

CHAPTER 1

SCOPE, METHODS, AND MATERIAL OF THE INVESTIGATION

Character and Extent of the Studies

The investigation reported in this monograph consisted of a series of separate studies, each concerned with one or more problems regarding BCG vaccination. Vaccines prepared, handled, or injected in different ways were given to comparable groups of children after preliminary screening with a tuberculin test. Two or three months after vaccination, and annually thereafter, tuberculin sensitivity was measured and the local vaccination lesions examined.

In order to give a comprehensive view of the scope of the investigation, the distinctive features of each study are summarized in table I (see pages 18-27). The studies have been grouped and numbered according to the principal subject under investigation :

<i>Code letter</i>	<i>Subject of study</i>
A	Storage of vaccine at different temperatures
B	Exposure of vaccine to light
C	Diluting vaccine
D	Dead (heat-treated) vaccine
E	Variations in preparation of vaccine
F	Variations in technique of intracutaneous injection of vaccine
G	Responses at different intervals after vaccination
H	Comparison of vaccines from different production centres

Plan of Studies

Observations of post-vaccination responses may be influenced by many complex variables. Therefore, the effect of changing a particular step in preparing or handling the vaccine was measured in relation to the results obtained when this change was not made. Thus, the effect of sunlight on vaccine was measured in relation to the results with part of the same batch not exposed to sunlight.

TABLE I. CHARACTER AND

Study number	Purpose	Vaccines used *		
	To study the effect of	Batch number	Strength in fractions of standard	Age in days (from harvest)
A I	Temperature and duration of storage of vaccine	869	4/1, 1/1	2-29
			1/1	4-29
			4/1, 1/1	8
A II	Temperature and prolonged storage of vaccine	933	1/1	8, 17-18, 49-50, 71-73, 202
				17-18, 49-50, 71-73
A III	Storage of vaccine at 42°C	964	1/1	12-19
B I	Exposure of vaccine to sunlight in Denmark	959	1/1	21-22
B II	Exposure of vaccine to light (sunlight, skyshine, and indoor daylight) in Egypt	967	1/1	11-13
				20-22
B III	Exposure of vaccine to light (outdoor shade and indoor daylight) in Egypt	970	1/1, 1/16	18-20
		142 †	1/1, 1/16	17-19
		970	1/1	28-36

* When not otherwise specified, all vaccines were produced by the Statens Serum Institut, Copenhagen, and stored at 2-4°C; the standard-strength vaccine contains 0.75 mg BCG organisms per millilitre. Vaccine described as heat-treated was placed in incubator at 80°C for two hours.

† Agousa Serum and Vaccine Institute, Cairo

EXTENT OF THE STUDIES

Further description of vaccine and/or techniques used		Study carried out in	Number vaccinated	Date of vaccination	Re-examinations at
Stored at 2-4°C	Vaccine used every 2-4 days	Denmark	3,766	16/11 to 13/12 1949	10 weeks, one year, two years
Stored at 20°C (first 2 days at 2-4°C)					
Stored at 37°C for 2 and 5 days					
Stored at 2-4°C	Denmark	738	24/2 to 6/9 1951	8 weeks, one year	
Stored at 20°C and 30°C (except for first 8 days and 1-3 days prior to use when stored at 2-4°C)					
Stored at 42°C for 0, 24, and 96 hours	Denmark	317	3/10 to 10/10 1951	9 weeks, one year	
Vaccine in 10-ml clear-glass ampoules exposed to sunlight in Copenhagen for 0, 1, 4, 12, and 23 hours; ampoules kept on ice during exposure	Denmark	287	7/9 to 8/9 1951	8½ weeks	
Heat-treated					
Vaccination carried out: (a) in direct sunlight (10-ml ampoules used up to 35, 75, or 135 minutes) (b) Indoors (10-ml ampoules used up to 30 or 115 minutes)	Egypt	540	23/10 to 25/10 1951	12½ weeks	
Vaccine in 10-ml clear-glass ampoules: (a) exposed in Egypt to sunlight (for 35, 75, or 135 and for 240 minutes) skyshine (for 240 minutes) indoor daylight (for 30 or 115 minutes) (b) stored at Statens Seruminstitut, Copenhagen	Denmark	399	1/11 to 3/11 1951	12 weeks	
Vaccination carried out: (a) outdoors in shade (1-ml clear-glass ampoules used up to 45 or 95 minutes) (b) indoors (1-ml clear-glass ampoules used up to 20 or 105 minutes)	Egypt	485	20/11 to 22/11 1951	12 weeks	
Vaccine in 1-ml clear-glass ampoules: (a) exposed in Egypt to: outdoor shade (for 45 or 95 minutes) indoor daylight (for 20 or 105 minutes) (b) airshipped to Egypt and back (c) stored at Statens Seruminstitut, Copenhagen	Denmark	267	30/11 to 8/12 1951	9 weeks	

TABLE I. CHARACTER AND

Study number	Purpose	Vaccines used *		
	To study the effect of	Batch number	Strength in fractions of standard	Age in days (from harvest)
C I	Diluting vaccine	876	1/1, 1/2, 1/4, 1/8	7-9
C II	Diluting vaccine	Special batch	1/1, 1/4, 1/16 1/64, 1/128, 1/256	5-6
			1/1, 1/4, 1/64, 1/256	16-17
D I	Addition of dead (heat-treated) vaccine to different strengths of living vaccine	912	See column "Further description"	6-8
D II	Addition of small fractions of living vaccine to different strengths of dead (heat-treated) vaccine	932	See column "Further description"	6-8

* See note * on page 18.

EXTENT OF THE STUDIES (continued)

Further description of vaccine and/or techniques used	Study carried out in	Number vaccinated	Date of vaccination	Re-examinations at																																			
	Denmark	837	16/1 to 18/1 1950	8½ weeks, one year, two years																																			
	Denmark	1,025	17/4 to 29/4 1950	9½ weeks, one year, two years																																			
Non-heat-treated and heat-treated vaccine added to make following combinations:	Denmark	500	21/9 to 23/9 1950	9½ weeks, one year, two years																																			
Strength in fractions of standard																																							
<table border="1"> <thead> <tr> <th>Non-heat-treated</th> <th>Heat-treated</th> <th>Total</th> </tr> </thead> <tbody> <tr> <td>1/1</td> <td>0</td> <td>1/1</td> </tr> <tr> <td>1/4</td> <td>0</td> <td>1/4</td> </tr> <tr> <td>1/16</td> <td>0</td> <td>1/16</td> </tr> <tr> <td>1/64</td> <td>0</td> <td>1/64</td> </tr> <tr> <td>1/256</td> <td>0</td> <td>1/256</td> </tr> <tr> <td>1/4</td> <td>3/4</td> <td>1/1</td> </tr> <tr> <td>1/16</td> <td>15/16</td> <td>1/1</td> </tr> <tr> <td>1/64</td> <td>63/64</td> <td>1/1</td> </tr> <tr> <td>1/256</td> <td>255/256</td> <td>1/1</td> </tr> <tr> <td>0</td> <td>1/1</td> <td>1/1</td> </tr> </tbody> </table>					Non-heat-treated	Heat-treated	Total	1/1	0	1/1	1/4	0	1/4	1/16	0	1/16	1/64	0	1/64	1/256	0	1/256	1/4	3/4	1/1	1/16	15/16	1/1	1/64	63/64	1/1	1/256	255/256	1/1	0	1/1	1/1		
Non-heat-treated					Heat-treated	Total																																	
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0	1/4	1/4																																					
	Denmark	522	15/2 to 17/2 1951	10 weeks, one year																																			

TABLE I. CHARACTER AND

Study number	Purpose	Vaccines used *		
	To study the effect of	Batch number	Strength in fractions of standard	Age in days (from harvest)
D III	Diluting vaccine containing a high proportion of dead organisms	975	1/1, 1/10, 1/100, 1/900	6-8
			1/1, 1/10, 1/100	6-8
D IV	Killing (or damaging) vaccines by heat, sunlight, or long storage	Special batch	1/1, 1/10, 1/100, 1/1,000, 1/10,000	15-29
			4/1, 1/1	15-29
			1/1, 1/100	15-29
			4/1, 1/1	15-29
		932	1/1	407-421
E I	Diluent, temperature and duration of storage of vaccine	Special batch	1/1	3-4, 10-11, 17-18, 24-25
	Duration of grinding of vaccine			3-4, 23
E II	Degree of growth of culture at time of harvest	Special batches	1/1	8-9
E III	Age of culture at time of harvest	Special batches	1/1	7
E IV	Age of culture at time of harvest (living and dead—heat-treated—vaccines used)	Special batches	1/1	9-11, 16-18, 19-21, 22-24
				19-21
E V	Transfer period of maintenance culture	Special batches	1/1, 1/16	10-12

* See note * on page 18.

EXTENT OF THE STUDIES (continued)

Further description of vaccine and/or techniques used	Study carried out in	Number vaccinated	Date of vaccination	Re-examinations at
Non-heat-treated	Denmark	331	13/12 to 15/12 1951	8½ weeks
Vaccine containing 1% non-heat-treated and 99% heat-treated organisms				
Not exposed to heat or light	Denmark	753	22/3 to 5/4 1952	7 weeks
Heat-treated				
Exposed to sunlight for 10 minutes				
Exposed to sunlight for 15 hours				
Not exposed to heat or light	Denmark	2,019	6/6 to 28/6 1950	11 weeks, one year, two years
Vaccine prepared in diluent with asparagin (diluted Sauton) and diluent without asparagin, stored at 2-4°C and 20°C				
Vaccine ground for 5 and 25 minutes	Denmark	226	28/9 and 29/9 1950	9½ weeks, one year
Culture flasks classified 11 days after seeding into: (a) those with least growth (b) those with intermediate growth (c) those with most growth Vaccine prepared from flasks of each group				
Cultures (seeded from same maintenance culture) harvested at 8, 11, and 14 days	Denmark	256	30/9 to 6/10 1950	9½ weeks, one year
Cultures (seeded from same maintenance culture) harvested at 8, 11, 14, and 21 days; non-heat-treated and heat-treated (all vaccines used on same days)	Denmark	567	20/9 to 22/9 1951	9 weeks
Culture harvested at 11 days: (a) 10% non-heat-treated and 90% heat-treated (b) 1% non-heat-treated and 99% heat-treated				
Vaccines prepared simultaneously from 11-day harvest of two maintenance cultures transferred for a period of ten months: (a) every 10-11 days (b) every 14 days	Denmark	346	26/2 to 28/2 1951	11 weeks, one year

TABLE I. CHARACTER AND

Study number	Purpose	Vaccines used *		
	To study the effect of	Batch number	Strength in fractions of standard	Age in days (from harvest)
F I	Injecting different volumes of vaccine	929	1/2	6-15
			1/4	6-15
			1/8	6-15
			1/12	6-15
F II	Depth of injection of vaccine	935	1/1	6-8
F III	Depth of injection of vaccine	959	1/1, 1/100	6-15
To compare				
G I	Responses at different intervals after vaccination	927	1/1, 1/16	6-11
			1/1	6-11
H I	Danish, French, Norwegian, and Swedish vaccines with regard to effect of duration of storage	Special batch	1/1	5, 17, 26, 37
			1/2	6, 18, 27, 38
			1/4	7, 28
		—	1/1	5, 17, 26, 37
			1/2	6, 18, 27, 38
		—	1/1	5, 17, 26, 37
			1/2	6, 18, 27, 38
			1/4	7, 28
		—	1/1	5, 17, 26, 37
			1/2	6, 18, 27, 38
			1/4	7, 28

* See note * on page 18.

EXTENT OF THE STUDIES (continued)

Further description of vaccine and/or techniques used	Study carried out in	Number vaccinated	Date of vaccination	Re-examinations at
Injection volume of vaccine: 0.05 and 0.10 ml	Denmark	951	25/1 to 3/2 1951	10½ weeks, one year
0.05, 0.10, and 0.20 ml				
0.10 and 0.20 ml				
0.30 ml				
Depth of injection of vaccine: (a) intracutaneous superficial (b) intracutaneous intermediate (c) intracutaneous deep (d) subcutaneous	Denmark	188	8/3 to 10/3 1951	10½ weeks, one year
Depth of injection of vaccine: (a) intracutaneous superficial (b) intracutaneous intermediate (c) intracutaneous deep (d) subcutaneous	Denmark	486	23/8 to 1/9 1951	10½ weeks, one year

Non-heat-treated	Comparable groups of the vaccinated examined 3½, 6, 8½, 11, and 17 weeks respectively after vaccination	Denmark	2,199	11/1 to 16/1 1951	3½ to 17 weeks, one year
Heat-treated					
<i>Vaccine produced by:</i> Statens Seruminstitut, Copenhagen	<i>BCG content of standard strength vaccine:</i> 0.75 mg/ml	Denmark	2,379	9/2 to 14/3 1950	9½ weeks, one year, two years
Institut Pasteur, Paris	1.00 mg/ml				
Nasjonalforeningens BCG Laboratorium, Bergen	1.00 mg/ml				
Bakteriologiska Laboratoriet, Sahlgrenska Sjukhuset, Gothenburg	0.50 mg/ml				

TABLE I. CHARACTER AND

Study number	Purpose	Vaccines used *		
	To compare	Batch number	Strength in fractions of standard	Age in days (from harvest)
H II	Danish and French vaccines	914	1/1	12
		436	1/1	9
			1/1	9
H III	Danish and Indian vaccines	Special batch	1/1, 1/4	7
		202	1/1, 1/4, 1/16	7
H IV	Danish and Indian vaccines used simultaneously in Denmark and India	Special batch	1/1, 1/10, 1/100	15-17
		248	1/1, 1/10, 1/100	15-17
		Special batch	1/1, 1/10, 1/100	16
		248	1/1, 1/10, 1/100	16
H V	Danish and Mexican vaccines used simultaneously in Denmark and Mexico	Special batch	1/1	10-12
			1/4, 1/16	10-11
		897	1/1	12
		69	1/1, 1/4, 1/16	10-12
		897	1/1	10-11, 24-25
		69	1/1	10-11, 24-25
H VI	Danish and Egyptian vaccines	968	1/1, 1/16	11-13
		140	1/1, 1/16	10-12

* See note * on page 18.

EXTENT OF THE STUDIES (concluded)

Further description of vaccine and/or techniques used		Study carried out in	Number vaccinated	Date of vaccination	Re-examinations at
Statens Seruminstitut, Copenhagen	0.75 mg/ml	Denmark	161	11/10 1950	9 weeks, one year
Institut Pasteur, Paris (liquid vaccine)	1.00 mg/ml				
Institut Pasteur, Paris (freeze-dried vaccine)	1.00 mg/ml				
Statens Seruminstitut, Copenhagen	0.75 mg/ml	Denmark	251	19/4 1950	9 weeks, one year, two years
King Institute, Madras	0.50 mg/ml				
Statens Seruminstitut, Copenhagen	0.75 mg/ml	Denmark	435	22/2 to 24/2 1951	6 weeks, one year
King Institute, Madras	0.50 mg/ml				
Statens Seruminstitut, Copenhagen	0.75 mg/ml	India	396	23/2 1951	6 weeks
King Institute, Madras	0.50 mg/ml				
Statens Seruminstitut, Copenhagen	0.75 mg/ml	Denmark	789	12/6 to 14/6 1950	11 weeks, one year, two years
Statens Seruminstitut, Copenhagen	0.75 mg/ml				
Laboratorio del BCG, Mexico City	0.25 mg/ml				
Statens Seruminstitut, Copenhagen	0.75 mg/ml	Mexico	341	12/6 to 27/6 1950	11 weeks
Laboratorio del BCG, Mexico City	0.25 mg/ml				
Statens Seruminstitut, Copenhagen	0.75 mg/ml	Egypt	515	30/10 to 1/11 1951	8½ weeks
Agouza Serum and Vaccine Institute, Cairo	0.75 mg/ml				

In some studies more than one problem was investigated at the same time. This was done when a greater amount of information could be obtained from one combined study than from separate studies of each of the problems. However, whenever vaccines were used that differed from the control vaccine in two or more ways, vaccines which differed from the control in each way individually were also included.

The essential feature of planning each study was that all variables except the factor being investigated should be randomly distributed so that significant differences between the groups should be due to this factor: the children receiving the different vaccines should be a random sample of the total group vaccinated in the study, and variations in the conditions or techniques of vaccination or retesting should be randomly distributed in all groups.

The application of these principles in the field was limited by certain practical considerations and by the nature of some of the problems studied. As a rule, all vaccines to be compared were given in rotation to all the children vaccinated in each school included in a study; post-vaccination examinations were carried out on the entire group simultaneously and in the same way. In the early period, when the work was carried out in many very small rural schools and the field teams were being trained in the special procedures of the investigation, all children within each school received the same vaccine (or one of two vaccines), but the different vaccines were used in rotation among the schools included in the study. The same procedure was followed when vaccine of the same batch was to be used at different times after preparation, or when the retesting was scheduled for varying times after vaccination. Under these conditions it was not possible to obtain strictly comparable conditions of vaccination or re-examination.

The number of children included in each group varied in the different studies from about 50 to 150. Large groups were used if very small differences were expected, or if detailed information on the frequency distributions of reactions was desired; small groups, if one factor was to be examined at various levels.

General Features of Field Work

The work was done by a small staff divided into teams usually composed of one doctor, two nurses, and one clerk.

Almost all of the doctors and nurses selected for the field teams had had previous experience in the international BCG-vaccination campaigns. Each new recruit worked under close supervision of the team doctor and a seasoned member of the team for the first few months. Although reasonably precise performance was usually obtained within the first two months, stability of operation took longer to be achieved. Team members frequently observed each other's performance, and independent observations of the same reactions by different observers afforded checks on the comparability of performance. The team clerks were trained statistical clerks.

The schedules were planned to give ample time for the work, so that accuracy was not sacrificed for the sake of speed. All procedures were carried out in a prescribed uniform way, and a single batch of tuberculin was used throughout all studies.

Each procedure within a single study was done by one person—one gave the tuberculin, one measured and described the reactions, one vaccinated—so that valid conclusions could be drawn from a reasonably small number of observations.

The intradermal route of administration was chosen for giving BCG and tuberculin because with it a *measured* quantity of vaccine or antigen could be injected, and because the resultant reactions can be described in quantitative terms.

The reactions were not interpreted as “positive” or “negative”, “take” or “no take”; they were simply measured and described. To minimize bias in making the observations the field personnel was kept unaware of the problems under study. The ampoules of vaccine were labelled in code to conceal their identity; and at each examination after vaccination all new children—and annually those previously classed as reactors—were examined, together with the vaccinated children, so that the tuberculin reactions read each day covered a wide range of size and density. The nurse or doctor making the observations did not know whether the child had been vaccinated; the findings were simply dictated to the clerk without opportunity to see the child’s record-card.

Field Facilities

In Denmark (where most of the work has been done) the established programme of tuberculin testing and vaccination in the schools was utilized as the framework into which the investigation was integrated. Through the sponsorship of the tuberculosis control officer of the area, and the co-operation of the school physicians, through-the-year examination of the schoolchildren in a given area (or group of schools) was replaced by tuberculin testing and vaccination within a short period by a single field-team.

Conditions in Denmark are almost ideal for such an investigation. Here BCG vaccination has been practised for many years; the medical profession almost unanimously support it, and the people readily accept it. Since the incidence of tuberculous infection is low, it was possible to obtain large numbers of uninfected children for vaccination, and to observe the development and course of post-vaccination allergy rarely complicated by superimposed tuberculous infection. The population is very stable. There is little migration about the country, or change of school within an area. It was expected—correctly—that co-operation would be excellent.

For the group as a whole, 90% of the children who were offered vaccination accepted, and approximately 90% of the vaccinated still in school participated at each subsequent re-examination. The main loss from the investigation was through children reaching school-leaving age; each year about one seventh of the total number of children reached this age and left school.

The investigation included nearly 42,000 pupils (almost all in the age-group 7-14 years) registered in the municipal schools of three separate areas. In each area all children registered in virtually all the schools were included in the "study population". One area was entirely rural, composed mainly of scattered villages and small farms; the schools were in general small, varying from 4 to 190 pupils; very few children had been previously vaccinated with BCG. The second area was mainly rural but included one suburban district, while the third was entirely suburban; many of the children in these two areas had been vaccinated before the beginning of the investigation.¹ In the suburban districts the schools were much larger, containing from 90 to 1,440 pupils.

A routine school-health activity could not be adapted to the needs of a research programme without adjustments on both sides. The school physicians relinquished their prerogatives for part of the routine supervision of the children's health; the teachers accepted the disruption of their schedules occasioned by the repeated visits; the children themselves were subjected to more frequent and extensive examinations. To keep the disturbance to schools and health authorities at a minimum, the field teams were made large enough to do the required work rapidly without loss of accuracy; every effort was made to suit the convenience of teachers and pupils; and the results of the tuberculin tests were promptly made known to the school physicians and the tuberculosis dispensaries.

Two important compromises had to be made between the routine school-health work and the research activity. The first was that no revaccinations were performed except on the children leaving school; this was essential if information on the duration of allergy was to be obtained. The other compromise, necessitated by using an established health activity as the framework for research, was that there were no unvaccinated controls. While for many purposes this lack is not of major importance, it undoubtedly detracts greatly from the value of some of the results.

Studies were also carried out in Egypt and in southern India (on study populations of between 3,000 and 4,000) and in Mexico City (nearly 2,000). In these countries again, use was made of existing health activities. In Egypt² and in Mexico the work was done in connexion with mass BCG-

¹ Only children with a negative history of previous BCG vaccination and no BCG scar are included in the material reported in this monograph.

² The field team was headed by Dr. Johannes Meijer.

vaccination programmes; in India, as part of an epidemiological investigation carried out by the TRO Field Research Station in Madanapalle.³ The age span of the children in Mexico City was very similar to that of the children in Denmark; in Egypt and India pupils up to 18 or 20 years were included.

Procedures and Techniques

Scheduling the field work

The first step in scheduling the field work was the calculation of the number of children to be included in the study. To the basic number desired for vaccination was added an estimate to cover the number that probably already reacted to tuberculin, those previously vaccinated or expected to refuse vaccination, and those expected not to attend for pre- or post-vaccination examinations.

In collaboration with the county tuberculosis officer and the school physicians, a group of schools was selected in an area containing the desired number of children. Before the actual schedule was prepared, each school was visited in order to obtain information regarding dates and hours of school attendance, local holidays, times when the testing would be convenient, and the number of children according to school-class and history of previous vaccination. On the basis of this information, the schedule was prepared. Similar information was obtained from the school each year before scheduling the annual re-examination.

Under usual field conditions one team could handle about 300 to 450 children per day. In areas where little or no BCG vaccination had previously been carried out, 80%-90% of the children tested were usually vaccinated. In areas where many of the children had already been given BCG, the proportion eligible for vaccination was of course smaller and the number of children to be handled correspondingly larger. Where teams could not handle in one day enough children to yield the desired number of vaccinations, the children were dealt with on successive days, and the small groups receiving each vaccine each day were combined in tabulating the results.

Testing and vaccination schedule

The pre-vaccination testing and vaccination were completed in three visits.

On the *first visit*, each child was examined for BCG-vaccination scar and questioned regarding previous history of vaccination. A dose of

³ Directed by Dr. Johannes Frimodt-Møller.

10 TU (tuberculin units) was given as the first test in all studies except three (C II, H I, and H III), in which 5 TU was given.

On the *second visit*, three or four days later, the first tuberculin test was read. Children failing to react according to specified criteria were vaccinated and tested with 100 TU.⁴ The criteria were defined in a special directive given to the team. During the first two years of the investigation, vaccination and the 100 TU test were given to children who did not react with at least 6 mm of induration to 10 TU, or with at least 5 mm of induration to 5 TU. Latterly, this limit was raised to 10 mm of induration to 10 TU.

On the *third visit*, three or four days later, the 100 TU test was read and the site of vaccination examined.

The first post-vaccination examination took place 10 weeks after vaccination, or as close to this time as possible. Because of holidays, etc., this interval varied from 6 to 12 weeks; it was from 8 to 12 weeks in all studies except two, where it was 6 and 7 weeks, respectively. For different subgroups within a single study, however, the interval between vaccination and re-examination was kept virtually constant. Subsequent examinations were carried out at approximately annual intervals after vaccination.

At each post-vaccination examination the vaccination lesion was observed and post-vaccination allergy was determined. For the first post-vaccination test, the same strength of tuberculin was used as for the pre-vaccination test. At the examination one year after vaccination, 10 TU was routinely given as the first tuberculin test. No revaccinations were done except on children leaving school, as they would be automatically lost to the follow-up programme.

In the first eleven studies, all children were routinely examined for enlargement or suppuration of the lymph-nodes two or three months and one year after vaccination.

Tuberculin test

Tuberculin was given by superficial intracutaneous injection into the dorsum of the forearm (the usual site in Denmark); the specified quantity (0.1 ml) was measured according to the markings on the barrel of the syringe.

During the first nine months of the investigation the injection was made at approximately the junction of the upper and middle thirds of the forearm; the first test (5 or 10 TU) was made on the left arm, and the second test (100 TU) on the right arm. After the summer of 1950 the site was changed to just below the midpoint between the elbow and wrist, because readings

⁴ In study D IV the 100 TU test was not given.

of the reaction in this area (particularly as regards density) are less influenced by the muscular development of the child than when the test is done over the muscle mass of the upper forearm. During the fall of 1951⁵ it became apparent that a test made at the same point as a previous test gave somewhat different results from one in a new, untested area. Thereafter no tuberculin test was made at a site that had been used previously. Studies are now under way to elucidate the influence of the site of injection.

Each tuberculin test was "read" after 3 or 4 days.⁶ First, the transverse diameter of erythema was measured. Then the reaction was felt and, if induration was present, its transverse diameter was measured. All measurements were made with a ruler calibrated in millimetres. Each palpable reaction was then classified into one of four categories of density, designated as types I to IV. Type I signifies the most dense reaction, characteristically raised, and usually with sharply demarcated borders. Reactions of type IV are the least dense, more a palpable swelling than an actual induration; types II and III are intermediate categories. Types I, II, and III represent reactions with clearly recognizable induration; reactions of type IV might easily escape notice unless the area is palpated with a light touch. While this classification is an arbitrary one, it provides a gross index of the relative firmness of reactions which range over a continuous scale from very dense to barely perceptible.

Finally, the presence or absence of additional features such as bullae and lymphangitis was noted.

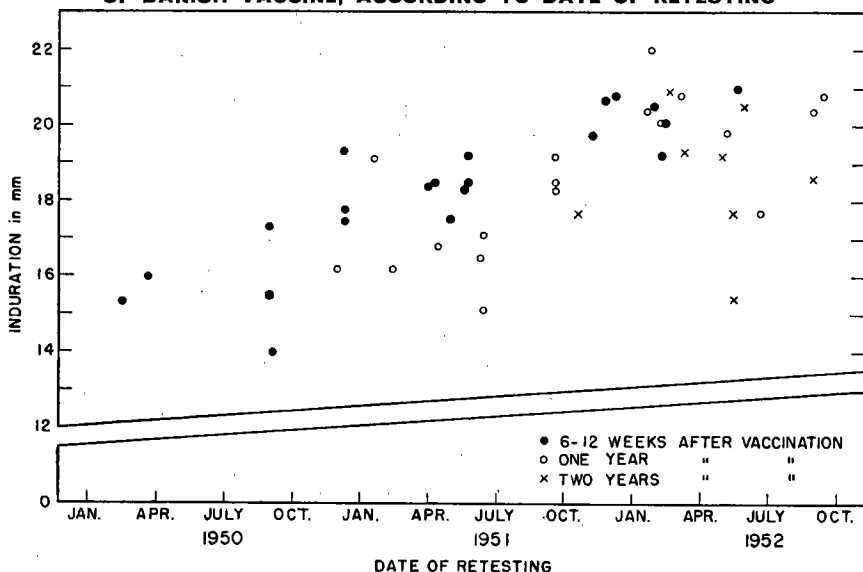
Long practice in carefully measuring reactions seems to be associated with larger readings. Fig. 1 shows the average size of induration of the reactions to 10 TU after vaccination with 23 batches of Statens Serum-institut standard-strength vaccine at all retesting periods, plotted according to date of testing. Despite large fluctuations there is a definite tendency towards an increased mean size with time. This is true both from batch to batch and for the same batch at the different testing periods. Moreover, this trend is present for the children reacting to the original tuberculin test with 6, or more, mm of induration and excluded from vaccination (not shown in the figure).

The tendency to measure reactions larger with growing experience was found for most of the nurses during training. Fig. 2 gives an example of before- and after-training differences in reading between a trainee and one of our experienced readers. The abscissa gives the mean size of the reactions as read by the two readers, and the ordinate the difference between the readings of the trainee and the TRO nurse. Before training

⁵ During the September/October testing of studies A I, D I, E II, E III, H II.

⁶ At the pre-vaccination testing and the first post-vaccination testing, the 5 or 10 TU tests were usually read at 3 days, but occasionally at 4 days. At the annual examination the 10 TU tests, with one exception (study E V), were read at 3 days. The 100 TU tests were read at either 3 or 4 days, as occasion permitted.

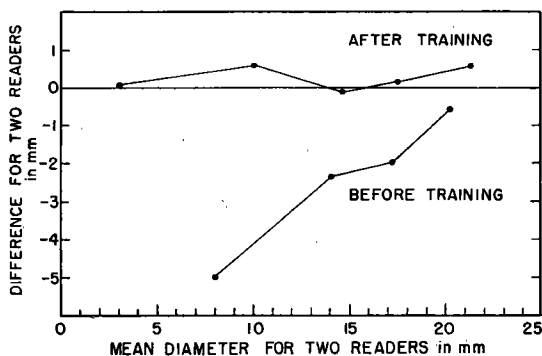
FIG. 1. MEAN DIAMETER OF MANTOUX REACTIONS TO 10 TU FOR 23 BATCHES OF DANISH VACCINE, ACCORDING TO DATE OF RETESTING



there was a pronounced difference between the two readers. The trainee read systematically smaller, though the difference varied for reactions of different sizes: reactions between 5 and 10 mm were on an average read 5 mm smaller, the very large ones less than 1 mm smaller. After training, the readings for the two nurses were remarkably close.

Even among persons with long experience small systematic differences persist. Fig. 3 gives the results of duplicate independent readings of the

FIG. 2. DIFFERENCES IN DIAMETER OF MANTOUX REACTIONS TO 10 TU, AS READ BY A TRAINEE AND AN EXPERIENCED READER, GROUPED ACCORDING TO SIZE OF REACTIONS



Detailed data are given in Appendix II, table 1, page 287.

same reactions by our present readers. This figure shows that between readers A and B, and between readers A and C, there is a difference of 1 mm or less, irrespective of the size of the reactions. (Data for fig. 2 and 3 are given in Appendix II, tables 1-3, pages 287 and 288.)

Vaccination

The vaccine was given intracutaneously in the

deltoid region of the left shoulder. The measured dose was 0.1 ml and all injections were made slowly and as superficially as possible (except when volume or depth of injection was under study).

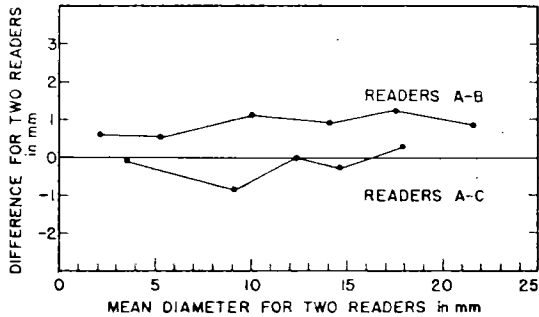
The development of a method for describing the vaccination lesion has proved difficult. At different intervals after vaccination there is considerable variation in the character of the lesion.

Moreover, at any one period, the stage of its development is not the same in all children. At the first post-vaccination examination (after two to three months), the widest transverse diameter of redness and of induration of the lesion was measured and recorded. If tissue destruction was present, the involved area was measured and described (ulcer, scab, etc.). Unusual features, such as subcutaneous abscesses, were noted. At the annual examinations, the size of the scar was measured and any unusual characteristics were described.

In presenting the data, the size of induration has, in general, been used to define the size of the lesion during the early months. In a few studies, where very weak fractional strengths of vaccine were used, a lesion was often clearly visible but no induration could be felt. In these studies,⁷ the size has been defined by the largest visible or palpable evidence of tissue alteration. One and two years after vaccination, the size of the superficial scar has always been used.

In the early studies the team physician examined the children for lymph-node involvement in the cervical and axillary regions on both sides. The number and size of palpable glands in each area were recorded, together with a brief description of the character of the nodes—adherence, tenderness, fluctuation, etc. being noted. This examination was recognized as unsatisfactory, both because it had to be carried out with the children fully dressed, and because the status of the lymph-nodes before vaccination was unknown. However, it was believed that gross enlargement of the glands should not often be missed, and that this finding on the side of vaccination might be evaluated by comparison with the findings on the opposite side in the

FIG. 3. DIFFERENCES IN DIAMETER OF MANTOUX REACTIONS TO 10 TU, AS READ BY TWO PAIRS OF EXPERIENCED READERS, GROUPED ACCORDING TO SIZE OF REACTIONS



Detailed data are given in Appendix II, tables 2 and 3, page 288.

⁷ C II, E V, G I, H III, H V

vaccinated children, and on the usual side of vaccination in the unvaccinated children examined at the same time. The frequency of glandular abscesses was found to be so low during the early studies that this examination was omitted from most of the later studies.

Supplies, Equipment, and Records

Tuberculin

The tuberculin used throughout these studies was the Statens Serum-institut purified protein derivative (PPD), batch number RT XIX-XX-XXI. This batch was used in the international mass campaigns from April 1948 to February 1950. The stock solutions (containing 1 mg PPD per millilitre of diluent) were usually prepared at least once a month. Dilutions were prepared at the Statens Serum-institut for each study and were kept constantly refrigerated until use, usually within two weeks of preparation. The 5 TU dilution contains 0.0001 mg of PPD/ml, the 10 TU 0.0002 mg/ml, and the 100 TU 0.002 mg/ml.

Vaccine

The vaccine routinely used in these investigations was prepared by the Statens Serum-institut. It was transported in refrigerated containers from the Institute to the field of operation, where it was stored either in a refrigerator maintained constantly at 2-4°C, or in an incubator at other desired temperatures.

The vaccine was supplied either in 10- or 1-ml ampoules. The latter have, in general, been considered preferable because with them the contents of the vials can be better mixed immediately before use and the vaccine is less exposed to light. When the damaging effect of light was recognized, 10-ml ampoules were always shielded in a wooden container during use, and syringes filled with vaccine were shielded from light.

A few vaccines from other BCG production centres have been made available for study through the courtesy of the directors of these laboratories.⁸ The vaccine was shipped in refrigerated containers from the home laboratory to the Statens Serum-institut, where it was handled in the same way as the Danish vaccine.

* Dr. Johannes Bøe, Nasjonalforeningens BCG Laboratorium, Bergen, Norway
Dr. Ismail El-Lamie, Agouza Serum and Vaccine Institute, Cairo, Egypt
Dr. Alberto León, Instituto del BCG, Mexico City, D.F., Mexico
Dr. K. S. Ranganathan, King Institute of Preventive Medicine, Madras, India
Dr. F. Van Deinse, Institut Pasteur, Paris, France
The late Dr. Anders Wassen, Bakteriologiska Laboratoriet, Sahlgrenska Sjukhuset, Gothenburg, Sweden

Equipment

One-millilitre glass tuberculin syringes (graduated in hundredths of millilitre) were used for both tuberculin testing and vaccination, with 25- or 26-gauge platinum needles. The syringes were pre-tested for leakage in a specially constructed apparatus⁹ and discarded during use if leakage occurred. Each syringe was provided with a small steel spring to keep the plunger in position when not touched. The syringes and needles used for each dilution of tuberculin, and for vaccinating, were kept entirely separate and were sterilized (by boiling in distilled water) in different containers. Between injections the needle was flamed, then a few drops of the contents of the syringe pressed out. When several vaccines were used on the same day, a different syringe was used for each. To identify the syringe to be used for each specific vaccine, a coloured rubber ring was placed about the syringe, the colour corresponding to the code letter on the label of the ampoule of vaccine. Thus, a red ring signified that this syringe must be used for the vaccine labelled with the code letter **R**.

Protocols, records, and processing of field data

Each study was carried out according to a written protocol describing in detail the materials to be used and procedures to be followed: what was to be done, with what, on whom, by whom, and when. Occasionally, changes from this plan were necessary. These deviations were described in appendices to the protocol. Since, in order to prevent biased observations, the field staff had to remain unaware of the purpose of the study, special directives were prepared for their guidance, specifying the steps in each procedure.

A copy of the standard record card is shown in fig. 4. The information for identifying child and family units was given by the school authorities and checked by the clerk at the first visit to the school. All records on the card were made in ink by the clerk at the dictation of the nurse or doctor making the examination. The absence (e. g., "0" induration), as well as the presence, of findings was recorded. If a mistake was made, a correction was written on the card; but erasures were not allowed. No cards were destroyed, and all were checked for completeness at each visit.

The body of the card is designed so that all procedures carried out on the same day are recorded on the same horizontal line. In addition to the date column, there are four sections for recording observations: the first section (columns 2-6) is for antigens other than tuberculin; the second and third sections (columns 7-10 and 11-14) are for the first and second tuberculin tests (the strength of tuberculin used is written on each card at the first visit to the school); the fourth section is for BCG vaccination. In all sections,

⁹ See Appendix III, page 303.

