Bull. Org. mond. Santé }
Bull. World Hlth Org. } 1950, 3, 1-24

BCG-VACCINE STUDIES *

1. Effect of Age of Vaccine and Variation in Storage Temperature and Dosage on Allergy Production and Vaccination Lesions Ten Weeks after Vaccination

LYDIA B. EDWARDS, M.D.

Chief of Field Studies, Tuberculosis Research Office, World Health Organization, Copenhagen

ANNA S. GELTING, cand. act.

Statistician, Tuberculosis Research Office, World Health Organization, Copenhagen

Manuscript received in September 1950

One of the most remarkable recent developments in the field of public health has been the widespread use of BCG vaccine for immunization against tuberculosis. Mass campaigns have been carried out on a national basis in several countries, and, under the international auspices of the Joint Enterprise, extensive programmes have been or are being conducted in Europe, Asia, North Africa, and the Middle East, and are now beginning in Latin America. By the end of July 1950, the Joint Enterprise had co-ordinated and directed the tuberculin-testing of approximately 20 million, and the vaccination with BCG of approximately 10 million, children and adolescents.

Despite this extensive worldwide application, the knowledge of BCG vaccine and its immediate and long-range effect is still very limited, and many practical problems so far unsolved have appeared in the course of recent vaccination programmes. Tests and criteria for the selection of candidates for vaccination, which had seemed satisfactory in one area or population, were found unsuitable in another. Variations in BCG vaccines have occurred, and unexplained failures to attain the expected very high percentages of conversions of the tuberculin reaction following vaccination have been reported. Increased demands for BCG have resulted in the establishment of additional laboratories for production, but adequate methods for evaluation and standardization of BCG vaccine are largely undeveloped.

* This is the first of a series of studies on BCG vaccination, planned as a co-operative undertaking of the Joint Enterprise, the Statens Seruminstitut, and the Tuberculosis Research Office, World Health Organization, Copenhagen. 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.

In an attempt to answer some of the most pressing problems of BCG vaccination, a series of studies was planned in the fall of 1949 as a co-operative undertaking of the Joint Enterprise, the Danish Statens Seruminstitut, and the Tuberculosis Research Office of the World Health Organization. The immediate objectives of the work included the development of practical methods for the assaying of BCG vaccine, the determination of the effects of a wide variety of factors on BCG, the comparison of vaccines produced in different laboratories, and the correlation of laboratory assays of BCG with results obtained in vaccinated human populations.

The first project undertaken in this investigation was a study of the effect of age of vaccine on post-vaccination allergy and local lesions, and how this effect might be modified by limited variations in storage temperature and dosage. The present paper, the first report from this co-operative BCG vaccine study, outlines the methodology used in the first project and describes the findings 10 weeks after vaccination.

Methods and Materials

General considerations

A practical method for field evaluation of a vaccine should fit certain requirements. It must be simple in execution and provide dependable results in a limited period of time without requiring the vaccination of an enormous number of individuals. Obviously, the most desirable criteria for assaying vaccines would be in terms of resistance of the vaccinated groups to tuberculous disease and death, but many difficulties, including the necessity for accurate diagnosis and long-term follow-up, render such methods impractical for routine field evaluation. Accordingly, the allergy produced by the vaccine and the local vaccination reactions have been chosen for study as an initial approach to vaccine evaluation.

Great emphasis has been laid on uniformity of techniques and operations and on the collection of accurate objective observations as free as possible from personal bias. The concept of " positive " and " negative " results has been avoided. Rather, the whole distribution of tuberculin and vaccination reactions is described in quantitative and qualitative terms. This method was considered to be of major importance since two groups might give similar results when divided into " positives " and " negatives " according to specified criteria, and yet present entirely different gradations of tuberculin sensitivity within the so-called " positive " and " negative " groups.

Plan of first project

A single lot of vaccine was used throughout the project, in the following combinations of dosage and storage temperature :

1. Vaccine of standard strength (0.75 mg/ml) stored constantly at 2-4°C.

2. Vaccine of standard strength (0.75 mg/ml) stored at 20°C (storage during the first 48 hours was at 2-4°C).

3. Vaccine of four times standard strength (3.00 mg/ml) stored constantly at 2-4°C.

Vaccinations were performed at 2- to 4-day intervals over a period of four weeks, the age of the vaccine being from 2 to 29 days (2, 4, 8, 10, 12, 15, 17, 19, 22, 24, 26, 29 days). Each of the three vaccine combinations described above was used each vaccination day on essentially comparable groups of individuals. (On the first vaccination day only the standard strength and the four times standard strength vaccine, both kept at 2-4°C, were used.)

Additional groups were vaccinated with 8-day-old vaccine of standard and four times standard strength, stored at 37°C for short periods of 2 days and 5 days. These groups will be discussed separately.

At the same time that the vaccine was used in the field, laboratory studies on the same lot of vaccine kept under similar conditions were carried out under the direction of Dr. K. Tolderlund in the Calmette Laboratory of the Statens Seruminstitut.

The pre-vaccination testing and vaccinations were carried out in November and December 1949.

The first comprehensive post-vaccination examinations were made 70-74 days after the vaccination. Tuberculin-tests were repeated and observations made on the reactions at the vaccination site and the regional lymph-nodes. Similar examinations are to be carried out at one year and at subsequent periods after vaccination.

Preparation and handling of the vaccine

The BCG vaccine used was part of the regular lot (No. 869) prepared 14 November 1949 by the Statens Seruminstitut, for routine use in Denmark and for the International Tuberculosis Campaign. An exact description of the vaccine will be included in the laboratory report of this project.

During the first 48 hours after preparation the vaccine remained under refrigeration. Some of the ampoules of standard strength vaccine were then transferred to an incubator at 20°C, the rest of the ampoules remaining in storage at 2-4°C. On each vaccination day, the vaccine was transported in thermos flasks at the temperature at which it had been stored. All vaccine ampoules were labelled in a code not known to the testing and vaccinating personnel.

Tuberculin

The tuberculin for pre- and post-vaccination testing was part of a lot of purified protein derivative (PPD) prepared by the Statens Seruminstitut (No. RT XIX-XX-XXI), and used in the international mass campaign from 1 April 1948 to 20 February 1950. Fresh dilutions were prepared

each week at the Institute and kept in the field under constant refrigeration. The dilutions used contained 10 tuberculin units (0.0002 mg) and 100 tuberculin units (0.002 mg) per 0.1 ml.

Composition of group studied

The investigation was carried out among the pupils of the municipal schools of a rural district of Denmark. The area is a homogeneous one composed of small farms, moorlands, villages, and small hamlets. The largest village in the area studied numbered about 900 inhabitants. The schools varied in size from 7 to 141 pupils. The age-span of the children examined was small, more than 99% of the children being born in the years 1935 to 1942 inclusive. The prevalence of tuberculin sensitivity was low, only 7.5% giving reactions of 6 or more mm of induration to the Mantoux 10 tuberculin unit (TU) test. Less than 1% of the non-reactors to Mantoux 10 TU gave reactions larger than 10 mm to the 100 TU test.

The extent of the study was as follows : [a]

Number of participating schools		90
Number of children registered as pupils in these schools		4,220
Number of children tested (Mantoux 10 TU test given and read)		4,074
Previous BCG	296	
No previous BCG—reactors [b]	283	
No previous BCG—non-reactors	3,495	
Number of children vaccinated		3,451
Number of vaccinated children retested at 10 weeks		3,270

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

Method of selection of comparable groups for vaccination

The participating schools were first divided into twelve groups, for vaccination on 12 different vaccination days. Each group contained approximately the same number of children and comprised six to nine schools, selected to give the widest possible geographical coverage compatible with convenient travel routes, day and hour of school attendance, etc. The groups were distributed at random over the study area. The schools in each group were subdivided into three subgroups [c] for vaccination with the three vaccine combinations,[d] each subgroup containing approximately the same number of children.

The composition of the 35 vaccination groups (three vaccine combinations on each of 11 vaccination days and two on the first) is given by age and sex in table IV. Variations in the age and sex distribution are present but there is no systematic variation with the

[a] The groups vaccinated with vaccine stored at 37°C not included.

[b] 6 or more mm of induration to Mantoux 10 TU

[c] For vaccination with 2-day-old vaccine, the schools were subdivided into only two subgroups, vaccine stored at 20°C not being used this day.

[d] In subsequent projects two of the vaccines under study were used on alternate non-reactors in each school.

factors under study. The frequency of reactors to the pre-vaccination Mantoux 10 TU test varied to some extent between the individual subgroups, but the variation was not systematic with age of vaccine. In general, the prevalence of reactors is so low in the area studied that, for practical purposes, natural infection need not be regarded as a significant factor in the production of allergy in vaccinated subjects tested 10 weeks after vaccination.

Organization of the field work

The field work was carried out under the auspices of the Statens Serum-institut, with the active co-operation of the County Tuberculosis Control Officer and the school physicians.

The pre-vaccination tuberculin-testing and vaccination schedule was as follows :

On the first visit the Mantoux test with 10 TU was given.

On the second visit 3 or 4 days later, the Mantoux 10 TU test was read. Children who failed to give reactions of at least 6 mm of induration were vaccinated and given a Mantoux test with 100 TU.

On the third visit 3 or 4 days later, both tuberculin-tests were read, and an examination was made of the site of vaccination.

The schedule of the post-vaccination examination 10 weeks later was the same as regards tuberculin-testing. In addition, on the first visit an examination was made of the vaccination site, and of the regional and contralateral lymph-nodes. No revaccinations were performed.

Throughout the project each technical operation was carried out by the same person ; only in rare instances of illness was a substitution necessary. All observations were made without knowledge of the previous history of the individual as regards vaccination, and of previous or other current findings. The recording of all observations on the record card was made by a secretary at the dictation of the observer.

Tuberculin-testing and vaccination techniques

The Mantoux test was given in the middle third of the dorsal aspect of the forearm as is the custom in Denmark. In reading the test careful measurements were made of the widest transverse diameter of the erythema and of the induration. In addition, the density of the reaction was classed as one of four types.[e]

Type I was used to describe a very dense reaction, characteristically elevated and usually sharply circumscribed. Type IV at the other end of the scale was used to describe a very soft reaction, one that was barely felt, with poorly defined borders, essentially more a palpable swelling than a definite increase in density. Type II and Type III were used to describe reactions falling somewhere between the two extremes, Type II being nearer to Type I, and Type III being nearer to Type IV.

[e] The pre-vaccination tuberculin reactions of Project I were not classified by type.

Vaccination was performed in the deltoid region of the left shoulder by the intracutaneous method.

In the examination of the vaccination site, measurement was made of the widest transverse diameter of erythema and induration, and the characteristics of the reaction, including ulcers, crusts, etc., were described.

Observations on lymph-node enlargement were made by the team physician who examined the axillary and cervical regions on both sides. The number, size, and characteristics of palpable glands were recorded.

Findings 10 Weeks After Vaccination

Reactions to the Mantoux 10 TU test

Ten weeks after vaccination, regardless of the vaccine used, very nearly 100% of the vaccinated children had become tuberculin reactors, a reactor being defined as one who shows 6 or more mm of induration. Of the 3,270 children retested, only 7, distributed in six different vaccination groups, failed to give such reactions, and 4 of these showed borderline reactions of 5 mm.

Differences in the degree of allergy developed are, however, clearly shown between the various vaccination groups if the whole spectrum of tuberculin sensitivity is taken into account. Since as yet no acceptable combined quantitative and qualitative scale of tuberculin sensitivity has been developed, the distributions by size and by type of reaction are analysed separately.

Size of reaction

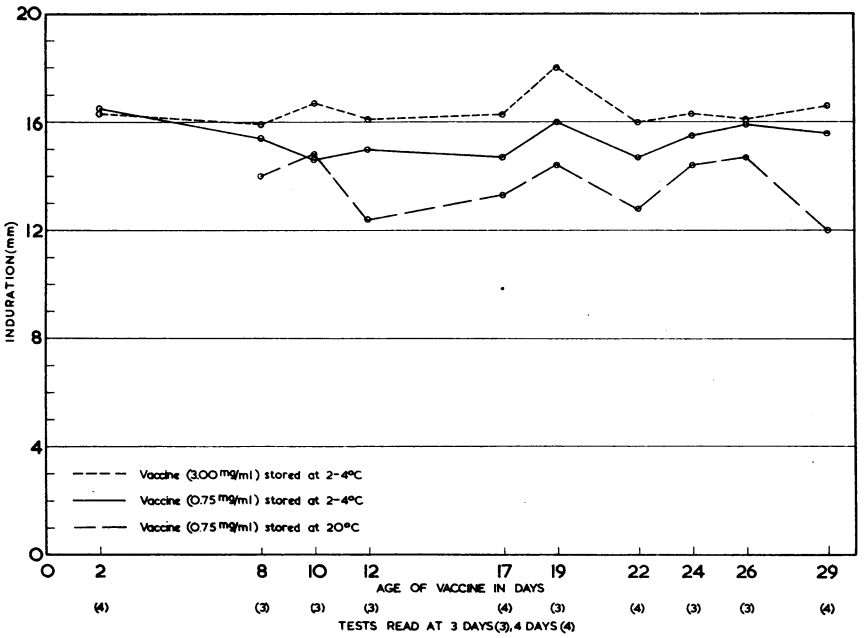
The arithmetic mean of the diameter of induration was selected as the quantitative measure to compare the post-vaccination reactions of the various vaccination groups.

For each of the 35 vaccination groups, the distribution by size of induration of the tuberculin reactions was found (table V). The patterns of these distributions were very similar and may be regarded as approximations to normal distributions (fig. 5). While the standard deviation was not constant, there was no systematic variation according to age of vaccine, storage temperature, or dosage.

Considering first only the effect of the age of the vaccine, no systematic variation in the mean size of reaction is observed with increasing vaccine age over the range from 2 to 29 days (fig. 1).[f] The fluctuations observed for each of the vaccine combinations at different ages are greater than can be explained by random variations, and their cause is not entirely clear. They are not explained on the basis of 3- as compared with 4-day reading

[f] The reading of the tuberculin-tests of the groups vaccinated with 4-day-old vaccine was delayed until the sixth day because of a snowstorm ; the groups vaccinated with 15-day-old vaccine were tested by error with a tuberculin dilution prepared two months previously. These groups have, therefore, been omitted from the analysis of the tuberculin reactions.

FIG. 1. MEAN SIZE OF INDURATION OF MANTOUX 10 TU REACTIONS 10 WEEKS AFTER BCG VACCINATION



of the tests. On two vaccination days (vaccine, 2-day old and 19-day old) a difference in vaccinator has been considered a possible cause. Other factors considered were minor variations in the constitution of the vaccinated groups, and variability in the day-to-day readings of the tests. These difficulties in evaluating the findings are stressed only as a warning against placing too much importance on minor variations.

While vaccine age, within the time limit of this study, had no demonstrable effect on the size of the post-vaccination tuberculin reactions, both dosage and storage temperature had a definite though limited influence. Increase in dosage was associated with increased size of reaction, and storage at 20°C as compared with 2-4°C, with diminished size of reaction. The arithmetic mean for the groups vaccinated with four times the standard dose of vaccine varied from 15.9 to 18.0 mm ; for the groups vaccinated with standard strength vaccine stored at 2-4°C from 14.6 to 16.5 mm ; and for the groups vaccinated with standard strength vaccine stored at 20°C. from 12.0 to 14.8 mm. The effect of increase in dosage and of storage at room temperature was apparent in the course of the first few days of the study, and the differences between the mean size of reaction for the three vaccine combinations remained essentially constant throughout the period of investigation.

Type of reaction

In general, the tuberculin reactions observed at the 10 weeks' post-vaccination testing were strong, most being classified as Types I or II. Increase in dosage of vaccine resulted in somewhat denser reactions, and storage at room temperature as compared to refrigeration in somewhat less dense reactions, but the differences were not striking (fig. 2).

FIG. 2. DENSITY OF INDURATION OF MANTOUX 10 TU REACTIONS 10 WEEKS AFTER BCG VACCINATION, CLASSED BY TYPES

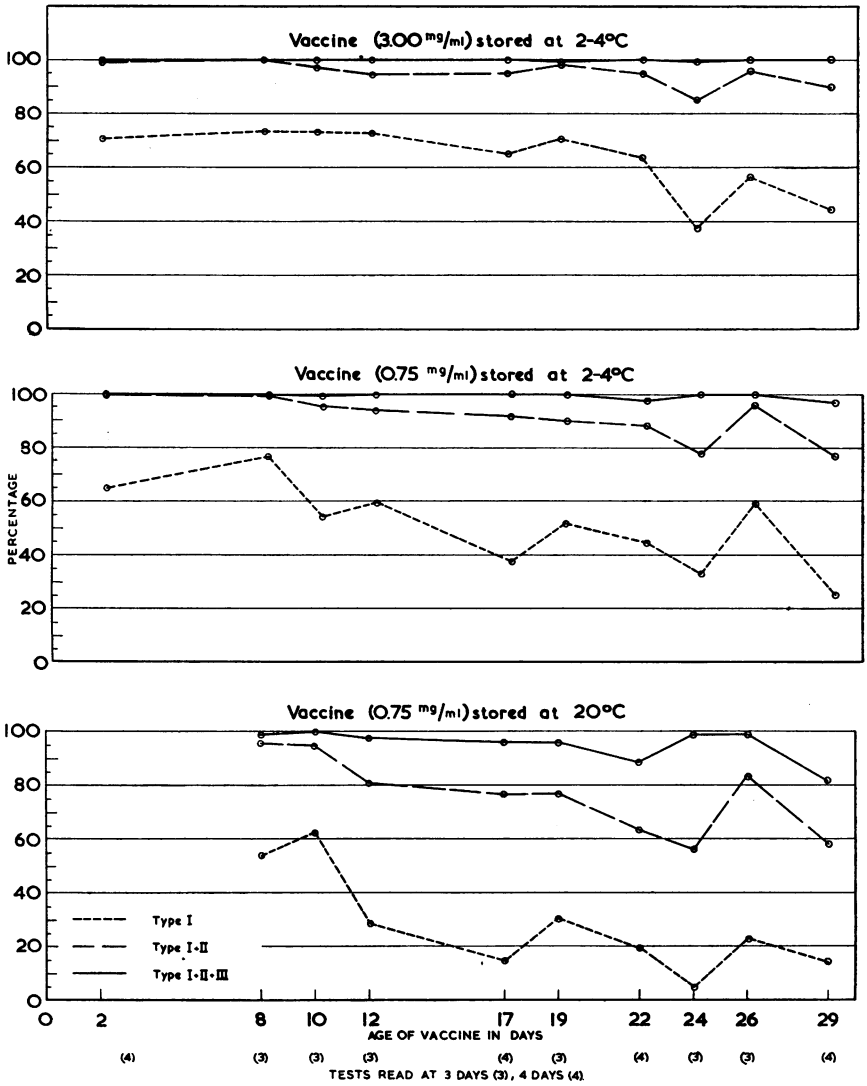
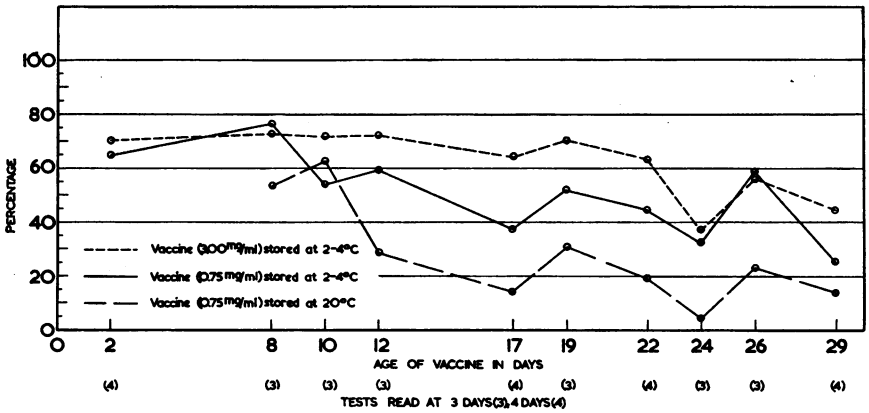


FIG. 3. FREQUENCY OF TYPE I INDURATION OF MANTOUX 10 TU REACTIONS 10 WEEKS AFTER BCG VACCINATION



A most interesting finding which developed from the study of the qualitative character of the post-vaccination reactions was that vaccine age had a demonstrable effect on the density of the reactions, an influence that could not be shown in this study when either percentage reactors or size of reaction was considered. As the vaccine increased in age, the resultant tuberculin reactions tended to become less dense. This change was least apparent with the vaccine of four times standard strength and greatest with standard strength vaccine stored at 20°C. This is shown in fig. 3 where the percentages of Type I reactions for each of the vaccination groups are presented.

The classification of reactions into the four arbitrary categories of density or palpability involves difficult subjective judgements, and stability in classifying reactions can be reached only after long experience. The individual reading the tests for this project was in the process of being trained for the work, and fluctuations in readings on different days are to be expected. Since, however, the reader had no knowledge of the vaccine used, the results for the groups studied on each day can be considered as being comparable.

Comparison of allergy in vaccinated and unvaccinated reactors

In selecting children for vaccination the arbitrary decision was made to consider those showing 6 or more mm of induration to the Mantoux 10 TU test as " natural " tuberculin reactors. Two hundred and eighty-three unvaccinated children fulfilled this criterion for reactors, and in this group the mean size of induration was 14.7 mm. While the findings for the pre- and the post-vaccination tests are not entirely comparable owing to difference in reader, it is apparent that the post-vaccination reactions approximated in average size to those seen in the natural reactors.

A comparison cannot be made of the qualitative aspects of the reactions since the pre-vaccination reactions of Project I were not classified by type. However, in subsequent projects, Types I and II together constituted from 80% to 94% of the total number of reactions observed in the unvaccinated "natural" reactors, findings not unlike the cumulative frequencies of these two types in the post-vaccination tests of Project I. It is of interest that the frequency of bullous and vesiculated reactions was about the same for the 283 unvaccinated reactors (8.1%) and the 939 children vaccinated with the fourfold dose (9.3%). Such reactions were much less frequent with standard strength vaccine stored at 2-4°C (3.1% among 985 vaccinated children) and fewest with the vaccine stored at 20°C (1.0% among 836).

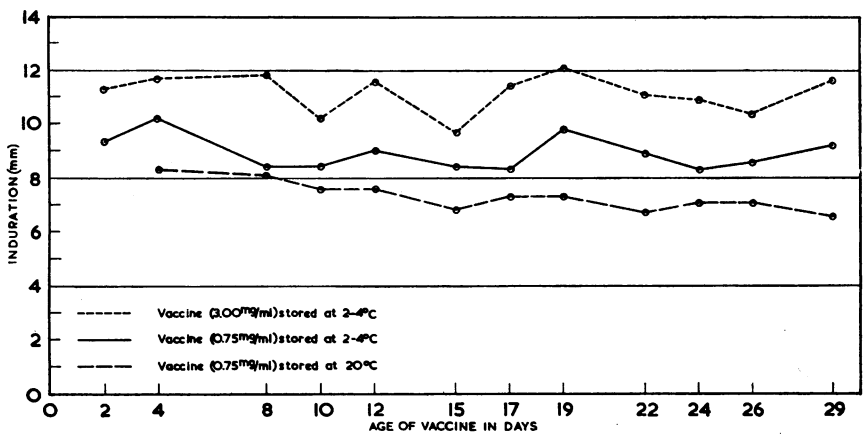
Local vaccination lesions

The size of the vaccination lesion has been defined as the size of the induration at the vaccination site, or in the few instances where induration was absent or impossible to measure accurately, as the size of the scab, ulcer, or scar.

The distribution by size of vaccination lesion, as well as the arithmetic mean and standard deviation is given in table VI for each of the 35 vaccination groups.[g] All these distributions differ systematically from a normal distribution by showing rightsided skewness. This skewness is not very marked, however, and shows no systematic variation with the factors under consideration. The standard deviation of the distribution was greatest for the stronger dose, and least for the standard strength vaccine stored at 20°C.

Age of vaccine up to 29 days has no apparent effect on the size of the vaccination lesion for vaccine of four times standard strength and standard strength vaccine stored at 2-4°C (fig. 4). For the standard strength vaccine

FIG. 4. MEAN SIZE OF VACCINATION LESIONS 10 WEEKS AFTER BCG VACCINATION



[g] The number of vaccination lesions examined exceeds by 74 the number of tuberculin reactions read.

stored at 20°C there seems to be a very slight tendency for the vaccination lesion to decrease in size with increasing age of vaccine.

Vaccine of four times standard strength gave larger local reactions than the standard strength vaccine, and storage at 20°C resulted in a decrease in size of lesion.

TABLE I. FREQUENCY OF UNHEALED ULCERS AND LOCAL ABSCESSES, 10 WEEKS AFTER BCG VACCINATION

Vaccine	Number of children examined	Unhealed ulcers			Local abscesses	
		Number	%	Mean size (mm)	Number	%
4 times standard strength (3.00 mg/ml) stored at 2-4°C	1,131	25	2.2	8.0	3	0.3
Standard strength (0.75 mg/ml) stored at 2-4°C	1,172	11	0.9	6.1	3	0.3
Standard strength (0.75 mg/ml) stored at 20°C	1,041	13	1.2	4.5	1	0.1

Frequency of inconvenient responses associated with vaccination

A practical consideration is the frequency of inconvenient responses to vaccination in relation to the factors under study. Such responses include ulcers persisting at 10 weeks, abscesses at the vaccination site, and enlargement and suppuration of the regional lymph-nodes. The very low frequency of such responses permits the calculation of satisfactory rates only for the three vaccine combinations without regard to age of vaccine. With the vaccine of four times standard strength, 2.2% of the children still showed unhealed ulcers 10 weeks after vaccination as compared with about 1% after standard strength vaccine (table I). The size of the ulcers was largest after the fourfold dose with a mean size of 8.0 mm, and smallest with vaccine stored at 20°C, mean size 4.5 mm. Abscesses at the vaccination site were rare in all groups (7 among the total group of 3,344 children examined).

In considering lymph-node enlargements, the tabulation presented in table II includes all enlargements classified as nut-sized or larger. The findings are presented for both right and left sides. It should be noted that the frequency of enlarged lymph-glands on the right side is uniformly low (0.2%, 0.3% and 0.2%). On the left, the side of vaccination, a marked difference is present according to the vaccine combination used. Almost 25% of those vaccinated with the fourfold dose showed enlarged glands, as compared with about 7% of those vaccinated with standard strength vaccine stored at 2-4°C, and 3% for the standard strength vaccine stored at 20°C. The enlarged glands were almost entirely found in the axillary area and were mainly classed as nut-sized. Glandular suppuration was

extremely uncommon with standard strength vaccine (only 3 among 2,213 vaccinated children), but somewhat more frequent with four times the dose (2.1%). It is of considerable interest that many of the walnut-sized glands with definite fluctuation were apparently asymptomatic, children, parents, and teachers being unaware of their presence.

Study on Effect of Storage of Vaccine at 37°C

To obtain preliminary information on the effect of short periods of storage of vaccine at a relatively high temperature, several small groups of children were given standard and four times standard strength vaccine stored for 2 and for 5 days at 37°C. The investigation was carried out in the same area, in similar schools and on children of the same age-group as in the study previously described.

The size of the vaccination groups was as follows :

	Number vaccinated	*Number retested*
Standard strength vaccine (0.75 mg/ml) stored at 37°C for 2 days	85	81
Standard strength vaccine (0.75 mg/ml) stored at 37°C for 5 days	102	100
Four times standard strength vaccine (3.00 mg/ml) stored at 37°C for 2 days	70	61
Four times standard strength vaccine (3.00 mg/ml) stored at 37°C for 5 days	59	57

The vaccine was from the same lot as was used in the previous studies (lot No. 869). During the first 48 hours after preparation it was stored at 2-4°C. Ampoules of both standard and four times standard strength were then transferred to storage at 37°C, some for 2, some for 5 days, then returned to refrigeration.

The vaccine was used when 8 days old, on one of the days that vaccination was performed with the three vaccine combinations in the main study. The post-vaccination tests were, however, made at 65 days, instead of at 74 days as in the main project and the findings, therefore, are not entirely comparable.

All the children in these groups gave post-vaccination tuberculin reactions of 7 or more mm of induration to the 10 TU test (table VII). These reactions approximated in size to those observed when vaccines of the same strength stored at 2-4°C. were used, but they were less dense in character. The vaccination lesions, although examined nine days earlier, were only slightly smaller in size than those observed in the main project (table VIII). The frequency of inconvenient responses is presented in table III. Apparently storage of vaccine for short periods at 37°C has only a relatively slight effect on early vaccination lesions and allergy.

TABLE II. LYMPH-NODE ENLARGEMENTS AND ABSCESSES OBSERVED 10 WEEKS AFTER BCG VACCINATION

	Number						Percentage					
	Vaccine						Vaccine					
	4 times standard strength (3.00 mg/ml) stored at 2-4°C		Standard strength (0.75 mg/ml) stored at 2-4°C		Standard strength (0.75 mg/ml) stored at 20°C		4 times standard strength (3.00 mg/ml) stored at 2-4°C		Standard strength (0.75 mg/ml) stored at 2-4°C		Standard strength (0.75 mg/ml) stored at 20°C	
Number examined	1,131		1,172		1,041		100.0		100.0		100.0	
	Left side	Right side	Left side	Right side	Left side	Right side	Left side	Right side	Left side	Right side	Left side	Right side
Enlarged lymph-nodes non-suppurative :												
Nut size	203	2	72	3	25	2	17.9	0.2	6.1	0.3	2.4	0.2
Date size	24	—	1	—	2	—	2.1	—	0.1	—	0.2	—
Walnut size	25	—	3	—	—	—	2.2	—	0.3	—	—	—
Total enlarged lymph-nodes non-suppurative	252	2	76	3	27	2	22.2	0.2	6.5	0.3	2.6	0.2
Enlarged lymph-nodes suppurative :												
Fluctuant	17	—	2	—	1	—	1.5	—	0.2	—	0.1	—
Incised or perforated	7	—	—	—	—	—	0.6	—	—	—	—	—
Total enlarged lymph-nodes suppurative	24	—	2	—	1	—	2.1	—	0.2	—	0.1	—

TABLE III. STUDY ON EFFECT OF STORAGE OF BCG VACCINE AT 37° C FINDINGS 9 WEEKS AFTER VACCINATION

	Standard strength (0.75 mg/ml) stored at:		4 times standard strength (3.00 mg/ml) stored at:	
	37°C for 2 days	37°C for 5 days	37°C for 2 days	37°C for 5 days
Mantoux 10 TU reactions :				
Number read	81	100	61	57
Arithmetic mean of size of induration (mm)	15.0	14.7	16.0	15.6
Local vaccination reactions :				
Number read	82	102	67	58
Arithmetic mean of size of induration (mm)	8.1	7.1	10.2	10.9
Ulcers (%)	0	0	0	0
Local abscesses (%)	0	0	3.0	0
Lymph-nodes :				
Number examined	82	102	66	58
Enlarged lymph-nodes non-suppurative (%)	7.3	2.0	7.6	12.1
Enlarged lymph-nodes suppurative (%)	0	0	1.5	0

DISCUSSION

It is a commonly accepted view that BCG vaccine should be kept cold, and used within a period of two weeks or less from the date of preparation. Further, when conditions prevent the use of fresh vaccine, it has been suggested that a stronger dose may be used to compensate for the influence of increased age of vaccine. The present investigation was undertaken to obtain more definite information on the effect of dosage and temperature and duration of storage on BCG vaccine.

The results of the post-vaccination examinations at 10 weeks are striking and unexpected. The decrease in allergy with increasing age of vaccine up to 29 days was extremely slight and apparent only in the qualitative character of the tuberculin reactions, not in their size. Storage at 20°C instead of at 2-4°C had surprisingly little effect on the level of allergy produced. Increase in dosage to four times the usual strength resulted in only slight increase in allergy although in a significant increase in inconvenient local and glandular responses.

In considering the significance of these results, certain points should be kept in mind. First, the findings reported are for 10 weeks after vaccination. While this early testing may be expected to reflect the findings a

year later, it is possible that differences will become apparent a year after vaccination which were not detectable at 10 weeks. Moreover, this early testing gives evidence only of a capacity to produce allergy but no information on the durability of the allergy produced.

A second consideration is that the lot of vaccine used in this study was apparently a strong one. Not only did essentially all the vaccinated children become reactors, but their reactions were strong, approximating to those observed in unvaccinated children considered reactors. It cannot be assumed that the effect of vaccine and storage temperature would be similar for a less potent vaccine.

Such considerations should temper with caution the interpretation of the reported results, and confirmation on other lots of vaccine and testing at longer intervals after vaccination should be awaited. The possible implications of these findings are, however, important. On the one hand, BCG vaccination would be a simpler and less expensive procedure if the age limit of the vaccine could be prolonged and refrigeration be proved unnecessary. On the other hand, if it is shown that, within the limits studied, age of vaccine and variations in storage temperature are not responsible for failure of the vaccine to produce conversion, it is urgent that other causes of such failure be sought.

TABLE IV. AGE* AND SEX DISTRIBUTION OF BCG-VACCINATED CHILDREN RETESTED AT 10 WEEKS

Age of vaccine (days)	Vaccine								
	4 times standard strength (3.00 mg/ml) stored at 2-4°C			Standard strength (0.75 mg/ml) stored at 2-4°C			Standard strength (0.75 mg/ml) stored at 20°C		
	Children born		Males	Children born		Males	Children born		Males
	1935 - 38	1939 - 42		1935 - 38	1939 - 42		1935 - 38	1939 - 42	
	%	%	%	%	%	%	%	%	%
2	46.5	53.5	53.5	33.6	66.4	48.6	.	.	.
4	46.8	51.9	41.6	33.9	66.1	58.9	65.5	34.5	52.9
8	51.9	48.1	45.7	48.5	51.5	50.5	38.4	61.6	60.5
10	48.5	50.5	49.5	44.7	55.3	53.4	48.7	50.4	42.5
12	52.3	47.7	51.2	45.1	54.9	53.7	54.2	45.8	47.0
15	56.0	44.0	37.4	22.7	77.3	48.5	30.4	69.6	54.9
17	47.0	53.0	45.2	57.7	42.3	53.2	51.6	48.4	46.0
19	42.6	56.4	53.5	40.0	60.0	52.0	46.2	53.8	61.5
22	43.3	52.2	64.4	48.2	51.8	49.4	55.2	43.7	58.6
24	41.9	57.1	49.5	41.6	58.4	43.4	39.3	60.7	46.4
26	43.7	55.2	42.5	48.5	50.5	51.5	49.5	50.5	49.5
29	47.3	52.7	58.1	37.4	62.6	53.5	54.7	45.3	50.0
Total	47.2	52.0	49.3	42.0	57.9	50.9	48.2	51.6	51.6

* 99.6 % of the children were born between the years 1935 and 1942 inclusive.

TABLE V. DISTRIBUTION BY SIZE AND BY TYPE OF INDURATION OF MANTOUX 10 TU REACTIONS 10 WEEKS AFTER BCG VACCINATION

Induration	Vaccine																	
	4 times standard strength (3.00 mg/ml) stored at 2-4°C												Standard strength (0.75 mg/ml)					
	Age of vaccine (days)																	Age of
	2	4*	8	10	12	15†	17	19	22	24	26	29	2	4*	8	10	12	15†
Size (mm)																		
0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7	1	—	—	—	—	—	—	2	—	1	—	—	—	—	—	—	—	—
8	1	1	—	—	—	—	—	—	—	—	—	—	1	1	2	2	1	—
9	1	—	2	—	1	—	3	1	—	—	—	1	—	—	1	4	—	6
10	3	4	2	1	3	10	4	—	4	4	1	3	5	6	1	7	1	7
11	2	4	6	3	2	4	2	3	1	3	4	2	2	7	6	12	5	12
12	4	2	5	7	4	12	7	2	9	4	1	6	6	4	5	7	4	9
13	10	7	6	8	4	16	6	3	7	3	4	1	12	3	13	10	11	9
14	14	6	7	9	11	14	15	3	12	13	13	5	20	4	17	15	12	17
15	14	11	9	15	12	11	11	8	9	14	16	9	15	8	16	8	15	9
16	7	8	10	8	10	5	11	9	9	13	9	12	13	3	4	13	15	6
17	8	7	9	11	10	7	10	11	16	13	15	9	14	6	10	4	6	7
18	14	4	5	6	10	5	17	13	9	9	10	7	14	4	10	4	3	8
19	5	2	8	7	7	3	13	13	3	11	4	3	13	—	8	6	5	2
20	3	3	3	8	7	1	4	9	3	7	5	7	8	6	2	2	3	1
21	2	5	3	5	4	—	5	8	2	5	4	1	4	1	3	4	1	1
22	5	4	4	5	1	—	3	7	—	3	—	2	4	1	3	1	—	1
23	—	4	1	2	—	2	1	4	1	2	1	2	2	—	1	2	—	—
24	2	1	1	2	—	—	1	1	3	—	—	1	5	—	1	2	—	—
25	1	2	—	1	—	1	—	2	1	—	—	2	1	—	—	—	—	—
26	—	—	—	1	—	—	—	—	—	—	—	1	1	—	—	—	—	1
27	2	1	—	—	—	—	1	2	—	—	—	—	—	—	—	—	—	—
28	2	—	—	—	—	—	—	—	1	—	—	—	—	1	—	—	—	—
29	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
30	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
31	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
Arithmetic mean	16.3	16.7	15.9	16.7	16.1	14.2	16.3	18.0	16.0	16.3	16.1	16.6	16.5	15.2	15.4	14.6	15.0	13.8
Standard deviation	4.1	4.4	3.5	3.5	2.9	3.0	3.6	3.8	3.5	3.2	2.6	3.7	3.5	4.4	3.2	3.7	2.5	3.5
Type																		
I	71	48	59	71	62	44	74	71	58	39	49	33	91	11	79	56	49	42
II	29	27	22	25	19	45	35	28	27	50	34	33	48	37	23	42	28	46
III	1	1	—	3	5	2	6	1	5	15	4	8	1	8	1	4	5	6
IV	—	1	—	—	—	—	—	1	—	1	—	—	—	—	—	1	—	2
No induration	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Total	101	77	81	99	86	91	115	101	90	105	87	74	140	56	103	103	82	97

* Read at 6 days, all other groups read at 3 or 4 days.

† Tested with tuberculin dilution prepared two months previously

Vaccine																
stored at 2-4°C						Standard strength (0.75 mg/ml) stored at 20°C										
vaccine (days)						Age of vaccine (days)										
17	19	22	24	26	29	2	4*	8	10	12	15†	17	19	22	24	26	29
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	1	1
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
—	—	1	—	—	—	.	—	—	—	1	—	1	—	—	—	—	1
—	—	—	—	—	—	.	—	—	—	—	—	—	2	—	—	—	4
—	—	1	—	—	—	.	—	—	—	1	—	1	—	—	—	—	2
—	—	—	1	—	1	.	2	3	1	5	3	2	—	—	1	—	4
2	—	—	1	—	—	.	3	6	5	9	10	7	—	6	—	3	5
6	1	4	1	4	2	.	3	4	9	15	11	17	9	14	7	5	7
10	3	5	6	4	6	.	12	10	8	6	18	15	5	11	7	7	8
13	2	7	9	7	10	.	6	8	11	8	8	11	8	16	8	12	6
9	2	11	11	13	11	.	11	10	6	8	13	13	15	9	7	8	3
24	7	14	18	5	14	.	10	11	14	10	12	18	14	7	13	13	5
6	6	14	13	9	10	.	16	10	12	5	6	10	16	8	15	8	6
10	9	8	15	15	10	.	8	4	10	3	5	8	10	5	9	4	3
7	5	7	12	12	9	.	4	8	13	5	4	5	11	8	5	8	3
7	7	4	9	13	8	.	2	4	9	3	5	8	7	2	3	7	1
9	1	1	5	8	5	.	2	2	5	—	5	5	4	—	3	5	3
5	4	3	3	5	5	.	2	1	5	4	2	2	2	1	4	4	1
1	1	3	3	—	4	.	2	—	1	—	—	—	1	—	2	5	1
2	2	1	4	—	1	.	2	1	2	—	—	1	—	—	—	—	—
—	—	1	1	1	1	.	1	2	—	—	—	—	—	—	—	1	—
—	—	—	—	—	—	.	—	—	1	—	—	—	—	—	—	—	—
—	—	—	1	1	—	.	1	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	2	—	—	—	—	—	—	—	—	—
—	—	—	—	1	1	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	1	—	—	—	—	—	—	—	—
—	—	—	—	1	—	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	1	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
14.7	16.0	14.7	15.5	15.9	15.6	.	14.3	14,0	14.8	12.4	12.9	13.3	14.4	12.8	14.4	14.7	12.0
3.1	2.9	3.2	3.1	3.4	3.6	.	3.4	3.8	3.6	3.3	3.1	3.2	2.9	2.6	2.9	3.6	4.0
42	26	38	37	59	25	.	30	46	71	24	17	18	32	17	4	21	9
60	19	37	51	36	51	.	48	36	36	43	55	77	48	38	43	55	28
9	5	8	25	4	20	.	8	3	6	14	28	24	20	22	36	14	15
—	—	2	—	—	3	.	1	1	—	2	2	5	4	10	1	1	12
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
111	50	85	113	99	99	.	87	86	113	83	102	124	104	87	84	91	64

TABLE VI. DISTRIBUTION BY SIZE OF INDURATION OF VACCINATION LESIONS 10 WEEKS AFTER BCG VACCINATION

Induration (mm)	Vaccine																	
	4 times standard strength (3.00 mg/ml) stored at 2-4°C												Standard strength (0.75 mg/ml)					
	Age of vaccine (days)												Age of					
	2	4	8	10	12	15	17	19	22	24	26	29	2	4	8	10	12	15
0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1	—	—	1
5	3	—	—	2	—	1	1	1	1	—	—	1	7	7	4	9	3	8
6	3	—	3	5	1	4	2	4	4	—	3	—	13	5	12	15	5	15
7	8	7	2	7	2	11	4	—	3	5	10	—	17	3	22	19	9	20
8	16	13	5	11	10	21	14	10	6	12	14	8	30	10	27	21	23	22
9	4	4	2	6	7	7	2	5	9	5	8	5	11	3	8	12	12	4
10	21	21	23	30	14	24	33	27	22	32	22	20	30	11	16	12	17	18
11	9	3	10	12	13	6	14	12	9	16	5	5	9	5	6	5	3	2
12	9	8	9	17	11	12	16	7	16	19	14	11	12	8	2	5	6	8
13	1	—	4	1	5	1	4	5	2	3	5	5	2	1	—	2	2	—
14	9	9	6	3	10	—	10	4	10	2	4	4	9	2	1	3	3	—
15	7	10	8	3	4	4	10	12	4	9	2	9	3	3	3	1	1	1
16	11	1	4	2	4	2	3	5	3	1	1	3	1	2	1	1	—	—
17	—	—	—	—	—	—	—	—	1	—	—	1	—	—	—	—	—	—
18	—	1	3	1	4	—	2	2	—	1	2	1	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—
20	5	4	—	—	1	1	2	3	—	1	1	1	1	3	—	—	—	1
21	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
22	1	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
23	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
24	—	1	—	—	—	—	1	3	—	—	—	—	—	1	—	—	—	—
25	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
26	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—
27	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
29	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—
31	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Total	107	84	81	100	86	94	118	103	91	106	92	74	146	65	103	105	84	101
Arithmetic mean	11.3	11.7	11.8	10.2	11.6	9.7	11.4	12.1	11.1	10.9	10.4	11.6	9.3	10.2	8.4	8.4	9.0	8.4
Standard deviation	3.7	3.9	3.2	2.4	2.9	2.6	3.0	4.5	2.9	2.4	2.9	2.8	2.7	4.1	2.2	2.4	2.1	3.4

Vaccine																	
stored at 2-4°C						Standard strength (0.75 mg/ml) stored at 20°C											
vaccine (days)						Age of vaccine (days)											
17	19	22	24	26	29	2	4	8	10	12	15	17	19	22	24	26	29
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	1	—	1	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	3	1	—	1
—	—	—	1	—	—	.	—	—	—	—	1	—	2	4	3	2	5
—	—	—	2	1	—	.	3	1	3	3	3	6	6	2	7	1	6
5	3	1	5	4	4	.	8	8	13	13	22	15	19	15	11	18	10
18	3	12	21	12	11	.	12	12	31	10	25	27	25	15	16	19	16
24	4	13	28	12	12	.	15	13	19	16	25	23	17	19	14	20	4
24	10	20	22	21	21	.	17	27	18	20	14	32	13	9	17	15	16
11	6	9	4	18	7	.	4	3	4	10	3	4	5	5	6	6	2
21	11	15	12	20	23	.	20	13	13	8	11	11	4	11	9	7	7
5	2	4	3	4	9	.	5	3	3	2	—	1	1	—	2	3	1
5	3	4	11	6	5	.	4	3	4	—	1	3	5	2	1	1	1
—	2	1	1	—	1	.	—	—	—	2	—	1	—	—	—	1	—
2	2	2	1	—	—	.	1	1	5	—	—	—	2	—	1	—	—
2	1	1	3	1	7	.	1	—	—	1	—	1	2	—	1	1	—
—	1	2	2	—	1	.	2	1	—	—	—	1	2	—	—	—	—
—	—	—	—	—	1	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	1	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
—	2	1	—	1	—	.	—	—	—	—	—	—	1	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	.	—	—	—	—	—	—	—	—	—	—	—
117	50	85	116	100	102	.	92	86	113	85	105	125	104	86	89	95	69
8.3	9.8	8.9	8.3	8.6	9.2	.	8.3	8.1	7.6	7.6	6.8	7.3	7.3	6.7	7.1	7.1	6.6
2.1	3.3	2.6	2.6	2.2	2.7	.	2.5	2.4	2.3	2.1	1.7	2.1	3.0	2.3	2.4	2.2	2.2

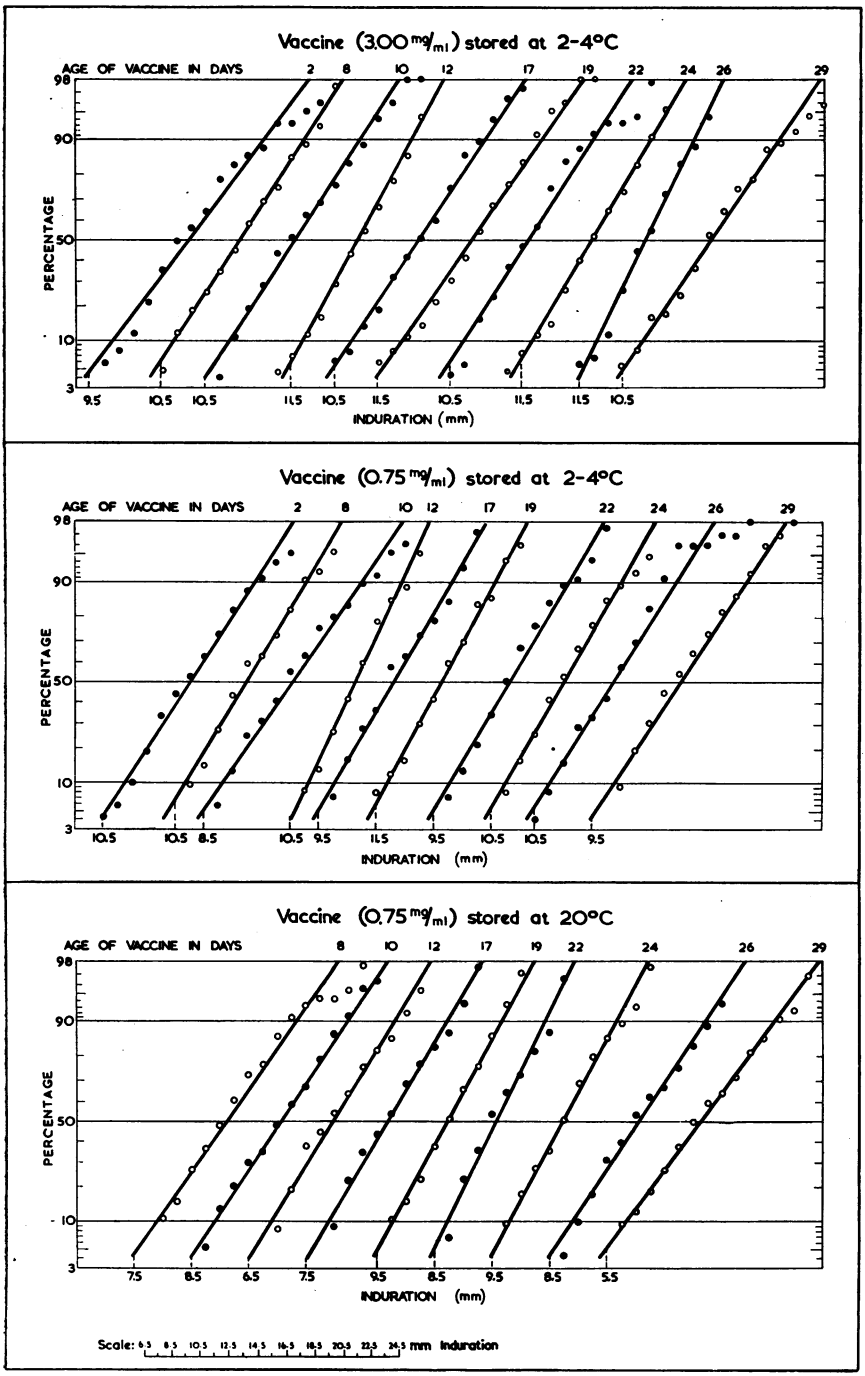
TABLE VII. DISTRIBUTION BY SIZE AND BY TYPE OF INDURATION OF MANTOUX 10 TU REACTIONS 9 WEEKS AFTER BCG VACCINATION

Induration	Vaccine			
	Standard strength (0.75 mg/ml) stored at :		4 times standard strength (3.00 mg/ml) stored at :	
	37°C for 2 days	37°C for 5 days	37°C for 2 days	37°C for 5 days
Size (mm)				
0	—	—	—	—
1	—	—	—	—
2	—	—	—	—
3	—	—	—	—
4	—	—	—	—
5	—	—	—	—
6	—	—	—	—
7	1	1	—	—
8	—	3	—	—
9	2	2	1	2
10	3	4	2	4
11	6	6	6	3
12	7	8	5	4
13	8	12	4	7
14	8	15	3	8
15	14	12	9	6
16	7	10	7	1
17	5	6	5	5
18	10	8	6	3
19	1	7	3	4
20	4	2	3	4
21	3	1	3	2
22	1	1	1	—
23	1	1	—	1
24	—	—	1	1
25	—	—	—	—
26	—	—	—	1
27	—	1	1	—
28	—	—	—	—
29	—	—	—	—
30	—	—	—	—
31	—	—	—	—
32	—	—	—	—
33	—	—	1	1
Arithmetic mean	15.0	14.7	16.0	15.6
Standard deviation	3.3	3.4	4.3	4.5
Type				
I	31	27	28	24
II	40	48	25	27
III	10	24	8	6
IV	—	1	—	—
Total	81	100	61	57

TABLE VIII. DISTRIBUTION BY SIZE OF INDURATION OF VACCINATION LESIONS 9 WEEKS AFTER BCG VACCINATION

Induration (mm)	Vaccine			
	Standard strength (0.75 mg/ml) stored at :		4 times standard strength (3.00 mg/ml) stored at :	
	37°C for 2 days	37°C for 5 days	37°C for 2 days	37°C for 5 days
0	—	—	—	—
1	—	—	—	—
2	—	—	—	—
3	—	—	—	—
4	2	6	—	—
5	4	21	—	2
6	13	23	8	—
7	13	14	5	4
8	25	19	16	9
9	4	1	2	2
10	12	13	17	17
11	—	—	—	1
12	7	2	5	13
13	—	—	1	—
14	2	2	3	2
15	—	1	5	3
16	—	—	1	3
17	—	—	—	—
18	—	—	1	—
19	—	—	—	—
20	—	—	2	—
21	—	—	—	—
22	—	—	1	—
23	—	—	—	—
24	—	—	—	2
Total	82	102	67	58
Arithmetic mean	8.1	7.1	10.2	10.9
Standard deviation	2.1	2.3	3.7	3.6

FIG. 5. CUMULATED DISTRIBUTIONS BY SIZE OF MANTOUX 10 TU REACTIONS 10 WEEKS AFTER BCG VACCINATION



A straight line indicates for each the normal distribution function with the same mean and standard deviation as observed.

ACKNOWLEDGEMENTS

These studies were carried out under the guidance and sponsorship of Dr. J. H. Holm and Dr. C. E. Palmer. The vaccine used was prepared by Dr. K. Tolderlund, who directed the simultaneous laboratory investigations and advised on technical aspects of the problem. Special thanks are due to Dr. Inger M. Thellesen and the nurses who carried out the field work, and to cand. act. K. Magnus and the staff who participated in the collection and statistical handling of the data.

The authors wish to acknowledge their very special indebtedness to Dr. G. Bindslev for his invaluable advice and assistance in the planning and execution of the field programme, and to the local health authorities, school physicians, teachers, and children whose splendid co-operation made this study possible.

SUMMARY

A series of field and laboratory studies of BCG vaccine was begun in the fall of 1949, as a co-operative undertaking of the Statens Seruminstitut, the Joint Enterprise, and the WHO Tuberculosis Research Office. The present paper, reporting results from the first project, deals with the effects of storage up to 29 days of standard strength vaccine kept at 2-4°C and 20°C, and of four times standard strength vaccine kept at 2-4°C.

Altogether 4,074 rural schoolchildren were tuberculin-tested, 3,451 vaccinated, and 3,270 examined and retested ten weeks later.

Irrespective of dosage, or temperature or duration of storage, essentially all the vaccinated children developed tuberculin reactions of 6 or more mm of induration to the Mantoux test with 0.0002 mg purified protein derivative (PPD). Increasing age of vaccine up to 29 days had no demonstrable effect on the size of the resultant tuberculin reactions, but was associated with decreasing density of reactions. Storage at 20°C instead of 2-4°C resulted in slightly smaller and less dense reactions. A fourfold increase in dosage resulted in a small increase in the size and density of the reactions, but also in a significant

RÉSUMÉ

Une série d'études relatives au vaccin BCG ont été amorcées à l'automne de 1949, sur le terrain et en laboratoire ; 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, qui fait état des résultats de la première étude, traite des effets de la conservation, jusqu'à 29 jours, de vaccins d'activité standard maintenus à une température de 2 à 4°C et de 20°C, et d'un vaccin d'activité quadruple, maintenu entre 2 et 4°C.

On a effectué une épreuve à la tuberculine sur un total de 4.074 élèves d'écoles rurales ; 3.451 d'entre eux ont été vaccinés et 3.270 ont été examinés et soumis à une nouvelle épreuve tuberculinique dix semaines plus tard.

Quelle qu'ait été la dose administrée, la température ou la durée de conservation du vaccin, pratiquement tous les enfants vaccinés ont réagi par des indurations de 6 mm ou plus à l'épreuve tuberculinique de Mantoux — effectuée avec 0,0002 mg de dérivé protéinique purifié (PPD). S'il n'a pas exercé d'effet décelable sur la dimension des réactions tuberculiniques, le vieillissement du vaccin jusqu'à 29 jours a eu, néanmoins, pour conséquence de diminuer le degré de palpabilité des indurations. La conservation à 20°C, au lieu de 2 à 4°C, s'est traduite par des réactions légèrement plus petites et moins intenses.

increase in the frequency of inconvenient local and glandular responses.

In addition to the main study, small groups of children were given standard and four times standard strength vaccines stored at 37°C for 2 and for 5 days. Nine weeks later all these children were tuberculin reactors according to current definition, although their reactions were less dense and slightly smaller than those found after vaccination with comparable strengths not subjected to high temperatures.

Even though the results reported suggest that duration and temperature of storage, within the limits studied, have little influence on post-vaccination allergy, two critical limitations must be considered. First, the vaccine used was apparently a very strong one, and it cannot be concluded that similar findings would be obtained with a less potent vaccine. Second, the allergy observed at ten weeks may be relatively temporary, and minor variations at the early testing reflect major differences at subsequent examinations.

L'administration d'une dose quadruple n'a provoqué qu'un léger accroissement de la dimension et de la palpabilité, mais elle a causé une augmentation sensible de la fréquence des réactions locales et ganglionnaires désagréables.

Indépendamment des expériences ayant fait l'objet de l'étude principale, il a été administré à de petits groupes d'enfants des vaccins de concentration standard et de concentration quadruple, conservés à 37°C pendant deux jours et cinq jours respectivement. Neuf semaines plus tard, tous ces enfants présentaient une tuberculino-réaction positive, suivant la définition courante ; toutefois, les indurations furent moins palpables et légèrement plus petites que celles constatées après vaccination avec des concentrations comparables d'un vaccin non soumis à des températures élevées.

Bien que les résultats enregistrés semblent indiquer que la durée et la température de la conservation, dans les limites étudiées, n'ont que peu d'influence sur l'allergie post-vaccinale, il convient de tenir compte, du point de vue critique, de deux éléments de nature à limiter la portée des conclusions. En premier lieu, le vaccin utilisé était apparemment très actif et l'on ne saurait affirmer qu'un vaccin moins actif donnerait des résultats semblables. En deuxième lieu, l'allergie observée après dix semaines peut être relativement temporaire ; de légères variations observées lors du premier examen de contrôle peuvent se traduire par des différences plus importantes lors d'examens ultérieurs.