BCG VACCINATION
BCG VACCINATION

Studies by the
WHO Tuberculosis Research Office,
Copenhagen

Report prepared under the direction of

LYDIA B. EDWARDS, M.D.
Chief of Field Studies

and

CARROLL E. PALMER, M.D., Ph.D.
Director

with the assistance of

KNUT MAGNUS, cand. act.
Assistant Statistician

Tuberculosis Research Office, World Health Organization,
Copenhagen

WORLD HEALTH ORGANIZATION
PALAIS DES NATIONS
GENEVA
1953
NOTE

Authors alone are responsible for views expressed in the Monograph Series of the World Health Organization.
COLLABORATORS

The investigations were undertaken under the joint auspices of the International Tuberculosis Campaign, the Danish Statens Seruminstitut, and the WHO Tuberculosis Research Office. The Tuberculosis Research Office was responsible for the planning and execution of the field programme, and the Statens Seruminstitut prepared and supplied the vaccine and tuberculin used and carried out the supplementary laboratory work. The salaries of the field personnel and their expenses were paid by the International Tuberculosis Campaign and later by the United Nations International Children's Emergency Fund.

The field staff, first headed by Dr. Inger Marie Thellesen, is now headed by Dr. Johannes Guld. The following doctors and nurses participated in the work at different times:

Dr. Johanne Weis Bentzon  Grethe Hagen  Solveig Midtgaard
Dr. Christian Rud  Lis Halkjør-Lassen  Elisabeth Nielsen
Agnete Andreasen  Birthe Johansen  Sigrid Nielsen
Helena Burzynska  Erna Kjølholm  Gudrun Kjær Poulsen
Edith Frederiksen  Minna Madsen  Signe Reinholdt

Dr. Knud Tolderlund, of the Statens Seruminstitut, was responsible for the preparation of the vaccine and tuberculin used and for the laboratory work. He was assisted by Dr. Inger Dragsted.

Dr. Phyllis Q. Edwards and Dr. I-Chin Yuan, of the Tuberculosis Research Office, assisted in preparing the manuscripts of the report, and Hans Jacob Kreyberg, cand. oecon., in compiling the statistical data.

Grateful acknowledgement is due to Dr. Johannes Holm, Director of the International Tuberculosis Campaign, and Dr. Georg Bindslev, Chief Physician, Krabbesholm, Denmark, for invaluable help and cooperation. Special mention also should be made of the assistance rendered by Chief Physicians, Dr. Gregers Falkenfleth and Dr. Tage Hyge, of Denmark.
## CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Introduction</td>
<td>13</td>
</tr>
<tr>
<td>1</td>
<td>Scope, methods, and material of the investigation</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>Pre-vaccination tuberculin sensitivity</td>
<td>39</td>
</tr>
<tr>
<td>3</td>
<td>Response to BCG vaccination</td>
<td>51</td>
</tr>
<tr>
<td>4</td>
<td>Effect of temperature and duration of storage of BCG vaccine</td>
<td>65</td>
</tr>
<tr>
<td>5</td>
<td>Effect of exposure of BCG vaccine to light</td>
<td>75</td>
</tr>
<tr>
<td>6</td>
<td>Variations in the technique of intracutaneous BCG vaccination</td>
<td>83</td>
</tr>
<tr>
<td>7</td>
<td>Variations in the preparation of BCG vaccine</td>
<td>97</td>
</tr>
<tr>
<td>8</td>
<td>BCG vaccine from different production centres</td>
<td>105</td>
</tr>
<tr>
<td>9</td>
<td>Effect of diluting BCG vaccine, and the significance of dead organisms</td>
<td>125</td>
</tr>
<tr>
<td>10</td>
<td>General summary</td>
<td>143</td>
</tr>
</tbody>
</table>

## APPENDICES

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>General notes to Appendices I and II*</td>
<td>148</td>
</tr>
<tr>
<td>I</td>
<td>Basic data for all studies</td>
<td>149</td>
</tr>
<tr>
<td>II</td>
<td>Additional data from selected studies</td>
<td>285</td>
</tr>
<tr>
<td>III</td>
<td>Measurement of leakage of tuberculin syringes</td>
<td>303</td>
</tr>
</tbody>
</table>

* Detailed contents of Appendices I and II will be found on pages 150-153 and 286, respectively.
FIGURES

1. Mean diameter of Mantoux reactions to 10 TU for 23 batches of Danish vaccine, according to date of retesting ................................................................. 34
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 .......... 34
3. Differences in diameter of Mantoux reactions to 10 TU, as read by two pairs of experienced readers, grouped according to size of reactions ........ 35
4. Standard field record-card .................................................................................. 38
5. Frequency distributions of diameter of pre-vaccination Mantoux reactions to 10 (or 5) TU among schoolchildren in Denmark, Mexico, Egypt, and India 41
6. Frequency distributions of diameter of pre-vaccination Mantoux reactions to 100 TU among schoolchildren in Denmark, Mexico, Egypt, and India who reacted with less than 6 mm of induration to 10 TU (or less than 5 mm of induration to 5 TU) ................................................................. 43
7. Frequency curves, by age, of diameter of pre-vaccination Mantoux reactions to 10 (or 5) TU among schoolchildren in Denmark, Mexico, Egypt, and India 44
8. Frequency curves, by age, of diameter of pre-vaccination Mantoux reactions to 100 TU among schoolchildren in Denmark, Mexico, Egypt, and India who reacted with less than 6 mm of induration to 10 TU (or less than 5 mm of induration to 5 TU) ................................................................. 47
9. Frequency distribution of diameter of Mantoux reactions to 10 TU (typed according to density), 6-12 weeks after vaccination ................................. 53
10. Frequency distribution of diameter of pre-vaccination Mantoux reactions to 10 TU ........................................................................................................... 53
11. Frequency distributions of diameter of Mantoux reactions to 10 TU at different intervals after vaccination (Study G I) ................................................................. 55
12. Frequency distributions of diameter of Mantoux reactions to 10 TU, 10 weeks, one year, and two years after vaccination (Study A I) ................................................................. 55
13. Frequency distribution of diameter of vaccination lesions 6-12 weeks after vaccination ........................................................................................................... 56
14. Frequency distribution of diameter of vaccination scars one year after vaccination ........................................................................................................... 57
15. Mean diameter of Mantoux reactions to 10 TU and of vaccination lesions 8-9 weeks after vaccination, according to temperature and duration of storage of vaccine (Studies A II and A III) ................................................................. 67
16. Mean diameter of Mantoux reactions to 10 TU, 8-11 weeks and one year after vaccination, for 7 batches of vaccine from four laboratories, according to duration of storage at 2-4°C ................................................................. 68
17. Mean diameter of scars one year after vaccination, for 7 batches of vaccine from four laboratories, according to duration of storage at 2-4°C ................................................................. 69
18. Mean diameter of Mantoux reactions to 10 TU one year after vaccination, for 3 batches of vaccine, according to duration of storage at 2-4°C and 20°C .......................... 70
19. Frequency distributions of diameter of Mantoux reactions to 10 TU one year after vaccination, for vaccines from three laboratories, according to duration of storage at 2-4°C (Study H I) .................................................. 71
20. Frequency distributions of diameter of Mantoux reactions to 10 TU, 8 weeks after vaccination, according to duration of storage of vaccine at 2-4°C, 20°C, and 30°C (Study A II) ................................................................. 72
21. Frequency distributions of diameter of Mantoux reactions to 10 TU, 9 weeks after vaccination, according to duration of storage of vaccine at 42°C (Study A III) .............................................................. 73
22. Frequency distributions of diameter of wheals according to volume of vaccine injected superficially .................................................................................. 86
23. Mean diameter of wheals according to volume of vaccine injected and depth of injection ................................................................. 87
24. Frequency distributions of diameter of wheals according to depth of injection of 0.1 ml of vaccine .................................................. 87
25. Frequency distributions of diameter of vaccination lesions, and frequency of local abscess and abnormal scar, according to depth of injection of vaccine (Studies F II and F III) .................................................. 93
26. Diameter of Mantoux reactions to 10 TU, 6-12 weeks after vaccination, for 21 batches of Danish vaccine: combined frequency distribution for all batches (A), and schematic curves for individual batches (B) .................................. 107
27. Relation between mean diameter of Mantoux reactions to 10 TU and of vaccination scars one year after vaccination, for 18 batches of Danish vaccine .......................... 110
28. Frequency distributions of diameter of Mantoux reactions to 5 or 10 TU, 6-12 weeks after vaccination, for vaccines from six laboratories .......................... 111
29. Frequency distributions of diameter of Mantoux reactions to 5 or 10 TU, 9½ weeks, one year, and two years after vaccination, for vaccines from three laboratories .............................................................. 112
30. Frequency distributions of diameter of vaccination lesions 6-12 weeks after vaccination, for vaccines from six laboratories .................................................. 113
31. Comparisons between single batches of non-Danish vaccines and concurrent batches of Danish vaccine .................................................. 115
32. Mean diameter of Mantoux reactions to 10 TU and of vaccination lesions, for a batch of Mexican (M) and a batch of Danish (D) vaccine, according to strength of vaccine (Study H V, Denmark) .................................................. 118
33. Mean diameter of Mantoux reactions to 5 or 10 TU and of vaccination lesions, for a batch of Indian (I) and a batch of Danish (D) vaccine, according to strength of vaccine (Study H III) .................................................. 119
34. Mean diameter of Mantoux reactions to 10 TU and of vaccination lesions 8½ weeks after vaccination, for a batch of Egyptian (E) and a batch of Danish (D) vaccine, according to strength of vaccine (Study H VI, Egypt) 121
35. Mean diameter of Mantoux reactions to 10 TU and of vaccination lesions 12 weeks after vaccination, for a batch of Egyptian (E) and a batch of Danish (D) vaccine, according to strength of vaccine (Study B III, Egypt) 121
36. Frequency distributions of diameter of Mantoux reactions to 10 TU, 7 weeks after vaccination, according to strength of vaccine (Study D IV) 126
37. Mean and standard deviation of frequency distributions of diameter of Mantoux reactions to 10 TU, according to strength of vaccine ................................. 127
38. Frequency distributions of diameter of vaccination lesions, and frequency of ulcer and scab, 7 weeks after vaccination, according to strength of vaccine (Study D IV) ........................................ 128
39. Mean diameter of Mantoux reactions to 10 TU and of vaccination lesions 7 weeks after vaccination, according to strength of vaccine (Study D IV) 128
40. Frequency distributions of diameter of Mantoux reactions to 10 TU, 7 weeks after vaccination, for living and dead (heat-treated) vaccine, according to strength of vaccine (Study D IV) ................................. 129
41. Frequency distributions of diameter of Mantoux reactions to 10 TU, for living and dead (heat-treated) vaccine, at different intervals after vaccination (Study G I) ........................................ 131
42. Mean diameter of Mantoux reactions to 10 TU, for living and dead (heat-treated) vaccine, according to strength of vaccine (Study D II) .............. 132
43. Mean diameter of vaccination lesions 10 weeks after vaccination, for living and dead (heat-treated) vaccine, according to strength of vaccine (Study D II) .......... 132
44. Frequency distributions of diameter of vaccination lesions 10 weeks after vaccination, for living and dead (heat-treated) vaccine, according to strength of vaccine (Study D II) .......... 133
45. Relation between size of Mantoux reactions to 10 TU and of vaccination lesions 10 weeks after vaccination, for different strengths of living and dead (heat-treated) vaccine (Study D II) ........................................ 134
46. Mean diameter of Mantoux reactions to 10 TU, 8½ weeks after vaccination, for living and mostly dead vaccine, according to strength of vaccine (Study D III) ........................................ 135
47. Mean diameter of Mantoux reactions to 10 TU, for living vaccine, according to strength, and for living and dead (heat-treated) vaccine mixed in different proportions (Study D I) ........................................ 136
48. Relation between size of Mantoux reactions to 10 TU and of vaccination lesions 8½ weeks after vaccination, for different strengths of living and mostly dead vaccine (Study D III) ........................................ 137
49. Relation between size of Mantoux reactions to 10 TU and of vaccination lesions 9½ weeks after vaccination, for different strengths of living vaccine, and for living and dead (heat-treated) vaccine mixed in different proportions (Study D I) ........................................ 137
50. Relation between size of Mantoux reactions to 10 TU and of vaccination lesions 7 weeks after vaccination, for different strengths of living, heat-killed, light-killed, and light-exposed vaccines, and for standard-strength vaccine stored for one year (Study D IV) ........................................ 139
TABLES

I. Character and extent of the studies ........................................... 18

II. Size and character of vaccination lesions at different intervals after vaccination (Study G I) ............................................. 57

III. Lymph-node findings in cervical and axillary regions 8½-11 weeks after vaccination ......................................................... 58

IV. Correlation between size of tuberculin reactions and of vaccination lesions 11 weeks after vaccination (Study E I) ............ 59

V. Extent of studies on effect of temperature and duration of storage of vaccine ................................................................. 66

VI. Size of tuberculin reactions and of vaccination lesions 8½ weeks after vaccination, and counts of viable bacillary units, according to duration of exposure of vaccine to sunlight (Study B I) .............. 76

VII. Size of tuberculin reactions and of vaccination lesions 12 weeks after vaccination (in Denmark and Egypt), and counts of viable bacillary units, according to exposure of vaccine to sunlight, skyshine, and indoor daylight in Egypt (Study B II) ...................................................... 77

VIII. Size of tuberculin reactions and of vaccination lesions 9-12 weeks after vaccination (in Denmark and Egypt), and counts of viable bacillary units, according to exposure of vaccine to outdoor and indoor daylight in Egypt (Study B III) ................................................... 79

IX. Counts of viable bacillary units, according to exposure of vaccine to sunlight and skyshine in Denmark and Egypt, December 1951 .... 80

X. Size of tuberculin reactions and of vaccination lesions according to strength and volume of vaccine injected (Study F I) ........... 89

XI. Size of tuberculin reactions and of vaccination lesions according to depth of injection of vaccine (Study F II) .................................. 91

XII. Size of tuberculin reactions and of vaccination lesions according to depth of injection of vaccine (Study F III) ......................... 92

XIII. Size of tuberculin reactions and of vaccination lesions according to time between transfers of maintenance culture used to prepare vaccine (Study E V) ...................................................... 98

XIV. Size of tuberculin reactions and of vaccination lesions according to harvest time of culture used to prepare vaccine (Studies E III and E IV) ................................................................. 99

XV. Size of tuberculin reactions and of vaccination lesions according to degree of growth of culture used to prepare vaccine (Study E II) ...... 100

— 11 —
XVI. Size of tuberculin reactions and of vaccination lesions according to duration of grinding of vaccine (Study E I) ........................................ 101
XVII. Size of tuberculin reactions and of vaccination lesions according to composition of vaccine diluent, temperature and duration of storage of vaccine (Study E I) ........................................ 101
XVIII. Extent of data on standard-strength vaccines from different laboratories 106
XIX. Size of tuberculin reactions 6-12 weeks, one year, and two years after vaccination, for 24 batches of Danish standard-strength vaccine . . . 108
XX. Size of vaccination lesions 6-12 weeks, one year, and two years after vaccination, for 24 batches of Danish standard-strength vaccine . . . 109
INTRODUCTION

This report describes an adventure in international research, planned to meet an international need and carried through with the help of health authorities, doctors, and laymen in many lands.

Tuberculosis constantly threatens the health and lives of peoples throughout the world, especially in areas with scanty medical resources. Because immediate full control of this disease is out of the question, hopes of prevention on a large scale have become centered on vaccination with BCG; and during the past few years millions of people have been tuberculin-tested and vaccinated in vast international campaigns sponsored and directed by international health agencies—at first the International Tuberculosis Campaign (ITC) * and later the World Health Organization and the United Nations International Children’s Emergency Fund.

Yet much remains to be learned about BCG vaccination. Despite laboratory evidence that it protects animals against virulent infection, and limited studies suggesting that it may also protect man, when the mass campaigns were started in the fall of 1947 there was little precise information about the vaccine itself, its variability, its keeping qualities, how it should be applied, and particularly its immediate and long-term effects. Nor was it known whether the methods used in Scandinavia and adopted for the campaigns could be applied indiscriminately to peoples in other parts of the world. It was therefore no surprise when serious problems were brought forward at the Conference on European BCG Programmes, September 1949—a meeting of national and international leaders of the mass-vaccination campaigns directed by the International Tuberculosis Campaign. One of the most serious problems was the unexplained failure, in some areas, of vaccination to induce what was deemed to be sufficient tuberculin allergy. The conference discussed possible causes—over-aged vaccine, inadequate refrigeration of stored vaccine, poor vaccination technique, and so on—but it soon became clear that the answer to this question (and to more fundamental questions concerning the production and evaluation of BCG-induced allergy) would not be found by discussion. The urgent need for scientifically controlled investigations on BCG vaccine and vaccination could no longer be denied.

Facilities for such research were at hand in Denmark; and WHO, through its Tuberculosis Research Office (TRO), in collaboration with the

* The International Tuberculosis Campaign, or “Joint Enterprise” as it was known in the official agreements, was created by the United Nations International Children’s Emergency Fund, the Danish Red Cross, the Norwegian Relief for Europe, and the Swedish Red Cross, for the purpose of assisting national governments in conducting mass BCG-vaccination.
Danish Statens Seruminstiuttet and the International Tuberculosis Campaign, agreed to conduct an intensive investigation of basic problems of tuberculosis immunization, with special reference to BCG. The Statens Seruminstiuttet, through its BCG section, offered to prepare the vaccine and carry out laboratory work. The ITC headquarters office in Copenhagen provided the necessary link between the every-day field problems in the international mass campaigns on the one hand, and the research programme on the other. The field work was readily integrated with the large-scale tuberculin testing and vaccination of Danish schoolchildren which was already in progress. Finally, the medical-statistical staff of TRO undertook to plan, coordinate, and direct the field research.

The programme has included short-term investigations of immediate practical problems arising in the field, together with more basic studies of the response of man to BCG vaccine. It is generally assumed that satisfactory vaccine properly injected will give rise to a good "take" at the site of vaccination and fairly long-continued tuberculin allergy. This implies not necessarily that allergy and immunity are closely related, but that an allergic person, compared with a non-allergic person, will react more promptly and strongly to contact with the infecting bacterium or its products, and so localize the infection more quickly and effectively—a response that may be important in the development of immunity. In the absence of a clear understanding—or even a yardstick—of immunity to tuberculosis, tuberculin allergy has been taken as the guide, however imperfect, to the effectiveness of BCG vaccination. The present world-wide programme of BCG vaccination underlines the need to learn more about tuberculin allergy and to improve the method of its measurement; and therefore in our work much emphasis has been placed on the study of post-vaccination tuberculin allergy. The local reaction at the vaccination site has also been carefully studied; the usefulness of these observations in children is not yet known, but it should be remembered that the potency of the vaccine is assessed by the local reaction in guinea-pigs.

Since this investigation has been done in schoolchildren, it has certain limitations. First, the groups studied were composed largely of children aged 7-14 years. From one point of view this is a distinct advantage, because it is with children of this age that most mass-vaccination campaigns are chiefly concerned. On the other hand, we have no experience with pre-school children; and there is reason to believe that the response to vaccination, particularly at the site of injection, may be different in the younger age-groups. Secondly, most of the studies were carried out in Danish children vaccinated with Danish vaccine; and work in other countries and with other vaccines indicates that the research programme must be extended racially and geographically to elucidate the different patterns of response among different peoples. A third, and possibly the most
important, limitation is the absence of an unvaccinated control-group. Such a group could not be formed within the framework of the school arrangements; and we have thereby lost an important control for several of the questions investigated.

This monograph sets out the work done during the first three years of the research programme, from November 1949 to September 1952. More than 40,000 schoolchildren in four different countries have been tuberculin-tested before vaccination, and over half of them have been vaccinated in 27 separate studies dealing with different vaccines and techniques. Tuberculin tests have been done, and the reactions at the site of vaccination examined, 6-12 weeks after vaccination in all studies, one year after vaccination in 20 studies, and two years after vaccination in 8 studies. Complete quantitative data for all of the tests and examinations, constituting a "source book" for ourselves and others working in this field, are given in tabular form as appendices. From these basic tables, we have selected material, grouped according to the subject under investigation, and prepared chapters illustrating and summarizing the findings that we regard as the most interesting and significant. (Not all of the material is reviewed in these chapters, which, however, include references to the appropriate "source" tables.) Some of the findings must be viewed as preliminary and the conclusions as tentative, pending further investigation. But many of the results are, we believe, based on solid foundations: some studies have been repeated so frequently and with such consistent findings as to warrant valid conclusions, even though often they are not in accord with prevalent views about BCG.

* * *

Since the end of the late world war there has been a quickening awareness among the family of nations that technical aid to underdeveloped countries, by promoting their well-being and thereby contributing to world peace, is not only a proper but a vital concern of the United Nations and its specialized agencies. Already, however, experience has shown that what is practicable in one country or region may not suit others with different environmental, economic, or social conditions. To apply the lessons learned in one area to a different people in a far land may require research extending beyond national or even continental boundaries, and for this the ordinary research institutions were not constituted and are not equipped. The investigation reported in this monograph illustrates a way in which a worldwide medical problem may be approached by coordinated international research.