Bull. Org. mond. Santé / *Bull. World Hlth Org.* 1950, 3, 279-300

BCG-VACCINE STUDIES

2. Effect of Variation in Dosage of BCG Vaccine on Allergy Production and Vaccination Lesions Nine Weeks after Vaccination

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Manuscript received in November 1950

In the fall of 1949 a series of studies on BCG vaccine was started as a co-operative undertaking of the Joint Enterprise, [1] the Danish Statens Seruminstitut, and the Tuberculosis Research Office of the World Health Organization. The first project undertaken was a study of the effect of age of vaccine on post-vaccination allergy and the local vaccination lesion, and how this effect might be modified by limited variations in storage temperature and dosage. The findings 10 weeks after vaccination formed the basis of the first report from this investigation.[2]

The second project in this series was devoted to the study of the effect on allergy production of variation in dosage. At the present time the dosage of BCG organisms in vaccines from different production centres is not the same, and within the same laboratory is changed from time to time to adjust the potency of the vaccine to desired levels. The actual relationship between variation in dosage and variation in the allergy produced is, however, not established.

Since in the first project it was found that increasing the dosage of the vaccine to four times standard strength resulted in a significant increase in inconvenient vaccination reactions, the strengths chosen for study in Project II were 1/1, 1/2, 1/4 and 1/8 standard strengths. The post-vaccination examination 8½ weeks later showed such minimal changes in allergy with these variations in dosage, that in a subsequent project a wide range of dosages from standard strength to 1/256 of this strength was investigated.

[1] The Joint Enterprise is the name given to the international mass BCG-vaccination programme carried out as a co-operative effort of UNICEF, the Danish Red Cross, the Norwegian Relief for Europe, and the Swedish Red Cross.

[2] *Bull. World Hlth Org.* 1950, 3, 1

The early post-vaccination findings of both projects are reported in this paper.

Methods and Materials

These studies were carried out among the schoolchildren of a rural district very similar to and adjoining the district where the first project of this investigation took place. The prevalence of tuberculous infection in the area was very low. The children were almost entirely in the age-group 6-14 years. The method of selection of groups for vaccination, and schedules and techniques were essentially the same as described in detail in the first report of this series.[3]

The tuberculin used for the Mantoux tests was part of the same lot of purified protein derivative (PPD)[4] as used in Project I and throughout these vaccine studies. In Project II a dilution containing 10 tuberculin units (TU) (0.0002 mg) per 0.1 ml was used as the first test and 100 TU (0.002 mg) given to those who did not react with at least 6 mm of induration to this test. In Project IV, 5 TU (0.0001 mg) were used as the first test and children showing less than 5 mm of induration were given the 100 TU dose. The tests were read at 3 or 4 days,[5] and again at 6 or 7 days. In reading the tests, the transverse diameter of the erythema and of the induration was measured. In addition, the density of the reaction was classed as one of four types, Type I being the most dense reaction, Type IV the least dense, Types II and III being intermediate categories.

The BCG vaccine was prepared by the Statens Seruminstitut in standard, and the desired fractional, strengths and kept constantly refrigerated until use. The vaccine used in Project II was part of the regular lot (No. 876) prepared 9 January 1950, while that used in Project IV was prepared 12 April specifically for this project. All ampoules were labelled in a code unknown to the testing and vaccinating personnel. Vaccinations were performed intradermally, the measured dose being exactly 0.1 ml of vaccine. The vaccination was given on the basis of the reaction to the first tuberculin-test (either 5 or 10 TU) at the same time, and on the same indication as the 100 TU test. Two strengths of vaccine were used in each school on alternate non-reactors. At the post-vaccination examination, the vaccination sites and regional and contralateral lymph nodes were routinely examined.

To permit maximum uniformity of technique each operation was carried out by the same person throughout each project with the exception that the vaccinations were performed by two vaccinators. The schedule was, however, so arranged that each vaccinator gave each strength of vaccine on each vaccination day.

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[3] *Bull. World Hlth Org.* 1950, 3, 1

[4] Statens Seruminstitut lot number RT XIX, XX, XXI

[5] Details given in the tables and graphs.

Plan and Extent of Project II

A single lot of vaccine was used in the following strengths :

(1) vaccine of standard strength (0.7500 mg/ml)
(2) vaccine of 1/2 standard strength (0.3750 mg/ml)
(3) vaccine of 1/4 standard strength (0.1875 mg/ml)
(4) vaccine of 1/8 standard strength (0.0938 mg/ml)

Vaccinations were performed on three consecutive days, the age of the vaccine being 7, 8, and 9 days. Each of the four strengths was used each vaccination day on essentially comparable groups of children.

The pre-vaccination testing and vaccinations were carried out in January 1950. The post-vaccination examinations were made 59 days after the vaccination.

The extent of the study was as follows :

Number of participating schools		20
Number of children registered as pupils in these schools		1,084
Number of children tuberculin-tested (10 TU test given and read)		1,046
Previous BCG	72	
No previous BCG—reactors	61	
No previous BCG—non-reactors	913	
Number of children vaccinated [6]		837
Number of vaccinated children retested at 8½ weeks (10 TU test given and read)		810

Findings of Project II

Regardless of the dosage used, all vaccinated children retested with 10 TU 8½ weeks after vaccination showed reactions of at least 6 mm of induration (table II). The effects of vaccination with the different strengths were not, however, entirely alike. As the dosage of the vaccine decreased, the mean size and the density of the Mantoux reactions also decreased and the vaccination lesions became smaller (table III), but the decrease was relatively slight when the range of decrease in dosage is considered. As shown in table I, the mean size of the tuberculin reactions dropped from 16.0 mm after vaccination with standard strength vaccine to 14.2 mm after 1/8 standard strength, and the frequency of the most dense reactions (those classed as Type I) decreased from 36.2% to 17.8%. The mean size of the local vaccination lesions decreased from 10.6 to 7.2 mm.

As was found in Project I, the distributions by size of Mantoux reactions were approximately normal, while the distributions by size of induration of local vaccination lesions showed a right-sided skewness.

[6] Vaccinated includes (as everywhere in this paper) only children without a previous BCG vaccination.

TABLE I. PROJECT II. SUMMARY OF FINDINGS 8½ WEEKS AFTER VACCINATION

Strength of vaccine	Number vaccinated	Mantoux 10 TU reactions							Local vaccination reactions	
		Number retested	Arithmetic mean of induration (mm)	Distribution by type of induration (%)					Number examined	Arithmetic mean of induration (mm)
				Type I	Type II	Type III	Type IV	Total		
1/1 standard	215	207	16.0	36.2	49.8	12.6	1.4	100.0	211	10.6
1/2 standard	202	194	15.1	32.4	46.9	18.6	2.1	100.0	201	9.5
1/4 standard	230	224	15.1	27.7	49.1	20.1	3.1	100.0	230	8.4
1/8 standard	190	185	14.2	17.8	37.8	40.6	3.8	100.0	189	7.2

Inconvenient reactions associated with vaccination were rare after all strengths of vaccine. Only one local abscess was observed, this occurring in a child vaccinated with the 1/2 standard strength. There were no glandular abscesses, and even moderate enlargement of the regional lymph nodes was uncommon.

Plan and Extent of Project IV

A single lot of vaccine was used in the following strengths :

(1) vaccine of standard strength (0.7500 mg/ml)
(2) vaccine of 1/4 standard strength (0.1875 mg/ml)
(3) vaccine of 1/16 standard strength (0.0469 mg/ml)
(4) vaccine of 1/64 standard strength (0.0117 mg/ml)
(5) vaccine of 1/128 standard strength (0.0059 mg/ml)
(6) vaccine of 1/256 standard strength (0.0029 mg/ml)

Vaccinations were performed on four different days, the vaccine ages being 5-6 days, and 16-17 days. On the two first vaccination days all 6 dilutions were used, while only 1/1, 1/4, 1/64 and 1/256 standard strengths were used on the two last vaccination days.

The pre-vaccination testing and vaccination were carried out in April and May 1950. The post-vaccination examinations were made 65 to 67 days after the vaccination.

The extent of the study was as follows :

Number of participating schools		23
Number of children registered as pupils in these schools		1,290
Number of children tuberculin-tested (5 TU test given and read)		1,242
Previous BCG	70	
No previous BCG—reactors	80	
No previous BCG—non-reactors	1,092	
Number of children vaccinated		1,025
Number of vaccinated children retested at 9½ weeks (5 TU test given and read)		956

Findings of Project IV

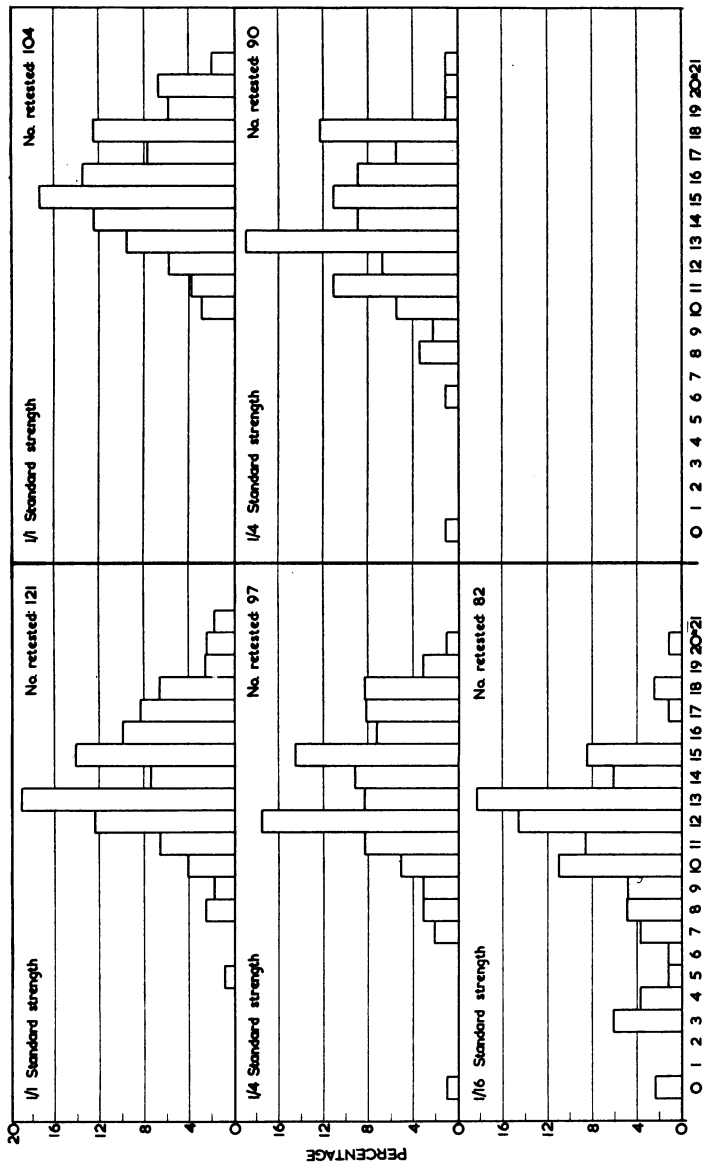
Nine and a half weeks after vaccination 956 or 93.3% of the vaccinated children were retested with 5 TU ; 151 gave reactions of less than 5 mm of induration. The frequency of these small reactions increased with decreasing dosage, but even after vaccination with 1/256 standard strength vaccine more than 60% of the children reacted with at least 5 mm of induration. 142 of the 151 children were retested with 100 TU.

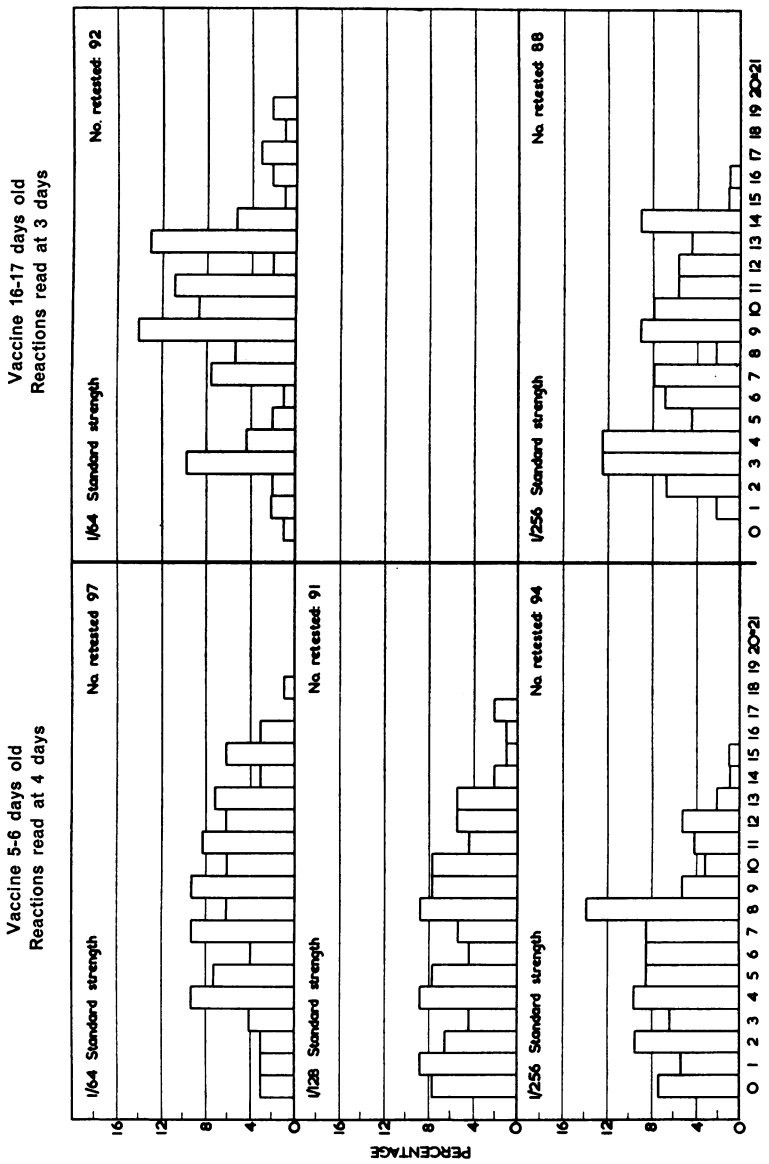
Fig. 1 shows the frequency-distribution by size of Mantoux 5 TU reactions 9½ weeks after vaccination for the different vaccine strengths.

FIG. 1. PROJECT IV. FREQUENCY-DISTRIBUTION BY SIZE OF INDURATION OF MANTOUX 5 TU REACTIONS 9½ WEEKS AFTER VACCINATION

Vaccine 5-6 days old
Reactions read at 4 days

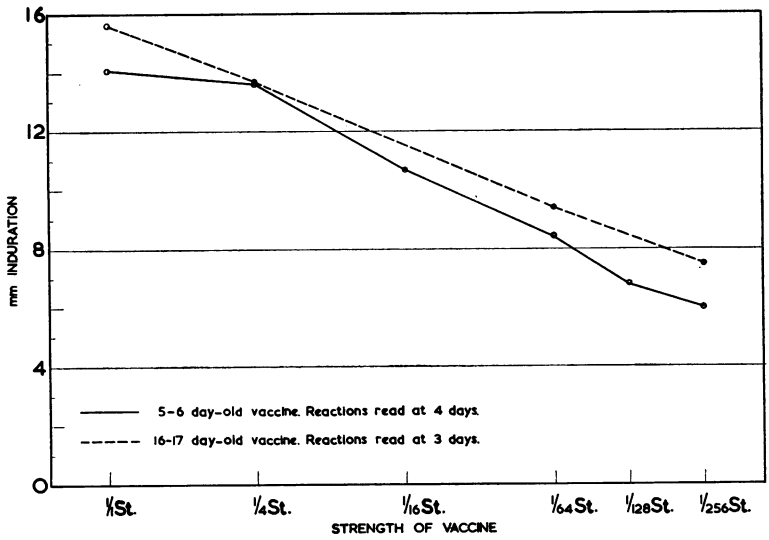
Vaccine 16-17 days old
Reactions read at 3 days




Vaccine 5-6 days old
Reactions read at 4 days
Vaccine 16-17 days old
Reactions read at 3 days
1/64 Standard strength
No. retested 97
1/64 Standard strength
No. retested 92
1/128 Standard strength
No. retested 91
1/256 Standard strength
No. retested 94
1/256 Standard strength
No. retested 88
PERCENTAGE
0 4 8 12 16
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20+21
mm INDURATION

As the dosage decreases the whole range of reactions shifts in the direction of zero, and the distribution, which for the stronger dosages is approximately normal, becomes asymmetric, with more and more of the reactions cumulated at the smallest sizes. The mean size decreases with decreasing dosage (fig. 2) from 14.2 mm (15.6 mm) [7] to 6.0 mm (7.6 mm) after vaccination with 1/1 and 1/256 standard strength respectively. The relation between mean size and the logarithm of the dosage is approximately linear. The standard deviation of the distribution increases with decreasing dosage until cumulation of reactions at the smallest sizes becomes marked (table IV).

FIG. 2. PROJECT IV. MEAN SIZE OF INDURATION OF MANTOUX 5 TU REACTIONS 9½ WEEKS AFTER VACCINATION



The variation with dosage of the density of the 5 TU reactions is illustrated in fig. 3 for the vaccine 5-6 days old, and 16-17 days old. As the dosage decreases the reactions become progressively less dense. Types I and II together constitute 85% (92%) of all reactions after vaccination with the standard strength vaccine but only 12% (23%) after the 1/256 standard strength. The soft indefinite Type IV reactions are rare after vaccination with the standard strength vaccine, constituting 3% (0%) of the total reactions, but become increasingly frequent as the dosage decreases : after the 1/256 strength they constitute 64% (41%) of all reactions.

The result of the testing with 100 TU is illustrated in fig. 4 which shows the frequency-distribution by size of induration for the different vaccine

[7] The figures in brackets refer to the vaccine 16-17 days old.

FIG. 3. PROJECT IV. DENSITY OF INDURATION OF MANTOUX 5 TU REACTIONS 9½ WEEKS AFTER VACCINATION, CLASSED IN TYPES

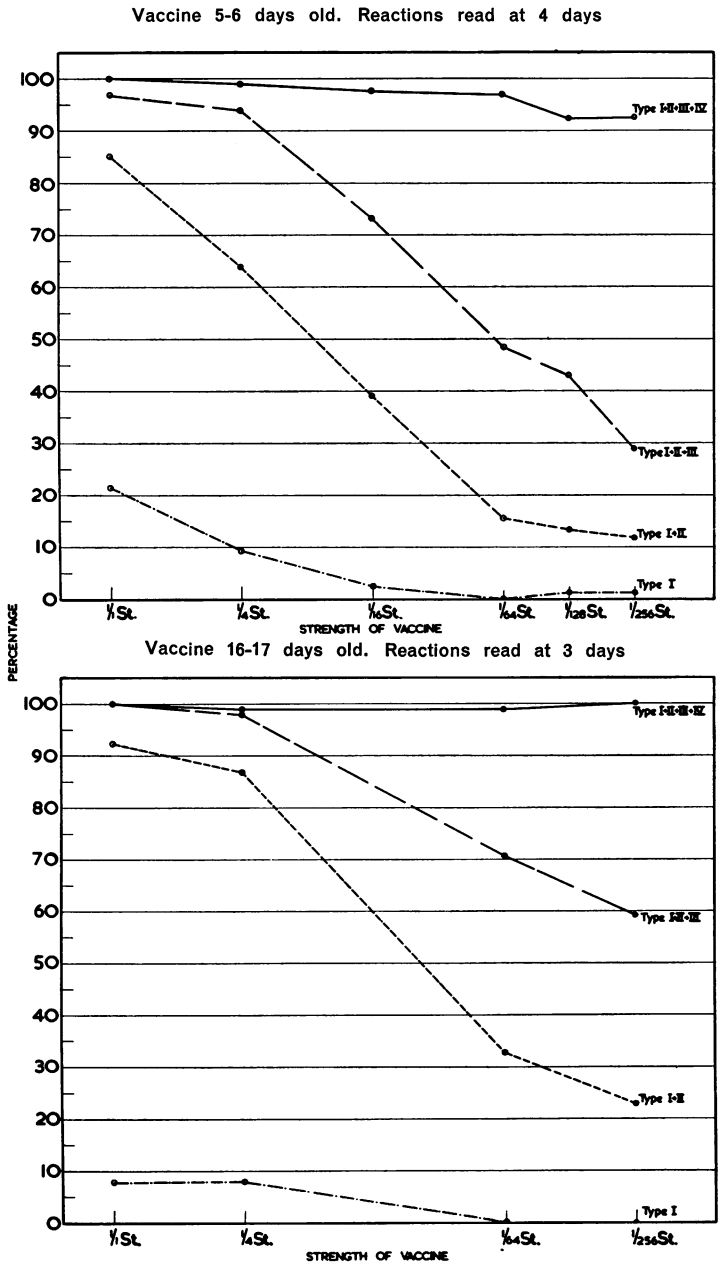


FIG. 4. PROJECT IV. DISTRIBUTION BY SIZE OF INDURATION OF MANTOUX 100 TU REACTIONS AT PRE-VACCINATION TESTING AND AT POST-VACCINATION TESTING

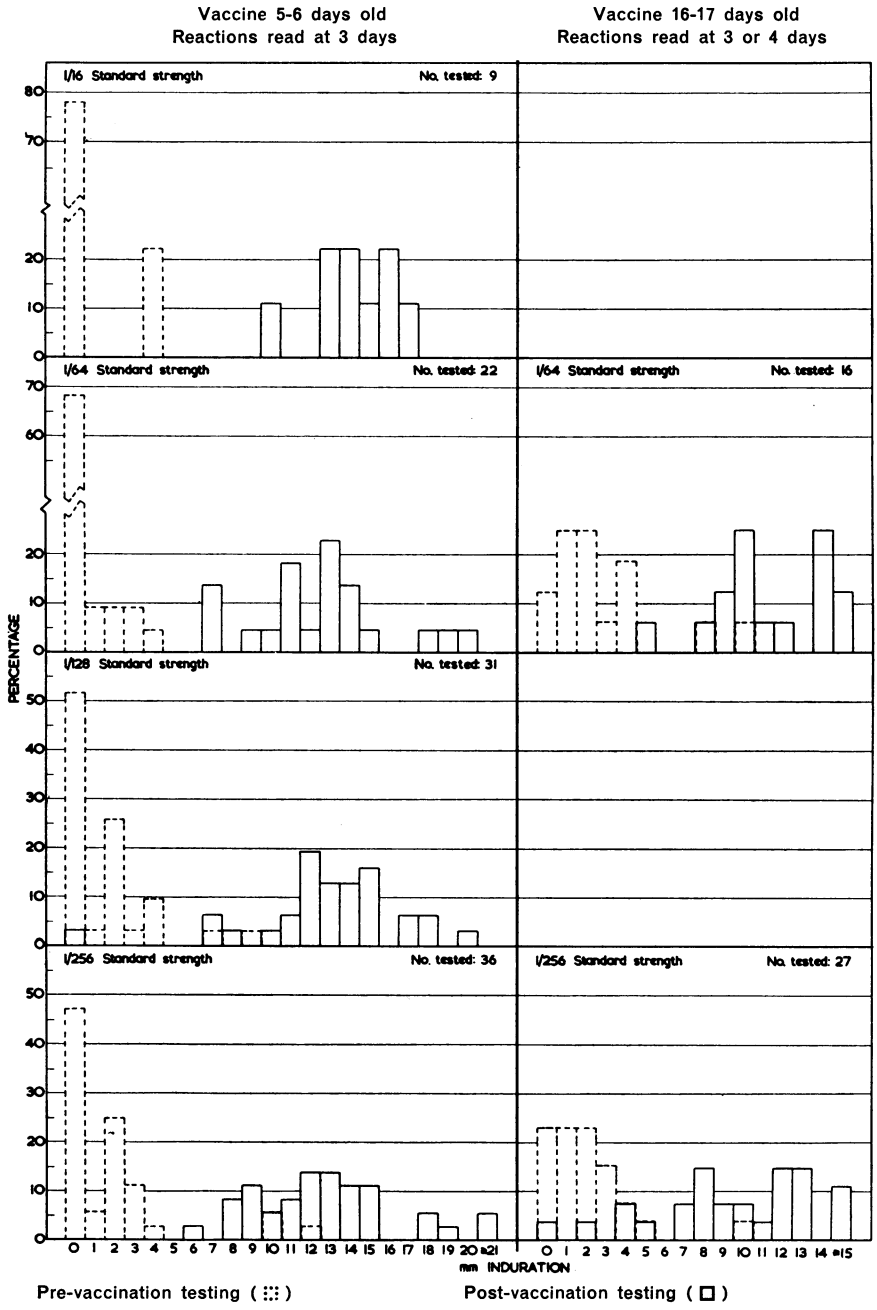
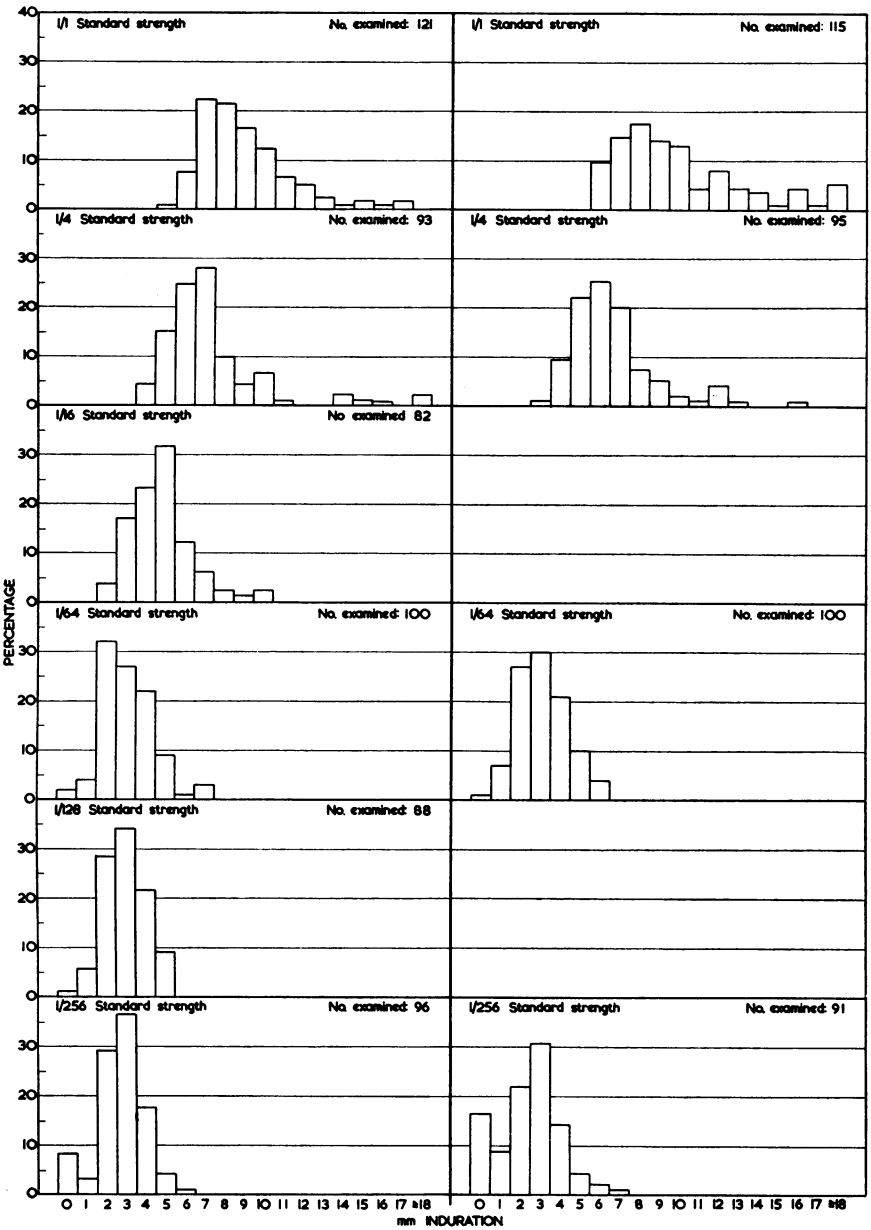


FIG. 5. PROJECT IV. DISTRIBUTION BY SIZE OF VACCINATION LESIONS 9½ WEEKS AFTER VACCINATION



strengths (table V). For purposes of comparison the corresponding distributions of 100 TU reactions observed in the same children at the pre-vaccination testing are given in the same graph. The post-vaccination reactions are strikingly at variance with those found at the pre-vaccination testing. The mean size of the former varies between 9.4 and 14.2 mm, of the latter between 0.7 and 2.8 mm.

Comparing the two vaccine ages, it will be noted that the mean size of 5 TU reactions developed after vaccination with vaccine 16-17 days old is uniformly larger than the corresponding mean after vaccination with vaccine 5-6 days old. Similarly, the oldest vaccine gave the densest reactions.

This result is not in accordance with what was found in Project I, where the mean size of the tuberculin reactions seemed to be independent of age of vaccine within the time limits of the study, and density decreased with increasing vaccine-age. It is probable that the differences here are due to the fact that the tuberculin reactions were read after different intervals, at four days for the children vaccinated with vaccine 5-6 days old, at three days for those vaccinated with the vaccine 16-17 days old. Several observations in this material indicate that this explanation may be correct. First, while the older vaccine gave larger reactions to the 5 TU test than the younger, the opposite is true for the reactions to 100 TU where the intervals of reading the tests were reversed, when the 5 TU test was read at three days, the 100 TU test was read at four days, and vice versa. Secondly, all 5 TU reactions were also read at seven days, and these readings do not demonstrate the tendency toward stronger reactions after older vaccine.

In the first two projects the size of the vaccination lesion was defined as the size of the induration. In this project, a sizable proportion of the children vaccinated with small dosages of BCG showed only scars or residual inflammatory changes without induration. The largest measure of visible or palpable change at the vaccination site has, therefore, been used in this project to define the size of the vaccination lesion.

All children vaccinated with 1/1, 1/4, and 1/16 standard strength vaccine showed local lesions (fig. 5 and table VI). Less than 2% of those vaccinated with 1/64 and 1/128 strength, and 8% (16%) of those vaccinated with 1/256 standard strength showed no local evidence of vaccination. The mean size of the lesions decreased with decrease in dosage from 8.9 mm (10.1 mm) to 2.7 mm (2.5 mm).

Only 1 local abscess was observed, in a child vaccinated with standard strength vaccine 16-17 days old. No glandular abscesses were found, and no enlargement of the regional lymph-nodes exceeding the size of a pea.

Discussion

The most significant finding from the present study is that the progressive decrease in dosage of BCG, obtained by using successively greater dilutions of vaccine, results in a progressive decrease in the level of post-vaccination allergy. Within the range of dilutions used, from standard to 1/256 standard strength vaccine, all of the vaccinated children showed some degree of acquired sensitivity to tuberculin. There is no evidence from these findings to indicate how post-vaccination tuberculin reactions may be divided into two groups—one called " positive ", presumably indicating a successful vaccination, the other " negative ", indicating an ineffective vaccination. If the wide range of BCG-produced allergy is to be a measure of immunity to tuberculosis, it remains as a most critical problem to determine the nature of the relation between that allergy and immunity.

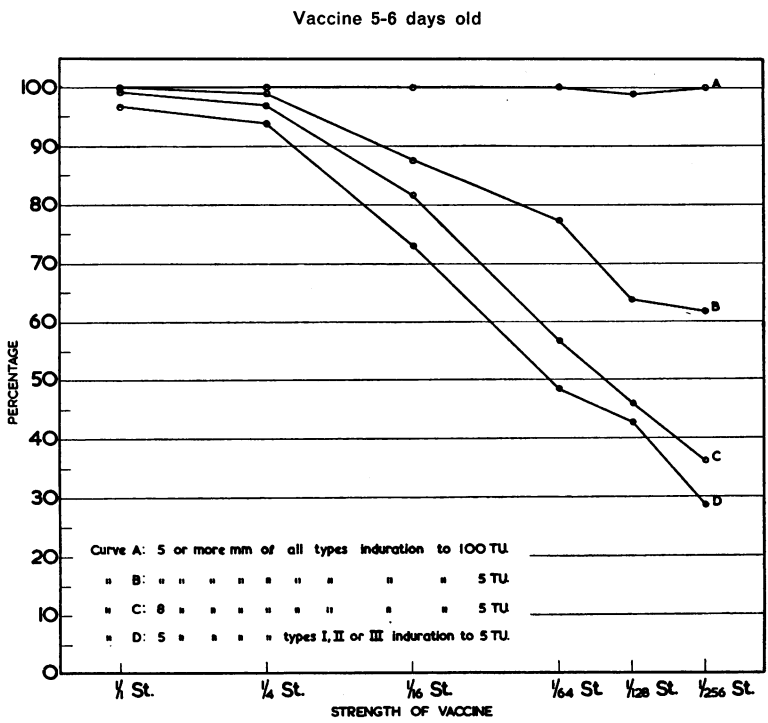
These findings have an important bearing on the methodology of evaluating the allergy-producing capacity of a vaccine. The usual method is to determine the percentage of " positive " reactors among the vaccinated, at specified periods after vaccination. It is worth while considering the theoretical justifications of such a method.

In the unvaccinated, the purpose for using the tuberculin-test is to separate the infected from the non-infected. The classification of reactions into two groups, the " positive " and the " negative ", is based on the assumption that the positive reactors are infected persons, and the negative, uninfected. A division of the range of post-vaccination reactions, however, does not represent a division of the vaccinated into BCG-infected and non-BCG-infected persons, but an arbitrary dichotomy at a specified level of allergy, the significance of which is unknown : a division which, moreover, gives no indication of the distribution of reactions above and below this level.

The inadequacy of evaluating vaccines by the percentage-positive method, as commonly used, may be illustrated by considering the findings of these studies in such terms. Fig. 6 shows for each of the vaccine strengths used in Project IV the percentage of the vaccinated that would be considered positive according to 4 criteria : 5 mm of all types induration to 100 TU, 5 mm of all types induration to 5 TU, 8 mm of all types induration to 5 TU and 5 mm of Types I, II or III induration to 5 TU. The minimum criteria used for curves A and B differ solely with respect to tuberculin dilution used, for curves B and C solely with respect to size of reaction, and for curves B and D solely with respect to density of reaction.

While for the stronger vaccines these changes in criteria make little difference, for the weaker vaccines the effect is striking. For instance, for the 1/256 standard strength vaccine the frequency of " positive " reactors according to the four criteria are 100%, 62%, 36%, and 29%. It is apparent

FIG. 6. PROJECT IV. FREQUENCY OF " POSITIVE " REACTORS ACCORDING TO FOUR DIFFERENT CRITERIA FOR A " POSITIVE " REACTION



that, unless both the dosage of tuberculin used and the criterion for a positive reaction are clearly defined, the results may be misleading. Of particular interest is the difference found according to whether Type IV reactions are included or excluded as induration. In practice, some readers consider these soft reactions as true induration while others include them in the " negative " group. Regardless of method of analysis used, it is obviously not sufficient to characterize a reaction solely by size of induration; it is necessary also to define the induration itself.

The efficiency in determining differences between the allergy produced by different vaccines in terms of percentage " positive " depends on the criterion used, and the level of tuberculin sensitivity produced by the vaccines. If a very weak level of allergy is chosen as the minimum criterion for a " positive " reaction, great variation in the allergy-producing capacity of vaccines may be missed. For instance, in this material if reactions of 5 mm of induration to the 100 TU test are considered positive, no difference can be demonstrated between standard strength vaccine and 1/256 of this strength, both giving 100% reactors. In Project II, even using 8 mm of

induration to 10 TU as the criterion, it is not possible to show significant differences in percentage " positive " between standard strength vaccine and 1/8 of this strength.

With the present limited knowledge of the variations in the character of post-vaccination allergy, and of the significance of such variations, it seems advisable to base methods of evaluation on the entire distributions of post-vaccination reactions, both quantitative and qualitative, and not to attempt interpretation of such reactions. Such a method has the additional advantage that significant differences between vaccines may be shown regardless of the level of tuberculin sensitivity produced by the vaccines.

The relation, observed in this study, between dosage of BCG and mean size and density of the post-vaccination reactions suggests the possibility of developing a scale of tuberculin sensitivity against which post-vaccination allergy produced by other vaccines may be matched. The development of such a scale is, however, contingent upon a stable relationship between variation in dosage and post-vaccination allergy, independent of the vaccine used. Further studies are under way to obtain additional information on this important point.

It should be remembered that variation in dosage in this study was carried out without variation in the quantity of vaccine injected. It is possible that the relation of dosage and allergy would be quite different if the quantity of vaccine injected varied with dosage.

ACKNOWLEDGEMENTS

The authors wish to acknowledge their deep indebtedness to Dr. J. H. Holm and Dr. C. E. Palmer who directed these studies ; to Dr. K. Tolderlund who prepared the vaccines and directed the simultaneous laboratory studies ; to Dr. G. Bindslev, the health authorities, and school physicians whose support and co-operation made these studies possible.

Special thanks are due to Dr. J. Guld, Dr. J. Weis Bentzon and the team nurses who carried out the field work, and to cand. act. K. Magnus and the staff who participated in the statistical handling of the data.

TABLE II. PROJECT II. DISTRIBUTION BY SIZE AND BY TYPE OF INDURATION OF MANTOUX 10 TU REACTIONS 8½ WEEKS AFTER VACCINATION

Induration	Strength of vaccine			
	1/1 standard	1/2 standard	1/4 standard	1/8 standard
Size (mm)				
0	—	—	—	—
1	—	—	—	—
2	—	—	—	—
3	—	—	—	—
4	—	—	—	—
5	—	—	—	—
6	—	—	1	—
7	—	—	—	1
8	2	1	4	2
9	2	8	3	8
10	6	7	14	20
11	6	11	16	17
12	13	13	13	12
13	17	20	21	17
14	26	23	26	32
15	24	24	23	12
16	19	25	26	17
17	23	20	25	10
18	22	21	17	16
19	22	5	12	11
20	9	6	9	7
21	4	4	4	1
22	2	2	5	1
23	4	1	2	—
24	3	3	3	—
25	2	—	—	—
26	1	—	—	1
Arithmetic mean	16.0	15.1	15.1	14.2
Standard deviation	3.4	3.2	3.5	3.3
Type				
I	75	63	62	33
II	103	91	110	70
III	26	36	45	75
IV	3	4	7	7
Total	207	194	224	185

Reactions read at 4 days.

TABLE III. PROJECT II. DISTRIBUTION BY SIZE OF INDURATION OF VACCINATION LESIONS 8½ WEEKS AFTER VACCINATION

Induration (mm)	Strength of vaccine			
	1/1 standard	1/2 standard	1/4 standard	1/8 standard
0	—	—	—	—
1	—	—	—	—
2	—	—	—	1
3	—	—	1	5
4	—	2	2	10
5	1	8	15	29
6	7	12	26	32
7	22	19	43	33
8	30	33	50	30
9	24	37	31	24
10	33	36	20	11
11	24	15	19	6
12	24	11	9	6
13	17	10	7	1
14	6	8	5	1
15	8	5	1	—
16	6	2	—	—
17	1	1	—	—
18	1	—	—	—
19	4	—	1	—
20	2	1	—	—
21	—	1	—	—
22	1	—	—	—
Total	211	201	230	189
Arithmetic mean	10.6	9.5	8.4	7.2
Standard deviation	3.1	2.7	2.3	2.2

TABLE IV. PROJECT IV. DISTRIBUTION BY SIZE AND BY TYPE OF INDURATION OF MANTOUX 5 TU REACTIONS 9½ WEEKS AFTER VACCINATION

Induration	Vaccine 5-6 days old (Reactions read at 4 days)						Vaccine 16-17 days old (Reactions read at 3 days)			
	Strength of vaccine						Strength of vaccine			
	1/1 St.	1/4 St.	1/16 St.	1/64 St.	1/128 St.	1/256 St.	1/1 St.	1/4 St.	1/64 St.	1/256 St.
Size (mm)										
0	—	1	2	3	7	7	—	1	1	—
1	—	—	—	3	8	5	—	—	2	2
2	—	—	—	3	6	9	—	—	2	6
3	—	—	5	4	4	6	—	—	9	11
4	—	—	3	9	8	9	—	—	4	11
5	1	—	1	7	7	8	—	—	2	4
6	—	—	1	4	4	8	—	1	1	6
7	—	2	3	9	5	8	—	—	7	7
8	3	3	4	6	8	13	—	3	5	2
9	2	3	4	9	7	5	—	2	13	8
10	5	5	9	6	7	3	3	5	8	7
11	8	8	7	8	4	4	4	10	10	5
12	15	17	12	6	5	5	6	6	2	5
13	23	8	15	7	5	2	10	17	12	4
14	9	9	5	3	2	1	13	8	5	8
15	17	14	7	6	1	1	18	10	1	1
16	12	7	—	3	1	—	14	8	2	1
17	10	8	1	—	2	—	8	5	3	—
18	8	8	2	1	—	—	13	11	1	—
19	3	3	—	—	—	—	6	1	2	—
20	3	1	1	—	—	—	7	1	—	—
21	1	—	—	—	—	—	1	1	—	—
22	—	—	—	—	—	—	1	—	—	—
23	1	—	—	—	—	—	—	—	—	—
Arithmetic mean	14.2	13.6	10.7	8.4	6.8	6.0	15.6	13.7	9.4	7.6
Standard deviation	3.0	3.3	4.0	4.4	4.5	3.6	2.7	3.3	4.4	4.1
Type										
I	26	9	2	—	1	1	8	7	—	—
II	77	53	30	15	11	10	88	71	30	20
III	14	29	28	32	27	16	8	10	35	32
IV	4	5	20	47	45	60	—	1	26	36
No induration	—	1	2	3	7	7	—	1	1	—
Total	121	97	82	97	91	94	104	90	92	88

TABLE V. PROJECT IV. DISTRIBUTION BY SIZE AND BY TYPE OF INDURATION OF MANTOUX 100 TU REACTIONS 9½ WEEKS AFTER VACCINATION *

Induration	Vaccine 5-6 days old (Reactions read at 3 days)						Vaccine 16-17 days old (Reactions read at 4 days)			
	Strength of vaccine						Strength of vaccine			
	1/1 St.	1/4 St.	1/16 St.	1/64 St.	1/128 St.	1/256 St.	1/1 St.	1/4 St.	1/64 St.	1/256 St.
Size (mm)										
0	—	—	—	—	1	—	—	—	—	1
1	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	1
3	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	2
5	—	—	—	—	—	—	—	—	1	1
6	—	—	—	—	—	1	—	—	—	—
7	—	—	—	3	2	—	—	—	—	2
8	—	—	—	—	1	3	—	—	1	4
9	—	—	—	1	—	4	—	—	2	2
10	—	—	1	1	1	2	—	—	4	2
11	—	—	—	4	2	3	—	—	1	1
12	—	—	—	1	6	5	—	—	1	4
13	—	—	2	5	4	5	—	—	—	4
14	—	—	2	3	4	4	—	—	4	—
15	—	1	1	1	5	4	—	—	1	3
16	—	—	2	—	—	—	—	—	—	—
17	—	—	1	—	2	—	—	—	—	—
18	—	—	—	1	2	2	—	—	—	—
19	—	—	—	1	—	1	—	—	1	—
20	—	—	—	1	1	—	—	—	—	—
21	—	—	—	—	—	1	—	—	—	—
22	—	—	—	—	—	—	—	—	—	—
23	—	—	—	—	—	—	—	—	—	—
24	—	—	—	—	—	1	—	—	—	—
Arithmetic mean	—	—	14.2	12.5	12.9	12.8	—	—	11.5	9.4
Type										
I	—	—	—	—	—	—	—	—	—	—
II	—	1	4	5	13	6	—	—	1	1
III	—	—	4	11	10	22	—	—	9	17
IV	—	—	1	6	7	8	—	—	6	8
No induration	—	—	—	—	1	—	—	—	—	1
Total	—	1	9	22	31	36	—	—	16	27

* Mantoux 100 TU test given to children showing reactions of less than 5 mm induration to the Mantoux 5 TU test.

TABLE VI. PROJECT IV. DISTRIBUTION BY SIZE OF VACCINATION LESIONS 9½ WEEKS AFTER VACCINATION

Lesion (mm)	Vaccine 5-6 days old						Vaccine 16-17 days old			
	Strength of vaccine						Strength of vaccine			
	1/1 St.	1/4 St.	1/16 St.	1/64 St.	1/128 St.	1/256 St.	1/1 St.	1/4 St.	1/64 St.	1/256 St.
0	—	—	—	2	1	8	—	—	1	15
1	—	—	—	4	5	3	—	—	7	8
2	—	—	3	32	25	28	—	—	27	20
3	—	—	14	27	30	35	—	1	30	28
4	—	4	19	22	19	17	—	9	21	13
5	1	14	26	9	8	4	—	21	10	4
6	9	23	10	1	—	1	11	24	4	2
7	27	26	5	3	—	—	17	19	—	1
8	26	9	2	—	—	—	20	7	—	—
9	20	4	1	—	—	—	16	5	—	—
10	15	6	2	—	—	—	15	2	—	—
11	8	1	—	—	—	—	5	1	—	—
12	6	—	—	—	—	—	9	4	—	—
13	3	—	—	—	—	—	5	1	—	—
14	1	2	—	—	—	—	4	—	—	—
15	2	1	—	—	—	—	1	—	—	—
16	1	1	—	—	—	—	5	1	—	—
17	2	—	—	—	—	—	1	—	—	—
18	—	—	—	—	—	—	2	—	—	—
19	—	—	—	—	—	—	1	—	—	—
20	—	1	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	3	—	—	—
23	—	—	—	—	—	—	—	—	—	—
24	—	—	—	—	—	—	—	—	—	—
25	—	1	—	—	—	—	—	—	—	—
Total	121	93	82	100	88	96	115	95	100	91
Arithmetic mean	8.9	7.4	4.8	3.1	3.0	2.7	10.1	6.6	3.1	2.5
Standard deviation	2.3	3.2	1.6	1.3	1.1	1.2	3.6	2.2	1.3	1.6

SUMMARY

A series of field and laboratory studies of BCG vaccine were begun in the fall of 1949, as a co-operative undertaking of the Danish Statens Seruminstitut, the Joint Enterprise, and the WHO Tuberculosis Research Office. The present paper, the second of this series, reports the findings of two studies dealing with the effect of variation in dosage of BCG vaccine on allergy production and vaccination lesions. The studies were carried out among rural schoolchildren, 6-14 years of age, altogether 2,288 children being tuberculin-

RÉSUMÉ

Une série d'études relatives au vaccin BCG ont été amorcées, sur le terrain et en laboratoire, en automne 1949 ; il s'agissait d'un travail entrepris conjointement par le Statens Seruminstitut de Copenhague, l'Œuvre Commune et le Bureau de Recherches sur la Tuberculose de l'OMS. Le présent article, le deuxième de la série, expose les résultats de deux études dont l'objet était de déterminer les effets que peut avoir, sur l'apparition de l'allergie et sur les lésions vaccinales, la variation de la concentration du vaccin

tested, 1,862 vaccinated, and 1,766 examined and retested approximately nine weeks later.

In the first of these studies, vaccine 7-9 days old was used in standard (0.75 mg/ml), 1/2, 1/4 and 1/8 standard strengths, approximately 200 children being vaccinated with each strength ; 8½ weeks later all the vaccinated children reacted with at least 6 mm of induration to a Mantoux test with 10 TU. The mean size of reactions decreased from 16.0 to 14.2 mm with decrease in dosage, the reactions became less dense, and the mean size of induration of vaccination lesions decreased from 10.6 to 7.2 mm.

Because of the limited effect on tuberculin sensitivity of decreasing the dosage of vaccine from standard strength to 1/8 of this strength, a second study was carried out using a wide range of dosages from standard to 1/256 standard strength, the vaccine being used when 5-6, and 16-17 days old. Approximately 100 children were vaccinated with each vaccine strength at each vaccination period. At the post-vaccination examination 9½ weeks later, children failing to react with at least 5 mm of induration to the 5 TU test were given 100 TU. The proportion receiving the 100 TU test increased from 0 % after the standard strength vaccine to about 40 % after the 1/256 standard strength.

With decreasing dosage the mean size of reactions to the 5 TU test decreased from about 15 mm to about 7 mm, and the reactions became less dense. For those

BCG. Ces études ont été effectuées sur des enfants âgés de 6 à 14 ans et fréquentant les écoles rurales. Au total, 2.288 enfants ont été soumis à une épreuve à la tuberculine ; 1.862 d'entre eux ont été vaccinés, 1.766 ont été examinés et soumis à une épreuve tuberculinique postvaccinale environ neuf semaines plus tard.

Pour la première de ces études, on a utilisé des vaccins âgés de 7 à 9 jours et présentant les degrés de concentration suivants : concentration standard (0,75 mg/ml), la moitié, le quart et le huitième de la concentration standard. Avec chacune de ces concentrations, on a vacciné approximativement 200 enfants ; 8 ½ semaines plus tard, tous les enfants vaccinés ont réagi par des indurations d'au moins 6 mm au Mantoux 10 UT. On a constaté que l'emploi des concentrations décroissantes donnait lieu à des réactions moins palpables et dont la dimension moyenne allait en diminuant (de 16,0 à 14,2 mm), de même la dimension moyenne de l'induration des lésions vaccinales (de 10,6 à 7,2 mm).

En raison de la faible différence observée dans les réactions à la tuberculine provoquées par la concentration standard du vaccin, d'une part, et par sa dilution au huitième, d'autre part, on a procédé à une seconde étude, pour laquelle on a utilisé des vaccins âgés de 5 à 6 jours et de 16 à 17 jours, à des degrés de dilution s'échelonnant entre la concentration standard et 1/256 de celle-ci. Lors de chaque série de vaccinations, chacune de ces dilutions a été administrée à une centaine d'enfants. Les enfants qui, au moment de l'examen postvaccinal effectué 9 ½ semaines plus tard, n'avaient pas réagi par une induration d'au moins 5 mm à l'épreuve à 5 UT, ont été soumis à une nouvelle épreuve à 100 UT. La proportion des enfants qui ont subi l'épreuve à 100 UT a varié entre 0 %, pour ceux qui avaient reçu le vaccin de concentration standard, et 40 %, pour ceux qui avaient reçu le vaccin à 1/256 de la concentration standard.

Parallèlement à la baisse du degré de concentration, on a observé une diminution de la dimension moyenne des réactions à l'épreuve à 5 UT (de 15 mm environ

receiving the 100 TU test the post-vaccination reactions were strikingly at variance with those found at the pre-vaccination testing. The mean size of the former varied between 9.4 and 14.2 mm, of the latter, between 0.7 and 2.8 mm.

The reactions to 5 TU were slightly stronger for the vaccine 16-17 days old than for the vaccine 5-6 days old. This was thought to be due to the fact that the post-vaccination tuberculin-tests for the vaccine 5-6 days old were read at four days, and for the vaccine 16-17 days old at three days.

The mean size of vaccination lesions fell with decrease in dosage from about 9 mm to below 3 mm.

The study shows that a progressive decrease in dosage of BCG, obtained by dilution of the vaccine, results in a quantitative diminution of post-vaccination allergy. A corresponding change in the qualitative characteristics of the allergy was also observed. Within the range of dilutions used, from full to 1/256 standard strength, all the vaccinated children showed an effect of vaccination by acquiring some degree of sensitivity to tuberculin. There is no evidence from the study to indicate how to separate " positive " from " negative " post-vaccination tuberculin reactions. If the wide range of BCG-produced allergy is to be a measure of immunity to tuberculosis, it remains as a most critical problem to determine the nature of the relation between that allergy and immunity.

à 7 mm environ), les réactions étant aussi moins palpables. Chez les enfants soumis à l'épreuve à 100 UT, les réactions post-vaccinales ont été extrêmement différentes de celles qui avaient été enregistrées lors de l'épreuve tuberculinique prévaccinale. Dans celle-ci, la dimension moyenne de la réaction était comprise entre 0,7 et 2,8 mm, alors que, dans l'épreuve postvaccinale, elle a varié entre 9,4 et 14,2 mm.

Les réactions à l'épreuve à 5 UT ont été légèrement plus fortes avec du vaccin âgé de 16 à 17 jours qu'avec du vaccin de 5 à 6 jours, ce que l'on attribue au fait que, lors de l'emploi du vaccin de 5-6 jours, la lecture des résultats de la réaction tuberculinique postvaccinale a été faite après quatre jours, tandis qu'elle a été effectuée après trois jours dans les cas où le vaccin utilisé datait de 16 à 17 jours.

La baisse du degré de concentration a entraîné une diminution de la dimension moyenne des lésions vaccinales (de 9 mm à moins de 3 mm).

L'étude a montré que l'emploi de concentrations décroissantes de BCG, obtenues en diluant le vaccin, se traduit par une diminution quantitative de l'allergie postvaccinale. On a également observé une modification correspondante des caractères qualitatifs de l'allergie. A l'intérieur de la gamme des dilutions utilisées, s'échelonnant entre la concentration standard et 1/256 de celle-ci, tous les enfants ont accusé l'effet de la vaccination en acquérant une certaine sensibilité à la tuberculine. Les résultats de cette étude n'ont pas fourni le moyen de distinguer les réactions tuberculiniques postvaccinales « positives » et « négatives ». Si la gamme étendue des réactions allergiques produites par le BCG doit permettre de mesurer l'immunité à la tuberculose, un problème essentiel reste à élucider : la nature de la relation existant entre l'allergie et l'immunité.