

STUDIES ON THE CHANGE OF TUBERCULIN REACTION
REPEATEDLY EXAMINED AT THE SAME SITE OF THE
SKIN OF A BCG VACCINATED GROUP OF SCHOOL CHILDREN

1. RESULTS OBTAINED 1 AND 3 MONTHS AFTER BCG
VACCINATION

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(Received for publication February 6, 1960)

Introduction

It has been recognized in Japan that the number of persons whose tuberculin reaction is difficult to assess is recently increasing. In these cases the color tone of the reaction is not fresh red as was formerly seen but dark red or pale brown and the margin of the reaction area is not discernible from the unreacted area. Moreover, it is often found that when the tuberculin reaction is repeatedly examined the reactions of the same person change from time to time: some times positive and other times negative. As such cases are frequently found in BCG vaccinated groups this causes the difficulty in finding out the positive conversion caused by the infection of virulent tubercle bacilli and it is believed by Yanagisawa¹⁾ and others²⁾⁻¹⁵⁾ that this modification of tuberculin reaction is caused by the repeated tuberculin tests.

However, the opinions on the mode of change of the tuberculin reaction is not yet unequivocal and it is thought necessary to analyse the phenomena in detail.

Materials and Methods

1) **Tuberculin used:** Old tuberculin Lot No. 17** was diluted in 1:2000 with 0.5% phenol physiologic saline and 0.1 ml of the solution was injected intracutaneously.

2) **BCG used:** Dried BCG vaccine Lot No. 10034** was suspended in the solution No. X-55*** in the concentration of 0.5 mg/ml and 0.1 ml of the suspension was

** produced by the National Institute of Health, Tokyo.

*** produced by the Japan BCG producing Co., Ltd.

injected intracutaneously on the outer side of the left upper arm. In order to prevent spontaneous agglutination the preparation of the suspension and its preservation during vaccination were always done in cool state. The viable count of the BCG vaccine was 2.6×10^7 /ml, and, therefore, the inoculum to each person was 2.6×10^6 .

3) **Vaccination:** Children in the age group of 6 to 12 and attending a primary school in Kyoto which had been under tuberculosis control for 20 years by us were tuberculin tested at the flexor side of left forearm. Negative (less than 5 mm diameter in redness) and pseudo-positive (less than 10 mm in diameter) reactors were again tuberculin tested at the same site of the skin 2 months later. One week later, moreover, these persons were tuberculin tested at the flexor side

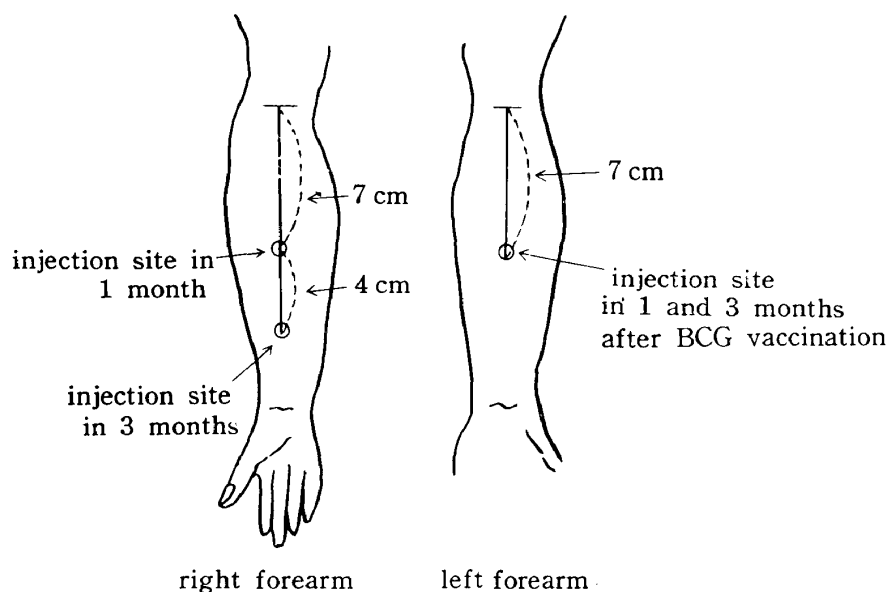


Fig. 1. Injection sites of tuberculin.

of right forearm. The number of those who were negative on both tests or at least pseudo-positive in one test and negative in another were 251. BCG was vaccinated to these 251 children.

4) **Tuberculin test:** One month after vaccination tuberculin test was done at the flexor sides of both forearms 7 cm distal part from the middle of each elbow. And 3 months after vaccination tuberculin test was again done at the same site of the left forearm and 11 cm distal (4 cm apart from the original injection site) part of the right forearm from the elbow. (see Fig. 1)

The results were read at 24 and 48 hour on the two diameters (rectangular) of the redness, induration, vesicle formation and double redness. To express the grades of tuberculin reaction the following index was adopted.

negative: less than 5 mm diameter in redness.

pseudo-positive: 6 to 9 mm diameter in redness.

positive: more than 10 mm diameter in redness.

Among the positives

+ : redness alone

++ : redness with induration

+++ : double redness and/or vesicle formation.

Results

Table 1 shows the 48 hour readings of the tuberculin reaction performed after 1 and 3 months of BCG vaccination.

Table 1. Tuberculin reaction after BCG vaccination.
(251 cases)

Time	Injection site	Tuberculin test					positive reaction in redness	induration	
		—	±	+	++	+++			
1 month after BCG vaccination	right forearm	No. of cases	2	5	3	217	24	244	241
		%	0.8	2.0	1.2	86.4	9.6	97.2	96.0
	left forearm	No. of cases	2	6	6	214	23	243	237
		%	0.8	2.4	2.4	85.2	9.2	96.8	94.4
3 months after BCG vaccination	right forearm	No. of cases	30	20	3	194	4	201	198
		%	12.0	8.0	1.2	77.2	1.6	80.0	78.8
	left forearm	No. of cases	4	21	49	177	0	226	177
		%	1.6	8.4	19.5	70.5	0	90.0	70.5

Note: Examined 48 hrs. after tuberculin injection.

After 1 month of vaccination the difference in tuberculin reaction between right and left arms was not observed: the positive rate in redness of right arm was 97.2% and 96.8% in left arm and the rate of induration was 96.0% in the right and 94.4% in the left arm. However, the difference became apparent after 3 months: the positive rate in redness of right arm was 80.0% and 90.0% of left; the rate of induration, 78.8% and 70.5%, respectively.

Table 2 (a) and (b) show the difference in tuberculin reactions 1 month after BCG vaccination between 24 and 48 hour values of the right (a) and left (b) arms. In the tables the numbers enclosed by the broad lines indicate that the values of 24 and 48 hour were equal. In the table 2 (a) it is shown that in right forearm 37 cases were stronger in 48 hour value than in 24 hour and 4 cases were

the reverse, and in the left forearm (Table 2 (b)) 37 and 5 were the case, respectively; that is, 48 hour value is usually stronger than 24 hour value.

Table 2 (c) and (d) show the difference in tuberculin reactions between right and left forearms at 24 (c) and 48 (d) hour. It may be said that the difference is not remarkable.

Similar observations were made by measuring the diameters of redness. Fig. 2 shows the diameters of redness at 24 and 48 hour in the right forearm.

Table 2. Tuberculin reaction, 1 month after BCG vaccination.

(a) Comparison of 24 and 48 hour values in right forearm.

48 hr. \ 24 hr.	-	±	+	++	+++	No. of 48 hr. > 24 hr.
-	0	2	0	0	0	37
±	2	3	2	12	0	
+	0	0	1	0	0	
++	0	0	0	203	21	
+++	0	0	0	2	3	
No. of 24 hr. > 48 hr.	4					

(c) Comparison of injection sites at 24 hour value.

right \ left	-	±	+	++	+++	No. of right > left
-	2	2	0	0	0	8
±	0	14	1	3	0	
+	0	0	0	0	0	
++	0	3	0	221	2	
+++	0	0	0	0	3	
No. of left > right	3					

(b) Comparison of 24 and 48 hour values in left forearm.

48 hr. \ 24 hr.	-	±	+	++	+++	No. of 48 hr. > 24 hr.
-	1	2	0	1	0	37
±	1	4	4	8	1	
+	0	0	0	0	0	
++	0	0	2	203	21	
+++	0	0	0	2	1	
No. of 24 hr. > 48 hr.	5					

(d) Comparison of injection sites at 48 hour value.

right \ left	-	±	+	++	+++	No. of right > left
-	2	0	0	0	0	8
±	0	5	0	1	0	
+	0	0	3	3	0	
++	0	0	0	210	4	
+++	0	0	0	3	20	
No. of left > right	3					

The dots plotted on the diagonal line indicate both 24 and 48 hour values are equal, and the dots on the left side of the line indicate that 24 hour value is larger than 48 hour and vice versa. In the figure the dots on the left side of the line are 73 and 166 are on the right side. Therefore, it may be said that there is a tendency of increase in diameter of redness with lapse of time between 24 and 48 hours. The same is true in the left forearm as shown in Fig. 3.

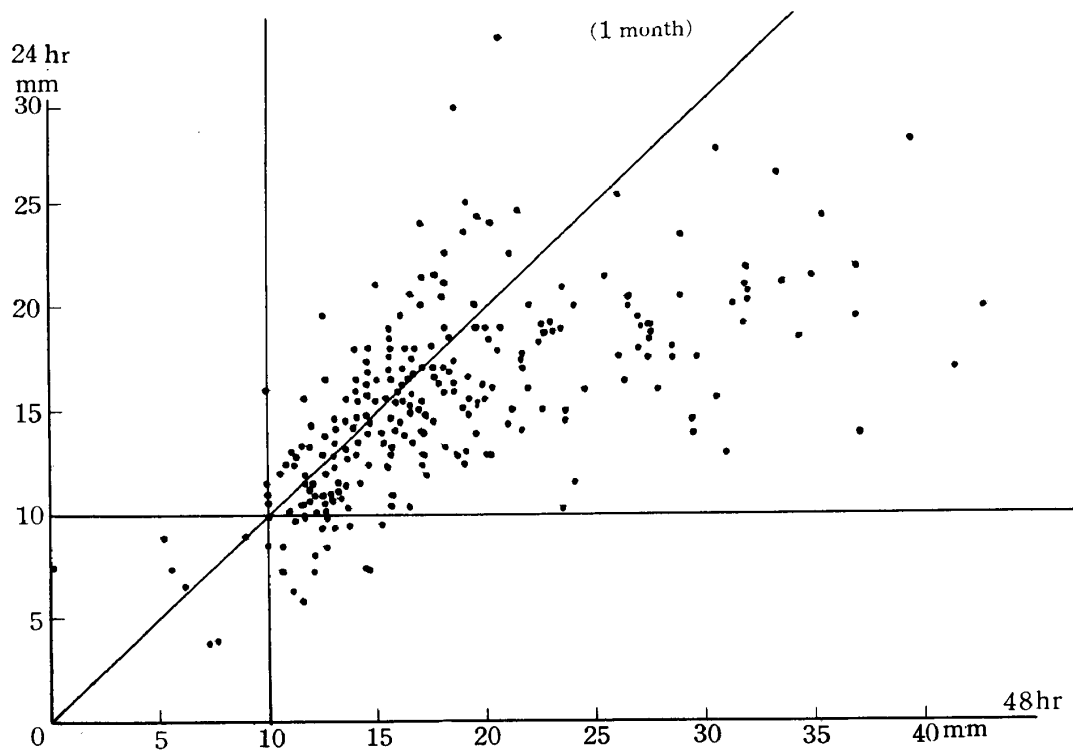


Fig. 2. Comparison of 24 and 48 hour values in redness in right forearm.

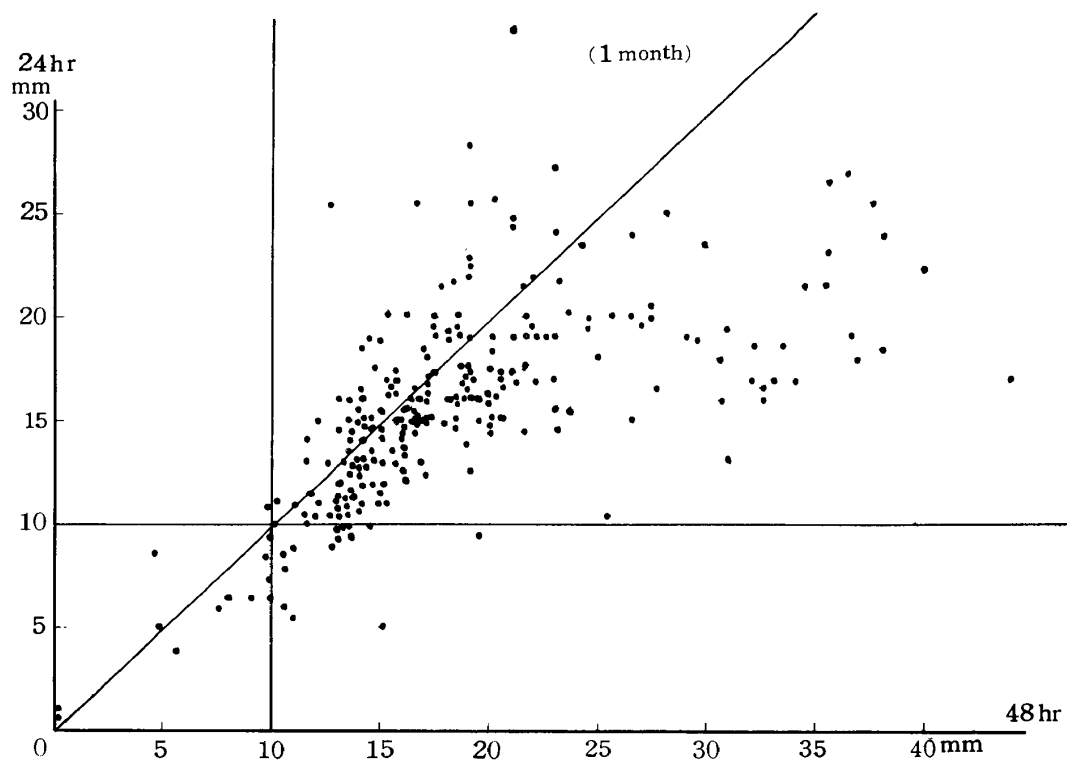


Fig. 3. Comparison of 24 and 48 hour values in redness in left forearm.

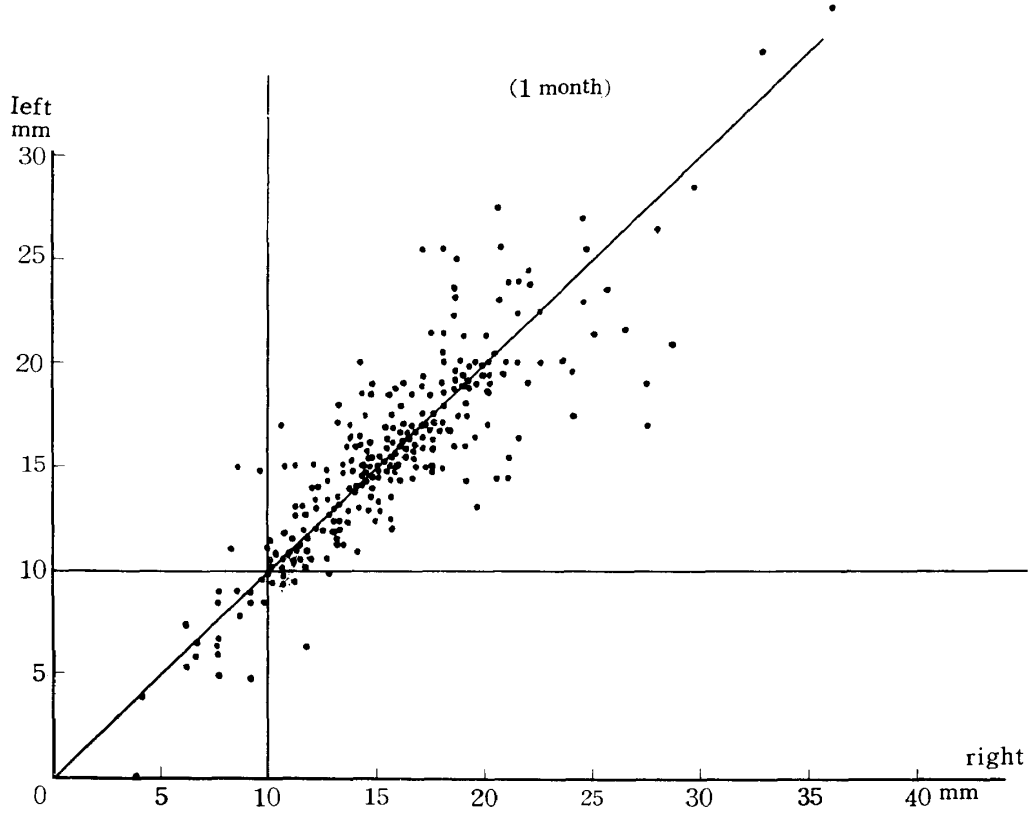


Fig. 4. Comparison of injection sites in redness at 24 hour value.

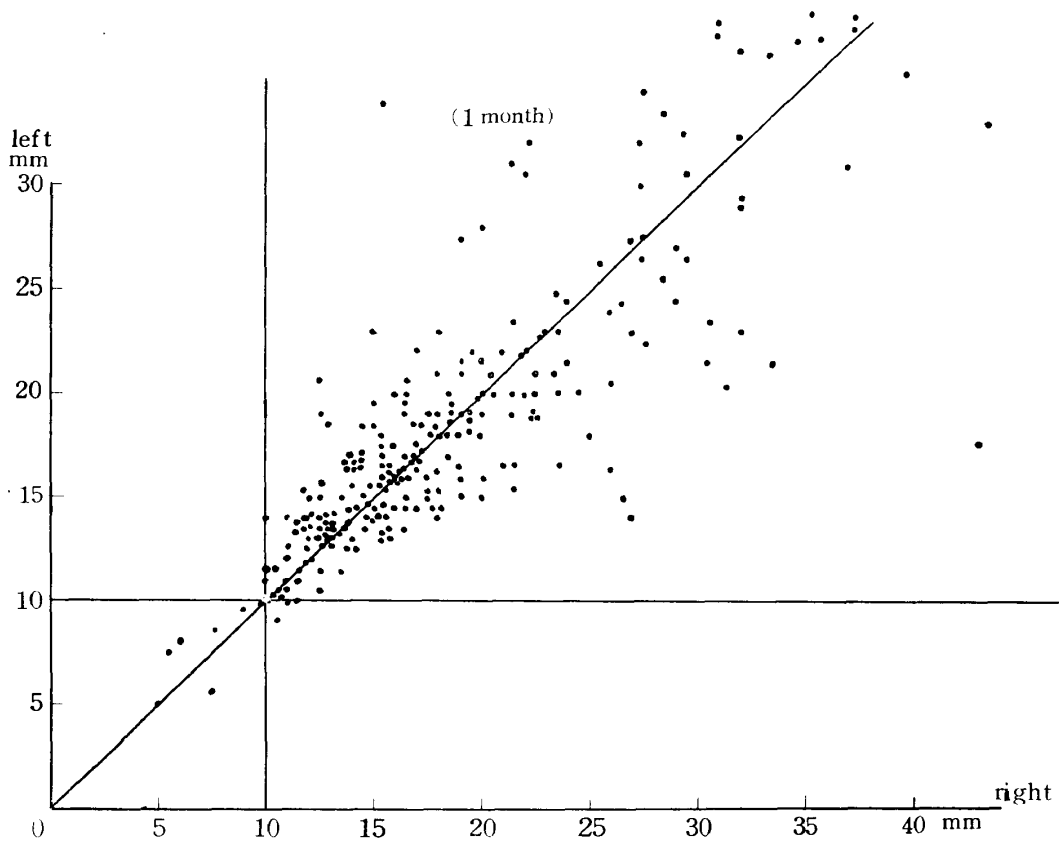


Fig. 5. Comparison of injection sites in redness at 48 hour value.

It is clear from Fig. 4 and 5 that no remarkable difference in the diameter of redness is observed between left and right arms at 24 (Fig. 4) and 48 (Fig. 5) hours.

From the results mentioned above it may be said that the reaction 1 month after BCG vaccination is bigger at 48 hour than at 24 hour regardless of the arm used, and there is no difference between right and left forearms.

The results in 3 months after BCG vaccination are shown in Table 3 and Fig. 6, 7, 8, and 9.

Table 3. Tuberculin reaction, 3 months after BCG vaccination.

(a) Comparison of 24 and 48 hour values in right forearm.

48 hr. \ 24 hr.	-	±	+	++	+++	No. of 48 hr. > 24 hr.
-	20	7	0	8	0	52
±	6	13	2	30	0	
+	0	0	1	2	0	
++	2	2	0	153	3	
+++	0	0	0	1	1	
No. of 24 hr. > 48 hr.	11					

(c) Comparison of injection sites at 24 hour value.

right \ left	-	±	+	++	+++	No. of right > left
-	0	0	0	0	0	0
±	1	0	0	0	0	
+	2	0	0	0	0	
++	31	43	2	98	0	
+++	1	8	1	62	2	
No. of left > right	151					

(b) Comparison of 24 and 48 hour values in left forearm.

48 hr. \ 24 hr.	-	±	+	++	+++	No. of 48 hr. > 24 hr.
-	0	0	0	0	0	1
±	0	0	1	0	0	
+	0	2	0	0	0	
++	3	17	41	113	0	
+++	1	2	7	64	0	
No. of 24 hr. > 48 hr.	137					

(d) Comparison of injection sites at 48 hour value.

right \ left	-	±	+	++	+++	No. of right > left
-	3	0	0	1	0	40
±	8	6	0	7	0	
+	13	5	3	28	0	
++	6	9	0	158	4	
+++	0	0	0	0	0	
No. of left > right	41					

Table 3 (a) shows that 52 were stronger in reaction at 48 hour value than at 24 hour and 11 were the reverse in the right forearm, indicating the increase of reaction with lapse of time similarly as had been in 1 month after vaccination.

While, on the contrary, in the left forearm where the injection of tuberculin was performed at the same site as had been done in 1 month the number of which 48 hour values were stronger than 24 hour was only one and the number

of the reverse was 137 as seen in Table 3 (b), indicating clearly the stronger reaction at 24 hour than at 48 hour.

When the reaction was compared between right and left forearms at 24 hour the reaction is clearly stronger in left arm (151 cases) than in right arm (no case) as seen in Table 3 (c). But at 48 hour the difference became obscured as seen in Table 3 (d).

Similar results were obtained when the diameter of redness was compared as seen in Fig. 6, 7, 8, and 9: that is, in the right forearm 48 hour value was usually larger than 24 hour value (Fig. 6) and the reverse was true in the left forearm

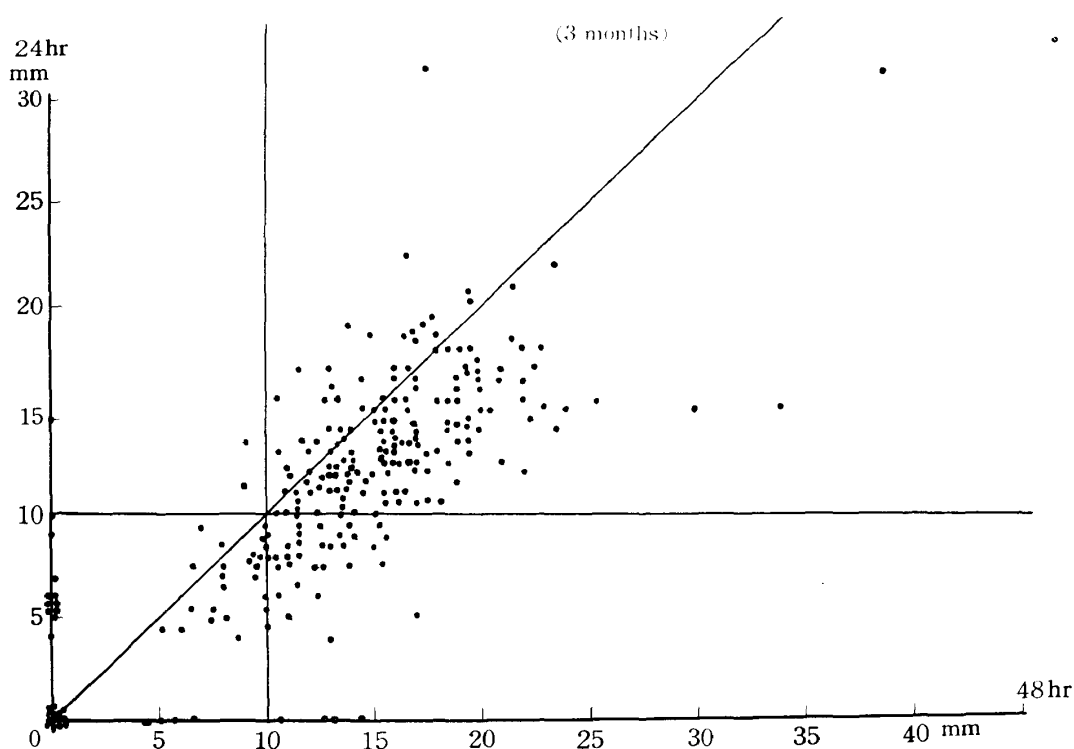


Fig. 6. Comparison of 24 and 48 hour values in redness in right forearm.

(Fig. 7); and while the value in the left arm was remarkably larger than in right arm (Fig. 8) at 24 hour, this difference became obscure at 48 hour and even larger in right arm (Fig. 9): the cases of $\text{left} > \text{right}$ were 96 and $\text{right} > \text{left}$ were 142.

This shows that in the left arm where the tuberculin reaction was tested at the same site in 3 months as in 1 month the maximum reaction was observed at 24 hour and the reaction was decreased at 48 hour; while, in the right arm where the second reaction (3 months) was performed 4 cm apart from the first (1 month) the maximum reaction was observed at 48 hour both in 1 and 3 months.

When the larger diameter at either 24 or 48 hour in each forearm 3 months after BCG vaccination was adopted, the reaction of left arm was clearly larger than right arm as seen in Fig. 10.

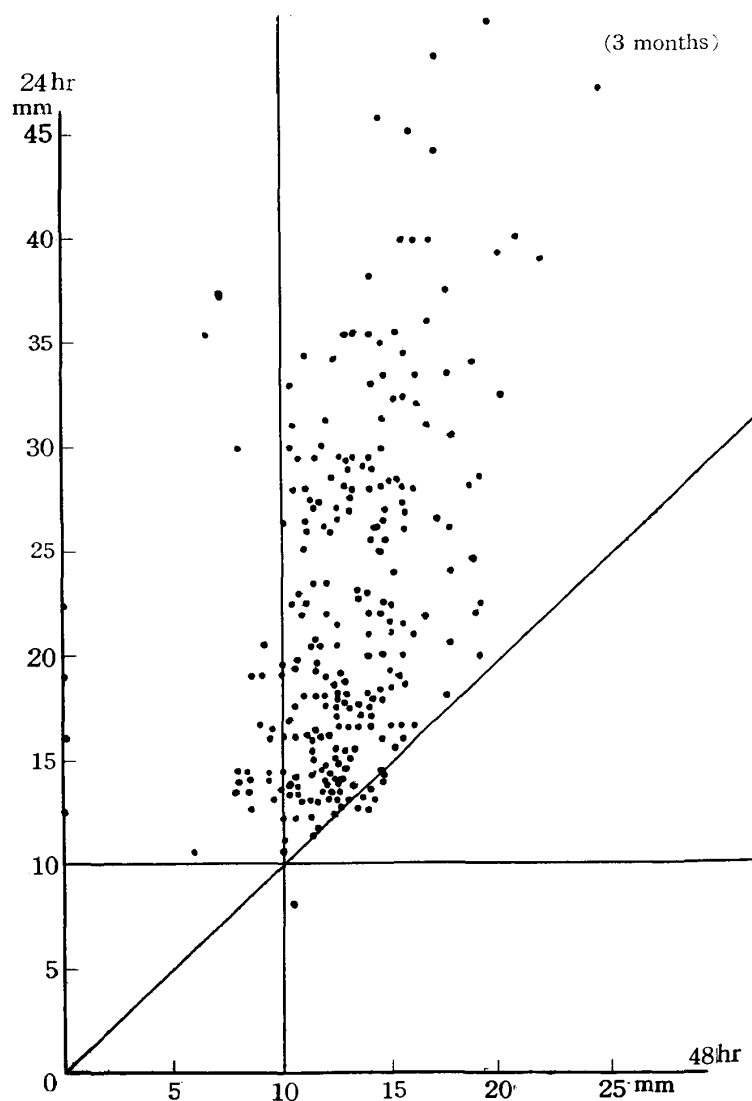


Fig. 7. Comparison of 24 and 48 hour values in redness in left forearm.

Discussion

When the positive rate and the induration rate of tuberculin reaction 1 month after BCG vaccination is compared to the preceding investigation¹⁶⁾, the present one is markedly high in both positive rate and induration rate. Although the difference in the subjects or the lot of tuberculin may play a part in this, one of the reasons may be in the high viable units of BCG preparation used in the present study compared to the former: in the former report we used BCG preparation containing only 4.2×10^5 viable units, about 1/5 of the present one.

According to the Japan BCG Research Council¹⁷⁾ and Obayashi¹⁸⁾, the correlation between the viable units of BCG and the strength of allergy after vacci-

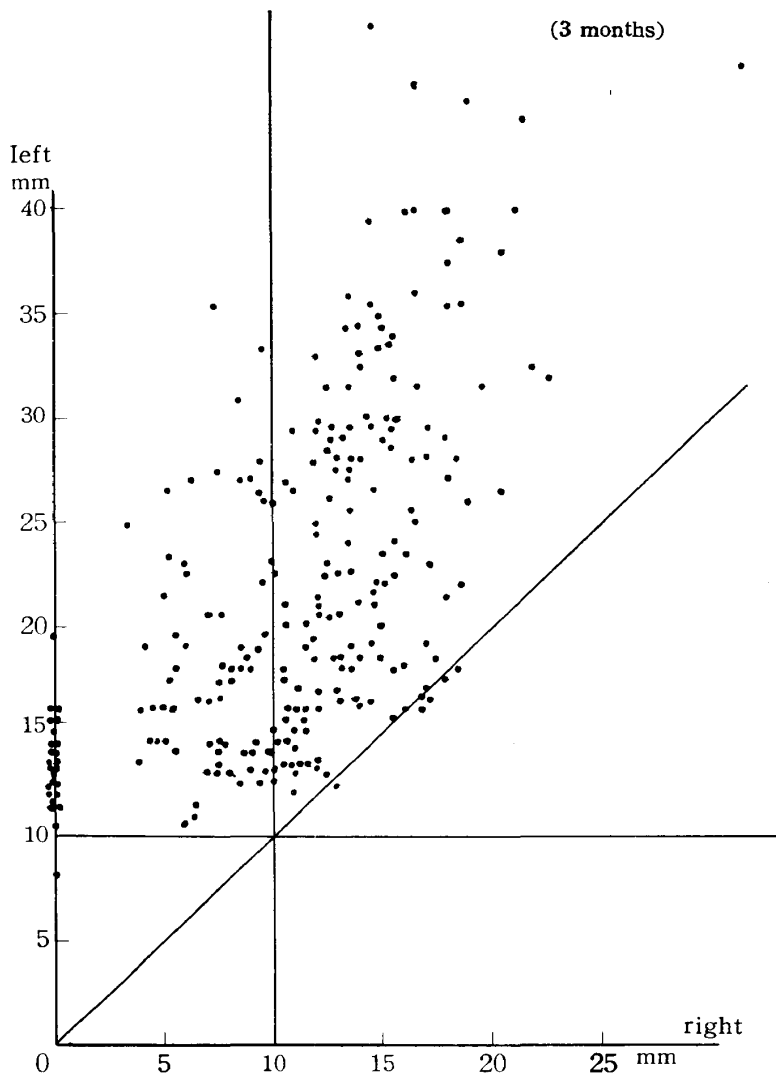


Fig. 8. Comparison of injection sites in redness at 24 hour value.

nation exists, although Krohn¹⁹⁾ denied it. It is also possible to expect the increase of positive conversion rate and the longer duration of tuberculin reaction by the recent improvement of BCG preparation. Therefore, it may be necessary the differential criterion be pursued to find out the positive conversion caused by the infection of virulent tubercle bacilli in BCG vaccinated groups from that caused by BCG vaccination alone.

The tuberculin reaction in 1 month after BCG vaccination was typical, increasing in reaction with time from 24 to 48 hour in both arms. In 3 months the reaction in the right arm where the injection site was different from 1 month was similar as in 1 month, but in the left arm where the tuberculin test was performed at the same site as in 1 month the 48 hour value was weaker than 24 hour and this is the reason why the difference in right and left arm was observed.

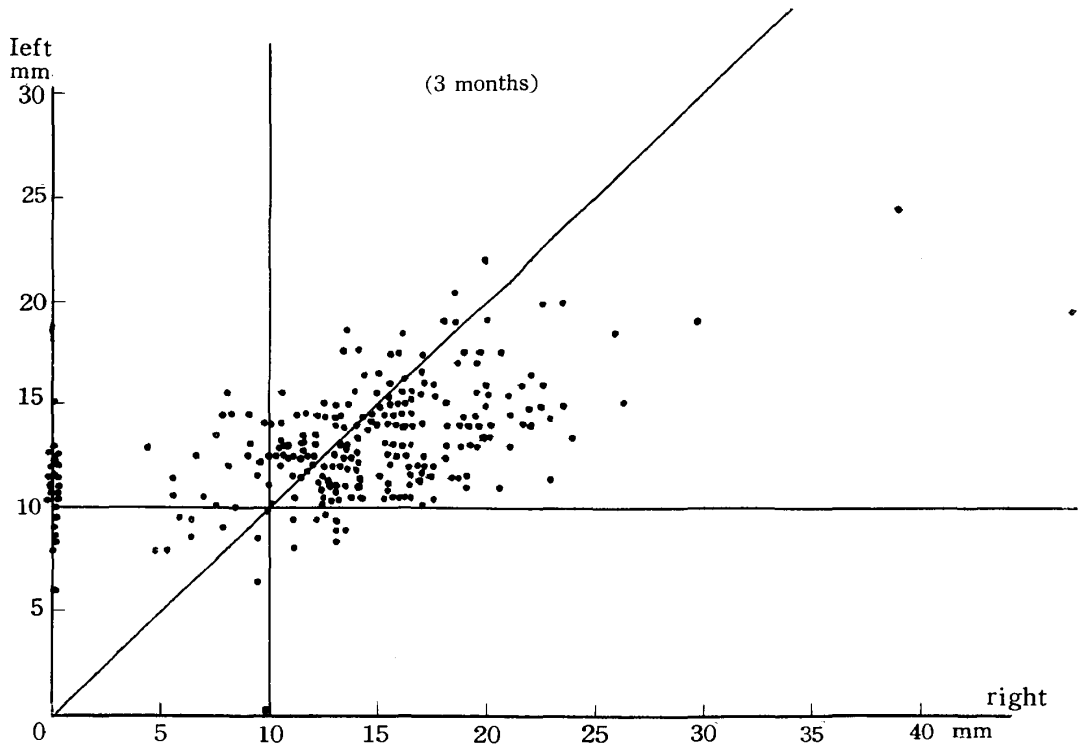


Fig. 9. Comparison of injection sites in redness at 48 hour value.

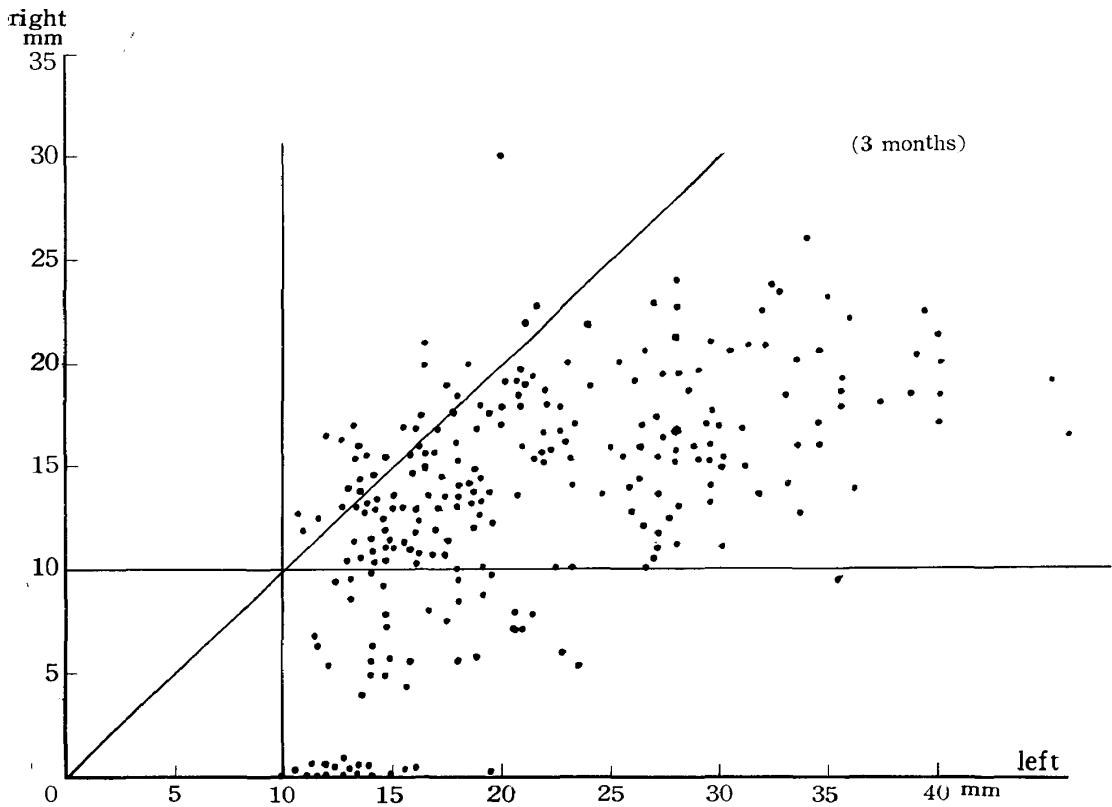


Fig. 10. Comparison of injection sites in redness, when the larger diameter at either 24 or 48 hours is adopted.

Such a change in reaction at the repeated site has been observed by other workers comparing the values of 24 and 48 hours¹⁾²⁾³⁾⁷⁾ or tracing the processes⁴⁻⁶⁾⁸⁾¹⁰⁾ and is called as the "accelerated reaction." Bachi¹³⁾¹⁴⁾ observed this histologically. Masumura¹⁵⁾ observed this by PPD-s, while others by old tuberculin. But in these preceding researches only a small number of cases are clear in their sites of tests and the number of repetition of tuberculin test. This is the reason why the present investigation was undertaken to elucidate the problem more precisely.

The first problem to be solved is whether the repeated tuberculin tests done before the positive conversion might influence the mode of tuberculin reaction after conversion or not. In our country the tuberculin test is usually done in the middle part of the flexor side of left forearm. Therefore, in the present investigation the pupils other than the first year grade have been tested more than 6 times in the middle part of the flexor side of the left forearm and all pupils of the first year grade were tested twice before BCG vaccination in the same site and once in the right forearm. If the previous tuberculin tests would influence the reactions after the positive conversion, the difference between right and left arm 1 month after vaccination and the difference between 1 and 3 months in the right arm should be observed. But there recognized no difference between them—all were typical reactions increasing in strength with time up to 48 hours and were clearly different from the reactions of the left forearm in 3 months. This indicates that the repeated tuberculin tests before the positive conversion may not influence the mode of tuberculin reaction after the positive conversion. Therefore, the results of 1 month after vaccination in the present investigation are to be regarded as those of the primary site and those of the left forearm of 3 months are to be those of the first repeated site, that is, the second test at the same site.

From the results of 3 months after vaccination it may be said that "the accelerated reaction" will appear as fast as the second time of repeated tuberculin tests.

Moreover, when the 48 hour value alone was compared between the new and the repeated sites. Terada⁶⁾ recognized the difference at the second test but Maeda³⁾ and Honzawa¹⁰⁾ reported that 3 or 4 times of repetition were necessary to observe the difference. From the present investigation it may be said that, although the 48 hour value itself showed little difference between the new (right arm) and the repeated (left arm) tests, there was some difference in positive rate, induration rate and in 40 per cent of the cases there was some change in color tone of the reaction. Therefore, it is thought that the influence by repeti-

tion of tuberculin reaction appears from the first repetition at 48 hour value too.

It is interesting in the 3 month reaction that the 24 hour value of the left arm is remarkably stronger than that of the right arm and at 48 hour the reactions of both arms had little difference: that is, in the right arm (the new site) the maximum height of the reaction (48 hour value) can not attain that of the left arm (the repeated site, 24 hour value). This is clearly shown in Fig. 10. When the larger diameter at either 24 or 48 hour values is adopted—this actually means in the left arm the 24 hour value is adopted and in the right arm the 48 hour value—the values of left arm are clearly stronger than the right arm.

Therefore, it may be said that at the repeated site the reaction is not only accelerated (accelerated reaction) but also intensified (augmented reaction) compared to the new site. But the opinions on this problem are controversial. Honzawa¹⁰⁾ stated that the repeated sites reacted stronger than the new sites in groups of which tuberculin reaction had been weak but in groups of exhibiting strong tuberculin reaction the new sites reacted stronger than the repeated sites. Terada⁶⁾ reported that the repeated sites were stronger in reaction than the new sites but Ikegami⁷⁾ insisted the repeated sites were weaker in adults. One of the reasons of the controversy may lie on the fact that these workers read the reaction at 48 hours. If the 24 hour values as well as 48 hour were read as in the present investigation, it may be clear that the repeated sites react stronger than the new sites even in the strongly reacting group.

There are several investigations concerning the distance between the primary and secondary injection sites which might not influence the secondary reaction^{3) 7) 11)}. In our cases 4 cm was the maximum distance in the forearm of school children in the secondary injection. And most of the diameters of the reaction in the first tuberculin test were within 4 cm and there was no accelerated reaction in the right forearm. Therefore, it may be reasonable to say that if the distance between the first and second injection sites were more than 4 cm, the influence of the previous test may be negligible.

Summary

Tuberculin reaction was successively examined using 2000 times diluted old tuberculin one and three months after BCG vaccination in school children.

The second tuberculin test (3 months after vaccination) was done on the same site as the first test in the left forearm and on the different site in the right forearm. The results were read after 24 and 48 hours.

(1) On the repeated sites of the left forearm the reaction appeared and decreased its intensity faster than the new sites of the right forearm (accelerated

reaction). Moreover, the reaction of the repeated sites was stronger than the new sites (augmented reaction).

(2) The tuberculin tests done in negative stadium do not influence the mode of reaction after the acquisition of allergy.

(3) Both accelerated and augmented reactions appear at the second tuberculin test (the first repetition) on the same site.

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- Note: Except for No. (4) and (19) all references are in Japanese.