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NATURALLY ACQUIRED TUBERCULIN SENSITIVITY IN NETHERLANDS NEW GUINEA

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SYNOPSIS

The author presents the results of some studies on tuberculin sensitivity that were carried out in Netherlands New Guinea in connexion with the WHO/UNICEF-assisted BCG-vaccination programme which was introduced there in May 1956.

The data obtained suggest that non-specific sensitivity to tuberculin is rather widespread in the country and that most of the medium-sized reactions (diameter of induration, 12-14 mm) to the conventional 5 TU Mantoux test are probably caused by infection with allergy-producing organisms other than tubercle bacilli. The implications of these findings with regard to both tuberculin testing and BCG vaccination in areas where such non-specific sensitivity is common are discussed.

In May 1956 a WHO/UNICEF-assisted BCG-vaccination programme was started in Netherlands New Guinea—a programme that aimed at covering at least 100 000 of the population [1] during the first three years of its operation.

In the course of this campaign, tuberculin studies were carried out with the object of finding possible causes for the large differences in the frequency distributions according to size of the reactions to standard 5 TU (tuberculin units) tests observed in different geographical areas. Some of the results of these studies that are of more general interest are discussed in the present paper.

The subjects studied can be divided into three main groups:

1. The reaction to 5 TU and, if indicated, to 100 TU of human PPD (purified protein derivative) in different populations.

2. The reaction to different kinds of tuberculin—namely, human PPD and avian PPD—in different concentrations.

3. The influence of variations in the concentration of tuberculin and the volume of diluent used in the test.

[1] Netherlands New Guinea covers an area of 163 000 square miles (about 420 000 km²) and has an estimated total population of 700 000. The population under administration is 370 000.

Materials and Techniques

Tuberculin

Both the human and the avian tuberculin were obtained from the Statens Seruminstitut, Copenhagen (human PPD batch RT XXII and avian PPD), and were received in stock solutions containing 50 000 TU per ml. Dilutions in phosphate buffer, pH 7.38, were prepared locally once a week and kept in a refrigerated condition until used. Fresh dilutions were used for each working day.

Syringes

All syringes were pre-tested for leakage by the method of Guld & Rud (1953), and only those that leaked less than 0.2 ml after being subjected to an internal pressure of 5.5 kg per cm^2 during 6 minutes were used. After being used for approximately 300 injections the syringes were retested and only those that conformed to the above-mentioned requirement were used again.

All syringes were properly marked in order to avoid possible exchange of one for another. For avian tuberculin only new syringes were used.

Reduction of volume effect

To reduce the possible influence of a " volume effect " (Guld, Magnus & Magnusson, 1955; Magnus et al., 1956) only one-half to one-third of each bottle of tuberculin was used. Owing to the shortage of personnel, it was not feasible to use four bottles in rotation (WHO Tuberculosis Research Office, 1957a).

Injection technique

The amount injected was measured according to the markings on the barrel as accurately as possible (Guld, 1954). A standard tuberculin test, using 5 TU of human PPD per 0.1 ml, was always given in the right forearm, and if a duplicate test was carried out, it was given in the left forearm. Only new sites were used (WHO Tuberculosis Research Office, 1955c).

Reading of reactions

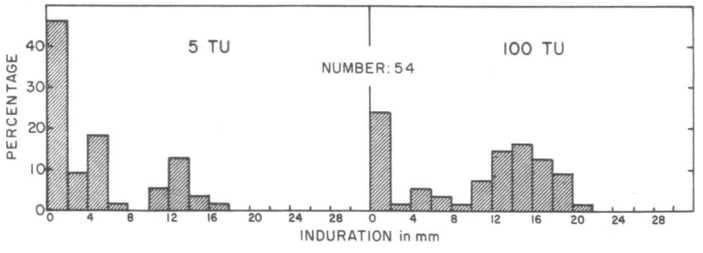
Reactions were read after 72 hours, with the exception of one small group of 100 TU tests, which were read after 48 hours. The size of all reactions (mm of induration) was carefully measured but no attempt was made to classify the reactions as either positive or negative. Comparative tests were always read independently; the reactions on the right arm were read first and those on the left arm were not measured until a large number of right-arm ones had been examined.

Results

1. *Reactions to 5 TU and 100 TU of human PPD in different populations* [1]

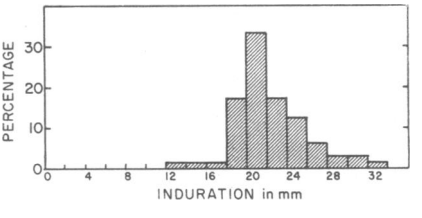
(*a*) *In a population where clinical tuberculosis is unknown.*[2] 54 adult male labourers from a district in the central highlands where clinical tuberculosis is unknown arrived in Hollandia by air. They were tested on arrival at the airport with both 5 TU and 100 TU of human PPD.

FIG. 1. DISTRIBUTION OF REACTIONS TO DUPLICATE TESTS WITH 5 TU AND 100 TU OF HUMAN PPD AMONG 54 UNSKILLED MALE WORKERS FROM A DISTRICT IN THE CENTRAL HIGHLANDS WHERE CLINICAL TUBERCULOSIS IS UNKNOWN



The distribution of reactions according to size is given in Fig. 1. It can be seen that, in the case of the 5 TU tests, there is an accumulation of medium-sized reactions (average induration, 13 mm), whereas, in the case of the 100 TU tests, there are many medium-sized to large reactions. No unfavourable reactions to 100 TU were observed. Fluoroscopic examination a few days after arrival did not reveal any abnormalities.

FIG. 2. DISTRIBUTION OF REACTIONS TO 5 TU OF HUMAN PPD AMONG 63 PATIENTS SUFFERING FROM CLINICAL TUBERCULOSIS



(*b*) *In patients suffering from clinical tuberculosis (bacteriologically confirmed diagnosis only).* In April 1957 and again in April 1958 all patients admitted to the tuberculosis wards of the Central Hospital at Hollandia were tested with 5 TU of human PPD. For obvious reasons, 100 TU

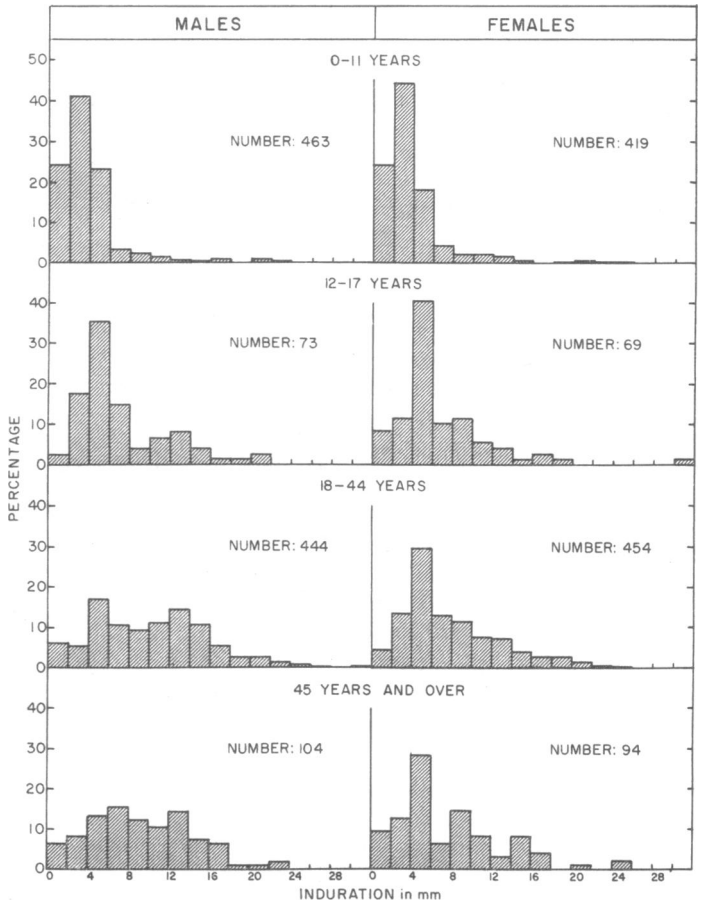
[1] It should be noted that the Government of Netherlands New Guinea employs one Dutch qualified medical officer per 8000 inhabitants under administration. In addition to receiving such medical attention as is necessary and feasible, the people are given a regular physical examination at least once a year. These yearly check-ups are well organized and are attended by 90-99% of the population.

[2] A district medical officer was appointed in this remote district in 1951 and intensive medical patrolling has been carried out since. From 1956 onwards, regular physical examinations (with 85% attendance) were undertaken. No case of tuberculosis has ever been found. However, 15 out of 61 children from this district who attended a boarding-school in Fakfak on the coast became tuberculin-positive after an average stay of 1½ years. Of these 15, five have already developed active tuberculosis. Under the national Quarantine Act (1954), these children are taken care of in an institution on the coast.

was not given. The distribution by size of the combined results is unimodal and more or less symmetrical in shape, with a mode of 20-21 mm (see Fig. 2).

(*c*) *In a population where clinical tuberculosis is rare.*[1] 98% of the total population of the district of Kamtoek Gressie, where clinical tuberculosis is rare, were examined in February 1957. The distribution of 5 TU reactions according to size for different age and sex groups is given in Fig. 3.

FIG. 3. DISTRIBUTION OF REACTIONS TO 5 TU OF HUMAN PPD AMONG THE GENERAL POPULATION OF KAMTOEK GRESSIE, A DISTRICT WHERE CLINICAL TUBERCULOSIS IS RARE

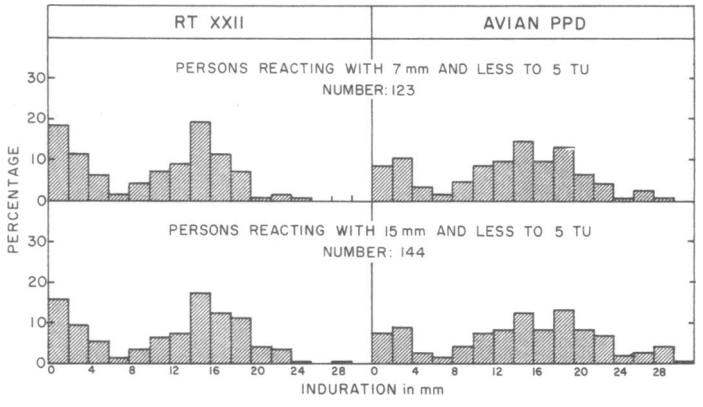


[1] Medical patrols were intensified after the Second World War, and regular medical examinations of the entire population have been undertaken since 1954. Only occasional cases of tuberculosis are seen, and in most instances they can be traced back to previous, usually recent, contact with the town population of Hollandia.

Large reactions, such as those observed in tuberculosis cases, are seen to be rare, but there is an accumulation, especially in males, of medium-sized reactions, with a mode of approximately 13 mm. There is a definite increase in such reactors after the age of 11 years.

In three villages where the entire population had 5 TU reactions of 15 mm or less, 100 TU tests with human and with avian PPD were given to the entire population. No unpleasant reactions were observed. The distribution of 100 TU reactions according to size is given in Fig. 4. The correlation between the 5 TU and 100 TU reactions is shown in Appendix Tables 1 and 2.

FIG. 4. DISTRIBUTION OF REACTIONS TO DUPLICATE TESTS WITH 100 TU OF HUMAN PPD AND 100 TU OF AVIAN PPD FOR DIFFERENT CLASSES OF REACTORS TO 5 TU OF HUMAN PPD AMONG THE GENERAL POPULATION OF KAMTOEK GRESSIE

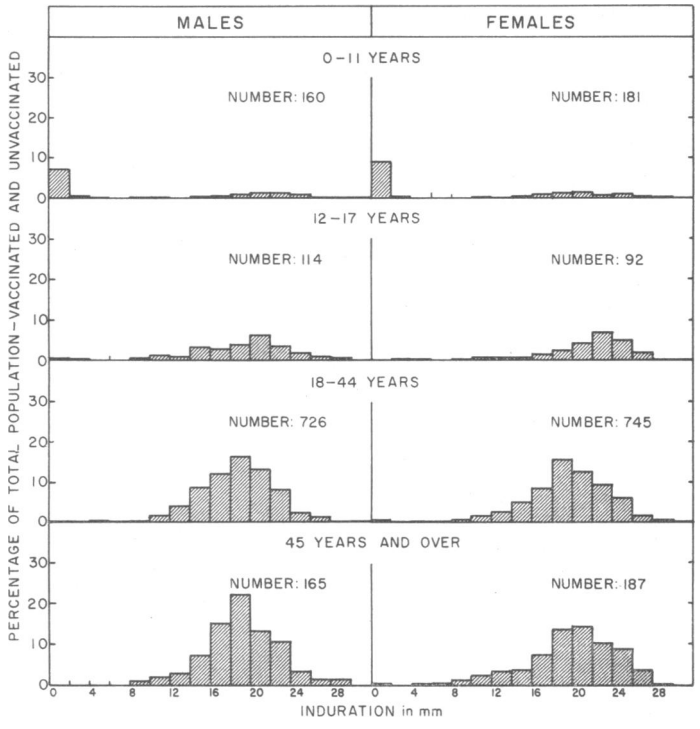


(*d*) *In a district where clinical tuberculosis is common.* At the beginning of 1958, the population of Sentanie, where previous general examination had shown clinical tuberculosis to be common, was subjected to an intensive mass case-finding survey. BCG had been given to non-reactors to tuberculin in 1956 and again in 1957 in the national mass campaign.

7% of the males and nearly 9% of the females excluded from vaccination in 1956 showed X-ray evidence of active pulmonary tuberculosis. Tubercle bacilli were isolated in 23% of these by culturing one swab only.

The frequency distribution of 5 TU reactions according to size for different age and sex groups is given in Fig. 5 for the people excluded from vaccination in 1956 and for those born after the campaign, expressed as percentages of the total population (vaccinated and unvaccinated). They show a high frequency of large tuberculin reactions. Owing to the limited time available, it was not possible to do 100 TU tests.

FIG. 5. DISTRIBUTION OF REACTIONS TO 5 TU OF HUMAN PPD AMONG THE GENERAL POPULATION OF SENTANIE EXCLUDED FROM VACCINATION OR BORN AFTER THE MASS CAMPAIGN



2. *Reactions to avian and to human tuberculin in different concentrations*

Since work carried out in India by the WHO Tuberculosis Research Office (1955b) had shown that people with small reactions to 5 TU of human tuberculin gave somewhat larger reactions to avian tuberculin of the same strength, comparative studies on naturally acquired sensitivity to human and avian tuberculins were undertaken in several places in Netherlands New Guinea.

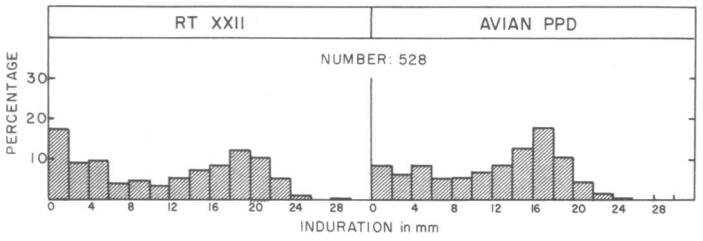
As the results of all these studies were of the same nature, only the figures obtained in one study, where 100 TU tests as well were carried out on large numbers of the group examined, will be given here.

In this particular study 528 schoolchildren (375 males and 153 females), 6-17 years of age, received duplicate tests with 5 TU of human and avian PPD.[1] None of these children had been vaccinated previously with BCG.

As the distribution of 5 TU reactions according to size for different age and sex groups had the same characteristics, only the combined results

[1] All duplicate tests were given by the same person.

FIG. 6. DISTRIBUTION OF REACTIONS TO DUPLICATE TESTS WITH 5 TU OF HUMAN PPD AND 5 TU OF AVIAN PPD AMONG UNVACCINATED SCHOOLCHILDREN AGED 6-17 YEARS IN FAKFAK



for all tests are given in Fig. 6. The actual numbers are presented in Appendix Table 3.

Fig. 6 shows that the reactions to both the human and the avian PPD tended to separate into two groups, one with large reactions (mode: 18-20 mm and 16-18 mm, respectively, with the human and the avian PPD) and one with small reactions (0-5 mm). Reactions of medium size were, however, rather frequent. As can be seen from Appendix Table 3, both the medium-sized and the small reactions to 5 TU of human PPD were relatively somewhat smaller than those to 5 TU of avian PPD.

It may be of interest to present also the reactions to 5 TU of human and of avian PPD in 26 patients suffering from clinical tuberculosis (bacteriologically confirmed diagnosis only). These are shown in Appendix Table 4. Here, the reactions to 5 TU of avian PPD are seen to be somewhat smaller than those to human PPD of the same strength.

100 TU tests were given to all schoolchildren who reacted with 14 mm or less to 5 TU of human PPD. The results of these tests were classified according to the children's reactions to 5 TU of human and of avian PPD. In Fig. 7 the frequency distribution of reactions, according to size, to 100 TU tests with human and with avian PPD is shown for each class separately. The actual numbers are presented in Appendix Tables 5 and 6.

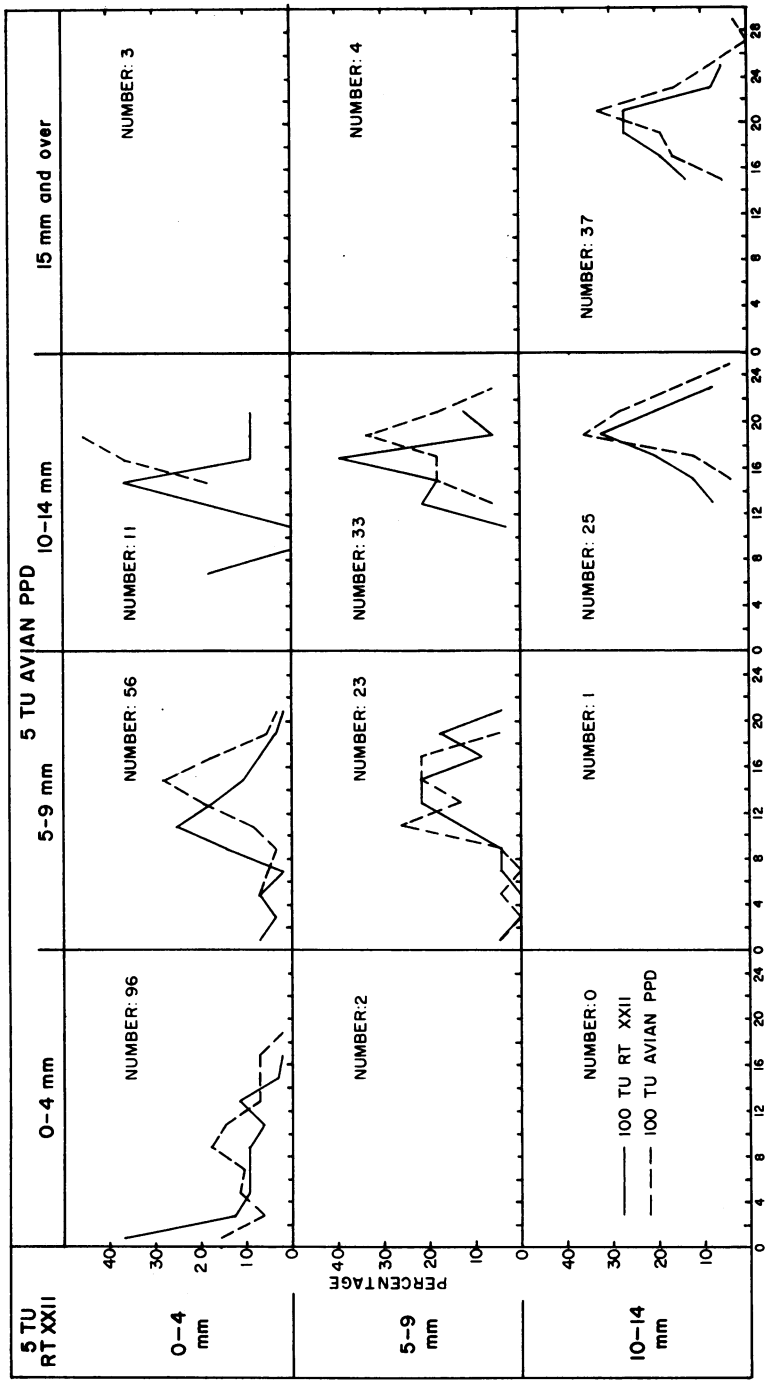
From Fig. 7 it can be seen that the mean size of the 100 TU reactions increased with the increase in size of the 5 TU reactions, but that the difference in mean size between the 100 TU reactions to human PPD and the 100 TU reactions to avian PPD decreased.

It would seem, therefore, that while a homologous tuberculin evokes larger reactions in previously infected individuals than a heterologous one at low concentrations, this tendency may well be less marked (or even absent) at higher concentrations.

3. *Influence of variations in the concentration of tuberculin and the volume of diluent used in the test*

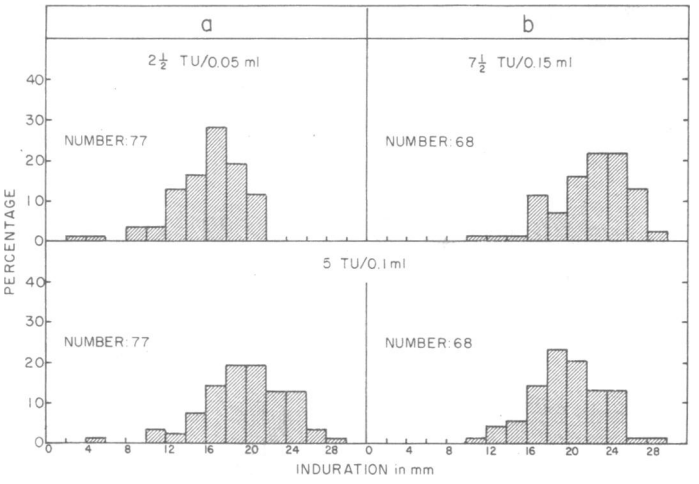
In order to determine the influence of such variations on the distribution of tuberculin reactions according to size of induration, two groups of

FIG. 7. DISTRIBUTION OF REACTIONS TO DUPLICATE TESTS WITH 100 TU OF HUMAN PPD AND 100 TU OF AVIAN PPD FOR DIFFERENT CLASSES OF REACTORS TO 5 TU OF HUMAN PPD AND 5 TU OF AVIAN PPD AMONG SCHOOLCHILDREN AGED 6-17 YEARS IN FAKFAK



people, previously excluded from vaccination, were given duplicate tests with different concentrations of tuberculin and different volumes of diluent. One group received 0.1 ml and 0.05 ml of a dilution containing 5 TU per 0.1 ml, and the other received 0.1 ml and 0.15 ml of the same dilution.

FIG. 8. DISTRIBUTION OF REACTIONS TO TWO SETS OF DUPLICATE TESTS WITH HUMAN PPD AMONG SAMPLES OF THE GENERAL POPULATION OF SENTANIE PREVIOUSLY EXCLUDED FROM VACCINATION



a. 5 TU in 0.1 ml and 2½ TU in 0.05 ml
b. 5 TU in 0.1 ml and 7½ TU in 0.15 ml

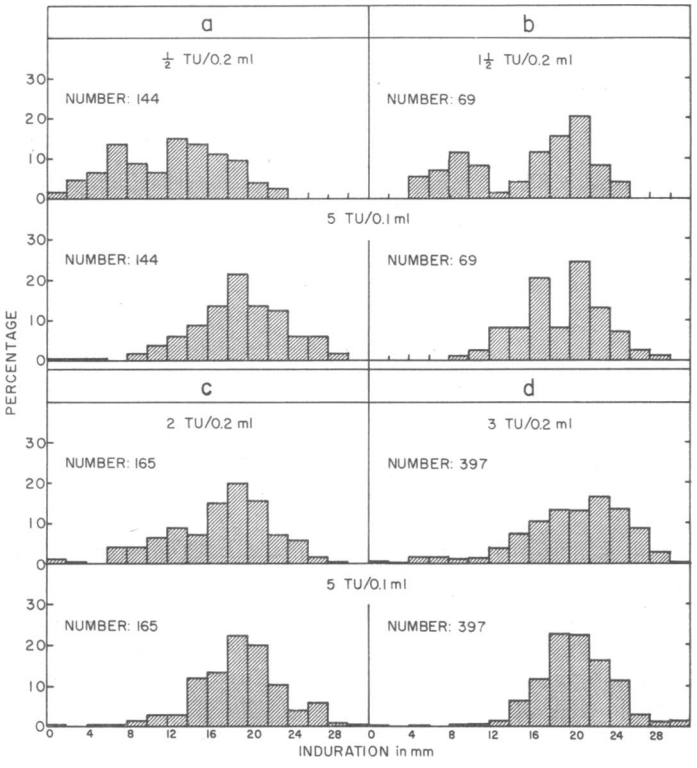
Fig. 8, in which histograms are given for both sets of duplicate tests (5 TU in 0.1 ml compared with 2½ TU in 0.05 ml, and 5 TU in 0.1 ml compared with 7½ TU in 0.15 ml), shows that a reduction in the volume of fluid—and hence in the dose of tuberculin, because the same stock dilution containing 5 TU per 0.1 ml was used—caused a considerable decrease in the size of the reactions, especially in the stronger tuberculin reactors, whereas an increase in the volume of fluid (and tuberculin) resulted in an increase in the size of all reactions, but particularly in the stronger reactors to 5 TU in 0.1 ml.

In order to establish whether this result was caused by the change in TU rather than by the change in the volume of fluid injected, a number of experiments was made in which one factor was kept constant and the other changed. It could be demonstrated that an increase in the volume of fluid (dose of tuberculin not changed) resulted in an increase in the size of the larger reactions, whereas a decrease in the volume of fluid (dose of tuberculin not changed) resulted in a decrease in the size of the larger reactions. Although the figures for the medium-sized reactions were too

small for definite conclusions to be drawn, it seemed that such reactions showed an opposite trend to that described above for the larger reactions.

The above observations are illustrated in a number of histograms from another experiment, in which both the dose of tuberculin used and the

FIG. 9. DISTRIBUTION OF REACTIONS TO FOUR SETS OF DUPLICATE TESTS WITH HUMAN PPD AMONG SAMPLES OF THE GENERAL POPULATION OF SENTANIE PREVIOUSLY EXCLUDED FROM VACCINATION

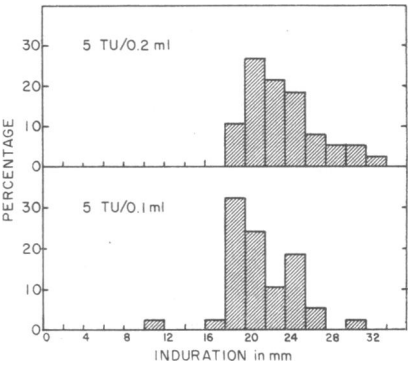


a. 5 TU in 0.1 ml and ½ TU in 0.2 ml
b. 5 TU in 0.1 ml and 1½ TU in 0.2 ml
c. 5 TU in 0.1 ml and 2 TU in 0.2 ml
d. 5 TU in 0.1 ml and 3 TU in 0.2 ml

volume of fluid injected were varied. Four sets of duplicate tests were compared: 5 TU in 0.1 ml on the one hand and ½, 1½, 2 or 3 TU in 0.2 ml on the other (see Fig. 9). It seems that as the amount of tuberculin injected in the greater volume of diluent (0.2 ml) decreased (3 → 2 → 1½ → ½TU), the distribution of reactions according to size of induration tended to

separate into two groups, one with reactions equal in size or a little smaller than those obtained with the standard 5 TU in 0.1 ml, and one with very much smaller reactions. However, reduction of the amount of tuberculin beyond a certain concentration resulted in a considerable decrease in the larger reactions also and hence in a less clear separation into the two groups.

FIG. 10. DISTRIBUTION OF REACTIONS TO DUPLICATE TESTS WITH 5 TU OF HUMAN PPD IN 0.1 ml AND 5 TU OF HUMAN PPD IN 0.2 ml AMONG 37 PATIENTS SUFFERING FROM CLINICAL TUBERCULOSIS



For purposes of comparison, the results of duplicate tests, in which 5 TU in 0.1 ml was compared with 5 TU in 0.2 ml, in a number of patients suffering from tuberculosis (bacteriologically confirmed diagnosis only) are given in Fig. 10 and Appendix Table 7. It can be seen that the reactions to 5 TU in 0.2 ml tend to be larger than those to 5 TU in 0.1 ml.

Discussion

It has been common practice in recent years to express the reactions of a population to a tuberculin test in terms of frequency distributions according to size of induration (or erythema) rather than in terms of percentages positive and negative (Edwards, Palmer & Magnus, 1953; WHO Tuberculosis Research Office, 1955a).

In European and North American countries these frequency distributions repeatedly show a break at 6-8 mm if a standard test of 5 TU of human PPD per 0.1 ml is used. The population studied for naturally acquired tuberculin sensitivity can thus be divided into two groups, one of which gives large reactions, with a mode of 16-20 mm, and one small reactions. If the latter group is given a second injection, of a much higher concentration of tuberculin (for example, 100 TU), again in Europe and North America the reactions obtained are fairly small (Edwards, Palmer & Magnus, 1953; Edwards & Palmer, 1953).

In addition, it has been observed that if patients from a tuberculosis hospital are tested with a low dose of tuberculin, the distribution of the reactions according to size corresponds very closely to the distribution of the large reactions in the general population (WHO Tuberculosis Research Office, 1955a). Consequently it is assumed that the latter represent that part of the population which has been infected with tubercle bacilli.

In many Asian and African countries, however, the natural break at 6-8 mm in the frequency distribution of reactions to 5 TU according to size of induration is very often unmarked or even absent (WHO Tuberculosis Research Office, 1955d, 1956, 1957b, 1957c, 1958). If in these countries 100 TU tests are given to people who react with 7-9 mm induration or less to a 5 TU test, a considerable number of large reactions are obtained. It has been assumed that this type of reactivity, commonly called " low-grade sensitivity " or " non-specific sensitivity ", is not caused by *Mycobacterium tuberculosis* var. *hominis*, but by another organism, which possibly has some antigenic relationship with tubercle bacilli (Edwards, Palmer & Magnus, 1953).

In the BCG trial carried out by the Medical Research Council of Great Britain (1956), the annual incidence of tuberculosis among people who reacted with 5-14 mm induration to a 3 TU intradermal Mantoux test was found to be 0.78 °/₀₀. Among persons who reacted only to 100 TU, a similar annual incidence—namely, 0.74 °/₀₀—was observed, whereas among individuals who gave 3 TU reactions of 15+ mm, the annual incidence was 2.93 °/₀₀. The annual incidence of tuberculosis in the tuberculin-negative, unvaccinated control group was 1.94 °/₀₀.

Observations of the same character have been made in the USA in a study of tuberculosis in student nurses (Palmer, Shaw & Comstock, 1958), and it has been suggested that a fairly large number of persons who react with 5-14 mm induration to 3 TU have not been infected with tubercle bacilli but have acquired their senstiivity through infection with some other organism (Palmer, 1957). It has further been suggested that this unknown infection might have induced a certain amount of immunity as well as allergy.

Our findings in the present paper seem to favour the concept that many reactions as large as 15-16 mm are not caused by infection with *Mycobacterium tuberculosis* var. *hominis*, but are evoked by an agent as yet unknown. Such reactions do occur in districts where tuberculosis is presumably rare,[1] but people suffering from clinical tuberculosis give even larger reactions. On the other hand, reactions to 5 TU of the same size as those observed in tuberculous patients are absent in districts where clinical tuberculosis is unknown, are rare in districts where it is rare, and are frequent in districts where it is common.

In 1955 it was reported that in India weak reactors to 5 TU of human PPD gave somewhat larger reactions to the same dose of avian tuberculin (WHO Tuberculosis Research Office, 1955b). This observation, which has also been made elsewhere—for example, the Philippines (Edwards & Krohn, 1957)—is borne out by the present study. Assuming that a homologous tuberculin evokes larger reactions than a heterologous one of the same concentration, it therefore seems likely that many of the 5 TU reactions of 15 mm or less were not caused by an infection with tubercle bacilli.

[1] See the footnotes on pages 643 and 644.

To confirm this, we retested certain people with 100 TU of human and of avian PPD—namely, all individuals who reacted with 15 mm induration to 5 TU in a number of small villages where the entire population reacted with less than 16 mm; and all schoolchildren, aged 6-17 years, who reacted with 14 mm or less to 5 TU in a group where 16+ mm reactions were rather frequent. No unpleasant reactions to the 100 TU dose were observed.

Reactions to 100 TU of avian tuberculin were generally speaking a little larger than those to 100 TU of human tuberculin, but the difference in reaction size between the two 100 TU tests decreased with increasing reaction to 5 TU. This observation seems to indicate that the specificity of the tuberculin test decreases when higher concentrations of tuberculin are used.

An increase in the specificity of the tuberculin test might be the explanation of our observations, which confirm earlier reports (Guld, Magnus & Magnusson, 1955; WHO Tuberculosis Research Office, 1957a), on the influence of reducing the concentration of tuberculin and increasing the volume of diluent injected. It was found that down to a certain concentration, doubling the volume injected resulted in a separation of the reactions of persons not previously selected for vaccination into two groups. Below this concentration, however, the larger reactions also decreased markedly, so that the separation became less distinct. Apparently, the mean size of the medium-sized reactions is closely correlated with the amount of tuberculin injected, whereas the mean size of the large reactions is positively correlated with the volume of diluent injected.

In interpreting the effect of concentration and dilution on the distribution of reactions according to size of induration, on the one hand, and the difference in response to human and avian tuberculin of the same strength, on the other, we must consider the possibility that both are an expression of the same phenomenon. For the purpose of discussion, it seems of advantage to treat separately the influence of different concentrations of tuberculin and the influence of an increased volume of diluent on the distribution of reactions according to size of induration.

Let us presume that a tuberculin prepared from a mycobacterium is made up of a number of different components, and that tuberculins prepared from different mycobacteria may have some of these components in common. Let us further presume that the induration measured in a standard tuberculin reaction actually is the sum of the reactions of the tissue to all the individual components to which the tissue has been sensitized through previous contact with a mycobacterium. A person infected with human tubercle bacilli will then react to all the components of human PPD, but to only some of the components of avian PPD. Naturally his reactions to avian tuberculin will be smaller, because the actual concentration of the reacting agent in this tuberculin is lower, although the same weight of tuberculin was injected. In the same way we may expect that if

human tuberculin is injected into an organism sensitized not with human bacilli, but with some other mycobacterium (or related organism), the organism will react only to those components of human tuberculin that are common to the sensitizing agent. The antigenic relation between this agent and the tubercle bacillus—in so far as it is represented in the tuberculin—will then be reflected in the size of the tuberculin reaction actually observed. If, on the other hand, a homologous tuberculin or a tuberculin more closely related antigenically than human PPD to the sensitizing agent is injected, a larger reaction will be obtained.

The effect of decreasing the dose and increasing the volume seems to indicate that there is a critical concentration of tuberculin below which the reaction of the tissue is suddenly very much reduced. This threshold will be high if the antigenic relationship between the organism from which the tuberculin was prepared and the organism that sensitized the tissues is weak, and will be minimal when the relationship is maximal, that is, when a homologous tuberculin is used. The use of such critical concentrations in countries where both specific and non-specific tuberculin sensitivity is prevalent will therefore result in a reduction in the size of all reactions—a reduction that will be comparatively small for persons infected with the homologous agent but will be comparatively large for persons infected with the heterologous one. An increase in the volume of diluent will bring more tissue into contact with the tuberculin and thus, in the case of critical concentrations, will further increase the difference in response to the test in persons sensitized to tuberculin by infection with different mycobacteria.

Conclusions

1. Our findings have shown that there is a relation between the distribution of reactions to 5 TU of human PPD and the number of cases of tuberculosis in the community. In populations where clinical tuberculosis is common, the right-hand side of the graph showing the distribution of reactions to 5 TU according to size of induration is dominated by reactions of considerable size (18+ mm). On the other hand, in populations where clinical tuberculosis is rare or absent, the right-hand side of the distribution graph is dominated by reactions of medium size (mode, 12-14 mm).

2. There is a difference in response to avian tuberculin between persons who react with 14+ mm induration to 5 TU of human PPD and persons who react with less than 14 mm.

3. Finally, it seems that the effect of the concentration of tuberculin and the volume of fluid injected is different in strong and weak reactors.

In other words, there are quite a number of differences between strong and weak tuberculin reactors. If we assume that the population is homogeneous and that therefore the response to infection has, within narrow

limits, the same characteristics in all individuals, it seems likely that most of the strong reactors have obtained their allergy through infection with tubercle bacilli, whereas the medium reactors (mode, 12-14 mm) have obtained their allergy through infection with some other organism.

If, on the other hand, the above assumption is wrong, and the population can be divided into two groups, one of which is likely to develop active tuberculosis and the other, through some inborn resistance, is very unlikely to do so, the difference observed may be due to a difference in immune response. General reports on the behaviour of recently introduced clinical tuberculosis in districts where the disease was previously unknown do not favour this last theory.[1] We are therefore inclined to believe that the first assumption is more likely to be correct.

In conclusion, therefore, we feel that most of the medium-sized tuberculin reactions (mode, 12-14 mm) to the conventional 5 TU Mantoux test in Netherlands New Guinea are probably caused by an agent as yet unknown.

Implications

(*a*) *With regard to BCG vaccination*

The aim of a BCG-vaccination campaign is to increase specific resistance to infections with tubercle bacilli. This does not necessarily imply that everybody who has not had previous contact with tubercle bacilli must be selected for vaccination. The possibility must be considered that other factors than infection with tubercle bacilli operate in the community and cause reactions to human tuberculin which may differ more in quality than in quantity (measured after 5 TU) from those caused by infection with tubercle bacilli. It then remains to be demonstrated whether or not such an allergy-producing factor has induced a specific immunity similar to that conferred by BCG.

If the allergy and immunity obtained through this hypothetical infection are related to each other in the same way as they are presumed to be in persons vaccinated with BCG, it might be unnecessary to vaccinate individuals with reactions to 5 TU of human PPD of over a specified size, not because they are presumably infected with tubercle bacilli, but because they are either infected or have naturally acquired immunity. In countries where the type of allergy described in this paper is prevalent, the absence of such immunity could then be the criterion for vaccination.

If, on the other hand, the allergy and immunity obtained through our hypothetical infection are related one to another in a different way, or are quite unrelated, it might be important to offer BCG to all persons who have not had previous contact with tubercle bacilli. In this instance, there

[1] See footnote 2 on page 643.

would be a great need for a more specific tuberculin test than the one used at the moment in mass BCG-vaccination campaigns.

(*b*) *With regard to assessing a local tuberculosis problem*

There is little doubt that the conventional 5 TU Mantoux test is, in many places in the world, a rather unsatisfactory tool for estimating the percentage of the population infected with tubercle bacilli. If a more specific tuberculin test could be designed, it would be of great interest, especially to countries where, owing to the inaccessibility of large areas, tuberculin testing is one of the few diagnostic techniques that can be practised. It seems that a test using a lower concentration of tuberculin in a greater volume of diluent might prove more satisfactory than the conventional one. More studies on the effect of concentration and dilution are needed before final conclusions can be drawn.

(*c*) *With regard to tuberculin-testing techniques in general*

Generally speaking, it is difficult to obtain syringes that are satisfactory for tuberculin-testing work. Many of the syringes on the market leak to such an extent that it is impossible to do any reliable work with them. If a technique could be designed whereby a concentration of tuberculin was used to which largely only previously infected individuals react, and whereby the size of the reaction was mainly determined by the size of the wheal produced through intracutaneous injection, the problem of leakage could easily be overcome. A reliable evaluation of different studies could then be obtained by comparing the percentages of positive reactors.

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RÉSUMÉ

L'auteur présente les résultats d'une étude sur la sensibilité à la tuberculine effectuée en Nouvelle-Guinée néerlandaise, au cours de programmes de vaccination par le BCG, auxquels participaient l'OMS et le FISE. De la tuberculine PPD humaine (RT 23) et de la PPD aviaire ont été utilisées. Les réactions étaient lues après 72 heures, la taille de l'induration étant prise comme critère.

La répartition des réactions à 5 UT de PPD humaine chez les sujets tuberculeux est comparée à celle d'une population où la tuberculose est très fréquente, rare ou absente.

Dans le premier cas, la répartition est unimodale (mode 20-21 mm) et plus ou moins symétrique. Des réactions de cette dimension-là sont fréquentes non seulement parmi les malades, mais dans les populations où la tuberculose est répandue. Elles sont rares là où la maladie est peu fréquente, et absentes lorsque la maladie est pratiquement inconnue. Cependant, dans ce dernier cas, on obtient des réactions dont le mode est 13 mm environ. Des réactions de cette taille sont fréquentes aussi dans les régions où la tuberculose est rare, et moins fréquente lorsque la tuberculose est assez répandue.

Les tests parallèles avec la PPD humaine et la PPD aviaire ont montré que les sujets à réactions faibles ou moyennes à 5 UT de PPD humaine présentaient des réactions plus fortes à 5 UT de PPD aviaire. C'était l'inverse chez les sujets à fortes réactions.

On a déduit de ces résultats que les réactions à 5 UT dont le mode est 13 mm n'étaient pas dues à une infection par *Mycobacterium tuberculosis* var. *hominis*, mais à un agent dont l'identité est encore inconnue.

D'autres résultats suggèrent que la spécificité du test à la tuberculine diminue à mesure qu'augmente la quantité de tuberculine administrée. Ces données ont conduit à étudier l'influence de la concentration de la tuberculine et du volume du diluant. En diminuant la quantité de tuberculine et en augmentant le volume du diluant, on obtient des résultats qui font supposer une relation entre la concentration de la tuberculine et la spécificité du test. L'auteur propose une explication à ces divers phénomènes:

Chaque tuberculine est constituée de plusieurs composants. Certains sont communs aux tuberculines provenant de diverses variétés de *Mycobacterium tuberculosis*. L'induration produite dans un test standard sera plus importante avec une tuberculine homologue qu'avec une tuberculine hétérologue (à concentration égale). Si l'on réduit progressivement la concentration de la tuberculine, on atteint un seuil de sensibilité au-delà duquel le tissu ne réagit plus. Cette concentration limite sera plus faible pour une tuberculine homologue que pour une tuberculine hétérologue, et d'autant plus faible que la tuberculine sensibilisante et la tuberculine d'épreuve seront plus étroitement apparentées. A cette concentration critique, le volume du diluant aura une grande importance: un volume élevé mettra la tuberculine au contact d'une plus grande surface de tissu. En utilisant la concentration limite de PPD humaine sous un fort volume de diluant, on provoquera une réaction chez les individus infectés par *M. tuberculosis* var. *hominis*. Les sujets qui sont devenus allergiques sous l'effet d'autres micro-organismes ne réagiront pas, car la tuberculine humaine est pour eux hétérologue et le seuil de réaction est par conséquent plus élevé.

Si cette hypothèse se vérifiait, dans d'autres parties du monde où l'on rencontre une sensibilité non spécifique à la tuberculine, on pourrait mettre au point un test plus spécifique, donc plus satisfaisant que le test à 5 UT/0,1 ml actuellement en usage.

REFERENCES

Edwards, L. B. & Krohn, E. F. (1957) *Amer. J. Hyg.*, **66**, 253
Edwards, L. B. & Palmer, C. E. (1953) *Lancet*, **1**, 53
Edwards, L. B., Palmer C. E. & Magnus, K. (1953) *BCG vaccination*, Geneva (*World Health Organization: Monograph Series*, No. 12)
Great Britain, Medical Research Council (1956) *Brit. med. J.*, **1**, 413
Guld, J. (1954) *Acta tuberc. scand.*, **30**, 16
Guld, J., Magnus, K. & Magnusson, M. (1955) *Amer. Rev. Tuberc.*, **72**, 126
Guld, J. & Rud, C. (1953) *Brit. med. J.*, **1**, 368
Magnus, K., Guld, J., Waaler, H. & Magnusson, M. (1956) *Amer. Rev. Tuberc.*, **74**, 297
Palmer, C. E. (1957) *Bull. int. Un. Tuberc.*, **27**, 105
Palmer, C. E., Shaw, L. W. & Comstock, G. W. (1958) *Amer. Rev. Tuberc.*, **77**, 877
WHO Tuberculosis Research Office (1955a) *Bull. Wld Hlth Org.*, **12**, 63

WHO Tuberculosis Research Office (1955b) *Bull. Wld Hlth Org.*, **12**, 85
WHO Tuberculosis Research Office (1955c) *Bull. Wld Hlth Org.*, **12**, 197
WHO Tuberculosis Research Office (1955d) *Data for the assessment of naturally acquired tuberculin sensitivity in seven countries in Asia*, Copenhagen (mimeographed report)
WHO Tuberculosis Research Office (1956) *Tuberculosis survey in the Somalilands*, Copenhagen (mimeographed report)
WHO Tuberculosis Research Office (1957a) *Bull. Wld Hlth Org.*, **17**, 203
WHO Tuberculosis Research Office (1957b) *Tuberculin sensitivity survey in Mauritius*, Copenhagen (mimeographed report)
WHO Tuberculosis Research Office (1957c) *Tuberculosis survey in Nigeria*, Copenhagen (mimeographed report)
WHO Tuberculosis Research Office (1958) *Tuberculosis survey in Basutoland, Bechuanaland and Swaziland*, Copenhagen (mimeographed report)

APPENDIX TABLE 1. DISTRIBUTION OF REACTIONS TO 5 TU AND 100 TU OF HUMAN PPD AMONG THE ENTIRE POPULATION OF THREE VILLAGES IN KAMTOEK GRESSIE

Reaction to 5 TU (induration in mm)	Reaction to 100 TU (induration in mm) 0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	Total	%
0	6	3	2	—	—	—	—	—	1	1	—	—	—	—	—	—	13	9.0
1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
2	10	7	2	—	1	1	2	—	—	—	—	—	—	—	—	—	23	37.5
3	4	4	3	1	1	2	2	6	4	4	—	—	—	—	—	—	31	
4	2	—	1	1	2	6	6	10	3	1	—	1	—	—	—	—	33	29.8
5	—	—	—	—	—	—	—	6	3	—	—	1	—	—	—	—	10	
6	1	—	—	—	1	—	1	1	2	—	—	—	—	—	—	—	6	9.0
7	—	—	—	—	—	—	—	1	1	3	1	—	1	—	—	—	7	
8	—	—	—	—	—	—	—	—	2	2	2	—	—	—	—	—	6	7.6
9	—	—	—	—	—	—	—	1	1	1	2	—	—	—	—	—	5	
10	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1	1.4
11	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1	
12	—	—	—	—	—	—	—	—	—	1	—	1	—	—	—	—	2	2.8
13	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	2	
14	—	—	—	—	—	—	—	—	—	1	—	1	—	—	—	—	2	2.8
15	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1	—	2	
Total	23	14	8	2	5	9	11	25	18	16	6	5	1	—	1	—	144	
%	16.0	9.7	5.6	1.4	3.5	6.3	7.6	17.4	12.5	11.1	4.2	3.5	0.7	—	0.7	—		100

APPENDIX TABLE 2. DISTRIBUTION OF REACTIONS TO 5 TU OF HUMAN PPD AND 100 TU OF AVIAN PPD AMONG THE ENTIRE POPULATION OF THREE VILLAGES IN KAMTOEK GRESSIE

Reaction to 5 TU of human PPD (induration in mm)	Reaction to 100 TU of avian PPD (induration in mm) 0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30+	Total	%
0	2	3	2	1	—	1	1	2	—	1	—	—	—	—	—	—	13	9.0
1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
2	4	4	2	1	3	3	3	2	—	1	—	—	—	—	—	—	23	37.5
3	3	5	—	—	1	4	3	6	2	2	4	1	—	—	—	—	31	
4	1	1	—	—	2	3	4	6	7	4	2	1	1	1	—	—	33	29.8
5	—	—	—	—	—	—	1	2	2	1	2	1	—	—	1	—	10	
6	1	—	—	—	—	—	—	—	1	4	—	—	—	—	—	—	6	9.0
7	—	—	—	—	—	—	—	—	—	3	—	2	—	2	—	—	7	
8	—	—	—	—	—	—	—	—	—	1	2	—	2	—	1	—	6	7.6
9	—	—	—	—	—	—	—	—	—	2	—	2	—	—	1	—	5	
10	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	1	1.4
11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1	
12	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1	—	2	2.8
13	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1	2	
14	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1	—	2	2.8
15	—	—	—	—	—	—	—	—	—	—	—	1	—	1	—	—	2	
Total	11	13	4	2	6	11	12	18	12	19	12	10	3	4	6	1	144	
%	7.6	9.0	2.8	1.4	4.2	7.6	8.3	12.5	8.3	13.2	8.3	6.9	2.1	2.8	4.2	0.7		100

APPENDIX TABLE 3. DISTRIBUTION OF REACTIONS TO DUPLICATE TESTS WITH 5 TU OF HUMAN PPD AND 5 TU OF AVIAN PPD AMONG 528 SCHOOLCHILDREN AGED 6-17 YEARS

Reaction to 5 TU of human PPD (induration in mm)	Reaction to 5 TU of avian PPD (induration in mm) 0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	Total	%
0	40	18	12	9	4	2	3	2	2	—	—	—	—	—	—	92	17.4
2	4	15	18	4	5	2	1	—	—	—	—	—	—	—	—	49	9.3
4	2	1	15	12	11	3	7	—	—	—	—	—	—	—	—	51	9.7
6	—	—	—	3	4	10	2	1	1	—	—	—	—	—	—	21	4.0
8	—	—	1	2	5	5	8	3	1	1	—	—	—	—	—	26	4.9
10	—	—	—	—	1	—	3	8	6	—	1	—	—	—	—	19	3.6
12	—	—	—	—	—	3	7	7	8	2	1	—	—	—	—	28	5.3
14	—	—	—	—	—	4	6	8	12	7	1	1	—	—	—	39	7.4
16	—	—	—	—	—	3	6	13	14	7	2	1	1	—	—	47	8.9
18	—	—	—	—	1	1	3	18	19	13	7	2	—	—	—	64	12.1
20	—	—	—	—	—	4	—	7	18	18	6	1	1	—	—	54	10.2
22	—	—	—	—	—	—	1	—	12	6	5	4	—	—	—	28	5.3
24	—	—	—	—	—	—	—	1	1	2	1	1	—	—	—	6	1.1
26	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
28	—	—	—	—	—	—	—	—	1	1	1	—	—	—	—	3	0.6
Total	46	34	46	30	31	37	47	68	95	57	25	10	2	—	—	528	
%	8.7	6.4	8.7	5.7	5.9	7.0	8.9	12.9	18.0	10.8	4.7	1.9	0.4	—	—		100

APPENDIX TABLE 4. DISTRIBUTION OF REACTIONS TO DUPLICATE TESTS WITH 5 TU OF HUMAN PPD AND 5 TU OF AVIAN PPD AMONG 26 PATIENTS SUFFERING FROM CLINICAL TUBERCULOSIS

Reaction to 5 TU of human PPD (induration in mm)	Reaction to 5 TU of avian PPD (induration in mm) 6	8	10	12	14	16	18	20	22	24	26	28	Total
12	—	1	—	—	—	—	—	—	—	—	—	—	1
14	—	—	—	—	—	—	—	1	—	—	—	—	1
16	—	1	—	—	—	—	—	—	—	—	—	—	1
18	—	—	—	2	2	—	2	1	—	—	—	—	7
20	—	—	—	2	1	3	4	—	1	—	—	—	11
22	—	—	—	—	—	1	1	1	—	—	—	—	3
24	—	—	—	—	—	—	—	1	—	—	—	—	1
26	—	—	—	—	—	—	1	—	—	—	—	—	1
Tota	—	2	—	4	3	4	8	4	1	—	—	—	26

APPENDIX TABLE 5. DISTRIBUTION OF REACTIONS TO 100 TU OF HUMAN PPD FOR DIFFERENT CLASSES OF REACTORS TO 5 TU OF HUMAN PPD AND 5 TU OF AVIAN PPD AMONG SCHOOLCHILDREN AGED 6-17 YEARS

Induration (mm) to 5 TU of		Reaction to 100 TU of human PPD (induration in mm)														Total	Not tested *
human PPD	avian PPD	0	2	4	6	8	10	12	14	16	18	20	22	24	26		
0-4	0-4	35	12	9	9	9	6	11	3	2	—	—	—	—	—	96	—
	5-9	4	2	4	1	8	14	10	6	4	2	1	—	—	—	56	7
	10-14	—	—	—	2	—	—	2	4	1	1	1	—	—	—	11	2
	15+	—	—	—	—	—	2	—	1	—	—	—	—	—	—	3	—
Total		39	14	13	12	17	22	23	14	7	3	2	—	—	—	166	9
5-9	0-4	—	1	—	—	—	—	—	—	1	—	—	—	—	—	2	—
	5-9	1	—	—	1	1	3	5	5	2	4	1	—	—	—	23	1
	10-14	—	—	—	—	—	1	7	6	13	2	4	—	—	—	33	1
	15+	—	—	—	—	—	—	—	2	1	1	—	—	—	—	4	—
Total		1	1	—	1	1	4	12	13	17	7	5	—	—	—	62	2
10-14	0-4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5-9	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1	—
	10-14	—	—	—	—	—	—	2	3	5	8	5	2	—	—	25	4
	15+	—	—	—	—	—	—	—	5	7	10	10	3	2	—	37	1
Total		—	—	—	—	—	—	2	8	13	18	15	5	2	—	63	5
Grand total		40	15	13	13	18	26	37	35	37	28	22	5	2	—	291	16

* Mainly absentees from a day-school

APPENDIX TABLE 6. DISTRIBUTION OF REACTIONS TO 100 TU OF AVIAN PPD FOR DIFFERENT CLASSES OF REACTORS TO 5 TU OF HUMAN PPD AND 5 TU OF AVIAN PPD AMONG SCHOOLCHILDREN AGED 6-17 YEARS

Induration (mm) to 5 TU of		Reaction to 100 TU of avian PPD (induration in mm)															Total	Not tested *
human PPD	avian PPD	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28		
0-4	0-4	15	6	11	10	17	14	7	7	7	2	—	—	—	—	—	96	—
	5-9	—	—	4	3	2	5	11	16	10	3	2	—	—	—	—	56	7
	10-14	—	—	—	—	—	—	—	2	4	5	—	—	—	—	—	11	2
	15+	—	—	—	—	—	—	—	1	—	1	—	—	1	—	—	3	—
Total		15	6	15	13	19	19	18	26	21	11	2	—	1	—	—	166	9
5-9	0-4	—	1	—	—	—	—	—	—	1	—	—	—	—	—	—	2	—
	5-9	1	—	1	—	1	—	6	3	5	5	1	—	—	—	—	23	1
	10-14	—	—	—	—	—	—	2	6	6	11	6	2	—	—	—	33	1
	15+	—	—	—	—	—	—	—	—	—	3	—	1	—	—	—	4	—
Total		1	1	1	—	1	—	8	9	12	19	7	3	—	—	—	62	2
10-14	0-4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	5-9	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1	—
	10-14	—	—	—	—	—	—	—	1	3	9	7	4	1	—	—	25	4
	15+	—	—	—	—	—	—	—	2	6	7	12	6	3	—	1	37	1
Total		—	—	—	—	—	—	—	3	10	16	19	10	4	—	1	63	5
Grand total		16	7	16	13	20	19	26	38	43	46	28	13	5	—	1	291	16

* Mainly absentees from a day-school

APPENDIX TABLE 7. DISTRIBUTION OF REACTIONS TO DUPLICATE TESTS WITH 5 TU OF HUMAN PPD IN 0.1 ml AND 5 TU OF HUMAN PPD IN 0.2 ml AMONG 37 PATIENTS SUFFERING FROM CLINICAL TUBERCULOSIS

Reaction to 5 TU in 0.1 ml (induration in mm)	Reaction to 5 TU in 0.2 ml (induration in mm)											Total
	14	16	18	20	22	24	26	28	30	32	34	
10	—	—	—	1	—	—	—	—	—	—	—	1
12	—	—	—	—	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—	—	—	—	—
16	—	—	—	1	—	—	—	—	—	—	—	1
18	—	—	3	5	3	1	—	—	—	—	—	12
20	—	—	1	1	2	2	3	—	—	—	—	9
22	—	—	—	1	2	1	—	—	—	—	—	4
24	—	—	—	1	1	2	—	1	1	1	—	7
26	—	—	—	—	—	1	—	1	—	—	—	2
28	—	—	—	—	—	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—	—	1	—	—	1
32	—	—	—	—	—	—	—	—	—	—	—	—
Total	—	—	4	10	8	7	3	2	2	1	—	37