EVALUATION OF THE SIZE OF MANTOUX TEST AFTER BCG VACCINATION ON INFANTS AND CHILDREN ATTENDING THE PAEDIATRIC DEMONSTRATION UNIT AT KENYATTA NATIONAL HOSPITAL, NAIROBI, AND THE MATERNAL AND CHILD HEALTH CLINIC AT THE COAST GENERAL HOSPITAL, MOMBASA

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by

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This dissertation is my original work and has not been presented for a degree at any other University.

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SUMMARY

This study has evaluated the Mantoux test in children who were successfully vaccinated with BCG at birth as indicated by the presence of a scar (1). Groups of children were evaluated at progressive intervals, 3, 6, 8, 12, 24, 36 and 48 months after immunisation. The purpose of the study was firstly to determine what size of positive reaction after Mantoux testing could be attributed solely to the immunisation and what size should be considered indicative of active infection and secondly to determine the appropriate age for booster BCG immunisation, taking into account the waning of the Mantoux reaction with time.

This study, using 1TU (one tuberculin unit) of PPD (purified protein derivative) has shown that 4 mm and 5 mm reactions were most characteristic of BCG allergy in the children studied and that reactions beyond 10 mm were extremely rare. This would suggest that Mantoux reactions more than 10 mm in size in children below the age of 4 years should be considered as highly suspicious of tuberculous infection.

There was a significant reversion of the Mantoux test reaction to negative (66%) by one year of age, there being a greater number of negative than positive results, suggesting that booster BCG should be given at this age in areas of high prevalence of tuberculosis such as Kenya.

INTRODUCTION - OBJECTIVES AND AIMS

"In Medicine one must pay attention not to plausible theorizing but to experience and reason together .... I agree that theorizing is to be approved, provided that it is based on facts, and systematically makes its deductions from what is observed ..... But conclusions drawn from unaided reason can hardly be serviceable; only those drawn from observed fact."

(Hippocrates: Precepts)

This study based on factual material is in accordance with the above view.

Mycobacterium tuberculosis infection is still a major problem in tropical paediatric practice and the determination of tuberculin sensitivity has always been a useful diagnostic procedure in young children (2).

The interpretation of the Mantoux test in non vaccinated children is well established. Grzybowski (3) using 5 tuberculin units (5TU) divides the reactions into positive, doubtful and negative according to the diameter of induration: the reactions are generally considered to be positive when they equal or exceed 5 mm, those

which show induration between 1 and 4 mm. in diameter are usually designated doubtful, and negative reactors are those showing no induration whatsoever. The committee (4) on diagnostic skin testing of the American Thoracic Society (using 5 TU of PPD) recommends the following interpretation:

1. 10 mm or more of induration = positive reaction. This is a positive tuberculin test and almost always reflects sensitivity resulting from infection with mycobacterium tuberculosis.

2. 5 mm through 9 mm of induration = doubtful reaction. This is a low grade reaction regarded as non-specific. The correct interpretation of a doubtful reaction is not easy and must vary from country to country (4). It is often aided by the injection of PPD (purified protein derivative) from tuberculoid bacilli (5). A reaction to tuberculoid PPD greater than to human PPD may generally be considered indicative of a non-tuberculous infection (5). In areas where infection with other mycobacteria is uncommon or in situations where the person being evaluated is at a high risk, such as a child who is a household contact of an elderly person, the interpretation of a reaction in the 5 mm to 9 mm range should be the same as for a 10 mm (or more) reaction, i. e., the judgement is that such a person is infected(6).

3. 0 mm through 4 mm of induration = Negative reaction. This reflects either a lack of tuberculin sensitivity, or a low-grade sensitivity which most likely is not due to mycobacterium tuberculosis infection. No repeat test is necessary unless there are other suggestive clinical signs of tuberculosis.

The interpretation of the Mantoux test in BCG vaccinated children is often difficult because of multiple factors (7). Aside from the individual biologic variations in reactivity, the diameter of the reaction is dependent on:

1. the source of the vaccine: Ashley (8) using two types of PPD 5TU (Connaught 5TU and 5TU supplied by the United States Public Health Service) showed that the results gave the impression that Connaught 5 TU, under the conditions used tended to give larger reactions than the corresponding product from the United States Public Health Service (8).

2. The method of inoculation: It has been said of the Mantoux test that it was the only means by which sensitiveness to tuberculin could be measured so accurately that two successive tests could be compared (9). The different strengths of the Mantoux test are the accepted standard by which all other tests are judged. It is the only test in which a measured dose of tuberculin is injected into the skin.

3. Viable counts in the vaccine: Ashley (8) and Siebenmann's study has shown a difference between the conversion rate following vaccines of different viable counts. That difference although not considered statistically significant created the impression that a low viable count of a BCG vaccine could result in a low tuberculin sensitivity.

4. The age at the time of vaccination: Joncas (7) considering two groups of children, one group vaccinated at birth and the second group vaccinated at 6 years showed that the percentage of conversion after vaccination varied from 83.7% in those vaccinated at birth, to 90% in those vaccinated at age 6 years. When tested 1 year after vaccination 58.1% of the infants vaccinated at birth had negative reactions, but of those vaccinated at age 6 years only 33.3% had negative reactions. Furthermore, the individual maximum diameter of the reaction 1 year after vaccination did not exceed 11 mm in those vaccinated at birth, whereas it reached 20 mm in some children vaccinated at age 6 years. The relative frequency of reversion to negative in infancy was concluded highly significant.

5. The time elapsed since vaccination (9) and the effect of supervening infection by atypical mycobacteria or a virulent mycobacterium tuberculosis (3) are considered important factors.

The results of Joncas's study (7) as that of Lifschitz's (11) clearly showed that when children, vaccinated with BCG at birth are skin tested with 5 TU of PPD at age one year or older, the reaction can be interpreted. If the diameter of induration is more than 10 to 12 mm, the reaction cannot be ascribed to BCG vaccination and is highly suggestive of supervening infection with mycobacterium tuberculosis or occasionally atypical mycobacteria. Also both studies noted a waning of the skin test reaction to tuberculin in the absence of supervening tuberculosis infection in children one year or more after BCG vaccination at birth.

Are children in our environment closely following these two observations so that a similar value can be given to the Mantoux test after a successful BCG vaccination at birth, this vaccination being common practice in most tropical areas with adequate medical coverage?

The objectives of this study were therefore: first to facilitate the interpretation of Mantoux test in children 4 years of age and below, who had a successful BCG vaccination at birth and second, to determine an appropriate age for a booster BCG vaccination. The need for a booster once the Mantoux test has reverted to negative is based on the work of Heimbeck (12) and Irvine (10) who concluded that allergy is an expression of immunity and that they seem to appear and disappear at more or less the same time.

MATERIALS AND METHODS

Under material and methods, the following items are considered: population, equipment, procedure and lastly reading of the Mantoux reaction.

1. Population

A total number of 296 children were Mantoux tested. These children were distributed into 7 groups according to the time elapsed after a successful BCG vaccination at birth as tabulated below.

Table I

Groups	Period elapsed after BCG vaccination in months	Number of individuals
I	3	30
II	6	37
III	8	62
IV	12	50
V	24	50
VI	36	34
VII	48	33

Each infant or child was assessed clinically and given a Mantoux test which was read at 48 hours, as it is known that pseudo reaction will have disappeared by 36 hours, that the maximum reaction is seen by 48 hours and that the reaction will start fading by 96 hours.

The Mantoux test was given only once and was not repeated or given to children with a previous history of Mantoux testing; repetitions of this test is known to cause a boosting effect on the size of subsequent reactions (13).

Infants and children were selected on the basis of:

1. a clinical assessment of good general conditions excluding from the study children with significant constitutional symptom of disease.

2. a good nutritional status as indicated principally by appropriate weight for age, using the modified Havard weight chart.

3. a negative history of tuberculosis contact, children with factors which are known to influence Mantoux test reactions; for example measles, whooping cough, chicken pox, lymphomas, sarcoidosis, adrenal corticosteroid therapy or immunnosuppressive drugs (14, 15, 16, 17) were excluded from the study.

2. Equipmen

1) Syringe: one milliliter glass tuberculin syringe, graduated in hundredths of a milliliter; the syringe was pretested for leakage and was sterilised by boiling in distilled water.

2) Needles: Neolus sterile disposable Terumo needles; non toxic, non pyrogenic "25G 5/8" - 0.50 x 16 mm. were used.

3) Tuberculin: Purified protein derivative (PPD) tuberculin, one tuberculin unit (1 TU) per 0.1 ml from the Medical Research Laboratory was used (see Appendix). One tuberculin unit strength was used because firstly it was the only one available and secondly it is the one in use in Kenyatta National Hospital and at the Coast General Hospital where the study was carried out. The PPD was stored in a refrigerator at 2^{o}C to 4^{o}C between the sessions and protected from light; the amount remaining in the syringe after each session was discarded.

4) Procedure: The Mantoux test, first described by Mantoux in 1908, is made by injecting one tenth of a milliliter of a standard dilution of tuberculin between the layers of the skin "intercuteneous injection" (19). It relies on the delayed hypersensitivity that develops in a person following mycobacterial infection and it is the most sensitive indicator of tuberculosis infection. The delayed

hypersensitivity reaction which is a cell mediated reaction or type IV in Gell and Coombs classification (20), is manifested, clinically, by an induration at the site of antigen injection in a sensitized person; such a person is a "reactor". A reactor is not synonymous with infection with mycobacterium tuberculosis. Infection with other mycobacteria, which are distributed worldwide, may result in cross-reaction. In general larger reactions with a given dose of antigen are more likely to be specific, i.e. due to infection with the mycobacterium from which the antigen was derived. The specific quantity (0.1 ml) is measured according to the marking on the barrel of the special tuberculin syringe. The technique used was that described by Irvine (10). The injection was made into the skin, on the front of the right forearm. The limb was gripped with the left hand and the skin where the injection was to be made drawn tight. After cleansing the skin with normal saline, the needle was introduced into the skin with the barrel upwards, so that the hole could be seen. As soon as the point was in the skin and before the hole was covered, the skin was gently lifted up by the needle, holding the syringe parallel with the skin, the needle was slipped in between the layers of the dermis until the hole was just out of sight. The injection was made close to where the left thumb was stretching the skin; as soon as the needle was properly in, the left thumb was shifted on to the base of the needle to grip it against the forearm. The plunger was then pressed down until one tenth of a milliliter had been injected, a circular wheal about 6 mm

in diamet ·, dimpled by hair follicles resulted. No dressing was required.

5) Reading: Readings were done 48 hours after the Mantoux test was given. The transverse and longitudinal diameters of the induration were measured and measuremen ts were made with a ruler calibrated in millimeters - Reactions were then classified using Edward's criteria (21), i. e., each palpable reaction was classified into one of four categories of density, designated as type I to IV. type I signified the most dense reaction, characteristically raised, and usually with sharply demarcated borders. Reactions of type IV were the least dense, more a palpable swelling than an actual induration. Type II and III are intermediate categories. Types I, II and III represented reactions with clearly recognizable induration. Reaction of type IV might easily escape notice unless the area was palpated with light touch. In cases where it was difficult to be sure that the induration was present, the elbow was first bent to a right angle to relax the skin and the finger was run lightly over the area, where a slight edge could be felt. If this failed, the technique was to pinch up a piece of skin away from the area and to feel the chickness of the fold; then to pinch up the area itself and see if it felt thicker; if still in doubt the reaction was regarded as negative (10).

RESULTS

The measurements of reaction to Mantoux using 1 TU of PPD after successful BCG vaccination during the neonatal period are presented in the following tables and discussed.

Each table deals with infants or children at the same interval of time from successful BCG at birth. The tables are numbered from two through eight and the intervals of time in months as follows: 3 months, 6 months, 8 months, 12 months, 24 months, 36 months, 48 months.

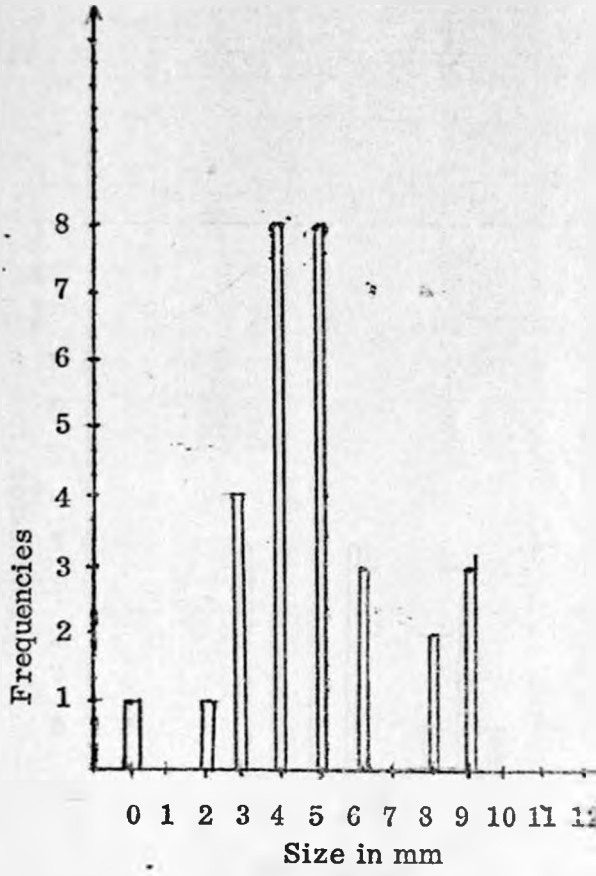
Table II.

Figure I Infants Mantoux tested 3 months after successful BCG vaccination (scar present) during the neonatal period.

Total number = 30

Frequency distribution of sizes of reactions.

Sizes (mm)	Frequencies
10	0
9	3
8	2
7	0
6	3
5	8
4	8
3	4
2	1
1	0
0	1



Comments:

5 mm and 4 mm sizes presented the highest frequencies.

No reaction above 9 mm was observed.

Reaction of 3 mm and below did not show a clear induration and were considered as type IV, i.e., as negative.

Reactions were then divided between positives and negatives.

Positive reactions comprising those of 4 mm and above numbered 24, (80%).

Negative reactions comprising those of 3 mm and below numbered 6, (20%).

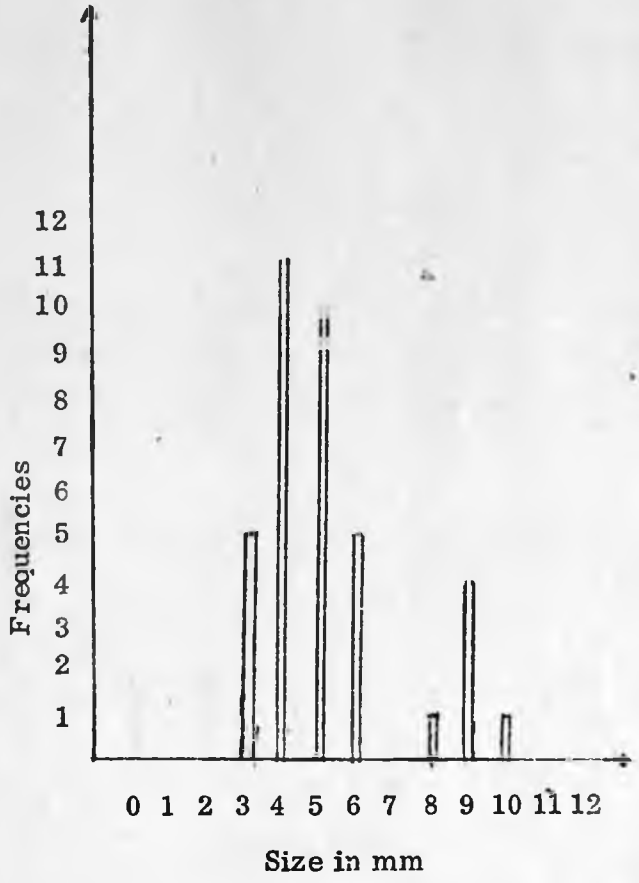
Table III

Figure II Infants Mantoux tested 6 months after successful BCG vaccination (scar present) during the neonatal period.

Total number = 37

Frequency distribution of sizes of reactions

Sizes (mm)	Frequencies
10	1
9	4
8	1
7	0
6	5
5	10
4	11
3	5
2	0
1	0
0	0



Comments:

5 mm and 4 mm sizes presented the highest frequencies.

One infant had a reaction of 10 mm.

Reactions of 3 mm and below did not show a clear induration and were considered as type IV, i.e. as negative.

Reactions were then divided between positives and negatives.

Positive reactions comprising those of 4 mm and above numbered 32, (86%).

Negative reactions comprising those of 3 mm and below numbered 5, (14%).

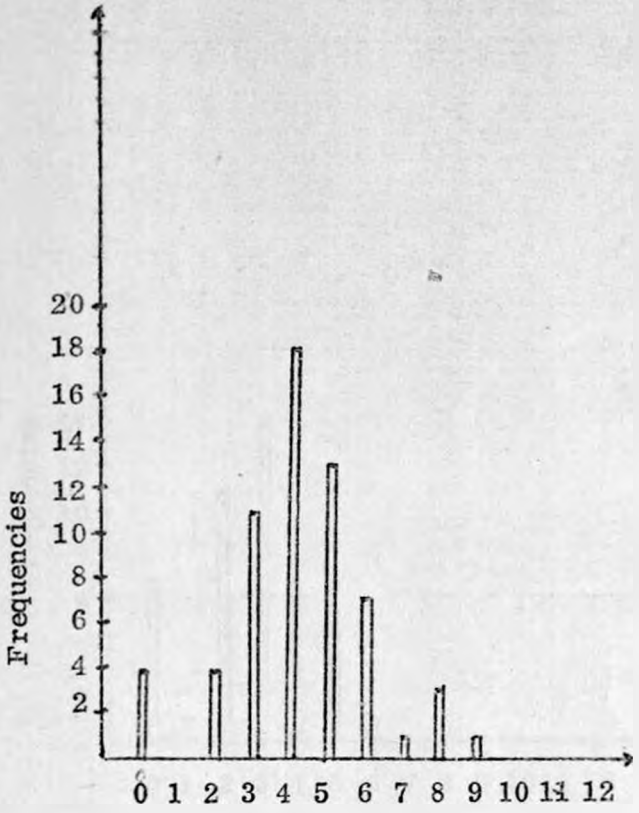
Table IV

Four I.. Infants Mantoux tested 8 months after successful BCG vaccination (scar present) during the neonatal period.

Total number = 62

Frequency distribution of sizes of reactions

Sizes (mm)	Frequencies
10	0
9	1
8	3
7	1
6	7
5	13
4	18
3	11
2	4
1	0
0	4



Comments:

5 mm and 4 mm sizes presented the highest frequencies.

Reactions of 3 mm and below did not show a clear induration and were considered as type IV, i. e. as negative.

Reactions were then divided between positives and negatives.

Positive reactions comprising those of 4 mm and above numbered 43, (70%).

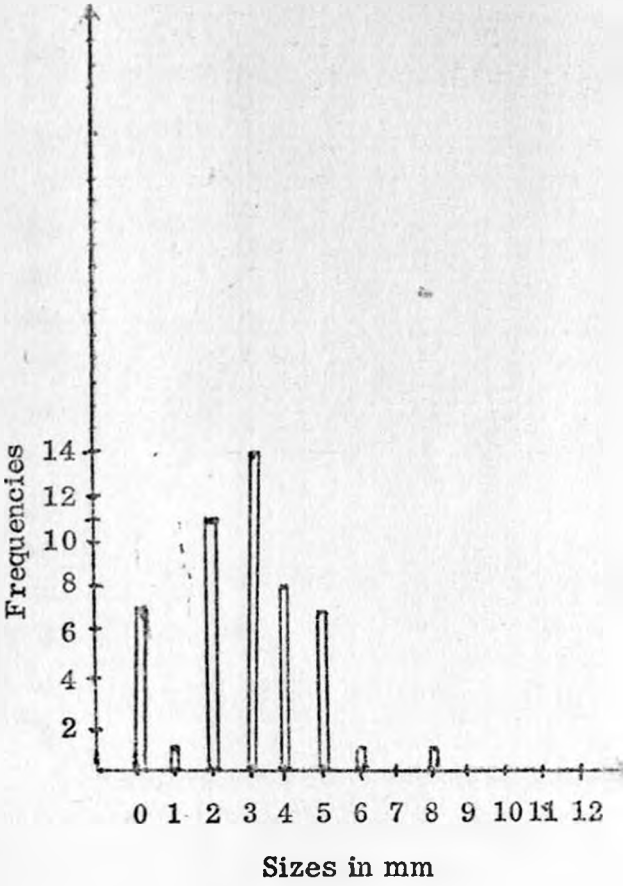
Negative reactions comprising those of 3 mm and below numbered 19, (30%).

Table V

Figure IV. Children Mantoux tested one year after successful BCG vaccination (scar present) during the neonatal period.

Total number = 50
Frequency distribution of sizes of reactions

Sizes (mm)	Frequencies
10	0
9	0
8	1
7	0
6	1
5	7
4	8
3	14
2	11
1	1
0	7



Comments:

There was a shift of the higher frequencies to the left, with a greater concentration of the 3 mm and 2 mm sizes than at earlier ages.

Reactions of 3 mm and below did not show a clear induration and were considered as type IV, i.e. as negative.

Reactions were then divided between positives and negatives.

Positive reactions comprising those of 4 mm and above numbered 17, (34%).

Negative reactions comprising those of 3 mm and below numbered 33, (66%).

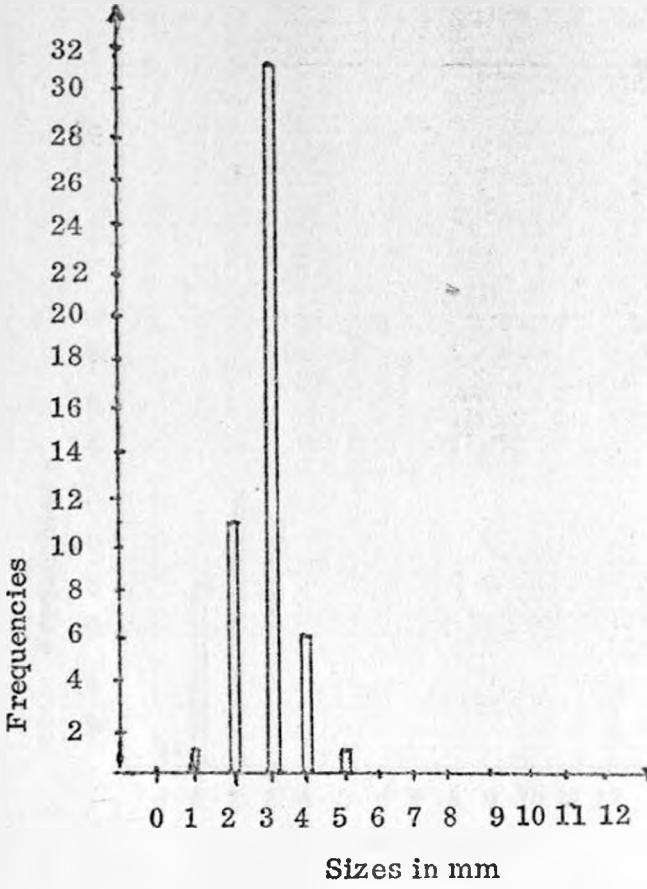
Table VI

Group V Children Mantoux tested 2 years after successful BCG vaccination (scar present) during the neonatal period.

Total number = 50

Frequency distribution of sizes of reactions.

Sizes (mm)	Frequencies
10	0
9	0
8	0
7	0
6	0
5	1
4	6
3	31
2	11
1	1
0	0



Comments:

There was a shift of the higher frequencies to the left, with a greater concentration of the 3 mm and 2 mm sizes than at earlier ages.

Reactions of 3 mm and below did not show a clear induration and were considered as type IV, i.e. as negative.

Reactions were then divided between positives and negatives.

Positive reactions comprising those of 4 mm and above numbered 7, (14%).

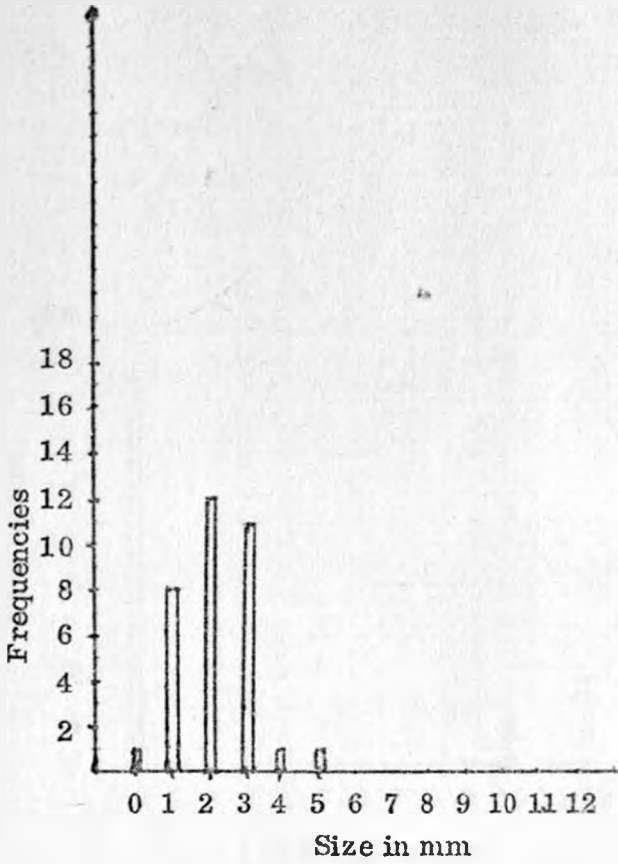
Negative reactions comprising those of 4 mm and below numbered 43, (86%).

Table VII

Figure VI Children Mantoux tested 3 years after successful BCG vaccination (scar present) during the neonatal period.

Total number = 34
Frequency distribution of sizes of reactions.

Sizes (mm)	Frequencies
10	0
9	0
8	0
7	0
6	0
5	1
4	1
3	11
2	12
1	8
0	1



Comments:

There was a shift of the higher frequencies to the left, with a greater concentration of the 1- 2- and 3 mm sizes than at earlier ages.

Reactions of 3 mm and below did not show a clear induration and were considered as type IV, i.e. as negative.

Reactions were then divided between positives and negatives.

Positive reactions comprising those of 4 mm and above, numbered 2, (6%).

Negative reactions comprising those of 3 mm and below numbered 32, (94%).

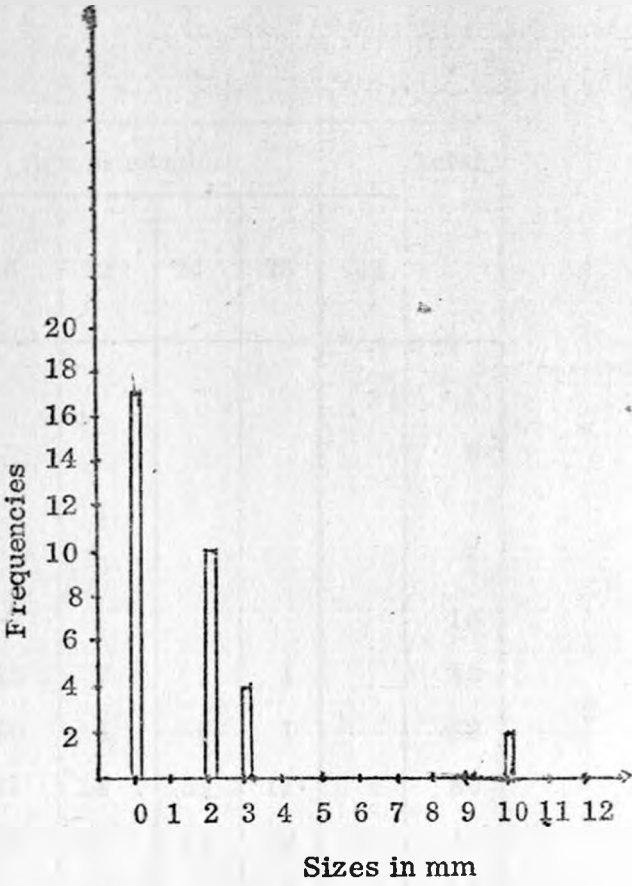
Table VIII

Figure VII Children : Mantoux tested 4 years after successful BCG vaccinatic (scar present) during the neonatal period.

Total number = 33

Frequency distribution of sizes of reactions

Sizes (mm)	Frequencies
10	2
9	0
8	0
7	0
6	0
5	0
4	0
3	4
2	10
1	0
0	17



Comments:

There was a shift of the higher frequencies to the left, with a greater concentration of 0 and 2 mm sizes than at earlier ages.

Reactions of 3 mm and below did not show a clear induration and were considered as type IV, i.e. as negative.

Reactions were then divided between positives and negatives.

Positive reactions comprising those of 4 mm and above numbered 2. 2 (6%).

Negative reaction comprising those of 3 mm and below numbered 31, (94%).

Statistical Analys s of the results by
Mr. Gemert - Statistician (Medical Research Centre)

Table IX.

Mantoux test sizes		Age in months							Total
Trans-formed scores	mm	3	6	8	12	24	36	48	
6.48	10		1					2	1
6.16	9	3	4	1					8
5.83	8	2	1	3	1				7
5.47	7	0	0	1	0				1
5.10	6	3	5	7	1				16
4.69	5	8	10	13	7	1	1		40
4.24	4	8	11	18	8	6	1		52
3.73	3	4	5	11	14	31	11	4	80
3.15	2	1	0	4	11	11	12	10	49
2.41	1	0	0	0	1	1	8	0	10
1.00	0	1	0	4	7	0	1	17	30
Number of children		30	37	62	50	50	34	31	294

Frequencies distribution of Mantoux test sizes at 3, 6, 8, 12, 24, 36, 48 months.

Table X

	Age in months						
	3	6	8	12	24	36	48
$\sum x$	147	193	260	146	145	74	32
$\bar{x}$	4.90	5.22	4.19	2.92	2.90	2.18	1.03
S_x^2	4.37	3.67	3.47	3.01	.50	1.06	1.43
S_x	2.09	1.92	1.86	1.74	.71	1.03	1.20
	on transformed scores						
$\sum x$	135.95	174.64	359.74	173.96	182.82	108.04	68.42
$\bar{x}$	4.52	4.72	4.19	3.48	3.66	3.18	2.05
S_x^2	1.04	.578	1.15	1.41	.16	.492	1.40
S_x	1.02	.76	1.07	1.19	.40	.70	1.19

Statistical comparison of the Mean Mantoux reaction sizes at different ages with testing being done on the transformed values, instead of x, $y = \sqrt{x} + \sqrt{x+1}$. The transformed scores ($y = \sqrt{x} + \sqrt{x+1}$) achieved the object to make the estimate of the variance independent of the mean. The results of the analysis were applicable to the observed mean. (Table X and page 22).

$\bar{x}_3 = 4.90 \quad \bar{x}_6 = 5.22$

$T = .64 \quad P > .50$

$\bar{x}_{3+6} = 5.07 \quad \bar{x}_8 = 4.19$

$T = 2.10 \quad P = .05$

$\bar{x}_8 = 4.19 \quad \bar{x}_{12} = 2.92$

$T = 3.10 \quad P < .01$

$\bar{x}_{12} = 2.92 \quad \bar{x}_{24} = 2.90$

$T = .73 \quad P > .50$

$\bar{x}_{12+24} = 2.91 \quad \bar{x}_{36} = 2.18$

$T = 1.63 \quad P = .10$

$\bar{x}_{36} = 2.18 \quad \bar{x}_{48} = 1.03$

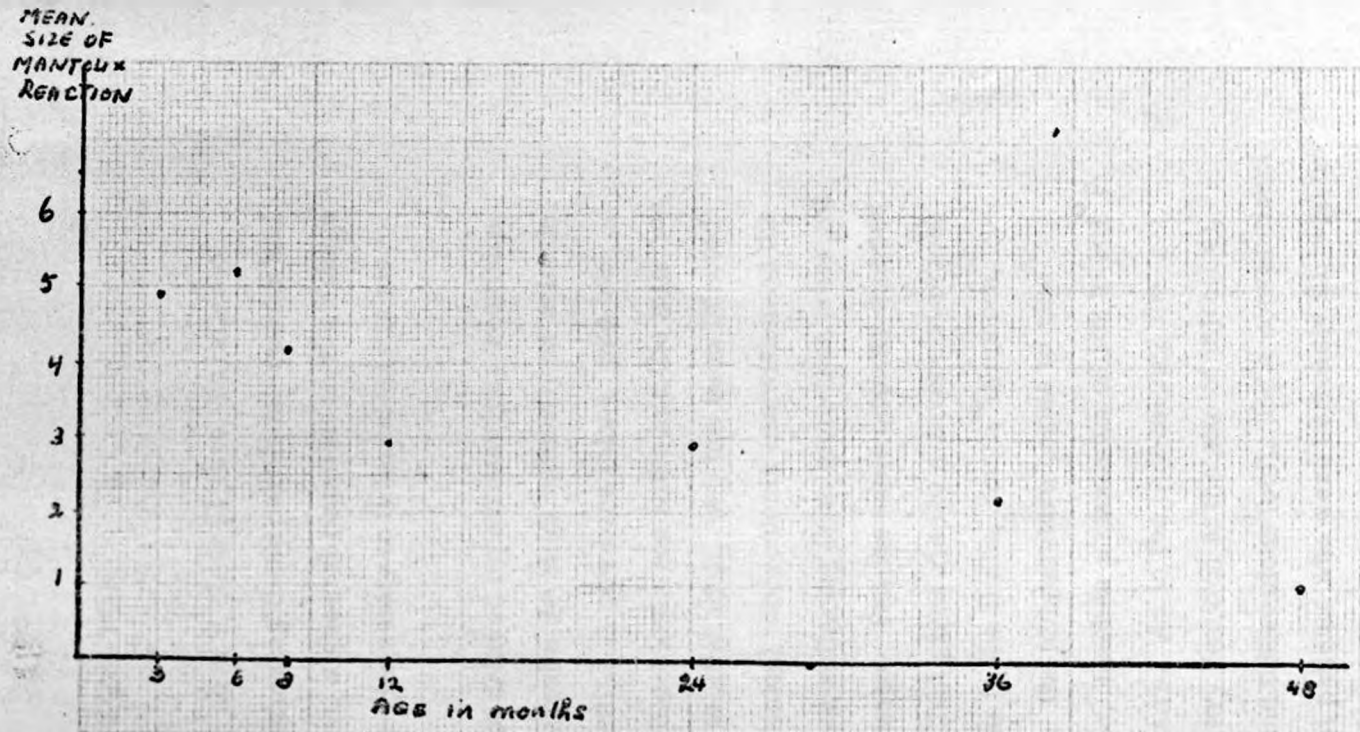
$T = 3.78 \quad P < .01$

The conclusions are:

. The difference between the age groups regarding the size of the Mantoux reaction leads to the following "groupings" of ages:

Ages in months	3 + 6	8	12 + 24	36	48
Means	5.17	4.2	2.9	2.2	1.0

. The means of the age groups were clearly different $P << .01$ (see graph).


MEAN SIZE OF MANTOUX REACTION
6
5
4
3
2
1
3
6
9
12
24
36
48
AGE in months

DISCUSSION

The results of this study show a clear picture, parallel to that of Joncas's (7). Before discussing these particular results and before coming to any conclusions, a general comment on the linkage between tuberculin reactivity and immunity to tuberculosis is given.

It is said that the state of delayed hypersensitivity requisite for eliciting the reaction to tuberculin develops naturally in animals infected with living tubercle bacilli. A new born infant, who has never come into contact with tuberculosis can withstand the injection of one ml of old tuberculin. A healthy adult, who has been infected but who is not suffering from clinical tuberculosis can withstand the injection of about 0.01 ml without suffering from more than transient malaise and slight pain in the limbs. But the same quantity injected into a clinically tuberculous patient gives rise to a severe reaction, characterised by malaise, pains in the limbs, cough, dyspnoea, rigor, vomiting and high fever; the temperature begins to rise about 4 hours after the injection, reaches a maximum of 102 to 104^{o}F and passes off after 12 to 15 hours. As in the Guinea-pig, the reaction is local (an inflammatory reaction at the site of injection), focal (an acute congestion around tuberculous foci), and a constitutional reaction (22). It was found that patients who were seriously ill, who had a short history or the pneumonic form of disease and who were apparently resisting badly, had a lower degree of sensitivity. In confirmation of the preceding observation, Woodruff (23) found that in pulmonary tuberculosis patients, the number of organisms in the sputum varied inversely with the degree of sensitivity to tuberculin. Turner (24) following up 191 treated cases of pulmonary tuberculosis

observed that relapse was commonest among those with the highest and those with the lowest degree of allergy. In relation to prognosis the figures of the British Medical Research Council's committee are among the most convincing (25). It was found that in children about to leave school at 14 - 15 years of age, the annual incidence of tuberculosis in the first $2\frac{1}{2}$ years of observation was 3.50 per 1,000 among those giving positive reactions to 3 tu (15 mm induration or more), 0.77 per 1,000 in those reacting weakly to 3 tu, and 0.73 per 1,000 in those reacting only to 100 TU (25). Though in childhood a positive reaction may become negative with complete healing of the lesion (26), most observers would agree with Hardy's (27) conclusion that tuberculin sensitivity tends to persist for a long time, even in the absence of re-infection. The allergic condition is often maintained by the persistence of tubercle bacilli in quiescent or healed lesions. This view is supported by Stead (28) and confirmed by animal experiments. Kanai (29) found that in mice streptomycin dependent strains survived without multiplication for a long time provided no streptomycin was given. The study of the cellular mechanisms that are characteristic of delayed hypersensitivity and immunity shows that in animals in which the development of immunity is stimulated by the injection of a vaccinal strain, delayed allergy always preceded immunity. Its most characteristic feature is the sensitivity of monocytes to tuberculin. When virulent tubercle bacilli are injected into such an animal, the macrophages accumulate around them and, as a result of products liberated from the growing bacilli, become "activated". This process is marked by the production of new lyzozomes and other organelles of digestive anzymes and possibly also of bactericidins. The cells are stimulated to proliferate, to

emmigrate from t e blood stream, to show focal accumulation and become transformed into epitheloid cells (30). According to Mackaness (31) immunity is dependent on this activation of the monocytes. Delayed hypersensitivity by itself is not enough. The cells must be in contact with the living organisms before they can become immune. This view, it will be seen, regards the delayed hypersensitivity state as a prerequisite of the immune state. Delayed hypersensitivity may exist without immunity, but immunity cannot exist without delayed hypersensitivity. Not all workers would agree with this conclusion. It is generally assumed that satisfactory vaccine properly injected will give rise to a good "take" at the site of vaccination and fairly long-continued tuberculin allergy (32). This does not imply necessarily that allergy and immunity are closely related, but that an allergic person, compared with a non-allergic person, will react more promptly and strongly to contact with infecting bacterium or its products, and so localises the infection more quickly and effectively, a response that may be important in the development of immunity. In the absence of a clear understanding or even a yard stick of immunity to tuberculosis, tuberculin allergy has been taken as the guide, however imperfect, to the effectiveness of BCG vaccination. The results of this study show a net predominance of the 4 mm and 5 mm sizes of the Mantoux test. One may say that this size of reaction is most characteristic of the BCG influence in particular; these findings may allow one to suggest an interpretation of the Mantoux test reaction with 1 TU of PPD, in children with a successful BCG vaccination during the neonatal period.

This interpretation may be written as follows:

1. 0 through 3 mm: "Sub optimum" reaction, these sizes were usually not associated with a clear induration and they were considered as negative reactions in this study.

2. 4 mm through 5 mm, "optimum" reaction, characteristic of successful BCG allergy alone.

3. 7 through 10 mm, "maximum" reaction, higher size of successful BCG allergy, here is a doubtful zone, and one should take into account the context, the history and other factors before making any decision on interpretation.

4. 11 mm and more "supra maximum" reaction, this reaction should be considered beyond successful BCG allergy alone.

The shifting of the peak of higher frequencies to the left, with predominance of small sized reactions as time elapsed, was very characteristic in this study as in that of Joncas's (7). Statistical analysis and testing showed a clear difference between the age groups regarding the mean size of the Mantoux reaction; this observation allowed "groupings" of ages with means which were not significantly different; three months and six months were groupable so were one year and two years. This type of grouping may be helpful in a survey study. A significant drop was noted from six months to eight months ($P = .05$) and from 8 months to 12 months, the difference was further increased ($P = .01$). Taking into account that reactions of 3 mm and below did not show a clear induration and

were considered as negative reactions, there was a definite reversion of the ratio positive: negative, negative reactions becoming predominant by one year and thereafter. This waning of the tuberculin reaction was clearly significant in this study as in Joncas's (7) and in Lifschitz's (11). Whatever the mechanism of this waning of the tuberculin reaction may be, whether related to an immunological immaturity (7) or to a complete healing of the lesion (27), most workers tend to believe that involution of the delayed hypersensitivity reaction does not interfere in any way with the acquired immunity (12). Joncas (7) in his study showed that children negative to 5 TU of PPD, when skin tested with 100 TU of PPD, gave positive reactions and that the incidence of tuberculosis after exposure was lowest in groups of individuals negative to 5 TU but positive to 100 TU of PPD. Apart from the boosting effect arising with successive tuberculin skin testing (13) which was not taken into account, this conclusion did not compare the group reacting to 100 TU to a group reacting optimally. As previously stated, in tuberculosis treated patients, relapses were commonest among those with the highest and those with the lowest degrees of allergy (24). One can easily assume that the lowest degree of allergy implies a poor degree of immunity. A child who becomes negative to 1 TU of PPD and who reacts positively to 100 TU of PPD may in fact have a few "memory cells" enabling a quicker response to a second challenge, if he is compared to a totally non-immune child; but if the former child is compared to one with an "optimum" reaction to 1 TU of PPD, few will disagree that the challenge will be taken better by the latter. In other words the acquired resistance is best in a child reacting optimally.

Tuberculosis infection appears to be severer during the early years of life. This so-called childhood type, common in the tropics presents thin-walled abscesses filled with caseous pus in the lungs and massive involvement of the hilar and mediastinal glands (32); miliary tuberculosis, bronchopneumonia, pericarditis, and extensive extrapulmonary dissemination is frequent from the glandular focus. Many of the children are under one year but also frequently older children with associated protein calorie malnutrition are involved. These findings have justified the BCG vaccination of newborns in countries of high prevalence of tuberculosis infection.

Protection conferred by BCG vaccination is limited, even after a successful BCG an infant is likely to be infected and suffer complications if exposed to an open contact (33). Immunity conferred by successful BCG during the neonatal period is not as satisfactory as that obtained later in life, due to immaturity of immunological mechanisms (7).

These observations and remarks suggest a need for maintenance of a good acquired resistance to tuberculosis principally during the early years of life. The only satisfactory means of assessing this important requisite so far, is the waning with time of the tuberculin test reaction.

Taking into account this waning of the Mantoux test reaction after successful BCG vaccination during the neonatal period, this study has considered the appropriate period for re-vaccination or booster vaccination as being 12 months after the initial one.

The following few remarks further support the above view:

The attendance of children at the paediatric demonstration unit at Kenyatta National Hospital was satisfactory up to one year of age.

The immunity resulting from successful BCG vaccination at one year is likely to be good enough to give satisfactory cover until the school entry.

The low reactivity for the few children with positive reactions by one year makes the pre-testing before BCG vaccinations unnecessary, because the accelerated reaction (Koch's phenomenon) is unlikely to occur.

CONCLUSIONS

The results of this study allow the following conclusions:

1. For the first objective, which was to facilitate the interpretation of the Mantoux test in children successfully BCG vaccinated during the neonatal period, that the 4 mm and 5 mm reaction sizes are the characteristic ones for the BCG allergy alone.

2. For the second objective, which was to determine the appropriate age for booster BCG in children successfully BCG vaccinated during the neonatal period, that one year after the initial successful BCG vaccination is the time appropriate for booster.

RECOMMENDATIONS

The recommendations of this study are:

1. Mantoux test readings up to 10 mm may be seen after successful BCG vaccination during the neonatal period.

   Reactions above this size should be considered to imply infection in children below 4 years of age.

2. By the age of 1 year, the majority of children successfully BCG vaccinated during the neonatal period are Mantoux negative.

   The giving of booster BCG vaccination at one year of age should therefore be considered.

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APPENDICES

I PPD

Purified protein derivative is a filtrate of heat killed cultures of tubercle bacilli that have been grown on synthetic media and precipitated with trichloracetic acid or neutral ammonium sulfate.

PPD.S was prepared by Dr. F. Seibert in 1939 from Batch No. 49608 and sent to the Division of Biologic Standards, National Institute of Health, Bethesda, Maryland, as the US Standard; it was adopted by the World Health Organization as the international PPD. Tuberculin in 1952. All other PPD tuberculins should be designated by lot number and producer.

Today, PPD tuberculins are standardized as bio equivalent to PPD-S rather than by protein weight; since PPD is more specific and better standardized, it is the preferable antigen. Currently available products have been stabilized by the addition of polysorbate 80, called Tween 80, to prevent the absorption of the tuberculo-protein onto a glass or plastic surface.

Tween 80 is Polyoxyethylene sorbitan mono oleate and is a non ionic emulsifying agent which stabilizes oil - in water emulsions.

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