactions to 20 TU.

The addition of the 20 TU test further enhances the allergy but mostly among those with intitial reactions of 8-13 mm to the I TU. The enhancing effect increases with increase in age especially among those with 10 mm or bigger reactions to 20 TU. It is likely that the enhancing effect is more in communities with high prevalence of non-specific allergy. The above findings seem to be supported in the light of a much larger material.

Not all persons in a group show boosting. Even among those with 10 mm and larger reactions to 20 TU, only about 40% show an increase in allergy of 5 mm or more from their initial reactions, and are mainly responsible for the increase in mean allergy of the whole group at the second test.

Some important implications of the finding on the design of surveys and in the common applications of repeat tuberculin tests are pointed out.

ACKNOWLEDGEMENTS

The authors are grateful to the field team for their hard work: Mr. Krishnamurthy, Team Leader; Mr. Shashidhar, 1 TU Reader; Mr. Nagaraj, 20 TU Reader ; Mr. Parthasarthy, 1 TU Tester ; Mr. Shanmugam and Mr. Shrinivasamurthy, 20 TU Testers.

To Dr. J. Guld, WHO, Geneva for suggesting the study. To Mrs. Shirley H. Ferebee, Tuberculosis Program, United States Public Health Service and to members of the TCC, NTI, specially Dr. J.O.O'Rourke, Mr. I.B.R. Thorup, Dr. D. Savic and Dr. G.D. Gothi for their helpful suggestions and valuable criticism.

To the Statistical Section especialy Mr. Dwarkanath, Miss R. Rajalakshmi, Mr. C. Satyanarayana and Miss. N. Parimala for help in analysis.

To Mr. Rangaswamy for the figures.

To Miss K.R. Prameela for the secretarial help.

REFERENCES

Annual Report of the BCG Vaccine Laboratory, Madras (1962-63).

D'Arcy Hart, Mitchell, D. N., and Ian Sutherland, (1964) *Brit. Meet. J.,* \, 795-803.

Ferebee, Shirley H., and Mount, Frank W,, (1963) *Amer Rev Resp Dis.,* 1, 118.

Geigy, J. R., S.A., (1956) *Doccumenta Geigy Scientific tables,* Fifth Edition, Basle, 47.

Kul Bhushan, (1958-59) Indian Council of Medical Research, New Delhi. Annual Report of All India BCG Assessment.

Magnus, K., and Edwards. L. B. (1955) *Lancet, 2,* 643.

Magnus, Knut., (1957) *Bull. Wld Hlth Org.,* 17, 249-254.

Marc Daniels, Frank Ridehalgh, and Springett, V. H., (1948) *Report on the Prophit Tuberculosis Survey,* London, Lewis H.K. and Co., Ltd., 72-76.

Ranganathan, K.S., (1951) Proceedings of the VIII Tuberculosis Workers' Conference (Hyderabad! 54-60.

Raj Narain, Kul Bhushan, and Subratnanian, M. (1961) *Ind. Jour. Tuber., 9,* No. 1, 15-29.

Raj Narain, Geser, A., Jambunathan, M. V., and Subramanian, M., (1963) *Bull. Wld Hlth Org., V),* 641-644.

Raj Narain, Nair, S.S., Chandrasekhar, P., and Ramanatha Rao, G., (1965) *Ins. Jour. Tuber.,* Vol. XII No. 1,

Rich, Arnold, R., (1951) *The Pathogenesis of Tuberculosis.* Second Edition, U.S.A., Charles, C., Thomas, 405.

APPENDIX TABLE 1—A

Correlation of 1 TU reactions at 0 and at 2 months for those with reactious of (0-9) mm to 20 TU

Reactions to 1 TU at 2 months

Reaction to 1 TU at 0 month	0	1	2	3	4	5	6	7	8	9	10	11	12	13
0	27	1	6	13	4	8	1	3	—	—	—	—	—	—
1	—	—	1	1	1	1	—	—	—	—	—	—	—	—
2	14	—	6	9	6	3	5	2	—	—	—	—	—	—
3	9	—	2	5	3	5	1	3	1	—	—	—	1	—
4	6	—	3	4	1	3	2	—	—	—	—	—	—	—
5	9	—	1	2	—	1	1	4	1	—	—	—	—	—
6	—	—	—	1	—	—	—	1	—	1	—	—	—	—
7	1	—	—	—	—	2	2	1	1	—	—	—	—	—
8	2	—	—	—	—	—	—	—	1	—	—	—	—	—
9	—	—	—	—	—	1	—	—	—	—	—	—	—	—
10	1	—	—	—	—	—	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	69	1	19	35	15	24	12	14	4	1	—	—	1	—

Appendix B

APPENDIX TABLE 1—B

Correlation of 1 TV reactions at 0 and at 2 months for those with reactions of ≥ 10 mm. to 20 TU

Reaction to 1 TU at 2

Reaction to 1 TU at 0 month	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30+	Total
0	11	—	1	1	2	1	2	3	1	1	2	—	2	1	1	3	1	3	1	1	—	—	—	1	—	—	—	1	—	—	—	40
1	2	—	—	—	—	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4
2	8	—	2	3	1	5	3	2	4	2	2	1	—	—	—	2	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	37
3	5	—	—	3	—	—	3	4	5	2	2	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	1	27
4	10	—	—	2	1	1	—	4	5	—	4	—	—	3	1	—	2	—	1	1	1	1	—	—	—	—	—	—	—	—	—	37
5	3	—	2	1	1	3	2	4	2	—	2	1	1	—	2	—	—	1	2	1	—	—	—	—	—	2	1	—	1	—	1	33
6	2	—	—	1	2	4	1	5	2	1	—	1	—	—	—	2	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1	23
7	7	—	—	3	3	3	1	3	2	—	1	—	2	—	—	3	2	1	—	1	—	—	1	1	—	1	—	1	—	—	—	36
8	—	—	—	—	1	1	1	—	2	1	—	—	1	1	—	1	—	1	1	—	—	—	—	—	1	1	1	1	—	—	—	15
9	—	—	—	—	—	1	1	—	1	—	—	—	—	—	1	1	—	2	—	1	—	—	1	—	—	—	1	—	1	—	1	12
10	1	—	—	1	—	—	—	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	6
11	—	—	—	—	—	—	—	—	—	1	—	—	1	—	—	1	—	—	—	—	2	1	—	1	—	—	—	—	—	—	—	7
12	—	—	—	—	1	—	—	—	—	—	—	—	1	1	—	2	—	1	—	—	—	—	—	3	—	—	1	—	—	—	1	11
13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1	1	—	—	—	1	—	—	—	4
TOTAL	49	—	5	15	12	19	15	26	25	8	14	3	8	6	6	15	5	11	6	5	3	2	3	8	3	5	4	4	2	—	5	292

APPENDIX TABLE 2

CORRELATION OF I TU REACTIONS AT 0 AND AT 2 MONTHS

INDURATIONS TO I TU AT 2 MONTHS (MM.)

INDURATIONS TO I TU AT 0 MONTH (MM.)

	0	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	TOTAL
00	68	1	10	15	8	11	4	10	1	1	2	--	2	2	1	4	1	3	1	1	--	--	--	2	--	1	--	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	120
01	2	--	1	1	1	2	1	--	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	9
02	32	--	13	20	11	12	10	5	6	2	2	1	--	--	--	2	--	1	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	118
03	22	--	6	12	5	8	5	11	7	8	2	--	1	2	--	--	--	--	--	--	--	--	--	2	1	--	--	--	--	1	--	--	--	--	--	1	--	--	--	--	--	--	--	89
04	19	--	5	8	2	4	6	6	5	--	5	1	--	4	2	--	2	--	1	1	1	1	--	--	--	--	--	1	--	--	--	--	--	1	--	--	--	--	--	--	--	--	--	75
05	19	1	4	4	5	6	3	10	3	--	3	1	2	--	3	--	--	1	2	1	1	--	--	--	--	2	2	--	1	--	--	--	1	--	--	--	--	--	--	--	--	--	--	73
06	3	--	1	3	2	5	5	13	5	4	2	1	--	2	1	2	--	1	--	--	--	--	--	1	--	--	--	--	--	--	--	1	--	--	--	--	--	--	--	--	--	--	--	52
07	9	--	1	2	3	7	3	5	4	3	3	1	3	--	--	4	2	1	--	1	--	--	1	2	1	1	--	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	59
08	3	--	--	--	1	2	2	1	4	1	--	1	2	1	--	1	--	1	1	--	--	--	--	--	1	2	1	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	26
09	1	--	--	--	--	3	1	--	1	--	--	--	--	--	1	2	--	3	--	1	--	--	1	--	--	--	1	--	1	--	--	--	1	--	--	--	--	--	--	--	--	--	--	16
10	3	--	--	1	--	1	--	1	--	--	2	1	1	--	--	1	--	--	--	--	1	1	--	--	1	3	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	17
11	--	--	--	--	--	--	--	--	--	1	1	--	1	1	--	1	--	--	--	--	2	1	--	2	--	--	--	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	11
12	--	--	--	--	1	--	1	--	--	--	--	--	2	1	--	3	2	1	--	--	1	--	--	3	--	--	1	--	--	--	--	--	1	--	--	--	--	--	--	--	--	--	--	17
13	--	--	--	--	--	--	--	--	--	--	--	--	--	--	2	--	--	--	--	--	--	--	1	1	--	--	--	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	5
14	--	--	--	--	--	--	--	1	--	--	2	--	--	--	2	--	--	--	--	--	--	--	1	--	--	--	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	8
15	--	--	1	--	1	1	--	--	--	--	1	--	--	--	--	3	--	1	2	1	2	1	--	--	2	--	--	--	--	--	--	--	1	--	--	--	--	--	--	--	--	--	--	17
16	--	--	--	--	--	--	--	1	--	1	--	1	1	--	--	2	--	--	--	--	--	--	--	--	1	--	1	--	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	9
17	--	--	--	--	--	--	--	--	1	--	1	1	1	--	3	1	--	1	2	--	1	1	--	1	1	--	1	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	17
18	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	1	1	--	--	--	1	--	--	1	--	1	--	1	--	--	--	--	--	--	--	--	--	--	--	7
19	--	--	--	--	--	--	1	--	--	--	1	--	--	--	--	1	--	--	--	--	--	--	--	3	--	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	8
20	1	--	--	--	--	--	--	--	--	--	--	--	1	--	--	1	--	--	--	1	--	2	--	1	2	3	1	--	--	--	2	--	--	--	--	--	--	--	--	--	--	--	--	15
21	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	--	1	--	--	--	2	--	1	2	--	--	2	2	--	1	--	--	--	--	--	--	--	--	--	--	--	--	--	12
22	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	1	--	1	--	2	2	1	1	1	--	--	--	--	--	--	--	--	--	--	--	--	--	10
23	--	--	--	--	--	--	--	1	--	--	--	--	--	--	--	--	--	--	--	1	1	--	--	--	--	--	1	3	--	1	1	--	--	--	--	--	--	--	--	--	--	--	--	9
24	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	--	--	--	--	1	--	4	--	2	2	--	1	--	--	2	--	--	--	--	--	--	--	--	--	--	13
25	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	2	--	--	--	--	--	1	--	1	--	--	--	--	--	1	--	--	--	--	--	--	--	--	--	--	7
26	--	--	--	--	--	--	--	--	--	--	--	1	--	--	--	--	--	--	--	--	--	--	--	3	--	1	3	3	1	--	--	--	--	1	--	--	--	--	--	--	--	--	--	13
27	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	2	--	--	1	2	2	2	2	4	--	1	--	--	--	--	--	--	--	--	--	--	--	17
28	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	--	--	1	--	--	1	--	1	3	1	--	--	--	--	--	--	--	--	--	--	--	1	--	--	--	11
29	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	1	--	1	--	1	1	--	--	--	1	--	--	--	1	2	--	--	--	--	--	--	--	11
30	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	--	--	--	--	--	--	1	1	3	--	1	--	--	--	1	--	1	--	--	--	--	--	--	--	--	--	10
31	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	--	--	--	--	--	--	--	--	2	--	2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	5
32	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	--	--	--	1	--	1	--	1	2	--	--	--	--	1	--	--	--	--	--	--	--	7
33	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	-1	--	2	--	--	--	--	--	--	--	--	--	--	--	--	3
34	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	--	--	--	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	--	--	3
35	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
36	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	--	--	--	--	--	1	--	--	--	--	--	--	--	--	--	--	--	--	2
37	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	--	--	--	1	--	--	--	--	--	--	--	2
38	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	--	--	--	--	--	1	--	--	--	--	--	--	--	--	--	--	--	--	2
39	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	--	--	--	--	--	--	--	--	--	--	--	--	--	1
40	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
41	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
42	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	--	--	--	--	--	--	--	--	--	--	--	1
TOTAL	163	2	42	€7	40	61	43	64	38	16	27	9	17	13	I6	15	12	12	13	I3	14	11	10	25	19	23	29	26	12	9	I 3	4	8	6	13	4	8	6	1	3	2	2	--	--	920

"TREATMENT OF PREVIOUSLY UNTREATED PULMONARY TUBERCULOSIS WITH ETHIONAMIDE AND ISONIAZID"

J.L. BHATIA and HARBANS LAL
(Medical College, Amritsar)

Ethionamide is a relatively new drug which has recently been introduced in the treatment of pulmonary tuberculosis. Pharmacologically it is 2-Ethyl Isothionicotinamide. Though its chemical structure shows great resemblence to isoniazid it has no cross-resistance with it. Therefore, Ethionamide has mainly been used as a second line drug in the treatment of drug resistant cases. (Brouet 1958, British Tuberculosis Association 1961, 1963 Somner and Brace 1962, Aquinas 1963),

In vitro experiments Ethionamide has been found to be twice as active as streptomycin (Rist et al 1958). It has the additional advantage that it is administered orally. Thus, theoretically, replacement of streptomycin by Ethionamide would appear to have definite advantages, even in previously untreated drug sensitive cases. Chicov and others (1961), Rist (1962), and British Tuberculosis Association (1964) have tried Ethionamide with Isoniazid in previously untreated drug sensitive cases and compared the results with PAS and Isoniazid in controlled trials. Encouraged by their results we decided to try this combination on about 25 previously untreated patients for a period of one year when drug supplies were promised through the courtesy of Messers Themis Pharmaceuticals. Unfortunately, after the trial had begun, the supplies became irregular and scanty and only 13 patients could complete the drug regimen for a period of 6 months. After this period 12 patients were switched on to PAS and isoniazid and only one patient (13th) is continuing Ethionamide and Isoniazid upto now (9 months period). This paper deals with the results of treatment with Ethionamide and Isoniazid for a period of 6 months on 13 patients.

Material and Methods

A total of 15 patients were admitted initially into the study. They were selected from the outdoor patients presenting at our T.B. centre and formed part of a larger study under the I.C.M.R. to study the prevalence of primary drug resistance. All the patients confirmed to the following criteria ;—

(i) were above 12 years of age;
(ii) were suffering from pulmonary tuberculosis of more than minimal extent;
(iii) had either not taken any previous chemotherapy or at the most for not more than 10 days.

All the 15 patients were admitted indoors into the R.B. Sir Gujjarmal Kesra Devi T.B. Sanatorium, Amritsar and the following investigations were undertaken;—

A careful history was taken and a thorough clinical examination was made. A special effort was made to elicit the history of any previous anti-T.B. chemotherapy. This questioning was repeated again after a period of about 6 weeks when it was thought that the patient might have developed confidence and would therefore be more co-operative in telling the truth.

A full plate X-ray of chest was taken at the time of admission and repeated once a month. Sputum examination for A.F.B. was done by smear and culture method. Drug sensitivity studies could not be undertaken. A record of weight was maintained. Blood examinations for total and differential leucocyte counts, haemoglobin and sedimentation rate were done initially and every month. Routine liver function tests were done initially and every fortnight (Serum bilirubin, thymol turbidity and flocculation and urine urobilinogen). Transminase estimations could not be done. General progress of the patient was recorded and any untoward symptoms were carefully enquired into.

Treatment and Dosage :—One Ethionamide 250 mgm sugar coated tablet (Rigenicid-Themis) and one Isoniazid 150 mgm (Themina-Themis) were administered twice daily immediately after breakfast and after dinner. Patients were also given multivitamins, B Complex and iron tablets by mouth.

Out of a total of 15 patients, two had to be excluded from the study. One of these was found to have had previous treatment with anti-T.B. drugs at the time of second interogation. The other became home sick and left the sanatorium against medical advice. The results of the remaining 13 patients who completed 6 months of treatment in the sanatorium with Ethionamide and Isoniazid are presented below :—

Sex and Age:—8 out of 13 patients were male and 5 female patients. Average age for the male patients was 30-33 years (range 16-36 years) and for female patients 30 years (range 20-55 years).

General Condition:—All patients were moderately ill excepting two patients (one male

and one female) who were very ill and emaciated.

X-Ray and Sputum;—Results are given in Table-1. 5 patients had involvement of 3 lung zones and 6 patients had multiple cavities.

All patients showed improvement on radiological examination. None showed any deterioration on treatment. The final assessment was made on completion of 6 months treatment. 5 patients showed considerable and

TABLE I

Assessment		Male	Female	Total
Number of zones involved (x-ray)	1	1	—	1
	2	3	—	3
	3	3	2	5
	4	—	—	—
	5	2	2	4
	6	—	—	—
Extent of cavi-*tation	No. cavity	1	2	3
	Single cavity	3	1	4
	Multi-ple cavity	4	2	6
Sputum for AFB by smear & concentra-tion	Positive	7	5	12
	Negative	1	—	1

*Maximum size of cavity was 2" in diameter & minimum ¼" in

Sputum was positive on smear in 12 cases. Thus all patients had moderate to far advanced disease.

Results of Treatment

All patients experienced a feeling of well being within one month of treatment. Majority of them became afebrile during the second month. Only two patients who were admitted in severely ill condition remained febrile till the end of 3rd month.

Changes in radiographic appearances towards improvement are given in Table-If.

TABLE II

Assessment	Male	Female	Total
Considerable	3	2	5
Moderate	4	2	6
Slight	1	1	2
Total	8	5	13

6 moderate improvement. Changes in cavitation are shown in Table III. 5 patients had complete disappearance of cavities and 5 others showed reduction in their size and number.

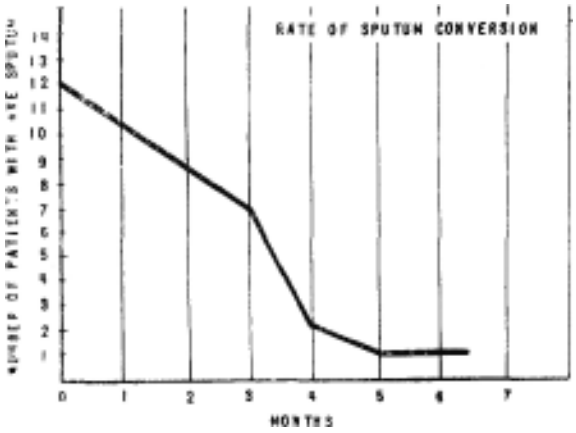
TABLE III

Assessment	No. of patients	Disappearance of cavity	Reduction in size and number
Single cavity.	4	3	1
Multiple cavity.	6	2	4
Total	10	5	5

Sputum conversion:—Sputum got converted in the majority of patients by the end of 4 months. At the end of 6 months it was positive only in 1 case by smear and concentration though by culture it was still positive in 3. The rate of sputum conversion by direct

smear and concentration is shown in the following graph.

Rate of sputum conversion by direct smear and concentration.



Changes in weight and B.S.R. All but one patient showed gain in weight. Average gain in weight was 17.5 Ibs (range 13-23 Ibs). In the one patient who did not gain weight sputum also remained positive on smear.

B.S.R. fell in all patients except one whose sputum remained positive. His B.S.R. rose by 19 m.m. average fall in B.S.R. was 55.6 mm westergren. (Range 3-114 mm). A marked fall in B.S.R. was noticed in those whose initial B.S.R. was very high.

Toxic effects:—No toxic effect was noticed clinically in any of these 13 patients. They seemed to tolerate Ethionamide in the dosage given quite well. None complained of any garlic smell or bad taste inspite of repeated questioning. In 8 patients results of cephalin cholesterol floeculation were + or + + at the end of 24 hours but these figures became normal after some time. Clinical jaundice was not seen in any patient. In one woman patient aged 60 years whose urine did not show any sugar at the time of admission to the study started having glycosuria after 6 months of treatment with Ethionamide and Isoniazid. Her fasting blood sugar at this time was found to be 266 mgm%. Unfortunately blood sugar estimation was not done initially before the start of drug regimen. Her diabetes was stabilized with insulin. Her glycosuria most probably started under the drug treatment as the urine was normal initially.

Discussion.

The results of this very small study have shown that 500 mgm of Ethionamide and 300 mgm of Isoniazid is quite effective in the treatment of newly diagnosed cases. At the end of 6 months treatment negative cultures were obtained in 77% of patients while sputum conversion by concentration was upto 92%. These results are still more impressive in view of the advanced nature of the disease treated and marked improvement noticed on radiological examination *vide* Tables-II and III. These results are comparable to those obtained at Hongkong where 72% of total patients initially accepted became culture negative at 24 weeks.

The acceptability of Ethionamide in this smal) series was quite good and there were no complaints of gastrointestinal disturbances or bad taste or odour as reported from the western countries. This may be due to racial differences as on the whole Indian patients seemed to tolerate Ethionamide quite well. The dose of 500 mgm was small as compared to these western reports where I g or 750 mgm had been given but in view of the smaller weight of our patients the dosage worked out to 11 mgm per K G. of body weight. With the same dosage (11 mgm 1 K.G.) Hong Kong patients also tolerated the drug quite well though they received medicament in a single dose in the evening while our patients received the drug twice daily. The tablets were sugar coated and this may also have had some effect.

No toxic effects except the appearance of diabetes in one patient were seen in this small series. The cost of 500 mgm Ethionamide (Rigenicid) for the patient in the Indian Market at present is about 88 paise while that of Streptomycin is 69 paise. Most patients have to get injections of Streptomycin privately for which the patient incurs an additional expenditure of at least 25 paise. Thus the actual cost of medicament in the case of Ethionamide and Streptomycin is about the same. In addition there is an obvious advantage for oral medication in rural areas where daily injections may not be easy.

But the effectiveness of Ethionamide and Isoniazid as reported from the controlled trials is equivient to PAS and Isoniazid which is known to be less effective than daily Streptomycin and Isoniazid. It was our impression also that the effectiveness of Ethionamide and Isoniazid combination was less than that of Streptomycin and Isoniazid though no controlled trial was done. Thus Ethionamide is not likely to be used routinely in place of Streptomycin. But in special circumstances where PAS is contraindicated because of intoleration or drug allergey and Streptomycin injections are not possible, Ethionamide is likely to be welcomed as a useful addition in the arma-

mentarium against tuerculosis without greatly increasing the cost of treatment.

SUMMARY

13 previously untreated patients of pulmonary tuberculosis have been treated with Ethionamide and Isoniazid for a period of 6 months. 77% conversion of sputum on culture was obtained.

ACKNOWLEDGEMENT

Our thanks are due to M/S Themis Pharmaceuticals who very kindly supplied Rigenicid and Themina samples free of cost for the above trial.

REFERENCES

1. Aquinas, Sister M; (1963) Tubercle (Lond) 44,76.
2. British Tuberculosis Association (1961) Tubercle (Lond) 42,2 69.
3. British Tuberculosis Association (1963) Tubercle (Lond) 44, 195.
4. British Tuberculosis Association (Hong Kong Tuberculosis Treatment Service (1964) Tubercle, (Lond) 45, 299.
5. Brouet, G. (1958) Bull. Uni. Int;Tuber; 28, 216.
6. Chicov, J; Hetrick, G; Lablou, M; Le Hir, M; Mercier, A; Neel, R, Risk, N; and Vicet. J. (1961) Rev. Tuberc (Paris) 25,1031.
7. Rist, N. (1962) Rev. Tuberc (Paris) 26,740.
8 Somner, A.R., and Brace, A.A.. Tubercle. (Lond,) (1962), 43, 345.

SOME OPERATIONAL FACTORS INFLUENCING THE RELATIVE UTILITY OF CULTURE METHOD OF DIAGNOSIS OF PULMONARY TUBERCULOSIS

K. P. RAO, S. S. NAIR, N. COBBOLD AND N. NAGANATHAN
(National Tuberculosis Institute, Bangalore)

Laboratory diagnosis of pulmonary tuberculosis is based fundamentally on revealing the presence of tubercle bacilli in sputum by direct microscopy, culture and/or animal inoculation. Direct microscopy is simpler, expeditious and lends itself to wider application and in many developing countries is still the limit in detecting tubercle bacilli. Culture method followed by tests for identifying the bacilli is recognised as the most accurate, reliable and technically superior method (Cancela Freijo, Holm, Levin Brandon and Me Millan, Mayer, Galland and Kobrinsky, Gifford, Monkinley and Hunter, as quoted by Darzins 1958), but being very expensive it remains the exclusive privilege of few laboratories in larger cities or those devoted to tuberculosis research in these countries. Though technically superior to direct microscopy, the efficacy of culture method depends on the laboratory methods employed and operational factors. The influence of the former has been recognised already and the methods standardised. But the role of operational factors on the relative utility of culture method over direct microscopy in different practical situations such as epidemiological survey, active community case finding, organisation of diagnostic services and evaluation of diagnosis and treatment in Tuberculosis Control Programmes, has not received the attention it deserves. The importance of culture has to be investigated for these three situations as they form sequential methods in planning, execution and evaluation of National Tuberculosis Control Programme. The present paper deals with a systematic study of data from four investigations to understand the influence of some of the operational factors on culture.

2.0 Materials and methods

2.1 Study 1

A longitudinal survey is being carried out in a randomly selected rural population in 3 taluks of Bangalore District. The nearest taluk headquarters is at a distance of 17 miles and the farthest 45 miles from Bangalore. The first round of the longitudinal survey was started in May 1961 and completed by September 1963. The material from this round is analysed in the present paper. Two sputum specimens, spot and overnight, were collected at intervals of 24 to 48 hours from persons having abnormal X-ray shadows among persons aged 5 years or more. The specimens were collected by house-to-house visit, stored in an insulated box containing an ice-tin immediately after collection and transported to NTI laboratory for investigations. The specimens on arrival at the laboratory were refrigerated, if they were not immediately processed. The interval between collection of specimens in the field and culture in the laboratory was 1 to 7 days. The two specimens from each individual were treated independently and examined by the following techniques for demonstrating tubercle bacilli. A smear was stained and examined first by Fluorescence microscopy and later on the same smear by ZN method. Each specimen was cultured on two slopes of Lowenstein-Jensen medium without potato starch (Jensen, 1955) after homogenising with 4% sodium hydroxide, diluting the homogenate with sterile distilled water and centrifuging at 4000 r.p.m. for 20 minutes. All positive cultures were identified by sub-culturing and observing growth at room temperature, rate of growth at 37°C, pigment production in dark and after exposure to light, catalase and peroxydase reactions, niacin production and sensitivity to isoniazid, streptomycin and P-amino salicylic acid (PAS).

2.2 Study 2

In a mass case-finding programme in Tumkur District, (the district headquarter town situated 45 miles from Bangalore) two specimens, a spot and an overnight, were collected from individuals of age 20 years or more with symptoms suggestive of pulmonary tuberculosis and positive tuberculin reactors below the age of 20 years, voluntarily reporting with symptoms. The specimens were examined by ZN microscopy and cultured by simple swab method (Nassau 1954, O'Hea, 1957) on the same day of collection, at Tumkur District Laboratory. These specimens were also transported to the NTI Laboratory in an insulated box containing ice-tin. The specimens were then stored in the refrigerator at NTI, if they were not processed immediately on arrival. The two specimens were treated independently and examined by Fluorescence microscopy and cultured by swab method. The results of these examinotions at NTI only are considered in this paper. Interval between collection of specimens in the field and culture in the NTI Laboratory was 1 to 7 days. All positive cul-

tures were identified as in study 1. The first specimen was collected on 12-3-1962 and the last on 6-3-1963.

2.3 Study 3

A technical assessment of sputum examination by ZN microscopy performed by non-specialized health staff of peripheral health facilities in Bangalore District (the study is under progress) is being carried out since October 1963. The nearest centre is 4 miles and the farthest is 30 miles from the NTI Laboratory. A spot specimen of sputum is collected by auxiliary health staff at each health facility daily from patients who have symptoms suggestive of pulmonary tuberculosis. Waxed paper cups with loose lids are being used for specimen collection. All smears examined by ZN method at each centre and the corresponding specimens are collected twice a week and transported to NTI laboratory for re-examination of smears, examination of a fresh duplicate smear and culture by swab method. All positive cultures are identified as in study 1. No refrigeration facilities are available in any of these centres and the specimens are not transported in an insulated box. However, specimens on receipt at the NTI laboratory are refrigerated if there is delay in culturing of specimens. The interval between collection from patients and culture ranges from 1 to 7 days. The material collected upto 9th September 1964 is included in this report.

2.4 Study 4

An operational and technical assessment of District Tuberculosis Programme at the end of one year of its launching was carried out in Anantapur District, Andhra Pradesh (the district headquarter is 130 miles from Bangalore). A sample was taken from all patients put on treatment between 1-1-1962 and 31-1-1962 and those diagnosed in that month, but had not collected drugs upto 15-8-1962. Spot specimens collected in the field were stored without any refrigeration whatsoever until transported to the NTI Laboratory, where specimens were refrigerated, if not immediately, examined on arrival. The specimens were then examined by both Fluorescence and ZN microscopy and cultured by swab method. All culture positives were further identified as in study 1. The interval between collection and culture was 1 to 7 days. The field collections were made from 20-8-1962 to 11-1-1963.

2.5 Materials included id studies 1 to 3 were collected during all seasons, but from areas having equitable and cool climate throughout the year. Study 4 was conducted in a hotter area. Part of the material was collected during the fairly hot autumn and remaining in mild winter.

2.6. The positive smears were graded as follows. except in study 3:

(+) = 1-3 bacilli in whole smear after prolonged search for atleast 10 minutes.

\+ = 1-5 bacilli in most fields.

++ = More than 5 bacilli in most fields.

+++ = Masses of bacilli in most fields.

The same nomenclature was used for both Fluorescence and ZN microscopy.

In study 3 the grading was—

<50 = Actual number.

\+ = ≥50 bacilli.

++ = Masses of bacilli.

Culture positives were classified as follows:

<20 = Actual number of colonies.

\+ = 20-100 colonies.

++ = More than 100 discrete colonies.

+++= Confluent growth.

3.0. Findings.

3.1. Groups of sputa examined after different intervals:

The main purpose of analysing the data according to interval between collection of sputum and laboratory examinations is to study how far the interval can influence culture results. If by chance the specimens cultured after longer intervals included more positives, any deterioration due to time lag will not be brought out by the analysis. If, on the other hand, these included less positives it may be wrongly concluded that time lag has adverse effect on the specimens. In order to study this "sampling effect", the percentage positive on microscopy after different intervals between collection and examination (Table 1) could be used as this interval has no effect on direct microscopy in which both dead and living acid fast bacilli are taken into account in classifying positive smears.

The percentage positive on direct smear did not differ much in the first two studies indicating that the groups of smears examined after different intervals did not differ much with respect to the proportion of positive specimens. In study 3, the specimens examined after 1-2 days had smaller proportion of positives compared to those examined after 3-4 days, but the difference just fell short of statistical significance. In

TABLE 1

Direct smears examined and percentage positive according to interval between collection and examination.

Study No.	Type of collection.	Direct smear technique.	Interval between collection and examination†. (1-2) days		(3-4) days		(5+) days		All days	
			No. read	Percentage positive.	No. read	Percentage positive.	No. read	Percentage Positive	No read	Percentage Positive
1	2	3	4	5				9	10	11
1.	Spot	ZN	3553	1.2	2814	1.2	550	1.6	6917	1.2
		Fl	3553	1.4	2814	1.2	550	1.6	6917	1.3
	Overnight	ZN	4209	1.1	1793	2.2	210	1.4	6212	1.4
		Fl	4209	1.2	1793	1.9	210	1.9	6212	1.4
2.	Spot	Fl	1475	2.5	1014	2.1	233	1.7	2723	2.3
	Overnight	Fl	945	2.8	1032	3.5	183	2.7	2160	3.1
3.	Spot	ZN	386	9.6	382	13.4	381	10.2	1149	11. 1
4.	Treated	ZN	24	12.5	103	23.3	47	36.2	174	25.3
		Fl	24	16.7	103	30.1	47	38.3	174	30.5
	Not treated	ZN	22	72.7	62	50.0	87	506	171	53.2
		Fl	22	77.3	62	61.3	87	51.7	171	58.5

† Direct smear examination and inoculation of culture slopes were done on the same day

study 4, there was a trend of increase with intervals for treated cases and one of decrease for not treated cases. This sampling effect has to be kept in mind while interpreting the culture results for study 4.

3.2. Effect of interval on culture results :

Table 2 gives the percentage positive on culture after different intervals between collection and inoculation of culture, for each of the 4 studies. In studies 1 and 2, where the specimens were transported in an insulated box containing icetin, the interval between collection and culture did not make any difference. In study 3, the percentage culture positive was significantly lower for the interval of J-2 as compared to 3-4 days. In study 4, there was not much difference for the treated eases, but the untreated cases showed smaller percentage positive with increase in interval. However, this fall in percentage from one interval to the next was not statistically significant. Further, it is interesting to note that in both these instances where larger differences were observed, the percentage culture positive in the 3 intervals showed the same type of variation as percentage direct smear positive in Table 1. This indicates that the differences observed were due to the sampling effect explained earlier. Thus there is no evidence to show that interval between collection and culture (from 1 to 7 days) has any effect on the detection of sputum positive cases by culture, even in the latter two studies in which no insulated box was used to transport the specimens and questions the need for such refrigeration.

3.3. Influence of preliminary screening on case yield:

Percentage of direct smear positives in Table 1 and percent- ge positive on culture in Table 2 showed wide variations between the studies. These percentages were dependent on the method of selection of persons for collection of sputum. Among "Case-finding" examinations (studies 1 to 3) the lowest yield per specimen examined was from survey material and highest

TABLE 2

Cultures read and percentage positive according to interval between collection and inoculation of culture slopes.

No.	collection	Interval between collection and inoculation of culture. (1-2) days		(3-4) days		(5 +) days		All days	
		No read	Percentage positive.	No. read	Percentage positive.	No. read	Percentage positive.	No read	Percentage positive.
1	2	3	4	5	6	7	8	9	10
1.	Spot	3548	2.1	2814	2.3	550	2.9	6912	2.3
	Overnight	4193	2.7	1786	2.9	210	2.4	6189	2.7
2	Spot	1475	3.8	1014	3.2	233	3.0	2722	3 5
	Overnight	945	3.9	1032	4.1	183	4.3	2160	4.0
3.	Spot	383	9.4	382	14.1	380	10.3	1145	11.3
	Treated	24	33.3	103	32.0	47	38.3	174	33.9
4.	Not treated	22	81.8	62	61.3	87	52.9	171	59.6

from the Bangalore District Programme where only those persons who came to the dispensary with symptoms like prolonged cough were examined. Among already diagnosed cases in Anantapur (study 4) the percentages positive on smear and culture were much higher, particularly among untreated cases.

Studies 1 and 2 aimed at the detection of largest possible number of sputum positive cases in the community. In study 1, all those aged 5 years or more who showed any type of abnormality in the X-ray were eligible for sputum collection. In study 2, sputum was collected from symptomatics among age 20 years or more and tuberculin reactors below 20 years. A comparison of the percentage of positive smears in these two studies in Table 1 shows that higher yield per smear examined may be available when persons are screened by symptoms. In Table 2 also, the percentage of culture positives is higher in Study 2 compared to Study 1. However this does not take into account the variations in prevalence of disease in the two areas, the proportion of sputum positive cases detected in the community and more sensitive method of culture used in study 1. Nevertheless it is suggestive that sputum examination of symptomatics among X-ray normals may be advantageous in tuberculosis surveys. A clear answer to this question can only be obtained from a study in which both these methods are used in the same area.

3.4 Influence of type of sputum collection :

The type of collection may influence the yield of sputum positive cases in mass case-finding. In studies 1 and 2 both spot and overnight collections of sputa have been made. Last columns of Tables 1 and 2 show that the yields from microscopy and culture were consistently more for the overnight collection; but the differences were not statistically significant.

3.5 Contamination of culture at different intervals :

Table 3 shows the number and percentage of cultures contaminated according to the interval between collection and inoculation of culture, in the four studies. The percentage contaminated did not increase with longer intervals in the first 3 studies. In study 4, some deterioration was observed particularly among the treated cases, but the numbers were too small to provide reliable conclusions. Thus an interval of upto 7 days may not adversely influence the contamination rate even when boxes without ice-tin are used to transport the specimens. Rajnarain et al (1963) reported that among 931 contaminated cultures included in their study, neither the interval of 1 to 13 days between collection and inoculation of cultures nor seasonal variations affected the contamination rate.

The contamination rate was consistently higher for overnight specimens in both studies I

TABLE 3

Percentage of contaminated cultures according to interval between collection and inoculation culture.

Study No.	Type of collection.	Interval between collection and inoculation of culture: (1-2) days Total cultured	(1-2) days Percentage contaminated by culture.	(3-4) days Total cultured	(3-4) days Percentage contaminated by culture.	(5+) days Total cultured	(5+) days Percentage contaminated by culture.	All days Total cultured	All days Percentage contaminated by culture.
1	2	3	4	5	6	7	8	9	10
1.	Spot	3548	4.1	2814	4.1	550	2.4	6912	4.0
	Overnight	4193	5.9	1786	4.3	210	3.3	6189	5.3
2	Spot	1475	2.1	1014	2.6	233	3.4	2722	2.4
	Overnight	945	5.1	1032	2.0	183	3.8	2160	3.5
3.	Spot	383	6.3	382	5.5	380	7.4	1145	6.4
4	Treated	24	—	103	—	47	12.8	174	3.5
	Not treated	22	—	62	1.6	87	5.7	171	3.5

and 2, the only exception being those put on culture after 3-4 days in study 2. These differences were also statistically significant for the total material from studies 1 and 2 and for those examined after 1-2 days in study 2. Thus the type of collection is an operational factor influencing the contamination rate. Probably with more emphasis on proper instructions on how to collect and preserve overnight sputum the contamination rate could be reduced.

The last column of Table 3 shows that the percentage contaminated was significantly more in study 1 as compared to study 2, for both types of collections. The same trend was shown during the two shorter intervals, the difference being statistically significant except for overnight specimens examined after 1-2 days. For the longest interval of 5 days or more study 2 shows higher contamination rate but the differences were not statistically significant. The higher contamination rate observed in study 1 is possibly the effect of centrifugation of those contaminants surviving the process of decontamination with 4% sodium hydroxide. In studies 2, 3 and 4 there is no such effect as sputum swab culture technique was employed. Comparatively higher rate of contamination observed in study 3 was perhaps, due to occurrence of extraneous contaminants in specimens collected in waxed cartons without proper screw-lids and kept exposed for long periods in the peripheral health facilities before examination. Another factor could be that in study 3 spot specimens were collected by semi-skilled staff with a short period of training whereas in all other studies, collection was done by NTI trained staff. Thus from the point of view of prevention of extraneous contaminants, the spot specimens in study 3 may not qualitatively differ from overnight collections. This again emphasizes the importance of strict adherence to proper techniques of specimen collections.

3,6. Influence of interval on cases detected by culture only:

One of the important contributions of culture examination is that of detecting some sputum positive cases not demonstrable by microscopy. To study this, the number of cultures done and the percentage positive among them which were direct smear negative, for different intervals, are shown in Table 4.

In the first two studies in which insulated box containing ice-tin has been used, the additional contribution made by culture did not differ significantly except for overnight specimens of study 1, examined after 5 or more days as compared to those examined after (1-2) days. In studies 3 and 4 larger differences were observed between the intervals but these were not statis-

TABLE 4

Percentage positive on culture only according 10 interval between collection and inoculation of culture

Study No.	Type of collection	Direct smear technique.	Interval between collection and inoculation of culture (1-2) days: Total cultured	(1-2) days: Percentage position, live by culture only	(3-4) days: Total cultured	(3-4) days: Percentage positive by culture only	(5+) days: Total cultured by culture only	(5+) days: Percentage positive	Alldays: Total cultured	Alldays: Percentage positive by culture only
1	2	3	4	5	6	7	8	9	10	11
	Spot	ZN	3548	1.3	2814	1.4	550	1.6	6912	1.3
		Fl	3548	1.2	2814	1.2	550	1.3	6912	1.2
	Overnight.	ZN	4193	1.8	1786	1.3	210	0.9	6189	1.7
		Fl	4193	1.7	1786	1.2	210	0.5	6189	1.5
2.	Spot Overnight	Fl	1475	1.4	1014	1.3	233	1.3	2722’	1,4
		Fl	945	1.4	1032	0.8	183	1.6	2160	1.1
3.	Spot	ZN	383	1.6	382	2.9	380	1.6	1145	2.0
4.	Treated	ZN	24	20.8	103	8.7	47	4.3	174	9.2
	Not treated	Fl	14	16.7	103	2.9	47	2.1	174	4.6
		ZN	22	9.1	62	12.9	87	3.4	171	7.6
		Fl	22	4.5	62	3.2	87	2.3	171	2.9

tically significant. Positives on culture only, expressed as a percentage of those positive on direct smear, showed similar results; the differences being significant only for overnight specimens of study 1. The reason for this stray significant result is not clear. Though the differences between the percentages for treated cases in study 4 were large, these were not statistically significant probably because of the smaller numbers involved. Even if the differences could be real, it has to be remembered that among these cases the percentage positive on direct smear was higher among specimens examined after longer intervals (see Table-1). Consequently these can be assumed to include more advanced cases among whom positives by culture only will be less as pointed out under discussion of Table-9 and this could most probably account for the differences observed. Thus the contribution to sputum positives by culture examination was not generally influenced by the interval between collection and inoculation of culture. This is in conformity with the finding from Table 2 that the total yield from culture was not influenced by this interval.

3.7. Influence of interval on viability:

In the absence of any knowledge about the population of bacilli at the beginning and at the time of examination no direct study on the influence of interval on viability could be made. However, some indirect evidence can be had by comparing the degree of positivity of cultures carried out at different intervals after collection (Appendix Table).

The proportion with lower degrees of positivity did not increase with longer intervals, as one would expect with loss in viability. Column 10 shows that among those positive on culture

only a large proportion (about 50%) had smaller number of bacilli. Even among this group the proportion with lower degree of positivity did not increase (Cols 6-8) with interval, indicating that interval did not appreciably influence the viability of the organisms. Similar results were observed even in studies 3 and 4, in which specimens were transported without insulated boxes.

Another indirect evidence could be obtained by considering the confirmation by culture of direct smears with 1-3 bacilli (scanty positives). With loss of viability and with increase in interval smaller percentages of these only may become positive on culture. The relevant data are given in Table 5.

The percentage confirmed by culture does not decrease with increase in interval. These results confirm the findings from Appendix Table that there is no evidence to indicate any serious loss of viability with interval upto 7 days.

TABLE 5

Percentage confirmed by culture out of scanty positives on direct smear according to interval between collection and inoculation of culture*

Study No.	Type of collec-	D.S tech- nique	(1—2) days D.S. +ve tion	Percent- age confirm- ed by culture	(3 — 4) days D.S. +ve	Percent- age confirm- ed by culture	(5+) days D.S. +ve	Percent- age confirm- ed by culture	All days D.S. +ve	Percent- age comfirm- ed by culture
1	2	34		5	6	7	8	9	10	11
1.	Spot	ZN	15	46.7	9	44.4	5	60.0	29	48.3
	Over- night	ZN	19	47.4	13	23.1	—	—	32	37.5
1.	Spot	Fl	20	30.0	4	50.0	1	100.0	25	36.0
	Over- night	Fl	16	56.3	5	20.0	1	100.0	22	50.0
2.	Spot	Fl	3	100.0	2	50.0	—	—	5	80.0
	Over- night	Fl	1	—	3	66.7	—	—	4	50.0
3.	Spot	ZN	2	50.0	2	50.0	1	—	5	40.0
4.	Treated	ZN	1	100.0	3	100.0	2	100.0	6	100.0
	Not treated	ZN	2	100.0	5	100.0	8	87.5	15	93.3
All studi- es	Spot		43	46.5	25	64.0	17	76.5	85	57.6
	Over- night		36	50.0	21	28.6	1	100.0	58	43.1
	Both		79	48.1	46	47.8	18	77.8	143	51.7

* 1—3 bacilli in whole smear after prolonged search for at least 10 minutes.

3.8. Influence of interval on culture confirmation of direct smear positives:

Another important contribution of culture examination is the confirmation of direct smear positives. This aspect is of considerable importance to eliminate false positives. Whether this function of culture examination will be influenced by the interval between collection and culture has been studied in Table 6.

There was no adverse effect for the longer intervals in any of these studies. The numbers available were rather small, especially for the interval of 5 days or more. This finding also is in conformity with the results from Table 2.

It will be observed from Table 6 (Column 11) that confirmation of direct smear positives is less in studies 1 and 3. To study this further, the numbers confirmed and not confirmed by culture among scanty positives and others, are shown in Table 7. It will be observed that the percentage confirmed by culture was generally smaller for scanty positives. Thus, at least in study 1, the scanty positives may account for the lower confirmation of direct smear positives. This may be due to loss of viability among scanty positives to an extent not demonstrable by culture. But Table 5 and Appendix Table show that there is no appreciable loss of viability with increase in interval for specimens with fewer bacilli (see 3.7). The real reason may be that a large proportion of the scanty positives may be false positives. This aspect is being studied further and will be reported later on.

Table 7 also shows that some smears with higher degrees of positivity ("others") were not confirmed by culture. This was particularly high in" study 3, where 9 out of 19 such specimens gave contaminated culture. Even after excluding contaminated cultures there were still an appreciable number of non-confirmed smears. The interval between collection and

TABLE 6

Number of direct smear positives and percentage confirmed by culture according to interval between collection and inoculation of culture

Study No.	Type of collection	D.S. Technique	Interval between collection and inoculation of culture (1-2) days		(3—4) days		(5+) days		All days	
			Total DS positive	Percentage confirmed by culture	Total DS positive	Percentage confirmed by culture	Total DS positive	Percentage confirmed by culture	Total DS positive	Percentage confirmed by culture
1	2	3	4	5	6	7	8	9	10	11
1.	Spot	ZN	42	71.4	33	81.8	9	77.8	84	76.1
		Fl	49	69.4	34	94.1	9	100.0	92	81.5
	Overnight	ZN	48	75.0	39	69.2	3	100.0	90	73.3
		Fl	51	80.4	34	85.3	4	100.0	89	83.1
2.	Spot	Fl	37	94.6	21	90.5	4	100.0	62	93.5
	Overnight	Fl	26	92.3	36	94.4	5	100.0	67	94.0
3.	Spot	ZN	37	81.1	51	84.3	39	84.6	127	83.5
4.	Treated	ZN	3	100.0	24	100.0	17	94.1	44	97.7
		Fl	4	100,0	31	96.8	IK	94.4	53	96.2
	Not treated	ZN	16	100.0	31	96.8	44	97.7	91	97.8
		Fl	17	100.0	38	94.7	45	97.8	100	97.0

inoculation of culture for these cases are given in Table 8.

It will be observed that the loss of viability resulting from longer intervals is not responsible for this phenomenon. Another possibility may be loss of viability due to presence of chemotherapeutic drugs in the specimens collected from those undergoing treatment.

3.9 Other factors which influence the contribution of culture examination:

Method of selection for sputum examination :

Preceding sections have shown that the interval between collection and culture is not an important factor influencing the contribution of culture examinations. The variation between

TABLE 7

Numbers confirmed by culture among "scanty positives" and "others" in studies 1 and 3.*

	STUDY 1								STUDY 3	
	Spot collection				Overnight collection				Spot collection	
	ZN		Fl.		ZN		Fl.		ZN	
	Scanty Others	posi-	Scanty posi- Others lives.	lives.	Scanty Olhers	posi- lives.	Scanty posi- Olhers tives.		Scanty posi- Others lives.	
Confirmed by culture.	14	50	9	66	12	54	11	63	3	103
Not confirmed by cullure.	15	5	16	I	20	4	11	4	2	19
Cullure conla-minated (out of 2 above).	1	—	—	—	2	—	3	1	1	9

*With higher grades of positivity by smear.

TABLE 8

Not confirmed smears with higher degrees of positivily according to interval between collection and inoculation of culture

Study-	Type of	Direct smear	Interval			Tolal
			1 -2 days	3-4 days	5 days or more	
1	2	3	4	5	6	7
1	Spol	ZN	4	1	—	5
		Fl	1	—	—	1
	Over-night.	ZN	2	2	—	4
		Fl	3	—	—	3
3	Spot	ZN	3	3	4	10

TABLE 9

Percentage positive on culture only among total culture positives

Study No.	Type of collection.	Direct smear technique.	Culture positives only		
			Total Culture positive	Number	Percentage
1	2	3	4	5	6
1.	Spot	ZN	157	93	59.2
		Fl	157	82	52.2
	Over-night.	ZN	169	103	60.9
		Fl	169	95	56.2
2.	Spot	Fl	95	37	38.9
	Over-night.	Fl	87	24	27.6
3.	Spot	ZN	129	23	17.8
4	Treated	ZN	59	16	27.1
		Fl	59	8	13.6
	Not treated	ZN	102	13	12.7
		Fl	102	5	4.9

studies have also indicated that some operational factors are much more important in this respect. Some idea regarding these factors can he had from Table 9.

Column 6 of the table shows that the additional contribution from culture* was highest (52-60%) in study 1 in which all persons of age 5 years or more with any type of abnormality in the X-ray were referred for sputum collection. In study 2, in which all persons of age 20 years or more with symptoms such as prolonged cough or chest pain etc. were referred for sputum examination, the contribution was less but still substantial (28-39%). In the Bangalore District Programme (study 3) in which sputum was collected from suspected tuberculosis cases (symptomatics) among general patients who report to health facilities, it was only about 18%. Among already diagnosed untreated cases this was less (5 to 13%), but among treated cases this increased to 27%. Thus the additional contribution from culture depends upon the method of screening for sputum examination. Methods which presumably yield cases in earlier stage of disease benefit more from culture examination.

Method of microscopy :

The relative efficiency of microscopy methods influences the estimation of additional yield of positives by culture. In studies 1 and 4 where two methods have been used, the percentage positive on culture only (Table 4 col. 11) and percentage of positives added by culture to microscopy (Table 9 col. 6) were consistently higher for ZN method, but the diffe-

* This is shown as percentage positive on culture only to total culture positives. This is preferred to calculation of percentage added to direct smear positives in view of the limitations of direct microscopy—false positives and false negatives.

According to the second method of calculation the false negatives of direct microscopy are added to the numerator and subtracted from the denominator, thereby considerably exaggerating the real effect. False positives of direct microscopy tends to under-estimate the additional contribution by culture as these are added to the denominator. Both these defects are eliminated in the method used in this paper for calculating additional contribution by culture.

rences were not statistically significant. This indicates that fluorescence microscopy may be superior to ZN method.

Resistance to anti-tubercular drugs :

Table 10 shows the percentage positive by culture only, separately for sensitive cases and those resistant to any of the three major anti-tubercular drugs—INH, Streptomycin and PAS.

The total number tested for sensitivity at different intervals were too small to give a reliable picture of the effect of interval. However, those found by culture only were generally smaller for longer intervals for resistant cases. In view of the implications of such a finding a similar analysis was carried out for 74b resistant cases. This showed that the percentage positive on culture only did not vary much for intervals of 0, 1, 2, 3 and 4 days between collection and initiation of culture.

Last column of the table gives an interesting finding. In studies 1 and 2, the percentage positive by culture only was less for resistant cases. In study 3 the percentage was nearly equal for both sensitive and resistant cases.

TABLE 1

Percentage positive on culture only according to interval between collection and inoculation of culture and sensitivity I resistance of tubercle bacilli in specimens.*

				Interval between collection and inoculation of culture							
	Type of	D.S-	Sensitive	(1-2) days		(3-4) days		(5+) days		All days	
Study No.	collection.	tech nique-	or resistant.	Total cul-tured	% cult 4-only	Total cul-tured	% cult+ only	Total cul-tured	% cult-f only	Total cul-tured	% cult-t-only
1	2	3	4	5	6	7	8	9	10	II	12
1.	Spot	ZN	S	66	59.1	S3	67.9	14	64.3	133	63.2
			R	*8*	75.0	11	18.2	*2*	—	21	38.1
		Fl	S	66	56.1	53	60.4	14	50.0	133	57.1
			R	*X*	62.5	11	9.1	2	—	21	28.6
	Over-night	ZN	S	93	69 9	43	53.5	3	33.3	139	64 0
			R	18	61.1	8	12.5	1	—	27	44.4
		Fl	S	93	68.8	43	48.8	3	—	139	61.1
			R	18	44.4	8	12.5	1	—	27	33.3
2.	Spot	Fl	S	42	38.1	21	52.4	6	33.3	69	42.0
			R	5	—	7	14.2	—	—	12	8.3
	Over-night	Fl	S	II	72.7	9	44.4	1	100.0	21	61.9
			R	—	---	2	50.0	1	---	3	33.3
3.	Spot	ZN	S	"20	15.0	40	22.5	26	15.4	86	18.6
			R	15	26.7	13	7.7	13	15.4	41	17.1
4	Trea-ted	ZN	S	1	100.0	5	60.0	6	_	12	33.3
			R	7	57.1	28	21.4	12	16.7	47	25.5
		Fl	S	1	—	5	20.0	6	_	12	8.3
			R	7	57.1	28	7.1	12	8.3	47	14.9
	Not treated	ZN	S	13	15.4	30	20.0	39	5.1	82	12.2
			R	5	—	8	25.0	7	14.3	20	15.0
		Fl	S	13	7.7	30	6.7	39	5.1	82	6.1
			R	5		8	—	7	—	20	—

* Resistance to any one of the three drugs—INH, Streptomycin or PAS.

There was no clear pattern for study 4. If the results of studies 1, 3 and 4 are combined for spot collections, the percentage added by culture to ZN microscopy was 36.4 for sensitive cases and 23.3 for resistant cases. The corresponding percentages for spot collections and addition by culture to Fluorescence miscroscopy for studies 1, 2 and 4 combined were 37.5 and 14.0 respectively. In both instances, the percenage positive on culture only was significantly smaller for resistant cases. This may be due to most of these cases being advanced and discharging larger number of tubercle bacilli.

4.0 Discussion

In judging the relative utility of culture method in different practical situations it is extremely important to consider several operational factors. These factors can be classified into two groups:

(i) factors which affect the viability of tubercle bacilli in the specimen collected such as transportation and storage, contamination and presence of antitubercular drug in specimen due to current chemotherapy.

(ii) factors which influence the quantum of viable bacilli in the specimen, at the time of collection, such as method of selection for examination, type of collection (spot or over-night) and previous chemotherapy.

Transportation and Storage:

Sula et al (1960) concluded that "Storage, handling-and transport can deteriorate the cultivability of tubercle bacilli to an extent as to make their contribution unrewarding". The results of the studies reported here do not show any such deterioration in the utility of the culture method after transportation and storage. The inverstigation by Sula et al differs from these studies in 3 important aspects viz.,

(1) the interval between "collection" and inoculation of culture was 15 to 18 days in the former, as compared to 1 to 7 days in the latter,

(2) more emphasis has been given to loss of viability in the former, as against the end result (positive or negative) in the latter. (The material of Sula et al—e.g. Appendix Table 1 does not always show statistically significant end results, except for very weak suspen sions).

If not to-day at least in the near future, adequate facilities for culture examination are likely to be available in most of the developing countries and the effect of transport and storage for shorter intervals would be of more practical interest. If the utility of culture is not diminished upto an interval of 7 days (as in the present studies) it could still be profitably used in many developing countries. Hong Kong Government Tuberculosis Service/British Medical Research Council (1964) ha\e also reported that climatic changes and time of transit upto 7 days did not influence viability. The effect of the conditions of transportation could not be studied systematically in the present investigation. All the studies included material drawn during all seasons. The numbers were not large enough to study the effect of seasons. The interval between collection and examination consists of the time for transportation plus period under refrigeration in the laboratory which could not be analysed separately. Further even when refrigeration was provided during transportation, by insulated ice boxes, the effect was not likely to last for more than 24 hours. Moreover all specimens, irrespective of transportation in "refrigerated box" or not, were kept in refrigerator soon after receipt in the laboratory. Even so, the observation from these studies that refrigeration during transportation, of the type used and for short periods, may not be of any use, is of so great practical importance that it would be worthwhile to study this more systematically. Transportation without refrigeration under different climatic conditions and for varying intervals may have to be studied under Indian Conditions.

Contamination:

Adequate refrigeration may not be possible during transportation and can create favourable conditions for fast growing concomittant bacterial flora to multiply. Higher contamination rate was not observed with the increase of interval between collection and culture in spite of the fact that in study 3 the specimens were kept at room temperature in peripheral health facilities and no insulated box was used for transportation to the main laboratory. However contamination was uniformly more frequent in overnight specimens than in spot specimens. These findings show that contamination is introduced at the time of collection and that there is no deterioration of specimens in this respect with storage and transportation upto an interval of 7 days. Sources of contaminants can be broadly classified into (1) concomittant bacterial flora in the pathological specimens, (2) extraneous bacterial flora appearing at the time of collection, under unfavourable environments. Such extraneous contaminants are more frequent in overnight specimens, and being predominently spores and

Fungi are possibly more resistant to preculture treatment of specimens and more damaging to obtain maximum yield of positive cultures. Therefore utmost care should be exercised in the technique of collection of specimens for culture.

Type of collection

Overnight specimens have shown uniformly higher percentage of positives by both microscopy and culture in studies 1 and 2, where both types of collections were made. This is so inspite of the higher contamination rate observed for overnight specimens.

With reduction in contamination, overnight specimens are likely to give even larger number of culture positives and thus be definitely superior to spot specimens in case-finding programme, if operationally feasible. In so far as the type of collection influences the yield from direct microscopy also, the use of results from direct microscopy for international comparability of tuberculosis morbidity as suggested by Stybio (1964) has to be modified to take this into account. Further, from comparisons of results of ZN and Fluorescence methods available at NT1 (which is being reported separately) it is felt that this is another factor which may affect international comparability on the basis of direct microscopy results.

Effects of selection for sputum examination:

The most important factor which affects the relative utility of culture method is the method of selecting persons for sputum examination. Where such methods are more liberal and consequently may include larger proportion of early cases (which presumably excrete fewer bacilli) the additional contribution by culture is very high. Among highly selective groups, such as those diagnosed as tuberculosis cases, the culture method is unrewarding. Other methods of screening gave intermediate results. These findings are in conformity with those reported by Styblo (1964). He observed 22% of cases by microscopy during preventive investigation and *46%* in investigations on account of symptoms. The proportion of cases positive on microscopy was larger in the present studies, for all methods of preliminary selection: 39-48% in study 1, 61-72% in study 2, 81% in study 3 and 73-86% and 87-95% respectively in treated and untreated cases in study 4. It is possible that in both types of investigations reported by Styblo the cases were at a comparatively early stage of disease than could be expected under conditions in South India.

The above results also lead to two important conclusions. In tuberculosis service programmes covering symptomatic persons the introduction of culture methods for case detection is unrewarding. This justifies the limiting of sputum case-finding to direct microscopy in organising a tuberculosis service as a first step of the National Tuberculosis Control Programme in India. But, for bacteriological examination of persons who are likely to excrete fewer bacilli, culture method becomes invaluable. In the context of the National Tuberculosis Programme, India, such a situation may arise in at least the following:

(1) Evaluation of the programme.
(2) Change of drug regimen depending on change in bacteriological status of the patient.
(3) Further evolution of the programme in order to detect cases in earlier stage of disease, to effect control of disease.

In order to make this possible in the near future it will be necessary to establish facilities for culture examination so that specimens collected could be examined within one week. If transporation of specimens without refrigeration under different climatic conditions is not likely/ to detract the value of culture method, these centres can tackle effectively all the 3 situations mentioned above by getting specimens from rural health facilities. On the other hand, if transportation without refrigeration is harmful or if end results of culture are likely to deteriorate even after refrigeration, with longer intervals, simpler methods may have to be found for areas which are so far away from culture facilities. The search for such simpler methods as suggested by Sula et al is even otherwise worthwhile in view of its enormous practical advantages to the developing countries.

Summary:

Laboratory diagnosis of pulmonary tuberculosis by culture is a well established, technically superior method compared to direct microscopy. But its relative utility may vary in different practical situations. This has been studied in four situations using data of the following studies conducted by the National Tuberculosis Institute, India (NTI):

(1) A "prevalence survey" in which two sputum specimens, spot and overnight, were collected from each person of age 5 years or more, showing abnormal X-ray shadows in 70 mm photo-flurogram.

(2) A community case-finding programme in which persons of age 20 years or more with symptoms suggestive of pulmonary tuberculosis and those tuberculin positive reactors below 20 years voluntarily reporting symptoms were eligible for sputum examination; two specimens, a spot and an overnight, were collected from each individual.

APPENDIX TABLE

Distribution of culture positives according to degree of positivity of culture

(Spot specimens only)

		All culture positives			Positive on culture only					
Study Number	Degree of positivity	1-2 days	3-4 days	5+ days	1-2 days	3-4 days	5+ days	D.S.+ Cult.+	D.S.- Cult.+	Remarks
1	2	3	4	5	6	7	8	9	10	11
1.	< 20 Colonies (34.2)	26	21 (32.3)	4 (25.0)	25 (54.3)	20 (52.6)	4 (44.4)	2 (3.1)	49 (52.7)	DS by ZN
	+	20 (26.3)	14 (21.5)	4 (25.0)	16 (34.7)	12 (31.6)	2 (22.2)	8 (12.5)	30 (32.3)	
	++	10 (13.2)	15 (23.1)	6 (37.5)	2 (4.3)	5 (13.2)	(33.3)	21 (32.8	10 (10.8)	
	44-4 (26.3)	20	15 (23.1)	2 (12.5)	3 (6.5)	1 (2.6)	—	33 (51.5)	4 (4.3)	
	Total	76	65	16	46	38	9	64	93	
1. (13.2:	< 20 Colonies (34.2)	26	21 (32.3't	4 (25.0;	25 •59.5)	20 (60.6)	4 (57.1!	2 (2.7)	49 (59.8)	DSbvFI.
	4 (26.3)	20	14 (21.5;	4 '25.0	14 ;33.3;	9 (27.3)	2 (28.6)	13 (17.3)	25 (30.5)	
	++	10	15 (23.1,	6 (37.5:	—	3 (9.1)	1 . (14.3'	27 (36.0)	4 (4.9)	
	+ 4+ (26.3;	20	15 (23.1)	o U2.5)	3 [7.1)	1 (3.0)	—	33 (44.0)	4 (4.9)	
	Total	76	65	16	42	33	/	75	82	

(3) Evaluation of sputum diagnosis by direct microscopy in a District Tuberculosis Programme in which specimens were collected from all those voluntarily visiting peripheral general health facilities of the district and having symptoms suggestive of pulmonary tuberculosis.

(4) Assessment of a District Tuberculosis Programme after a year of its commencement in which specimens were collected from patients completing treatment for varying periods and those diagnosed but not initiating treatment.

In all these studies the specimens collected in the field were transported to NTI laboratory for bacteriological examination. The specimens were subjected to field conditions of storage and transport and there was an interval of 1-7 days between collection of specimens in the field and inoculation of culture slopes in the laboratory.

The main findings are:

(1) Additional cases detected by culture depended on preliminary selection of cases for examination; maximum in study 1 and progressively diminishing for studies 2, 3 and 4 in that order.

(2) This additional contribution by culture was not influenced by the interval of 1-7 days indicating that there was no appreciable loss of viability. Further, (a) the proportion confirmed by culture among specimens with lower degree of positivity on smear did not decrease with interval and (b) the proportion of cultures with lower degrees of positivity did not increase with interval, both of which could be expected with loss in viability.

(3) The interval of 1-7 days between collection and inoculation of culture did not adversely affect the yield of positive cultures.

(4) Contamination rate did not increase with the interval, but was dependent on type of collection, emphasizing the need for strict adherence to the correct techniques of collection in the field.

(5) In using direct microscopy for international comparability, the type of collection of specimens and the technique of examination by microscopy has to be uniform.

(6) Whereas culture is unrewarding in a tuberculosis survice programme as a first step in developing the national tuberculosis control programme, it becomes invaluable for evaluation of the programme, changing drug regimen on the sputum results and in further evolution of the programme in order to achieve control of the disease.

Further study on the transportation of sputum to central laboratories, without refrigeration and in different climatic conditions has been suggested. On the basis of such a study it may be possible to develop regional laboratories that can offer facilities of culture examination economically and profitably for large areas.

Acknowledgements

The authors gratefully acknowledge the members of the Technical Co-ordination Committee of the National Tuberculosis Institute, in particular Dr. Nagpaul, Director, Dr. Raj Narain, Epidemiologist and Dr. Savic, WHO Medical Officer, for their helpful criticism and advice. We also express our thanks to Dr. J. Guld, (Medical Officer,) and Mr. Sundaresan (Statistician) WHO, Geneva, for their valuable comments.

To Miss Rajalakshmi, Statistical Assistant, who helped in analysis of all the statistical data we express our appreciation.

All the studies were conducted with the active co-operation of the staff of different sections of the National Tuberculosis Institute to whom we are thankful.

REFERRENCES

Darzins, E. (1958) *The Bacteriology of Tuberculosis.* P. 256.

Hong Kong Government Tuberculosis Service/British Medical Research Council (1964; *Tubercle.* 45. 77-95.

Jensen, K.. (1955) *Bull. int. Un. Tuberc.* 25, 89.

Nassau. E. (1954), *Tubercle, Colonial Supplement No. 3.*

O'Hea, A. .1. (1957), *F. Path., Bact.,73,* 389.

Raj Narain, A. Gesar, M. V. Jambuaathan and Subramanian, *Ind. Jour. Tuberc.,* **10**, P. 85-116/*Bull/. Wld. Hlth. Org.,29,* P. 641-664.

Styblo, K. (1964), *Bull. Int. Un. Tuberc.,* 34, 49-52.

Sula, L., Sundaresan T. K. and Langerova, M (1960). *Bull. Wld. Hlth. Org.,23.* 635-651.

THIACETAZONE JAUNDICE

Case Reports

R.K. NARANG and J.N, SARIN
(G.S.V.M. Medical College, Kanpur)

Thiosemicarbazones were one of the earliest drugs to be tried in the treatment of tuberculosis but fell into disrepute due its toxicity. Since the East African/British MRC Trial (1963), it has again emerged as a potent companion drug to isomazid and has been extensively used in developing countries. The present workers present three cases of jaundice which was thought to be due to thiacetazone.

Case Report

Case No. 1 : Mrs. G.R. , a 29-year old Hindu female was admitted to the hospital on March 27, 1965. She had been extensively treated for pulmonary tuberculosis during the last four years with streptomycin, isoniazid and PAS. On clinical examination she was thin built, anaemic and had pitting oedema on the feet. Hb was 8 Gm%, Serum proteins were 4.5 Gm% with albumin 2.2Gm% and globulin "2.3Gm%. Smear examination of the sputum was strongly positive for AFB. A skiagram of the chest showed extensive cavitary disease in both the lungs. As the newer antituberculosis drugs were not available in the hospital she was placed on streptomycin I Gm, isoniazid 300 mg and thiacetazone 150 mg. daily along with high protein diet and iron tablets. On July 17, 1965 jaundice was noted for the first time. In the proceeding three days she had lost her appetite completely and had compalined of recurrent vomiting. Liver was enlarged seven fingers below the costal margin, was firm with smooth surface and sharp margin and was tender. Total leucocyte count was 11,400 per cu. mm. with polymorphs 75% and lymphocytes 25%. Vanden Berg was direct positive, serum bilirubin 3.6 mg%, cephalin cholesterol floculation test 2 plus positive and alkaline phosphatase 13 units. There were bile salts and pigments in the urine and urobilinogen test was positive. A histopathologic examination of the tissue obtained on liver biopsy, done with vim Silverman needle, showed fatty infiltration of the liver. All antituberculosis drugs were completely withdrawn and the patient placed on supportive measures only. She had received 150 mg. thiacetazone daily for a total of 102 days. The jaundice and the liver enlargement gradually regressed but the patient had become very weak and emaciated. She finally expired on August 29, 1965, The patient had extensive pulmonary tuberculosis and had a poor prognosis but, it was thought, that the jaundice hastened her end.

Case No. 2 : M.S., 40 years, Muslim male was a case of pulmonary tuberculosis and chronic hypertrophic emphysema. He had been treated with streptomycin, PAS and isoniazid for the last three years but this treatment had failed to close a giant cavity in the left upper zone and his sputum had emained positive. So, the apicoposterior segment of the upper lobe was removed in January, 1965. During the operative period the patient was treated with streptomycin 1 Cm., isoniazid 300 mg. and thiacetazon 150 mg. daily and he was advised to continue the same treatment after operation. On July 14, 1965, during a routine check up, icterus was noted; he had no gastrointestinal complaints. He had received thiacetazone for a total period of 190 days. All antituberculosis drugs were stopped and he was admitted to the hospital for investigations. Leucocyte count was 6,400 per cu. mm. with neutrophils 70%, lymphocytes 28% and eosinophils 2%; Hb was JO gm%, serum proteins 5 Gm% and globulin 2 Gm%; Serum bilirubin was 2.4 mg%: Vanden Berg was direct positive, alkaline phosphatase was 20 units and cephalin cholesterol floculation 2 plus. Urine contained bile salts and pigments and urobilinogen test was positive. A histopathological examination of the liver tissue obtained on needle biopsy showed that "normal architecture of the liver was disorganised. Parenchyma showed marked fatty infiltration. There was infiltration by lymphocytes in the portal tracts and proliferation of fibrolasts". Diagnosis: Fatty infiltration with early cirrhosis of the liver. Unfortunately the patient developed a tension pneumothorax on the right side during liver biopsy and expired two months later due to empyema and bronchopleural fistula. Autopsy was refused by the relatives.

Case No. 3 : R.L., 30 years, Hindu male had been treated with various combinations of streptomycin, isoniazid and PAS for the last six years. He had bilateral advanced pulmonary tuberculosis. His sputum was constantly positive for AFB. Since he could not afford the new antituberculosis drugs, thiacetazone 150 mg. along with streptomycin 1 Gm. and isonazid 300 mg. per day was started on December 5, 1964. He reported for check up

on January 16, 1965 and was found to have jaundice. He had no gastrointestinal symptoms. Liver was enlarged 1 finger below the costal margin, bad a smooth surface and was tender. He had received thiacetazone for 42 days. All the drugs were withdrawn. Serum bilirubin was 1.8 mg%, alkaline phosphatase 35 units, cephalin cholesterol floculation 1 plus. The urine contained bile salts and pigments; urobilinogen test was positive. Liver biopsy was refused by the patient. Jaundice and the liver enlargement regressed in a fortnight.

DISCUSSION

The three cases under discussion are out of 131 cases treated with thiacetazone under the care of the senior author. 53 of these were previously untreated cases and were given a combination of isoniazid 300 nig. and thiacetazone 150 mg. per day. The remaining 78 were cases of chronic advanced lesion and had been extensively treated with streptomycin, isoniazid and PAS and were retreated with streptomycin, isoniazid and thiacetazone due to non-availability of second line drugs. It is significant that all cases of jaundice were noted in the latter group (giving an incidence of 3.8% of jaundice cases in this group and an over-all incidence of 2.2%). All the three cases came from the low income group, had chronic pulmonary tuberculosis of a long duration and had poor nutritional status. It is suggested that chronic malnutrition due to low protein intake and katabolism due to the effect of the disease and chronic anoxia made the liver susceptible to toxicity due to thiacetazone. Pines (1964) found a high incidence of hepatotoxicity in cases treated with a combination of isoniazid and thiacetazone. He did not find evidence of liver damage when thiacetazone was given with drugs other than isoniazid and suggested that isoniazid itself gave rise to hepatotoxicity and potentiated the toxicity of thiacetazone. Liver damage due to isoniazid is a possibility as Rubin et al (1952) have been able to produce jaundice and fatty degeneration of liver in dogs with prolonged high doses of isoniazid but this is very uncommon in man. For example, Reynolds (1962) could collect only 9 cases in the literature, including one case of his own, while Urban and Bahrs (1963) found no adverse effect on liver due to long-term isoniazid treatment. However, it cannot be denied that when two potentially toxic drugs are given together, the chances of toxicity will be high. But in view of the evidence presented above, malnutrition and chronic anoxia appear to be more important factors. The case of acute yellow atrophy reported in the East African/British MRC trial (1963) occurred in a severely malnutrited patient. In patients with a short history and without any previous medication, treatment with isoniazid and thiacetazone is rarely complicated by jaundice. Our experience in this respect agrees with that of others (e.g. East African/British MRC, 1963, Deshmukh et al. 1963, Sikand et al, 1963, Menon, 1965). But in chronic drug resistant cases the use of thiacetazone should be discouraged. Not only it is ineffective but is likely to do positive harm to the patient by producing serious toxic reactions.

Clinically, it is very difficult to differentiate cases of jaundice due to infective hepatitis from those due to drug toxicity. A histopathological examination of the liver tissue obtained on needle biopsy of the liver is very useful in this respect. Infective hepatitis could be excluded by this procedure in two of our cases; the third patient refused the biopsy.

On the evidence presented in this paper, it is difficult to say if thiacetazone jaundice is a toxic or hypersensitivity phenomenon. The dose of thiacetazone was 150 mg. per day in all the patients including those who did not develop jaundice. The duration of treatment before jaundice was noticed was 42, 10*1* and 190 days respectively. The usual stigmata of hypersensitivity like fever, rash, adenopathy. joint pains and eosinophilia were absent. In any case buclizine hydrochloride 30 mg, per day (included in the proprietery preparation of isoniazid and thiacetazone used in these cases) was ineffective in preventing thiacetazone jaundice.

SUMMARY

Three cases of jaundice due to thiacetazone are reported. Infective hepatitis was excluded by liver biopsy in two cases. All the three cases were chronic advanced drug resistant cases of pulmonary tuberculosis and had malnutrition. The incidence of thiacetazone jaundice in such cases was 3.8%. No case of jaundice occurred in thiacetazone treatment of previously untreated patients. It is suggested that chronic malnutrition and chronic anoxia are important factors in predisposition to thiacetazone, hepatotoxicity.

REFERENCES

Deshmukh, M.D., Master, T.B,, and Kulkarni. K.G., (1963): *Current Medical Practice, 7.* 491.
East African/British MRC Second Thiacetazone Investi gation (1963): *Tubercle Land.* 44, 301.
Menon, N.K., (1965): *Ibid,* **46**, 19.
Pines, A. (1954) : *Ibid,* **45** 188.
Reynolds, E. (1966): *Ibid.* **43**, 375.
Rubin. B., Hassert, O.J.. Thomas, B.G.G., & Burke. J.C. (1952): *Amer. Rev. Tubcrc.,* **65**, 392.
Sikand, B.K., Goyal, S.S., and Mathur, G.P.: (1965): *Indian, J. Tuberc. **11**,* 38.
Urban, H. and Bahrs. G. (1963): *Amer. Rev. Resp Dis.,* **87**, 319.

LETTER TO THE EDITOR

The following letter is reproduced here to stimulate interest in the persons working on the bacteriology of Tuberculosis. Our workers have shown some interest in Bovine Tuberculosis but no significant control study has been undertaken on the material from extra Pulmonary Tuberculosis lesions affecting bones, joints, glands and serus membranes. This letter written by a former UNICEF Officer in India who was primarily concerned with only supply operations shows the keen interest he has in the problem of Tuberculosis in our country.

It is time that the subject of bovine Tuberculosis in man is thoroughly studied and such doubts as expressed in this letter are laid to rest. To the best of our information, there is neither significant Tuberculosis in cattle nor bovine Tuberculosis in human beings in India.

*

A Letter from Moscow (USSR) to the Edi(or, Indian Journal of Tuberculosis

The subject of this letter is, of course, TB — the question I have had discussed already with you—bovine TB in India. It is very well known or at least rather firmly believed that in India bovine TB does not play any significant role in human pathology.

Two reasons are normally given as explanation of this phenomenon.

1. Most of the milk for human consumption in India is produced by buffaloes, which are immune to TB.

2. Most of the milk used for consumption is boiled before use as a matter of routine. (I do not think I should give you references in respect of these two statements—they are well known).

It occurs, however, that these two factors may not play such a significant role as it is generally believed—in certain parts of the country at least. Firstly, there is a good lot of cows in the country and cows' milk is being consumed in sufficiently big part of the country to cause concern in respect of contamination of milk with TB bacilli. Secondly, having no desire to disprove the statements regarding the prevalence of the custom to boil milk before consumption, I believe that a good lot of milk is consumed in a raw form as milk or curds.

The rather high probability of bovine TB infection in certain parts of the country occurred to me while analysing some statistical data, which were kindly given to me by Professors of Paediatrics from some medical colleges. The following table represents the percentage breakdown of all TB cases diagnosed in outpatients' departments of three Paediatric Departments of medical colleges.

As could be seen from this table abdominal form of TB are of a significant importance in all three places though in Agra it presents one-third of all TB cases seen in OPD. While being aware that not all cases with abdominal TB lesions are to be attributed to elmentary infection, it seems to be possible to use the figures of relative frequency of abdominal TB among children as an indication of the possible role the bovine TB plays in human pathology.

In the next Table (2) also computed on the basis of data obtained from Paediatric Departments of Medical Colleges, the relative frequency of abdominal TB is shown among inpatients—children. It can be seen that in Kanpur, Jabalpur, Agra and Jaipur children with abdominal form of TB constitute a sizeable part of all TB cases (above 10%). Similarly high incidence on abdominal TB among

TABLE 1

	Nagpur 1962-63 & 1964	Baroda 1963-64	Agra 1964
Total number of TB children seen in OPD.	1101	619	677
Pulmonary TB	746—67.8%	248—40.0%	264-39.0%
TB Meningitis	52- 4.8%	211-34.6%	101—14.9%
Abdominal TB	78 - 7.0%	75-12.0%,	232—34.2%
TB Lymphadenitis	214—19.4%	48- 7.6%	—
Other forms of TB	11— 1.0%	37— 5.8%	80—11.9%

TABLE 2 *Distribution of different forms of TB among inparients of Paediatric Departments of the Following Medical College**

	Bombav 1960	Nigpur 1962-64	Kanpur 1964	Madras 1962-63	Hyderabad 1962-64	Jab a 'pur 1962-64	Aera 1963-64	Jaipur 1963-64	M mgalore 1963-64	Baroda 1953-64
Total number of TB cases -Inpatients, children.	216	299	175	1160	953	35?	286	216	317	286
Pulmonary TB	56—25.9%	96-32.1%	37-21.2%	562-48.3%	103-10.8%	170-47.9%	57-20.0%	84-38.9%	175-55.2%	*67-23.2%*
Plural TB	10- 4.6%	20- 6.7%	*9*- 5.2%	35- 3.0%	16- 1.6%	8- 2.2%	8- 2.8%		*1* • 2.2%	9- 3.2%
TB Meningitis	107-^19.2%	164-54.8%	90-51.2%	543 ... 47.0%	463-48.7%	93-26.2%	118-26.2%	110-51.0%	•14-29.6%	197-69.1%
Abdominal TB	13- 6.0%	13- 4.4%	20-11.4%	16- 1-4%	35- 3.6%	42-11.8%	55-19.2%	22-10.1%	13- 4.2%	4- 1.4%
Other TB forms	30-143%	6 2.0%	19-10.8%	4 0.3%	336-35.3%	42-11.9%	48-16.8%		28- 8.8%	9 - 3.1%

Bombay -J.B. Hospital for children, .)..!. Group of Hospital -Prof. P. Udani

Madras—Egmore Hospital, Madras Medical College—Prof. Achar Hyderabad

Nilaufer Hospital for children—Prof. Harish Chandra Other hospitals beiong to

Medical Colleges in respective cities.

inpatients in Indore Medical College is reported by N. Bhandary and K. Kaul (Indian J. Child Health-1961, V. 10 Nil P. 549-551). It should be noted that in other places mentioned in the table 2 children with abdominal TB constitute less than 5% of the total TB group.

Such a difference can hardly be considered as an accidental one- apparently there is certain rule in it. Geographically it seems that higher incidence of abdominal TB among children is found in northern parts whereas it is lower in southern parts. It is difficult to say at this stage what factors play the role to cause such a difference. The source of milk may be of importance in some areas the number of cows as against buffaloes is much higher (I could have only an impression to this effect while travelling in India) which may mean an increased reservoir of infection with bovine TB. The difference may be in cultural patterns— customs regarding the use of milk may vary in respect of boiling milk, using curds and other products of unboiled milk etc.

I have just found a reference in a German TB Journal (Ftschr Fur Tuberk 1965 Vol. 123 Helf 4/6) an article of Dr. H.N. Knipping about TB problem in India where he quotes an article describing the Bovine TB features in India. 25% buffaloes in Lahore area and 2.8% of cows in Madras and Patna area suffered from TB. With these conflicting information and impressions, the question of Bovine TB in India deserves scientific study and the bearing it could have on epidemiology of Tuberculosis in India.

Sd/- V. Tatochenko

Institute of Paediatrics, AMS,
Lomonosovsky P.R. 2/40,
Moscow, U.S.S.R.

BOOK REVIEW

Pulmonary Tuberculosis — by **R.** Vishwanathan. Asia Publishing House, Asaf Ali Road. New Delhi. Price Rs. 16.50.

In his foreword to the book, Sir Geoffrey Marshall has stated that the author in the restricted compass of less than 150 pages has given a lucid and well balanced survey of the whole subject of Pulmonary Tuberculosis. It is remarkable that the author has been able to give such a panoramic view in such a small canvas. In fact, there is not a single aspect of the tuberculosis problem which he has left untouched. The book contains a wealth of information on all aspects of tuberculosis. The subjects of epidemiology, tissue and immunological reactions to infection and pathogenesis of tuberculosis have been given due and jusifiable emphasis as they are often neglected both by students and practitioners. The value of the book is enhanced by the fact that references have been made not only to work done in foreign countries but also to the work done in India. The personal experience of the author extending for a period of 30 years on the subject of tuberculosis as a clinician, as a teacher and as an administrator has contributed in no small measure to the academic excellence which the book has attained.

No doubt, views and opinions of other experienced workers have been freely referred to and discussed in order to make ihe book more comprehensive. Central aspects of tuberculosis have been adequately dealt with, though briefly. Considering the importance of the tuberculosis problem as a public health menace in India, it is hoped that in the next edition of the book tuberculosis control programme emphasising in particular the role of voluntary agencies will be given more space. This suggestion however is not given as a criticism of the book. Considering the growing importance of mycobacteriosis and finding of atypical tuberculin reactions in a large section of the population, the subject could have been dealt with in greater detail.

Altogether it is an excellent book which can be read with immense profit not only by under graduate students but also by specialists and practitioners. We can confidently recommend it as a textbook in medical colleges. The book should find a place in every medical library in India and abroad. B.M.C.

NEWS & NOTES

1967 Workers' Conference

The 22nd TB and Chest Diseases Workers' Conference will be held in Hyderabad early in January, 1967. Dr. Khushdeva Singh, the Chairman of the Standing Technical Committee of the Association, will preside over the conference,

The programme of the conference will include Symposia on "Tuberculosis in Industry" and "Drug default—its psychological, social, economic and administrative factors". There will also be panel discussions on "Prevalence of drug resistance" and "Clinical significance of Drug resistance" and "Emergencies of Chest Practice". The conference will also include sessions on "BCG Vaccination" and "Type of tuberculosis developing among BCG vaccinated persons and the course of disease among them" and on "Economics of Health".

Those who wish to contribute papers on the above subjects should send the same to the Secretary-General, Tuberculosis Association of India, 3, Red Cross Road, New Delhi latest by 15th June, 1966.

New TB Seal Design

The Tuberculosis Association of India held its Seal Design contest for the 17th Seal Sale Campaign recently. Out of the 90 Seal designs entered in the competition, the design submitted by Shri B.B. Banerjee of New Delhi was selected by a Committee. The Seal depicts a few human forms, highly stylised and grouped with an attractive colour scheme in a balanced pattern. Shri Banerjee has been awarded the prize of Rs 500 for the design.

Award Of Seal Sale Trophy

The Association's Trophy for the highest Seal Sale Collections was awarded to Andhra Pradesh TB Association. The Committee appointed to adjudicate as to which Association should receive the Trophy decided on the basis of State Association reports of collections that the Andhra Pradesh Association had taken 21,00,000 Seals for the 15th Campaign and collected Rs. 1,45,000 which works out to Rs. 402.96 per 1,00,000 population.

The award was made in accordance with the decision made by the Association to institute a Trophy every year from 1966 to the State TB Association which makes the largest Seal Sale Collections in proportion to the population of the concerned State.

Chest & Heart Conference, Eastbourne, 1967

The Chest and Heart Association, London will be holding a Chest & Heart Conference at Eastbourne from 4th to 7th April, 1967. This conference will be open to doctors and non-medical workers in the chest and heart field. The conference will include a number of main sessions, smaller meetings devoted to scientific and clinical matters and also group discussions.

Eminent chest specialists from all parts of the world will take part in the conference.

For details, write to the Conference Secretary, Chest & Heart Association, Tavistock House North, Tavistock Square. London W, C. I.

l.C.M.R. Research Scheme

The Indian Council of Medical Research has launched a new scheme for enabling research workers in Medical Colleges to devote their time entirely to research for a limited period.

This scheme is intended primarily for experienced scientists, who are actively engaged in research in the field of Medicine and allied sciences; have attained some eminence in their field of study and are anxious to devote their entire attention for a limited period so as to be able to pursue the important leads already obtained by them.

According to this scheme, the selected research worker will be treated as having been seconded to the Research Cadre of the l.C.M.R. for the duration of the scheme which will normally be one year but may be extended to two years. The salary and allowances, which the research worker would have drawn in his permanent post, will be met by the l.C.M.R. while the parent office would have to safeguard his/her seniority in service and promotion in the permanent cadre, The Council will make a grant of Rs. 5,000 per annum towards contingent expenditure and will also provide the research workers with one or two research assistances/fellows.

The selected research worker may elect to work at the institution, where he is employed or in other institution of his choice. This provision has been made to enable the research worker to migrate to an institution where better facilities are available for work in his line. If the research worker has to change his venue, the Council will bear the travelling allowance of the worker and the members of his family. Those interested may write to the Director-General, Indian Council of Medical Research, Medical Enclave, Ansari Nagar, NewDelhi-16.

The Indian Journal of Tuberculosis

ABSTRACTS

Vol. XIII March 1966 Abst. No, 2

Chemotherapy of Pulmonary Tuberculosis in Adults. The Choice of Drugs in Relation to Drug Susceptibility:—

A Statement of the Commitee of Therapy—The Choice of Drugs in relation to Drug Susceptibility.

Amer. Rev. Besp. Dis.,

Sept., 65, Vol. 92, No. 3.

Bacteriological control of previously untreated pulmonary Tuberculosis can be obtained with drugs alone.

Chemotherapy failures occur as a result of errors in:

1. The choice of Drugs.
2. Administration of Drugs.
3. Patients' failure to take the prescribed drugs regularly for a long period.

Treatment failures are associated with presence of bacilli resistant to drugs used. Treatment should be so planned that resistance will not be missed or ignored.

I. Susceptibility-Testing to, Isoniazid, P.A.S. and Streptomycin

The susceptibility of micro-organism to Isoniazid, Para-aminosalicylic acid and Streptomycin should be checked before and during treatment to detect both initial and acquired resistance which may interfere with effective therapy.

A. The Need for Susceptibility Testing to the Primary Drugs

Adequate laboratory facilities for evaluating susceptibility to the primary drugs should be made available to all Physicians and Clinics treating patients with tuberculosis.

B. Recommendation Procedures

First positive culture in all patients be tested for susceptibility and cultures obtained after three months of treatment should be tested for drug susceptibility. Susceptibility tests should be repeated at intervals of about four weeks in those whose secretions remain positive.

Resected specimens provide an added opportunity to evaluate drug susceptibility of the bacilli received from the actual lung tissue. Drug concentration should be: Isoniazid: 0.2, 1, 5 r/ml P.A.S. : 1, 5, 25 r/ml Streptomycin: 2.5, 5, 10, 100 r/ml Periodic testing of susceptibility of standard strains of H 37 RV to appropriate concentration of Drugs should be done.

Even with good laboratories a number of limitations must be recognised.

1. Despite some variations in results due to technical factors, including differences in media, size of inoculum and other details, the procedures are sufficiently accurate to be of great clinical value.
2. Isoniazid resistance in Vitro does not preclude Isoniazid's having some limited Clinical effects.
3. The susceptibility of bacteria from different lesions in the same lung may vary. Thus it is possible that important resistance may occasionally be missed.
4. Laboratory susceptibility to Isoniazid and perhaps to other drugs may persist occassionally despite prolonged use of the drugs without evident clinical effectiveness.

C. Species identification Of Resistant Organism

Finding of unexpected drug resistance especially to drugs which have not been employed in the patients' treatment, such cultures should be thoroughly examined for the morphologic and chemical features of the various forms of atypical mycobacteria.

II. The Choice of Drugs in Treatment

Before susceptibility tests, treatment should be started with drugs to which it is anticipated the bacilli will be susceptible.

A. Minimal and Non-Cavitary. Moderately Advanced Pulmonary Tuberculosis

In patients with minimal non-cavitary, moderately advanced tuberculosis previously untreated, combined use of Isoniazid and P.A.S. is recommended.

B. Cavitary Pulmonary Tuberculosis

The use of three drug regimen may be important both because of the risk of initial

resistance to one of the drugs used and because development of resistance during therapy is further reduced.

The addition of streptomycin results in an increased number of toxic reactions, the frequency and severity of which depends largely upon the age of the patient, the daily dose and duration of administration.

III. Therapy With Streptomycin Isoniazid and PAS

Streptomycin:—is given 1 Gm. daily for a period of six to twelve weeks or more depending on Clinical response.

Bi-weekly or thrice weekly regimens resulted in excessive rate of drug resistance.

In elderly patients or in those 'with pre-existing bearing impairments or renal damage. Streptomycin should be given in smaller doses daily or not at all.

Streptomycin may be even more important for patients for whom P.A.S. or Isoniazid cannot be used because of intolerance or resistance. In these cases, Streptomycin may be given for longer period o four to six months or more because of toxicity. This may be given daily in small dosage in preference to intermittent therapy.

Isoniazid:— Daily dosage of Isoniazid for adults is usually 5 mgm per Kilo of body weight about 300 mgm daily given orally in two to three doses. Higher dosage may be recommended in patients with severe or acute disease or if response is inadequate. With higher dosage 50 - 100 mgm pyridoxine daily prevents peripheral neuritis.

Para-Aminosalycylic Acid

The daily dose of P.A.S. is 200 mgm per Kilo of body weight usually in three divided doses after meals. It is usually given as sodium salt. If sodium restriction is indicated a sodium free form of P.A.S. should be used.

Duration of Therapy

Chemotherapy should be administered continuously for a minimum of two years. After completion of combined Chemotherapy it is possible that further protection can be given to patients with a high risk of relapse, such as certain diabetes, silocotics and open negative cases by prolonging indefinitely the administration of Isoniazid alone.

Patients' Cooperation

The need for taking drugs regularly must be frequently emphasized to the patients. Urine tests for P.A.S. and Isoniazid mav be done to assist the Clinicians in detecting failure to take these medications.

IV. Toxicity of Streptomycin, Isoniazid and P.A.S.

Streptomycin:—Toxic reactions may manifest itself in transient or permanent vestibular damage especially in older patients.

Loss of hearing is seldom a clinical problem but periodic audiogram or other hearing tests are advised.

Renal damage is uncommon, however it is advisable to perform periodic urine analysis and blood urea nitrogen. Paraesthesia and malaise following injection does not lead to permanent damage. Hypersensitivity to Streptomycin may occur and is manifested by fever, rash or other allergic manifestations.

Para-Amino Salicylic Acid:—commonly produces gastrointistinal symptoms. Hypersensitivity reaction may manifest itself by appearance of rash, fever and in some instances such as headache, malaise or sore throat. Drugs should be stopped if there are serious side effects.

Isoniazid:—Toxicity is often manifested as symmetrical, Peripheral, Polyneurites, which usually developes in mal-nourished patients (alcoholics, Cirrhotics) or patients receiving large doses of Tsoniazid.

Pyridoxine 50-100 mgm daily may be given to prevent it.

Isoniazid occasionally has a transient effect on memory and ability to concentrate and rarely may lead to Psychosis. Central Nervous symtoms may be encountered more frequently in epileptic patients receiving Isoniazid. After hypersensitivity reaction to Isoniazid, Streptomycin or P.A.S it is possible to desensitize the patients by starting with minute doses and progressively increasing doses of the drug to therapeutic levels.

It is important to stay below the dose that causes any symptom. If difficulty is encountered a brief period of steroid therapy may be valuable.

V. Surgery During Initial Chemotherapy

If surgical procedure is indicated, necessary drug changes should be coordinated with the contemplated surgery. If Laboratory or clinical evidence of drug resistance is present, therapy should be changed to ensure that two effective drugs are given during and after surgical period.

Management of Tuberculosis due to Organism Resistant to one or more of the Primary Drugs

The therapy of patients with resistant

organism often involves the use of more toxic drug? and it is best to give such treatment in a Hospital. Patients with resistant organisms present additional problems, social, psychologic and physical. Many have had difficulty in cooperating with medical care and are less able to tolerate important therapeutic agents. Their lesions are usually extensive with cavitation and often seen to have poor resistance to disease.

A. Drug Susceplibitty Testing

Cultures showing resistance to Primary drugs should be examined for susceptibility to other drugs although these are less standardized and difficult to perform.

Treatment of Patients with Resistant Organisms

1. If resistant to one drug is demonstra ted, that drug should be discontinued and treatment continued with two or more drugs to which the organism are susceptible.

a. In the presence of Isoniazid resistance, Isoniazid is frequently continued because of a possible remaining antimicrobial effect and the negligible hazard of toxic side effects.

If Isoniazid resistance is found in the pretreatment culture (with susceptibility to Streptomycin and P.A.S.)daily Streptompcin, P.A.S. and possibly one other drug may be used.

Patients who continue to discharge tubercle bacilli during drug treatment and in whom resistance to Isoniazid has occurred during therapy should be given a new combination of two or more drugs.

b. If Streptomycin resistant is found, Isoniazid and P.A.S. should be used with the possible addition of a third drug.

c. If P.A.S. resistance is present, Isoniazid and daily Streptomycin should be used.

2. If resistance to two primary drugs is found initially or subsequently, two or more drugs to which the organism is fully suscep tible should be given.

3. When resistance to Streptomycin and Isoniazid or to all three primary drugs is present, the situation is more difficult, reliance must be placed entirely on two or three other appropriate drugs.

C. Dosage and Toxicity of Secondary Drugs

Cycloserine:—The dosage is 1.0 Gm. daily in divided doses orally. Pyridoxine 100 mgm should be given to prevent seisures. Dilantin 100 mgm thrice daily may be added if needed.

Close clinical observation for evidence of agitation or other personality changes is important.

Pyrazinamide:—Atoial dose of 3 Gm. daily (40 mgm per Kilo) may be given in three portions of 1 Gm. each. (About 3% developed Jaundice).

Bromosulphalein elevation occurs in 15%. Ssrum glutamic oxalacetic transminase (SGOT) elevations are more common,

Viomcycin: — dose is 1 Gm. twice a day. twice weekly. Vestibular auditory or renal damage may occur.

Kunamycin: — 1 Gm. intramuscular thrice weekly.

Audiogram should be done every week.

Ethonamide:—1 Gm. in divided doses orally.

Anorexia, nausea, vomiting, metallic taste and Jaundice is a rare complication. Serum glutamic oxalacetic transminase are useful in early detection of impaired liver function.

D. Clinical Indications for Change of Chemotherapy

Roentgndgraphic or bacteriological worsening of disease.

E. Surgery in Relreatment Patients

All patients being treated • for resistant organism should be evaluated for surgery as achievement of' a permanently satisfactory status without surgical intervention is much less certain than, it is in patients receiving treatment for the first time. **Duration of Therapy in Patients with Resistant Organisms**

Treatment should be continued over as long a period as possible for good results.

Prolonged use of Isoniazid alone after termination of other therapy may be of some value.

H.B.D.

Ethambutol in the Retreatment of Pulmonary Tuberculosis

Bobrowitz I.D.; Gokulanathan, K. S
Dis. of the Chest; 1965, 48, 239.

Sixty-four'hard core' patients-individuals with chronic, infectious, advanced, drug resistant pulmonary tuberculosis-were treated with Ethambutol (EMB). All were re-treatment cases. In a group of 17, EMB was used alone and in the other 47 it was combined with one other second line drug not previously used in that patient. P.Z.A. was the most commonly used companion drug. All patients continued on INK.

Clinical improvement was usually rapid, significant and lasting. When EMB was used alone,

bacteriological and X-ray improvement though early was not lasting and therefore EMB alone is recommended only for short term use like "Umberalla" for surgery etc. Of the 47 patients on EMB combined treatment, 75% achieved sputum conversion, mostly within 3 months. Bacteriological relapse has been noted only in 2 patients. Cavity closure occurred in 4 patients and open negative healing in 5 others. As most of the cases were chronic and the cavities were multiple and with thick fibrotic walls, only bacteriological rather than X-ray improvement could be expected. There were only 2 deaths, one from coronary thrombosis and one in a patient with liver disease who had successfully completed treatment with EMB. Drug toxicity was noticed in 2 patients only. They complained of blurring of vision, difficulty in reading and inability to differentiate the colours fully. In both cases the disability disappeared when the drug was discontinued. There was no other evidence of toxicity, intolerance or side effects. The dosage of EMB recommended is 25 mg/kg for 60 days and 15 mg/kg thereafter.

S.P.P.

Retreatment of Drug Resistant Tuberculosis at Batty State Hospital

Raymond F. Corpe; Frank A. Blalock.
Dis. of the chest; 1965, 48, 305

Results of treatment of 107'treatment failure' cases of pulmonary tuberculosis with a life expectancy of about one year with Ethambutol (EMB) are reported. The treatment was started with EMB, Ethionamide and INH; and when sensitivity results were available, one of the four drugs—cycloserine, Viomycin, PZA and Kanamycin—were added in the light of sensitivity result. Sputum conversion was obtained in 84% cases; 70% having been converted by the 4th month. The conversion occurred earlier in coloured than in white patients, and in younger than in older patients. The death rate was high —15% but it was attributable mostly to the ravages of the pre-existing disease. There was significant co-relation between sputum conversion and X-ray findings. There was no true toxicity to EMB. Six patients complained of temporary blurring of vision but it disappeared in two, following temporary interruption of drug. In 4 others the disability disappeared when the drug was permanently withdrawn. The bacteriological relapse occured in 6% with an average duration of follow up for one year.

S.P.P.

Hearing loss in Infants of Tuberculous Mothers Treated with Streptomycin during Pregnancy.

Robinson G. C., & Cambon K. G.
New Eng.J Med. 1964, 271, 949.

Evidence is produced o show that streptomycin and dihydrostreptomycin can filter through the placenta and reach the foetus during pregnancy if these drugs are administered to the mother. Cases are reported where loss of hearing has been ceased in the foetus as a result of the mother's treatment though such an event is not frequent

SP.P.

The Results and Follow-up Study of the Ambulatory Chemotherapy of Pulmonary Tuberculosis.

The Joint Research Committee on Chemotherapy of the Kekkaku Yoboka.
Kekkaku; 1964, 39, 500.

The results of treatment have been analysed tor relapse in 576 cases of pulmonary tuberculosis who had ambulatory treatment during the past 8 years. Some of the cases were treated with triple drug schedules (Streptomycin, PAS and INH) and some with PAS and INH only. The drug regime employed, the age of the patient and the extent and type of lesion at the start of treatment did not seem to influence the relapse rates. The duration of treatment between 6 to 17 months was inferiorto that of 24 to 35 months. Higher rate of relapse was also seen if the lesion at the *end of* treatment was not exclusively fibrotic.

S.P.P.

Izoniazid Prophylaxis in Contacts of Persons with Known Tuberculosis

Ovid B. Bush; J.R., Masamitsu Sugimoto; Youshihiko Fujii and Frank A. Brown, JR.
Amer. Rev. ofResp. Dis. 1965, 92, 732.

A double blind study on chemoprophylaxis was started in Osaka in 1957 in collaboration with the U.S.P.H. service programme. A total of 2,205 household contacts of 650 index cases were included. Of these 0.6% did not complete initial examination and 1.9% were found to have active tuberculosis on initial examination and were therefore excluded from the study. One thousand one hundred and forty two contacts were given INH for one year and the remaining 1,096 were give a placebo. Nearly 55% contacts took INH regularly throughout the entire year and in 16% the regularity and accept-

ability of treatment was less than 25%. During the year of medication, 8 contacts in the INH group and 11 in the placebo group developed active tuberculosis This difference is not significant and is contrary to the U.S.P.H. service study result.

S.P.P.

A New Sign for Evaluating Pulmonary Cavities: The "Wall Sign"

Christoforidis A.J.; Nelson S.W.; & Pratt P.C.
Radiology; 1964, 83, 460.

A new sign for evaluating the status of cavitary lesions of the lung has been observed during bronchography. When barium sulphate suspended in a solution of normal saline containing 1.75 per cent sodium carboxyrrethylcellulose enters a cavity, subsequent roentgenograms may outline its wall for a long period of time (positive "wall sign"). This is caused by phagocytosis of the medium by the macrophages in the wall of the cavity when it is infected and ulcerated. On the other hand, there is prompt and complete elimination of the contrast medium from uninfected cavities. Thus, the presence of a positive 'wall sign' invariably indicates that the cavity is infected, whereas the negative wall sign is indicative of an uninfected non-ulcerated cavity. Of 18 cavitary lesions demonstrating the positive wall sign, 4 were abscesses, 5 were infected bronchogenic cysts, and v were tuberculous cavities. In the group of 9 cases with the negative wall sign, 7 were brochogenic cysts and 2 were open healed epithelialized tuberculosis cavities without histological evidence of ulceration in the wall.

S.P.P.

An Immediate Skin Test for the Diagnosis of Active Pulmonary Tuberculosis.

Harry Glenchur, Byron E. Fossieck, JR., and Milton Silverman Amer. Rev. ofResp. Dis. 1965, 92, 741

Mycobacterium tuberculosis (H37Ra) bacillary extract was separated by column chromatography under near physiologic conditions. One of these fractionated products (17) was Hssayed as a potential immediate skin-test antigen for diagnosis of and screening for active tuberculosis. One hundred and six patients with active pulmonary tuberculosis were tested, and a positivity test rate of 90.6 per cent was obtained. The time of onset of the positive reactions suggested an Arthus' phenomenon. Sixty one control patients (both PPD negative and positive) were all negative to this test

S.P.P.

Tuberculin Reactions in Malnourished Children
Harland, P.S.EG.
The Lancet; 1965, (ii), 719.

Children with subnormal growth due to a protein-deficient diet showed a significantly lower ($P < 0.01$) Tuberculin reaction after BCG vaccination, The reaction showed impairment even when the test was repeated after 4 months. The degree of impairment was directly related to the weight of the child as a percentage of its expected weight for age. The impairment however was not absolute, and was overcome by increasing the test dose of Tuberculin to 20 T.U. and improvement in nutrition and weight of the children.

S.P.P.

Spontaneous Pneumothorax
Lynn, R.B.
Dis. of the Chest; 1965, 48. 251.

Datas are presented with regard to 106 patients of radiologically proved spontaneous pneumothorax in Canada. Of these 97 were males and 9 females, age varying from new born to 79 years. The onset of symptoms was associated with exertion in 13 patients only. Chest pain and dysponea (85% and 70% respectively) were the most common presenting symptoms and 3 patients were asymptomatic. Hydrothorax was the most common complication, present in nearly l/3rd cases. Of the associated diseases bullous emphysema was commonest (43 patients), next was asthma (12 patients). Pulmonary tuberculosis was present only in 5 cases. Conservative treatment aiming at early and rapid re-expansion of the lung with some form of pleurodesis (hyper-tonic glucose solution is recommended) is the treatment of choice. However, if it fails or the attacks recur or become disabling, thoracotomy with bullectomy is recommended.

SP.P.