

## STANDARDIZATION OF A NEW BATCH OF PURIFIED TUBERCULIN (PPD) INTENDED FOR INTERNATIONAL USE

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### SYNOPSIS

A new batch of PPD tuberculin, RT 23, prepared at the request of UNICEF by the Statens Seruminstitut, Copenhagen, has been compared with previous batches of tuberculin from the Statens Seruminstitut and with the International Standard for the Purified Protein Derivative of Mammalian Tuberculin. The comparisons were made by intradermal tuberculin testing. The majority of tests were carried out in human subjects with various types and levels of tuberculin sensitivity—namely, tuberculous patients in the Netherlands; BCG-vaccinated as well as spontaneously tuberculin-sensitive army recruits in the Netherlands; BCG-vaccinated schoolchildren in Denmark; population groups in the tropics (Mauritius and Nigeria) including a high proportion of persons with low-grade sensitivity. In addition, comparisons were made in guinea-pigs sensitized in different ways.

For these comparisons, RT 23 was diluted both with phosphate-buffered saline alone (as were the other preparations) and with phosphate-buffered saline containing 0.05% Tween 80 as a stabilizing agent.

The results were found to differ significantly with the type and level of sensitivity of the groups tested. Thus, while the potency ratio of RT 23 and the International Standard is about 2:1 in BCG-vaccinated persons, it is about 1:1 in persons with naturally acquired tuberculin sensitivity. RT 23 in diluent containing Tween 80 elicited larger reactions than did RT 23 in ordinary diluent; however, when reactions of the same size

were compared, a difference in character was observed: the reactions to RT 23 diluted with Tween 80 were softer and less frequently bullous.

On the basis of the results—in terms of reaction size—obtained in patients and in non-vaccinated recruits, it is suggested that one unit of RT 23 should be defined as 0.02  $\mu\text{g}$  of the dry substance; for use as a first or single dose in routine testing, 0.02  $\mu\text{g}$  of RT 23 in diluent stabilized with Tween 80, or 0.06  $\mu\text{g}$  in diluent without Tween 80, is recommended.

A large new batch of purified tuberculin (PPD), designated RT 23, has been produced by the Statens Seruminstitut, Copenhagen.<sup>1</sup> Its total weight in the dry form is 670 g; 500 g of this batch is the property of UNICEF. If used for intradermal testing only, this very large quantity should cover world requirements for many years.

In view of its future importance the new batch has been made the subject of extensive biological assays. One of the objects of these assays was to standardize the new product against the International Standard for the Purified Protein Derivative of Mammalian Tuberculin (PPD-S); another was to compare it with two older purified products (RT 19-20-21 and RT 22) from the Statens Seruminstitut. A direct comparison with the last-mentioned products was considered important because they have been widely used in international work, but were not standardized in terms of the International Standard at the time of issue, being issued before the establishment of the Standard.

A further purpose of the assays was to examine the relative potencies of dilutions of RT 23 prepared with and without Tween 80. Recent experiments have shown that purified tuberculin in high dilution loses a large—and variable—proportion of its active substances by adsorption to the container. This adsorption, it has been found, may be prevented by addition to the dilution of a small quantity of the detergent Tween 80.<sup>2</sup> Thus, a tuberculin dilution to which Tween 80 has been added will show a higher potency than a dilution of the same concentration not containing this detergent. The introduction of Tween 80 as a stabilizing agent, therefore, will require the recommendations for dosage to be changed.

In the present report the results of the assays are given and suggestions made concerning the concentrations in which the new purified product should be used.

### Principles of Standardization

Intradermal testing was used throughout, both for the assays made in guinea-pigs and for those made in human subjects. This method was preferred because the tuberculins to be compared are primarily intended for intradermal use: if the ratio of potency for two tuberculins differs with

<sup>1</sup> See article on page 829 of this number of the *Bulletin*.

<sup>2</sup> See article on page 799 of this number of the *Bulletin*.

the testing methods, the ratio observed for intradermal testing will be the one of practical interest.

As regards the selection of the doses (expressed in terms of weight of dry substance) used in the present experiments, this was in the case of RT 23 based on preliminary assays (unpublished) performed in animals as well as in human subjects, and in the case of the older products (PPD-S, RT 19-20-21, RT 22) on assays and comparisons made in previous years.

From such previous experience it was expected that the tuberculins might be qualitatively different in the sense that doses found to be of equal potency in one kind of population would show different potencies in another population (Nissen Meyer, 1952; WHO Tuberculosis Research Office, 1955b). It was therefore decided to perform the comparisons in a variety of populations—guinea-pigs as well as human beings—with different types of allergy, so as to avoid drawing conclusions of too limited validity. The guinea-pigs were sensitized in three different ways. Thus, one group of animals was inoculated with virulent human tubercle bacilli, one with BCG vaccine and one with killed avian tubercle bacilli. The human population groups included tuberculous patients and army recruits in the Netherlands, BCG-vaccinated schoolchildren in Denmark, schoolchildren in Nigeria and villagers in Mauritius.

In each of the different populations, a “parallel line assay” was made. In this type of design several doses (usually two or three) are used of each tuberculin product under comparison. It is then possible to estimate a dose-response function for each product, that is, to express the size of the tuberculin reaction as a function of the dose of tuberculin. If the dose-response curves for two or several tuberculin products (as estimated in the same assay) are parallel (and straight), the potency ratios for these products may be computed arithmetically from the experimental data. For a general description of this and other biological assay methods, see, for example, Finney (1952).

In the assays made on guinea-pigs five tuberculin products were compared, two doses (dilutions) being used of each product and each animal being tested with all ten dose-product combinations. The range covered by the two doses from each product was fairly wide, corresponding to a ratio of 10 : 1 or 20 : 1. The guinea-pig assays in this respect differed from the assays on human subjects where a rather narrow range of doses was used, the ratio of the largest dose to the smallest dose not exceeding 4 : 1.

One reason why a narrow range of doses was used in all the assays in human populations was that the present assays were mainly aimed at determining the ratios of potency at the dose level routinely used for intradermal testing in humans. It is now widely accepted that a single intradermal test with a moderate dose of purified tuberculin (usually corresponding to about 5 tuberculin units (TU)) will produce a definite reaction in nearly all persons infected with tuberculosis without causing too many unpleasant reactions

under ordinary circumstances. However, even if it had been desirable to use a wide range of doses for the assays in human subjects, it would not have been feasible: the assays were made in the course of ordinary tuberculin-testing programmes and in these a high frequency of unpleasant reactions such as might have resulted from the use of higher doses would scarcely have been tolerated by the public, or by the authorities.

In human subjects each assay was thus designed to provide a ratio of potency for a particular type of population and for a particular dose level (about 5 TU). Four or five tuberculin products were tested in each assay, three doses per product being used in some assays, and two doses in the rest of the assays. The doses were spaced in geometric progression, in the ratios 1 : 2 : 4 or 4 : 6 : 9.

In most of the assays on human subjects, the participants received two tests each, a common, constant reference test and a variable test with a dilution selected at random among those to be assayed. In one study population (Dutch army recruits, see Table 1), a routine test was used as the reference test (this routine test had to be given in any case because the reactions to the assay tests, with varying doses of different products, were not considered acceptable for practical purposes—selection of “tuberculin reactors” for further examination). The reactions to the reference test were utilized for the analysis of the assay results, in two different ways. First, the reaction to the reference test was used for the discrimination of different levels of tuberculin sensitivity—for example, for selecting the definitely tuberculin-positive persons for further separate analysis. It would have been difficult, had no reference test been given, to make legitimate use of the reactions to the assay tests for the double purpose of first selecting the “positives” and then comparing their reactions to different tuberculins or doses. For instance, with a relatively weak dilution of tuberculin, persons with a low degree of sensitivity would be classified as “negative” more frequently than they would with a strong tuberculin dilution, and the potency of the weak dilution would be overestimated because the potency estimate would tend to be based on persons with rather strong allergy only. The use of a constant reference test, however, makes it possible to define entirely comparable subgroups of “positives” for the comparison of the various assay tuberculins.

Besides being used for the discrimination of different levels of allergy, the results of the reference test were also used for quantitative adjustment of the assay results, that is, they were used as “concomitant observations” in Fisher’s sense (Fisher, 1949). Although the subgroups—resulting from random assignment of the test subjects to the various assay tuberculins—are “comparable”, they will show random differences in degree of tuberculin sensitivity, and part of these random differences (which are irrelevant for the comparison aimed at) may be revealed and eliminated by means of the results of the reference test: the regression of the size of the assay reaction

on that of the reference reaction is computed, and the estimate of the mean size of the assay reactions adjusted accordingly. This method of computation differs from that used by Fisher mainly in that a separate regression coefficient is computed for each subgroup (a subgroup being defined as all subjects within a population group tested with the same assay dilution). A small loss of information results, but the validity of the procedure is somewhat clearer than when a common regression coefficient is used: when the assay tuberculins are qualitatively different the "true" value of the regression might differ from one subgroup to another.

In the two tropical populations (Mauritius and Nigeria) a constant reference test was given to all, in addition to the assay test in each person, so as to permit the selection of persons with low-grade tuberculin sensitivity for separate analysis. Again within each of the categories "high-grade" sensitivity and "low-grade" sensitivity, the results of the reference test were used as "concomitant observations" for further adjustment of the assay results.

No simultaneous reference test was given in the assay in BCG-vaccinated Danish schoolchildren, but for about half of these children the results of a routine tuberculin test, given one or two years previously, were available. These tests results were also used in the analysis as "concomitant observations".

A different design was used in an assay in tuberculous patients: two assay tests were given to each patient, but no reference test. Eight assay dilutions were used in all possible pairings, and the analysis made as for a balanced, incomplete block design ( $k = 2$ ,  $t = 8$ ,  $b = 28$ ) (Cochran & Cox, 1950).

The dilutions and products used in the various assays are specified in Table 1. The strength of the dilutions is expressed in terms of micrograms of dry substance per 0.1 millilitre of diluent ( $\mu\text{g}$  per 0.1 ml), so as to avoid confusion with "TU's". As regards PPD-S, the International Standard for the Purified Protein Derivative of Mammalian Tuberculin, one unit is referred to as 0.02  $\mu\text{g}$ .

It can be seen from Table 1 that the dilutions prepared with Tween 80 are relatively less concentrated. It was known, from preliminary, unpublished assays in human subjects, that dilutions prepared with Tween 80 are several times stronger than dilutions of corresponding concentration prepared without Tween, presumably because the addition of Tween prevents an otherwise heavy adsorption of tuberculin to the container. Thus the figures given in Table 1 for each assay dilution indicate only the amount of tuberculin originally dissolved per unit volume of diluent and not the actual strength. As regards the dilutions prepared without Tween the actual strength is reduced owing to adsorption.

When the assays were designed it was anticipated that in future practice RT 23 would always be used with Tween 80, and the dilutions of RT 23

**TABLE 1. DILUTIONS OF PURIFIED TUBERCULIN USED IN THE VARIOUS ASSAYS ( $\mu\text{g}$  OF DRY SUBSTANCE PER 0.1 ml)**

Population	Number of persons	Assay dilutions					Products and dilutions used for reference test
		PPD-S	RT 19-20-21	RT 22	RT 23	RT 23 with Tween 80	
Tuberculous patients, Netherlands	316	0.05 0.1	0.05 0.1	— —	0.05 0.1	0.015 0.03	—
Army recruits, Netherlands	5857	0.067 0.1 0.15	0.067 0.1 0.15	0.067 0.1 0.15	0.067 0.1 0.15	0.02 0.03 0.045	RT 22 0.067 $\mu\text{g}$ per 0.1 ml (= 5 TU)
Schoolchildren, Denmark	610	0.05 0.1 0.2	0.05 0.1 0.2	0.05 0.1 0.2	0.05 0.1 0.2	0.01 0.02 0.04	RT 19-20-21 0.1 $\mu\text{g}$ per 0.1 ml (= 5 TU)
Tropical populations	1068	0.1 0.2	0.1 0.2	— —	0.1 0.2	0.03 0.06	RT 19-20-21 0.1 $\mu\text{g}$ per 0.1 ml (= 5 TU)
Guinea-pigs	—	0.04 0.4	0.04 0.4	0.04 0.4	0.04 0.4	0.01 0.2	—

with Tween were therefore given double weight in the assays, that is, they were used for twice as many tests as the other assay dilutions. The only exception is the assay made in tuberculous patients; owing to a mistake in the planning, double weight was given here to the strongest dilution of RT 23 with Tween and the strongest dilution without Tween. With this exception, all results for dilutions with Tween may be considered to have half the variance of the corresponding results for other dilutions.

Much emphasis was put on a strictly random allocation of the assay dilutions to the subjects to be tested. Thus, the dilutions were allocated completely at random rather than by any rotational scheme. For each assay, lists were prepared which gave the order in which the assay dilutions were to be used, this order being derived from tables of random numbers for statistical use.

### Study Populations

#### *Guinea-pigs*

The guinea-pig assays were carried out in the Tuberculosis Department of the Statens Seruminstitut, Copenhagen. Two identically designed assays

were made at an interval of two months. 72 white guinea-pigs weighing 380-480 g were used per assay. The animals were divided into three groups of 24 animals, which were sensitized with living human tubercle bacilli, living BCG, and killed avian tubercle bacilli, respectively.

The first group was injected intraperitoneally with  $10^{-4}$  mg of living virulent human tubercle bacilli, strain E 10883, suspended in phosphate buffer of pH 7.4. The second group was vaccinated intracutaneously with four simultaneous injections of 0.1 ml of BCG vaccine containing 0.375 mg (moist weight) of bacilli per ml (BCG Department, Statens Serum Institut, Copenhagen). The third group was sensitized subcutaneously by two simultaneous injections of 0.25 ml of a suspension of dried, heat-killed avian tubercle bacilli, strain V 64, in Bayol F (light paraffin oil). The suspension contained 4 mg (dry weight) of bacilli per ml.

The animals were tested six weeks after inoculation in the first assay and five weeks after inoculation in the second.

#### *Tuberculous patients*

This part of the standardization was carried out by the Organization for Health Research TNO, The Hague, in co-operation with the WHO Tuberculosis Research Office. The tests were given and read by nurses from the Organization for Health Research, with considerable experience in tuberculin testing for research as well as for routine purposes.

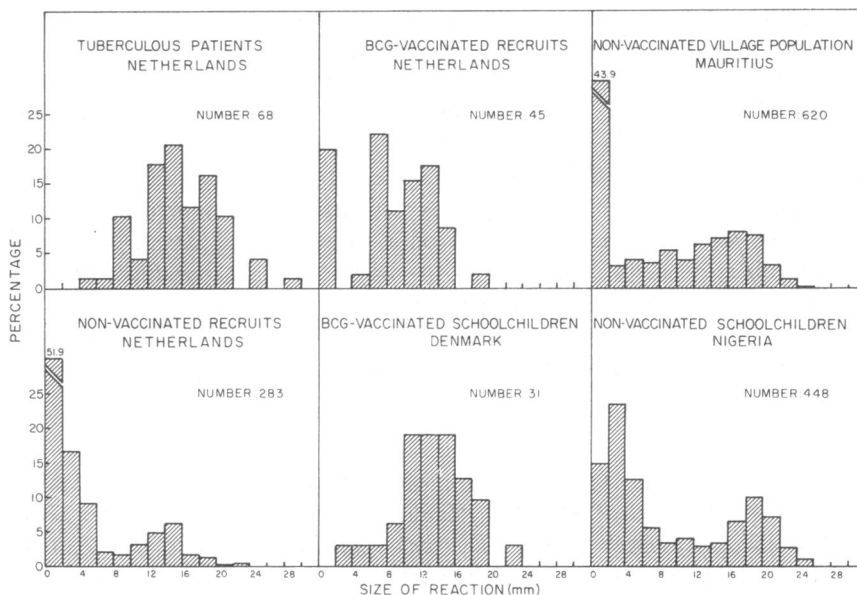
The testing was done in November 1957 and comprised nearly all the patients in two sanatoria in the Netherlands—namely, 121 patients in the Green Cross Sanatorium in Delft and 195 patients in the sanatorium "Zonnegloren" at Soest. A few patients were not tested because they appeared not to be suffering from tuberculosis. An indication is given in Fig. 1 (top left) of the distribution of tuberculin sensitivity among the patients tested. The histogram shows the distribution by size of the reactions to tests with a 5 TU dilution of RT 19-20-21 ( $0.1 \mu\text{g}$  per 0.1 ml), which was one of the assay dilutions. As the patients tested with this dilution were selected at random, the distribution may be regarded as representative of the total group of patients.

#### *Army recruits*

This study was also carried out by the Organization for Health Research TNO, in co-operation with the WHO Tuberculosis Research Office. As in the above study, the field work was done by a special team from TNO with many years' experience in tuberculin testing.

In the Netherlands Army, recruits are routinely given a single test with a 5 TU dilution of the purified tuberculin RT 22 ( $0.067 \mu\text{g}$  per 0.1 ml). Permission was obtained for giving two tests to each recruit—namely, the routine test and an additional test with one of the dilutions to be assayed.

**FIG. 1. DISTRIBUTIONS BY SIZE OF REACTIONS TO INTRADERMAL TESTS WITH 5 TU OF RT 19-20-21, AMONG THE DIFFERENT POPULATION GROUPS STUDIED**



On the day of testing, each recruit was asked if he had been vaccinated with BCG and was examined for BCG scars. Doubtful cases of previous BCG vaccination were, as a rule, recorded as vaccinated rather than as non-vaccinated. Two groups of recruits were examined, 3132 in June 1957 (including 431 with a positive or doubtful anamnesis of previous BCG vaccination) and 2725 in August 1957 (including 379 with a positive or a doubtful BCG anamnesis). The distribution of tuberculin sensitivity was practically the same in June and August, and the results from the two groups have therefore been combined in this report. The distribution by size of the reactions to 5 TU of RT 19-20-21 is shown in Fig. 1, separately for non-vaccinated and vaccinated recruits. An extensive report on other tuberculin studies in the Netherlands, and on the technique used, is given by Griep & Bleiker (1957).

#### *BCG-vaccinated schoolchildren*

This assay was carried out by the WHO Tuberculosis Research Office field team in Copenhagen in April and October 1957 in connexion with the annual testing of children in Copenhagen suburban schools.

Altogether, 610 children in five schools were tested. Only children known to have been BCG-vaccinated were included. A single test with



one of 15 assay dilutions was given to each child, the dilutions being allocated at random. No reference test was used, but 306 of the children had been tested one or two years previously (after BCG vaccination) by the same team. The distribution of tuberculin sensitivity is illustrated in Fig. 1, which gives the reactions of the children tested with a 5 TU dilution of RT 19-20-21.

### *Tropical populations*

One assay was carried out on the island of Mauritius in September-October 1957 by the WHO Tuberculosis Control Project. It included a village population of 620 persons (children under 4 years of age and BCG-vaccinated persons were excluded).

Another assay was made on 448 children aged 6-9 years, in three schools in Ibadan, Nigeria, in November 1957. The testing was done by the WHO Tuberculosis Survey Team in West Africa.

The primary purpose of these two assays was a comparison of the different tuberculin preparations in persons with low-grade tuberculin sensitivity.<sup>1</sup> Each person was given a test with one of eight assay dilutions, and also a reference test with a 5 TU dilution of RT 19-20-21, the latter for the purpose of selecting persons likely to have a low-grade sensitivity. The distribution of tuberculin sensitivity, as determined by the size of reaction to the reference 5 TU test, is given in Fig. 1, separately for the villagers in Mauritius and the Nigerian schoolchildren.

## **Tuberculins : Products and Dilutions**

### *Products*

*PPD-S* was prepared by Florence Seibert and co-workers (Seibert & Glenn, 1941). The method used involved sterile filtration and ultrafiltration of heat-killed synthetic-medium cultures, followed by precipitation in a semi-saturated, neutral solution of ammonium sulfate, seven times repeated. This tuberculin has been widely used for epidemiological investigations by the United States Public Health Service (see, for example, Palmer, Ferebee & Petersen, 1950). A portion of the batch was in 1952 adopted as the International Standard for the Purified Protein Derivative of Mammalian Tuberculin (WHO Expert Committee on Biological Standardization, 1952) and is now maintained at the Statens Seruminstitut, Copenhagen; it is distributed in freeze-dried form in ampoules, each containing 10 mg of tuberculin and 4 mg of buffer salt. In 1953, one unit of this Inter-

<sup>1</sup> A low-grade sensitivity to tuberculin—appearing as small or medium-sized reactions to a low dose of tuberculin (and as large reactions to a high dose)—has been found to be highly prevalent in many tropical and subtropical areas, including Mauritius and Nigeria. This type of sensitivity is held to be non-specific, i.e., unrelated to infection with tubercle bacilli (Palmer, Ferebee & Petersen, 1950; WHO Tuberculosis Research Office, 1955a).

national Standard was fixed at 0.000028 mg, i. e., 0.00002 mg of tuberculin plus 0.000008 mg of buffer salt (WHO Expert Committee on Biological Standardization, 1953). In the present report, however, the quantity of PPD-S is given in terms of net weight, without buffer salt, one unit of the International Standard being referred to as 0.02  $\mu$ g.

For each of the assays to be reported a stock solution containing 50 000 TU per ml was made up by dissolving the contents of one ampoule in 10 ml of phosphate-buffered saline without Chinosol.

*RT 19-20-21* (RT XIX-XX-XXI, RT 19-21) was prepared at the Statens Seruminstitut, Copenhagen, according to the method described by Lind (1947). (Sterile filtration and ultrafiltration of synthetic-medium culture, precipitation with trichloroacetic acid, dehydration with ether.) It was standardized against RT VII—the reference PPD tuberculin of the Statens Seruminstitut—and one unit of RT 19-20-21 was defined as 0.00002 mg of the dry substance. The batch was used for nearly all the tuberculin testing in the International Tuberculosis Campaign in Poland, Czechoslovakia, Hungary, Yugoslavia, Austria and Greece during the years 1948-50. In addition, it has been used during the last nine years for investigations of tuberculin sensitivity and post-vaccination allergy conducted by the WHO Tuberculosis Research Office, WHO BCG-assessment teams and WHO tuberculosis survey teams. Further, most of the studies on the instability of purified tuberculin in high dilution reported elsewhere in this issue<sup>1</sup> have been carried out with this product.

*RT 22* was prepared in 1949-50 at the Statens Seruminstitut, Copenhagen, by the same technique as used for RT 19-20-21. It was issued in the form of ready-made dilutions and also in the form of stock solutions for further dilution in other laboratories. It has been used in all the BCG mass campaigns conducted under the auspices of WHO between August 1950 and July 1958, for testing of approximately 214 million persons (Christensen, 1957; and unpublished material). When standardized by the Statens Seruminstitut before issue, it was estimated to contain 1 TU in 0.000013 mg, that is, to be 50% stronger than RT 19-20-21. Later comparisons in human subjects (WHO Tuberculosis Research Office: unpublished material) did not clearly confirm the greater strength of RT 22, but for practical reasons no changes were made in the definition of one unit of this tuberculin.

*RT 23* has been issued from the Statens Seruminstitut since July 1958, the recommended dosage for a single or first test being 0.02  $\mu$ g in 0.1 ml of phosphate-buffered saline with 0.05% Tween 80. This dosage is designated 1 TU. The method of preparation of RT 23 has been described earlier in this issue.<sup>2</sup>

<sup>1</sup> See articles on pages 765 and 783.

<sup>2</sup> See article on page 829.

### *Preparation of dilutions*

The dilutions used in this study were prepared in such a way as to reduce the chance of gross random errors due to adsorption of tuberculin to glass. In order to reduce the possibility of systematic errors due to inter-dilution variations, at least two independently prepared batches of each tuberculin dilution were used in each group. To minimize the effect of inter-ampoule variations, a considerable number of ampoules of each dilution were used.

The dilutions were prepared by diluting stock solutions containing 1 mg per ml (about 50 000 TU per ml) of the various preparations in two steps to the desired concentration. Phosphate-buffered saline, pH 7.38,<sup>3</sup> alone and phosphate-buffered saline containing 0.05 ‰ Tween 80 were used as diluents. All dilutions (except the stock solution of PPD-S) contained 0.1 ‰ Chinosol as preservative.

The glassware used in the preparation of the dilutions had not been used previously.

In most assays the dilutions used had been prepared a few weeks previously and stored at 2-4°C. The dilutions used in the animal studies had been stored for 2-5 days only; the dilutions used in the tropical populations had been prepared 6-10 weeks previously.

## Testing and Reading of Reactions

### *Assays in guinea-pigs*

Ten tests with ten different tuberculin dilutions were given to each animal, the dilutions being allocated at random to the various test-sites. The testing procedure was the same as for human subjects—intradermal injection of 0.1 ml of the appropriate tuberculin dilution. The testing for each assay was completed in 3 days, 24 animals—8 from each of the 3 antigen groups (living human tubercle bacilli, BCG, and killed avian tubercle bacilli)—being tested per day. Two sets of the ten tuberculin dilutions had been prepared and half of the animals in each antigen group were tested with one set and half with the other. (No significant variations were found between the two sets of dilutions.) The reactions were read after 24 and 48 hours, each time by two readers. The longitudinal and transverse diameters of erythema were measured and recorded. The data given below are based on the average of the 24-hour readings of the two readers (the readings made at 48 hours gave similar results).

### *Assays in human subjects*

For each test, 0.1 ml of the appropriate tuberculin dilution was injected intradermally on the dorsal aspect of the forearm. When two tests were

<sup>3</sup> The composition of this buffer is described in the article on page 799 of this number of the *Bulletin*.

given to the same person, they were placed symmetrically, one on each forearm. In the Danish schoolchildren the test was given at a site on the forearm that had not been used for testing in previous years, so as to avoid the effect of local sensitization (WHO Tuberculosis Research Office, 1955b). In the assays conducted in the Netherlands, a freshly boiled syringe and needle were used for each injection, according to the TNO routine field technique. In the other assays (Denmark, Nigeria and Mauritius) the routine technique of WHO/TRO was followed: only syringes pre-tested for leakage were used and the sterilization between injections was limited to flaming of the needle-point. Throughout all assays separate syringes were used for each tuberculin product.

The reactions were read after 3 days, or in a few cases 4 days. The reaction was palpated and the transverse diameter of induration measured and recorded. In addition, the presence of bullae or vesicles was noted. In the assays in the Netherlands and Denmark (but not in Nigeria and Mauritius) an estimate was also made of the relative firmness of the palpable reactions; the reactions were classified into four types, type I being the most dense reaction and type IV representing barely perceptible, very soft reactions (Palmer, Ferebee & Petersen, 1950).

The tuberculin ampoules were labelled in code so that the identity of the assay dilutions would be unknown to the field personnel. As the nurses who read the reactions were not allowed to see the record cards (they dictated the results of their readings to a secretary) they did not know whether any two tests had been made with the same or with different dilutions.

A record card was filled in for each person tested, the standard cards of the respective field teams being used. As regards the assays in the Netherlands, copies of the cards were sent to the WHO Tuberculosis Research Office. From the assays in Africa and Denmark the original cards were forwarded to the Tuberculosis Research Office for analysis.

### Results in Guinea-pigs

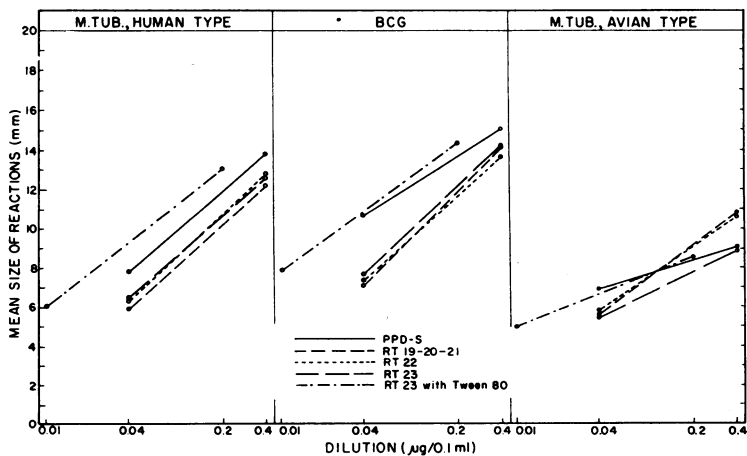
The two experiments gave the same results, though the reactions obtained in the second experiment were systematically smaller than those obtained in the first experiment. This systematic difference is most likely due to the shorter interval between vaccination and testing in the second experiment—5 weeks as compared with 6 weeks in the first experiment.

Table 2 shows the mean size of reactions and the regression coefficient for each of the tuberculin preparations. The regression varies significantly within the two groups sensitized with BCG and with avian tubercle bacilli. This makes a "standardization" impossible. However, it is possible to calculate the potency ratio between any two preparations for a given dose

**TABLE 2. MEAN SIZE OF REACTIONS (IN mm) IN GUINEA-PIGS TO ALL DOSES AND REGRESSION COEFFICIENT FOR EACH OF FIVE TUBERCULIN PRODUCTS**

Product	Experiment number	Mean size of reactions to all doses ( $\bar{y}$ )			Regression coefficient ( $b$ ) for doubling of doses		
		Animals sensitized with			Animals sensitized with		
		human tubercle bacilli	BCG	avian tubercle bacilli	human tubercle bacilli	BCG	avian tubercle bacilli
PPD-S	I	11.1	13.3	8.2	2.02	1.22	0.54
	II	10.6	12.6	7.9	1.51	1.40	0.71
RT 19-20-21	I	9.6	11.0	8.8	1.95	1.90	1.60
	II	8.6	10.3	7.6	1.80	2.26	1.56
RT 22	I	10.4	11.2	8.8	2.13	1.84	1.45
	II	8.8	9.9	7.6	1.73	1.97	1.44
RT 23	I	10.3	11.6	7.8	2.01	1.75	1.14
	II	8.8	10.3	6.5	1.74	2.08	0.93
RT 23 with Tween 80	I	10.2	11.6	7.2	1.88	1.49	0.88
	II	9.0	10.5	6.4	1.38	1.44	0.74

For RT 23 in dilution with Tween 80 the doses are 0.01  $\mu\text{g}$  and 0.2  $\mu\text{g}$ .  
For the other products the doses are 0.04  $\mu\text{g}$  and 0.4  $\mu\text{g}$ .

**FIG. 2. MEAN SIZE OF REACTIONS TO 10 ASSAY DILUTIONS IN GUINEA-PIGS SENSITIZED IN THREE DIFFERENT WAYS**

**TABLE 3. POTENCY RATIOS AND 95% FIDUCIAL LIMITS FOR FIVE TUBERCULIN PRODUCTS MEASURED IN THREE GROUPS OF GUINEA-PIGS SENSITIZED WITH HUMAN TUBERCLE BACILLI, BCG AND AVIAN TUBERCLE BACILLI**

Sensitization of animals	Experiment number	PPD-S					RT 23 with Tween 80							
		RT 19-20-21		RT 22		RT 23		RT 23 with Tween 80		PPD-S		RT 23		
Human tubercle bacilli	I	0.58	0.73	0.77	0.95	0.76	0.95	2.03	2.58	0.47	0.60	0.28	0.35	0.45
		0.46	0.46	0.62	0.62	0.61	0.61	1.60	1.60	0.37	0.29	0.22	0.29	0.29
	II	0.44	0.57	0.47	0.62	0.47	0.62	1.24	1.74	0.74	1.07	0.28	0.36	0.40
		0.34	0.34	0.36	0.36	0.36	0.36	0.89	0.89	0.51	0.24	0.22	0.24	0.24
BCG	I	0.40	0.52	0.42	0.55	0.47	0.62	1.27	1.74	0.98	1.60	0.26	0.33	0.42
		0.31	0.31	0.32	0.32	0.36	0.36	0.93	0.93	0.60	0.22	0.20	0.22	0.25
	II	0.45	0.56	0.37	0.48	0.44	0.55	1.03	1.43	1.01	1.56	0.28	0.34	0.36
		0.36	0.36	0.29	0.29	0.35	0.35	0.74	0.74	0.66	0.21	0.23	0.23	0.23
Avian tubercle bacilli	I	1.11	1.48	1.18	1.63	0.70	1.04	1.18	2.01	1.66	6.11	0.59	0.81	0.72
		0.83	0.83	0.86	0.86	0.47	0.47	0.69	0.69	0.45	0.46	0.43	0.46	0.31
	II	0.77	1.02	0.78	1.07	0.34	0.59	0.70	1.39	1.53	3.96	0.49	0.67	0.58
		0.58	0.58	0.57	0.57	0.20	0.20	0.35	0.35	0.59	0.37	0.36	0.36	0.21

The potency ratios relative to PPD-S (left-hand side of table) have been calculated for a dose of PPD-S of 0.1 µg (=5 TU).  
The potency ratios relative to RT 23 with Tween 80 (right-hand side of table) have been calculated for a dose of RT 23 of 0.03 µg in dilution with Tween 80.

of one of the preparations. Thus, the left half of Table 3 shows the potency ratio (with 95% fiducial limits) for each preparation against PPD-S, corresponding to a dose of PPD-S of 0.1  $\mu\text{g}$  (5 international units); and the right half of Table 3 shows the potency ratio for each preparation against RT 23 in dilution with Tween 80, corresponding to a dose of 0.03  $\mu\text{g}$  of RT 23 with Tween 80.

In Fig. 2 the mean reactions (means of both experiments) are shown graphically according to the source of sensitization of the animals.

In animals sensitized with human tubercle bacilli or BCG, RT 23 without Tween 80 shows about the same activity as RT 19-20-21 and RT 22, but is definitely weaker than PPD-S. When diluted with buffer containing 0.05 ‰ Tween 80, RT 23 is stronger than the other preparations (diluted without Tween 80).

In animals sensitized with avian tubercle bacilli, RT 23 without Tween 80 is weaker than the other preparations, while RT 23 with Tween 80 appears to be as active as these preparations.

### Results in Human Populations

#### *Tabulation of experimental data*

The results of the assays have been tabulated in the appendix tables. For each assay (except that made in tuberculous patients) the results are arranged with a separate appendix table for each assay dilution. In each table those tested with a specified assay dilution are distributed according to the sizes of their reactions to this dilution (vertical scale) and to the reference test (horizontal scale). In addition, the reactions to the assay dilution are, for each size-category, distributed by type of induration (degree of firmness) and by presence or absence of bullae and vesicles (horizontal scale).

The data from the assay in tuberculous patients (Appendix Table 1) are given separately for each pairing of assay dilutions (each patient was tested with 2 out of 8 different assay dilutions).

#### *Analysis of reaction size*

For purposes of analysis, some of the study populations have been subdivided. Thus, the tropical populations have each been divided into three groups according to size of reaction to the reference test: namely, those with a reaction of 12 mm or more, those with reactions of 4-11 mm, and those with reactions of 0-3 mm. It was assumed that the first group would mainly consist of persons with specific sensitivity, that the second group would include a high proportion of persons with "non-specific"

sensitivity and that the third group would be composed of both persons with "non-specific" sensitivity and non-sensitive persons.

The non-vaccinated Dutch army recruits have been classified into "tuberculin-positive" (a reaction of 8 mm or more to the reference test) and "tuberculin-negative" (a reaction of 7 mm or less). There is presumably little or no "non-specific" sensitivity in the Netherlands and the "tuberculin-positive" group was therefore assumed to consist almost exclusively of persons with "specific" sensitivity and has been analysed as a whole; no analysis has been made of the tuberculin-negative group. The army recruits with a definite or doubtful anamnesis of BCG vaccination have been analysed both as a whole and by sub-groups. The subgroup with reactions of 4-11 mm to the reference test may be assumed to represent BCG-induced allergy; of the persons with reactions of 0-3 mm, some may not have been vaccinated and some may not have been effectively vaccinated; those with reactions of 12 mm or more have not been analysed separately, mainly because they are so few (this subgroup probably includes some persons who are infected with tuberculosis).

The BCG-vaccinated schoolchildren tested in Copenhagen have a stronger and more uniform tuberculin sensitivity than the BCG-vaccinated recruits. It is unlikely that any of these children were infected with tuberculosis: they had been vaccinated only a few years previously and lived in an environment with a low infection risk. The results for these children have been analysed as a whole. It should be noted, however, that the results for about one half of the children have been adjusted according to a previous tuberculin test.

Table 4 shows for each population or subgroup of population the mean reactions to the different assay dilutions. In addition, the table gives an estimate of the variance of each distribution and each mean. Section A of this table contains the results for the tuberculous patients. Both unadjusted (left-hand part) and adjusted (right-hand part) means are given. The latter have been computed from the 56 means given in Appendix Table 1 (the 56 means were treated as single observations in a balanced incomplete block design where  $k = 2$ ,  $t = 8$  and  $b = 28$ ; for details of this analysis, see Cochran & Cox, 1950). The results for the other populations are set out in section B. The figures in the left-hand part of this section are unadjusted and refer to the reactions to the assay dilutions only,  $\bar{y}$  being the mean reaction size,  $s_1^2$  the variance of the distribution of reactions and  $\frac{1}{n} \cdot s_1^2$  the estimated variance of  $\bar{y}$ . The right-hand part gives the corresponding adjusted data, the adjustment being based upon the results of the reference test. This adjustment is made as follows. For the population group or subgroup concerned, the mean size of the reference reactions is computed separately for each assay dilution,  $\bar{x}$ , as well as for all assay dilutions combined,  $\bar{\bar{x}}$ . Next is computed the regression coefficient,  $b$ , of the reactions



to the assay dilution on the reactions to the reference test. If  $y$  is the unadjusted mean size of reactions to one assay dilution, the adjusted value is obtained from  $y - b(x - \bar{x})$ . Finally,  $s_2^2$ , the variance of the distribution relative to the regression line, and  $\frac{1}{n} \cdot s_2^2$ , the estimated variance of  $y - b(x - \bar{x})$ , are given.<sup>1</sup>

The last line for each population group gives the total number of persons in the group, weighted means of the variances  $s_1^2$  and  $s_2^2$ , the mean of all reactions to the reference test,  $\bar{x}$ , and means of the variances  $\frac{1}{n} \cdot s_1^2$  and  $\frac{1}{n} \cdot s_2^2$ . As mentioned previously, RT 23 with Tween 80 was in principle allocated to twice as many persons as each of the other tuberculin products; the mean values of  $\frac{1}{n} \cdot s_1^2$  and  $\frac{1}{n} \cdot s_2^2$  have been computed so as to be directly valid for the assay dilutions without Tween 80, being twice the estimate for dilutions of RT 23 with Tween 80.

<sup>1</sup> The variance of  $y - b(x - \bar{x})$  is more correctly estimated as the sum of two terms, the first of which is  $\frac{1}{n} \cdot s_2^2$ . The second term varies with the size of  $(x - \bar{x})$  but has an average close to  $\frac{1}{n \cdot (n-1)} \cdot s_2^2$ , on condition that  $\bar{x}$  is the mean of a sample selected strictly at random from a population with mean  $\bar{x}$ ; this condition is fulfilled in the present case. Because the second term is relatively small, it has been neglected in the computations.

**TABLE 4. MEAN SIZE OF REACTIONS (IN mm) TO THE VARIOUS ASSAY DILUTIONS, TOGETHER WITH ESTIMATES OF VARIANCE, SEPARATELY FOR EACH GROUP OR SUBGROUP OF POPULATION**

**A. Tuberculous Patients (Netherlands)**

Products and dilutions (µg/0.1 ml)		Number of tests given (n)	Unadjusted figures			Adjusted figures	
			Mean size of reactions	Mean square of deviations (s. <sup>2</sup> )	$\frac{s_1^2}{n}$	Mean size of reactions	Estimated variance of adjusted means
PPD-S	0.05	69	14.6	17.74	0.26	14.6	
	0.1	72	17.6	20.54	0.29	17.5	
RT 19-20-21	0.05	69	12.3	14.82	0.21	12.4	
	0.1	68	15.2	20.31	0.30	16.1	
RT 23	0.05	70	14.2	19.02	0.27	14.4	
	0.1	105	17.7	15.39	0.15	17.8	
RT 23 with Tween 80	0.015	69	14.7	27.51	0.40	14.6	
	0.03	110	16.8	16.35	0.15	16.7	
Total/Means . . .		632			0.25		0.16

**TABLE 4. MEAN SIZE OF REACTIONS (IN mm) TO THE VARIOUS ASSAY DILUTIONS, TOGETHER WITH ESTIMATES OF VARIANCE, SEPARATELY FOR EACH GROUP OR SUBGROUP OF POPULATION (continued)**

**B. Other Populations**

Products and dilutions ( $\mu\text{g}/0.1 \text{ ml}$ )	Number of persons tested (n)	Unadjusted figures			Mean size of reactions to reference test ( $\bar{x}$ )	Regression of y on x (b)	Adjusted figures			
		Mean size of reactions ( $\bar{y}$ )	Mean square of deviations ( $s_1^2$ )	$\frac{s_1^2}{n}$			Mean size of reactions ( $\bar{y}-b(\bar{x}-\bar{x})$ )	Mean square of deviations from regression line ( $s_2^2$ )	$\frac{s_2^2}{n}$	
<b>Non-vaccinated recruits, Netherlands (Reactions to reference test : 8 mm or more)</b>										
PPD-S	0.067	65	15.0	16.32	0.25	12.5	0.8	14.9	9.71	0.15
"	0.1	46	15.4	15.80	0.34	12.5	0.5	15.4	14.67	0.32
"	0.15	53	17.0	15.00	0.28	12.3	1.0	17.0	9.25	0.17
RT 19-20-21	0.067	40	11.5	8.41	0.21	12.2	0.6	11.6	6.14	0.15
"	0.1	51	13.8	8.90	0.17	12.4	0.5	13.8	7.35	0.14
"	0.15	48	15.2	13.80	0.29	12.8	0.7	14.9	10.25	0.21
RT 22	0.067	40	12.2	9.26	0.23	11.8	0.7	12.5	6.01	0.15
"	0.1	45	13.2	10.69	0.24	13.0	0.7	12.7	6.41	0.14
"	0.15	42	14.9	19.84	0.47	12.1	0.9	15.2	13.08	0.31
RT 23	0.067	43	14.4	6.30	0.15	12.0	0.4	14.6	5.46	0.13
"	0.1	45	14.9	13.19	0.29	12.1	0.5	15.0	11.90	0.26
"	0.15	49	17.0	17.01	0.35	12.6	0.2	17.0	17.21	0.35
RT 23 with Tween 80	0.02	98	14.6	18.66	0.19	12.2	0.6	14.6	16.67	0.17
"	0.03	93	16.2	11.82	0.13	12.6	0.7	16.0	8.33	0.09
"	0.045	97	17.3	15.51	0.16	12.2	0.7	17.4	11.97	0.12
Total/Means . . .		855		13.87	0.29	12.4 ( $= \bar{x}$ )			10.74	0.22

TABLE 4. B. Other Populations (continued)

Products and dilutions ( $\mu\text{g}/0.1 \text{ ml}$ )	Number of persons tested	Unadjusted figures				Mean size of reactions to reference test	Regression of y on x	Adjusted figures		
		Mean size of reactions	Mean square of deviations	$s_1^2$	$s_2^2$			Mean size of reactions	Mean square of deviations from regression line	$\frac{s_2^2}{n}$
<b>Vaccinated recruits, Netherlands (Total)</b>										
PPD-S	0.067	41	6.4	25.55	0.62	6.1	1.1	7.3	6.71	0.16
"	0.1	41	7.0	35.72	0.87	6.0	1.1	7.9	10.01	0.24
"	0.15	52	9.1	34.84	0.67	6.7	1.2	9.2	7.93	0.15
RT 19-20-21	0.067	57	5.7	19.20	0.34	6.5	0.8	6.0	5.29	0.09
"	0.1	45	8.1	24.33	0.54	6.8	0.9	8.1	8.03	0.18
"	0.15	46	9.2	25.87	0.56	7.1	0.9	8.9	7.52	0.16
RT 22	0.067	38	7.2	18.71	0.49	6.6	0.8	7.4	6.75	0.18
"	0.1	39	8.3	18.63	0.48	7.0	0.9	8.1	7.29	0.19
"	0.15	42	10.6	25.42	0.61	6.8	0.7	10.6	16.69	0.40
RT 23	0.067	49	9.9	20.06	0.41	7.4	0.6	9.6	13.32	0.27
"	0.1	41	10.7	18.12	0.44	7.6	0.5	10.3	12.91	0.31
"	0.15	44	11.8	26.40	0.60	7.0	0.9	11.7	10.79	0.25
RT 23 with Tween 80	0.02	92	7.9	41.67	0.45	7.0	1.1	7.6	14.30	0.16
"	0.03	91	9.6	45.54	0.50	7.4	1.1	8.9	13.86	0.15
"	0.045	92	9.3	37.99	0.41	6.2	1.1	10.0	16.85	0.18
Total/Means . . .		810		30.38	0.67	6.8 (= $\bar{x}$ )			11.30	0.25
<b>Vaccinated recruits, Netherlands (Reactions to reference test : 0-3 mm)</b>										
PPD-S	0.067	14	1.2	5.72	0.41	1.6	1.0	1.0	4.79	0.34
"	0.1	16	1.2	3.27	0.20	1.4	0.8	1.3	2.49	0.16
"	0.15	14	1.9	8.29	0.59	1.3	0.8	2.0	7.91	0.56
RT 19-20-21	0.067	18	1.4	2.49	0.14	1.7	0.4	1.3	2.35	0.13
"	0.1	13	2.0	7.00	0.54	1.3	0.9	2.1	6.42	0.49
"	0.15	11	3.0	9.60	0.87	0.5	2.5	5.4	5.90	0.54
RT 22	0.067	10	1.7	2.46	0.25	1.5	0.7	1.6	1.53	0.15
"	0.1	7	1.7	9.91	1.42	0.9	2.0	2.8	6.40	0.91
"	0.15	14	6.8	23.41	1.67	2.1	2.0	5.5	19.25	1.38
RT 23	0.067	12	6.3	17.52	1.46	1.7	1.5	6.0	15.72	1.31
"	0.1	8	7.9	17.84	2.23	1.6	1.2	7.6	18.85	2.36
"	0.15	14	6.8	25.87	1.85	1.6	2.4	6.4	16.97	1.21
RT 23 with Tween 80	0.02	26	2.0	7.52	0.29	1.7	1.0	1.7	5.98	0.23
"	0.03	22	0.9	1.61	0.07	0.9	0.4	1.1	1.48	0.07
"	0.045	31	3.8	18.81	0.61	1.4	0.6	3.9	18.89	0.61
Total/Means . . .		230		10.60	0.86	1.4 (= $\bar{x}$ )			9.09	0.73

TABLE 4. B. Other Populations (continued)

Products and dilutions ( $\mu\text{g}/0.1 \text{ ml}$ )	Number of persons tested (n)	Unadjusted figures			Mean size of reactions to reference test ( $\bar{x}$ )	Regression of y on x (b)	Adjusted figures			
		Mean size of reactions ( $\bar{y}$ )	Mean square of deviations ( $s_1^2$ )	$\frac{s_1^2}{n}$			Mean size of reactions ( $\bar{y}-b(\bar{x}-\bar{x})$ )	Mean square of deviations from regression line ( $s_2^2$ )	$\frac{s_2^2}{n}$	
<b>Vaccinated recruits, Netherlands (Reactions to reference test : 4-11 mm)</b>										
PPD-S	0.067	25	8.6	11.33	0.45	8.0	0.8	8.2	8.14	0.33
"	0.1	19	9.1	14.44	0.76	7.4	0.7	9.2	12.68	0.67
"	0.15	31	10.6	10.97	0.35	7.5	0.9	10.7	6.90	0.22
RT 19-20-21	0.067	32	6.9	11.19	0.35	7.4	1.0	7.0	5.93	0.19
"	0.1	27	10.0	8.54	0.32	8.0	0.7	9.7	6.46	0.24
"	0.15	24	9.7	13.88	0.58	7.4	1.0	9.8	9.20	0.38
RT 22	0.067	22	8.5	8.07	0.37	7.1	0.5	8.7	6.94	0.32
"	0.1	28	9.3	8.97	0.32	7.8	0.6	9.1	7.96	0.28
"	0.15	23	12.1	15.40	0.67	8.0	0.6	11.8	14.08	0.61
RT 23	0.067	23	10.1	9.26	0.40	7.0	1.0	10.6	5.22	0.23
"	0.1	24	10.5	12.52	0.52	7.2	0.6	10.7	10.68	0.44
"	0.15	21	13.3	9.71	0.46	7.9	0.7	13.0	6.94	0.33
RT 23 with Tween 80	0.02	47	8.0	30.34	0.65	7.1	1.7	8.7	16.83	0.36
"	0.03	49	10.8	27.17	0.55	7.6	1.3	10.7	18.42	0.38
"	0.045	49	11.2	24.43	0.50	7.4	1.2	11.3	16.76	0.34
Total/Means . .		444		16.45	0.69	7.5 (= $\bar{\bar{x}}$ )			11.29	0.48
<b>Vaccinated schoolchildren with previous test, Denmark</b>										
PPD-S	0.05	17	12.5	9.52	0.56	16.9	0.3	12.3	8.62	0.51
"	0.1	20	14.8	16.06	0.80	16.9	0.7	14.3	7.68	0.38
"	0.2	21	16.8	18.25	0.87	17.4	0.6	16.0	12.50	0.60
RT 19-20-21	0.05	15	11.5	18.83	1.26	14.7	0.6	12.3	13.16	0.88
"	0.1	15	13.5	13.55	0.90	15.1	0.6	14.2	5.21	0.35
"	0.2	21	16.5	10.06	0.48	16.5	0.3	16.4	9.70	0.46
RT 22	0.05	20	12.5	25.11	1.26	14.8	0.6	13.3	11.85	0.59
"	0.1	16	14.6	10.40	0.65	16.2	0.5	14.5	9.28	0.58
"	0.2	16	17.0	24.67	1.54	16.2	0.8	16.9	10.89	0.68
RT 23	0.05	15	11.7	20.07	1.34	15.4	0.5	12.1	14.28	0.95
"	0.1	17	16.9	19.06	1.12	16.8	0.8	16.5	5.07	0.30
"	0.2	16	18.7	8.89	0.56	16.8	0.9	18.2	3.76	0.24
RT 23 with Tween 80	0.01	35	11.7	45.47	1.30	16.5	0.8	11.3	30.74	0.88
"	0.02	29	15.0	16.31	0.56	16.3	0.7	14.9	10.40	0.36
"	0.04	33	15.2	26.45	0.80	15.4	1.0	16.0	10.11	0.31
Total/Means . .		306		20.78	1.22	16.2 (= $\bar{\bar{x}}$ )			12.13	0.71

TABLE 4. B. Other Populations (continued)

Products and dilutions ( $\mu\text{g}/0.1 \text{ ml}$ )		Number of persons tested (n)	Unadjusted figures			Weighted means for children with and without previous test*
			Mean size of reactions ( $\bar{y}$ )	Mean square of deviations ( $s^2$ )	$\frac{s_1^2}{n}$	
		<b>Vaccinated schoolchildren without previous test, Denmark</b>				<b>All vaccinated schoolchildren, Denmark</b>
PPD-S	0.05	17	9.5	15.64	0.92	11.3
"	0.1	17	11.9	24.06	1.42	13.4
"	0.2	18	13.9	20.53	1.14	15.2
RT 19-20-21	0.05	14	9.6	28.55	2.04	11.3
"	0.1	16	12.6	21.85	1.37	13.6
"	0.2	19	15.4	23.81	1.25	16.0
RT 22	0.05	19	12.0	13.89	0.73	12.8
"	0.1	18	12.6	18.14	1.01	13.8
"	0.2	16	15.4	32.79	2.05	16.3
RT 23	0.05	18	13.2	14.38	0.80	12.5
"	0.1	16	13.8	24.44	1.53	15.5
"	0.2	14	17.4	14.71	1.05	17.9
RT 23 with Tween 80	0.01	41	10.7	22.65	0.55	11.1
"	0.02	24	13.8	13.45	0.56	14.5
"	0.04	37	15.2	19.53	0.53	15.7
Total/Means		304		20.35	1.22	Variance of weighted means: 0.45

\* The weighted means in this frame are obtained from two previous sets of figures : adjusted mean values for vaccinated schoolchildren with previous test and unadjusted mean values for vaccinated schoolchildren without previous test. The inverse of the variances ( $1/0.71$  and  $1/1.22$  respectively) were used as weights. The variance corresponding to the weighted means is computed as  $\frac{1.22 \cdot 0.71}{1.22 + 0.71}$  (= 0.45).

TABLE 4. B. Other Populations (continued)

Products and dilutions ( $\mu\text{g}/0.1 \text{ ml}$ )	Number of persons tested	Unadjusted figures				Mean size of reactions to reference test	Regression of y on x	Adjusted figures		
		Mean size of reactions	Mean square of deviations	$\frac{s_1^2}{n}$	Mean size of reactions			Mean square of deviations from regression line	$\frac{s_2^2}{n}$	
	(n)	( $\bar{y}$ )	( $s_1^2$ )		( $\bar{x}$ )	(b)	( $\bar{y}-b(\bar{x}-\bar{x})$ )	( $s_2^2$ )		
<b>Non-vaccinated village population, Mauritius (Reactions to reference test : 0-3 mm)</b>										
PPD-S	0.1	28	0.5	1.44	0.05	0.0	—	(0.5)	(1.44)	(0.05)
"	0.2	25	1.2	7.08	0.28	0.3	2.0	1.0	4.68	0.19
RT 19-20-21	0.1	30	1.4	5.69	0.19	0.2	1.1	1.3	5.14	0.17
"	0.2	39	0.9	4.60	0.12	0.0	—	(0.9)	(4.60)	(0.12)
RT 23	0.1	21	2.5	8.36	0.40	0.4	2.2	2.0	5.57	0.27
"	0.2	27	3.7	17.54	0.65	0.3	1.8	3.5	16.27	0.60
RT 23 with Tween 80	0.03	69	1.0	3.94	0.06	0.3	0.1	1.0	3.99	0.06
"	0.06	54	0.6	2.89	0.05	0.1	1.5	0.7	2.56	0.05
Total/Means . .		293		5.59	0.21	0.2 (= $\bar{x}$ )			4.98	0.18
<b>Non-vaccinated village population, Mauritius (Reactions to reference test : 4-11 mm)</b>										
PPD-S	0.1	10	6.7	20.01	2.00	7.9	0.6	6.5	20.06	2.01
"	0.2	14	8.5	18.27	1.30	8.4	0.3	8.3	19.32	1.38
RT 19-20-21	0.1	16	9.0	15.73	0.98	8.3	1.5	7.9	3.80	0.24
"	0.2	10	9.6	28.27	2.83	7.2	2.0	10.4	16.69	1.67
RT 23	0.1	15	9.7	10.50	0.70	6.3	1.0	11.1	5.68	0.38
"	0.2	11	13.6	7.06	0.64	7.6	1.0	13.6	2.22	0.20
RT 23 with Tween 80	0.03	14	5.1	31.98	2.28	7.4	0.6	5.2	32.79	2.34
"	0.06	21	7.7	36.83	1.75	7.6	1.7	7.7	21.97	1.05
Total/Means . .		111		22.12	2.46	7.6 (= $\bar{x}$ )			15.62	1.94
<b>Non-vaccinated village population, Mauritius (Reactions to reference test : 12 mm or more)</b>										
PPD-S	0.1	20	15.7	17.91	0.90	16.4	1.0	15.8	12.25	0.61
"	0.2	18	19.4	19.78	1.10	16.3	1.2	19.6	8.32	0.46
RT 19-20-21	0.1	27	16.1	9.41	0.35	16.6	0.6	16.0	5.15	0.19
"	0.2	30	17.4	10.79	0.36	16.6	0.9	17.2	4.34	0.14
RT 23	0.1	17	17.8	10.81	0.64	17.3	0.6	17.3	7.76	0.46
"	0.2	23	19.0	13.18	0.57	15.4	0.9	20.0	7.02	0.31
RT 23 with Tween 80	0.03	33	16.8	12.27	0.37	16.3	0.9	17.0	7.53	0.23
"	0.06	48	18.6	14.25	0.30	16.7	0.8	18.4	8.91	0.19
Total/Means . .		216		13.26	0.66	16.5 (= $\bar{x}$ )			7.56	0.38

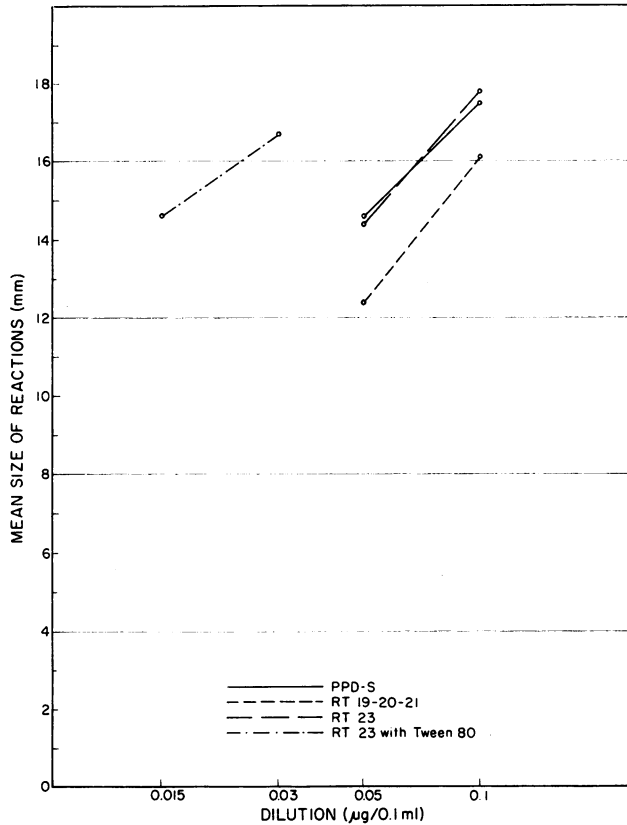
TABLE 4. B. Other Populations (concluded)

Products and dilutions ( $\mu\text{g}/0.1 \text{ ml}$ )	Number of persons tested (n)	Unadjusted figures			Mean size of reactions to reference test ( $\bar{x}$ )	Regression of y on x (b)	Adjusted figures			
		Mean size of reactions ( $\bar{y}$ )	Mean square of deviations ( $s_1^2$ )	$\frac{s_1^2}{n}$			Mean size of reactions ( $\bar{y}-b(\bar{x}-\bar{x})$ )	Mean square of deviations from regression line ( $s_2^2$ )	$\frac{s_2^2}{n}$	
<b>Non-vaccinated schoolchildren, Nigeria (Reactions to reference test : 0-3 mm)</b>										
PPD-S	0.1	15	2.1	2.78	0.19	1.4	0.9	2.1	1.63	0.11
"	0.2	20	2.6	5.50	0.28	1.3	0.8	2.8	4.74	0.24
RT 19-20-21	0.1	20	2.6	6.05	0.30	1.2	0.2	2.6	6.35	0.32
"	0.2	15	3.1	8.84	0.59	1.5	1.8	3.0	4.48	0.30
RT 23	0.1	21	4.3	7.93	0.38	1.5	1.4	4.3	5.28	0.25
"	0.2	15	6.7	29.21	1.95	1.6	3.0	6.4	16.69	1.11
RT 23 with Tween 80	0.03	30	1.8	3.11	0.10	1.6	0.5	1.8	2.81	0.09
"	0.06	37	3.0	12.19	0.33	1.6	1.6	2.8	8.87	0.24
Total/Means . .		173		8.96	0.54	1.5 (= $\bar{x}$ )			6.28	0.36
<b>Non-vaccinated schoolchildren, Nigeria (Reactions to reference test : 4-11 mm)</b>										
PPD-S	0.1	14	6.6	13.34	0.95	6.3	0.6	6.6	13.49	0.96
"	0.2	10	6.4	9.38	0.94	5.5	1.1	7.4	3.78	0.38
RT 19-20-21	0.1	14	7.6	6.11	0.44	6.5	1.0	7.4	1.58	0.11
"	0.2	9	10.0	11.50	1.28	6.1	1.5	10.4	10.42	1.16
RT 23	0.1	10	11.1	13.66	1.37	6.8	1.0	10.7	5.01	0.50
"	0.2	12	12.2	10.20	0.85	7.8	0.5	11.5	9.31	0.78
RT 23 with Tween 80	0.03	20	6.8	27.92	1.40	6.6	1.6	6.4	8.43	0.42
"	0.06	29	6.3	21.58	0.74	5.7	1.4	7.2	12.98	0.45
Total/Means . .		118		16.36	1.44	6.4 (= $\bar{x}$ )			9.01	0.74
<b>Non-vaccinated schoolchildren, Nigeria (Reactions to reference test : 12 mm or more)</b>										
PPD-S	0.1	15	19.3	9.77	0.65	18.7	0.8	18.9	3.02	0.20
"	0.2	12	20.9	17.17	1.43	19.6	1.4	19.0	4.11	0.34
RT 19-20-21	0.1	16	19.0	14.40	0.90	19.6	0.7	18.0	3.87	0.24
"	0.2	23	21.7	8.94	0.39	18.7	0.7	21.4	5.36	0.23
RT 23	0.1	19	19.9	7.76	0.41	17.7	0.8	20.3	4.45	0.23
"	0.2	15	21.8	6.46	0.43	18.6	0.5	21.6	5.53	0.37
RT 23 with Tween 80	0.03	33	18.8	11.08	0.34	17.0	0.9	19.9	3.43	0.10
"	0.06	24	20.2	9.15	0.38	17.5	0.9	20.9	4.58	0.19
Total/Means . .		157		10.29	0.71	18.2 (= $\bar{x}$ )			4.27	0.28

*Results in terms of reaction size*

The adjusted mean values given in Table 4 are shown graphically in Fig. 3-7. In each of these figures, the dilutions of tuberculin are plotted along the abscissa (logarithmic scale) and the adjusted mean sizes of reac-

**FIG. 3. MEAN SIZE OF REACTIONS \* TO 8 ASSAY DILUTIONS IN TUBERCULOUS PATIENTS IN THE NETHERLANDS**



\* Adjusted mean values; the standard error is 0.4 mm for each mean.

tions along the ordinate. The curves illustrate what may be called dose-response functions, and the graphical presentation makes it more easy to spot peculiar features and differences between the tuberculins.

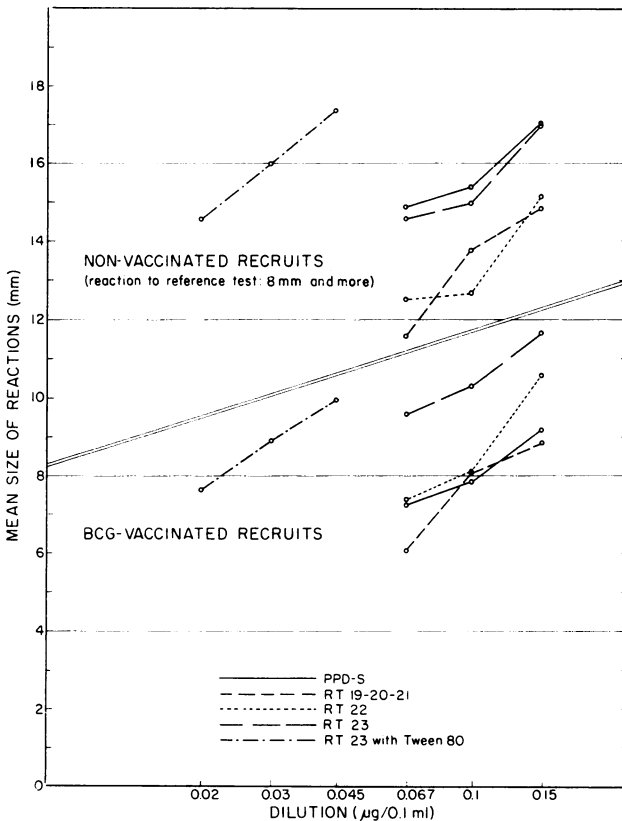
To judge from the graphs, the curves tend to be parallel in all instances (at least there is no systematic deviation from parallelism) and they also tend to be straight, that is, curvature is not very pronounced or always to the same side. To make certain, an analysis of variance has been made for



each set of means in Table 4 (using a logarithmic function of the strength of dilution, and attaching, as before, double weight to the mean sizes of reactions to RT 23 with Tween 80). In practically all instances the deviations from parallelism and from linearity were found to be non-significant. The only exception was the subgroup of children in Nigeria with reactions to the reference test of 12 mm or more; here the probability of deviation from parallelism was computed to be more than 97.5%. However, when so many sets of figures are tested at a time an extreme fractile of 97.5% cannot be considered truly significant.

Since there is no apparent non-parallelism or curvature, it is permissible to compute the potency ratios, with their confidence or fiducial limits, for each assay. This has been done and the results are given in Table 5. Of

**FIG. 4. MEAN SIZE OF REACTIONS \* TO 15 ASSAY DILUTIONS IN BCG-VACCINATED AND NON-VACCINATED RECRUITS IN THE NETHERLANDS**



\* Adjusted mean values; the standard error is, for dilutions without and with Tween 80, respectively, 0.5 mm and 0.3 mm for non-vaccinated recruits, and 0.5 mm and 0.4 mm for vaccinated recruits.

TABLE 5. POTENCY RATIOS AND 95% FIDUCIAL LIMITS FOR DIFFERENT POPULATIONS AND SUBGROUPS OF POPULATION

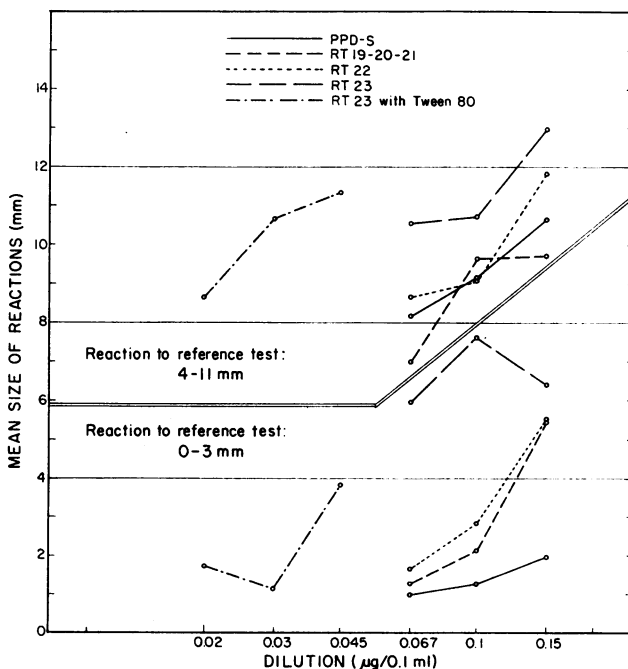
Population and subgroup of population	PPD-S = 1				RT 23 with Tween 80 = 1											
	RT 19-20-21		RT 22		RT 23		RT 23 with Tween 80		PPD-S		RT 19-20-21		RT 22		RT 23	
Tuberculous patients, Netherlands	0.66	0.53			1.02	0.84	1.24	3.65	0.41	0.27	0.22	0.18			0.34	0.28
Non-vaccinated recruits, Netherlands (Reaction to reference test: 8 mm or more)	0.48	0.36	0.62	0.50	0.64	0.37	1.15	4.30	0.35	0.17	0.14	0.10	0.18	0.11	0.26	0.21
Vaccinated recruits, Netherlands (Total)	0.88	0.66	1.14	1.22	1.62	0.94	3.18	5.34	0.30	0.21	0.27	0.16	0.37	0.23	0.53	0.42
Vaccinated recruits, Netherlands (Reaction to reference test: 0-3 mm)	1.70	1.04	3.45	1.95	4.25	1.20	29.00	7.54	0.23	0.13	0.66	0.25	0.80	0.29	1.48	0.82
Vaccinated recruits, Netherlands (Reaction to reference test: 4-11 mm)	0.83	0.57	1.17	1.16	1.69	0.83	2.93	5.91	0.24	0.17	0.20	0.14	0.37	0.20	0.44	0.33
Vaccinated schoolchildren, Denmark	1.10	0.78	1.55	1.37	1.95	0.98	2.73	7.90	0.17	0.13	0.19	0.14	0.32	0.23	0.32	0.24
Tropical populations (Reaction to reference test: 0-3 mm)	1.52	0.49	53.00		24.24			17.00	0.30	0.06	0.46	0.18			7.34	1.48
Tropical populations (Reaction to reference test: 4-11 mm)	2.33	1.21	11.00		6.76	2.79	121.00	5.10	0.36	0.20	0.84	0.46			2.45	1.00
Tropical populations (Reaction to reference test: 12 mm or more)	1.00	0.71	1.41		1.94	1.38	3.08	6.48	0.22	0.15	0.22	0.15			0.42	0.32

course, it is not in any way proven that the dose-response curves are parallel, and the computed potency ratios should be considered valid only within the range of doses actually used in the assays. The 95% fiducial limits have been computed according to Fieller's theorem (see Finney, 1952).

It is apparent from Fig. 3-7, as well as from Table 5, that the results in terms of potency ratio are not the same from assay to assay. This is particularly evident when one compares the results from BCG-vaccinated persons (Dutch recruits and Danish schoolchildren) with those from "naturally infected" persons (tuberculous patients and non-vaccinated recruits in the Netherlands). In the "naturally infected" subjects, PPD-S is of the same strength as RT 23 without Tween 80 and definitely stronger than RT 19-20-21 or RT 22; whereas in BCG-vaccinated subjects, PPD-S is definitely weaker than RT 23 without Tween 80 and not stronger than RT 19-20-21 or RT 22.

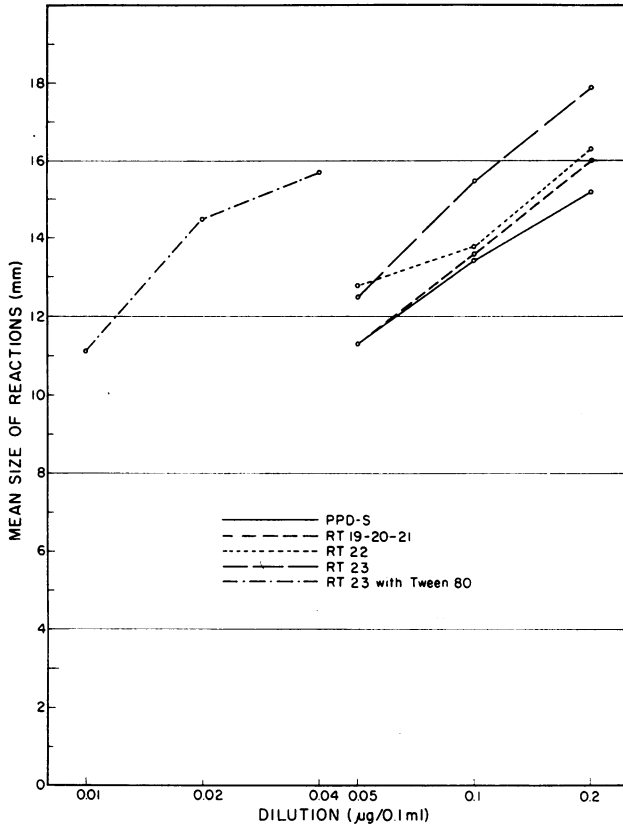
A similar pattern is found in the two tropical populations (Nigeria and Mauritius). Here RT 23 without Tween 80 and RT 19-20-21 are more

**FIG. 5. MEAN SIZE OF REACTIONS\* TO 15 ASSAY DILUTIONS IN BCG-VACCINATED RECRUITS IN THE NETHERLANDS, GROUPED ACCORDING TO SIZE OF REACTION TO THE REFERENCE TEST**



\* Adjusted mean values; the standard error is, for dilutions without and with Tween 80, respectively, 0.7 mm and 0.5 mm for persons with reference reactions of 4-11 mm, and 0.9 mm and 0.6 mm for persons with reference reactions of 0-3 mm.

**FIG. 6. MEAN SIZE OF REACTIONS \* TO 15 ASSAY DILUTIONS IN BCG-VACCINATED SCHOOLCHILDREN IN DENMARK**



\* Adjusted mean values; the standard error is 0.7 mm for dilutions without Tween 80 and 0.5 mm for dilutions with Tween 80.

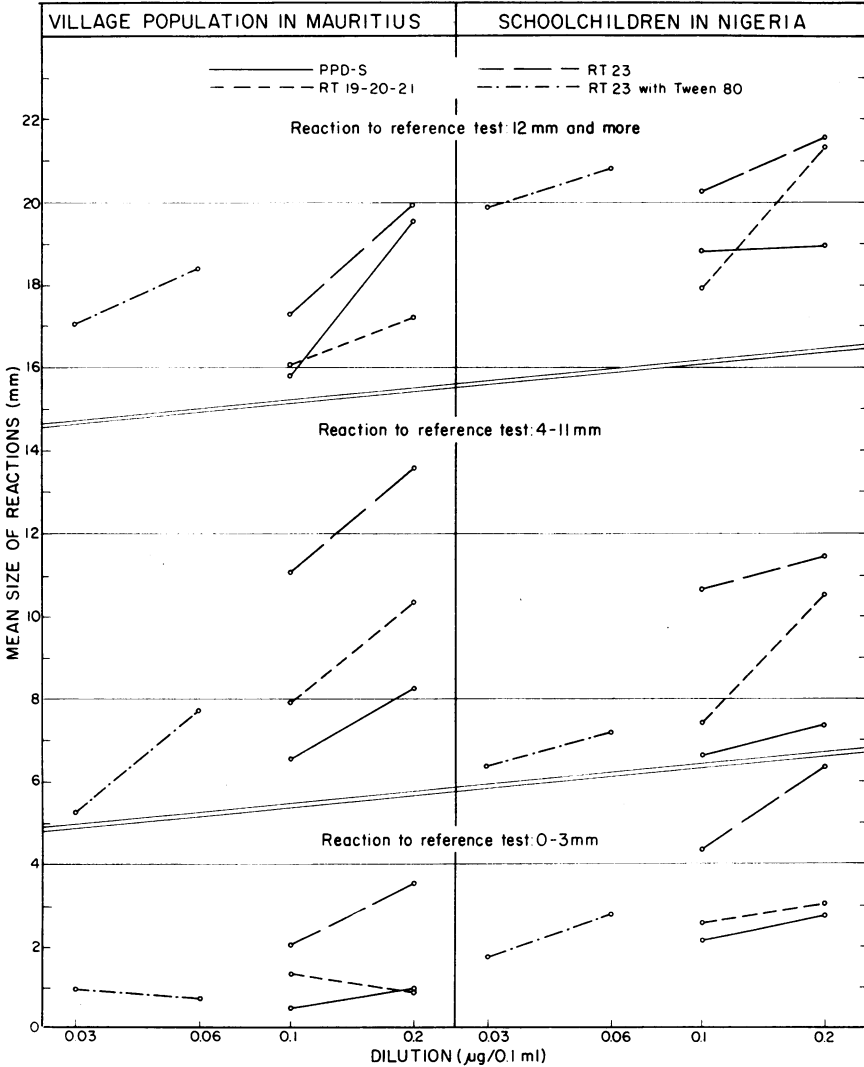
potent, relative to the International Standard, in persons with weak allergy (11 mm or less) than in persons with strong allergy (12 mm or more). It is particularly striking that RT 23 with Tween 80 clearly differs from RT 23 without Tween 80 in this respect: the preparation containing Tween is relatively more potent in persons with strong allergy.

The results in human populations differ from the results in guinea-pigs; for example, RT 23 without Tween 80 is weaker than PPD-S in BCG-vaccinated guinea-pigs, but stronger than PPD-S in BCG-vaccinated recruits and schoolchildren. (The interpretation is not quite unambiguous in this case because erythema rather than induration was recorded for the reactions in guinea-pigs.)

The results of the present standardization thus cannot be summarized

in terms of potency ratios, that is, it is not possible to state that a certain amount of a particular tuberculin equals another, given amount of the International Standard.

**FIG. 7. MEAN SIZE OF REACTIONS\* TO 8 ASSAY DILUTIONS FOR TWO TROPICAL POPULATIONS, GROUPED ACCORDING TO SIZE OF REACTION TO THE REFERENCE TEST**



\* Adjusted mean values; for dilutions without Tween 80 the standard errors are 0.6 mm, 1.4 mm, and 0.4 mm for persons in Mauritius with reference reactions of 12 mm or more, 4-11 mm, and 0-3 mm, respectively; the corresponding standard errors for Nigeria are 0.5 mm, 0.9 mm, and 0.6 mm; the standard errors for dilutions with Tween 80 are in all cases 0.7 times those given for dilutions without Tween 80.

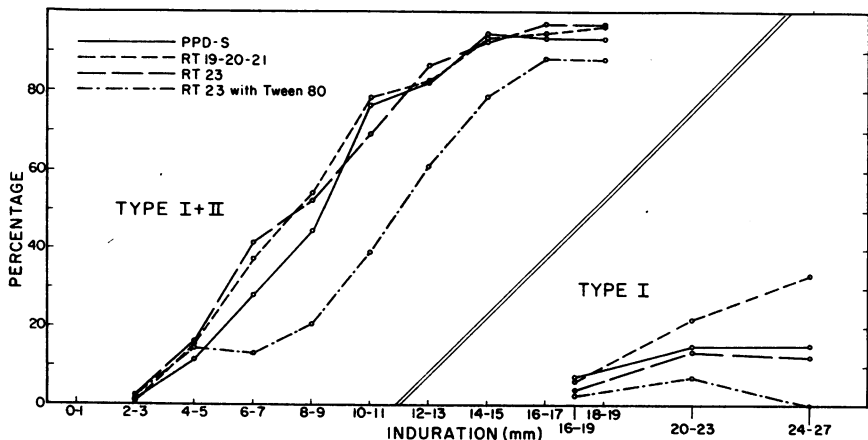
*Results in terms of reaction type*

In the assays in the Netherlands and Denmark each reaction, in addition to being measured, was classified according to degree of density. This classification, introduced by Palmer (see Palmer, Ferebee & Petersen, 1950), includes four categories or "types". Type I is a clearly demarcated, very firm infiltration and type IV a soft, ill-defined induration, while types II and III are intermediate categories.

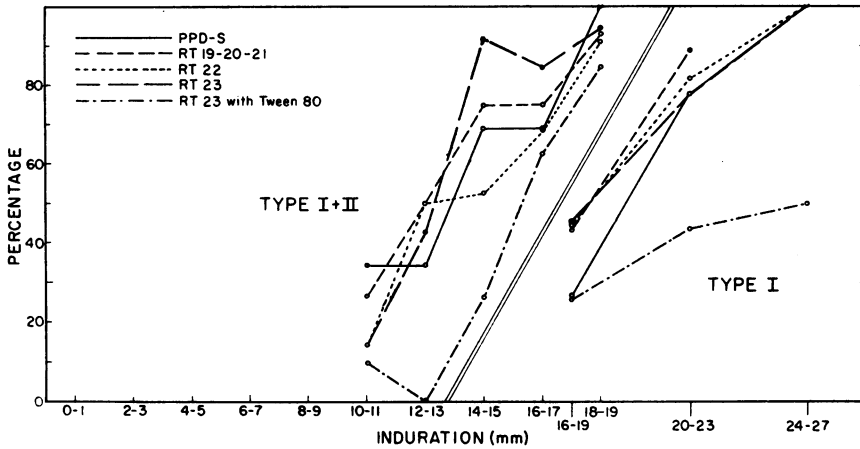
Detailed results of the classification by type are given in the appendix tables. One striking feature of the type distribution is illustrated in Fig. 8 and 9, which show the regression of type of reaction on size of reaction. In Fig. 8, which is based on the data from the Netherlands (those for tuberculous patients as well as those for vaccinated and non-vaccinated recruits), the distribution by type is shown for each reaction size; groups of 2 or 4 mm have been used, rather than 1-mm groups, to obtain reasonably smooth curves. Of the two sets of curves in the figure, the one to the right shows for each size category the proportion of reactions that has been classified as type I. A separate curve is given for each tuberculin product, the reactions to the different dilutions of each product having been combined. The other set of curves, to the left, shows in a similar way the percentage of reactions of type I and type II combined. For clearness of presentation, only the steepest part of the curves is shown. Fig. 9, which is arranged in the same way as Fig. 8, shows the regression of type of reaction on size of reaction for BCG-vaccinated schoolchildren in Denmark.

It is immediately seen from Fig. 8 and 9 that, for a certain reaction size, RT 23 with Tween 80 gives a larger proportion of soft reactions than the

**FIG. 8. RELATIVE FREQUENCIES OF FIRM AND SOFT REACTIONS, ACCORDING TO SIZE OF REACTION, FOR ALL ASSAY DILUTIONS IN RECRUITS AND TUBERCULOUS PATIENTS IN THE NETHERLANDS**



**FIG. 9. RELATIVE FREQUENCIES OF FIRM AND SOFT REACTIONS, ACCORDING TO SIZE OF REACTION, FOR ALL ASSAY DILUTIONS IN BCG-VACCINATED SCHOOLCHILDREN IN DENMARK**

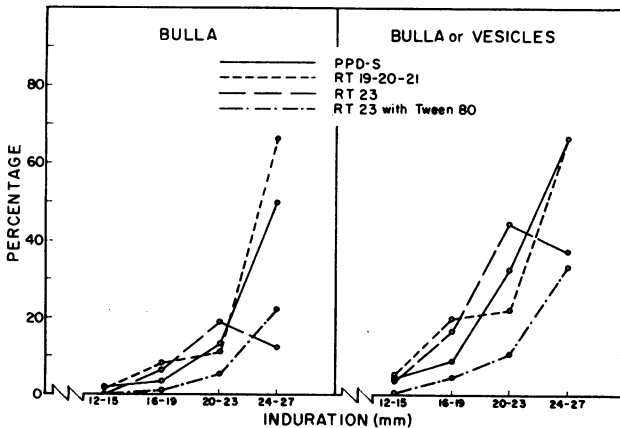


other tuberculin preparations. A potency ratio based on the type (degree of firmness) of the reaction would be different from the potency ratio based on the size of the reaction, as far as dilutions with or without Tween 80 are concerned.

#### *Frequency of bullous reactions*

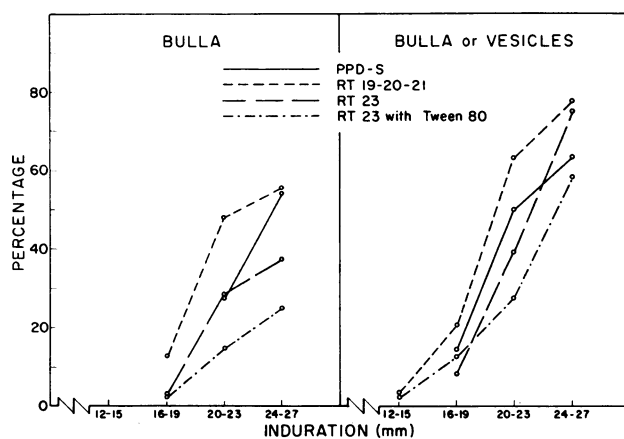
The frequency of bullous reactions and of vesicles is shown in Fig. 10 and 11, for each reaction size (4-mm groups have been used to obtain

**FIG. 10. RELATIVE FREQUENCIES OF BULLOUS REACTIONS, ACCORDING TO SIZE OF REACTION, FOR ALL ASSAY DILUTIONS IN NON-VACCINATED RECRUITS AND TUBERCULOUS PATIENTS IN THE NETHERLANDS**



smoother curves). Of the two sets of curves in each figure, that to the left shows the frequency of bullae only, that to the right the frequency of reactions with bullae and/or vesicles. Fig. 10 shows the results from tuberculous patients and from non-vaccinated recruits in the Netherlands, and Fig. 11 the results from the two tropical populations. Vaccinated persons are not included here because practically no bullous reactions were observed in this category.

**FIG. 11. RELATIVE FREQUENCIES OF BULLOUS REACTIONS, ACCORDING TO SIZE OF REACTION, FOR ALL ASSAY DILUTIONS IN TWO TROPICAL POPULATIONS**



Again, there are clearly qualitative differences between the products; RT 23 with Tween 80 produces fewer bullous reactions for a given reaction size than do the other products. This feature is most marked in the two populations in the Netherlands, but is also observed in the tropical populations.

### Discussion

To sum up, it seems almost impossible to express the potency of a purified tuberculin product as a fraction or multiple of that of another purified tuberculin: the ratio of potency for two products varies with the population in which the comparison is made and with the aspect of the tuberculin reaction utilized for the comparison. Thus, the potency ratio between the RT products and PPD-S is not the same in BCG-vaccinated and in non-vaccinated persons, the potency ratio for RT 23 and PPD-S is not the same in BCG-vaccinated human subjects and in BCG-vaccinated



guinea-pigs, and even the stabilizing effect of the addition of Tween 80 to the dilution is not merely quantitative: the potency ratio between RT 23 with Tween 80 and RT 23 without Tween 80 differs according to whether the size of the reaction or the density of the reaction is used to estimate the potency; this potency ratio also varies with the population tested, perhaps with the source or with the strength of allergy.

The existence of such "qualitative" differences between tuberculin products is not a new discovery. Jensen et al. (1938) have described the different appearance of reactions to Old Tuberculin (OT) and to PPD prepared according to the original method of Florence Seibert, and have pointed out that purified tuberculin cannot really be standardized in terms of the International Standard for Old Tuberculin, that at best it can be stated that certain doses of the two products may be considered equipotent for practical purposes under certain conditions. These authors, and later Holm & Lind (1947), therefore suggested that an international standard for PPD should be established in addition to that held for OT.

A large batch of purified tuberculin, intended as standard and designated PPD-S, was produced by Seibert and her co-workers (Seibert & Glenn, 1941). A part of this batch was in 1952 officially declared the International Standard for the Purified Protein Derivative of Mammalian Tuberculin (WHO Expert Committee on Biological Standardization, 1952). However, PPD-S had been prepared by a new technique which in some respects differed from that used for the previous batches of PPD and for the Danish RT products; Seibert & Glenn (1941) had stated that it was about twice as strong as their old standard PPD. It was first demonstrated by Nissen Meyer (1952) that PPD-S is qualitatively different from RT 19-20-21: testing BCG-vaccinated and non-vaccinated children with a low level of allergy, he found RT 19-20-21 to be relatively stronger in the former group and PPD-S to be relatively stronger in the latter group. (The reader is referred to Nissen Meyer's paper for a general discussion of the comparison of tuberculin preparations that may be "qualitatively different".) His findings were confirmed in later investigations by the WHO Tuberculosis Research Office (1955b) in which PPD-S and RT 19-20-21 were found to be equipotent in BCG-vaccinated persons, but of different strengths (PPD-S being stronger) in tuberculin-sensitive, non-vaccinated persons. These results correspond closely to what was found in the present series of assays.

It seems regrettable that the same technique was not used for the preparation of these important tuberculin products.

One purpose of the present assays is to standardize RT 23 against PPD-S, that is, to determine the amount of RT 23 that corresponds in biological activity to one unit of the International Standard for PPD. The results show this to be impossible. Yet it is desirable to define a given amount of RT 23 as one unit, if only for the practical purpose of making it easier to remember doses and to record tuberculin tests. It is also desirable that one

unit of RT 23 be so defined that, even if it is not strictly speaking equipotent to one unit of PPD-S, it is at least of about the same strength for the most common practical purposes. Such a statement about equal strength for practical purposes was made by Madsen, Holm & Jensen (1942) when they suggested that one unit of OT should be defined as 1/100 mg of the International Standard for Old Tuberculin and one unit of PPD as 0.00002 mg of a reference PPD. These doses were considered roughly equivalent because, as previously reported by Jensen et al. (1938), they elicited the same percentage of positive reactions in non-tuberculous hospital patients. However, we consider it preferable to use the criterion of equal reaction size (regardless of type of reaction, etc.) for the establishment of "equal" doses of PPD-S and RT 23. The precision of a comparison of the frequencies of positive reactions depends on the discrimination between traumatic reactions and weak allergic reactions—a difficult procedure, which is strongly dependent on both the observer and the particular distribution of allergy in the population used for the testing. This has already been discussed by Holm (1934), who pointed out that the most precise results are obtained by comparing the reactions corresponding to the steepest part of the dose-response curve, that is, fairly large reactions rather than almost negative reactions. Another reason for using the reaction size as the criterion is that in recent years the tendency has been to measure distributions of allergy in terms of distributions of reactions by size, rather than in terms of percentages of reactors to various doses.

Furthermore, we think that since the tuberculins concerned have been prepared from human strains of the tubercle bacillus, and since the primary purpose of tuberculin testing is to measure the prevalence of specific tuberculous infection, the definition of the unitage of RT 23 should be based on testing in persons with allergy of presumably specific origin, rather than on testing in persons with BCG-induced allergy or low-grade allergy of presumably non-specific origin.

A much more difficult question is that of the diluent to be used when tuberculins are standardized. One unit of the standard is defined as a specified amount of the dry substance; this definition, however, does not specify which diluent should be used, which is unfortunate in view of the fact that the potency of tuberculin has been found to vary with the diluent. Thus Jensen et al. (1938), diluting tuberculin to the same concentration with physiological saline and with phosphate buffer, found the saline dilution to be less potent, possibly by as much as 25-50%, than the phosphate-buffer dilution and concluded that: "This undoubtedly is due to differences in the hydrogen ion concentration. . . , and in practice has to be taken into consideration in the preparation of tuberculin dilutions by the international standard with 1/100, 1/10 and 1 mg per 0.1 cc."<sup>1</sup> However, dilutions far

<sup>1</sup> The present authors have found, in experiments mostly unpublished, that adsorption of tuberculin is less pronounced in buffered diluent than in pure saline.

more potent than the phosphate-buffer dilution may be obtained by addition to the diluent of other stabilizing agents than buffer—for example, Tween 80. Thus the possibility of adding a stabilizing agent must also be considered in deciding on the composition of the diluent to be used for the International Standard. One might perhaps assert that in any standardization the dilutions of the standard and of the test tuberculin should always be prepared with the same diluent.

As regards the present standardization this would have to be based on the comparison of PPD-S and RT 23 in buffer diluent, since dilutions of PPD-S with Tween 80 were not included in the present assays. It is not known whether the effect of Tween 80 is absolutely the same for PPD-S and RT 23, but comparative testing in BCG-vaccinated guinea-pigs suggests that it is at least of the same order.<sup>1</sup>

Thus, according to the results obtained in naturally infected persons (tuberculous patients and non-vaccinated recruits in the Netherlands) with tuberculin diluted in buffer without Tween 80, one unit of RT 23 may be defined as 0.00002 mg of the dry powder—a round figure close to the obtained estimates. But this definition, which agrees with the terms in which the International Tuberculin Unit is defined, has nevertheless a serious drawback: dilutions of tuberculin for practical use will have to be labelled according to the amount of tuberculin used for the preparation, no account being taken of the type of diluent used, that is, of the biological strength of the dilution.

As seen in Table 5 the potency ratio of RT 23 with and without Tween 80 varies between 2 : 1 and 4 : 1 (except for persons with low-grade allergy in the tropics and BCG-vaccinated recruits with weak allergy) and a potency ratio of 3 : 1 might be accepted as an appropriate approximation for practical purposes. But to label two dilutions, one of which is three times stronger than the other, as containing the same number of tuberculin units would without doubt involve a sacrifice of the interests of the ordinary user of tuberculin. To him, the terms “unit” and “standardization” mean that he does not have to bother with the batch-to-batch variations of biological products, because a given number of units can always be expected to give the same response in the same kind of population. It is therefore regrettable that the definition of the International Tuberculin Unit does not include the diluent to be used—for example, in such terms as: “one unit is 0.000028 mg of the International Standard dissolved in 0.1 ml of diluent of a defined composition” and “*n* units is *n* times 0.000028 mg dissolved in 0.1 ml of such a diluent”.

The present assays were also designed to estimate the dose of RT 23 that would correspond to the doses formerly used in WHO-sponsored BCG campaigns. It should first be noted that one unit of RT 19-20-21 or RT 22 does not correspond in potency to one unit of the International

<sup>1</sup> See Fig. 4 in article on page 799 of this number of the *Bulletin*.

Standard, except in BCG-vaccinated persons. According to the results from tuberculous patients and non-vaccinated recruits, "5 TU" of RT 19-20-21 is approximately equipotent with 3 TU of the International Standard. RT 22 has been held to be about 50% stronger than RT 19-20-21 (0.000013 mg of RT 22 being defined as one unit), but according to the present results it would seem to be only 20%, if at all, stronger. Thus "5 TU" of RT 22 probably corresponds to 2 or 2.5 TU of the International Standard.

If 0.00002 mg of RT 23 is considered equipotent with one unit of the International Standard PPD (both tuberculin in buffer diluent), then 0.00006 mg of RT 23 in buffer diluent without Tween 80 or 0.00002 mg of RT 23 in buffer diluent with Tween 80 should correspond fairly well to 3 TU of PPD-S, to 5 TU of RT 19-20-21 and perhaps to 6 or 7 TU of RT 22; and we think that these doses of RT 23 may confidently be used instead of the "5 TU test" as used at present.

Dilutions of "second dose" strength, that is, dilutions containing up to 100 TU per 0.1 ml, were not tested in human subjects in the present investigation. Such strong doses are today used for research purposes more often than for routine testing, and it seems preferable for each research worker to find for himself the particular dose that will serve his purpose. Testing in guinea-pigs<sup>1</sup> indicates that the dose-response curve for tuberculin in dilutions with Tween 80 is not steeper than the curve for tuberculin in dilutions without this detergent. It is thus very likely that Tween-tuberculin also may be given in a second dose 10 or even 20 times larger than the first dose without fear of too many unpleasant reactions.

Much information is still needed about the properties of RT 23, especially when used in dilutions stabilized with Tween 80. Thus, the assay results obtained in the tropics in persons with low-grade allergy are very striking and definitely call for further studies; in such persons the addition of Tween 80 to the diluent has no effect whatsoever. The most tempting explanation is perhaps that of "non-specificity". There is reason to believe that low-grade allergy is caused, not by infection with tubercle bacilli of human type, but by some other antigenically related organism. There is also reason to believe that PPD is composed of at least two fractions of different adsorbability,<sup>2</sup> one that has a low adsorbability and is found even in ordinary dilutions of PPD and one that is highly adsorbable and is therefore found mainly or exclusively in stabilized dilutions of PPD. A possible explanation of the present findings is that these fractions differ also with regard to specificity; thus it might be assumed that the highly adsorbable fraction is the more specific one, in the sense that it does not elicit reactions in persons whose allergy is of non-specific origin.

However, the possibility of an entirely different interpretation must be considered. It has been demonstrated that the reactions elicited with

<sup>1</sup> See Fig. 4 in article on page 783 of this number of the *Bulletin*.

<sup>2</sup> See article on page 783 of this number of the *Bulletin*.

tuberculin in Tween 80 diluent are larger but not appreciably firmer or more elevated than those obtained with tuberculin in ordinary diluent. Presumably a difference in size is easily recognized in the case of fairly large and firm reactions, even if it is not accompanied by a difference in firmness. However, the same may not be true for very weak reactions: differences in size between small, soft reactions may not be recognizable if they are not associated with differences in firmness. The interpretation that Tween 80 has no perceptible effect on weak reactions in general, rather than on non-specific reactions only, is perhaps confirmed by the findings in BCG-vaccinated persons: in vaccinated recruits with very weak allergy—as in persons in the tropics with low-grade allergy—there is no appreciable difference between the reactions to RT 23 with Tween and without Tween (see Table 5). The observation, made in several population groups, that reactions to Tween-tuberculin when distributed by size show a relatively larger dispersion than the reactions to ordinary tuberculin points in the same direction (see Table 4). It is not easy to explain the “enhancing” effect of Tween 80, though. It might be an effect *in vivo* of Tween on the development of the reaction, but this hypothesis is not consistent with the finding that Tween 80 had no *in vivo* effect on the size of the reaction in guinea-pigs in experiments designed to investigate this very point.<sup>1</sup>

The wide scope of the present investigations has been justified by the character of the results: if the standardization had been carried out in only one kind of population the results would have been of very limited applicability. It should be noted, in particular, that the results of a standardization in guinea-pigs cannot be expected to be applicable to human subjects.

A problem that requires increasing attention when a thorough standardization is made of each new batch of tuberculin is that of how to acquaint the user with those batch-to-batch differences that cannot be eliminated by adjusting the strength of dilution. With each new batch, new instructions concerning dosage, etc., may have to be issued. The best solution to this problem is no doubt to prepare a batch of tuberculin large enough to cover the global demand for tuberculin for a generation or two. The new batch—RT 23—corresponds to a total of 33 000 000 000 doses of 0.00002 mg each. Even assuming that as much as two-thirds of it will be lost in handling in the field or used for stronger tests, and even assuming that it will be used in all countries of the world (after all, in no country will the entire population be tested every year), the new batch should suffice for quite a number of years and would thus seem to go a long way towards solving the above-mentioned problem.

<sup>1</sup> See Table 6 in article on page 799 of this number of the *Bulletin*.

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## RÉSUMÉ

Un nouveau lot de tuberculine (RT 23), préparé par le Statens Seruminstitut à la demande du FISE, a été comparé avec de précédents lots de tuberculine et avec l'Étalon international de Dérivé protéinique purifié de Tuberculine de Mammifères. Pour ces essais, RT 23 a été dilué dans du soluté salin avec tampon phosphate (comme les autres préparations) et dans ce même diluant additionné de Tween 80 (0,05 ‰) comme agent stabilisateur. Les réactions à la tuberculine inoculée par voie intradermique ont été comparées.

Des essais antérieurs avaient montré que des tuberculines qui paraissaient de même activité lorsqu'elles étaient appliquées à un certain groupe de population, présentaient des différences lors des tests sur d'autres collectivités. C'est pourquoi les études ont porté sur divers groupes de populations, de divers types et niveaux d'allergie, de sorte que les résultats reposent sur des bases assez larges. Ces groupes sont les suivants: des sujets atteints de tuberculose, aux Pays-Bas; des recrues des Pays-Bas, vaccinées au BCG ou naturellement sensibles à la tuberculine; des écoliers danois vaccinés au BCG; des collectivités de régions tropicales — île Maurice et Nigeria — parmi lesquelles une forte proportion de personnes faiblement sensibles à la tuberculine. A titre de comparaison, on a procédé à des essais sur des cobayes sensibilisés de diverses façons.

Les comparaisons ont été faites selon une technique comportant l'essai de deux ou trois doses différentes pour chaque tuberculine. Dans la plupart des cas, les sujets humains ont été soumis chacun à deux tests: l'un de référence toujours le même, l'autre avec l'une des tuberculines à l'étude. Les résultats du test de référence ont servi de critère de classement des populations en groupes correspondant à des niveaux différents de sensibilité à la tuberculine, sur lesquels les résultats des essais ont été étudiés séparément.

Les résultats variaient de façon significative selon le type et le niveau de sensibilité des groupes testés. Ainsi, le rapport entre l'activité de RT 23 et celle de l'Étalon international est d'environ 2:1 chez les personnes vaccinées au BCG; il est de 1:1 chez les personnes présentant une sensibilité à la tuberculine naturellement acquise. La tuberculine RT 23, dans un diluant contenant du Tween 80, provoque des réactions plus fortes que si le diluant ne contient pas de Tween 80. A dimensions égales, les réactions provoquées par la tuberculine avec Tween 80 sont plus souples et moins souvent bulleuses. L'accentuation de la réaction due au Tween 80 ne se manifeste que chez les sujets fortement ou assez fortement allergiques, mais n'apparaît pas chez les sujets peu sensibles. Les résultats ne sont pas les mêmes, s'il s'agit d'hommes ou de cobayes. C'est ainsi que le RT 23 sans Tween 80 provoque des réactions plus faibles que l'Étalon international chez les cobayes vaccinés au BCG, mais plus fortes que ce même Étalon chez les écoliers et les recrues.

Sur la base de ces résultats — exprimés en fonction des dimensions de la réaction — il est proposé que l'unité de RT 23 soit définie comme correspondant à 0,00002 mg de substance sèche. Cette définition, comme celle de l'Étalon international, ne tient pas compte du diluant. Pour l'usage courant là, où l'on utilisait «un test à 5 UT», il est proposé d'utiliser à l'avenir 0,00002 mg de RT 23 (1 UT) dans un diluant tampon contenant du Tween 80, ou 0,00006 mg (3 UT) dans un diluant tampon sans addition de stabilisateur.

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## APPENDIX TABLES

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The batch of PPD whose standardization is described in this paper is estimated to contain some 33 000 million single doses—in other words to be large enough to cover world needs for mass tuberculin-testing for ten years or more. It is proposed that this batch, which has been standardized against the batch of PPD used by the WHO Tuberculosis Research Office in many countries during the last few years as well as against the International Standard for PPD, be established as an international reference preparation. It is hoped that it will be accepted and used by all countries in the future, so that valid comparisons can be made of the results of studies and surveys and of the tuberculin testing carried out in mass case-finding and BCG-vaccination campaigns in different parts of the world. To stimulate world-wide acceptance of the new batch, details of its biological characteristics should be given as wide a circulation as possible. These details are therefore presented in the following appendix tables, which will enable interested workers in the various countries to relate the computations given in the text of the paper to the original data from which they are derived, to study the distributions of reactions in the type of population they are specially concerned with, and to compare the results of their own investigations on a particular population group with the findings in other groups.



**APPENDIX TABLE 1. SIZE AND CHARACTER OF REACTIONS TO TWO ASSAY TESTS IN EACH OF 316 TUBERCULOUS PATIENTS IN THE NETHERLANDS, GROUPED ACCORDING TO 28 COMBINATIONS OF 8 ASSAY TESTS**

Induration (mm)	Type of reaction	Pre-ence of bullae (B) and vesicles (V)	Induration (mm)	Type of reaction	Pre-ence of bullae (B) and vesicles (V)
Replication 1 Block 1 *					
0.03 µg/0.1 ml RT 23 with Tween 80			0.05 µg/0.1 ml RT 23		
1	0	0	1	0	0
9	III	0	9	II	0
12	II	0	13	II	0
12	II	0	11	II	0
12	III	0	12	II	0
14	II	0	14	II	0
16	II	0	14	II	0
16	II	0	15	II	0
17	IV	0	8	III	0
20	II	V	23	II	V
21	II	0	12	II	0
23	II	V	22	II	V
17	II	0	19	II	0
20	II	B	19	II	0
16	II	0	13	II	0
17	II	0	16	II	0
Mean:			Mean:		
15.2			13.8		

Replication 1 Block 3					
0.05 µg/0.1 ml PPD-S			0.015 µg/0.1 ml RT 23 with Tween 80		
23	I	V	17	II	V
4	III	0	4	III	0
19	II	0	17	II	0
13	II	0	14	III	0
21	II	V	13	II	0
11	II	0	17	II	0
6	IV	0	2	IV	0
14	II	0	11	II	0
14	II	0	17	III	0
17	II	0	19	II	0
Mean:			Mean:		
14.2			13.1		

Replication 1 Block 4					
0.1 µg/0.1 ml RT 19-20-21			0.05 µg/0.1 ml RT 19-20-21		
7	III	0	5	III	0
9	III	0	7	II	0
13	II	0	9	II	0
15	I	0	15	II	0
21	III	0	7	IV	0
25	II	0	17	II	0
15	II	0	14	II	0
Mean:			Mean:		
15.0			10.6		

Replication 1 Block 2					
0.1 µg/0.1 ml RT 23			0.1 µg/0.1 ml PPD-S		
19	II	0	23	II	0
16	II	0	17	II	0
18	II	0	19	II	0
22	I	B	25	I	V
12	III	0	13	III	0
18	II	0	13	II	0
12	II	V	20	II	V
16	II	0	16	II	0
14	II	0	25	II	0
19	III	0	21	II	0
20	II	0	19	II	0
25	II	B	24	II	B
26	I	V	22	I	0
20	II	0	12	II	0
16	II	0	13	II	0
14	II	V	13	II	0
15	II	0	15	II	B
Mean:			Mean:		
17.8			18.2		

Replication 2 Block 5					
0.03 µg/0.1 ml RT 23 with Tween 80			0.1 µg/0.1 ml RT 23		
19	II	0	28	II	V
19	I	0	20	II	0
22	I	V	21	I	B
24	II	0	23	I	V
14	III	0	19	III	0
15	II	0	19	II	0
16	II	0	18	II	0
17	II	0	22	—	B
24	II	0	20	II	0
19	II	0	22	II	0
19	II	0	18	II	B
16	II	0	23	II	0
15	II	0	20	II	0
14	II	0	17	II	0
Mean:			Mean:		
18.1			20.7		

\* Replication and block numbers refer to the statistical design (see page 860).

**APPENDIX TABLE 1. SIZE AND CHARACTER OF REACTIONS IN 316 TUBERCULOUS PATIENTS, NETHERLANDS (continued)**

Replication 2 Block 6					
0.05 µg/0.1 ml RT 23			0.1 µg/0.1 ml PPD-S		
18	II	0	19	II	0
5	III	0	6	II	0
20	II	0	23	II	0
6	II	0	11	III	0
13	III	0	11	IV	0
24	II	0	22	II	0
13	II	0	23	II	0
16	II	B	25	II	B
15	II	0	15	II	0
Mean:			Mean:		
14.4			17.2		

Replication 3 Block 9					
0.03 µg/0.1 ml RT 23 with Tween 80			0.1 µg/0.1 ml PPD-S		
21	I	0	18	I	0
17	II	0	14	II	0
17	II	0	17	II	V
16	II	0	17	II	0
16	II	0	12	II	0
19	II	0	20	II	0
19	II	0	22	III	0
20	II	0	24	I	B
22	II	0	21	II	V
8	II	0	12	II	0
14	II	0	15	II	0
15	III	0	22	II	0
16	II	0	24	II	B
17	III	0	18	III	0
18	II	V	23	II	V
Mean:			Mean:		
17.0			18.6		

Replication 2 Block 7					
0.05 µg/0.1 ml PPD-S			0.05 µg/0.1 ml RT 19-20-21		
23	II	V	16	II	V
10	II	0	12	II	0
13	II	0	14	II	0
19	II	0	11	II	0
10	II	0	8	II	0
13	II	0	9	III	0
11	II	0	13	II	0
Mean:			Mean:		
14.1			11.9		

Replication 3 Block 10					
0.05 µg/0.1 ml RT 23			0.1 µg/0.1 ml RT 23		
15	II	0	21	II	0
18	II	0	19	II	0
15	II	0	24	II	0
13	II	0	12	II	0
19	II	0	20	II	0
15	II	0	15	II	0
16	II	0	15	II	0
13	II	0	17	II	0
17	II	0	19	II	0
9	II	0	12	II	0
11	II	V	18	II	V
18	II	0	18	II	0
13	II	0	19	I	V
9	II	0	16	II	0
Mean:			Mean:		
14.4			17.5		

Replication 2 Block 8					
0.015 µg/0.1 ml RT 23 with Tween 80			0.1 µg/0.1 ml RT 19-20-21		
9	III	0	11	II	0
21	II	0	24	I	B
13	II	0	15	II	0
19	II	0	20	II	0
Mean:			Mean:		
15.5			17.5		

**APPENDIX TABLE 1. SIZE AND CHARACTER OF REACTIONS IN 316 TUBERCULOUS PATIENTS, NETHERLANDS (continued)**

Replication 3 Block 11					
0.05 µg/0.1 ml PPD-S			0.1 µg/0.1 ml RT 19-20-21		
20	II	0	19	II	0
15	II	V	20	II	V
11	II	0	18	II	V
15	II	0	19	II	V
15	II	V	18	II	0
12	II	B	28	—	B
17	II	0	20	II	0
12	II	0	12	II	0
Mean: 14.6			Mean: 19.2		

Replication 4 Block 14					
0.05 µg/0.1 ml RT 23			0.015 µg/0.1 ml RT 23 with Tween 80		
15	II	0	21	II	0
15	II	0	21	II	0
14	II	0	17	II	0
18	II	0	19	I	0
18	II	0	14	II	0
8	I	0	8	I	0
13	II	0	15	II	0
19	II	V	18	II	0
Mean: 15.0			Mean: 16.6		

Replication 3 Block 12					
0.015 µg/0.1 ml RT 23 with Tween 80			0.05 µg/0.1 ml RT 19-20-21		
20	II	0	18	II	0
19	II	0	15	II	0
20	II	0	19	II	0
15	III	0	8	II	0
3	IV	0	4	II	0
18	II	0	15	II	0
1	—	0	4	III	0
18	II	0	18	I	B
Mean: 14.2			Mean: 12.6		

Replication 4 Block 15					
0.1 µg/0.1 ml RT 23			0.1 µg/0.1 ml RT 19-20-21		
23	II	B	12	II	V
15	II	0	14	I	0
15	II	0	14	I	0
15	II	0	13	III	0
22	I	V	19	II	V
19	II	B	18	II	V
12	II	0	16	II	0
4	II	0	5	II	0
22	II	0	17	II	0
10	II	0	11	III	0
9	II	0	9	II	0
20	I	0	18	I	0
15	I	0	13	II	0
16	III	0	15	II	0
14	II	0	19	II	0
17	II	0	15	II	0
Mean: 15.5			Mean: 14.2		

Replication 4 Block 13					
0.03 µg/0.1 ml RT 23 with Tween 80			0.05 µg/0.1 ml PPD-S		
19	II	0	16	II	0
16	II	0	14	II	0
16	II	0	14	II	0
15	III	0	13	III	0
11	II	0	9	III	0
21	II	0	14	I	0
19	II	V	17	II	V
18	III	0	19	III	0
18	II	0	16	II	0
18	II	0	11	II	0
21	II	0	15	II	0
12	II	0	11	II	0
14	II	0	12	II	0
15	III	0	17	III	0
18	III	0	11	III	0
Mean: 16.7			Mean: 13.9		

Replication 4 Block 16					
0.1 µg/0.1 ml PPD-S			0.05 µg/0.1 ml RT 19-20-21		
17	I	0	15	I	0
19	II	0	14	II	0
14	III	0	9	II	0
19	II	0	17	II	0
25	II	0	15	II	0
16	II	0	11	II	0
17	I	0	14	II	0
20	II	0	18	II	0
Mean: 18.4			Mean: 14.1		

**APPENDIX TABLE 1. SIZE AND CHARACTER OF REACTIONS IN 316 TUBERCULOUS PATIENTS, NETHERLANDS (continued)**

Replication 5 Block 17					
0.03 µg/0.1 ml RT 23 with Tween 80			0.015 µg/0.1 ml RT 23 with Tween 80		
19	II	0	23	II	0
18	II	0	16	II	0
17	II	0	17	II	0
17	II	B	11	II	B
16	II	0	13	II	0
16	I	V	14	II	0
14	II	0	10	II	0
14	II	0	10	III	0
13	II	0	11	III	0
13	II	0	10	III	0
3	IV	0	3	II	0
21	II	0	18	II	0
17	II	0	16	II	0
19	II	0	17	II	0
19	II	0	23	II	0
20	II	0	18	II	0
Mean:			Mean:		
16.0			14.4		

Replication 5 Block 20					
0.1 µg/0.1 ml PPD-S			0.1 µg/0.1 ml RT 19-20-21		
12	II	0	9	II	0
21	III	0	20	III	0
20	I	0	14	I	0
12	II	0	12	II	0
8	II	0	8	III	0
13	II	0	12	II	0
12	III	0	8	III	0
12	III	0	13	II	0
Mean:			Mean:		
13.8			12.0		

Replication 5 Block 18					
0.05 µg/0.1 ml RT 23			0.05 µg/0.1 PPD-S		
10	II	0	14	III	0
14	II	0	13	II	0
9	II	0	10	II	0
13	II	0	13	II	0
18	II	B	18	II	0
12	III	0	10	II	0
10	II	0	14	II	0
14	II	0	9	II	0
22	II	V	18	II	V
18	II	0	29	II	0
Mean:			Mean:		
14.0			14.8		

Replication 6 Block 21					
0.03 µg/0.1 ml RT 23 with Tween 80			0.1 µg/0.1 ml RT 19-20-21		
22	II	0	18	II	0
22	II	0	20	II	0
27	II	B	25	II	B
11	IV	0	14	III	0
13	II	0	15	II	0
13	III	0	13	III	0
14	II	0	11	II	0
16	II	0	14	II	0
16	III	0	17	II	0
17	III	0	13	III	0
19	II	0	16	II	0
19	II	0	20	II	0
19	II	V	17	II	V
18	II	0	17	II	0
21	II	0	17	II	0
12	III	0	12	II	0
19	II	0	18	II	0
Mean:			Mean:		
17.5			16.3		

Replication 5 Block 19					
0.1 µg/0.1 ml RT 23			0.05 µg/0.1 ml RT 19-20-21		
18	II	0	14	II	0
24	II	0	14	II	0
20	II	V	18	II	V
17	II	0	11	II	0
14	III	0	12	III	0
19	II	0	9	III	0
19	II	0	10	II	0
22	II	0	13	II	0
15	II	0	9	II	0
17	II	0	9	II	0
14	II	0	10	III	0
18	II	0	19	II	0
20	II	0	12	II	0
13	I	0	12	II	0
17	II	0	13	II	0
16	II	0	14	II	0
19	I	0	22	I	0
Mean:			Mean:		
17.8			13.0		

Replication 6 Block 22					
0.05 µg/0.1 ml RT 23			0.05 µg/0.1 ml RT 19-20-21		
16	II	0	12	II	0
13	II	0	20	II	0
12	II	0	11	II	0
16	II	V	13	II	0
21	II	0	13	II	0
Mean:			Mean:		
15.6			13.8		

**APPENDIX TABLE 1. SIZE AND CHARACTER OF REACTIONS IN 316 TUBERCULOUS PATIENTS, NETHERLANDS (concluded)**

Replication 6 Block 23					
0.1 $\mu\text{g}/0.1$ ml RT 23			0.05 $\mu\text{g}/0.1$ ml PPD-S		
17		0	12		0
16		0	14		0
12		0	9		0
20		0	14		0
19		0	17		0
21		0	11		0
21		0	21		0
22		0	20		0
16		0	15		0
24		0	16		0
16		0	15		0
Mean:	18.5		Mean:	14.9	

Replication 7 Block 26					
0.05 $\mu\text{g}/0.1$ ml RT 23			0.1 $\mu\text{g}/0.1$ ml RT 19-20-21		
14		0	14		0
11		0	14		0
12		0	14		0
11		0	9		0
14		0	17		0
12		0	9		0
8		0	12		0
21		V	18		0
Mean:	12.9		Mean:	13.4	

Replication 6 Block 24					
0.1 $\mu\text{g}/0.1$ ml PPD-S			0.015 $\mu\text{g}/0.1$ ml RT 23 with Tween 80		
21	I	0	17		0
24		0	20		0
21		0	25		0
20		0	17		0
21		0	13		0
16		0	17		0
14		0	9		0
Mean:	19.6		Mean:	16.9	

Replication 7 Block 27					
0.1 $\mu\text{g}/0.1$ ml RT 23			0.015 $\mu\text{g}/0.1$ ml RT 23 with Tween 80		
24		0	17		V
21		B	14		0
22		0	12		0
13		0	10		0
17		0	13		0
16		0	15		0
11		0	4		0
18		0	15		0
11		0	12		0
14		0	15		0
19		V	14		0
17		0	13		0
15		0	10		0
18		0	20		0
14		0	21		0
20		0	19		0
Mean:	16.9		Mean:	14.0	

Replication 7 Block 25					
0.03 $\mu\text{g}/0.1$ ml RT 23 with Tween 80			0.05 $\mu\text{g}/0.1$ ml RT 19-20-21		
20		0	11		0
22		B	9		0
12		0	7		0
13		0	12		0
13		0	11		0
15		0	8		0
16		0	9		V
17		0	15		0
18		0	10		0
19		0	10		0
20		0	14		0
18		0	14		0
23		0	17		0
26		0	15		0
10		0	10		0
14		0	11		0
15		0	8		0
Mean:	17.1		Mean:	11.2	

Replication 7 Block 28					
0.1 $\mu\text{g}/0.1$ ml PPD-S			0.05 $\mu\text{g}/0.1$ ml PPD-S		
15		0	15		0
20		0	18		0
18		0	18		0
11		0	10		0
17		0	18		0
17		0	18		0
15		0	13		0
19		0	20		0
Mean:	16.5		Mean:	16.2	













**APPENDIX TABLE 2. DISTRIBUTION OF 5047 NON-VACCINATED RECRUITS, NETHERLANDS (continued)**

		Reaction to the reference test 0.067 µg/0.1 ml of RT 22																						Total	Reactions to the assay test									
		Induration (mm)																							by type						with vesicle			
																									I	II	III	IV	with bulla					
Reaction to the assay test 0.15 µg/0.1 ml of RT 19-20-21		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Total	I	II	III	IV	with bulla	with vesicle			
0	102	8	12	4	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	127	—	—	—	—	—	—	—	—	
1	15	1	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	21	—	—	—	—	—	—	—	—	
2	13	1	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	17	—	—	—	—	—	—	—	—	
3	15	1	4	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	23	—	—	—	—	—	—	2	15	
4	4	3	2	5	3	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	18	—	—	—	—	—	—	1	4	18
5	3	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7	—	—	—	—	—	—	3	6	9
6	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	3	4
7	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	2	—	1
8	—	—	—	3	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6	—	—	—	—	—	—	1	2	—
9	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	5	—
10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	9	—	—	—	—	—	—	—	5	4
13	—	—	—	—	—	—	1	—	—	—	—	3	1	—	—	—	—	—	—	—	—	—	—	—	5	—	—	—	—	—	—	—	5	—
14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	1	2	—
15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—	—	—	—	—	—	2	—	—
16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8	—	—	—	—	—	—	—	6	2
17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6	—	—	—	—	—	—	—	6	—
18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	3	—
19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	3	—
20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	1	—
21	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—	—	—	—	—	—	—	1	—
Total	152	16	29	16	7	3	2	2	2	2	6	3	4	9	6	4	4	5	4	—	1	—	—	—	280	—	—	—	—	—	—	—	—	—















APPENDIX TABLE 2. DISTRIBUTION OF 547 NON-VACCINATED RECRUITS, NETHERLANDS (continued)

Reaction to the assay test Induration (mm)	Reaction to the reference test 0.067 µg/0.1 ml of RT 22 Induration (mm)																						Total	Reactions to the assay test					
																								by type				with bullae	with vesicle
																								I	II	III	IV		
0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22							
232	22	21	11	2				1					1										290						
1	25	3	2																				30						
2	32	7	2	5	1	1																	48						
3	24	2	7	6	2		1																42	45					
4	8	4	6	4	1	2						1											26	30					
5	4	4		2																			10	14					
6	3		3	2				1															9	8					
7					1				1														3	3					
8				1		1				1													4	4					
9						1		1															3	2					
10							1																3	1					
11								1		1													4	2					
12									1	1		2	1					1					4	4					
13									1	1	3	1											7	5					
14									1	2	4	2	2				1						14	2					
15										2	2		1	1									6	4					
16											3	4	3	3									13	5					
17										1	1	3		1	1	1							8	4					
18								1				5	1	2									11	8					
19										2	4	5		2									14	11					
20										2	1		1	1									5	10					
21												1			1	2							4	5					
22																1							1	4					
23								1															1	1					
24															1								1	1					
Total	328	42	41	30	6	4	4	4	5	4	11	17	22	18	5	6	5	3	1	1	1	1	557						











APPENDIX TABLE 3. DISTRIBUTION OF 810 BCG-VACCINATED RECRUITS, NETHERLANDS (continued)

Reaction to the assay test 0.067 µg/0.1 ml of RT 19-20-21 Induration (mm)	Reaction to the reference test 0.067 µg/0.1 ml of RT 22 Induration (mm)																			Total	Reactions to the assay test by type				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		19	I	II	III	IV
0	5	—	1	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
2	1	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	
3	—	1	—	3	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	
4	—	1	—	1	2	1	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1	4	3	
5	—	—	—	—	1	2	—	1	1	—	—	—	1	—	—	—	—	—	—	—	—	3	3	—	
6	—	—	—	—	—	—	1	2	1	—	—	—	—	—	—	—	—	—	—	—	1	1	2	—	
7	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	1	1	—	
8	—	—	—	—	—	—	1	1	—	1	1	—	—	—	—	—	—	—	—	—	—	2	2	—	
9	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1	—	
10	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
11	—	—	—	—	1	—	—	—	—	—	1	1	—	1	—	—	—	—	—	—	—	—	—	—	
12	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	4	1	
13	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1	—	
14	—	—	—	—	—	—	—	—	1	1	1	—	—	—	—	1	—	—	—	—	—	—	3	1	
15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1	—	
Total	6	2	2	8	4	5	3	4	5	3	3	1	1	2	1	1	1	1	—	—	57				



**APPENDIX TABLE 3. DISTRIBUTION OF 810 BCG-VACCINATED RECRUITS, NETHERLANDS (continued)**

Reaction to the assay test Induration (mm)	Reaction to the reference test 0.067 µg/0.1 ml of RT 22 Induration (mm)																			Total	Reactions to the assay test by type				
																					Total	I	II	III	IV
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18						
0	2	3	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6	—	—	—	—
1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
6	1	—	1	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7	—	—	—	—
7	—	—	—	—	1	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—
8	—	—	—	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—
9	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—
10	—	—	—	—	—	—	—	2	1	1	—	—	—	—	—	—	—	—	—	—	4	—	—	—	—
11	—	—	—	—	—	—	—	—	1	—	1	—	—	—	—	—	—	—	—	—	3	—	—	—	—
12	—	—	—	—	—	—	1	1	1	—	—	—	—	—	—	—	—	—	—	—	4	—	—	—	—
13	—	—	—	—	—	—	—	—	1	—	1	—	—	—	1	—	—	—	—	—	4	—	—	—	—
14	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—
15	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—
16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—
Total	4	4	2	3	2	2	3	4	6	2	3	5	1	—	1	2	—	—	—	1	45	—	—	—	—

APPENDIX TABLE 3. DISTRIBUTION OF 810 BCG-VACCINATED RECRUITS, NETHERLANDS (continued)

Reaction to the assay test Induration (mm)	Reaction to the reference test 0.067 µg/0.1 ml of RT 22 Induration (mm)																			Total	Reactions to the assay test by type				
																					I	II	III	IV	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18						19
0	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—
1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—
3	1	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—
4	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—
5	—	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—
6	—	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—
7	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—
8	—	—	—	—	1	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	4	—	—	—	—
9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	1	—	—	—	2	—	—	—	—	—	—	—	—	3	—	—	—	—
12	—	—	—	—	1	1	—	3	—	1	—	—	—	—	—	—	—	—	—	—	8	—	—	—	—
13	—	—	—	—	—	—	1	1	—	—	1	—	—	—	—	—	—	—	—	—	3	—	—	—	—
14	—	—	—	—	—	—	—	—	1	—	—	1	—	1	—	—	—	—	—	—	3	—	—	—	—
15	—	—	—	—	—	—	—	1	—	—	1	—	—	—	—	—	—	—	—	—	2	—	—	—	—
16	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	4	—	—	—	—
17	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1	—	—	—	—
Total	8	1	2	—	1	5	4	3	3	3	2	3	7	1	2	—	—	—	—	—	46	—	—	—	1





**APPENDIX TABLE 3. DISTRIBUTION OF 810 BCG-VACCINATED RECRUITS, NETHERLANDS (continued)**

Reaction to the assay test Induration (mm)	Reaction to the reference test 0.067 µg/0.1 ml of RT 22 Induration (mm)																			Total	Reactions to the assay test by type				
																					I	II	III	IV	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18						19
0	2	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—
1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	1	1
5	—	—	—	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	1	1
6	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1	—	—
7	—	—	—	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	2	—	1	1	—
8	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	2	—
9	—	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	2	1	—
10	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	—
11	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
12	—	—	1	—	—	—	1	—	2	—	—	—	—	—	—	—	—	—	—	—	5	—	4	1	—
13	—	—	—	1	—	1	—	—	2	—	—	—	—	—	—	—	—	—	—	—	4	—	4	—	—
14	—	—	1	—	1	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	4	—	2	1	1
15	—	—	—	—	—	—	—	2	1	—	—	2	—	—	1	—	—	—	—	—	6	—	6	—	—
16	—	—	—	1	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	3	—	2	1	—
17	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1	—	—
18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1	—	1	—	—
Total	3	—	4	7	4	1	1	2	2	4	8	1	—	4	—	—	1	—	—	—	42	—	—	—	—





APPENDIX TABLE 3. DISTRIBUTION OF 810 BCG-VACCINATED RECRUITS, NETHERLANDS (continued)

	Reaction to the reference test 0.067 µg/0.1 ml of RT 22 Induration (mm)																			Total	Reactions to the assay test																				
																					by type																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		19	I	II	III	IV																
Reaction to the assay test 0.15 µg/0.1 ml of RT 23 Induration (mm)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	2																				
	2																																								
	1																																								
	2	1																				2																			
	3																																								
	4	1	1																			2																			
	5																																								
	6		1	1																		2																			
	7		1																			1																			
	8			1																																					
	9							1														1																			
	10					1																1																			
	11					2	1															1																			
	12			1	1		2	1				1										3																			
	13	1		3			1	1														6																			
	14							1			3											6																			
	15									1												5																			
	16										1											1																			
	17											2	1									3																			
	18									1			1		1							3																			
	19										1											2																			
	20														1							2																			
																						1																			
Total	5	1	3	5	1	4	1	4	2	3	1	5	4	2	2	1	1	1	1	1	1	44																			



**APPENDIX TABLE 3. DISTRIBUTION OF 810 BCG-VACCINATED RECRUITS, NETHERLANDS (continued)**

	Reaction to the reference test 0.067 µg/0.1 ml of RT 22 Induration (mm)																			Total	Reactions to the assay test by type				
																					I	II	III	IV	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18						19
0	8	1	3	2	4	3	1	—	—	—	—	—	—	—	—	—	—	—	—	—	22	—	—	—	—
1	—	—	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—
3	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6	—	—	—	—
4	—	—	1	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6	—	—	—	—
5	—	—	—	—	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—
6	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
7	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—
8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—
9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7	—	—	—	—
13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6	—	—	—	—
14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—
15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—	—	—	—
16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	—	—	—	—
17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—
18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—
19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—
20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
21	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
Total	9	1	5	11	7	8	4	7	6	5	9	1	5	2	5	4	2	—	—	1	92	—	—	—	—





















































**APPENDIX TABLE 5. DISTRIBUTION OF 620 NON-VACCINATED VILLAGERS, MAURITIUS (continued)**

Reaction to the assay test 0.03 µg/0.1 ml of RT 23 with Tween 80 Induration (mm)	Reaction to the reference test 0.1 µg/0.1 ml of RT 19-20-21 Induration (mm)																								Total	Reactions to the assay test		
	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	with bulla	with vesicle
48	—	1	2	1	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	59	—	—
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	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	61	—	2	6	1	2	3	2	1	1	3	1	2	2	6	3	6	4	2	4	2	2	—	—	—	116	—	—



















**APPENDIX TABLE 6. DISTRIBUTION OF 448 NON-VACCINATED SCHOOLCHILDREN, NIGERIA (concluded)**

Reaction to the assay test		Reaction to the reference test 0.1 µg/0.1 ml of RT 19:20:21 Induration (mm)																								Total	Reactions to the assay test			
		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	with bulla	with vesicle	
0	10	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16	1	1	
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
2	2	7	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	14	1	1	
3	3	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	4	1	4	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	5	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	6	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	8	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
21	21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
22	22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
23	23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
24	24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
25	25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Total	Total	12	15	10	13	3	6	1	2	2	2	1	1	3	1	6	7	2	2	1	1	2	2	1	1	1	90			