THE INDIAN JOURNAL OF MEDICAL RESEARCH

Vol. 72, (Suppl.) July 1980

ISSN 0367—9012

Tuberculosis Prevention Trial, Madras

INDIAN COUNCIL OF MEDICAL RESEARCH
NEW DELHI

Indian J Med Res 72 (Suppl), July 1980

CONTENTS

Indian J Med Res 72 (Suppl.), July 1980, pp 1-74

Trial of BCG Vaccines in South India for Tuberculosis Prevention

Tuberculosis Prevention Trial, Madras*

The protective effect of BCG vaccination in man has been evaluated in a number of controlled trials. In these trials, the protection observed varied from none to 80 per cent. In view of these conflicting results, a large scale BCG trial was planned in India and the protective effect of BCG vaccination evaluated in a controlled, double-blind, community trial near Madras in south India in a population of about 360,000 persons. In this trial, all individuals aged 1 yr and above were tested with 3 IU of PPD-S and 10 units of PPD-B, and simultaneously, BCG vaccines and placebo were allocated randomly to all those aged 1 mo and above. All individuals aged 10 yr and above were X-rayed, and from such persons whose photofluorograms were interpreted as abnormal two specimens of sputum were collected and bacteriologically examined. Intensive efforts were made, by means of regular follow-up surveys every 2½ yr and more frequently, by selective case-finding among suspects and further by maintaining permanent diagnostic services for symptomatics, to identify all new cases of tuberculosis occurring in the community. Mutually exclusive random samples of the population were retested with tuberculin at 2½ mo, 2½ and 4 yr after the intake in order to evaluate the tuberculin sensitivity over time in the study population. The study population was characterised by a high prevalence of tuberculous infection nd disease as also by a very high prevalence of nonspecific sensitivity. This report presents findings of the first 7½ yr of follow-up. The tuberculin sensitivity induced by BCG vaccination was highly satisfactory at 2½ mo but waned considerably between 2½ mo and 2½ yr with no further waning in sensitivity thereafter. Incidence of infection was high in the study population. However, incidence of bacillary disease was more frequent among initial tuberculin reactors, especially among the older persons, than among non-reactors of whom the majority were in the younger age groups. The distribution of new cases of pulmonary tuberculosis among those not infected at intake did not show any evidence of a protective effect of BCG. Certain hypotheses that may explain the findings have been discussed.

*The Tuberculosis Prevention Trial, Madras, is a project under the auspices of the Indian Council of Medical Research, New Delhi in collaboration with the World Health Organisation, and (until June 1976) the Centre for Disease Control, US Public Health Service, USA. This report was prepared by Dr G. V. J. Baily, Project Director, Dr Raj Narain, formerly Project Director, Dr S. Mayurnath, Assistant Project Director, Shri R. S. Vallishayee, Senior Statistician and Dr J. Guld, Consultant, World Health Organisation. Bacteriological investigations were carried out during intake at the Union Mission Tuberculosis Sanatorium (Director at that time : Dr J. Frimodt-Møller), Madanapalle, and later at the Tuberculosis Research Centre (Director : Dr S. P. Tripathy), Madras

WHILE the protective effect of BCG vaccine against tuberculosis can be demonstrated experimentally through vaccination of suitable animals and subsequent challenge with *Mycobacterium tuberculosis*, evidence of the protective effect in man is best obtained by means of controlled clinical trials. A controlled clinical trial should as far as possible be, double blind, and the allocation of treatments random.

A number of such controlled BCG trials have been carried out in recent decades with very conflicting results. A trial in a population of American Indians[1] in the late 1930's indicated a high protection (of the order of 80 per cent) and so did a trial among British school-leavers[2,3] started in 1950. In these trials the populations were followed for twenty and fifteen years respectively. Of the four trials in the United States, started between 1947 and 1950, the one carried out in Puerto Rico[4] indicated a modest protective effect estimated at 31 per cent while two trials in Alabama and Georgia[5,6] and one in Illinois[7] failed to show any protection. Also started around 1950, a trial in south India[8,9] indicated moderate protection. For a detailed review and discussion of these trials the reader is referred to Sutherland[10].

An explanation offered at an early date[11] for these contradictory results was that sensitisation by mycobacteria other than *M. tuberculosis* causing nonspecific sensitivity to tuberculin, might interact with, and possibly mask, the protective effect of BCG vaccination. But it has also been suggested that the potency of the vaccines used may have varied[12] either in terms of simply the dose, or more likely in terms of differing antigenic value of the strains of BCG used.

Vaccines for the different trials were prepared in different laboratories from cultures that had been propagated for many years and had therefore been subject to mutation and selection of mutants (in fact, the original BCG strain is not, strictly speaking, a 'strain' according to present standards, since it was not derived from a single colony but from a whole culture[13]). Differences in immunogenicity and virulence among the strains used in some of the trials could be demonstrated in experimental animal models and the potency differences observed in these models were closely associated with the observed differences in protection[14-17]. Thus, the strain could indeed be an important variable. This is also suggested by the fact that, for the trials conducted in Georgia, Alabama and Illinois in which BCG vaccination appeared ineffective[5-7], the vaccine had been prepared by the same laboratory. On the other hand, vaccine from this laboratory proved highly effective in another trial among Chicago infants[18]. In another trial, among British school-leavers[3], two very different vaccinations with BCG and vole bacillus vaccines (the former administered by injection, the latter by multiple puncture) appeared to give the same level of protection. The actual relevance of the vaccine strain *vis-a-vis* the protective effect in man therefore remains problematic.

Very little is known about the correlation of dose of BCG and protection against tuberculosis in man. On the other hand, the dose-response function for delayed hypersensitivity in man is well known : roughly speaking, a ten-fold decrease of the dose may be expected to yield, in post-vaccination tuberculin testing, reactions some 2-5 mm smaller in

diameter[19,20]. In complete contrast, the dose-response function in the guineapig is zero over a wide range (i.e., from the standard dose down to 10^{-5} of that dose), if only sufficient time is allowed after vaccination. This has been documented with great precision for delayed hypersensitivity[20] but also seems to be true for protection[21]. A retrospective analysis of the British trial indicated that the variation in the protective effect for batch-to-batch variations in viable units over a range of 4:1 was too small to be statistically significant[22]. However, vaccines from different laboratories vary more than that and vaccinations are often carried out with vaccines that are severely damaged in terms of reduction in viable counts at the time of vaccinations either due to poor storage or faulty handling.

It is now generally accepted that low-grade tuberculin sensitivity is nonspecific, i.e., due to sensitization by mycobacteria not identical with *M.tuberculosis,* yet antigenically related [23-27]. Such nonspecific tuberculin sensitivity is highly prevalent in most tropical areas and exists also in some subtropical areas[28]. Studies of delayed hypersensitivity to mycobacterins prepared from various mycobacteria indicate that the causative organism(s) may be antigenically close to *M. avium* and to Runyon's groups II and III[29-32]. It has been suggested that such sensitization could be associated with protection against tuberculosis and possibly mask the protective effect of BCG vaccination. That hypothesis was confirmed in experiments in mice[33] and in guineapigs[11]. This was also borne out in a surveillance programme of naval personnel[34] and in a retrospective study of a rural population in south India[35]. In their extensive series of experiments, in guineapigs, Palmer and Long[11] were able to demonstrate a protective effect of *M. avium,* of *M. kansasii,* and of a scotochromogen, inferior to the protective effect of BCG but still quite significant. In order to simulate the vaccination with BCG of persons having nonspecific sensitivity, they superimposed BCG vaccination on infection with *M. avium* or 'atypical' mycobacteria and found, comparing with animals vaccinated with BCG alone, that the protective effects were not additive : "Cutaneous tuberculin sensitivity and anti-tuberculosis effects which follow BCG vaccination are the same regardless of whether or not a previous mycobacterial infection had already produced some tuberculin sensitivity and anti-tuberculous effects". The authors offered this phenomenon as an explanation for the contradictory results of the American trials from 1947 and the British trial, pointing out that the trials with low or no protection were carried out in populations "saturated early in life with infections with atypical mycobacteria". In those trials in which BCG vaccination proved ineffective, or had a low effectiveness, the presence of nonspecific sensitivity was indeed demonstrated, but detailed quantitative analyses showed that nonspecific sensitivity alone could not have masked completely the effect of a vaccine that would have had a high efficacy in a nonsensitised population[12,36]. Also, in the trial in Puerto Rico in which a modest protection was observed, this applied equally to the population with and without low-grade sensitivity[37]. In India, nonspecific sensitivity is highly prevalent except in a few areas situated at higher altitudes[38-40]. Thus while knowledge of the protective effect of BCG would be desirable among both kinds of populations, i.e., with a

high prevalence of nonspecific sensitivity and without, the former would be far more relevant in India, as in most other tropical countries.

Finally, it should be mentioned that one factor, the presence or absence of tuberculous infection in the trial subjects was in fact considered in relation to selection of subjects in all the trials. However, in view of the very rationale for vaccination against tuberculosis, this factor was implicitly assumed to play such an important role that no attempts were made to investigate it further; infected subjects have invariably been excluded from vaccination trials. The WHO Expert Committee on Tuberculosis, in its 8th report[41], recommended the omission of tuberculin test for screening before vaccination "....in countries where BCG vaccination is essential to the effective control of tuberculosis, where cost is of major importance, and where prior tuberculin test would considerably reduce the coverage". Such direct vaccination is being practiced extensively in many developing countries including India. Judging from the size of accelerated vaccination lesion, it appears that a vaccine dose of BCG is equivalent to a few units of tuberculin only. A number of studies in recent years have shown that vaccination of persons infected with *M. tuberculosis* results in vaccination scars that are slightly larger than in uninfected persons. No other significant effects have been demonstrated. Such studies have been reviewed by Ten Dam[42] and Egsmose[43]. An entirely different possibility is that BCG might increase the specific immunity in people infected so many years ago that their naturally acquired immunity (from the original, virulent infection) might have waned. A sufficiently large study including elderly people with a high incidence of tuberculosis would illuminate this question.

Neither the empirical findings of the field trials nor other epidemiological observations gave any indication of further relevant factors, but lack of detailed epidemiological knowledge could well have been important in this respect. The apparently conflicting trial results, as well as the fact that the vaccines employed in the trials cannot be reproduced, called for further and more systematic research. To some extent, this had become easier because of the technical progress made meanwhile in the preparation of BCG vaccine. Freeze-drying techniques developed made it possible to prepare a stable product from any BCG strain, and to maintain the strains as seed lots without the risk of further genotypic alterations[44]. Accordingly there was a need to undertake further field trials with vaccines prepared from different seed lots and in different dosages, and to conduct these trials in populations where nonspecific tuberculin sensitivity was present and in others where it was absent; the incidence of tuberculosis in the study population should be fairly high, and BCG vaccination should not be a current public health measure. A trial satisfying most of these conditions was organised in south India by the Indian Council of Medical Research (ICMR), New Delhi, in cooperation with the World Health Organisation and the Center for Disease Control, US Public Health Service, in a population where nonspecific tuberculin sensitivity was highly prevalent. Unfortunately, a contrasting area in which nonspecific tuberculin sensitivity was of low prevalence, or even absent, could not be identified in India.

The objectives of the present trial were

to obtain : (1) a precise estimate of the protective effect of BCG vaccination against tuberculosis in the non-infected, (2) the effect of BCG vaccination in persons already infected,(3) the protective effect of different strains of BCG, (4) the influence of dosage of BCG on the protective effect and (5) epidemiological data on tuberculosis in the community.

2. History of the BCG trial

In 1963, the Government of India took up the question of conducting a BCG trial in India. There was still some controversy in the country about the use of BCG, and it was felt that the problem had to be settled by a controlled field study under Indian conditions. The WHO secretariat was consulted and it was agreed that such a trial involving a large population and followed over a number of years would require substantial resources and thus need funding from special sources. The project was initially financed largely from the United States PL 480 funds, supplemented by a WHO research grant. Later the WHO grant was supplemented by grants from the Danish government. When the PL 480 grants ceased in 1976, the main funding of the project was done from research grants from the Department of Science and Technology, Government of India. As from 1979 the main funding of the project is being done by the Indian Council of Medical Research. Administratively, the project was originally a section of the National Tuberculosis Institute, Bangalore, and from 1966, a separate project under the Indian Council of Medical Research.

A research protocol for a controlled field trial (with placebo and with 2 doses and 2 strains of BCG) was accepted by the Indian Council of Medical Research in 1968. As it was envisaged that the project would function as a section of the National Tuberculosis Institute it was decided, for operational convenience, to conduct the study in an area in south India, not far from the Institute. Most of south India has been repeatedly covered with BCG, with a coverage which, while rarely above 30 per cent among the younger age-groups, was still high enough to interfere seriously with a controlled trial. Two areas, however, had been kept free of BCG vaccination programme over the years : part of Bangalore District in Karnataka State, and all of Chingleput District (near Madras city) in Tamil Nadu State.

With access to data already collected in Bangalore District, by the National Tuberculosis Institute[45,46], and after the project had carried out sample surveys of its own in Chingleput District[47], it became clear that the prevalence of tuberculosis was 50 to 100 per cent higher in the latter place, which accordingly was selected for the trial. A contributory justification for selecting this area was its high prevalence of leprosy: the hope at that time was to examine simultaneously the possible preventive effect of BCG against leprosy, already the subject of studies elsewhere with conflicting results[48-50].

The sample surveys in Chingleput District were carried out in Kanchipuram Taluk, located in the southern part, and Ponneri Taluk. located in the northern part of the District, each survey covering villages totalling 10,000 to 12,000 persons. The prevalence of bacillary tuberculosis in persons 10 yr and more was estimated at 9 per 1000 in Ponneri and 7.5 per 1000 in Kanchipuram and it was therefore decided to include part of Ponneri Taluk,

the whole of Trivellore Taluk (comprising Kadambathur, Trivellore, Poondi and Ellapuram panchayat unions), and part of Tiruttani Taluk (Tiruvalangadu panchayat union). However, during intake it turned out that the cooperation of the population in Ponneri Taluk was vastly inferior and eventually Ponneri was abandoned for the trial.

3. Study design

Factorial design — The experimental design used in the study may be described as a "factorial design" which is an integral element in the now classical experimental methodology developed by Fisher[51] and others. In such a design, two or more factors are varied simultaneously, usually in such a way that each level of each factor is tested equally often for any combination of levels of the other factors. If the effect of one factor depends on the level of another factor, this design will ensure against false generalisations which might have been made from an emperical estimate obtained under one particular set of conditions. It is desirable for an optimal factorial design that it should be 'balanced', i.e., contain all possible combinations of the various factors under investigation.

The design can be considered to be a factorial design, the factors being the vaccine strain (and placebo) and the vaccine dosage. These factors were combined in a single experiment by allocating one of the following treatments, randomly to any individual.

(a) Strain 1331, 0.1 mg; (b) Strain 1331, 0.01 mg; (c) Strain 1173 P2, 0.1 mg; (d) Strain 1173 P2, 0.01 mg; (e) Placebo.

It will be observed that the use of four equal subgroups (a), (b), (c) and (d) above is superior to a design (with the same total population) with, say, only the first three subgronps. With four equal sub-groups, the strain effect would be (a + b) — (c + d) and the dose effect (a + c) — (b + d)*. With only three subgroups the strain effect would be (a—c) and the dose effect (a — b). In the former design, every person contributes to either estimate, in the latter only two-thirds contribute to either estimate.

It is also an advantage to have a common control group for all the vaccinated groups obtaining an average protection of (e) — k (a+b+c+d), where k is a factor adjusting for group size. Otherwise to carry out two trials e.g., one with (a) above versus a control group (e) and the other (a), (b), (c), (d) above but without controls would be a distinctly inferior design both in economic and in logical terms.

Allocation of vaccines — It was decided to make the allocation completely random i.e., without any attempt at balanced or stratified allocation or by cluster. With a study of this size (*see*, Size of population) the proportion of persons thus allotted to different vaccines within each stratum will differ very little and not only the allotment itself but also the later analysis is greatly simplified. In the analysis of the follow-up it is permissible to disregard the 'sampling error' in allocating different vaccines. The results seen as morbidity (or mortality), even when cumulative over the years, would rarely exceed one in a hundred; thus the sampling error would be very much larger for the numerator (number of cases) than for the hundred times larger denominator (number vaccinated) and it can be shown that

*The last 'independent contrast', (a—b—c+d), is interaction of strain and dose

under these circumstances the variance of the rate will increase by only one per cent if the number vaccinated is not counted but deduced from the *a priori* probability of being vaccinated. One minor deviation was made from the complete individual randomisation, in that the two BCG strains were allocated randomly to clusters each consisting of persons vaccinated from one set of three ampoules : two ampoules with 0.1 and 0.01 mg of the same strain and one placebo ampoule. In reducing the number of ampoules used simultaneously to three rather than six, the strain factor was chosen for confounding with clusters. For the entire study since the number of such clusters was expected to be large (about 1000 clusters for each strain) it was presumed that this factor would also be well randomized.

Size of population — In the earlier trial in India by Frimodt-Møller and associates[8], the protection is quoted as 60 per cent, with a confidence interval (at the 95 per cent level) of 14 to 84 per cent. The confidence interval varies, roughly speaking, with the square root of the number of cases. There were 30 new (incidence) cases, in this earlier trial, arising among 11,000 initially non-infected persons. But even with 1000 cases similarly distributed in a new trial, i.e., 715 cases among a non-vaccinated half of the population and 285 among the vaccinated, the confidence interval (at the 95 per cent level) would still be from 54 to 65 per cent. It follows that the trial should be as big as financial resources, qualified manpower and operational conditions permit. It was expected that out of all new cases a sizeable fraction would belong to those tuberculin negative at the time of vaccination. In the longitudinal survey of the National Tuberculosis Institute, Bangalore[52], during the first 1½ yr of observation 19 (28 per cent) cases out of a total of 67 incidence cases originated from the tuberculin negatives. It was thus expected that in a study population of 250,000 persons with an annual incidence of 2 cases per 1000, already the first 2½ yr should yield 1250 cases out of which 350 would be previously negative and thus contribute to the main purpose of the trial, yielding a significant if not a very precise estimate.

4. Choice of BCG strains, vaccine dosages and placebo

Of the several strains of BCG that exist and are being used for vaccination, only strains fulfilling the requirements formulated by the WHO Expert Committee on Biological Standardization[44] were considered. The most fundamental of these requirements is that a strain must exist as a well-defined and well-examined 'primary seed lot', so that the vaccine used in the trial can be reproduced at any future time. Preferably, the residual virulence in man should be well-known from previous wide use in vaccination programmes. A number of such strains exist and are known from studies in recent years to differ among themselves in many respects, as mentioned in the Introduction. The question was whether to select only one strain or whether to include several with widely differing properties. The most tempting was no doubt strain 172 (the 'Tokyo' strain), which has an exceptional resistance to freeze-drying (T. Hashimoto, personal communication), and to adverse storage conditions[53], but which combines a high allergenic potency in man[54] with a comparatively low activity in certain experimental animal studies[55-57].

But while the inclusion of widely different strains would have been of scientific interest, it would have implied a corresponding increase in the study population. In a final compromise, two strains were selected that are quite alike in terms of *in vivo* activity though their *in vitro* properties differ to some extent. In the unlikely event that one of the two strains turn out to be better than the other, this in itself would be a result of great practical importance. The strains selected were 'strain 1331' and 'strain 1173 P2' referred to commonly as the 'Copenhagen' and the 'Paris' strains respectively.

Strain 1331 — There were two particular reasons for including this strain: it has been used in India since 1966 for all routine production of BCG vaccine and it is derived from a strain which was used in the successful British trial in 1950-52. The history of this strain is as follows :

In November 1931, the Statens Seruminstitut in Copenhagen received a new BCG culture, labelled 423, from Institut Pasteur, Paris, and abandoned the one used previously as being too virulent. Over the following years, apart from the cultures kept for seed in the routine production, several other lines were propagated for varying periods of time on either liquid Sauton medium or on bile-potato. (Here and in the following, the word 'line' is used for a separate series of sub-cultures not suspected of being genetically different from the mother strain). In 1949, a line that during the previous five years had been kept exclusively on bile-potato was transferred to Sauton via one passage on Sauton-potato, and kept on liquid Sauton ever since by transfer every tenth to eleventh day. Three parallel lines were kept with staggered intervals so as to allow production on one particular weekday every week. The liquid vaccine supplied for the British trial must have consisted of virtually equal parts of these three lines. During the period 1950—1958 nearly all cultural transfers were made by the late Dr K. Tolderlund, who aimed at keeping the growth characteristics of the strain constant by always selecting 'typical' parts of the previous culture for seeding the next; no change was observed in the morphology of the strain during these years (K. Bunch-Christensen, personal communication). In October 1958, an ampoule of liquid vaccine from Copenhagen (routine batch number 1331) was transferred, in the Japan BCG laboratory, Tokyo, to glycerol bile-potato and was kept on this medium with a total of 45 serial transfers, until September 1960 when it was transferred via one passage on Sauton-potato back to Sauton, the second passage of which was freeze-dried with 1 per cent sodium glutamate, as one batch comprising 5 drying lots (T. Sawada, personal communication). The batch was later transferred to Copenhagen and was examined extensively both as vaccine and as seed material. The viability was found to be rather low, indicating a survival after freeze-drying of approximately 5 per cent. But as a seed it was found to be very suitable, yielding vaccines that were indistinguishable from those produced from the lines maintained traditionally by serial sub-cultures on Sauton (K. Bunch-Christensen, personal communication). The batch was adopted in 1966 as the 'Copenhagen' seed lot. It is stored in deep-freeze, and tests over the years have indicated no significant loss of viability.

Strain 1173 *P2* — This strain is distri-

buted from the Institut Pasteur, Paris, and is currently used in more than 20 BCG production laboratories in all parts of the world. It was derived in 1961 from a single colony that (out of 30 colonies examined) yielded cultures that (especially in terms of pigmentation) "had properties most closely representative of the usual BCG, that is, the Paris BCG strain" (J. Augier, F. Kosloff, personal communication). This strain, 1173 P, was lyophilized and the second batch, 1173 P2, is now the primary seed lot of the 'Paris' strain. Only a small number of ampoules are left of this batch, so that for all ordinary production purposes secondary seed lots are used, three passages removed from the primary lot.

The strain shows a high and consistent activity in several animal models and a high allergenic potency in man, as demonstrated in the studies already quoted.

Dosage—Nominal dosages of vaccines, from reputed laboratories, have varied in recent years between 0.2 and 0.015 mg (semi-dry weight), and deterioration under field conditions further widen the range. As mentioned earlier, a previous study[22] failed to show any difference in protection for an inadvertant variation in strength of vaccine of 4 : 1. It was decided for the present study to use doses of 0.1 and 0.01 mg, the former dose considered to be the strongest that could be used in young children without an undue complication rate, the latter a compromise that (judging from BCG induced tuberculin sensitivity) might still be expected to add measurable protection in persons with naturally acquired low-grade sensitivity.

Placebo — A double-blind technique, with placebo 'vaccination' of the control group, was considered essential to ensure identical treatment of the vaccinated and the controls. No serious attempt was made at finding a placebo that would result in a skin lesion simulating a BCG lesion. It was felt that to find a substance that would cause the same kind of scar yet be biologically innocuous would have been difficult. An innocuous substance (Dextran 500, Pharmacia, batch To 852, 0.625 mg per 10 ml ampoule) was therefore used, freeze-dried to form a powder of the same appearance as freeze-dried vaccine.

5. Preparation and testing of BCG vaccines

Vaccine preparation — The vaccines used in the trial were prepared as follows. An ampoule of the seed lot was reconstituted with 1-2 ml of Sauton medium and seeded on Sauton-potato. After 3 to 4 wk a loop of the pellicle was transferred to liquid Sauton medium from where, after 10 days, transfer was made to a second Sauton medium from which the harvest for vaccine production was collected after 6 days (for 1173 P2) or 7-8 days (for 1331). The microscopic morphology, the *p*H, and the sterility of the culture were examined for each flask and flasks with abnormal findings discarded.

Liquid surplus was removed in a Birkhaug apparatus, pooling harvests from 3 or 4 flasks, and a suitable amount of the moist bacterial mass was transferred to a ball-mill for homogenisation and dilution with distilled water with sodium glutamate, 4 per cent, to predetermined concentrations in terms of moist weight. These suspensions were then dispensed in ampoules, frozen immediately afterwards, and kept in deep-freeze till the day of freeze-drying. Several lots might thus

be prepared from one batch, each lot dried on a different day, but all originating from a single suspension. Freeze-drying was performed over 20 h and the ampoules hand-sealed under vacuum immediately after drying. In Copenhagen, suspensions of 20 and 2 mg per ml were dispensed with 0.5 ml per 10 ml ampoule, resulting in ampoule contents of 10 and 1 mg respectively. In Madras, 5 ml ampoules were used throughout, containing 5 and 0.5 mg respectively.

The vaccines were released for use only after satisfactory completion of all routine tests in the production laboratories, i.e., tests for viability, for safety and for non-contamination.

Vaccine lots — Vaccines and placebo were supplied 6 times to the trial thrice from Copenhagen and Madras each. Each supply comprised one batch of 1331 and one of 1173 P2, each in one or two drying lots for each of the two dosages.

For preparation of vaccine from 1331, the primary seed lot was used in all cases. For 1173 P2, a special seed lot, 1173 P2S dated April 1968, was prepared in Copenhagen from the French seed lot 1173 P2 lot 5. 1173 P2 lot 5 is 6 passages removed from P2. The first of the six batches from strain 1173 P2 used for vaccination in the trial was P2S itself (though a separate drying lot) ; the other five were prepared with P2S as seed lot, three passages removed. Thus the 1173 P2 vaccines used in the trial were 9-12 passages removed from P2.

Of the primary seed lot 1331, close to 2000 ampoules, of 1173 P2, 80 ampoules, of 1173 P5, 300 ampoules and of 1173 P2S, 2000 ampoules are still available.

Randomization and labelling of ampoules — The four vaccines and the placebo ampoules were packed by the production laboratories in cardboard boxes of three ampoules each. Boxes were numbered A 001—A 999, B 001—B 999, etc., and for each box number the strain (either 1331or 1173 P2) was selected at random by means of a table of random digits (there was thus no stratification and therefore no equalization of the total number of boxes of one and the other strain). Each box contained an ampoule with 0.1 mg BCG per 0.1 ml (after reconstitution), an ampoule with 0.01 mg per 0.1 ml (of the same strain) and a placebo ampoule. A random permutation of the digits 1, 2 and 3 was allotted to each box, for random coding of the three ampoules. From the randomized scheme thus obtained (see Table 1), sheets of labels were typed, each sheet for one kind of vaccine only, and the labels were (after the sheets

TABLE 1 — SPECIMEN COPY OF RANDOMISED SCHEME FOR ALLOCATION OF AMPOULES TO BOXES

Box No.	Strain	Ampoule number		
		0.1 mg	0.01 mg	Placebo
A-501	Danish	1	3	2
A-502	French	2	3	1
A-503	French	2	3	1
A-504	French	2	3	1
A-505	Danish	2	1	3
A-506	French	3	1	2
A-507	Danish	2	1	3
A-508	French	3	2	1
A-509	Danish	2	1	3
A-510	French	3	2	1
A-511	French	3	2	1
A-512	Danish	2	3	1
A-513	French	2	3	1
A-514	French	1	3	2
A-515	Danish	1	3	2
A-516	French	3	2	1
A-517	Danish	2	3	1
A-518	Danish	1	2	3
A-519	Danish	2	3	1

had been photocopied, see Fig. 1) applied to one lot of vaccine at a time. After all the lots of a particular supply had been labelled, they were packed as described above, three in each box, at which occasion errors such as use of the same code number for two ampoules would be revealed.

Custody of codes — The complete set of codes (illustrated in Table 1) is kept by the production laboratories. The project's statistical staff received during the earliest stage of the follow-up, copies of the codes for samples of population in whom the immediate effect of vaccination was assessed (see next para), but these copies were destroyed in each case as soon as the analysis of the assessment was completed. Later, the codes as they appeared on the photocopies of labels (Fig. 1) were punched and transferred to tape outside India and copies of this tape stored in Geneva (WHO) and New Delhi (ICMR). All further decoding of the vaccination status of cases (and other strata of the population) is done by transmitting the recorded vaccine ampoule code numbers for all diagnosed cases to New Delhi from where the desired statistical tabulations are returned to the project. Thus the vaccination status of the individual patient will remain unknown to the project staff.

Assessment of vaccination lesions in man — As a running check on gross errors in the allocation and registration of vaccinations, a 10 per cent cluster sample was randomly selected for examination of vaccination lesions 2½ months (av. 75 days, range 62-99 days) after vaccination. For selecting the 10 per cent sample, each panchayat, which is an administrative unit consisting of 2-3 villages, was divided into 10 clusters of households. Households in one of these clusters, selected at random, constituted the 10 per cent sample. All persons in the selected clusters of households, except for those registered after intake, were eligible for lesion reading. In each person the diameter of tissue destruction, i.e., pustula, open ulcer, scab or scar, was measured. The examination was performed without reference to the pre-vaccination tuberculin status, and indeed without access to the coded vaccination record; thus the examiner did not know whether any two persons had been vaccinated from the same ampoule. The coverage obtained were of the order of 80-90 per cent.

For the vaccine ampoules used in the clusters thus assessed, the project obtained a decoding from the respective production laboratories, and the distribution of lesions by size was tabulated for each ampoule. The proportion of gross errors revealed by this procedure was extremely small, and thus the allocation procedure could be deemed to be satisfactory.

After 2½ yr, an additional post-vaccination examination was carried out in another cluster sample, of 5 per cent. The sampling methodology was as follows: firstly, one out of every 4 panchayats was selected at random and then in the selected panchayat two out of the ten blocks of households were randomly selected giving a 5 per cent random sample. If by chance one of the two blocks selected had already been selected earlier for the 2½ month retesting, it was just passed over and the next block in the random order selected. All individuals in the households included in these blocks were eligible for retesting except children born after intake. The remaining clusters, 85 per cent, were included for examination after four years. For these two later examinations of lesions,

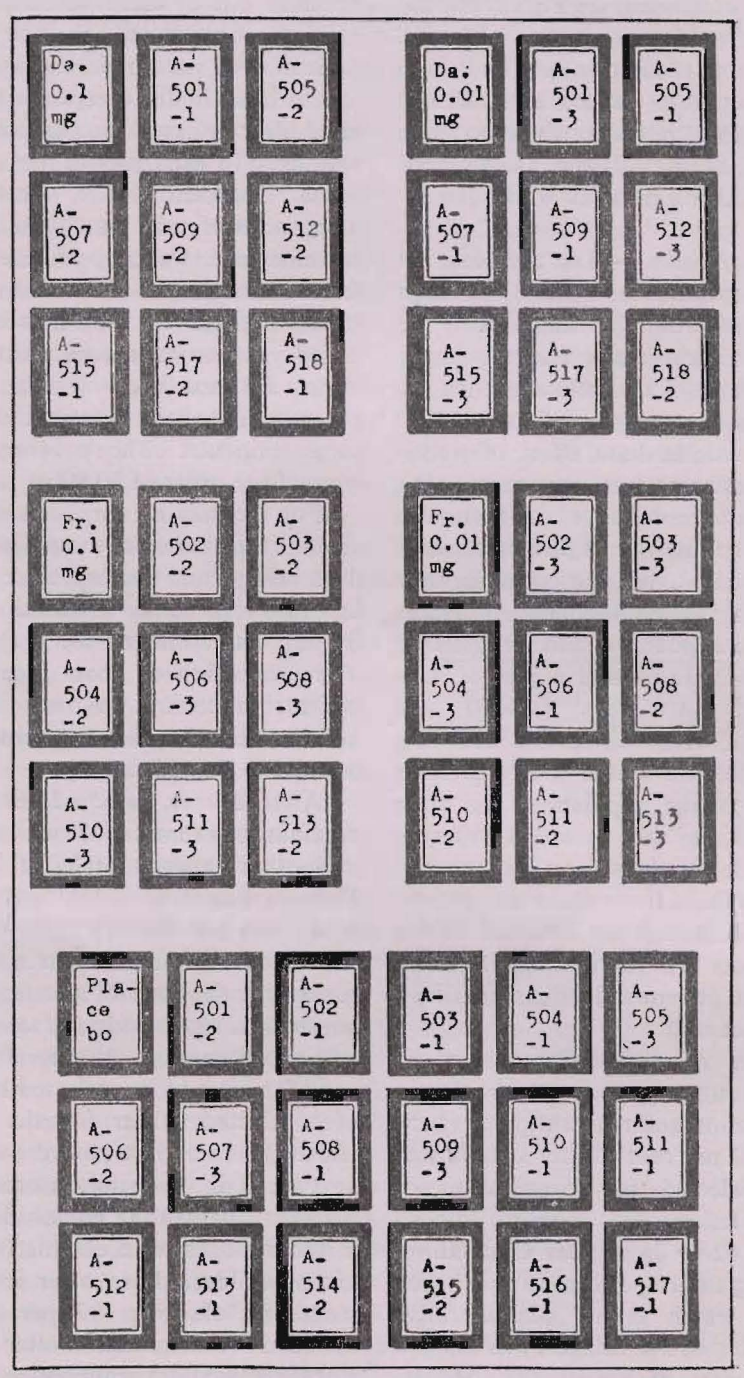

Fig. 1 — Specimen sheet of labels for ampoules

the distributions of scars by ampoule were tabulated by computer by the statistical department of the Indian Council of Medical Research, New Delhi.

In a random sample of BCG boxes, representing persons examined for lesions at each of the three examinations mentioned above, the distribution of scars (presence or absence) for each of the two vaccine doses and the placebo was seen. The proportion of lesions (scars) observed was 98.5 per cent for the 0.1 mg dose of vaccine, 85 per cent for the 0.01 mg dose of vaccine, and 10 per cent for persons given the placebo.

Three statistical tests were constructed for picking out exceptionally unlikely distributions of lesions by ampoule. The deviation from a proportion of 98.5 per cent scars for the 0.1 mg dose of vaccine, 85 per cent for the 0.01 mg dose of vaccine and 10 per cent for the placebo, was assumed to vary from ampoule to ampoule according to the binomial distribution, and this assumption was tested by means of the chi-square test; further, the distribution by size of lesion (scar) was presumed not to have a larger mean for the ampoule with 0.01 mg dose of vaccine than for the ampoule with 0.1 mg dose of vaccine, from same box, and this was tested by means of a one-tailed t-test. The distributions of lesions for the three ampoules of each box were tested for disagreement with each of these four hypotheses, both at a probability level of one in ten thousand and a probability level of one in a thousand. Out of a total of 2744 boxes, nine boxes fell outside the one in ten thousand level of probability, and an additional 3 boxes outside the one in a thousand probability. The nine boxes are much beyond the expected value (one in ten thousand out of 2744, or 0.3 box) while the additional 3 boxes between the one in thousand and the one in ten thousand level, quite correspond to expectations. Correspondingly, 501 persons (out of 260,000) recorded as having been vaccinated from any of the nine boxes have been classified as 'vaccination uncertain', while persons from the last 3 boxes have been accepted for the main purpose of the trial.

To what extent the nine errors originated in the production laboratories (e.g., wrong labelling) and to what extent in the field (e.g., filling a syringe from the wrong ampoule) is not clear from inspection of the actual distributions, but also obviously of little interest considering the rarity of such errors.

After excluding persons 'vaccinated' with vaccines or placebo from the nine boxes, it would still be of interest to study, for the rest of the boxes, the proportion of persons without a BCG scar, though vaccinated, and with a BCG scar, though given the placebo. The frequency of such (non-sampling) errors is shown in Table 2. Only persons between 1 mo to 29 yr are considered, as those 30 yr and above were not eligible for examination at 4 years. The magnitude of these errors was not much. However, for each of the three treatments, the frequency of errors at $2\frac{1}{2}$ and 4 yr was significantly more than that at $2\frac{1}{2}$ months ($P<0.01$).

Analysis of the data by age showed that the frequency of persons without a scar, though vaccinated (with either 0.1 or 0.01 mg dose) was the highest in the youngest age groups. This is not surprising and has been reported earlier; for instance Patel and Shaw[58] reported that a number of new born vaccinated infants did not develop a scar; nor is it surprising that a significantly higher proportion of

TABLE 2 — PROPORTION OF PERSONS WITHOUT A BCG SCAR, THOUGH VACCINATED, AND WITH A BCG SCAR, THOUGH GIVEN THE PLACEBO AMONG THOSE AGED 1 MO TO 29 YR

Treatment given	Interval from intake		
	2½ mo	2½ yr	4 yr
	0.1 mg of BCG		
No examined	6384	1758	23583
No without a scar	14	32	250
Percentage	0.2	1.8	1.1
	0.01 mg of BCG		
No examined	6330	1735	23630
No without a scar	340	218	2900
Percentage	5.4	12.6	12.3
	Placebo		
No examined	6650	1741	23833
No with a scar	183	118	1667
Percentage	2.8	6.8	7.0

persons given the 0.01 mg dose of BCG as compared to 0.1 mg dose did not develop a scar. But the greater frequency of vaccinated persons with no scar at 2½ yr and 4 yr as compared to that at 2½ months can only mean that the vaccination scar in a few persons did fade after the interval of 2½ yr and was no longer, at least easily, discernible. The frequency of such persons at 4 yr was not higher than that at 2½ yr suggesting that the maximum fading took place within the first 2½ yr.

On the other hand, the greater frequency of scars among those given placebo, at 2½ and 4 yr as compared to at 2½ months has a different explanation. It is likely that causes other than BCG vaccination (i.e., small boils or injuries at the vaccination site) gave rise to scars and these were mistaken for BCG scars. If so, the frequency of such non-specific (i.e., non-BCG) scars should not only be more after the longer intervals (as seen in Table 2) but also increase with age. Analysis of the data by age showed that this was indeed the case at 2½ and 4 yr but not so at 2½ months. Probably at 2½ months the scar was still 'fresh' and well marked (and in some cases even showing a scab, pus or a crust) and not likely to be mistaken for a nonspecific scar.

However, the important point to stress is that the magnitude of these errors as reflected by the results of lesion reading at 2½ mo was not much. In fact, these could be overestimates as they comprise not only errors of vaccination but also those of lesion reading. Thus, it can be safely concluded that after excluding persons 'vaccinated' from the nine boxes, as mentioned above, the results of lesion reading tallied well with the records of 'vaccination' and the allocation of 'vaccines' to the population can be considered to be satisfactory.

Post-vaccination allergy and local skin lesions soon after vaccination — Post-vaccination tuberculin testing was carried out in the first instance simultaneously with the reading of lesions (scars) 2½ mo after vaccination. (Retesting after longer intervals will be described in Chapter 15). Of the clusters selected for lesion reading a sub-sample of one-fifth (2 per cent of the total population) was selected (at random) for this testing. The coverage for retesting was lower than for the lesion reading. For persons below 15 yr of age and with an initial tuberculin reaction of 0-7 mm the coverage was 56 per cent for those vaccinated and 73 per cent for those given placebo (the retest may have been less acceptable for

those vaccinated because at that stage most of them still had a non-healed lesion after vaccination).

While this sample is representative for the study population (being a random sample) and permits valid comparisons of the two strains used, the 6 supplies are not strictly comparable because each supply has been used at a different time and in an arbitrarily selected fraction of the study area. For the last (sixth) supply, few persons were vaccinated and very few retested.

The retest dose was (as for the initial tuberculin testing) 3 IU of PPD-S. The reactions (transverse diameter of induration) were read after 3 to 4 days, without reference to the previous tuberculin reaction or vaccination lesion. The results have been analysed separately for 5-yr age-groups but no significant age trend was found. Post-vaccination tuberculin reactions, and local lesions, in children 1 month to 14 yr of age, tuberculin negative at intake (diameter of reaction 0-7 mm) are shown in Table 3, separately for each of the 12 batches.

As expected, both tuberculin reactions and skin lesions are definitely smaller for the 0.01 mg vaccine dose. Otherwise, there is no indication of significant batch-to-batch variation.

A direct comparison of two supplies, one from each laboratory — The second supply from Copenhagen and the first supply from Madras were used simultaneously in nearly the whole of Trivellore town (*see* Chapter 7), boxes from the two laboratories being allocated at random. (Trivellore had been divided

TABLE 3 — MEAN SIZE OF TUBERCULIN REACTIONS (2 PER CENT RANDOM SAMPLE OF THE POPULATION EXCLUDING 8 BLOCKS IN TRIVELLORE) AND LOCAL SKIN LESIONS (10 PER CENT RANDOM SAMPLE), 2½ MONTHS AFTER VACCINATION, AMONG THOSE TUBERCULIN NEGATIVE (REACTION 0-7 MM) BEFORE VACCINATION AND AGED 1 MO TO 14 YR

Production laboratory and strain	Date of Preparation	Tuberculin reaction size (mm)				Lesion size (mm)			
		Dose, 0.1 mg		Dose, 0.01 mg		Dose, 0.1 mg		Dose, 0.01 mg	
		No exam.	Mean size	No exam.	Mean size	No exam.	Mean size	No exam.	Mean size
Cph 1331	Mar 68	42	14.5	59	9.9	255	5.8	262	3.7
Cph 1173	Apr 68	31	15.0	27	10.9	237	5.4	190	3.7
Cph 1331	Nov 68	32	13.8	17	10.5	379	4.8	379	3.1
Cph 1173	Oct 68	57	15.5	60	12.8	409	5.2	375	3.6
Cph 1331	Nov 69	5	16.2	8	8.1	218	5.4	216	3.6
Cph 1173	Nov 69	2	14.0	4	9.0	125	5.3	127	3.6
Mds 1331	Sep 68	18	12.7	15	10.2	67	4.7	57	3.3
Mds 1173	Sep 68	6	18.7	9	9.4	73	5.1	75	3.4
Mds 1331	May 69	11	11.5	23	8.4	144	5.1	190	3.2
Mds 1173	May 69	10	14.0	9	9.8	201	5.4	192	3.3
Mds 1331	Apr 70	—	—	—	—	17	5.5	12	2.8
Mds 1173	Apr 70	—	—	—	—	16	5.2	13	1.6
Total		214	14.5	231	10.6	2141	5.2	2088	3.4

into nine blocks, and one of these had already been included for the 10 per cent random sample referred to above. The eight remaining blocks were used for the present comparison). All children from this area were offered retesting after 12 wk to obtain a direct and precise comparison of the two supplies. (Since all children were offered the test, this area could not be included in independent retestings at the later dates). The results are given in Table 4 separately for each dose of each of the four batches.

The tendency for the batches from Copenhagen to give a stronger response than the batches from Madras is so slight in magnitude that no biological significance can be attached to it. In conclusion, the results of both Tables 3 and 4 indicate a high uniformity in the potency of the batches used, irrespective of production laboratory or strain.

A comparison of two batches in Danish school children — Of the three batches prepared in Copenhagen from strain 1331, the first (batch no. 28) and the third (batch no. 46) were assessed in tuberculin-negative, 7-yr old school children in Denmark. The tuberculin test both before and after vaccination was an intradermal test of 2 units of RT 23 with Tween 80. The re-examination was carried out 8-10 wk after vaccination. For batch 28, the mean reaction size (in 66 children) was 16.2 mm (standard deviation 3.9) while the BCG lesions averaged 6.0 mm. For batch 46, the mean reaction size (in 117 children) was 18.5 mm (standard deviation 3.7) and the mean lesion size 6.6 mm[59,60]. (For a direct comparison of post-vaccination allergy in Indian and Danish children, using identical vaccines and tuberculin in both places, *see* Vallishayee and associates[61]).

In vitro *quality control* — In addition to the *in vitro* quality control tests done routinely at the production laboratories, it was decided to repeat these examinations, for all the batches of vaccines used in the trial, elsewhere than in the production laboratories. Randomly selected samples of all the six supplies were sent to different laboratories. These laboratories were instructed as part of their reference services for WHO, to carry out quality control tests. Tables 5, 6 and 7 give the supply numbers and the names of the production and investigating labo-

TABLE 4 — COMPARISON OF THE FIRST SUPPLY FROM MADRAS AND SECOND SUPPLY FROM COPENHAGEN IN CHILDREN OF AGE 1 MO TO 14 YR AND WITH AN INITIAL TUBERCULIN REACTION OF 0-7 MM, IN 8 BLOCKS OF TRIVELLORE

Production laboratory and strain	Date of preparation	Dose, 0.1 mg			Dose, 0.01 mg		
		No exam.	MRS (mm)	MLS (mm)	No exam.	MRS (mm)	MLS (mm)
Cph 1331	Nov 68	318	17.6	5.0	317	12.6	3.4
Cph 1173	Oct 68	252	17.9	5.2	271	14.6	3.6
Mds 1331	Sep 68	305	16.0	4.9	277	13.2	3.5
Mds 1173	Sep 68	253	16.8	5.0	265	12.8	3.4

MRS, mean reaction size; MLS, mean lesion size

TABLE 5 — ESTIMATES OF VIABLE UNITS (MILLIONS PER ML) IN LABORATORIES OUTSIDE[a] THE PRODUCTION LABORATORY

Supply No.	Product. lab. and strain	Date of prep.	Date sample shipped	Investigating laboratory: Moscow 1 mg/ml	Moscow 0.1 mg/ml	Prague 1 mg/ml	Prague 0.1 mg/ml	Madras/Budapest[b] 1 mg/ml	Madras/Budapest[b] 0.1 mg/ml	Copenhagen 1 mg/ml	Copenhagen 0.1 mg/ml
I	Cph 1331	Mar 68	Apr 69	2.5	cfl.[c]	6.9	0.04			8.6 — 6.0	not done
	Cph 1173	Apr 68	Apr 69	4.4	cfl.	0.0	0.0			5.4 — 7.8	0.4 —0.4
II	Cph 1331	Nov 68	Apr 69	5.5	cfl.	1.5	0.008	6.0 — 5.3	0.6 —0.3	5.5 — 5.1	0.4 —0.6
	Cph 1173	Oct 68	Apr 69	4.3	cfl.	1.8	0.007	5.0 — 3.4	0.7 —0.4	8.2 — 8.7	0.7 —0.7
III	Mds 1331	Sep 68	Apr 69	7.0	cfl.	1.8	0.03	6.7 — 6.0	0.8 —0.5	5.7 — 8.1	0.6 —0.6
	Mds 1173	Sep 68	Apr 69	11.0	cfl.	3.2	0.000	9.7 — 6.7	0.7 —0.4	8.9 —13.1	0.6 —0.6
IV	Cph 1331	Nov 69	Aug 70	2.48— 2.45	0.20—0.12	3.44— 2.63	0.16—0.06	2.10— 3.89	0.30—0.25	3.08— 3.28	0.24—0.26
	Cph 1173	Nov 69	Aug 70	2.53— 1.94	0.15—0.18	4.28— 4.57	0.31—0.30	4.55— 3.72	0.38—0.31	2.35— 2.20	0.22—0.22
V	Mds 1331	May 69	Aug 70[d]	7.08— 8.20	0.42—0.37	8.72—11.14	0.82—0.72	12.02—13.04	0.96—0.72	7.24— 7.06	0.66—0.59
	Mda 1331	May 69	Sep 70							9.08— 9.50	0.63—0.47
	Mds 1173	May 69	Aug 70[d]	5.80— 4.54	0.47—0.50	14.14—13.70	0.64—0.62	10.54— 8.66	0.62—0.59	5.66— 7.54	0.40—0.35
	Mds 1173	May 69	Sep 70							6.64— 5.30	0.47—0.47
VI	Mds 1331	Apr 70	Oct 71	6.56	0.53—0.50	13.06—10.84	0.85—0.73	6.34— 8.64	0.72—0.60	7.46— 6.60	0.51—0.65
	Mds 1173	Apr 70	Oct 71	2.26	0.24—0.27	8.00— 9.12	0.50—0.58	2.14— 3.34	0.29—0.32	3.74— 3.70	0.36—0.32

[a]Data for investigations in Copenhagen of batches prepared in Copenhagen are included for reference. The sample examined with those shipped in April 1969 was taken from remaining stock in Copenhagen. For the samples shipped in August 1970, however Copenhagen vaccine was returned from Madras, and the data shown refer to this returned sample; [b]In this column, the upper half (vaccines prepared 1968, first 6 lines) shows results from Madras, while the second half (last 8 lines) shows results from Budapest; [c]For the first 6 batches, Moscow reported "confluent growth" for the low-dose ampoules; [d]The shipment sent in Aug. 1970 was 3 wk delayed during air transport. Additional samples were therefore sent in September 1970

ratories. The first three supplies were in April 1969 sent from Madras to the BCG Laboratory in Prague and the BCG Control Laboratory in Moscow. Samples of the 3rd supply (produced in Madras) were also sent to the BCG Laboratory in Copenhagen. In 1970 and 1971 samples of the last three supplies were sent to Prague, Moscow, Budapest and Copenhagen. Thus in the case of the fourth supply (produced in Copenhagen) the vaccine was re-examined there after storage in Madras.

Examinations of opacity and dry weight (desiccation till constant weight of BCG filtered off from the resuspended vaccine) were compatible with a 10:1 ratio for the two strengths. The actual data are not shown here.

Reconstituted, undiluted liquid from placebo ampoules was seeded on solid egg medium. No growth of mycobacteria was observed. Tests of vaccines on several media showed no significant contamination with microorganisms other than mycobacteria.

Results of viability tests are shown in Tables 5, 6 and 7. In Table 5 estimates of viable units based on counts of colonies on solid media (L—J, Ogawa) are shown in terms of millions per ml of the concentrations used in the field trial. In many cases, laboratories made two estimates for each sample. Such double estimates are shown with a hyphen. Table 6 shows the oxygen-uptake, a test of the enzymatic activity of the live BCG organisms, measured in a Warburg apparatus. Table 7 shows the germination rate, which is a semiquantitative estimate of the percentage of bacilli that are judged to have started germinating, by observation through the microscope of live BCG seeded 1-4 days ago on solid medium.

TABLE 6 — ESTIMATES OF OXYGEN UPTAKE IN LABORATORIES OUTSIDE[a] THE PRODUCTION LABORATORY

Supply No.	Product. Lab. and strain	O_2 uptake (µl) of 30 mg BCG during 1 hr	O_2 uptake (µl) of 20 mg BCG during 3 hr	
		Prague	Madras	Copenhagen
I	Cph 1331	18—23		36
	Cph 1173	13—16		26
II	Cph 1331	27—26	21	38
	Cph 1173	21—19	23	73
III	Mds 1331	11— 9	25	11
	Mds 1173	15—13	25	34
IV	Cph 1331	25		49
	Cph 1173	18		34
V	Mds 1331	23		17
	Mds 1331			23
	Mds 1173	15		11
	Mds 1173			11
VI	Mds 1331	Not available		18—21
	Mds 1173	13		26—26

[a,b] as in Table 5; Details of sample, mg/ml preparation and shipment same as in Table 5

For a further description of these various methods see WHO/TB/Techn. Guide/ 77.9 (Ref. 62).

In judging the data on viability, the relative crudeness of these methods should be kept in mind. The colony counts do not measure number of bacteria, but number of 'units', i.e., bacteria or clumps, ond is thus strongl dependant on the deggree ro clumping. The assesssment of aermination is free of this objection, but is at the same time a more subjective method. The estimation of oxygen-uptake (which can be rather precise if carried out on large samples) is in this

TABLE 7 — ESTIMATES OF GERMINATION[a] RATE IN LABORATORIES OUTSIDE[b] THE PRODUCTION LABORATORY

Supply No.	Lab and strain	Prague 48 h	Prague 72 h	Madras 48 h 1	Madras 48 h 0.1	Madras 72 h 1	Madras 72 h 0.1	Copenhagen 48 h 1	Copenhagen 48 h 0.1	Copenhagen 72 h 1	Copenhagen 72 h 0.1
I	Cph 1331	25	50					50	not done	75 — 90	not done
	Cph 1173	20	50					50 — 75	25 — 50	75 — 90	50 — 75
II	Cph 1331	20	40	25	25	50 — 75	25—50	(25)— 50	25	75	50 — 75
	Cph 1173	5	30	50	25—50	50 — 75	50—75	50	50	75 — 90	75
III	Mds 1331	20	50	25—50	25	50 — 75	25—50	10 — 25	10	25 —(75)	25 —(50)
	Mds 1173	20	70	50	25—50	75—(90)	50—75	50 — 75	25 — 50	75 — 90	50 — 75
IV	Cph 1331							25 —(50)	50	50 —(75)	75
	Cph 1173							(25)— 50	50	75	75 —(90)
V	Mds 1331							25	25	50	50
	Mds 1331							(10)— 25	10 —(25)	50	25—(50)
	Mds 1173							25 —(50)	(10)— 25	75	25 — 50
	Mds 1173							25 —(50)	10 — 25	50 — 75	25 — 50
VI	Mds 1331							50	25 — 50	75	50 — 75
	Mds 1173							10 — 25	10 — 25	50	50

[a]Estimated percentage of germinating mycobacteria out of total. For intervals with one limit in bracket, the estimate is closer to the limit not in bracket;

[b,c] as in Table 5; Details about sample (mg/ml) preparation and shipment same as in Table 5

case somewhat imprecise because only a limited number of vaccine ampoules could be used for each test.

For the samples shipped in April 1969, there seem to have been mishaps in estimating viable units, in both Moscow and Prague (Table 5). In Moscow the weaker vaccine gave confluent growth, although the stronger gave results as expected. The most obvious explanation is a systematic mistake in the specification of the dilution procedure. In Prague, the results are very variable, and the weak vaccines disproportionately weaker than the strong vaccines. Some were without growth. Very likely, the colony count system failed because of a faulty solid medium. Fortunately the Prague Laboratory made at the same time determinations of oxygen uptake and germination rates, and these results are quite within the expected range. It should further be noted that the second supply (produced in Copenhagen) gave adequate colony counts in Madras, and that the third supply (produced in Madras) gave adequate colony counts in Copenhagen. For all other vaccines, the estimates obtained are compatible with the assumption that the vaccines were of uniform strength. This agrees with the rather uniform post-vaccination allergy (Tables 3 and 4), and there is thus *a priori* no reason to analyze the trial separately either for various batches or for each of the two production laboratories.

6. Tuberculins

The nomenclature for skin test materials from mycobacteria for demonstration of delayed hypersensitivity is still not clarified. It is generally agreed that *M. bovis* and *M. avium* are species in their own right and not sub-species of *M. tuberculosis*. Thus it does not seem reasonable to talk of, e.g., avian tuberculin. Yet an International Standard for avian tuberculin is still in existence. Runyon[63] has proposed 'mycobacterins' as a comprehensive name for all these substances.

In the earliest studies of low-grade sensitivity[24,64,65] (for a review see Edwards and Edwards[66]), use was made of different doses of tuberculin (e.g., 5 units and 100 units) and quantitative measurement of reactions. Palmer and Strange Petersen[23] suggested that reactions must be taken to be nonspecific if they are weak or if they can be elicited only with a strong dose of tuberculin; also, that specific and non-specific reactions are often overlapping, so that reactions of intermediate size cannot be classified (also see Nyboe[28]). The introduction of double testing with a tuberculin and simultaneously with a mycobacterin prepared from a different mycobacterial species[29,32,67,68] appeared to be a significant improvement, as shown by Edwards and co-workers[68] in their study of such double testing among patients.

Tuberculin—There can be little doubt that the batch of tuberculin named PPD-S, prepared by Seibert and co-workers in 1939 by precipitation with ammonium sulphate[69], is superior to the other available tuberculins. It appears to be slightly more specific than acid-precipitated tuberculin[70] and to be less prone to adsorption[71]. A part of the original PPD-S was used by Palmer and co-workers in their many epidemiological studies; dilutions made from this part (supplied by the Antigen Production Laboratory, Atlanta) were also used during the earliest phase of the present trial. Another part of this batch was adopted as the International Standard for Purified Protein Derivative (PPD) of Mammaliau Tuberculin[72]. Two ampoules of this tuberculin, each ampoule containing 2,500,000 IU, were made available to the study. These were reconstituted, diluted in phosphate buffer (*p*H 7.25), dispensed in ampoules and again freeze-dried (in the BCG department, Statens Seruminstitut, Copenhagen) each ampoule containing 500 IU and 1/6000 g mole phosphate buffer salt.

Mycobacterin — Based on a number of unpublished pilot studies it was felt that mycobacterins prepared from avian, Gausse or Battey strains would all serve equally well as a choice for atypical mycobacterin. The decision to use PPD-B, prepared from an atypical strain from the Battey hospital, USA (now identified as *M. intracellulare*), was based mainly on the consideration that there was already extensive epidemiological and clinical experience with this preparation[73]. While the American authors have tended always to compare equal doses by weight of different mycobacterins it was decided for the present purpose to use the strongest acceptable dose of PPD-B so as to reveal even weak degrees of sensitivity. Thus it was decided to use 10 'units' of PPD-B in the study, rather than the 5 'units' previously used by Palmer.

Products and dosages — Four lots of PPD-S and eleven lots of PPD-B were used in the trial. During the first 6 months of the trial, PPD-S was supplied from Atlanta, Georgia, USA, in ready-made dilution with a nominal content of

0.0001 mg or 5 'units' per 0.1 ml. During the remaining two years of intake the International Standard was supplied from Copenhagen in ampoules each containing 500 IU.

Before using the tuberculin from Copenhagen it was compared with the tuberculin from Atlanta. The results are shown in Tables 8 and 9. It will be observed that 5 IU, in freeze-dried form, received from Copenhagen was stronger than 5 'units' of PPD-S ready-made solution received from Atlanta. So the comparison was repeated, this time using 2.5 IU from Copenhagen against 5 'units' from Atlanta. The latter was now slightly stronger than the former. It was therefore decided to use 3 IU from Copenhagen as an equivalent dose to 5 'units' from Atlanta.

PPD-B supplied from the Antigen Production Laboratory, Atlauta, was used in the trial except during the last 3 months. For the major duration of this period, batch 100616, lot II was used. During the last 7 months, PPD-B prepared from batch Poly B, lots IV-VI was used. The results of a parallel line assay showed that they were not different in potency. On two occasions when shipments of PPD-B from Atlanta were delayed, an available supply of freeze-dried PPD-B with gelatine was used for short periods. PPD-B (poly B) in freeze-dried form was used towards the end of the intake for about 3 months. Most of it was freeze-dried in Copenhagen but some in the BCG Laboratory in Madras.

Before using the freeze-dried PPD-B from Copenhagen, comparison was made between this and the one in dilution received from Atlanta. Correlation of reactions among indviduals simultaneously tested with the same doses by weight, showed practically no difference between the two preparations. It was therefore decided to retain the same dose by weight for freeze-dried PPD-B also.

Possible variation among the different lots of tuberculin as well as PPD-B was examined in yet another way. The mean size of reaction in 4 categories of persons were plotted for each panchayat arranged in time order as shown in Fig. 2. Panchayats where more than one lot has been used, have been left as gaps. Supplies of new lots have been shown as vertical continuous lines for lots of PPD-S and vertical broken lines for lots of PPD-B. It is seen from Fig. 2 that although there is variation in tuberculin response in different parts of the trial area, these changes cannot be identified with change of lots. Thus it was confirmed that the potency of the different lots of PPD-S and PPD-B used was uniform.

7. Study area

The area selected for the study included 209 contiguous panchayats and one town, with a total population of about 360,000 persons in Chingleput District of Tamil Nadu State in south India. Chingleput District with an area of 8170 km^2 and a population (1971) of about 2,900,000 is situated on the east coast. Administratively the district is divided into seven taluks. For further administrative and developmental purposes a taluk is divided into 3-5 panchayat unions, each with 40-70 panchayats. Each panchayat (commune) consists of one or more villages with their hamlets with populations from below one thousand to several thousands.

The study area selected included the whole of Trivellore Taluk (comprising Kadambattur, Trivellore, Poondi and

TABLE 8 — REACTIONS TO 5 IU OF PPD FROM COPENHAGEN AND 5 "UNITS" OF PPD-S FROM ATLANTA AMONG PERSONS AGED 10 YR OR MORE

Reaction (in mm) to 5 IU of PPD (from Copenhagen) Freeze-dried : 16-27/9/68	Reaction (in mm) to 5 "nnits" of PPD-S (from Atlanta) Date of preparation: 10-6-68																	Total
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32+	
0	11	1	3	4	2													21
2	1	2	5	2														10
4	2	7	10	18	1	1												39
6	1	3	7	9	7	1	1											29
8		2	3	4	6			1										16
10			1	10	2	1												14
12		1	3	6	3	2	3	1										19
14		1	4	3	5	4	4	1										22
16			3	6	4	7	8	4	7	2	1							42
18				1	1		5	7	14	8	2	2	1					41
20					1	1	1	1	10	13	5	9	4					45
22				1		1		3	8	10	6	6	6	1				42
24							1		1	6	3	3	8	3				25
26										2		7	5	1				15
28													2	6	2			10
30																		
32+																	1	1
Total	15	17	39	64	32	18	23	18	40	41	17	27	26	11	2		1	391

Ellapuram panchayat unions) and part of Tiruttani Taluk (Tiruvalangadu panchayat union). Situated in the study area is the town, Trivellore (40 km west of Madras City), with a population of about 25,000. For operational convenience of the trial, the town was divided into nine blocks. For the purposes of this trial the panchayat was taken as the main population unit.

TABLE 9 — REACTION TO 2.5 IU OF PPD FROM COPENHAGEN AND 5 "UNITS" OF PPD-S FROM ATLANTA AMONG PERSONS AGED 10 YR OR MORE

Reaction (in mm) to 2.5 IU of PPD (from Copenhagen) Freeze-dried : 16-27/9/68	Reaction (in mm) to 5 "units" of PPD-S (from Atlanta) Date of preparation: 10-6-68																	Total
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32+	
0		4	2	3	3			1										13
2	2	6	12	4	1													25
4	2	3	13	15	4	2	4	1										44
6		1	4	6	12	4	3	1										31
8			2	4	4	1	2											13
10					1		4	2										7
12					1	2	2	5	3									13
14						2	4	4	5	2								17
16							1	7	4	12	5	5		1				35
18				1				3	12	5	12	14	7					54
20								2	4	9	5	18	8	2				48
22									1	2	8	5	13	7	2			38
24									1	4	3	13	8	9	3	1		42
26										1	1	1	13	2	3	1		22
28														4	1	1	2	8
30																		
32+														1	2			3
Total	4	14	33	33	26	11	20	26	30	35	34	56	49	26	11	3	2	413

Each panchayat and town-block was given a three digit number, the first digit identifying the panchayat union and the next two identifying the panchayat or the town-block within the panchayat union. Fig. 3 shows the trial area divided into panchayat unions, their respective panchayats, and other salient features. Over 60 per cent of the population in the study area depend on agriculture based

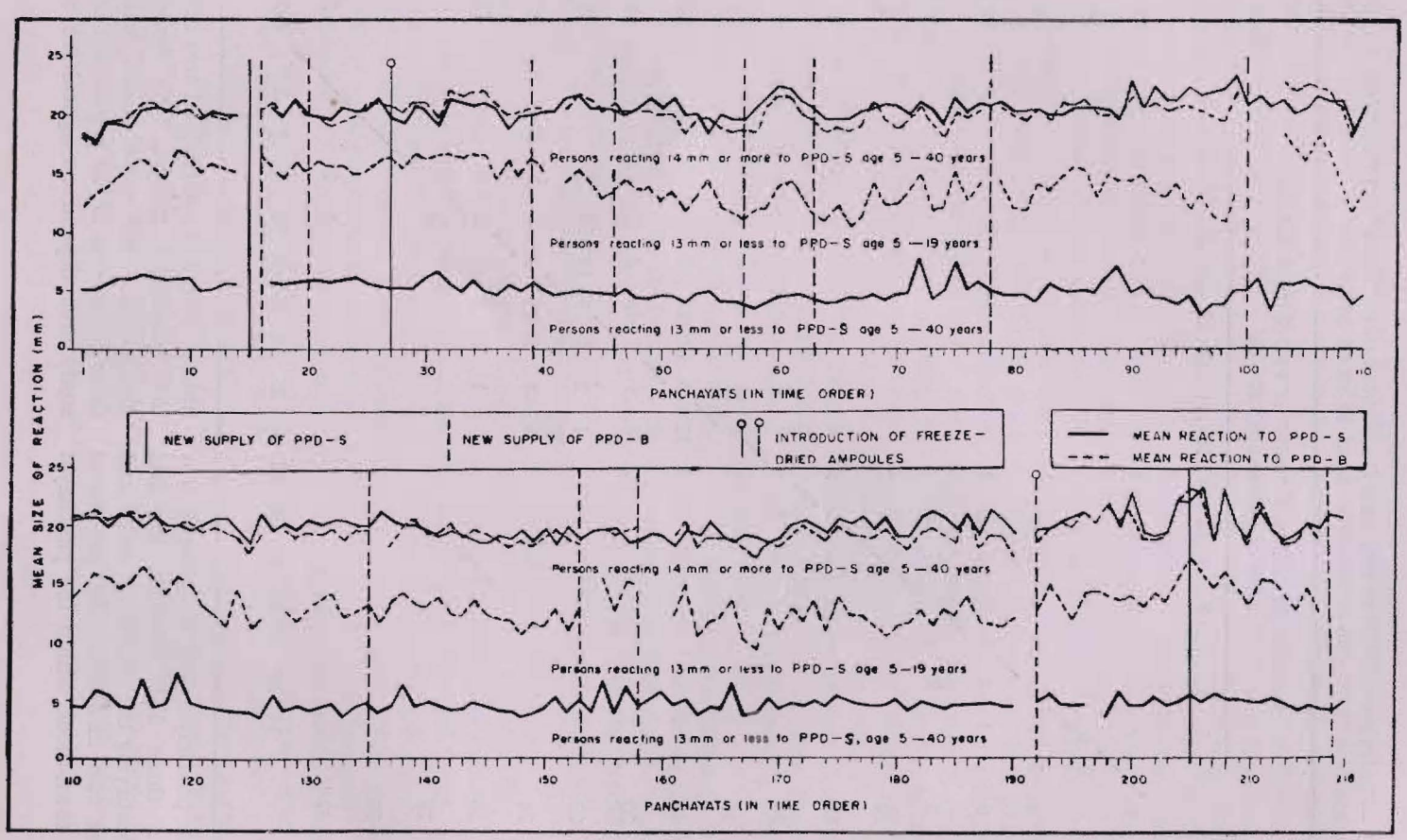

Fig. 2 — Variation with time, through the period of admission, in mean sizes of reactions to PPD-S and PPD-B, for selected categories of persons.

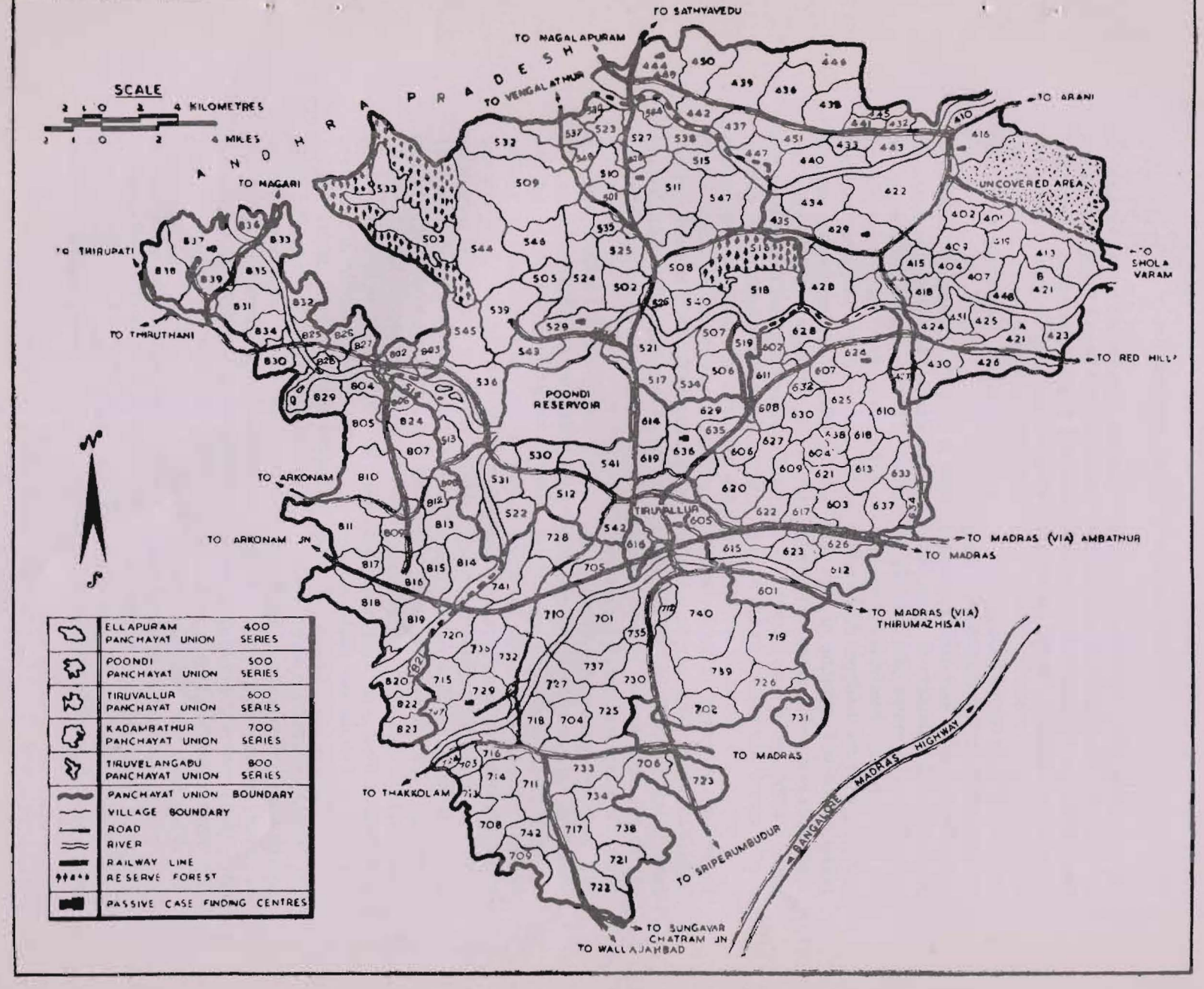

Fig. 3 — Map showing the BCG trial area

on seasonal rains, the other main occupation being small household industries. There are no parennial rivers in the area and the main-stay of irrigation are wells and dammed water reservoirs. The altitude is approximately 50 m above sea level.

Besides tuberculosis, there is a high prevalence of leprosy and filariasis among the population. There are 12 health facilities run by the Government of Tamil Nadu in the area. Of these, one is a hospital with 60 beds at Trivellore; the rest offer mainly outpatient care and carry out public health activities. There is also a church-managed hospital. Private practitioners of modern medicine are less than a dozen and are mainly in the town and two larger villages. There usually is, in every major village, at least one practitioner of indigenous medicine.

8. The intake

Intake was started in July, 1968, after completion of the preliminary investigations comprising selection of area, selection of biologicals and training of personnel. During the pre-intake period, 10 manuals* and work instructions, with concommitant cards and forms, had been developed and extensively tested. The intake lasted 2 yr and 8 mo and was completed in March, 1971.

The intake comprised the following main activities : (1) a complete census of the population; (2) skin testing for delayed hypersensitivity, vaccination (vaccine or placebo), and X-ray examination; (3) reading of skin test reaction; (4) X-ray reading; (5) collection of sputum samples from those identified as eligible on the basis of X-ray results; and (6) bacteriological examination of sputum samples.

Prior to starting the work in the area, public cooperation was sought by contacting all officials and other persons who could assist in obtaining cooperation of the people. The nature of activities and the services provided were carefully explained with emphasis on the casefinding activity. In addition, just before taking up each panchayat, a visit was made by a team leader to each village, when a rough map of the panchayat was drawn after a survey of the village.

The statistical section of the project supplied to the field teams individual cards—TPT 69 (Fig. 4)—each card marked with a random digit out of the numbers 1, 2, 3 (for the purpose of random allocation of vaccines and placebo). The field teams stamped seven-digit individuals numbers on these cards in the order in which the cards were received. The first three digits indicated the panchayat union and the panchayat within the panchayat union and the next four a serial number for each person in the panchayat. Independently of the individual numbers each household was given a household number which was painted on the doorpost.

Census of the population — Census was carried out on a household basis visiting each house in a systematic manner, so as not to miss any. A 'household' was defined as a group of persons who live together and have their main meals prepared in the same kitchen.

Census of each household comprised of obtaining the full composition through careful interview of the head of the house-

*Manual for testers and vaccinators, manual for census takers, instructions for filling the ondividual card, mannal for recording names, manual for X-ray technicians, manual for X-ray reading manual for sputum collection

hold or a responsible member, and recording, on individual cards, identification particulars viz., name, father's or husband's name, relationship to the head of the household, age, sex, occupation, census status, and if an individual had gone to another place, the place he has gone to—or if he were a visitor, the place he belonged to (for this the census taker was provided with a list of panchayats and villages in the study area). The members of a household would usually but not always be registered in a logical order with consecutive individual numbers. Some important aspects of census are given below.

Name — All interviews for census had to be conducted in the regional language—Tamil. Since the statistical machinery is based on English rather than Tamil letters, the names had to be recorded in the English alphabet. While Tamil is phonetic, English is not, also not when used for spelling of Tamil names. To permit alphabetical sorting and listing of names it was then necessary to introduce a standardized spelling. For this purpose a standard list of 300 common names in the area was prepared, on the basis of which spelling of any other name could be deduced. Thus the spelling 'Muurthi' was used also for a literate person who might himself spell his name 'Moorthy' or 'Murti'. Surnames (caste names) were mnemonically coded into three letter codes. The name was recorded in box 1.

Relationship : Relationship to the head of the household was recorded in box 8. Head of the household is a

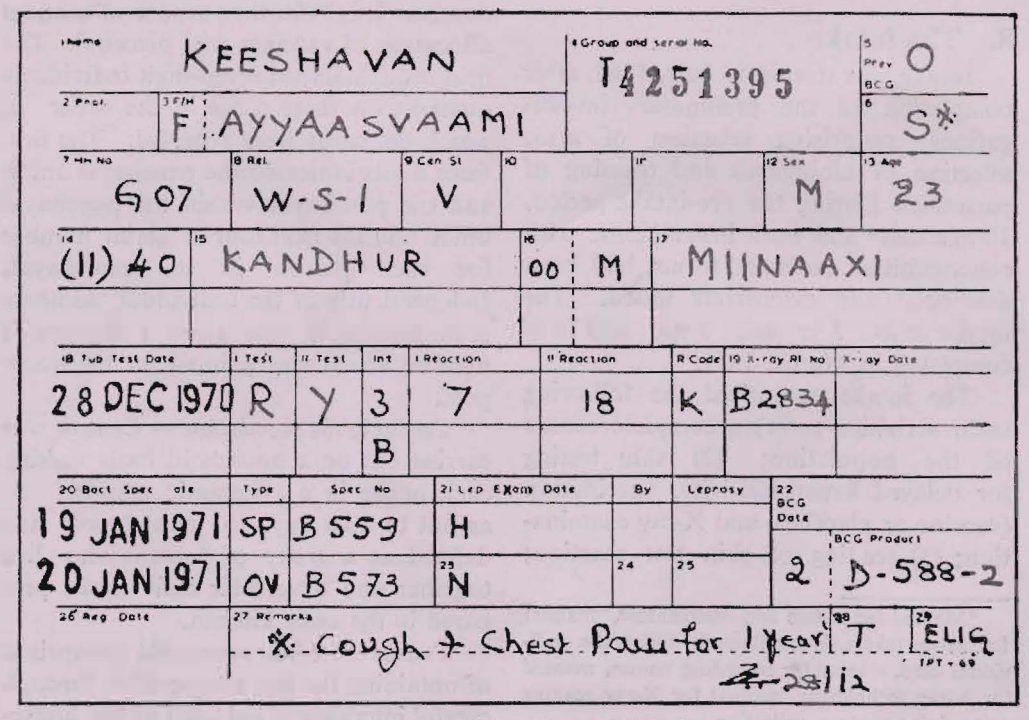

Fig. 4 — Individual card (TPT-69)

member of the household who is recognised as such by the other members of the household. He was identified as A and his wife as W (or if A was female, her husband as H). Brothers in the order of descending age were recorded as B, C, D, E, sisters as S, T, U, V, father F and mother M. Sons were indentified as 1, 2, 3, 4 or 5 and daughters 6, 7, 8 or 9. For example, 6-H-S would mean 'the sister-in-law of the eldest daughter of the head of the household'. In such a manner even the most complicated relationships could be coded and recorded. Codes also exist for homeless persons and visitors.

Census status : Census status was recorded, in box 9, as a single letter : P for a resident present at the time of census; A, absent resident; V, visitor staying overnight in the household; X, a casual visitor only, etc. (Similar but other codes exist for follows-up census status e.g., B for new-born; F, fate unknown).

Age : Age (recorded in box 13) if stated by a person was taken as the correct age. In many instances, the individual was unable to state his or his child's age. In these instances age was estimated after having a look at the person. In the words of the manual "the estimation is done by cross-examining the person, or in case of infants, the parents, with reference to important happenings in the past". For children aged below one yr, age was recorded in months and for the rest, in completed years.

Testing with PPD-S and PPD-B, 'vaccination', finger-printing and X-ray examination — An Examination Centre was set up at a convenient place in the village, for conducting tuberculin tests, 'vaccination', and X-ray examination. All individuals for whom the census was completed were sent to the examination centre with a chit showing their household numbers ; the individual cards were never given to the persons but taken to the X-ray centre by one of the team-members.

At the examination centre, the identification particulars, i.e., name, age, sex etc. of each individual were verified and the left shoulder examined for scars indicative of previous BCG vaccination.

Skin testing with PPD-S and PPD-B was offered to all individuals aged one year and above. The tests were given intradermally in the mid-dorsal aspects of the forearms. To avoid 'psychological bias' in reading of the reactions, the two tests were allocated randomly to right and left forearms, according to the last digit of the individual number being even or odd. Each tester was assisted by a clerk who would dictate the combination, check that it was correctly followed, and record accordingly.

'Vaccination' (i.e., vaccine or placebo) was offered to all individuals aged one mo and above. The vaccinator would use one box with three ampoules at a time, and would vaccinate the individual from the ampoule with the suffix (1, 2, or 3) corresponding to the random digit previously entered in the individual card. Also, the vaccinator had a clerk who would dictate the ampoule to be used, and check and record the ampoule number.

From the time the vaccine was manufactured till it was actually used in the field it was kept under refrigeration at 4°-8°C. In the field, to protect the vaccine from heat and sunlight, special precautions were taken : The vaccine ampoule was kept immersed in ice, in a closed insulated jug, right up to the moment of filling the syringe. Also, the syringes used for vaccinations were covered with

specially designed rubber tubings. Further-more, most vaccinations were carried out around sunset or later, or, if occasionally earlier, invariably in the shade.

Finger prints were taken on the reverse of the individual card immediately after 'vaccination'. For individuals aged 5 yr and above one dab and one roll finger print of the left index finger, and for children aged below 5 yr, a left palm-print were taken. (When a new case of tuberculosis was diagnosed in the follow-up of the population, the finger print obtained at the time of sputum collection was compared with the finger print obtained at the time of vaccination, as a final proof of the identity of the individual).

X-ray examination — All individuals aged 10 yr and above were eligible for a chest X-ray, 70 mm photofluorogram, postero-anterior view. The X-ray units operated on storage batteries and, being mounted in sturdy vehicles, could reach almost all villages in the study area. A clerk noted down, for each X-ray roll, individual numbers in the order of exposure. The individual number as stamped on the individual card of the person X-rayed was exposed on his photofluorogram.

Reading of skin test reactions — Because of the size of the study population and the duration of the intake it was necessary to employ more than one tuberculin reader. To ensure a uniform quality of readings the readers were intensively trained and standardised before they were introduced into the study. The standardization was in terms of intra and inter-reader consistency. Systematic periodical assessment of the readers ensured the continued uniform quality of readings.

Reading of the skin test reactions, in terms of transverse diameter of induration in mm, was carried out usually after three days, occasionally after four days, and in exceptional cases after two or five days. The reader and a clerk went from house to house. The reader dictated the size of reactions in right arm and left arm in that order to the clerk, who repeated and recorded the measurements. The reader would thus not know the order in which PPD-S and PPD-B had been allocated to the right and left forearms.

X-ray reading — The photofluorograms were processed at the field headquarters and read by two X-ray readers independently and 'blindly' at the project headquarters. The readers (there were several during the study, because of changes in personnel) were physicians already specialised in chest diseases, and were further given training in reading 70 mm films.

The reading was recorded by the reader only if abnormal, according to the following code. The individual number as seen on the film was also recorded.

0. Exposure technically inadequate

Extra respiratory

1.1 Cardiac abnormality
1.2 Vascular abnormality
1.3 Bony abnormality (e.g., scoliosis)

Respiratory, definitely extra-pulmonary

2.1 Very dense spot or spots in hilar region (calcifications)
2.2 Obliterated costo-phrenic angle and/or pleural scar and/or pleural calcification
2.3 Evidence of chest surgery
2.4 Enlarged mediastinal and/or hilar glands
2.5 Basal-parietal opacity, indicative of pleurisy with effusion (in any area)

2.6 Pneumothorax or hydropneumothorax
2.7 Special pathology not specified above

Special patterns

5.1 Uniformly dense, round opacity, single or multiple (e.g., cyst)
5.2 Atelectasis
5.3 Consolidation
5.4 Less dense opacity combined with cardiac abnormality (6 and 1.1 both present in one person)

Opacity or opacities in lung fields

3 Very dense and very well demarcated (e.g., calcification)
4 Dense and well demarcated (e.g., fibrosis)
6 Less dense opacity, or less well demarcated (e.g., infiltration)
7 Ill-demarcated or doubtful cavity
8 Well-demarcated cavity or cavities, each less than 4 cm (less than 6 mm on the 70 mm film)
9 At least one well-demarcated cavity more than 4 cm (more than 6 mm on the 70 mm film)

In case of multiple lesions, only the most serious was recorded. For the reading, 3, 4, 6, 7, 8 and 9 above, a second digit was recorded, as follows :

Total extent of opacities	Location		
	Single opacity	More opacities in one lung only	Both lungs
Less than one square centimeter (1.5 mm² on the 70 mm film) or linear bands	1	2	3
Less than one sixth of total area of lung fields	4	5	6
More than one sixth of total area of lung fields	7	8	9
Small spots, widely disseminated in both lungs	—	—	0

For a first digit reading of 1-9, the following alphabets were used as a third code (except calcification).

A — Abnormality other than tuberculosis; B — Tuberculosis, inactive ; C — Tuberculosis, possibly active ; D — Tuberculosis, probably active.

It may be noted that the above alphabet code has been used systematically in most Indian epidemiological mass surveys since 1955.

Umpire readings — The photofluorograms of all persons read as 'C' or 'D' by one of the two readers only, and those of persons whose sputum samples were positive on direct microscopy but negative on culture were reassessed by a third (umpire) X-ray reader.

Sputum collection — Individuals whose photofluorograms were classified as codes 5 to 9, or C or D even if not 5 to 9, by either reader were eligible for sputum examination. For all such individuals, the individual numbers were listed and sent to the field.

From each eligible individual, attempts were made to collect two samples of sputum, one a supervised 'spot' sample and the other, an overnight 'collection' sample. The spot sample was collected during the first visit of the sputum collector to the household of the individual, and the collection sample at the second visit, the next morning. If for any reason the collection sample was either not satisfactory or not obtained, a second spot specimen was collected. Whenever a specimen of sputum was classified as

'contaminated' on culture, attempts were made to obtain a third sample of sputum. All precautions were taken to establish the correct identity of eligible persons as also to see that the sputum specimens were as far as possible obtained from the bronchial tree and not mere saliva.

Sputum samples were collected in sterilised universal containers with aluminium screw caps. The bottles were supplied to the field teams by the laboratory (Union Mission Tuberculosis Sanatorium Laboratory, Madanapalle, initially and later Tuberculosis Research Centre Laboratory, Madras). These bottles had serial numbers engraved on the screw caps, e.g., E 001 to E 999 etc. At the time of sputum collection the bottles were used at random and not strictly according to serial order.

All sputum samples were stored under refrigerated condition from the moment of collection and transported under refrigeration till delivery to the bacteriological laboratory.

Bacteriological examination of sputum specimens — Sputum specimens were re-received at the bacteriological laboratory along with a form indicating the number of bottles and the dates of collection. The laboratory did not get the individual number or any other particulars along with specimens, except the 'bottle number' that was engraved on the screw-cap. The laboratory forthwith allotted a 'laboratory number' of its own to each specimen, recorded the bottle number and the respective laboratory number for each specimen in a register.

The following examinations were carried out for each sample :

Smear examination by fluorescence microscopy after staining a heat fixed smear by auramine phenol stain. The results were recorded and later directly punched in terms of either actual number of bacilli in the whole smear if not above 99, otherwise as 1+ to 4+. The information was reclassed by computer as follows (number of acid-fast bacilli seen) ;

0, no acid-fast bacilli seen; 1, 2, 3, 4, 5, 6-11, 12-24, 25-49, 50-99

$\geqslant$100/smear, 1-5/low power field (1+); 6-100/low power field (2+); $>$100/low power field but$<$100/high power field (3+); $\geqslant$100/high power field or clumps (4+)

with additional classes for results not available.

Sputum Culture—To 5 ml of a sputum sample, an equal volume of 4 per cent NaOH was added, shaken in a mechanical shaker for 20 min and centrifuged at 4000 rpm for 20 min. The supernatant fluid was carefully decanted and the deposit resuspended in sterile distilled water, recentrifuged and the deposit inoculated on 2 slopes of Lowenstein-Jensen(L-J)medium. The inoculated bottles were incubated for a maximum period of 9 wk. The bottles were examined every week for 8 to 9 wk unless a 'positive' or 'contaminated' result was obtained in the meantime. Culture bottles which did not show any growth at the end of 8 wk were reported as 'negative' and discarded.

All positive cultures were examined by an independent person for morphological characteristics for classification as 'typical tubercle bacilli' indicating *M. tuberculosis* (or *M. bovis*). Microscopic examination was performed on the bacilli from all cultures of doubtful colonial morphology and those which did not show acid-fast bacilli were discarded as contaminants, the rest, which showed acid-fast bacilli. reported as strains of atypical morphology.

Culture results were reported quantita-

tively in terms of number of colonies (up to 49 colonies), or 1+ (50-100 colonies), 2+ (more than 100 colonies), or 3+ (confluent growth). Out of the two slopes, the one with the higher growth was reported. The results were punched directly and reclassed by computer as follows :

no growth; growth in water of condensation; 1 colony; 2 colonies; 9 colonies; 10-14; 15-19; 20-24; 25-49; 50-100; more than 100 discrete colonies; confluent growth;

with further classes for contamination and other special cases.

Drug sensitivity tests : The following drug sensitivity tests were carried out on all strains with typical morphology. The standard sensitive strain H 37 Rv. was tested with each batch of culture. The following drug slopes were used :

Drug	Slopes containing (μg per ml)
Streptomycin	8, 16, 32, 64
Isoniazid	0.1, 0.2, 1, 5
Para-nitrobenzoic acid (PNB)	500

However, the PNB sensitivity test was not used in Madanapalle, where instead a catalase test was carried out. Up to March 1972, three weaker dilutions were used in addition for streptomycin and two for isoniazid, and PAS sensitivity was tested for 6 different concentrations.

After sensitivity test readings, the minimal inhibitory concentration (MIC) values were determined using a 20-colony end-point. Isoniazid sensitivity was reported as MIC while those of streptomycin and PAS as resistance ratios (RR).

The niacin test was performed on one of the control slopes from the drug sensitivity tests. Each bottle was first inspected for the presence of water of condensation; 0.5 ml distilled water was added to each culture bottle which had scanty or no water of condensation, and the culture bottle autoclaved. About 0.25 ml of the resultant extract was transferred to a test-tube, and 0.25 ml of 3 per cent alcoholic benzidine solution followed by 0.25 ml of 10 per cent aqueous cyanogen bromide solution were added. The results were reported as follows :

Pink or red precipitate 2+; Faint pink precipitate 1+; White precipitate W.

Growth at 25°C : The number of colonies were reported as for the primary cultures above. However, for the analysis of the present report even one colony was taken as 'growth'.

Definition of a strain of M. tuberculosis— Based on past experience of the laboratory, strains of *M. tuberculosis* were defined according to the flow-chart, shown in Table 10, which was part of the computer program. It will be noted that particular weight was given to the niacin reaction. Other tests came in mainly when the niacin test result and the PNB sensitivity were not available, as might happen especially if the secondary cultures failed.

Figures given in parenthesis in Table 10 show the proportion of cultures falling into each class of the table. These figures are based on a sample of about 7200 cultures. It is observed that, although the classification chart provided decisions for all combinations of results almost all the cultures that were classified as *M. tuberculosis* were niacin positive.

Runyon's classification — The class UMB is a mixture of well-classified strains other than *M. tuberculosis,* and of strains not definitely classifiable. Further analysis of the class UMB is not attempted in this report, although the laboratory on the

TABLE 10 — CRITERIA FOR CLASSIFICATION OF MYCOBACTERIAL STRAINS AS *M. tuberculosis* (*M. Tub*) OR UNIDENTIFIED MYCOBACTERIA (UMB)*

Results of PNB	Niacin (++)	Colony morphology : 'typical'			Colony morphology : 'atypical' or not available	
		Niacin (+)	Niacin not done	Niacin negative	Niacin (+)	Niacin negative or not done
Sensitive	*M. tub* (57.2)	*M. tub* (0.2)	*M. tub* (0.0)	(a) (0.0)	*M. tub* (—)	UMB (0.3)
Not done	*M. tub* (0.1)	*M. tub* (—)	(b) (2.5)	UMB (—)	(c) (—)	UMB (2.6)
Resistant	*M. tub* (0.4)	*M. tub* (0.0)	UMB (—)	UMB (0.9)	UMB (0.1)	UMB (35.7)

Figures in parentheses give percentage of cultures falling in each class; (—) indicates no frequency and (0.0), <0.5 per cent (a) If ≥ 10 colonies : go to (d); otherwise : go to (e); (b) If ≥ 10 colonies : *M. tub.*; otherwise : UMB; (c) If photo chromogen, scotochromogen or rapid grower : UMB; otherwise go to (e); (d) If growth at 25°C : UMB; otherwise (no growth or not done) : *M. tub.*; (5) If no growth at 25°C; *M. tub.*; otherwise (growth or not done) : UMB

*Unidentified mycobacteria includes such strains as are often referred to as 'atypical', as well as unclassifiable strains

basis of pigmentation and other criteria reported classification mainly according to Runyon.

9. Record linkage at intake

In a long term prospective study such as this, data for each individual are collected at different places and at different points of time requiring the application of the concept of 'record linkage'. With the large volume of data, such linkage would be extremely difficult if not impossible without the use of data processing machinery. It is also essential that there should be rigid rules for recording in field and laboratory which permit direct transfer of data on to punch cards without coding or copying by hand. Given below are some of the essential details of the different records used, transferring of data from records to punch cards and the record linkage achieved through data processing machinery. It will be noticed that there was virtually no manual coding or transfer of data from one record to another and data once collected was directly transferred on to punch cards, verified and for each individual all data linked by machinery.

Records — During intake, data for each individual was collected on one or more (depending upon the eligibility of the individual for different examinations) of the following 5 types of records, each independent of the others.

(i) Individual card (from the field) : showing identification particulars (such as name, age, sex etc.), tuberculin test results, code of BCG vaccine, X-ray roll number and sputum bottle numbers;

(*ii*) X-ray report (from the X-ray reader); giving the results of the X-ray;

(iii) Report giving sputum smear results (from the laboratory);
(iv) Report giving sputum culture results (from the laboratory);
(v) Report giving the results of identification and drug sensitivity tests (from the laboratory).

The field teams ensured that the individual cards were complete and correct before they were sent to the statistical section at headquarters. Otherwise 'field statistics' were avoided as far as possible, and speedy punching and checking of punched data relied upon to reveal omissions and errors. A routine was therefore introduced whereby, for each type of record, the presence of those kinds of errors and inconsistencies that were known to occur occasionally were checked systematically on the machine at the earliest possible moment and the data amended, wherever possible.

Machinery —Throughout the period of intake processing of punched cards was done on a tabulator (IBM 447) along with reproducing punch, collator and sorter.

Punch codes — With a tabulator and a mechanical sorter the standard 80-column punch-card limits flexibility where the information for a single person may fill several such cards. Very careful attention was therefore given to the construction of punch codes, both in terms of condensation of information and in terms of compatibility of cards with different types of information.

Two important punch codes are : one for punching the 'name card', which gives the individual's serial number, name, father's or husband's name, household number, family relationship, age and sex. The other is for the 'analysis card' which in addition to serial number, household number, age and sex contains all information essential for epidemiological analysis omitting the individual's name, family relationship and serial numbers of sputum bottles.

Much of the information in columns 1–28 is identical for the two cards, being data essential for both epidemiological analysis and for identification of the individual. The two types of cards can thus be kept separate and used for separate purposes, perhaps sorted and filed in quite different sequences.

Punched Cards

From the individual card — The first card punched for a person were the name card, the field-data card and the movement card. The field-data card is identical with the name card for the first 28 columns. Thereafter it contains the data from boxes 18–22 of the individual card (see Fig. 4) punched directly in the order in which they appear in the card. It will be seen that the field-data card contains X-ray roll number, sputum bottle numbers and BCG code but no results of X-ray or bacteriology. The movement card contains, in addition to the individual number, the contents of boxes 14–17 from the individual card (i.e., the identification particulars in the village of origin for visitors, settlers and newly married women), but is punched only if these boxes are filled in; two cards are punched if the two lines refer to different places or to different persons in the same place.

From the X-ray report — As soon as an X-ray film roll was read by both readers, an X-ray 'roll master card' was punched, with the X-ray roll number, identity of the readers, date of reading and panchayat number. Further, a card was punched, for each abnormal finding

directly from the X-ray readers' original entries. There may be two X-ray cards for a person (if judged abnormal by both readers) or one or none. X-ray results interpreted as normal were derived from the X-ray roll master card by reproducing the identity of the readers into the field-data card after matching by X-ray roll number; if there was a reader but no X-ray result, the reading was taken to be normal.

From the laboratory reports—From these reports results of microscopy (as they became available), culture (punched either as soon as they were found positive or after eight weeks, if negative) and drug sensitivity and identification tests were punched. These cards contained bottle numbers, but no individual numbers; individual numbers are not supplied to the laboratory to ensure that examination of the sputum specimen in the laboratory is independent of the knowledge of which two specimens belonged to the same individual. It was therefore necessary to prepare a separate set of 'bottle-cards' usually two-reproduced from each field-data card with individual number and bottle number. By sorting both these bottle-cards and the cards with bacteriological results according to bottle number, it is possible to collate corresponding cards and transfer the individual number to the card with the bacteriological results.

Analysis card — Finally, the analysis card was produced by transferring all the information of epidemiological interest from the other cards, after bringing the information into the required format. For instance, the tuberculin reactions were punched in the field-data card in the order of 1st test and 2nd test (right arm, left arm), which is more convenient for the punch clerk. The order of PPD-S, PPD-B (which is more convenient for statistical analysis) was obtained later by logical editing in the suitably programmed tabulator and automatic repunching of the data in the latter form in the analysis card.

10. Criteria for defining infection and disease

In epidemiological studies, criteria for determining an individual's allergic status, or change in status, are best based, not on uniform, 'accepted' rules, but on studies of the pattern of findings in samples representative of the population.

The definition of the allergic status of an individual depends on several factors such as the allergens used, their dosages, variations in reading of reactions and the characteristics of the population under study. Thus for each population the crietria for defining prevalence and incidence of infection will have to be evolved by studying the distributions of reactions obtained, in that poulation.

In defining a case of tuberculosis, while demonstration of tubercle bacilli (or histopathological evidence) is a fairly authentic criterion, interpretation of X-ray shadows is known to be equivocal and relatively subjective.

Criterion for defining a reactor to PPD-S — As discussed earlier, it is possible that some of the intermediate reactions to PPD-S are nonspecific cross-reactions to the organism(s) causing reactions to PPD-B[23,28]. The correlation of reactions to PPD-S and PPD-B is shown in Table 11. The distribution is very asymmetrical but scarcely permits conclusions concerning cross-reactions. Earlier studies[73] had shown that from results of dual testing in those with medium size indurations of 6–11 mm

TABLE 11 — CORRELATION BETWEEN SIZE OF REACTION TO PPD-S AND PPD-B FOR AGE-GROUP 1-14 YEARS

Size of reaction (mm) to PPD-S	Size of Reaction (mm) to PPD-B																
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30+	Total
0	1835	2828	2094	1649	1511	1545	1571	1585	1154	624	208	106	21	6	1	1	16739
2	1042	4022	4473	3259	3416	3099	3074	4002	2853	1709	558	219	86	18	6	1	31837
4	163	851	1715	1683	1494	1646	1842	3143	3400	2474	818	363	150	20	7	1	19770
6	26	75	314	385	497	482	678	1433	1968	1733	660	291	180	27	4	—	8753
8	10	18	36	67	90	153	241	608	926	889	400	238	114	18	3	—	3811
10	3	10	19	22	42	42	115	301	551	601	305	169	86	16	2	—	2284
12	5	5	12	17	21	54	43	242	360	441	245	114	67	17	2	—	1645
14	11	18	16	12	35	60	119	176	456	586	330	222	107	14	2	—	2164
16	7	11	21	16	47	51	101	289	391	665	426	213	118	30	2	—	2388
18	4	17	22	25	33	64	109	313	622	749	625	299	136	55	15	1	3089
20	1	14	12	12	22	37	56	224	510	847	405	285	182	39	9	1	2656
22	5	4	10	11	23	27	51	141	365	548	501	174	217	59	9	2	2147
24	1	7	9	11	26	21	41	115	270	429	523	338	213	88	25	1	2118
26	—	1	—	4	6	5	21	50	113	193	235	210	259	37	24	2	1160
28	—	—	—	1	4	1	3	14	30	58	72	97	86	57	4	4	431
30+	—	—	—	—	—	—	1	2	5	14	18	17	24	22	9	2	114
Total	3113	7881	8753	7174	7267	7287	8066	12638	13974	12560	6329	3355	2046	523	124	16	101106

to 5 TU PPD-S, those infected with *M. tuberculosis* could be clearly separated from those not so infected. But in our data, the classification of the study population as infected or non-infected on the basis of PPD-S and PPD-B testing proved impossible[40]. Therefore only the results of testing with PPD-S have been considered for defining a positive tuberculin reaction.

Fig. 5 (left half) shows the distributions of reactions to PPD-S in different age and sex groups in the study population up to the age of 34 yr. The distributions after the age of 34 yr (not shown) were very much similar to the distribution in age group 25-34 yr. The frequency of large reactions—with a mode at about 18 mm—is very small in the youngest age groups and gradually increases with age up to the age of 25 yr among males and up to 35 yr among females and remains the same thereafter. The separation between the two distributions, i.e., the presumably uninfected left-hand distribution and the presumably infected right-hand distribution, is far from sharp. Even for the age groups 1–4 and 5–9 yr there is much overlapping between the two classes. However the 'antimode', i.e., the dip between the two distributions, is mostly around 12 mm, which therefore is taken as a limit defining 'reactors' to PPD-S on the expectation that with this limit 'false positives' and 'false negatives' will tend to cancel each other.

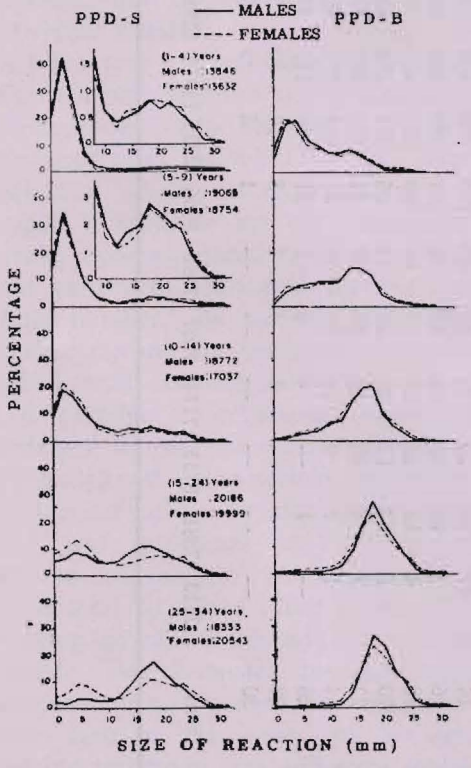

Fig. 5 — Distributions of reactions to PPD-S and PPD-B by age and sex

It may be noted that among more than 1000 patients having at least two positive cultures of *M. tuberculosis* each, only 1.4 per cent, and among nearly 800 patients having one positive culture of *M. tuberculosis* each, only 3.6 per cent had reactions to PPD-S smaller than 12 mm. This implies that the test thus defined is highly sensitive.

However, for studying the protective effect of BCG vaccination among the non-infected, only persons reacting with 0-7 mm to PPD-S have been considered as 'definitely not infected'.

Criterion for defining a reactor to PPD-B — Fig. 5 (right half) shows the distribution of reactions to PPD-B in different age and sex groups, also up to the age of 34 yr. The distributions after the age of 34 yr (not shown) were very much similar to the distribution in age group 25–34 yr. Even among children aged below 5 years, there is no point of separation between subjects who may be classified as reactors and those who may be classified as non-reactors. Among

those aged 15 yr or more the distributions of reactions are unimodal and may be regarded as consisting of reactors only. Such reactors would seem to have about 10 mm or larger reactions to PPD-B. Also the skewness among the three younger age groups is largely to the left of 10 mm and thus a reaction of 10 mm or more to PPD-B has been adopted as the criterion for defining a reactor to PPD-B.

Criteria for defining an X-ray case of tuberculosis—To obtain from our material an idea of the inter-reader variation, the correlation of the X-ray readings of two readers, who had read the largest numbers of photofluorograms during intake was studied. For the three most severe categories of X-ray readings, i.e., 7, 8 or 9, indicating evidence of a definite or doubtful cavity, reader I classified 160 in the above categories while reader II, 294. However, the readers were in agreement in only 130, or 40 per cent of the 324 picked out by either. If code 6, often indicating pulmonary infiltration, was added the agreement was of the order of 44 per cent.

Correlation of the readings of the same two readers by the alphabet code, i.e., codes C or D indicating possible or probable tuberculous aetiology, was also studied. Reader I classified 2913 according to the above two codes and reader II, 2748, but they agreed in only 1797 cases, or 47 per cent of the 3864 persons classified as C or D by either reader. Of the 2913 persons so classified by reader I, 21 per cent and, of the 2748 by reader II, 22 per cent yielded cultures positive for *M. tuberculosis*. The low agreement between the two readers and the relatively low bacteriological confirmation suggest that the evaluation of X-rays in a survey, especially based on a single photofluorogram appears to be unsatisfactory for arriving at a definition of an X-ray case of pulmonary tuberculosis.

In view of these differences in the X-ray readings between the readers no absolute criterion for definition of a 'case' of pulmonary tuberculosis based only on X-ray is available. In this report, persons (abacillary) for whom photofluorograms were classified, according to the alphabet code, as C or D by both readers are referred to as X-ray cases. Photofluorograms classified as belonging to category C or D by one of the readers only were submitted to a third reader. Persons for whom the photofluorograms were then classified as C or D by the third reader are also considered as X-ray cases.

Criteria for Defining a Bacillary Case of Tuberculosis

Cases positive on smear only—It will be recalled that for each individual eligible for sputum examination, attempts were made to collect two samples of sputum which were examined by direct smear and culture techniques.

Table 12 presents, for the 2608 cases positive on smear and/or culture at intake, correlation of the results of direct smear and culture in terms of the number of acid fast bacilli (AFB) on direct smear and the number of colonies on culture. Of the two sputum specimens collected from each individual, the specimen that showed the highest number of bacilli on smear and the specimen that showed the highest colony count on culture are considered for the correlation. It will be observed that out of 2608 individuals, 567 (22 per cent) were positive on smear only, while 869 (33 per cent) were positive on culture only. Of the 567 persons positive on smear but negative

TABLE 12 — CORRELATION BETWEEN RESULTS OF DIRECT SMEAR AND CULTURE FOR BACILLARY CASES DIAGNOSED AT INTAKE

No of colonies on culture	No of acid fast bacilli in smear								Total
	0	1-3	4-5	6-11	12-24	25-99	+/++	+++	
0		430	55	36	16	3	26	1	567
1-3	445	16	3	2	1	2	6	1	476
4-5	80	4	3	2	1		4		94
6-9	58	5	2	5	1	1	6		78
10-24	130	16	3	10	2	2	20	1	184
25-49	59	8	1	6	7	2	18		101
+	60	6	7	20	11	6	85		195
++	34	16	6	20	18	18	398	32	542
+++	3		1	3		6	218	140	371
Total	869	501	81	104	57	40	781	175	2608
UMB*		116	23	13	8	4	7	1	172

*Unclassified mycobacteria, irrespective of number of colonies; Most of these cases were culture negative on one of the two specimens

on culture, 430 (76 per cent) had only 1-3 bacilli while another 55 (10 per cent) had 4 bacilli.

Whether the 567 cases, positive on smear only, can be considered as bacillary cases is doubtful. The reproducibility was poor : only 6 per cent among males and 4 per cent among females showed bacilli on both smears examined. The X-ray confirmation (C or D by two readers) in such persons was also only 13 per cent. The problem of smear positive and culture negative cases has been examined by Raj Narain and associates[45], in another survey conducted not far from the area of the study, but using the same techniques. In that study also the findings were similar and it was suggested that cases showing only 1-3 bacilli on smear and negative on culture should not be considered as bacillary cases. For these reasons, smear positive and culture negative cases have been presented in the material after excluding all cases who had shown only 1-3 bacilli on the whole smear.

The above conclusions are in no way affected even when the result of the specimen giving the highest number of colonies on culture and the corresponding result of smear examination of the same specimen was correclated.

Cases positive on culture : It will be appreciated that in this report, culture positive cases mean those in whom the culture growths from sputum specimens have been classified as *M. tuberculosis* based on morphological characteristics of the colonies, niacin test and other identification tests (see Table 10).

All culture positive cases do not present uniform characteristics in terms of reproducibility of the culture result, confirmation by direct smear or by radiology. Thus several categories of culture

positive cases can be identified : those positive on culture on two specimens (sputum samples) and those positive on culture on only one specimen when more than one specimen is examined; those positive on both culture and smear and those positive on culture only.

Presented below are some of the characteristics of the culture positive cases in relation to the results of smear examination. Of the total 2041 culture positive cases diagnosed at intake 1852 cases for whom two cultures and two smears have been examined are included :

	Cul. pos. Smear pos.	Cul. pos. Smear neg.
Total cases	1097	755
Cul. pos. on 2 specimens	968	235
Cul. pos. on 1 specimen	129	520
C or D by 2 readers	989	492

It will be observed that among the 1097 cases that were positive on both culture and smear 968 (88 per cent) were positive on culture of two specimens while the corresponding proportion for those positive on culture only was 31 per cent i.e., 235 out of 755. Further, cases positive on culture and smear had X-ray confirmation in 989 (90 per cent) while those positive on culture only had X-ray confirmation in only 492 (65 per cent) of the 755 cases. Reproducibility of culture results as well as confirmation by other techniques were distinctly higher among cases positive on both culture and smear than among cases positive on culture only.

Another aspect in which the culture positive cases could be examined is by comparing the cases positive on one culture only with those positive on two cultures in terms of number of colonies observed on culture growth. Table 13 presents the distribution by number of colonies of 2041 cases, culture positive for *M. tuberculosis* at intake, separately for those positive on one culture only and for those positive on two cultures, and separately for three age groups. For persons with two positive cultures the one showing higher number of colonies is considered.

It will be observed that in the age group 10–24 yr, of the 65 cases positive on one culture only, 45 (69 per cent) had less than 6 colonies, whereas the corresponding proportion was 8 out of 86 (9 per cent) among those positive on two cultures. Among cases positive on one culture only and having less than 6 colonies, 26 (58 per cent) out of 45 had only one colony on the culture. The trend was very similar in all age groups.

Cases of Tuberculosis

The presentation above is not an exhaustive examination of the characteristics of cases of tuberculosis nor an attempt to evolve a definition of a case of pulmonary tuberculosis. There is little doubt that cases positive on two (or more) cultures can be considered as true cases of pulmonary tuberculosis. On the other hand cases positive on one culture only have characteristics that are markedly different from those positive on two or more cultures. It is quite likely that most of these cases are true cases of pulmonary tuberculosis being the so-called 'early cases', but some 'false cases' (mislabeled in the field or contaminated in the laboratory) would also be

TABLE 13 — DISTRIBUTION BY NUMBER OF COLONIES ON CULTURE AND BY AGE OF PERSONS CULTURE POSITIVE FOR *M. tuberculosis* AT INTAKE, SEPARATELY FOR PERSONS POSITIVE ON ONE CULTURE ONLY AND THOSE POSITIVE ON TWO CULTURES

No of colonies	Age group (in yr)												Total			
	10-24				25-44				45+							
	+ve on 1 culture		+ve on 2 cultures		+ve on 1 culture		+ve on 2 cultures		+ve on 1 culture		+ve on 2 cultures		+ve on 1 culture		+ve on 2 cultures	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
01	26	40.0	1	1.2	107	32.7	6	1.1	141	31.6	16	2.9	274	32.7	23	1.9
02	9	13.8	3	3.5	37	11.3	10	1.8	51	11.4	10	1.8	97	11.6	23	1.9
03	4	6.2	2	2.3	19	5.8	3	0.5	23	5.2	8	1.4	46	5.5	13	1.1
04	4	6.2	2	2.3	14	4.3	3	0.5	26	5.8	8	1.4	44	5.3	13	1.1
05	2	3.1	—	—	9	2.8	2	0.4	19	4.3	5	0.9	30	3.6	7	0.6
06-24	8	12.3	6	7.0	55	16.8	50	9.0	81	18.2	62	11.1	144	17.2	118	9.8
25-99	10	15.4	11	12.8	38	11.6	99	17.8	50	11.2	88	15.7	98	11.7	198	16.5
>100	2	3.1	41	47.7	32	9.8	217	39.0	40	9.0	210	37.5	74	8.8	468	38.9
Confluent growth	—	—	20	23.3	16	4.9	167	30.0	15	3.4	153	27.3	31	3.7	340	28.3
Total	65	100.0	86	100.0	327	100.0	557	100.0	446	100.0	560	100.0	838	100.0	1203	100.0

included here. All cases were put on treatment with anti-tubercular drugs and thus the study of the fate of these cases would not provide reliable evidence on whether some cases in either group were false cases and as such could be excluded for obtaining estimates of different epidemiological indices. In view of the above considerations, four categories of cases are presented separately in the material dealing with prevalence of diseases. (i) cases positive on two cultures, (ii) cases positive on one culture only (iii) cases positive on smear only, excluding those showing 1-3 AFB on the entire smear and (iv) abacillary X-ray positive cases (C or D by two readers).

11. Study population

During intake, the entire popualtion in the study area was registered, but only the *dejure* population has been considered for this report. A *dejure* population excludes visitors but includes permanent residents who were temporarily away. The registered population, the dejure population and among the *dejure* population, numbers tuberculin tested, 'vaccinated', X-rayed and sputum examined, in different age groups, are shown in Tables 14 and 15 for males and females respectively.

The age pyramid both for the total registered and the resident (*dejure*) population is shown in Fig. 6. It is observed

TABLE 14 — POPULATION REGISTERED AND COVERAGES OBTAINED FOR VARIOUS EXAMINATIONS BY AGE IN MALES

Age group	Population registered	*Dejure* population	No vaccinated	No test-read	No X-rayed	Sputum Examination		
						No eligible	Both specimens examined	Only one specimen examined
< 1 M	865	761			not eligible			
1-11 M	4605	4270	2301			not eligible		
1-4 Y	19469	18521	14135	14148			not eligible	
5-9 Y	23342	22701	19476	19340			not eligible	
10-14 Y	23152	22402	19724	19352	20027	965	868	31
15-19 Y	15254	14721	11923	11432	12115	673	578	34
20-24 Y	14651	13984	11012	10286	11236	851	680	77
25-29 Y	14965	14357	10848	10145	11163	1129	952	69
30-34 Y	13419	12988	9511	8987	9894	1280	1088	69
35-39 Y	12253	11997	8859	8432	9204	1467	1224	97
40-44 Y	9908	9708	7122	6856	7464	1438	1218	85
45-49 Y	9384	9233	6968	6743	7348	1639	1434	80
50-54 Y	7307	7181	5400	5232	5738	1465	1266	76
55-59 Y	5868	5761	4401	4303	4684	1343	1172	70
60-64 Y	4599	4501	3379	3298	3638	1107	974	50
65-69 Y	3007	2939	2209	2203	2466	833	732	45
70 + Y	3086	2993	1768	1885	2275	812	708	50
Total	185134	179018	139036	132642	107252	15002	12894	833

TABLE 15 — POPULATION REGISTERED AND COVERAGES OBTAINED FOR VARIOUS EXAMINATIONS BY AGE IN FEMALES

Age group	Population registered	*Dejure* population	No vaccinated	No test-read	No X-rayed	Sputum Examination		
						No eligible	Both specimens examined	Only one specimen examined
< 1 M	941	820			not eligible			
1-11 M	4789	4471	2459		not eligible			
1-4 Y	19091	18174	13880	13899	not eligible			
5-9 Y	23333	22592	19157	19020	not eligible			
10-14 Y	20440	19781	17568	17456	17832	1070	990	23
15-19 Y	14016	12879	10292	10239	10713	547	480	17
20-24 Y	17569	15880	10811	10894	12321	677	611	24
25-29 Y	16238	15372	11370	11550	12966	882	811	21
30-34 Y	12761	12363	9568	9702	10604	873	803	30
35-39 Y	11491	11235	9149	9200	9807	951	867	29
40-44 Y	9549	9344	7559	7514	7973	930	853	32
45-49 Y	8737	8487	6913	6811	7263	1073	978	33
50-54 Y	7277	6999	5375	5289	5687	881	789	39
55-59 Y	5698	5417	4169	4163	4471	858	752	43
60-64 Y	4189	3945	2626	2664	2993	642	568	31
65-69 Y	2704	2553	1641	1721	1976	458	388	30
70+ Y	2668	2523	882	1078	1442	424	359	33
Total	181491	172835	133419	131200	106048	10266	9249	385

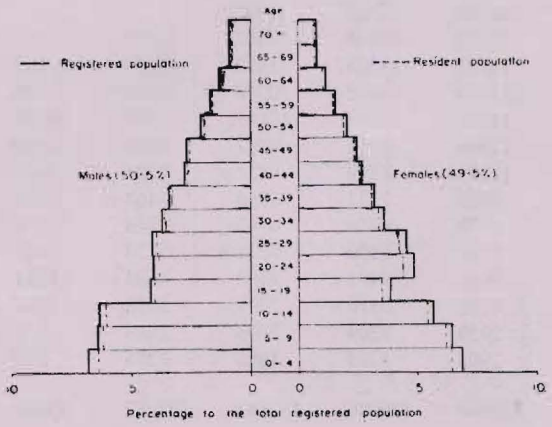

Fig. 6 — Total registered population and resident (*de jure*) population by age and sex.

that there are much fewer persons in the age group 15–19 yr than in the younger age groups. This may be due to a bias in age estimation, or among males due to emigration in search of employment or for education.

During intake a total of 366, 625 persons were registered. The *dejure* population consisted of 351, 853 persons (179 018 males and 172, 835 females). Of those eligible, 77.8 per cent were 'vaccinated'; 77.3 per cent tuberculin tested and read, 82.2 per cent X-rayed and 92.5 per cent sputum examined. Of those X-rayed, 11.8 per cent were eligible for sputum examination while 11.0 per cent had sputum examined by one or both specimens. 9680 (or 3.3 per cent of those examined for scars) persons who had a scar suggestive of previous BCG vaccination elsewhere have been excluded from analysis.

Prevalence of reactors to tuberculin (PPD-S)—Considering persons with 12 mm and bigger reactions to PPD-S, irrespective of the size of reaction to PPD-B, as infected with *M. tuberculosis,* the prevalence of infection in the study population by age and sex is shown in Table 16, and Fig. 7. The overall infection prevalence was 50 per cent; 54 per cent among males and 46 per cent among females.

The prevalence of infection rose rapidly from the youngest age group up to about 25 yr among males and 35 yr among females and then almost levelled off. At those ages, over 80 per cent of males and 70 per cent of females were infected. The infection prevalences were lower among females than among males for age groups beyond 15 yr.

'Sex difference' in reactions to PPD-S—Distribntions of reactions to PPD-S for

TABLE 16 — NUMBER AND PERCENTAGE OF REACTORS (≥ 12 mm) TO PPD-S BY AGE AND SEX

Age group (yr)	Males			Females		
	No of test-read	No reactors	%	No test-read	No of reactors	%
1-4	13937	681	4.9	13722	726	5.3
5-9	19091	2892	15.1	18788	2747	14.6
10-14	18789	5993	31.9	17049	4904	28.8
15-19	10655	5669	53.2	9720	4128	42.5
20-24	9538	6857	71.9	10286	5546	53.9
25-29	9632	7719	80.1	11136	6921	62.1
30-34	8707	7288	83.7	9425	6241	66.2
35-39	8266	7040	85.2	8980	6297	70.1
40-44	6760	5769	85.3	7356	5392	73.3
45-49	6657	5752	86.4	6687	4894	73.2
50-54	5195	4380	84.3	5215	3875	74.3
55-59	4278	3550	83.0	4128	2983	72.3
60-64	3280	2692	82.1	2654	1917	72.2
65-69	2192	1742	79.5	1711	1242	72.6
70+	1877	1510	80.4	1074	786	73.2
Total	128854	69534	54.0	127931	58599	45.8

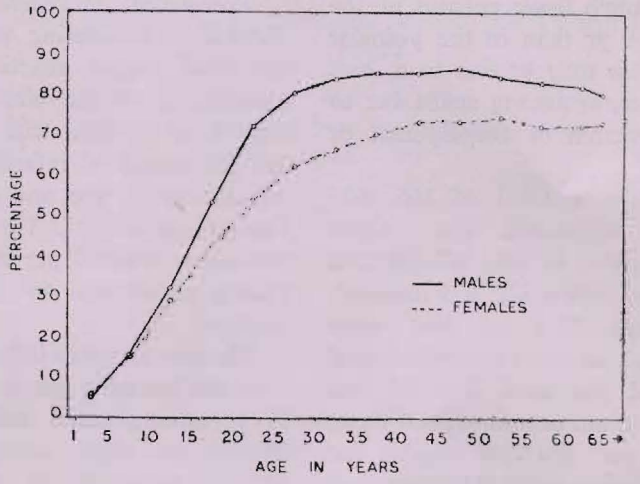

Fig. 7 — Prevalence of infection (12 mm or more to PPD-S) by age and sex

the two sexes in Fig. 5, left half, show practically no difference up to the age of 15 yr. After that age there is a remarkably constant difference. Frequency of small reactions (say, below 10 mm) is higher among females. Frequency, of large reactions (say, between 10 and 22 mm) is higher among males and of reactions larger in size than about 22 mm is again higher among females.

This phenomenon was also reported earlier[74,75]. WHO Tuberculosis Research Office[76] reported the same phenomenon and stated that reactions in women were larger and varied more in size than in men. The same phenomenon can also be seen, to a lesser degree, for PPD-B reactions. Its significance is not known.

Prevalence of reactors to PPD-B— Fig. 5, right half, shows the distributions of reactions to PPD-B as frequency polygons, in five age groups up to 35 yr. As explained in Chapter 10, considering persons reacting with 10 mm and above to PPD-B as reactors the prevalences by age and sex are shown in Table 17. It will be observed that even in age group 1-4 yr the prevalence was as high as 34.4 per cent and rose steeply so that by about 15 yr, almost everybody was a reactor. Further, unlike for PPD-S, there were no differences in the prevalences between males and females.

The absence of any difference in the prevalences between the two sexes suggest that this massive sensitisation is caused by environmental mycobacteria, which however, rarely cause lung disease in man in this population.

Also, because of this massive sensitisation, analysis of incidence of disease among individuals without nonspecific sensitivity bas not been feasible.

Prevalence of bacillary cases of tuberculosis — Tables 18 and 19 (males and females respectively) show the distribution by age and the corresponding prevalences for three categories of bacillary cases, i.e., cases positive on culture on two specimens, cases positive on culture on one specimen only, and cases positive on direct smear only (with more than three

TABLE 17 — NUMBER AND PERCENTAGE OF REACTORS (≥ 10 mm) TO PPD-B BY AGE AND SEX

Age group (yr)	Males			Females		
	No test-read	No of reactors	%	No test-read	No of reactors	%
1-4	13846	4708	34.0	13632	4746	34.8
5-9	19065	12750	66.9	18754	12684	67.6
10-14	18772	16869	89.9	17037	15161	89.0
15-19	10651	10358	97.2	9714	9210	94.8
20-24	9535	9408	98.7	10285	9911	96.4
25-29	9629	9518	98.8	11128	10842	97.4
30-34	8704	8641	99.3	9415	9190	97.6
35-39	8259	8185	99.1	8964	8772	97.9
40-44	6754	6677	98.9	7344	7183	97.8
45-49	6647	6553	98.6	6680	6511	97.5
50-54	5189	5093	98.1	5199	5035	96.8
55-59	4272	4174	97.7	4122	3964	96.2
60-64	3274	3172	96.9	2642	2522	95.5
65-69	2188	2122	97.0	1702	1618	95.1
70+	1881	1806	96.0	1075	1007	93.7
Total	128666	110034	85.5	127693	108356	84.9

bacilli on the entire smear). In all, 2041 culture positive cases were detected in a population of 206,609 persons X-rayed giving a prevalence (adjusted) of 1068 per 100,000; 1704 among males and 439 among females. The prevalence of cases culture positive on two specimens was 664 per 100,000 (1106 among males and 234 among females) and that of cases culture positive on one specimen only was 404 per 100,000 (598 among males and 205 among females). The prevalence of cases culture negative but smear positive (>3 AFB) was 101 per 100,000; 139 among males and 63 among females. For all categories of cases, prevalence of disease is lower among females than among males and increases as age advances in both sexes as also seen from Fig. 8. However, as the definition of a case becomes less rigorous the difference in prevalence between males and females also reduces.

It may be of interest for purposes of comparison with other prevalence surveys to study the prevalence of bacillary cases of tuberculosis, where cases are classified taking the results of both smear and culture into consideration. Table 20 presents the number of two categories of cases, i.e., those that are both smear and culture positive and those that are positive on culture only, distributed by age and sex. It will be observed, from comparison of data in Tables 18, 19 and 20, that the prevalence of culture positive and smear positive cases was similar to the prevalence as seen for cases positive on two cultures and the prevalence of cases culture positive and smear negative was similar to the prevalence as obtained for those positive on one culture.

TABLE 18 — PREVALENCE OF BACILLARY CASES ON CULTURE AND SMEAR, BY AGE, MALES

Age group (yr)	No X-rayed	No eligible for bact. exam.	Bacteriological specimen examined		Culture positive on two specimens		Culture positive on one specimen only		Smear positive (> 3 AFB), culture negative	
			twice	once	No	per 100,000	No	per 100,000	No	per 100,000
10-14	19437	939	846	28	2	11	5	28	9	50
15-19	11272	620	533	32	15	155	4	30	5	49
20-24	10408	781	628	69	44	526	31	281	9	97
25-29	10607	1082	912	66	86	962	35	300	15	156
30-34	9588	1239	1050	68	110	1354	50	495	10	116
35-39	9017	1436	1201	96	133	1764	84	900	8	98
40-44	7357	1417	1200	84	114	1830	70	930	14	210
45-49	7253	1617	1412	80	127	2005	63	834	18	269
50-54	5695	1455	1259	75	113	2293	79	1384	7	134
55-59	4657	1333	1164	70	96	2361	66	1397	11	255
60-64	3617	1102	970	50	63	1979	54	1516	12	358
65-69	2455	827	728	45	47	2175	43	1747	7	305
70+	2266	812	708	50	36	1822	47	2102	7	331
Total	103629	14660	12611	813	986	1106	631	598	132	139

The rates are based on the assumption that persons eligible for bacteriological examination, but not examined, have the same rates as those examined. Thus if

x = no. of persons X-rayed; e = persons eligible for bact. exam.; i = persons bact. exam. once; ii = persons bact. exam. twice; p = persons culture positive once; pp = persons culture positive twice;

then the rate of persons culture positive twice will be

$$\frac{pp}{ii} \cdot \frac{e}{x}$$

and the rate of persons being culture positive either once or twice

$$\frac{p+pp}{i+ii} \cdot \frac{e}{x}$$

so that the rate of persons culture positive once only is the latter minus the former

It may be recalled that the overall coverage for X-ray examination was 82.2 per cent and for sputum examination 92.5 per cent. To what extent the non-response groups have affected the prevalence is not known. Even in the hypothetical case that the non-response groups had a lower prevalence, it can be said that the population had a high prevalence of bacteriologically proved cases of tuberculosis, as compared to findings from similar surveys conducted in rural areas in India[52,74,77].

Prevalence of X-ray cases — Table 21 and Fig. 8 present the number of abacillary X-ray cases diagnosed and the

TABLE 19 — PREVALENCE OF BACILLARY CASES ON CULTURE AND SMEAR BY AGE, FEMALES

Age group (yr)	No X-rayed	No eligible for bact. exam.	Bacteriological specimen examined		Culture positive on two specimens		Culture positive on one specimen only		Smear positive (>3 AFB), culture negative	
			twice	once	No	per 100,000	No	per 100,000	No	per 100,000
10-14	17412	1046	968	23	3	19	3	17	6	36
15-19	10170	514	451	16	3	34	5	53	3	32
20-24	11654	642	579	23	19	181	17	148	2	18
25-29	12505	843	772	21	24	210	16	130	2	17
30-34	10300	843	774	30	27	286	22	213	7	71
35-39	9576	928	846	29	31	355	23	243	8	89
40-44	7805	915	840	32	32	447	27	346	4	54
45-49	7134	1053	960	33	30	461	30	431	8	119
50-54	5609	874	783	39	15	299	22	402	4	76
55-59	4434	851	745	43	14	361	15	345	6	146
60-64	2981	641	567	31	11	417	14	482	3	108
65-69	1964	457	387	30	4	241	8	429	5	279
70+	1436	423	358	33	4	329	5	349	3	226
Total	102980	10030	9030	383	217	234	207	205	61	63

Calculation of rates same as shown in Table 18

corresponding prevalences among the X-rayed population. The overall prevalence for both sexes was 1429 per 100,000; 1886 among males and 978 among females. Though there is an increase in prevalence as age increases, as seen also for the bacillary cases, the trend alone may not justify that these are all true cases of tuberculosis. There is a relatively larger number of X-ray cases in the very young (10–14 yr) and the very old (65 yr and more) compared to the bacillary cases in these age groups. The higher prevalences among young children may be due to reading of their X-rays as hilar adenitis and in whom the sputum specimens in almost all instances were abacillary. Among the old it was possibly due to the classification of healed tuberculous lesions seen on X-ray, as 'active'. On the other hand, it is also likely that a proportion of these shadows are simply nonspecific in nature, but radiologically classified as C or D.

12. Follow-up methodology

Follow-up of study population to diagnose new cases of tuberculosis that have developed after intake comprises three activities : resurvey, selective casefinding and passive casefinding. Samples of the population were also followed up at different intervals after intake for eliciting the status of tuberculin allergy (post-vaccination allergy) after 'vaccination'. The methods adopted are described below.

Techniques followed for X-ray and sputum examinations during follow-up were the same as those adopted at the time of intake.

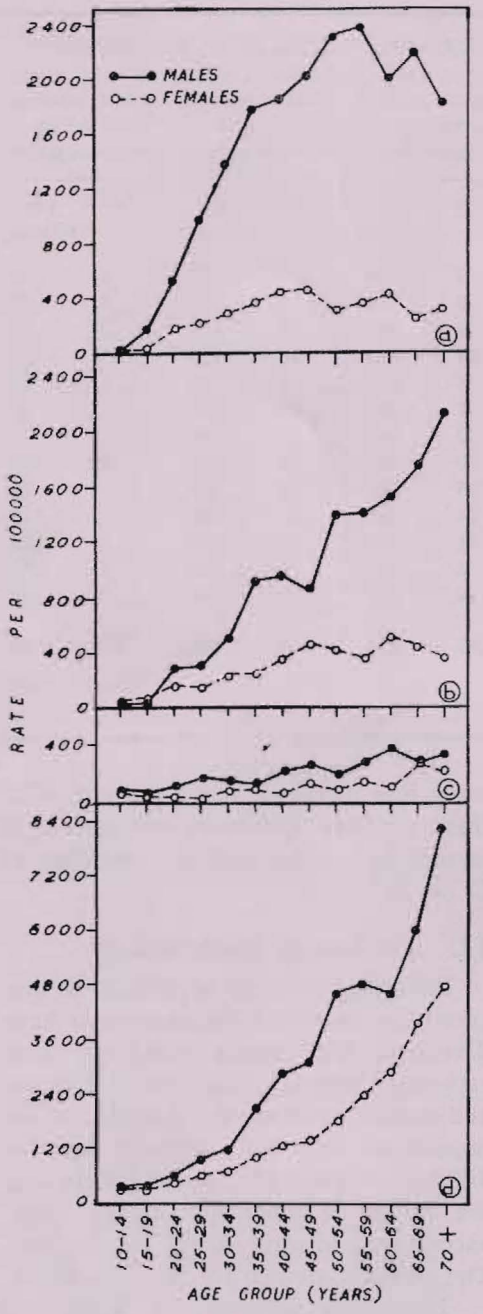

Fig. 8 — Prevalence of disease for 4 categories of cases, by age and sex. (a) cases culture + ve on 2; (b) culture + ve on one specimen only; (c) culture — ve, smear + ve (>3AFB) ; (d) Abacillary X-ray cases (C or D by two)

Resurvey : The resurvey activity is run continuously covering the study population systematically every $2\frac{1}{2}$ yr. Thus the interval between two consecutive surveys for each village would be almost exactly $2\frac{1}{2}$ yr.

As from the first resurvey round, eligibility for X-ray examination was extended down to persons aged 5 yr and above, so that at the 5 yr follow-up, (i.e., second resurvey) the cohort from intake was examined irrespective of age.

The resurvey activity comprises of updating census through registration of new entrants in the study population (new born, settlers, persons missed in the previous round, and visitors), X-ray examination, sputum collection, and finger-printing at the time of sputum collection but no tuberculin testing or BCG vaccination. The procedures for these examinations are the same as for intake.

Half-way through the second resurvey the eligibility criteria for examinations in the resurvey had to be changed for reasons of economy. It had also become clear at that time that the main target group of the trial, i.e., persons with smaller tuberculin reactions at intake, yielded a very small proportion of all new cases. Therefore efforts were concentrated on this group comprising persons with a reaction size of 0–15 mm to PPD–S at intake and in addition, irrespective of the size of reaction to PPD–S, those falling into a 'high risk' group (with suspect X-ray shadows or smear positive though culture negative in the previous examination). However, in a random sample consisting of one-third of all panchayats included in the study, all individuals aged 5 yr and above were examined according to the criteria adopted for the first resurvey.

Selective case-finding — At intervals of

TABLE 20 — PREVALENCE OF CASES POSITIVE ON BOTH CULTURE AND SMEAR AND CASES POSITIVE ON CULTURE ONLY AT INTAKE, BY AGE AND SEX

Age group (yr)	No X-rayed		Culture positive smear positive cases				Culture positive smear negative cases			
			Males		Females		Males		Females	
	M	F	No	per 100,000	No	per 100,000	No	per 100,000	No	per 100,000
10-14	19437	17412	1	6	4	24	6	33	2	12
15-19	11272	10170	13	127	4	43	6	58	4	43
20-24	10408	11654	42	452	19	174	33	355	17	156
25-29	10607	12505	79	824	27	230	42	438	13	111
30-34	9588	10300	113	1306	30	305	47	543	19	193
35-39	9017	9576	142	1744	35	388	75	921	19	210
40-44	7357	7805	101	1515	34	457	83	1245	25	336
45-49	7253	7134	118	1763	31	461	72	1076	29	431
50-54	5695	5609	103	1973	15	284	89	1705	22	417
55-59	4657	4434	87	2018	10	244	75	1740	19	463
60-64	3617	2981	58	1732	11	396	59	1762	14	503
65-69	2455	1964	48	2092	6	335	42	1830	6	335
70+	2266	1436	38	1796	3	226	45	2127	6	452
Total	103629	102980	943	994	229	237	674	710	195	202

For calculation of rates refer to footnote under Table 18
M, males; F, females

TABLE 21 — DISTRIBUTION OF ABACILLARY X-RAY CASES BY AGE AND SEX

Age group (yr)	Males			Females		
	Number X-rayed*	No of cases	Rate per 100,000	Number X-rayed*	No. of cases	Rate per 100,000
10-14	19351	60	310	17331	59	340
15-19	11183	39	349	10100	28	277
20-24	10216	61	597	11555	46	398
25-29	10346	97	938	12390	74	597
30-34	9265	113	1220	10185	67	658
35-39	8622	183	2122	9433	95	1007
40-44	6988	200	2862	7665	95	1239
45-49	6866	215	3131	6971	97	1391
50-54	5339	248	4645	5481	102	1861
55-59	4345	212	4879	4299	103	2396
60-64	3358	156	4646	2882	85	2949
65-69	2277	137	6017	1891	76	4019
70+	2088	170	8142	1377	66	4793
Total	100244	1891	1886	101560	993	978

*Persons sputum positive or for whom sputum results not available are excluded

7½ mo, or later (after second resurvey) 10 mo (i.e., three or two times between two surveys) every village was visited and any person with a suspect X-ray shadow or absent for X-ray at the previous examination was offered an X-ray examination. Also, each household was informed that persons suffering from chronic cough or chest pain should report for an X-ray examination. At the examination centre, persons were identified using population registers (see Chapter 13 and each person X-rayed. Later, from persons whose photofluorograms showed any abnormality samples of sputum were collected. For persons classified as bacteriological suspects (i.e., smear positive but culture negative) at the previous examination sputum was collected irrespective of the results of X-ray examination. At the time of sputum collection a fresh set of finger prints were obtained as described earlier.

Passive case-finding — A tuberculosis clinic was established by the project in the premises of the Government Hospital in Trivellore with a static X-ray unit and sputum collection facilities. Any person reporting there with symptoms suggestive of tuberculosis, whether referred by the hospital's out-patient clinic or by a private practitioner, or self referred, could be examined on any morning. In addition, the ten rural general peripheral health institutions in the study area were visited along with an X-ray unit every two to four weeks, for examination of symptomatics referred by the medical officers of those institutions or private practitioners in the area.

In this activity each symptomatic was carefully identified using population registers, an X-ray was taken and a spot-specimen of sputum obtained at the same time. The laboratory was requested to process only those sputum specimens collected from persons whose X-ray pictures showed any abnormality. Efforts were also made to obtain a second sputum specimen from persons with abnormalities on X-ray. The sputum specimens collected from others were discarded. At the time of each sputum collection finger prints were taken.

In time, this service became well known and popular, and accounted for about one-third of the total cases found.

Additional samples of sputum — Irrespective of the method of follow-up, efforts were made to obtain an additional specimen of sputum whenever a specimen was reported as contaminated by the laboratory. During visits to the villages for this purpose, efforts were also made to collect additional samples of sputum from those whose smears were positive (to guard against the possibility of the corresponding cultures getting contaminated) and from whom only one positive culture result was obtained. On this occasion also finger-prints were obtained as part of identification of the individual.

Repeat tuberculin tests — Repeat tuberculin tests were carried out in random samples of population groups at 2½ mo, 2½ yr and 4 yr after intake. (As described in Chapter 5, most of Trivellore town was not retested according to this design, i.e., testing was done only at 2½ mo). The retests were carried out mainly for the evaluation of tuberculin allergy at different intervals after vaccination. For each retesting a separate sample of population was selected so that no individual was tested more than twice i.e., once at intake and again at the time selected for retesting.

All retests were carried out with 3

IU of PPD-S injected at a different site than used for the first two tests at intake.

Retesting at $2\frac{1}{2}$ *mo* — First retesting was carried out $2\frac{1}{2}$ mo after the vaccination. The exact average interval was 75 days, the range being 62 to 99 days. A 10 per cent random sample of the population was selected for measuring the size of the vaccination lesion and recording its nature and of this, one-fifth (2 per cent sample) for assessing the size of post-vaccination allergy. The method of sampling is already described under Chapter 5.

Retesting at $2\frac{1}{2}$ *yr* — For the $2\frac{1}{2}$ yr retesting, a 5 per cent random sample of the population was drawn. The sampling methodology has already been described in Chapter 5.

Retesting at 4 *yr* — A ten per cent sample of the total population had been selected for lesion-reading at $2\frac{1}{2}$ mo and another 5 per cent sample for retesting at $2\frac{1}{2}$ yr. The remaining 85 per cent of the total population was included for retesting at 4 yr after intake. For this retesting, all individuals aged up to 30 yr at intake and who had been 'vaccinated' and children born after intake, were eligible.

Treatment of Cases

Cases diagnosed at intake or in any of the follow-up activities were put on domiciliary treatment with anti-tubercular drugs. Treatment was organised by the clinic run by the project or under the supervision of the clinic in any one of the ten general health institutions in the study area.

13. Record linkage at follow-up

Machinery — As the data for each person from more than one round occupied several punched cards, the tabulator was found inadequate and towards the end of intake it was replaced by a computer (IBM 1401–H).

Records, punched cards and punch codes — Except for details mentioned in the next three paragraphs, records and punch codes are virtually the same as used during intake.

For each resurvey or round of selective casefinding, the computer prints out by means of the punched name cards from the previous survey, new individual cards approximately of the form shown in Fig. 4, with all identification particulars but omitting details of previous examinations. New information for each individual (change in census stutus, household number or relationship, and X-ray roll number of the X-ray taken in that round) is entered in these cards in the field; the cards are returned daily and the information immediately punched.

Eligibility for sputum collection — The punched cards with the information from the individual cards and the punched cards with the concurrent X-ray readings are passed through the computer on a daily basis and the computer will for each person without X-ray abnormality, or ineligible for X-ray, punch an up-dated name card and an analysis card for the current round. For persons eligible for sputum collection the machine prints out an individual card with name and census status of the person and a request for collection. In the field, the serial numbers of the sputum containers used are entered in this card, and the numbers are punched and later collated with the laboratory results as described for intake (Chapter 9).

Additional examination and diagnosis— All cards for any person for whom sputum

collection has been requested are transferred to another computer program, run on a weekly basis, and kept there till all examinations for the current round have been completed, by which time the machine punches the updated name card and current analysis card. To the card deck for this weekly program are added examination results as they become available, especially bacteriological results. In each weekly run, the machine reads in all available information for one person at a time and determines whether any action is indicated. Possible actions of the machine are : request to the field for collection of a third sputum specimen (e.g., if a specimen is reported from the laboratory as being contaminated); request for umpire reading of X-ray (e.g., in case of any positive bacteriological finding but with X-ray readings not 'active', or negative); request for hospitalization and collection of additional 'spot' and 'overnight' sputum specimens (for a positive bacteriological finding on smear or on a single culture) ; request for putting a new case of tuberculosis on treatment (persons with two positive cultures). It may be noted that a case may well be diagnosed before all examinations are completed. In such a case the machine will print out a treatment card immediatey but await the results of other examinations (e.g., a third sputum specimen) before finalising and punching the analysis card.

It may be appreciated that with the procedures adopted, as described above, each investigation (X-ray reading, sputum examinations) was conducted independently and without reference to the results of other investigations. The results of the several investigations were collated in the (suitably programmed) computer and the classification of a case of tuberculosis was arrived at. Thus no bias was introduced in performing the different investigations and the establishment of the case classification of an individual was completely objective.

Filing of data — The updated name cards and the analysis cards are filed panchayat-wise. For each panchayat, at the end of five months by which time all results of examinations are expected to be available, if cards for a few individuals had not yet been finalised these were inspected by the statistical staff, to determine why they had not been finalized by the machine and necessary action taken for finalization. All cards were then checked by the machine for consistency and missing entries, and corrections made, as described earlier. If a person had been examined in a place other than the one where he was examined at intake a duplicate analysis card was made out with the individual number of the original panchayat and filed in the latter. The analysis cards were added to the main file (see below) already containing all previous analysis cards for each person, and the updated name cards were used for printing-out of individual cards for the next visit to the panchayat.

Identification of new cases of tuberculosis — For every new bacillary case of tuberculosis, as classified by the computer, a manual check was made to ensure that the diagnosis was correct according to the original records from laboratory and X-ray readers. It was also ensured that finger prints obtained from the case at the time of collection of the sputum specimen which turned out to be positive were identical with those obtained at intake (vaccination).

Data files — Some data files of punched cards have been created for the purpose

of epidemiological analyses and for the up-to-date census feed-back to the field. Four such important files are :

Main file : All analysis cards are filed in individual order and for each individual kept in a chronological order. This is a master file giving all information for each person included in the trial. This file is continuously kept updated. However, total number of cards in this file, at present, is more than 2 million, which is quite unwieldy for frequent handling.

Intake file : A separate file containing analysis cards of intake only is maintained. This gives all base-line information and can be used independently for analysis of intake data such as prevalences, without reference to the main file.

Case file : The main file has been duplicated for all persons who are culture positive for *M. tuberculosis* on at least one specimen or have a positive smear in any round. This small file is very useful to keep count of all incidence cases and to carry out any analysis on cases only. This file is kept continuously updated by adding cards of new cases as and when they are diagnosed.

Name file : A file consisting of all name cards is maintained. When fresh census data is available the old name cards are replaced by new name cards with updated census. Normally the entire file is updated once in $2\frac{1}{2}$ yr when a fresh census of the whole popnlation is taken. This file is used to print out population registers and to print out individual cards for resurveys and selective follow-up rounds.

Population registers—After each survey (i.e., once in $2\frac{1}{2}$ yr), several population registers are printed by using the name and movement cards. One register, for every panchayat, is printed after sorting the cards according to household, thus showing all members of a family together; another register, also for each panchayat, is printed after sorting all names alphabetically, including persons registered in a different panchayat but referred (through a movement card) to the particular panchayat; a third register (prepared after the intake only) is alphabetic in order, one for each panchayat union. Later if at the clinic, or at any one of the peripheral heath institutions, a person attends for whom the individual number will not be available his identity in the study is established as follows : first his name is located in the alphabetic register (which also gives household number, age and sex, and individual number) and then by referring to the corresponding household register his identity confirmed through verifying the names of other members of his household.

14. Incidence of infection and disease

Incidence of infection — It may be recalled that the entire study population, except children aged below one year, was eligible for testing with PPD-S and PPD-B at intake. In addition, repeat tuberculin tests with 3 IU of PPD-S were carried out in mutually exclusive random cluster samples of population at $2\frac{1}{2}$ mo (a 2 per cent sample), $2\frac{1}{2}$ yr (a 5 per cent sample), and 4 yr (an 85 per cent sample) after intake (see Chapters 5 and 12). The population of the greater part of Trivellore town, constituting 6 per cent of the study population, had all been included for retesting at $2\frac{1}{2}$ mo, and was therefore not included in the retestings at $2\frac{1}{2}$ and 4 yr from the results of which the incidence data given below

are obtained. The repeat tests were carried out mainly to study the status of allergy after BCG vaccination and not for the purpose of direct estimation of the incidence of infection. However, the information as available is presented below.

The results of $2\frac{1}{2}$ mo retesting would not provide data on incidence of infection as the duration after the first test was very short. It would be obvious that the incidence of infection as obtained from $2\frac{1}{2}$ year and 4 yr retest results could be studied only among those who had been 'vaccinated' with placebo. For this purpose, decoding of the data was done on a computer by the ICMR headquarters in Delhi and the tabulations, in the form of summary tables, were obtained by the project. Thus the vaccination status of individual persons remained unknown to the project staff.

Among the definite non-reactors (0-7 mm to PPD-S) administered placebo at intake, in the age group 1-14 yr, 1,238 and 19,397 children were included in the samples selected at $2\frac{1}{2}$ yr and 4 yr respectively. Of these, 877 (71 per cent) and 13,216 (68 per cent) respectively were test-read. In age group 15 yr and above the test-read coverages were 49 per cent at $2\frac{1}{2}$ yr and 45 per cent at 4 yr.

Criterion for defining a newly infected person — The methodology for defining a person as newly infected described by Raj Narain and associates[78] has been adopted in this material. Briefly, the difference between the size of reaction at the second test and that at the first test for each individual is obtained and these differences are studied as distributions. Only persons in whom the sum of the two reactions was 20-59 mm were included in the 'distribution of differences' for reasons explained in the report referred to.

Fig. 9 shows the distributions of differences of reactions as detailed above among children administered placebo at intake, separately for three age groups 1-4, 5-9 and 10-14 yr. The distributions of differences between reactions at $2\frac{1}{2}$ yr and at intake and those between reactions at 4 yr and at intake are respectively shown on the left half and right half of the figure. The distributions for other age groups were almost unimodal.

It will be observed that among children aged 1-4 yr, the distribution at the $2\frac{1}{2}$ yr retest (Fig. 9, left half) is bimodal, the antimode being at about 12 mm. This would mean that in this age group, there are two classes of individuals, one with an increase of 12 mm or more with the mode at 20 mm and another with an increase of less than 12 mm with the mode at 4 mm. Individuals belonging to the former class are regarded as those with an evidence of new infection, and are defined as the 'newly infected'. It may however be observed that though the distributions of the uninfected and the newly infected are not completely separate, the shapes of the two distributions being similar, the overlapping due to some 'not newly infected' being considered as 'newly infected' and vice-versa would be nearly equal and thus in the estimation of newly infected, the errors mostly get cancelled. In the age group 5-9 yr, the bimodality is less obvious, even less so in the age group 10-14 yr. In later age groups (not shown) it is hardly discernible.

At 4 yr also (Fig. 9, right half), among children aged 1-4 yr at intake, the distribution of differences in reactions to the two tests shows a similar phenomenon as at $2\frac{1}{2}$ yr except that the antimode is at 10 mm. Again, in age groups 5-9 yr

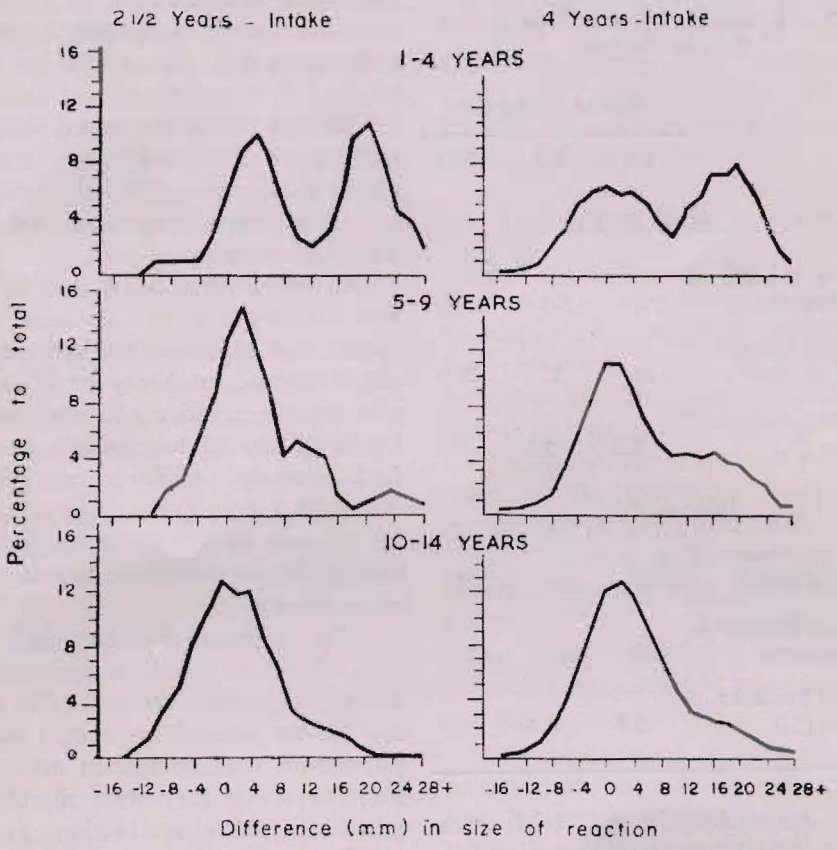

Fig. 9 — Distribution of differences between reactions to PPD-S at intake and at 2½ years, or at intake and at 4 years, among children given placebo

and above bimodality is not obvious.

Thus, in the following, estimates of the average annual incidence of infection are derived using the criteria of an increase of 12 mm or more for those retested at 2½ yr and an increase of 10 mm or more for those retested at 4 yr among persons reacting with 0-7 mm to PPD-S at intake.

Estimates of annual incidence of infection—Table 22 presents the incidence of infection in age groups 1-4, 5-9, and 10-14 yr among the placebo administered controls in the study population. At 4 yr, the sample was very much larger and the observed estimates of the average annual incidence of infection were of the order of 3, 4 and 6 per cent for the age groups 1-4, 5-9 and 10-14 yr respectively. It is observed that the incidence rates increased with age at intake. Although, at 4 yr, the study population was large, these estimates may not be considered as very precise, since in the population concerned the test used was not sufficiently specific, especially for the age goups 5-9 and 10-14 yr. Nevertheless, infection

TABLE 22 — INCIDENCE OF INFECTION IN THE PLACEBO GROUP

	Age at intake (yr)		
	1-4	5-9	10-14
Interval, ½ yr			
No with a reaction of 0-7 mm at intake	297	363	217
No with an increase of 12 mm or more	25	32	25
Av. annual incidence of infection (%)	3.5	3.6	4.8
Interval, 4 yr			
No with a reaction of 0-7 mm at intake	4518	5467	3231
No with an increase of 10 mm or more	486	908	690
Av. annual incidence of infection (%)	2.8	4.4	5.8

with *M. tuberculosis* in the trial area appears to have been at least as common as in other parts of India, if not more so.

Raj Narain and associates[78] described a method of calculating annual incidence of infection from age specific prevalence of infection. Applying this indirect method to the prevalence data on infection as presented in Table 16, the annual incidence of infection works out to 1.74 per cent in age group 1-4 yr, 2.42 per cent in age group 5-9 yr and 4.03 per cent in age group 10-14 yr. These rates are lower than those estimated by the direct method.

Incidence of disease — As described earlier, the study population is followed up by resurveys systematically once in every 2½ yr. In addition, selective casefinding is carried out at shorter intervals and passive casefinding on a regular continuous basis (for details *see* Chapter 12.

All new cases diagnosed during the first 2½ yr after intake by any of the above three approaches are referred to as incidence cases diagnosed during the 'first follow-up'.

An 'incidence bacillary case' during the first follow-up is an individual who at intake was examined by X-ray and was either normal on X-ray or if abnormal was sputum negative and who became a bacillary case of tuberculosis during the first follow-up. Children ineligible for X-ray at intake (i.e., those aged below 10 yr) and who became bacillary cases during the follow-up are also considered as incidence cases.

The incidence of tuberculosis during first follow-up period was estimated by considering the new cases, as defined above, that arose from among the study population who completed all examinations to which they were eligible, irrespective of BCG vaccination, and were abacillary at intake and examined during the follow-up. Thus children aged below one year who were not eligible for tuberculin testing would also be included.

Since the report essentially has bearings on protection offered by BCG, data on incidence of disease is presented as related to tuberculin sensitivity, i.e., size of reaction to PPD-S at intake. Further, in the present report, the incidence data pertaining only to the first follow-up and only for culture positive cases (and not for culture negative smear positive or X-ray cases) is presented. Incidence data for all the first three follow-ups and for all categories of cases will be presented in subsequent reports.

Incidence of bacillary cases during first follow-up — Table 24 presents the number of new bacillary cases, culture positive on at least two specimens, that occurred among the study population that was abacillary at intake. The cases are presented by age, sex, tuberculin sensitivity and X-ray results at intake, Table 24 presents the corresponding numbers of new cases culture positive on one specimen only.

Of the 213,307 persons followed up 1331 new bacillary cases developed during the first follow-up. Of these, 908 were culture positive on at least two specimens and 423 positive on one specimen only. The overall incidence of pulmonary tuberculosis confirmed by culture during the first follow-up was 624 per 100,000 or 250 per 100,000 per annum. Of the total 1331 cases, 985 occurred among males and 346 among females. The average annual incidence of disease among the 107, 619 males was 366 per 100,000 and among the 105,688 females, 131 per 100,000. Thus, the incidence of disease among males was nearly 3 times of that among females. The incidence of disease increased with age for both males and females.

Of particular interest in connection with the effect of BCG vaccination is the distribution of new cases as related to the tuberculin reaction at intake. Out of the total 1331 culture positive cases diagnosed during the first follow-up 52, 21, 85 and 1173 cases occurred from among those who at intake reacted with 0-7 mm, 8-11 mm, 12-15 mm and 16 mm or more respectively. Correspondingly, the annual incidence of disease (per 100,000) during the first follow-up works out to 21, 55, 192 and 563 respectively. The incidence of disease was much lower among those not infected at intake as compared to that among infected (even when considering that two-thirds were vaccinated ; see Table 26). A similar finding has also been reported from other surveys conducted in south India[9,52].

15. The effect of BCG vaccination

The primary objective of the study was to obtain a precise estimate of the protective effect of BCG vaccination in the non-infected, the main subsidiary objectives being to study the influences of dosage and strain on the protective effect. The design of the trial provided for the study of the protective effect through comparison of the incidence of disease among the vaccinated and among the controls. Further, the procedures adopted provided for the study of the protective effect against the development of tuberculosis especially cases positive on culture.

The rationale of BCG vaccination is to substitute the natural and potentially harmful primary infection with virulent tubercle bacilli by an artificial and innocuous primary infection with attenuated bacilli that have maintained the immunogenic properties but not the pathogenicity of the virulent bacilli. Among the immunogenic properties are the enhanced resistance to a subsequent exposure to virulent infection, but also immune reactions such as induction of delayed hypersensitivity.

Development of delayed hypersensitivity to mycobacterial antigens leading to its demonstration as cutaneous sensitivity to tuberculin has been extensively used for monitoring the immediate effects of BCG vaccination. The level of tuberculin sensitivity induced by BCG vacci-

TABLE 23 — NEW CASES OF TUBERCULOSIS, CULTURE POSITIVE ON AT LEAST TWO SPECIMENS, DURING THE FIRST FOLLOW-UP BY AGE, SEX, TUBERCULIN REACTION AND X-RAY RESULT (IRRESPECTIVE OF BCG VACCINATION) AT INTAKE

Age at intake (yr)	No followed-up		Number of new cases — X-ray result at intake: Ineligible or normal		Others		No followed-up		Number of new cases — X-ray result at intake: Ineligible or normal		Others	
	M	F	M	F	M	F	M	F	M	F	M	F
	0-7 mm to PPD-S						12-15 mm to PPD-S					
1-4	13651*	13410*	3	1	—	—	110	105	—	—	—	—
5-14	22929	21980	—	2	—	—	1770	1227	—	1	—	—
15-24	4455	5495	2	—	—	1	2155	1214	—	1	1	—
25-34	1770	4579	1	3	—	—	2019	1673	4	2	2	—
35-44	1121	2773	1	—	1	1	1547	1513	5	—	8	2
45-54	837	1737	1	—	—	—	1276	1128	7	—	10	1
55-64	607	872	1	1	1	1	790	624	2	—	1	3
65+	350	280	1	—	1	—	397	188	1	—	5	1
Total	45720	51126	10	7	3	3	10064	7672	19	4	27	7
	8-11 mm to PPD-S						16+ mm to PPD-S					
1-4	277	237	—	—	—	—	451	470	—	1	—	—
5-14	2647	2098	—	—	—	—	5842	5045	5	5	—	2
15-24	1560	1285	2	—	—	—	7389	5302	31	6	24	6
25-34	916	1576	2	2	1	—	9589	9165	36	15	58	22
35-44	684	1280	—	3	—	—	8565	8527	51	12	100	40
45-54	561	937	—	—	2	—	6835	6192	42	6	118	32
55-64	426	516	—	—	2	—	4086	3075	23	5	88	18
65+	205	166	—	—	1	1	1802	1019	10	2	48	6
Total	7276	8095	4	5	6	1	44559	38795	198	52	436	126

*Children aged less than 1 yr who were ineligible for tuberculin testing are also included

nation is an indication of the quality of the vaccination since this level is known to be correlated with the dose of (live) vaccine actually introduced into the skin[19]. Moreover, delayed hypersensitivity, as it reflects cell-mediated immunity, may be related to the protection afforded[79].

Thus, in the assessment of the effect of BCG vaccination in the present study, the development and duration of delayed hypersensitivity induced by BCG has been studied in addition to the incidence of tuberculosis among the vaccinated and among the controls.

TABLE 24 — NEW CASES OF TUBERCULOSIS, CULTURE POSITIVE ON ONE SPECIMEN ONLY, DURING THE FIRST FOLLOW-UP BY AGE, SEX, TUBERCULIN REACTION AND X-RAY RESULT (IRRESPECTIVE OF BCG VACCINATION) AT INTAKE

Age at intake (yr)	No followed-up		Number of new cases: X-ray result at intake: Ineligible or normal		Others		No followed-up		Number of new cases: X-ray result at intake: Ineligible or normal		Others	
	M	F	M	F	M	F	M	F	M	F	M	F
	0-7 mm to PPD-S						12-15 mm to PPD-S					
1-4	13651*	13410*	2	2	—	—	110	105	—	—	—	—
5-14	22929	21980	6	3	1	1	1770	1227	—	—	—	—
15-24	4455	5495	1	—	1	—	2155	1214	3	—	—	1
25-34	1770	4579	1	—	—	1	2019	1673	1	1	1	1
35-44	1121	2773	1	—	1	2	1547	1513	—	2	—	1
45-54	837	1737	—	—	2	—	1276	1128	1	—	3	1
55-64	607	872	1	—	—	—	790	624	1	—	3	1
65+	350	280	2	—	1	—	397	188	3	—	2	2
Total	45720	51126	14	5	6	4	10064	7672	9	3	9	7
	8-11 mm to PPD-S						16+ mm to PPD-S					
1-4	277	237	—	—	—	—	451	470	—	—	—	—
5-14	2647	2098	—	—	—	—	5842	5045	4	1	1	—
15-24	1560	1285	—	—	—	—	7389	5302	11	6	11	5
25-34	916	1576	—	—	—	1	9589	9165	19	13	24	14
35-44	684	1280	—	—	—	—	8565	8527	11	6	37	16
45-54	561	937	—	—	—	—	6835	6192	11	9	39	19
55-64	426	516	—	—	1	—	4086	3075	12	3	33	19
65+	205	166	1	1	1	—	1802	1019	3	4	25	5
Total	7276	8095	1	1	2	1	44559	38795	71	42	170	78

*See foot-note under Table 23.

BCG induced tuberculin sensitivity — Development of BCG induced tuberculin sensitivity was studied among all persons irrespective of age or initial tuberculin sensitivity. However, in the following, data is presented only for children aged 0-14 yr who were (definitely) tuberculin negative (0-7 mm to PPD-S) at intake.

As described earlier, repeat tuberculin tests with 3 IU of PPD–S were carried out in random samples of the population at 2½ mo, 2½ and 4 yr after vaccination. As already explained, there did not appear to be any significant differences

between the various batches of vaccines used. They have been therefore clubbed in presenting the development and duration of delayed hypersensitivity.

The results of retesting are presented in Table 25 and graphically, as frequency polygons, in Figs 10, 11 and 12, showing the distributions of reactions for the 0.1 mg dose of BCG, the 0.01 mg dose of BCG and the placebo respectively. The observations in the age groups 0-4, 5-9 and 10-14 yr (at intake) for the two doses of the vaccines are compared with the placebo to illustrate the level of tuberculin sensitivity induced and its development with time. It should be noted that in this evaluation any boosting of tuberculin sensitivity as a result of repeated testing[80] was avoided by testing different sample populations on each occasion.

Development of hypersensitivity among those vaccinated with the 0.1 mg dose of BCG appeared highly satisfactory showing unimodal, almost normal, distributions with mean reaction sizes of 16-17 mm. But after 2½ yr this pattern had changed considerably with the distributions showing a definite shift to the left, with mean reaction sizes of 9-12 mm suggesting a loss of sensitivity from 2½ mo to 2½ yr. There was no further loss of sensitivity seen from 2½ to 4 yr. However, the average level of sensitivity at 2½ and 4 yr still remained considerably higher than that in the placebo group. Both from Table 25 and Fig. 10 it is apparent that the degree of waning in tuberculin sensitivity from 2½ mo to 2½ yr varied with age being most pronounced in the youngest children. The significance of the phenomenon of waning in tuberculin sensitivity is not clear.

The findings are similar for those vaccinated with 0.01 mg dose of BCG also, though the mean size of indurations were smaller at all levels and even at 2½ mo the distributions tended to be bimodal.

The observation made in this study that tuberculin sensitivity apparently waned during the first few years after vaccination and then remained at the same level appears to be very different from the one made by Horwitz and Bunch-Christensen[54]. They reported that BCG induced allergy as elicited (using 2 TU of

TABLE 25 — MEAN SIZE OF POST-VACCINATION REACTIONS AT EACH OF THE 3 RETESTINGS AMONG CHILDREN REACTING WITH 0-7 MM AT INTAKE

Age at intake (yr)	Retested at 2½ mo		2½ yr		4 yr	
	No.	Mean	No.	Mean	No.	Mean
			0.1 mg BCG			
0-4*	500	16.1	334	9.1	5252	9.8
5-9	503	16.7	350	10.3	5518	11.3
10-14	344	17.4	227	12.1	3184	12.6
0 14	1347	16.7	911	10.3	13954	11.0
			0.01 mg BCG			
0-4*	469	12.8	328	6.5	5269	7.8
5-9	526	12.2	338	7.9	5439	9.1
10-14	366	13.7	199	9.0	3147	10.4
0-14	1361	12.8	865	7.6	13855	8.9
			Placebo			
0-4*	512	4.2	347	5.0	5261	6.2
5-9	586	5.2	363	6.3	5467	7.9
10-14	401	7.6	217	7.7	3231	9.2
0-14	1499	5.5	927	6.1	13959	7.6

*Children less than 1 mo-old are excluded

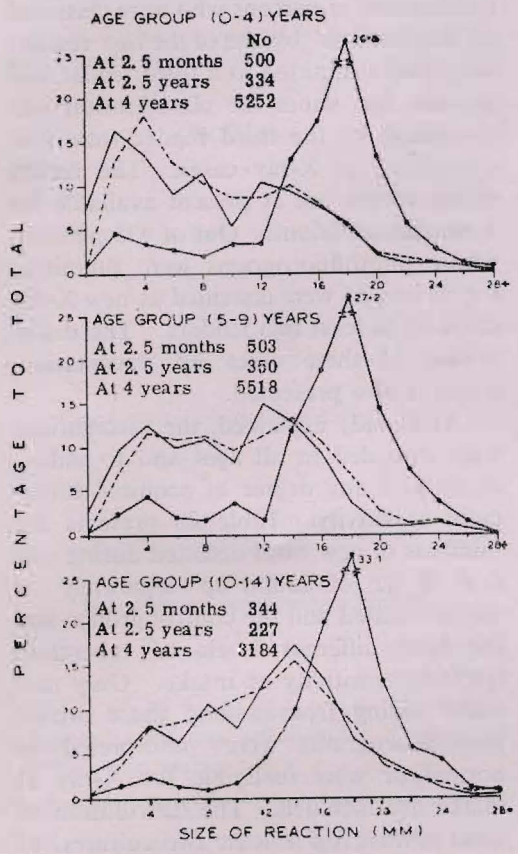

Fig. 10 — Post-vaccination allergy after 3 intervals of time among those with an initial size of reaction of 0-7mm (●——●, at $2\frac{1}{2}$ mo; —, at $2\frac{1}{2}$ yr; - - - -, at 4 yr) (persons vaccinated with the strong dose).

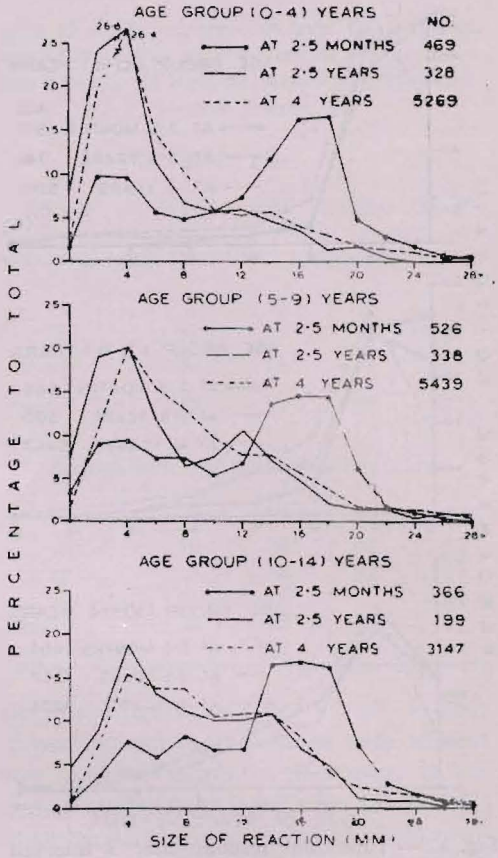

Fig. 11 — Post-vaccination allergy after 3 intervals of time among those with an initial size of reaction of 0-7mm (persons vaccinated with the weakdose)

RT 23 with Tween 80) at 2 mo after vaccination among school children aged 7 yr was almost unchanged after 5 yr. There were no unvaccinated controls in their study.

It should be noted that in the above presentation the sample at $2\frac{1}{2}$ mo included an area (the 8 blocks of Trivellore town previously mentioned) which was not represented in the samples at $2\frac{1}{2}$ yr and 4 yr. However, the conclusions drawn were in no way affected when the data was reanalysed (not presented) after excluding the results from this area at $2\frac{1}{2}$ mo.

Incidence of tuberculosis among the vaccinated and the controls — In studying the effect of BCG vaccination, in addition to culture positive cases, non-bacillary X-ray cases were also considered. For this purpose all new culture-negative X-ray cases (i.e., C or D by two readers) that occurred among persons who, at intake, were tuberculin negative (0-7 mm to PPD-S) and whose chest photofluoro-

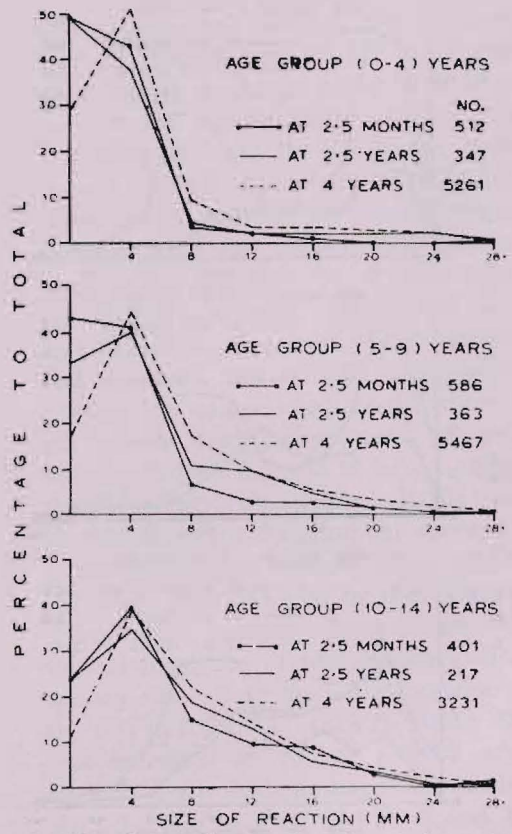

Fig. 12 — Tuberculin reaction after 3 intervals of time among those with an initial size of reaction of 0-7 mm (persons given the placebo)

grams were interpreted as normal, or were ineligible for X-ray, are being reviewed, along with matched controls, independently by two X-ray readers. For this, all available photofluorograms (from intake to the last available) of each person were submitted to each of the two readers for an independent review. The readers, on the basis of the review of photofluorograms, classified new cases of tuberculosis. Only persons so classified by both readers were considered as X-ray cases for the purpose of studying the effect of BCG vaccination. The photofluorograms of persons who were classified as 'tuberculosis' by one of the two readers only were submitted to a third reader and persons for whom the classification was confirmed by the third reader were also considered as X-ray cases. The results of the review are at present available for 2 panchayat unions. Out of 433 persons, whose photofluorograms were submitted for review, 55 were classified as new X-ray cases by at least two readers. The distribution of these cases by 'vaccination' status is also presented.

As already explained, the vaccinations were extended to all ages and to individuals with any degree of acquired tuberculin sensitivity. Table 26 presents the numbers of new cases detected during the first $7\frac{1}{2}$ yr of follow-up separately for the vaccinated and the control groups and for four different levels of tuberculin (PPD-S) sensitivity at intake. Only new cases arising from among those whose photofluorograms were interpreted as normal or were ineligible for X-ray at intake are included. The distribution of cases positive on at least two cultures, of cases positive on one culture only and of X-ray cases (based on review) are shown under (*a*), (*b*) and (*c*) respectively. Exact relative incidence figures are not yet available, but it may be taken that the denominators for the vaccine groups and the placebo group are the same. Thus it is clear at a glance that in any of the groups, BCG vaccination, over $7\frac{1}{2}$ yr, had had no effect in offering protection against the development of pulmonary tuberculosis. The very remote possibility of one strain being associated with a protective and the other with a harmful effect was examined. As no such difference was observed the results of both strains have been combined.

TABLE 26 — DISTRIBUTION OF NEW CASES DURING THE FIRST 7½ YR OF FOLLOW-UP, ACCORDING TO VACCINATION STATUS

Tuberculin reaction at intake	BCG, mg 0.1	0.01	Placebo	Total
Cases with at least two positive cultures				
0-7	37	37	28	102
8-11	14	17	14	45
12- 5	40	34	38	112
16 or more*	259	257	287	803
Cases with one positive culture only				
0-7	22	28	19	69
8-11	4	3	5	12
12-15	20	13	8	41
16 or more*	90	91	82	263
X-ray cases according to review**				
0-7	19	18	18	55

*only a part of this group was eligible for follow-up at 2nd and 3rd resurveys.
**follow-up period : 5 yr

TABLE 27 — DISTRIBUTION OF NEW CASES AMONG PERSONS REACTING WITH 0-7 MM AT INTAKE, SEPARATELY FOR 2½ YR ROUNDS, ACCORDING TO VACCINATION STATUS

Round	BCG, mg 0.1	0.01	Placebo	Total
Persons with at least two positive cultures				
I	4	9	2	15
II	13	15	8	36
III	20	13	18	51
Total	37	37	28	102
Persons with one positive culture only				
I	7	5	4	16
II	10	19	3	32
III	5	4	12	21
Total	22	28	19	69

It is seen that in all groups the large majority of cases occurred among those already infected at intake. In this connection it should be noted that in 2/3rds of the panchayats, persons with a reaction of 16 mm or more at intake were not eligible for active follow-up during later rounds.

The new cases arising from among the definite non-reactors, i.e., those reacting with 0-7 mm at intake, have been further distributed according to the time of the first observation of a positive culture as shown in Table 27. It is seen that there is a tendency for the number of cases to be actually higher among the vaccinated during the first two periods (5 yr) of follow-up. This tendency fails to attain statistical significance ($0.10 > P \geq 0.05$) if one considers only cases with at least two positive cultures. However, it becomes statistically significant ($P < 0.01$) if all bacillary cases, including cases positive on one culture only, are considered. The observation that this tendency appears to be more pronounced for the weak vaccine than for the strong vaccine is not statistically significant. The tendency for the number of cases to be higher among the vaccinated is not seen during the third period (5-7½ yr) of follow-up. In fact, among cases with one positive culture only, two-thirds of whom were aged less than 15 yr at intake, this tendency is reversed in that the number of cases is higher in the control group. However, this does not attain statistical significance.

All the 171 new bacillary cases arising from among the definite non-reactors at

intake are also distributed by vaccination status separately for two age-groups, 0-14 and 15+yr at intake as shown in Table 28. It should be noted that the majority (72 per cent) of the cases occurring among those aged 15 yr or more were culture positive on at least two specimens whereas the majority (67 per cent) of cases in the 0-14 yr age group were culture positive on one specimen only. The pattern of the distribution of cases seen in Table 28 remains almost the same as in Table 27.

Those reacting with 16 mm or more at intake have also been further distributed according to the time of the first observation of a positive culture as shown in Table 29. As already mentioned, the apparent decrease in the number of new cases with time is due to the fact that in 2/3rds of the panchayats, persons with reactions measuring 16 mm or more at intake were not eligible for active follow-up during later rounds. It is seen from Table 29 that the results are similar for all the three periods of follow-up and BCG vaccination did not seem to have any effect, either beneficial or harmful, on persons already infected at the time of vaccination.

Discussion

This report presents the findings of a controlled trial of the protective effect of BCG vaccination against pulmonary tuberculosis in a rural community in India during a $7\frac{1}{2}$ yr follow-up of the study population. The report also documents the design and the procedures of the study in detail, and presents the essential epidemiological features of the study population.

The study design, a form of factorial design, may be considered as superior to

TABLE 28 — DISTRIBUTION, BY AGE, OF THE 171 NEW BACILLARY CASES AMONG PERSONS REACTING WITH 0-7 MM AT INTAKE, SEPARATELY FOR $2\frac{1}{2}$ YR ROUNDS, ACCORDING TO VACCINATION STATUS

Round	Age at intake (yr)	BCG, mg 0.1	0.01	Placebo	Total
I	0-14	7	7	4	18
	15+	4	7	2	13
	Total	11	14	6	31
II	0-14	10	11	4	25
	15+	13	23	7	43
	Total	23	34	11	68
III	0-14	7	7	18	32
	15+	18	10	12	40
	Total	25	17	30	72

TABLE 29 — DISTRIBUTION OF NEW CASES AMONG PERSONS REACTING WITH 16 MM OR MORE AT INTAKE, SEPARATELY FOR $2\frac{1}{2}$ YR ROUNDS, ACCORDING TO VACCINATION STATUS

Round	BCG, mg 0.1	0.01	Placebo	Total
Persons with at least two positive cultures				
I	65	68	78	211
II	101	99	107	307
III	93	90	102	285
Total	259	257	287	803
Persons with one positive culture only				
I	36	29	30	95
II	24	33	28	85
III	30	29	24	83
Total	90	91	82	263

some other possible designs as every individual included in the study contributes to each one of the comparisons under study. Further, the design also enables the study of interactions between the factors (here, the dose and the strain of BCG).

The procedures adopted had the following main characteristics : the study was planned to obtain estimates of the protective effect of BCG vaccination especially against the development of pulmonary tuberculosis proved bacteriologically; the potency of vaccines used in the study were assessed through quality control tests, post vaccination allergy and also it was ensured that these vaccines would be available as seed-lots for any future use; the techniques adopted were standardised and the procedures of intake, follow-up and diagnosis of a case of tuberculosis ensured complete objectivity of each procedure; and finally, the follow-up procedures were systematic and intensive in an effort to identify and document all new cases of pulmonary tuberculosis occurring in the study population during follow-up.

The study population was characterised by high prevalences of tuberculous infection, nonspecific sensitivity and tuberculous (pulmonary) disease as well as high incidences of tuberculous infection and disease. The prevalence of nonspecific sensitivity was so high that even with the very large study popnlation adequate numbers of persons, without initial nonspecific sensitivity, among whom the effect of BCG vaccination could be studied separately, could not be obtained.

The results of the trial reported here show that BCG did not confer any protection against the development of pulmonary tuberculosis during the first $7\frac{1}{2}$ years after vaccination. Under the circumstances, the question of examining the significance of the differences in protective effect between vaccine strains or dosages does not arise.

Details of 8 BCG trials, including the present one, in which the protection observed varied from none to 80 per cent are given in Table 30. BCG vaccination has been the subject of controversy throughout its more than 50 yr long history. With this new result at hand, that BCG did not protect (until now) in this trial, it is perhaps appropriate to discuss some of the hypotheses that may explain the different findings.

As mentioned earlier, the influence of nonspecific sensitivity and potency of vaccine used were offered as the main possible explanations for the considerable differences in the estimates of protective effect observed in the trials conducted earlier. As a result while planning the present study, care was taken not only to select two of the best strains of BCG in use but also to ensure that these strains would be available as seed-lots for any future nse. Also, right from the stage of planning of the study it was intended to carry out a similar study in another area in India where the prevalence of nonspecific sensitivity was low or non-existent. Studies on the prevalence of mycobacterial sensitivity in various parts of the country revealed that only population in areas situated at high altitudes had lower prevalences of non-specific sensitivity though it was not completely absent. The population in these areas however, are very small in relation to the total population of the country. Carrying out another BCG trial in such small and scattered population groups would operationally be very difficult and, even if such a study were

TABLE 30 — PROTECTION OBTAINED IN EIGHT CONTROLLED TRIALS OF BCG VACCINATION AGAINST TUBERCULOSIS

Trial and subjects	Ref. no	Intake period	Duration of observation (yr)	Percentage protection
N. American Indians: (1 to 18 yr age)	1	1935-38	9-11	80
Chicago : infants	18	1937-48	12-23	75
Georgia : School children (6 to 17 yr age)	6	1947	20	Nil
Puerto Rico : general population (below 20 yr age)	4	1949-51	5½-7½	31
Georgia and Alabama : general population (age 5 yr and above)	5	1950	14	14
Great Britain : school children (14 to 15½ yr age)	3	1950-52	15	78
Madanapalle, south India : general population (all ages)	84	1950-55	9-14	30
Chingleput, south India : general population (all ages)	Present trial	1968-71	7½	Nil

to be carried out and it was shown that BCG did protect in the absence of nonspecific sensitivity, the applicability of the findings would indeed be very much limited for the country as a whole. This is possibly true of many other, and especially, developing countries of the world.

The above argument assumes that nonspecific sensitivity could have influenced and masked the protective effect of BCG vaccination. This, however, may not have been quite true as shown by the following. In their painstaking animal experiments, Palmer and Long[11] showed that in guineapigs, 'atypical' strains of mycobacteria conferred a lower protection than BCG : *M. kansasii* gave results close to those of BCG, while strains of the avian group (Runyon groups II and III) gave intermediate results, and rapid growers very little. The 'atypicals', isolated from the sputum specimens obtained from the present study population, representing the environmental mycobacteria, in all probability causing the nonspecific sensitivity in the study population, comprised 1.5 per cent of photochromogens, 68 per cent of groups II and III and 30.5 per cent of rapid growers. One would then expect that 'atypical' sensitization would result in an apparent reduction of the effect of BCG but not *abolish* the effect altogether. This hypothesis however, could not have been tested in the study population, because of the very high prevalence of nonspecific sensitivity, even if some (say, 30 per cent) protection had been observed in the study. However, the hypothesis has once been tested, retrospectively, in the study in Puerto Rico : vaccine and placebo had been allocated irrespective of the reaction to a high-dose test, and it turned out that there was the

same low protection in those positive and in those negative to the high-dose test[37,81]. It is thus unlikely, though not impossible, that nonspecific sensitivity was the sole factor responsible for the *complete lack* of protective effect of BCG observed in the present trial.

There is no doubt that BCG strains mutate in terms of colony morphology and in terms of antigenicity. For the Danish strain, the evidence available indicates that the strain used in this trial had not mutated from the strain used earlier in the British trial where a substantial protection was observed. Three circumstances favour the hypothesis that there was no genetic change in the strain between 1952 and 1960 (when the strain was made into a stable seed-lot). First, every year for the last 30 yr, the allergenicity of the Danish vaccine has been monitored in Danish school children and there has been no change over this period. Second, the methods used to propagate this strain between 1947 and 1960 were conservative, as advocated by Calmette. Third, a comparison of vaccines prepared from the seed-lot with the vaccines prepared from cultures maintained by culture transfers from 1958 to 1965, did not reveal any differences in morphology, antigenicity or virulence in animals, or in allergenicity in children. As regards the French strain, there was a mutation in pigmentation in the late 1950s, but the French workers feel that they succeeded in saving the original strain by back-selection. In the animal experiments conducted at the International Reference Centre for BCG, Copenhagen[56,57] several BCG strains including the French and Danish strains were studied for virulence for hamsters and for protective effect in bank voles. It was found that the residual virulence and the protective effect of the French strain were quite satisfactory; in fact, in terms of survival of bank voles as a function of vaccine dose, the French strain was the strongest of all strains used including the Danish strain. Also, in a recent experiment by D. W. Smith and co-workers (personal communication, 1979), the French strain protected guinea-pigs well, though significantly less than did the Danish strain. Thus, there is much circumstantial evidence that the strains used in this trial were 'genuine' BCG. A meeting of chiefs of quality control laboratories, held in Copenhagen in February 1978, concluded that the vaccines used in this trial were of good quality.

The methods and materials of the study were scrutinised at a meeting of experts held in Madras in October 1977, and it was concluded that there were no apparent flaws in the procedures followed in the study. With the very involved code for vaccine ampoules conceivably a true protection might have been masked by labelling or recording errors. However, since a blind reading of vaccination lesions in a very large proportion of the study population tallied with the records of the codes in 99.8 per cent, such recording errors cannot have influenced the results.

Vaccines prepared from the Danish strain were used in the British as well as in the present trial. In the study in British school-leavers during the first $7\frac{1}{2}$ yr of follow-up, the incidence of tuberculosis observed was higher among non-reactors than that among reactors. On the other hand, in the present study during a similar period of follow-up the incidence of bacillary disease among the non-reactors (0-11 mm) at intake was much

lower than that among the reactors. This observation remains the same even when persons in the age group 10 to 19 yr only, in the present study, whose age would be nearer to the age of the participants in the British trial, are considered. However, in addition to the differences in the age of the participants, the two studies are different in several other aspects, for instance, tuberculins used and definition of a case of tuberculosis, and are thus, not strictly comparable. Even so, the epidemiological situation in the present study, appears to be quite different from what was observed among the school-leavers in Britain. In the study population of the 'longitudinal survey', in a rural population of south India conducted from 1960 onwards by the National Tuberculosis Institute, Bangalore[52], the annual incidence of new bacillary cases, among those with an initial reaction of 0-9 mm to RT 23 (1 TU, with Tween 80), was estimated at 58 per 100,000 while in the present study among those reacting with 0-7 mm to 3 IU of PPD-S and belonging to the same age group (5 yr and above), it was 25 per 100,000, or among those reacting with 0-11 mm at intake, 31 per 100,000. Among the reactors, in the Bangalore study, the annual incidence was 291 and in the present study, 503 per 100,000. Although the data are not strictly comparable, incidence of tuberculosis among non-reactors to tuberculin in the present study, as well as in the Bangalore study appears to be very low in relation to that reported in the British trial and further, lower in the present study than in Bangalore district. The very much lower incidence among non-reactors and a considerably higher incidence among reactors in the present study is a striking finding. It has also been shown that in the present study the incidence of infection is very high. Thus, we are left with the impression that while incidence of infection is very high, those that are recently infected rarely progress to manifest disease. Incidence of tuberculosis is high but only among the middle aged and especially elderly men, individuals who must have been first infected many years ago.

The high incidence of infection, the very low incidence of disease among non-reactors associated with a high incidence of disease among reactors suggest that a large proportion of cases occur not as a result of primary infection but as a result of either endogenous reactivation or exogenous reinfection. If most of the cases do occur as a result of exogenous reinfection the question arises whether BCG can protect at all against such disease and whether this has any bearing on the difference observed in the protective effects between the British and the present trials. However, whether all or a large proportion of cases occurring among the initial non-reactors in the present trial, during the first $7\frac{1}{2}$ yr of follow-up, were also due to exogenous reinfection is difficult to determine.

It could be argued that the lack of protection as well as the tendency for the number of cases to be higher among the vaccinated (as shown in Chapter 15) is due to the vaccinated individuals being at an increased risk of developing tuberculosis if they were infected within 3 wk after vaccination, i.e., before the manifestation of cell-mediated immunity. This however, is unlikely and not borne out by the findings : persons contracting infection within a period of 3 wk after vaccination and then progressing into bacillary cases would be extremely few

and even then one would expect the surplns cases to be confined to the first period of follow-up only and not beyond. One other possibility that could be considered in this regard is that BCG itself might be the causative agent of lung tuberculosis, and that the positive cultures obtained in these cases were BCG cultures. However, this also is not borne out by the results: the results of niacin tests on the cultures of sputum specimens from all these cases were strongly positive (2+) indicating that the organisms were *M. tuberculosis* and not BCG, since niacin tests on BCG cultures of the Danish and French strains are either negative or weakly positive (trace reactions)[82].

The tendency for the number of cases to be higher among the vaccinated was not seen during the third period of follow-up. There was no difference in the number of new cases positive on at least two cultures developing iu the vaccinated and the control groups. But, for cases positive on one culture only, majority of whom were in the 0-14 yr age group, a tendency was seen for the number of cases to be higher in the control group. However, it remains to be seen whether this tendency will be confirmed in the fourth period of follow-up.

A local phenomenon that may be of relevance is the frequency of strains of *M. tuberculosis* that are of low-virulence in the guineapig. This phenomenon was exhaustively studied by Mitchison[83], who found these low-virulence strains to be susceptible to hydrogen peroxide, though they tended to be catalase-positive. Nothing seems to be known, however, about the epidemiological significance of these strains. They have been isolated from previously untreated patients admitted to the Tuberculosis Chemotherapy Centre, Madras, and they were niacin-positive and isoniazid sensitive. Evidence from elsewhere in India is scanty but compatible with the existence of these low-virulence strains in other parts of the country. Work is now underway in this project to study the distribution of such strains, especially in regard to age distribution, infection history, and fate of the patients. While there is as yet no indication of an association between the prevalence of these strains and the BCG results, it nevertheless seems worthwhile to investigate this phenomenon.

In conclusion, the present study has revealed that BCG, over a $7\frac{1}{2}$ yr did not show any protection against the development of pulmonary tuberculosis. Also, though the incidence of pulmonary tuberculosis was high in the study population, it was much less freqnent among initial non-reactors to tuberculin, the majority of whom were in the younger age groups, and who could be expected to benefit from BCG vaccination. However, it should be pointed out that results of the study may not be extrapolated to infants, since infant tuberculosis was not observed in the trial.

Acknowledgment

The authors are grateful to Professor V. Ramalingaswami, Director-General, ICMR, for according permission to publish the report and to Dr C. Gopalan, Professor P. N. Wahi and Dr B. L. Taneja, former Directors-General, ICMR, for their guidance and support; to Drs C. E. Palmer, G. W. Wijsmuller, S. H. Ferebee, P. Q. Edwards and L. A. Farer of the USPHS for their collaboration in the early phases of the study; to Dr K. L. Hitze, Chief, Tuberculosis and Respiratory Infections Unit, WHO,

Geneva ; to Dr B. N. M. Barua, Adviser in Tuberculosis, Government of India and Drs N. K. Menon and N. L. Bordia, former TB Advisors, Government of India, for their guidance and support ; to the Medical Officers, Drs A. S. Bagga, K. Neelakantachar, E. V. Venkatarama Gupta, P. Jayalakshmi, V. N. Ambalavanan and T. K. Narayanan for the services rendered to the project; to Dr D. R. Nagpaul, formerly Director, National Tuberculosis Institute, Bangalore, who was associated with the project in the early stages and to the Medical Officers of the same Institute, Drs G. D. Gothi, P. Chandrasekhar, G. C. Banerjee, Pyarelal, A. K. Chakraborthy, especially for reading photofluorograms; to the staff of the laboratories, especially Drs K. G. Kulkarni and R. Prabhakar and Sriyuts P. Venkataraman and B. N. Gopalan; to the staff of the statistical unit of the T. B. Prevention Trial especially Sriyuts K. Naganna, M. S. Krishnamurthy, A. M. Diwakara, A. M. Ramanathan, S. H. Raghavendra Rao, K. R. Kalyanam, S. Raja Rao, Smt. S. S. Murthy and Smt. M. P. Radhamuni who have diligently carried out the statistical work of the project. Special mention of thanks is due to the devoted work of the entire field staff especially Sriyuts D. L. Satyanarayana Rao, B. N. Appegowda, K. R. Rangaswamy, M. S. Krishnamurthy, A. N. Shasidhara, R. S. Nagabhushana Rao, D. S. Anantharaman, K. Sridhar, L. Vasu and K. Narasimha Rao. For the regular supply of vaccines and tuberculins thanks are due to Miss K. Bunch-Christensen, Chief, BCG Department, State Serum Institute, Copenhagen, Denmark, the Antigen Production Laboratory, Atlanta, Georgia, USA, and Dr J. C. Suri, formerly Director, BCG Vaccine Laboratory, Guindy, Madras. Thanks are also due to the Government of Tamil Nadu for cooperation; the State Tuberculosis Officers and the District Tuberculosis Officers, Kanchipuram, for the supply of anti-tuberculosis drugs for treating patients; the State T. B. Association for granting funds for building a clinic at Trivellore; the Medical Officers of the Taluk Hospital, Trivellore and the Medical Officers of the Public Health Institutions and the Private Medical Practitioners in the study area for cooperating in diagnosis and treatment of cases; the Superintendents, Government T. B. Sanatorium, Tambaram and the T. B. Hospital, Tiruvottiyur for having provided facilities for admitting seriously ill T. B. patients referred by the project and to the staff of the Thoracic Unit of the General Hospital and the Institute of Child Health, Madras, for their cooperation. The help of the secretarial and administrative staff of the project, throughout the study, is gratefully acknowledged. Special thanks are due to Smt M. Vijayalakshmi for secretarial assistance in the preparation of this report.

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Indian J Med Res 72 (Suppl), July 1980

IJMRAQ 72 (Suppl) 1-74 (1980)

CONTENTS

Printed and published by Shri Prem Chand for the Indian Council of Medical Research, New Delhi, at the Kapoor Art Press, A38/3, Mayapuri Industrial Area, New Delhi 110064

Regd. No. D(SW)008