Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1966, 34, 605-622

Problems Connected with Estimating the Incidence of Tuberculosis Infection*

RAJ NARAIN,[1] S. S. NAIR,[2] P. CHANDRASEKHAR [3] & G. RAMANATHA RAO [4]

Many problems have to be faced in the estimation of an apparently simple but valuable index—namely, the incidence of tuberculosis infection. Very little attention seems to have been paid to these problems so far.

Records from 50 villages in a district of South India, whose populations were tested with 1 TU of PPD RT 23 in Tween 80 diluent and retested after 18 months, have been examined for a reappraisal of existing methods. As a result, it has been found that some of these methods are subject to gross errors and that available figures are unreliable.

For estimating the newly infected, a new approach based on the drawing of a curve for the distribution of differences in reaction size from one round of tuberculin testing to another is presented. Further, it is shown that the newly infected probably constitute a homogeneous group with an increase in mean reaction size of about 24 mm and standard deviation of 4 mm. Accordingly, 98% of the newly infected show an increase in reaction size of 16 mm or more. There are others who show similarly large increases in allergy on a retest, even in the absence of infection. *The number of persons in the latter category rises with age and is likely to be greater in areas with a high prevalence of non-specific allergy.*

INTRODUCTION

The incidence or rate of new infection with *Mycobacterium tuberculosis* is an index of the risk of infection to which a community is exposed. An accurate estimation of incidence is of considerable importance in understanding the epidemiology of tuberculosis and in organizing control measures. It is, moreover, essential for the assessment and better planning of BCG vaccination programmes. The degree of protection against tuberculosis provided by BCG vaccination is likely to vary directly with the risk of infection in the community. Vaccination campaigns have been recommended for many age-groups—for example, the newborn, children of school-leaving age, and persons aged up to 25 years. The choice of the most suitable age for vaccination depends upon the risk of infection at different ages, among other factors. Further, it is possible that the epidemiology of tuberculosis morbidity may differ between communities with high and low risks of infection. Reports from Denmark and the USA show that about 75%-80% of the new cases in any year are found among those who have been previously infected (Palmer, Shaw & Comstock, 1958; Groth-Petersen, Knudsen & Wilbek, 1959). Frimodt-Møller, Jacob & Parthasarathy (1964) have also reported similar findings from Madanapalle, South India. A larger proportion of new cases may arise among the previously non-infected in communities with a high risk of infection. It is also possible that a high incidence of infection may occur in communities in which the tuberculosis morbidity is increasing.

There is a great need for simplification of survey techniques. Surveys of tuberculosis prevalence include tuberculin testing, X-ray and sputum examination. The latter two procedures are very costly, and need special equipment and a much longer period of training for the personnel concerned. It is possible that in any community there is a mathematical relationship between the prevalence of cases, radiologically diagnosed or bacteriologically con-

* From the National Tuberculosis Institute, Bangalore, India. This article has also been published in the *Indian Journal of Tuberculosis*, 1965, 13, 5.

[1] Epidemiologist.
[2] Senior Statistical Officer.
[3] Medical Officer, Epidemiological Section.
[4] Junior Statistical Officer.

firmed, and the prevalence or the incidence of infection. If so, it may be possible to use the considerably simpler tuberculin test to obtain a fairly accurate estimate of disease in a community. New infection, which represents recent events, may be better correlated with prevalence, and may provide more reliable estimates of this rate.

Not much is known about the incidence of infection in different age-groups in India. It has been estimated indirectly from age-specific prevalence rates of infection by Bogen (1957), Frimodt-Møller (1960) and Raj Narain et al. (1963). The incidence rates calculated by Bogen were almost identical for all age-groups, with an average of 5.3% per annum. The rates estimated by Frimodt-Møller varied from 0.7% to 6.2%, and those by Raj Narain et al. from 0.9% to 4.4% in different age-groups. The criterion for evidence of infection used by the first two authors was induration of 5-6 mm or more elicited by 5 or 10 TU of PPD RT 22. In India—at least in some parts—the proportion of intermediate reactors makes it difficult to draw the line of demarcation between positive and negative reactors. Raj Narain et al. based their calculations on a reaction of 10 mm or more to 1 TU of PPD RT 23 as indicative of infection with *Myco. tuberculosis*.

The incidence of infection can also be estimated directly by repeating the tuberculin test at a subsequent date (Daniels et al., 1948). On the basis of this method Frimodt-Møller (1960) concluded that the rate of infection with tubercle bacilli was probably about 4% for all ages. An increase in the size of induration from 4 mm or less on the first occasion to 10 mm or more on the second was taken as evidence of fresh infection. However, he considered an incidence rate of 4% to be on the high side and rather arbitrarily decided that the rate was 1%-2%.

These differing estimates of the incidence of infection underline the difficulties involved in accurately estimating such rates. In the present paper these difficulties are examined in the light of data available from some of the studies conducted at the National Tuberculosis Institute (NTI), Bangalore, and a new method of estimation is discussed.

MATERIAL AND METHODS

Material from three studies has been utilized:

Study 1 : As part of a Longitudinal Survey undertaken by the NTI, a random sample of 134 villages from Bangalore District has been surveyed once and a repeat survey is in progress. No previous tuberculin testing or BCG vaccination has been carried out in this area. After a complete census of the population had been taken, a tuberculin test with 1 TU of PPD RT 23 in 0.1 ml of buffered diluent containing 0.005% Tween 80 was given on two occasions (Rounds I and II), and each time the presence or absence of a BCG scar (definite or doubtful) on either shoulder of each person tested was recorded in order to exclude vaccinated persons who might have migrated from other areas. Those with indurations of 13 mm or less at Round I were offered a test with 20 TU of the same tuberculin in Tween 80 diluent. This second test was made within a week of the reading of the first 1 TU test. The longitudinal diameter of induration was recorded three or four days after each tuberculin test.

This report is based on the data from the first 50 villages, for which complete information for both rounds is available. The population concerned does not constitute a random sample, but this is probably unimportant, since the report is chiefly concerned with the development of a suitable method of estimating incidence, rather than with the calculation of actual rates by age and sex. In these villages Round I of the survey was carried out between May and December 1961 and Round II between November 1962 and July 1963. The interval between the two rounds was, on an average, about 18 months.

Study 2 : During a preliminary analysis of the above data, it became apparent that there was a general increase in the size of the tuberculin reactions elicited at Round II. A similar phenomenon has been observed in the USA (Ferebee & Mount, 1963). Towards the end of 1963, in another study carried out in some villages in the area of the Longitudinal Survey, tuberculin tests were performed using the same techniques, except that randomly selected control groups for the 1 TU and 20 TU tests were tested with a placebo only (Raj Narain et al., 1966b[1]). A retesting was carried out after two months in a random sample of half the houses in the villages. This study provided data on the enhancing of tuberculin allergy seen in repeat tuberculin tests.

Study 3 : While the Longitudinal Survey is in progress reader assessments are being carried out periodically to judge the standards of the tuberculin test readers. Inter- and intra-reader comparisons are made. The findings have been used to estimate the magnitude of reader variation.

[1] See article on page 623 of this issue.

Unless otherwise stated the data presented relate to the main study (Study 1) only.

Study population and coverage

Table 1 shows the coverage of the *de facto* population at each round for tuberculin tests only. After the exclusion of persons with BCG scars or those for whom the presence or absence of a scar was not recorded, tuberculin test readings at both rounds are available for 14 414 persons.

Although at each round in the surveys, the *de facto* population is examined, it may be better, for repeat surveys, to report on the *de jure* population only. The percentage coverage at each round is likely to be lower when the *de jure* population is considered, because those members of the population who are absent at the time of registration are eligible for testing but mostly not available, while the temporary visitors are available for testing but not eligible for the study. However, with the *de jure* population, the percentage of those tested and read at both rounds is likely to be greater, because the temporary visitors included in the *de facto* population at Round I are not likely to be available at Round II, while the temporary absentees who return during the actual duration of the survey and are tested at Round I are more likely to be available at Round II. With the *de jure* population (excluding those with BCG scars, etc.) 14 953 would have been available for the correlation, and 14 285 were common to the two populations.

During the interval between the two rounds, six of the villages were visited by mass BCG campaign teams. Mainly as a result of this visit, 492 persons had BCG scars at Round II as compared with 137 at Round I. The *de facto* population of these six villages was 4339 and 4402 in the two rounds respectively.

SOME BASIC PROBLEMS IN THE INTERPRETATION OF TUBERCULIN REACTIONS

What level of tuberculin reaction may be taken as evidence of infection ?

Histograms showing the distribution of tuberculin reactions to 1 TU in Round I for age-groups 0-4, 5-9 and 10-14 years, as well as for " all ages ", are presented in Fig. 1. The distribution for the age-group 0-4 years shows a clear division between the infected and the non-infected, the former consisting of all persons with reactions of 14 mm or more, and the latter of those with reactions of 9 mm or less. The division becomes more and more blurred with increasing age, till in the histograms for " all ages " it can no longer be seen, and the line of demarcation could be at any level between 8 mm and 14 mm. The absence of a sharp line of demarcation between infection and non-infection has been reported earlier

TABLE 1

DE FACTO POPULATION AND COVERAGE FOR TUBERCULIN TESTS IN ROUND I AND ROUND II

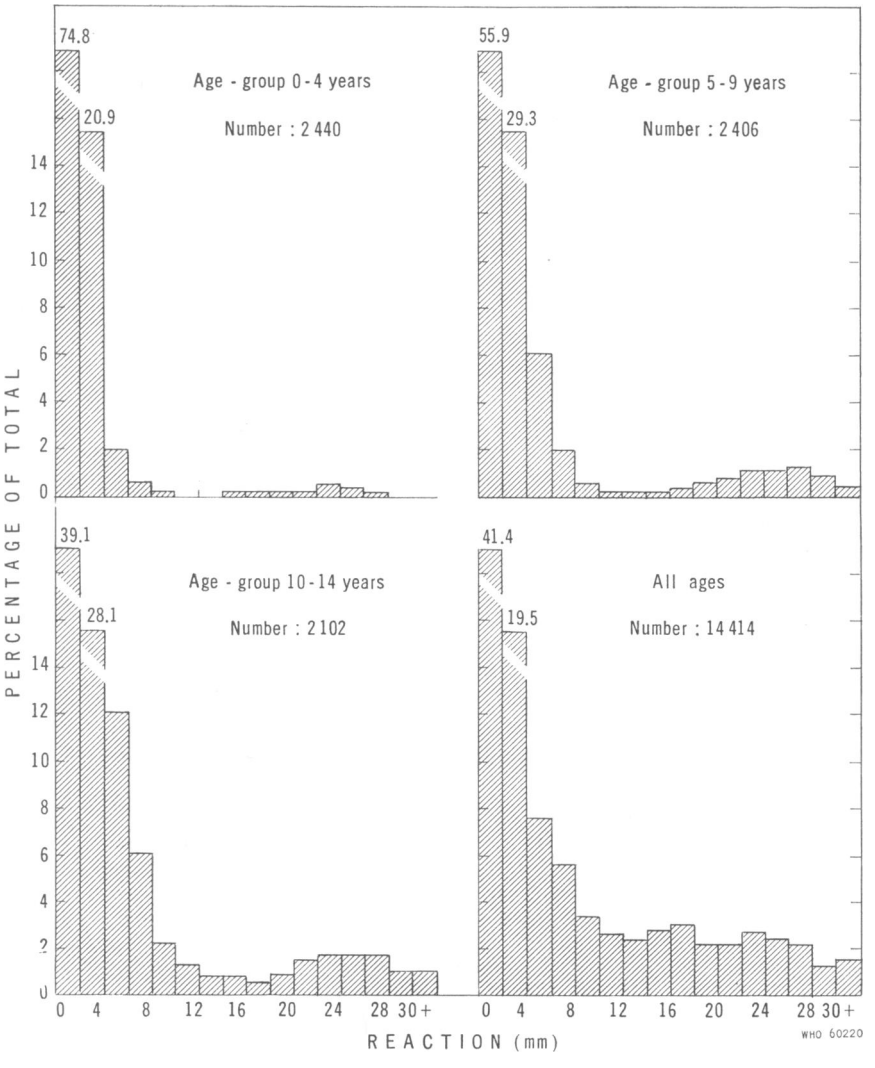
	Round I			Round II		
	Number of persons	Percentage eligible	Percentage of *de facto* population	Number of persons	Percentage eligible	Percentage of *de facto* population
De facto population	22 665	—	100.0	22 323	—	100.0
For 1 TU test:						
Tested	21 724	95.8	95.8	20 253	90.7	90.7
Read	20 423 [a]	94.0	90.1	18 910 [b]	93.4	84.7
For 20 TU test:						
Eligible	16 345	—	72.1			
Tested	15 199	93.0	67.1			
Read	13 183	86.7	58.2			

[a] Including 137 with BCG scars and 24 for whom the presence or absence of a scar was not recorded.

[b] Including 492 with BCG scars and 53 for whom the presence or absence of a scar was not recorded.

FIG. 1

REACTIONS TO 1 TU AT ROUND I, BY AGE



in detail (Raj Narain et al., 1963). When the entities " non-infected " and " infected " cannot be sharply defined, the definition of the " newly infected " also becomes difficult. Unless otherwise stated, persons with reactions of 9 mm or less at Round I have been taken as non-infected for reasons give elsewhere (Raj Narain et al., 1963). The results of the 20 TU test in such persons have also been used to define more precisely the non-infected group.

Margin of error in tuberculin test results

In any long-term survey variations in the technique of tuberculin testing and reading are unavoidable, even when well-trained technicians are employed, as in this study. Both inter- and intra-reader variations are involved in comparing the tuberculin test results in the two rounds. From Study 3 it is estimated that on an average inter- and intra-reader

variations between Round I and Round II are not likely to exceed ± 6 mm in more than 5% of the observations. The reading errors have an equal chance of being positive or negative, except at extreme ends of the distribution, where zero readings at Round I can only show an increase, and the very large reactions have a greater chance of showing only a decrease in allergy at a subsequent round (Raj Narain et al., 1966b[1]).

The effect of repeat tuberculin tests

A comparison of tuberculin allergy in the tested and the control group in Study 2 showed that at two months the mean reaction size in the previously tested group was 1.7 mm greater than in the controls. The enhancing of tuberculin allergy by the first test was much more marked in those with initial reactions of 8-13 mm to 1 TU or 10 mm or more to 20 TU. Further, the enhancing of allergy increased with age (Raj Narain et al., 1966b[1]).

Waning of allergy

Once established, anaphylactic hypersensitivity wanes with the passage of time, provided there is no further contact with the antigen (Rich, 1951). Waning is difficult to measure from the results of two tests because of the enhancing effect of the first test. It cannot be studied by comparing the results of a test at two points in time (as in Study 2), because the changes due to new infection and waning that take place during the interval act in opposite directions and maintain a fairly constant prevalence rate. It is possible that some idea of the extent of waning could be obtained from the results of repeat tests carried out after an interval long enough for the enhancing effect of the first test to become negligible or nil.

If the "boosting" of allergy by a previous tuberculin test is greater among those whose allergy has waned (Raj Narain et al., 1966 b[1]), this may also be a source of error in estimating the incidence of infection, especially in older age-groups, among whom a comparatively large number of persons are likely to show waned allergy.

METHODS OF ESTIMATING THE INCIDENCE OF INFECTION

Incidence rates can be calculated indirectly from age-specific prevalence rates of infection by a mathematical method, and directly from the results of tuberculin tests repeated after an interval. These methods and their limitations are discussed below.

Indirect method of calculating incidence

Three mathematical methods of calculating rates of incidence of infection from prevalence of infection in different age-groups have been reported earlier (Raj Narain et al., 1963). All three methods gave nearly identical results. A fourth method, leading to similar results but probably more appropriate to biological data than the three above-mentioned methods, has been used in the present paper (see Annex 1). In view of the uncertainty in defining the infected, different levels of tuberculin reaction have been regarded as evidence of infection. Incidence rates thus derived are presented in Table 2.

Sufficiently high levels of tuberculin reaction—namely, $\geqslant$ 18 mm and $\geqslant$ 22 mm—have been included in order to see the effect of regarding as infected only the definitely positive, and to examine their influence on incidence rates. Whatever level is considered, the incidence rates increase up to about 35 years of age. They then decrease in succeeding age-groups—so much so that after 55 years of age many of the values derived are on the negative side. This is probably due to the fact that the number of people in the higher age-groups who become tuberculin-negative owing to the waning of allergy is greater than the number who become positive as a result of new infection, thus making the formulae inapplicable to the higher age-groups. Similarly, the calculated incidence rates would be underestimates for the younger age-groups according to the effect of waning among them, the exact extent of which is not known.

In deriving these rates certain assumptions have been made:

(1) Each level considered represents the line of demarcation between infection and non-infection.

(2) The risk of infection has not materially changed during the life of the persons in the age-groups considered.

(3) Death-rates for the infected and the uninfected in the previous years have been the same.

(4) There is a uniform rate of infection within each age-group.

(5) Infected persons do not become tuberculin-negative.

These assumptions impose certain limitations, and in view of points (2), (3) and (5) the incidence rates

[1] See article on page 623 of this issue.

TABLE 2
ANNUAL INCIDENCE RATES DERIVED FROM AGE-SPECIFIC PREVALENCE OF INFECTION AT FOUR LEVELS OF TUBERCULIN REACTION [a]

Age-group (years)	Number of subjects	Annual incidence rates (%) according to different levels of reaction taken as evidence of infection			
		≥ 10 mm	≥ 14 mm	≥ 18 mm	≥ 22 mm
0-4	3 286	0.8	0.8	0.6	0.3
5-9	3 043	0.8	0.8	0.7	0.6
10-14	2 957	1.6	1.2	0.9	0.6
15-24	3 127	2.1	1.3	0.6	0.3
25-34	2 938	2.0	1.4	0.8	0.6
35-44	1 832	1.1	0.9	0.5	0.2
45-54	1 421	0.8	0.7	0.4	0.4
55-64	1 027	−0.2	−0.1	0.3	0.2
65 +	626	−0.1	0.1	−0.2	−0.2

[a] For method of calculation, see Annex 1.

for the younger age-groups might be expected to be more reliable than the rates for the older groups. In fact, for want of a better criterion, they may be so considered for comparison with the results obtained by other methods.

Direct methods of calculating incidence rates

For a direct measurement of incidence rates, tuberculin reactions at two points in time are required. Table 3 presents a correlation of 1 TU reactions for the 14 414 persons tested and read at both rounds. The reactions obtained at Round II show a general shift to the right, mainly as a result of the boosting effect of the previous tests in Round I (Raj Narain et al., 1966b[1]).

(1) *Conversion rates.* The simplest method is to regard as newly infected those tuberculin-negatives at Round I who became positive at Round II. The method has been used in many studies, notably the Prophit Survey (Daniels et al., 1948). Annual incidence rates thus calculated are shown in Table 4 for four different levels of tuberculin reaction taken as evidence of infection. For comparison, annual rates from Study 2 have also been included. The annual incidence rates in the two studies are entirely different, the rates in Study 2 being fantastically high. The latter, being based on small numbers, may be subject to a large variation. But even for the group " all ages " the rates are more than 11 times greater than those for Study 1, and there must be other reasons for such large differences. Boosting may be greater at two months than at 18 months. Even if boosting is equal at the two intervals, it is multiplied six times in Study 2 to obtain annual rates, and reduced by a third in Study 1. Even the rates from Study 1 are high, especially for the older age-groups, and are not compatible with the actual prevalence of infection in different age-groups, unless waning in these age-groups is high enough to account for the low prevalence of infection. Further, even when an induration of 18 mm is regarded as evidence of infection, the incidence rates do not conform to the observed prevalence of infection, at least for the older age-groups.

Similar exaggerated values derived from conversion rates have been reported by Ferebee & Mount (1963). The chief drawback of this method is that it equates the first and the repeat tuberculin test results and takes into account neither the boosting of the latter by the former, nor random variations such as reading errors.

(2) *Increase in allergy as evidence of new infection.* An alternative, with present techniques, is to regard as newly infected the negatives in Round I who show a definite increase in allergy at Round II.

[1] See article on page 623 of this issue.

TABLE 3
CORRELATION OF REACTIONS TO 1 TU AT ROUND I AND ROUND II

Reactions to 1 TU at Round II (mm)

Reactions to 1 TU at Round I (mm)

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	Total
0	1 404	135	492	618	557	408	262	158	130	72	90	67	68	51	49	52	38	20	19	25	24	12	15	16	18	20	9	5	8	4	13	5	6	2	—	5	5	—	1	1	—	—	—	—	1	—	—	—	—	—	—	—	4 885
1	300	44	117	175	155	104	51	29	15	4	14	9	6	6	5	6	7	2	4	1	4	—	3	1	1	2	8	—	1	—	2	—	2	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1 080
2	410	47	177	234	254	171	93	68	58	26	30	19	19	11	25	18	16	6	7	4	7	3	4	5	5	7	4	3	2	1	2	2	1	1	1	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1 743
3	172	23	81	131	146	134	79	52	46	24	30	16	21	13	14	17	10	4	1	6	4	5	1	3	4	7	3	3	3	2	3	1	—	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1 061
4	87	8	29	58	93	78	64	47	30	26	32	14	14	12	12	7	9	7	4	1	5	4	5	2	2	2	3	—	1	1	3	—	2	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	664
5	48	4	7	29	36	42	36	34	29	18	25	10	19	7	6	13	11	10	2	6	3	3	2	2	4	4	1	3	1	2	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	420
6	44	2	15	15	28	52	34	34	16	24	13	17	22	13	12	18	9	7	6	2	9	1	2	2	6	7	1	1	—	1	3	1	1	2	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	422
7	30	4	7	13	21	24	21	26	27	14	22	15	18	14	15	22	11	9	9	6	7	—	7	3	2	5	1	1	1	2	2	—	—	1	1	2	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	365
8	18	4	3	9	11	20	14	13	21	4	15	14	14	13	17	17	10	5	10	6	4	4	5	4	3	3	2	—	—	—	3	1	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	269
A 9	10	—	—	8	7	6	11	15	11	7	8	7	10	10	15	7	7	6	6	4	4	3	2	2	2	1	1	5	—	1	2	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	180 B
10	12	—	—	3	2	8	6	6	8	7	10	7	7	7	13	13	8	5	5	3	2	5	3	7	4	3	—	4	—	—	—	—	—	—	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	160
11	10	—	1	1	3	8	7	4	4	3	11	8	13	12	14	19	13	9	6	7	4	5	7	1	4	8	3	—	1	3	1	1	1	1	—	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	195
12	7	1	1	2	—	6	1	8	9	4	12	10	9	10	11	17	5	9	5	5	9	6	7	4	8	5	2	3	2	1	2	2	—	—	2	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	186
13	9	—	—	2	1	4	5	4	8	2	5	5	6	8	13	8	3	9	8	4	7	4	4	3	5	4	5	2	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	140
14	5	—	—	1	1	6	7	4	7	4	6	7	18	12	11	13	16	4	8	10	5	8	7	5	3	4	5	6	2	—	1	2	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	190
15	4	1	—	1	1	4	5	4	8	5	12	5	8	13	8	9	12	7	9	9	9	9	6	5	8	5	5	6	1	1	2	1	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	185
16	4	—	—	2	2	3	5	1	2	3	8	3	10	5	14	7	10	18	13	13	16	7	10	14	9	9	1	5	3	—	6	—	5	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	210
17	2	2	—	—	3	2	6	—	5	2	5	3	5	7	5	5	7	16	13	4	12	4	14	9	9	11	13	5	5	2	6	1	3	2	—	1	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	191
18	2	—	—	—	—	—	—	—	—	2	2	—	2	5	4	4	12	9	5	2	9	6	3	5	12	9	8	2	7	3	3	3	1	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	123
C 19	1	—	—	—	—	—	1	1	1	3	1	3	—	3	8	6	4	3	8	9	13	9	11	11	10	6	15	6	6	5	6	2	3	—	3	2	1	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	163
20	2	—	—	—	1	1	3	1	2	—	—	1	—	1	1	4	3	4	2	4	3	3	15	10	7	12	8	6	4	6	3	1	1	3	2	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	116
21	1	—	—	—	—	1	1	—	—	—	1	1	—	3	3	4	10	2	4	5	13	9	11	14	8	8	14	11	5	12	12	3	5	4	4	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	171
22	3	—	1	2	—	2	1	—	1	—	1	1	3	2	2	4	11	8	7	3	13	5	10	2	6	16	15	7	9	2	18	5	3	2	2	7	1	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	177
23	1	—	—	—	—	—	2	—	1	2	1	1	2	—	2	7	7	7	8	6	7	9	6	6	12	14	19	6	6	12	10	1	7	2	2	2	1	—	—	—	3	—	—	—	2	—	—	—	—	—	—	—	—	174
24	3	—	—	—	1	—	2	—	—	—	1	—	—	—	4	1	—	2	3	3	8	5	9	12	17	21	18	12	8	10	13	5	6	10	3	4	4	1	1	1	—	—	1	—	—	—	—	—	—	—	—	—	—	189
25	—	—	—	—	—	—	—	—	1	—	1	—	3	2	2	3	3	4	2	4	3	5	9	3	5	13	18	7	7	8	11	4	5	3	5	3	2	3	—	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	141
26	—	—	1	1	1	2	—	—	—	1	—	—	1	1	1	1	5	4	2	4	4	5	10	3	8	22	15	11	15	7	16	7	—	4	4	6	1	3	1	—	1	—	—	—	—	1	1	—	—	—	—	—	—	170
27	—	—	—	—	—	—	—	—	1	—	—	—	—	3	3	2	1	3	2	3	4	5	4	4	4	9	9	5	4	5	13	3	10	7	3	5	7	2	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	122
28	—	—	—	—	—	1	—	—	—	—	1	—	—	1	1	1	—	—	—	2	3	1	4	5	7	9	6	5	12	4	7	1	1	3	3	5	1	—	1	—	3	—	—	—	—	1	—	—	—	—	—	—	—	89
29	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1	2	1	—	3	5	4	6	3	3	2	3	2	2	5	1	3	1	3	2	1	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	58
30	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1	—	1	2	—	—	1	2	5	6	3	4	4	7	5	5	2	1	4	3	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	59
31	—	—	—	—	—	—	1	—	1	—	—	1	—	—	—	2	—	—	—	—	2	—	2	1	4	6	—	1	3	—	3	—	—	—	1	1	1	1	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	33
32	—	—	—	—	—	—	—	—	—	—	—	1	—	1	—	—	1	—	—	—	—	—	—	—	1	1	—	2	4	—	2	—	2	1	1	—	1	1	1	1	1	—	1	—	—	—	—	1	—	—	—	—	—	—	24
33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	1	—	1	1	2	2	1	3	1	—	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	17
34	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	1	1	2	1	2	—	2	1	2	1	2	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	18
35	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	3	1	1	—	—	—	—	—	—	—	2	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10
36	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	4
37	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1	1	—	—	—	1	—	1	—	—	—	—	—	—	—	—	—	5
38	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
39	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1
40	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
41	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
42	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
43	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
44	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
45	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
46	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
47	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
48	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
49	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
50+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1
Total	2 589	275	932	1 305	1 324	1 087	719	509	442	257	357	245	298	246	291	307	259	200	179	165	223	146	201	174	205	267	223	143	131	105	183	64	80	60	46	60	43	18	11	8	20	1	4	2	4	5	1	—	—	—	—	14 414

TABLE 4

ANNUAL INCIDENCE RATES AS ESTIMATED FROM CONVERSION RATES IN STUDIES 1 AND 2, BY AGE

Age-group (years)	Number tested and read at both rounds		Number uninfected (<8 mm) at Round I		Annual incidence rates (%) according to different levels of reaction taken as evidence of infection							
					≥ 8 mm		≥ 10 mm		≥ 14 mm		≥ 18 mm	
	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2
0-4	2 440	145	2 396	140	1.7	−4.2	1.5	—	1.1	—	1.1	4.2
5-9	2 406	150	2 243	134	4.8	45.0	3.9	30.6	2.6	17.4	0.3	4.2
10-14	2 102	170	1 799	137	10.9	171.0	8.1	87.6	4.6	47.4	2.3	31.2
15-24	1 899	110	1 318	66	17.3	263.4	13.1	232.2	8.6	160.8	4.8	90.0
25-44	3 392	217	1 861	81	23.5	222.0	16.2	235.2	12.5	198.0	8.4	156.6
45 +	2 173	126	1 022	49	25.1	220.2	21.8	196.2	14.6	180.0	11.8	111.0
All ages	14 412	918	10 639	607	11.9	123.6	9.3	104.4	6.7	84.0	4.8	60.6

The annual incidence rates corresponding to an increase in reaction size of at least 5, 10, 15 or 20 mm are shown in Table 5 for different age-groups. When an increase of 5 mm or more in reaction size is taken as evidence of new infection the annual incidence of infection is higher than that derived from conversion rates (see Table 4, data for Study 1). An examination of Table 3 shows that nearly 30% of the persons showing a rise of 5 mm or more at Round II are from the group with reactions of 9 mm or less at both rounds. It is only when the increase in reaction size reaches 15 mm or 20 mm that the annual incidence rates tend to be similar to those shown in Table 2 for the younger age-groups, at the 10-mm level. If the results of the two methods are at all comparable, then persons who are newly infected do show an increase in reaction size of 15-20 mm or more; otherwise the incidence rates shown in Table 2, even for the younger age-groups, must be assumed to be underestimates.

In Study 2, for the 649 persons (" all ages ") with reactions of 9 mm or less, annual rates of incidence

TABLE 5

ANNUAL INCIDENCE RATES BASED ON AN INCREASE IN REACTION SIZE OF AT LEAST 5, 10, 15 OR 20 MILLIMETRES AS EVIDENCE OF NEW INFECTION, BY AGE

Age-group (years)	Number tested and read	Number uninfected (≤9 mm) at Round I	Annual incidence rates (%) based on an increase in reaction size of at least:			
			5 mm	10 mm	15 mm	20 mm
0-4	2 440	2 398	4.4	1.5	1.1	0.8
5-9	2 406	2 255	10.7	3.6	1.9	1.3
10-14	2 102	1 846	18.6	5.9	2.5	1.5
15-24	1 899	1 415	26.5	9.1	3.5	1.6
25-34	2 058	1 297	32.6	15.1	6.8	3.2
35-44	1 334	745	33.8	14.8	6.4	3.4
45 +	2 173	1 131	36.9	19.6	10.8	4.9
All ages	14 412	11 087	19.5	8.0	3.8	1.9

based on an increase in reaction size of at least 15 mm or 20 mm work out to be 45.6% and 18.6% respectively. These are extremely high compared with the corresponding rates in Table 5. Though the material is limited, this finding could mean that even with a rise of 20 mm as evidence of infection, the effect of boosting is not eliminated at two months. In calculating annual rates this effect is multiplied six times (see page 610).

(3) *Distribution of the differences in 1 TU reactions from Round I to Round II.*[1] If new infection causes a distinct rise in tuberculin allergy which is greater than the combined rise due to boosting and reader variation, the distribution of the differences in 1 TU reactions from Round I to Round II should clearly indicate the newly infected. Such a curve (not shown here) was drawn for the entire study population in Table 3. The curve was symmetrical, with the mode showing a shift to the right corresponding to the general increase in reaction size at Round II, but the newly infected group was not seen distinctly on the right side of the curve. Probably the comparatively small numbers of newly infected were lost among the much larger numbers of those found infected or non-infected at both rounds. To exclude these two groups—which obviously do not contribute to the number newly infected—and at the same time to maintain the symmetry of the curve, the following method was adopted.

The diagonal line in Table 3 drawn from 0-mm reactions at both rounds to 50-mm reactions at both rounds passes through all the figures corresponding to the number of persons whose tuberculin reactions were the same at both rounds. Any line drawn across this diagonal from à given réaction at Round I to the same-sized reaction at Round II runs through figures which represent, on the upper side of the diagonal, the number of persons who showed increases in reaction size and, on the lower, the number who showed corresponding decreases. All figures in the triangle in the left upper corner formed by the line joining 9-mm reactions at either round (step-ladder line A) represent the number of persons whose average reaction was less than 5 mm at the two rounds and who can be safely excluded as definite non-reactors at both rounds. Similarly the figures in the triangle to the right of step-ladder line B represent the number of persons whose average reaction was 30 mm or more at the two rounds (or, in fact, who had a reaction of at least 10 mm at either round) and who can be excluded as definite reactors at both rounds. The distribution of the remaining persons, as presented in Fig. 2,[2] shows a small bulge in the right-hand tail of the curve. The exclusion of the definite reactors and non-reactors at both rounds has made the bulge somewhat prominent, since these omissions have resulted in an increase in the relative proportion of the newly infected. The bulge becomes quite prominent (Fig. 3, continuous line) when the left upper triangle is enlarged to include those persons with average reactions of less than 10 mm (step-ladder line C in Table 3).

The distribution of the differences in 1 TU reactions was also drawn separately for the excluded triangles in Table 3 and was observed to be unimodal with no bulge in either tail.

In Fig. 2 and 3 the distribution curve (continuous line) is fairly normal in shape, with the mode showing a shift of about 4 mm to the right. As the reading errors are likely to be equally distributed round about " 0 ", the shift of the mode is probably due to the boosting effect. The bulge in the right-hand tail represents a group that showed a distinct increase in allergy from Round I to Round II and, in all probability, consists of the newly infected.

Distribution curves were drawn for the different age-groups (Fig. 4). For the age-group 0-4 years the bulge has attained the status of a separate distribution on the right side. Non-specific allergy, waning and boosting are likely to influence this age-group to a much smaller degree, and the separate distribution on the right can reasonably be assumed to represent the newly infected. If this assumption is correct, the hypothesis that new infection results in a large increase in allergy is supported.

Curves for the other age-groups show two interesting features: (1) the bulge generally starts at about 16 mm for the different age-groups, and (2) the bulge in the right-hand tail becomes less prominent with increasing age. The latter finding could mean that there are others, besides the newly infected, who show a large increase in allergy and that their number increases with age, thus blurring the clear distinction seen in the age-group 0-4 years. Such persons are included in the calculation when the criterion of increase in allergy is used to distinguish the newly infected, and therefore estimates for the older age-groups tend to be too high.

[1] The authors are beholden to Dr J. Guld, World Health Organization, Geneva, for suggesting this method.

[2] To smooth out sharp fluctuations, 3-point moving averages have been used for observed curves in Fig. 2-6.

FIG. 2

DISTRIBUTION OF THE DIFFERENCES IN REACTIONS TO 1 TU FROM ROUND I TO ROUND II AMONG THOSE WITH AVERAGE REACTIONS OF 5-30 MILLIMETRES

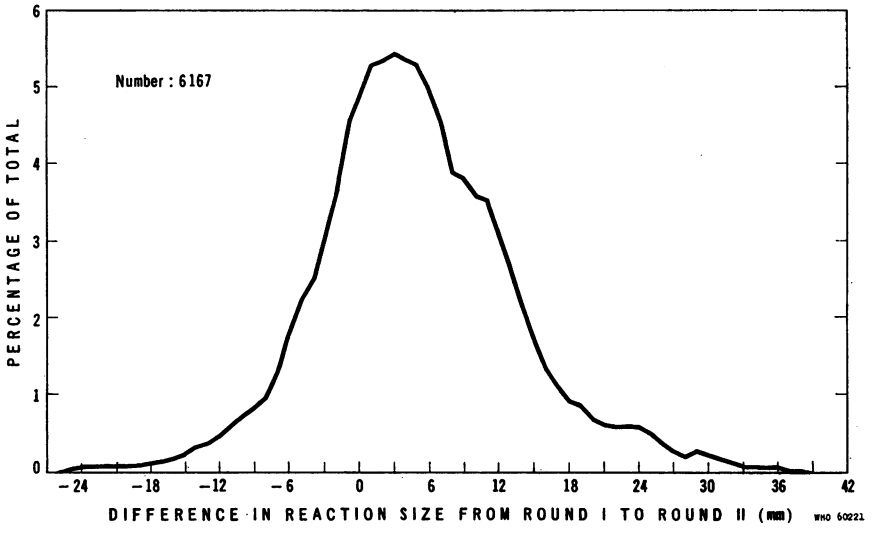
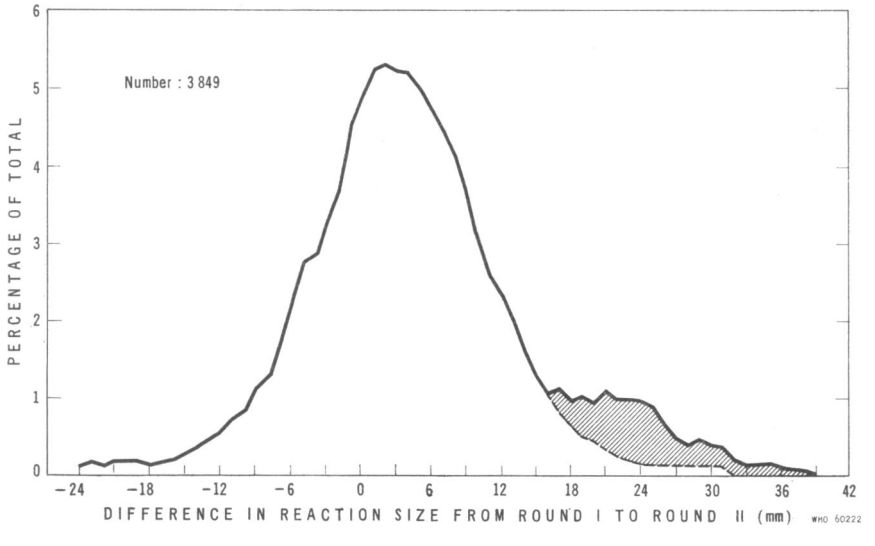


FIG. 3

DISTRIBUTION OF THE DIFFERENCES IN REACTIONS TO 1 TU FROM ROUND I TO ROUND II AMONG THOSE WITH AVERAGE REACTIONS OF 10-30 MILLIMETRES, AND A METHOD OF CALCULATING THE NUMBER NEWLY INFECTED



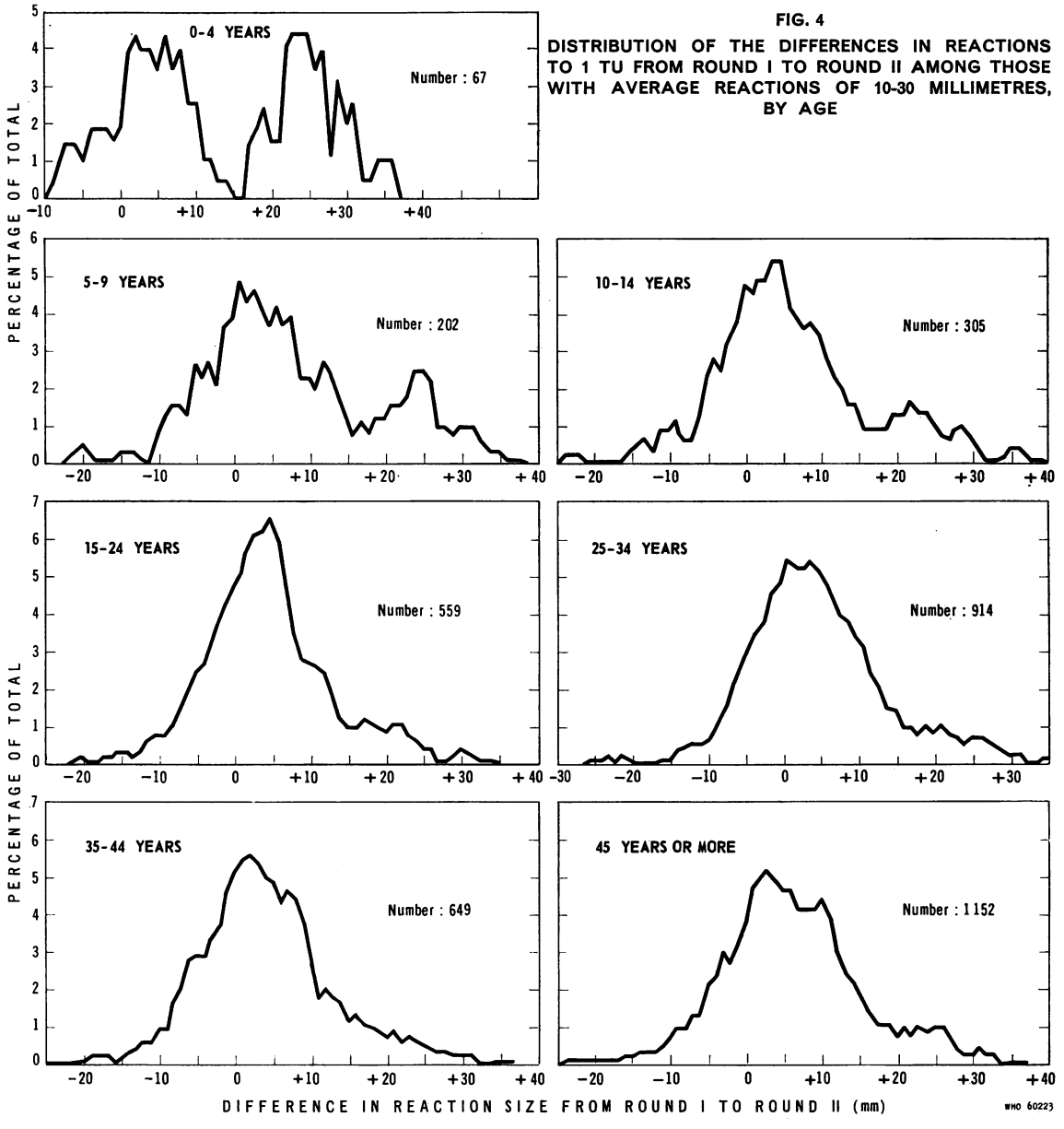


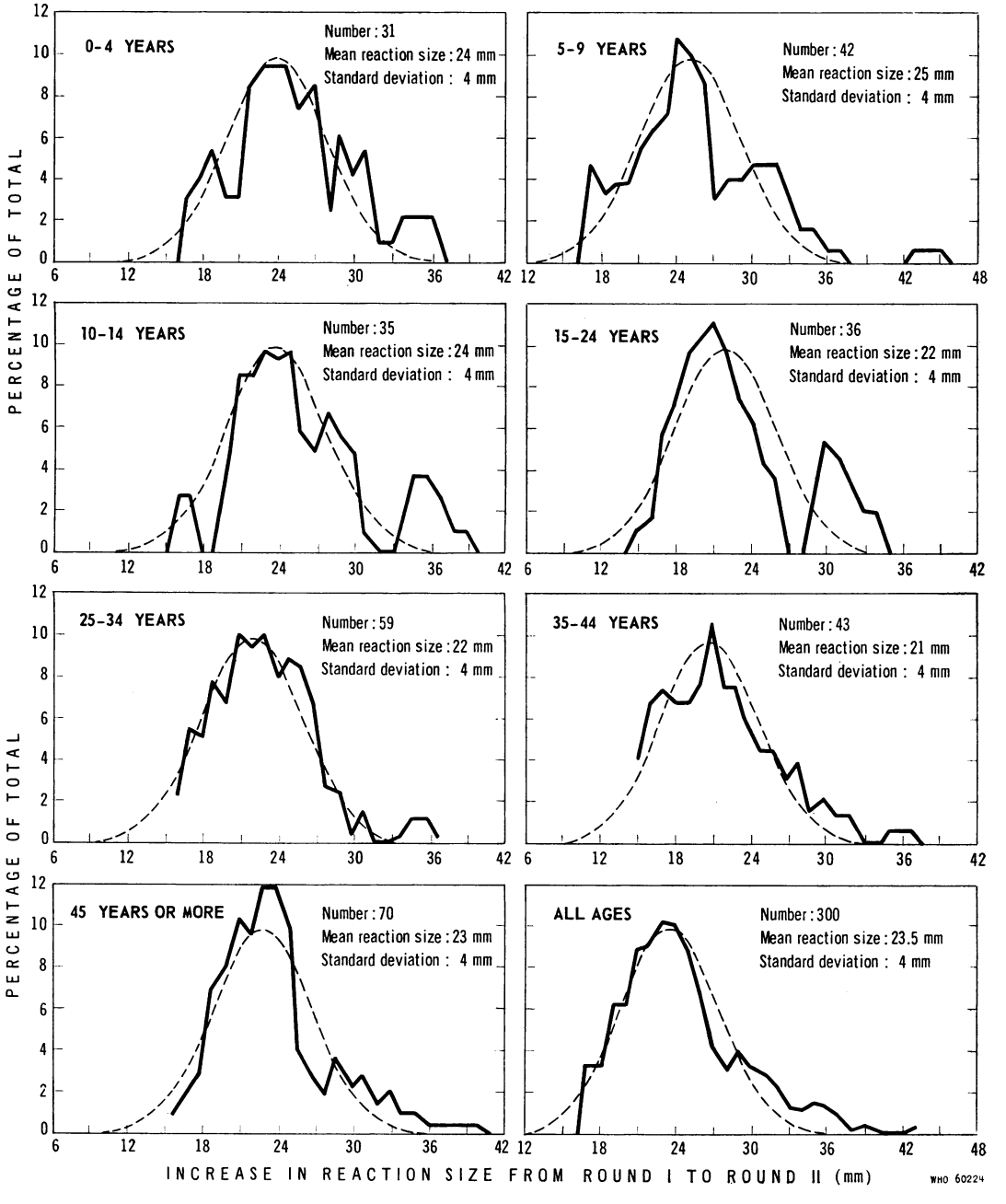
FIG. 4
DISTRIBUTION OF THE DIFFERENCES IN REACTIONS TO 1 TU FROM ROUND I TO ROUND II AMONG THOSE WITH AVERAGE REACTIONS OF 10-30 MILLIMETRES, BY AGE

The next problem is how to estimate the size of this group of newly infected persons. Many methods have been tried. One method is to project on the right-hand tail of the curve, starting from the beginning of the bulge, the corresponding portion of the left-hand tail (broken line in Fig. 3). The shaded area in the figure then represents the number of persons showing an increase in reaction size of 16 mm or more minus the number of those who could normally be expected to show such an increase as a result of experimental errors and causes other than new infection.

The frequency curves of such shaded areas for different age-groups and for all ages are shown in Fig. 5 (continuous line). In the age-groups 0-4, 5-9, 10-14 and 15-24 years the numbers are quite

FIG. 5

FREQUENCY CURVES AND BEST-FIT NORMAL CURVES FOR THE SHADED AREA ONLY (SEE FIG. 3), BY AGE[a]



[a] The figures for the mean reaction size and the standard deviation are those for the fitted curves.

small and the curves show large fluctuations. However, normal curves,[1] which fit fairly well the major portion of these frequency curves, are also shown in Fig. 5 (broken line). (For the method of fitting normal curves, see Annex 2.) These normal curves are based on a mean induration of about 24 mm and standard deviation of about 4 mm. Thus in each age-group the shaded area of the distribution of differences represents a homogeneous group with a normal distribution showing a mean increase of about 24 mm from Round I to Round II. Further, 98% of this group showed an increase of 16 mm (24 mm minus 2×SD) or more. This explains why the bulge starts at about 16 mm in the curves for the different age-groups in Fig. 4.

The frequency curves in Fig. 5 invariably reveal a small but distinct group that showed a larger increase in reaction size (mostly 32 mm or more). A study of these 25 persons showed that:

(1) all had reactions of 0-4 mm to 1 TU at Round I;

(2) 16 had reactions of 10 mm or more to 20 TU;

(3) 16 (not all in group (2) above) were females; and

(4) 15 were in the age-group 0-14 years.

A follow-up of these persons, as well as of others showing large changes in reaction size ($\geqslant$16 mm) between the two rounds, will be available when Round III of the Longitudinal Survey is completed.

The numbers newly infected, estimated in this manner for the different age-groups, added up to a total of 316. The corresponding figure obtained from the distribution of differences for all ages was 300. It is indeed remarkable that in spite of the small numbers in some age-groups and the rather crude and subjective manner in which the frequency in the shaded area was estimated such close results could be obtained.

FURTHER PROBLEMS

Influence of non-specific allergy

Boosting is probably the reason why persons other than the newly infected show a large increase in allergy. Since this effect is more marked among those with non-specific sensitivity (Raj Narain et al., 1966b [1]), the number of such persons should increase with an increase in non-specific allergy. It is difficult to define non-specific allergy. Intermediate reactions to 1 TU (Nyboe, 1960) are not as suitable for this purpose as are different grades of reaction to 20 TU. Over 50% of those with reactions of 0-4 mm to 1 TU show reactions of 10 mm and more to 20 TU and constitute nearly half the number of persons with this size of reaction to 20 TU. The numbers of persons with various grades of reaction to 20 TU and 1 TU and the percentages of these showing an increase in reaction size of 16 mm or 20 mm and more are presented in Table 6. The data are tabulated by age-group in order to eliminate the effect of age. A minimum increase of 16 mm (24 mm minus 2 × SD) and 20 mm (24 mm minus 1 × SD) have been considered, because 98% and 84% respectively of the newly infected will then be included. The percentage showing such increases in allergy elicited by 1 TU generally rises as allergy elicited by 20 TU increases (whatever the size of reaction to 1 TU), and is considerably greater among those who show reactions of 20 mm or more to 20 TU. This indicates that the 20 TU reactors are another group that may show large increases in allergy without new infection. If this is the only other group showing such increases in allergy, it should be possible to use an increase in reaction size of 16 mm or more as an approximate indication of incidence of new infection in very young age-groups and also, perhaps, in communities in which non-specific sensitivity is absent. A similar study in an area with a low prevalence of non-specific allergy may provide a more precise answer.

Estimation of the number exposed to the risk of new infection

Throughout this paper, for the calculation of incidence rates, all persons with reactions of 9 mm or less to 1 TU in Round I have been regarded as non-infected and therefore exposed to the risk of new infection. This does not take into account factors such as non-specific allergy and other experimental errors at Round I. Some other possibilities are the number of persons with reactions of 0-4 mm to 1 TU at Round I, the number with reactions of 0-4 mm to 20 TU at Round I, and the number of non-infected at both rounds (see Table 3, triangle to the left of step-ladder line C) plus the number newly infected. With these four sets of figures as the denominator and the number newly infected, as

[1] The increase in allergy after new infection is likely to be represented by a normal curve. The data in Fig. 6 (page 620) (from an unpublished study by the National Tuberculosis Institute, 1963) show that an increase in size of induration after BCG vaccination in schoolchildren, as measured with 1 TU of PPD RT 23 in Tween 80 diluent, has produced a normal curve with a mean induration of 4 mm and a standard deviation of 4 mm.

[1] See article on page 623 of this issue.

TABLE 6

PERCENTAGE SHOWING A RISE OF AT LEAST 16 OR 20 MILLIMETRES IN SIZE OF REACTION TO 1 TU, BY AGE AND BY SIZE OF INITIAL REACTION TO 20 TU AND 1 TU

Size of reaction to 20 TU (mm)	Age-group (years)	Reactions of 0-4 mm to 1 TU			Reactions of 5-9 mm to 1 TU		
		Number tested and read	Percentage showing an increase of at least:		Number tested and read	Percentage showing an increase of at least:	
			16 mm	20 mm		16 mm	20 mm
0-4	0-9	2 585	1.5	1.2	18		
	10-24	295	2.4	2.4	5		
	25 +	143	9.1	6.3	9		
	All ages	3 023	1.9	1.6	32 [a]		
5-9	0-9	553	1.3	0.9	10		
	10-24	242	3.3	2.5	12		
	25 +	99	6.1	3.0	3		
	All ages	894	2.3	1.6	25 [a]		
10-14	0-9	448	2.9	2.2	14		
	10-24	500	3.2	2.4	65	3.1	1.5
	25 +	243	7.0	5.3	42	2.4	–
	All ages	1 191	3.9	2.9	121	2.5	0.8
15-19	0-9	383	2.6	2.1	34	2.9	2.9
	10-24	711	2.7	1.8	198	3.0	1.0
	25 +	616	9.4	4.4	209	4.8	1.9
	All ages	1 710	5.1	2.8	441	3.9	1.6
20 +	0-9	157	14.6	8.9	35	5.7	2.9
	10-24	575	5.0	3.0	277	6.5	2.9
	25 +	857	15.1	8.6	489	9.8	5.3
	All ages	1 589	11.4	6.6	801	8.5	4.4

[a] The results for 32 persons who showed less reaction to 20 TU than to 1 TU and for some of the 25 persons who showed reactions of 5-9 mm to both the tests are likely to have been due to experimental errors associated with tuberculin testing. No percentages are presented for these two groups.

estimated from the shaded area in Fig. 5, as numerator, annual incidence rates in the different age-groups are presented in Table 7.

For the age-group 0-4 years the four sets of denominators are nearly equal, but as age increases the differences become larger. With reactions of 0-4 mm to 20 TU as the denominator, the incidence rates become extremely high for the older age-groups. The fourth set, which uses the results for both rounds, may have the advantage that the errors introduced at one or the other round are reduced considerably. It may be noted that incidence rates rise with age. This rise could be due to an actually higher incidence of infection as age increases or to the fact that the shaded area still includes some persons with a greater boosting of allergy by previous tuberculin tests, the numbers of such persons increasing with age.

The incidence rate for the age-group 0-4 years is calculated at 0.9% per annum with all four denominators, and at 0.8% with the indirect method, taking a 10-mm or 14-mm level of induration as evidence of infection (see Table 2, page 610). Is the rate of incidence of infection really so low in this age-group, in spite of a large number of open cases in the community (Indian Council of Medical Research, 1959; Raj Narain et al., 1963) and the utter lack of measures for the prevention of infection ? The answer must be in the affirmative. During Round I in the study villages, of 616 children below one year, only one had a reaction of more than 4 mm to 1 TU. The recorded reaction for this child was 16 mm at Round I and 0 mm at Round II. In Round II, none of the 578 children below one year (all of whom were born after Round I) had a reaction of more than 6 mm.

TABLE 7

INFLUENCE OF VARIOUS DENOMINATORS ON ANNUAL INCIDENCE RATES, BY AGE

Age-group (years)	Number exposed to risk of new infection as estimated by:				Annual incidence rates (%) using as denominator:			
	Reactions of 0-9 mm to 1 TU	Reactions of 0-4 mm to 1 TU	Reactions of 0-4 mm to 20 mm	Number of uninfected in both rounds + number of newly infected	Reactions of 0-9 mm to 1 TU	Reactions of 0-4 mm to 1 TU	Reactions of 0-4 mm to 20 TU	Number of uninfected in both rounds + number of newly infected
0-4	2 398	2 375	2 305	2 402	0.9	0.9	0.9	0.9
5-9	2 255	2 155	1 108	2 236	1.2	1.3	2.5	1.3
10-14	1 846	1 572	344	1 805	1.3	1.5	6.8	1.3
15-24	1 415	1 041	82	1 354	1.7	2.3	29.4	1.8
25-34	1 297	908		1 173	3.0	4.4		3.5
35-44	745	546	196	698	3.9	5.3	58.8	4.4
45+	1 131	835		1 037	4.1	5.6		4.8
All ages	11 089 [a]	9 433 [a]	4 035	10 691 [a]	1.8	2.1	5.0	1.9

[a] The figures for " all ages " in columns 2, 3 and 5 exceed the totals for the different age-groups by 2, 1 and 14 respectively. The last of these discrepancies is due to the difference of 16 in the number of newly infected, as estimated from the curve for " all ages " and from the curves for the different age-groups, minus the difference of 2 in column 2.

Identification of newly infected persons

The shaded area provides an estimate of the number newly infected but does not generally identify them. For a study of the development of disease among the newly infected such identification is necessary. This is, in fact, possible to achieve in the age-group 0-4 years, owing to the clear division in the distribution of differences, and a follow-up of this age-group may provide valuable information. For the older age-groups the only alternative, with the present methods, is to consider persons showing a sufficiently high increase in allergy (the actual figure may increase with age) so that the group thus selected will consist predominantly of the newly infected.

Need for testing and improving the method

The above method of estimating the newly infected is tentative and needs testing on a larger scale and on material from other areas. Further, the demarcation of the shaded area is subjective and the use of more objective methods, such as mathematical curves that best fit the observed frequency curves, should be tried.

In projecting the corresponding portion of the left-hand tail of the curve, in order to demarcate the shaded area in Fig. 3 (page 613), it has been assumed that the two tails of the curve are symmetrical and represent experimental errors and other factors to an equal degree. If the right-hand tail is larger than the left-hand in any of the age-groups, the number newly infected in such age-groups may be overestimated.

DISCUSSION

All persons of about the same age in a similar *milieu*, whether already infected, uninfected or showing non-specific allergy, may be assumed to have the same chance of meeting with new infection. But the tuberculin test cannot, with any degree of certainty, diagnose further infection in the already infected. The risk of infection can therefore be estimated only among the previously uninfected. The problem is, therefore, to estimate, at the beginning of a specific period, the number of persons previously uninfected and to estimate the number among them who develop primary infection during that period. Both of these estimates are, however, difficult to compute, since they are influenced by experimental errors associated with tuberculin testing and reading, the prevalence of non-specific allergy, the waning of allergy and chance variations. In addition, the results of a second tuberculin test are influenced by the boosting effect, which creates

further problems in estimating the number of those newly infected.

The method based on the distribution of differences (see page 612) eliminates the effect of all the above-mentioned factors, at least in the younger age-groups, and provides estimates of the number newly infected. But it is not possible, with this method, to identify the newly infected persons, except in the age-group 0-4 years.

Four methods have been suggested in Table 7 for estimating the number of non-infected persons. Of these, the last method has the advantage that it takes into account tuberculin test results at both rounds and therefore to a large extent overcomes errors associated with tuberculin testing and reading at one or the other round.

Whatever the estimate of the non-infected, the rates of incidence of infection rise with age. This is contrary to the findings of previous reports in which the rates rise up to a certain age and then show a decline (Frimodt-Møller, 1960; Raj Narain et al., 1963). Theoretically, there is no reason why the incidence of infection should decline after, say, 20-25 years of age. The risk of infection may increase up to a certain age, when contact with the community reaches a maximum, and then tend to remain the same. The continued rise in incidence after 45 years of age may be due to a greater degree of boosting in the older age-groups (Raj Narain et al., 1966 b [1]), to such an extent that this cannot be accounted for by the normal curve of differences. Previous reports (Magnus, 1957; Raj Narain, Kul Bhushan & Subramanian, 1961) have shown that the boosting of allergy by a tuberculin test is greater among persons vaccinated with BCG—so much so that Magnus (1957) suggested that BCG allergy could be maintained by repeated tuberculin tests. It is possible that waned allergy after infection with *Myco. tuberculosis* is more akin to allergy induced by BCG and shows greater boosting. The probably greater frequency of waning in the older age-groups may then be responsible—at least partly—for the continued rise in incidence rates in the older age-groups. In this context a systematic study of the waning of allergy becomes very important. Unfortunately, a direct measurement of waning must be based on two tests and is vitiated by boosting. Smaller doses of tuberculin or longer intervals between tests may have to be tried in an attempt to eliminate the boosting effect.

One of the methods of estimating incidence of infection—namely, taking a definite increase in allergy as evidence of primary infection—has the advantage that the newly infected can then be identified. This method became inapplicable because there were other persons, besides the newly infected, who showed larger increases in allergy, particularly in the older age-groups. In the preceding paragraph the possibility of the boosting of waned allergy in such persons was considered. Another possibility is that such large increases in allergy may result from the reinfection of persons with waned allergy by *Myco. tuberculosis* or perhaps other, closely related, organisms. Such increases may possibly be balanced by large decreases in allergy due to waning and thus maintain the symmetry of the curve of differences. In view of this symmetry the first assumption is tenable only if the number of persons showing a large decrease in allergy due to waning during the interval between the two tests (18 months) is nearly equal to the number showing a great boosting of waned allergy that existed at the time of the first test.

Obviously, further work is needed before it will be possible to devise a definite method of measuring the incidence of infection. Perhaps a follow-up, at Round III and possibly Round IV of the Longitudinal Survey, of the newly infected found at Round II would help to evaluate various criteria for evidence of new infection.

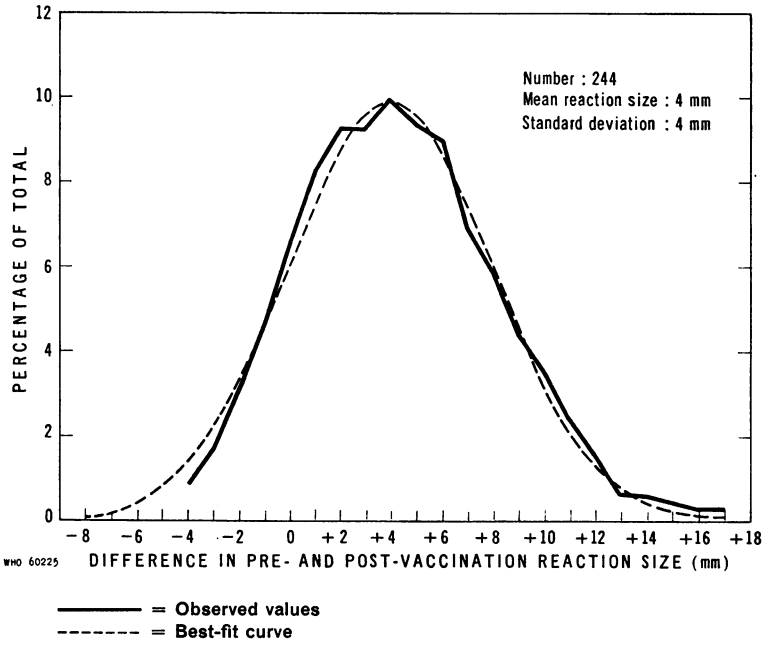
Another possible source of error may be mentioned. It has been suggested that only a large increase in allergy should be regarded as evidence of new infection. If there are any newly infected persons who fail to show such an increase, they may represent a source of error. It is known that after intradermal vaccination (infection) with BCG, a number of persons fail to become tuberculin-positive. Also, persons given the same vaccine show great variations in the resultant post-vaccination increase in allergy (Fig. 6). In another report by Raj Narain et al. (1966a),[2] it has been shown that a large number of children from households in which there are persons with bacteriologically confirmed tuberculosis are tuberculin-negative. It is possible that some of these children are infected and yet remain tuberculin-negative. It was reported that 14% of bacteriologically confirmed cases were tuberculin-negative (Raj Narain et al., 1963). Not all these negative results may be attributed to possible errors. The size and extent of this source of error, wherein

[1] See article on page 623 of this issue.

[2] See article on page 639 of this issue.

FIG. 6

DISTRIBUTION OF THE DIFFERENCES IN REACTIONS TO 1 TU BEFORE BCG VACCINATION AND SIX MONTHS AFTER VACCINATION



a person, after infection, may show an increase in reaction size of less than 16 mm, or even remain tuberculin-negative, are not known.

The present report has suggested the possibility that the incidence of infection may rise rather sharply in the older age-groups. The hypothesis of resistance to infection in the younger age-groups has been posed (Raj Narain et al., 1966a[1]). Is the higher incidence of infection in the older age-groups due to reduced resistance to infection? This supposition is similar to the suggestion that reduced resistance to disease in old age results in a higher prevalence of cases among the elderly. At any rate, if the incidence of new infection is really higher in the older age-groups, the persons concerned should not be excluded from mass BCG campaigns.

Apart from these conjectures, some implications of the findings of the study are clearly important:

(1) It is almost certain that all previous estimates of such an important index of the dynamics of tuberculosis in a community as the incidence of infection must be reviewed. The incidence rates found in the Prophit Survey (Daniels et al., 1948) may be gross overestimates.

(2) In estimating infection rates in surveys it is customary to exclude persons with previous BCG scars. The influence of previous tuberculin tests during BCG mass campaigns also needs consideration, and the methods of measuring infection rates (for prevalence or incidence) in areas in which there has been previous tuberculin testing may have to be changed.

(3) In clinical practice it may be hazardous to regard any increase in reaction size of less than 16 mm, on a retest, as evidence of new infection.

[1] See article on page 639 of this issue.

Annex 1

AGE-INFECTION CURVE AND ANNUAL INCIDENCE OF INFECTION

The usual feature of age-infection curves is that they rise steadily with age and level off after attaining a maximum. This suggests that infection in a community whose members are born uninfected gathers momentum with age.

The rates for annual incidence of infection based on the assumptions enumerated on page 609 can be obtained as follows:

For example, in the age-groups 0-4 and 5-9 years, the uninfected (i.e., those with indurations of less than 10 mm) form 97.9% and 94.0% respectively of the total. At birth none was infected and all were exposed to the risk of infection. At the mid-point of the age-group 0-4 years (2.5 years) 97.9% were at risk.

The change brought about in the proportion uninfected in any two age-groups may be assumed to follow the mathematical relationship:

$$q' = q\, e^{-rn},$$

where q and q' are the percentages uninfected in two consecutive age-groups, r is the annual rate of infection, and n is the interval between the mid-points of the two age-groups. In particular, for the newborn, the annual rate of infection is derived from the equation:

$$97.9 = 100\, e^{-2.5r},$$

which gives a value of 0.8% for r. For persons in the age-group 5-9 years, a similar value (0.8%) for r is obtained from the equation:

$$94.0 = 97.9\, e^{-5r}.$$

The rates for other age-groups may be similarly calculated.

Annex 2

METHOD OF FITTING A NORMAL CURVE FOR THE DISTRIBUTION OF DIFFERENCES IN TUBERCULIN REACTIONS FROM ROUND I TO ROUND II

The frequency distribution of differences in the size of tuberculin reactions from Round I to Round II (the measurement at Round II minus the measurement at Round I) was calculated for the group of persons represented by the shaded area in Fig. 3 (see page 613). To obtain a normal curve with the best fit, the mode was located with the help of the observed distribution, and an approximate value for the standard deviation was obtained from the spread of the distribution. With these trial values the frequencies of the expected normal curve were calculated. Other trial values of mode and standard deviation were adopted and the same procedure was repeated till a curve was obtained that gave a fairly good fit, as judged by the eye.

ACKNOWLEDGEMENTS

The authors would like to thank the field teams for their hard and painstaking work. They are also grateful to members of the Technical Co-ordinating Committee of the National Tuberculosis Institute, Bangalore, for their valuable suggestions and criticism, and to the Statistical Section of the Institute (especially Mr G. Dwarakanath, Mr K. Gundanna and Mrs M. J. Jayalakshmi) for help in compiling the tables.

RÉSUMÉ

L'organisation dans les meilleures conditions possibles de mesures de lutte contre la tuberculose et de campagnes de vaccination de masse par le BCG exige que l'incidence de la maladie soit connue. L'estimation de cet indice apparemment simple et très négligé soulève de grandes difficultés.

Des renseignements provenant de cinquante villages du district de Bangalore, en Inde méridionale, où des enquêtes sur la prévalence de la tuberculose ont été menées sur un échantillon de villages choisis au hasard, ont servi au calcul de l'incidence de l'infection par des méthodes classiques. Dans l'ensemble, les taux d'inci-

dence basés sur ces méthodes ne sont pas acceptables. La méthode courante des taux de conversion est sujette à de graves erreurs.

Les auteurs décrivent une nouvelle méthode d'estimation de l'incidence basée sur la distribution de la différence entre les réactions obtenues lors de deux épreuves tuberculiniques successives utilisant 1 UT de tuberculine. Les nouveaux infectés, suivant cette méthode, forment un groupe homogène à distribution normale (l'augmentation moyenne du diamètre des réactions étant d'environ 24 mm et la déviation standard de 4 mm). L'augmentation entre la première et la seconde induration a été de 16 mm ou plus pour 98% des sujets de ce groupe. Mais certains autres, en l'absence d'infection récente, ont présenté également un degré plus élevé d'allergie entre les deux réactions, résultant d'une potentialisation tuberculinique, d'une allergie non spécifique ou d'autres facteurs. Le nombre de ces personnes, qui constituent la principale source d'erreurs lorsque l'on évalue l'incidence en déterminant l'augmentation nette de l'allergie, s'accroît avec l'âge et semble plus élevé dans les régions à haute prévalence d'allergie non spécifique.

Les auteurs examinent les problèmes que pose l'estimation du nombre de sujets que l'on peut considérer comme exposés à une nouvelle infection. Malgré l'ampleur du problème de la tuberculose, le surpeuplement, la malnutrition et le peu ou l'absence de précautions capables de prévenir la diffusion de l'infection, les taux d'incidence obtenus sont très bas, même pour un pays comme l'Inde. L'absence quasi complète de nouveaux infectés parmi les enfants de moins d'un an, au cours des différentes phases de l'enquête, confirme cette constatation.

REFERENCES

Bogen, E. (1957) *Indian J. Tuberc.*, **4**, 80-85

Daniels, M., Ridehalgh, F., Springett, V. H. & Hall, I. M. (1948) *Tuberculosis in young adults*, London, Lewis, pp. 72-76

Ferebee, S. H. & Mount, F. W. (1963) *Amer. Rev. resp. Dis.*, **88**, 118

Frimodt-Møller, J. (1960) *Bull. Wld Hlth Org.*, **22**, 61-170

Frimodt-Møller, J., Thomas, J. & Parthasarathy, R. (1964) *Bull. Wld Hlth Org.*, **30**, 547-574

Groth-Petersen, E., Knudsen, J. & Wilbek, E. (1959) *Bull. Wld Hlth Org.*, **21**, 34

Indian Council of Medical Research (1959) *Tuberculosis in India. A sample survey 1955-57*, New Delhi (*Special Report Series*, No. 34)

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

Nyboe, J. (1960) *Bull. Wld. Hlth Org.*, **22**, 5-37

Palmer, C. E., Shaw, L. W. & Comstock, G. W. (1958) *Amer. Rev. Tuberc.*, **77**, 877

Raj Narain, Geser, A., Jambunathan, M. V. & Subramanian, M. (1963) *Bull. Wld Hlth Org.*, **29**, 641-664; *Indian J. Tuberc.*, **10**, 85-116

Raj Narain, Kul Bhushan & Subramanian, M. (1961) *Indian J. Tuberc.*, **9**, 15-29

Raj Narain, Nair, S. S., Ramanatha Rao, G. & Chandrasekhar, P. (1966a) *Bull. Wld Hlth Org.*, **34**, 639-654

Raj Narain, Nair, S. S., Ramanatha Rao, G., Chandrasekhar, P. & Pyare Lal (1966b) *Bull. Wld Hlth Org.*, **34**, 623-638

Rich, A. R. (1951) *The pathogenesis of tuberculosis*, 2nd ed., Springfield, Ill., Thomas, p. 405