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REPORT ON VISIT TO THE BCG PRODUCTION LABORATORY, KARACHI, PAKISTAN

by

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and
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CONTENTS

Page

Introduction 3

BCG Production Laboratory 5

1. Personnel 5
2. Laboratories 6
   - A. The premises 6
   - B. Equipment 10
3. The Animal Quarters 10
4. Special Precautions against Contamination 11
5. Preparation of Media 12
6. Technique of (A) Subculture or Strains and (B) Preparation of Vaccine 13
7. Control Tests 15
   - A. Sterility tests 15
   - B. Tests for viability 15
   - C. Innocuity test 19
   - D. Potency test 20
   - E. Test for tuberculin sensitivity 22
   - F. Determination of homogeneity 22
   - G. Determination of moisture content in the pressed culture . . 23
   - H. Tightness of sealed ampoules 23
   - I. Test for transmission of light 23
   - J. Testing in children for potency 23
8. Special Investigations 23

Page

9. Discussion 25

10. Recommendations 33

1. Personnel 33
2. Laboratories 34
3. The animal quarters 37
4. Special precautions against contamination 38
5. Preparation of Media 38
6. Technique of (A) subculture of strains and (B) preparation of vaccine 39
7. Control tests 40
Other recommendations 43

Conclusions 43

References 44

List of Personnel 46

Tables, Sketch

The background for my visit in May-June 1956 to the BCG Production Laboratory in Karachi was the demonstration of an increasing rate of secondary contamination of the batches of vaccine prepared by that Laboratory during the years 1953 to 1955. The reasons for the contamination were considered to be as follows:

1. A high concentration of contaminants in the air in the Laboratory due to a series of different factors.

2. Lack of sufficient electrical power and frequent interruptions of the electric current and the supply of water.

3. On the part of some of the staff members, a certain degree of inefficiency.

Reference should here be made to section VIII (pages 22-24) of the report on my visit,[16] where the three points mentioned above are discussed.

A series of recommendations were set up in my report aimed at:

(a) Preventing further infestation of the laboratories by white ants.

(b) Reducing the possibility of contaminating the air in the laboratories and keeping the rooms clean.

(c) Providing a constant and sufficient supply of electric power and water.

(d) Ensuring adequate sterilization of media and utensils.

(e) Establishing better and safer working conditions in the Laboratory.

(f) Attaching an assistant medical officer to the staff and maintaining constant supervision, education and the strictest discipline of the personnel.

(g) Modifying the techniques of maintaining the cultures and preparing the vaccine for the purpose of having available at any time a sufficient quantity of suitable culture and vaccine.

(h) Standardizing the method of vaccine production in order to ensure uniformity as regards the quality of the current batches of vaccine.

(i) Resuming tests for viability and potency of the vaccine and introducing some additional control tests.

(j) Requiring supplementary technical equipment.

It was concluded that I could not recommend the approval of the BCG Laboratory in Karachi until the alterations suggested by me had been carried out.

In May 1959 the Government of Pakistan informed the WHO Regional Office for the Eastern Mediterranean that my previous recommendations for the improvement of the Karachi Laboratory had been satisfied, and that the Government wished "to obtain WHO agreement to the use of the Karachi vaccine in the BCG Campaign". A proposal on the part of the Regional Director WHO/EMRO to make the necessary arrangements for the visit of a WHO consultant to the Laboratory was accepted by the Pakistan Government, and I was asked by WHO to examine the situation in Karachi. I visited Karachi for that purpose between 8 March and 5 April and between 30 April and 11 May 1960. It was agreed by WHO/EMRO that the assessment to be made by me should comprise both the premises, the staff and the work in the Laboratory in Karachi, including the vaccine product.

During my stay in Karachi all help and facilities were given to me by the Pakistan Ministry of Health and UNICEF, and I would like to express my thanks to the following:

Brigadier M. Sharif, Director-General of Health, Government of Pakistan.

Dr Ali Nawab Khan, Assistant Director-General of Health, Government of Pakistan.

Mr Mohammad Ibrahim Dhamee, Deputy Secretary, Ministry of Health, Government of Pakistan.

Dr M. Hasan, Director BCG Campaign.

Dr Shafique Ahmad, Officer in charge of the BCG Production Laboratory, Karachi.

Mr Knud Christensen, Resident Representative, UNICEF Mission in Pakistan.

In the following a survey of the status of the Karachi Laboratory as of May 1960 will be presented. The conditions will be set in relation to those in 1956, and the various problems attached to the Production Laboratory will be mentioned. References to my report in 1956 will have to be made rather often. The suggestions and page numbers indicated in brackets in the following refer to that report.[16]

BCG PRODUCTION LABORATORY

1. PERSONNEL

Reference should be made to the attached list of persons employed in the Laboratory.

In spring 1958 the previous Director of the Laboratory, Dr M. Hasan, resigned from his post. The present Chief of the Department, Dr Shafique Ahmad, has had a good education in bacteriological work, and in 1952-53 he was given a six months' WHO fellowship to study BCG vaccine production at the Statens Seruminstitut, Copenhagen and at the Statens BCG Laboratory in Bergen, Norway. For some time he acted as assistant chief in the BCG Laboratory in Karachi and later as officer-in-charge while the previous chief Dr Hasan was on leave. Dr Shafique works carefully, and his technical skill is very high.

The work of the Chief of the Laboratory claims his full activity. A very heavy responsibility rests on him, and in addition to his routine work on maintaining cultures and preparing vaccine, and to his administrative duties, he has to maintain constant supervision of the personnel and their work and the strictest discipline, a task which is too big for one man to cope with alone. Moreover, another qualified person should always be available for the purpose of taking over the Director's work in his absence. The Director should therefore be assisted by a deputy chief working full time in the Laboratory according to the requirements of WHO.[23] (Sugg. B, p.25).[16] The post as such has not yet been filled; but this question is being seriously considered by the Government.

As the activity of the Laboratory since its being closed down is rather limited and only comprises the maintenance of the cultures and the preparation of a batch of vaccine once or twice a week, it has been rather difficult to form an adequate opinion of the qualification of each staff member. However, I gained a favourable impression of the personnel, who work carefully and are very interested in their job.

2. LABORATORIES

A. The premises

The department consists of the following rooms (see attached sketch). The rooms are all used for the same purpose as in 1956 except rooms X, Z and ?.

Room		
Room	A	Changing room
"	B	Store room
"	C	BCG maintenance
"	D	Vaccine preparation
"	E	Ampoule filling
"	F	Ante-room 1
"	G	Ante-room 2
"	H	Director's office
"	I	W.C. for Director
"	K	Preparation of media
"	L	Technicians' office
"	M	Cleaning of glassware
"	N	Vacuum testing and packing of ampoules
"	O	Not in use
"	P	W.C. and shower for staff, except Director
"	PP	Gas plant
"	R	Sterilization
"	S	Stores
"	T	Cold storage
"	U	Cleaning of tuberculin bottles
"	V	Despatch of vaccine and tuberculin
"	W	Clerical staff
"	X	Office of the Director of BCG Campaign
"	Y	Tuberculin diluting and bottling
"	Z	Lumber room of Director of BCG Campaign
"	?	Three rooms belonging to the WHO/UNICEF BCG Survey Team

For some reason or other the window in the eastern wall of the room N has not been indicated on the sketches in the previous reports on the Laboratory prepared by Drs Timmerman,[12] Agerholm Christensen[3] or myself;[16] the window has a roller blind fitted outside.

Changes in the conditions of the Laboratory since 1956

An electrical power sub-station has been established in the closest proximity to the Laboratory, which is thus supplied with constant and adequate alternating current (AC). A constant and adequate supply of water is also available (Sugg. A and B, p. 25).[16]

In order to prevent further infestation of the Laboratory by white ants, frequent inspection of all outer and inner walls, tightening of new cracks, controls for tightness and recementing of all grooves between the tiles in the rooms outside the sterile section, have been arranged for. (Sugg. F, p. 26).[16] In fact, at present there are no signs of any fresh attack from white ants.

The wooden doors 3, 13 and 14 have been replaced by steel doors with thresholds and glass panes, and the wooden door 7 replaced by a wall with a fixed window. All the wooden doors have been fitted with self-closing hinges. Mats have been placed before doors 12 and 14, and there are shelves with sandals, to be used in the Laboratory only, in front of door 14. In anteroom F a mat soaked in lysol is placed in a metal tray. A wall has been erected in the corridor of the Tuberculin Dilution Section, thus separating the three rooms belonging to the TB Survey Team from the rest of the building. However, this wall only reaches two-thirds of the way up from the floor. (Sugg. G, 1-3, p. 27).[16]

New ceilings have been installed in the rooms outside the sterile section (except the Tuberculin Dilution Section) using compressed sheets of hardboard, and the grooves between the sheets are covered by wooden mouldings. (Sugg. H, p. 27).[16]

All ceilings, doors, windows, walls and the compressors on the verandah have been repainted (Sugg. R, p. 28),[16] and the walls have been given a better and smoother finish. (Sugg. O, p. 27).[16] The rooms appear to be clean and tidy except room R, the windows of which, as in 1956, are nearly always open.

The panes of the door 3 have been painted a greenish colour, and the door is covered with a black curtain; the hatches between the rooms C and K and rooms E and N have been blacked out (Sugg. J, p. 27),[16] only partially, however, as one of the double doors only is covered with a black curtain.

Better electric light for working has been arranged for above the tables in the three sterile rooms, in the ceilings of rooms N, in the corridor between the doors 12 and 14, and above the window in room H. (Sugg. I, p. 27).[16]

The two large autoclaves which have been idle since 1951 have been moved to room L. However, they have not yet been connected with the water pipes and the electric current. A second hot air sterilizer has been erected. (Sugg. D, p. 26).[16]

A special duct for inlet of air passing through the outer wall of room D to the air conditioning plant in that room has been installed.

The verandah surrounding the building has been cleared of rubbish (Sugg. S, p. 28).[16]

From the above, it would appear that the working conditions of the Laboratory have improved considerably. However, it still suffers from quite a number of deficiencies which will be stated here, the majority of which have been mentioned previously.[16]

1. The problem of the air conditioning system has not yet been solved. (Sugg. C, p. 25).[16] The present plant is the same as in 1956, apart from the alteration mentioned above; sometimes it works satisfactorily and is then able to cool down the temperature in the laboratories sufficiently. However, it has not functioned quite frequently due to leakage of the compressors and to non-availability of freon gas. Apparently the filters are able to sterilize the air. The sterilizing effect of the filters was examined several times in 1959 by Dr Shafique and once again by me during my present stay (see later section VIII C). It has been ascertained that a rather strong draught of air along the working benches is caused by the air conditioning system, which may involve the risk of obtaining secondary contamination. Furthermore, the plant suffers from the deficiencies mentioned in my report in 1956, i.e. its apparent inability to create a positive pressure (a possible leakage between doors and door frames may also be considered in this connection) and to supply air containing an adequate proportion of oxygen and carbon dioxide; the difficulty of obtaining airproof fitting between the cover and the frame of the filters of the plant; and the fact that cleaning of the filters and reloading them with fresh material still has to be done inside the sterile rooms. During my stay, an estimate of the cooling capacity of the present plant was made by the Executive Engineer of the Pakistan Public Works Department. This disclosed that the plant was not sufficiently big to fulfil the demands for cooling off the temperature of the Laboratory during the hotter season of the year.

2. Sterilization of media and utensils is still carried out in room R; the two large autoclaves have not been taken into use nor has a third one been erected (Sugg. D, p. 26);[16] the two small upright kerosene-heated autoclaves are therefore still used for sterilizing the media and various items.

3. The roof of the building is not waterproof (p. 11, paragraph 10).[16] On several occasions during my stay in Karachi, I experienced that during rainfall the water came pouring down through the ceilings of rooms L and N, through the roof in room R and the roof covering the veranda between room R and door 5, through the ceilings in the corridor between doors 12 and 14, in anterooms F and G and in rooms Y and V. The ceilings in the Tuberculin Dilution Section also need to be repaired (Sugg. H, p. 27),[16] and the ceiling and walls in different parts of the Laboratory need repainting.

4. Some grooves between the tiles in the western part of the veranda and in the corridor outside the rooms belonging to the TB Survey Team need refilling with cement, and the door frames at the entrance to room V and to one of the rooms belonging to the TB Survey Team need repairing.

5. Doors 2, 8 and 12 have not been supplied with thresholds and doors 5 and 6 not been bricked up. (Sugg. G 1, p. 27).[16]

6. No shower has been established in room B (Sugg. L, p. 27).[16]

7. For lighting the rooms in the laboratories, generally direct electric current (DC) is used: this applies also to the rooms in which additional stronger lighting (supplied with alternating current (AC)) has been provided for, as mentioned previously. Half the incubators in the sterile rooms are driven by DC, the other half by AC. The AC is only seldom interrupted. However, short or fairly long interruptions of the DC take place quite frequently, which is very unfortunate for the cultures in the incubators and for the work of the personnel. The electric light in room K is not sufficiently strong to work by. Quite a few tubes of fluorescent light are missing from the fittings, and one or two table lamps are needed to be used for counting colonies. Owing to their non-availability, sterilamps have not been fitted in rooms K, L, M and N. (Sugg. K, p. 27).[16]

B. Equipment

The technical equipment still needs to be supplemented and extra supplied.

Two incubators and one refrigerator have been out of function for a long time and the staff members have had difficulties in repairing these.

Thermographs have not been installed in the incubators, refrigerators, autoclaves and hot air sterilizers, and neither are maximum and minimum thermometers placed in the apparatus when in use. (Sugg. E, p. 26).[16]

No electrical pH-meter has been installed. (p. 32)[16]

Adequate burners for sealing ampoules are not available (see later sect. VI, B).

No spare parts for electric hair clipper are available and no further metal trays have been procured (see later sect. III). (p. 32)[16]

3. THE ANIMAL QUARTERS

These are arranged as in 1956 (see report 1956[16] and the attached sketch):

M: main entrance
I: yard, formed like an iron barred cage
II):
III): rooms both for breeding stock and test animals
W: water trough
T: water taps

Since 1956, when two compartments in room III for breeding the stock of guinea pigs only were available, four further compartments have been established. A new ceiling made of layers of cardboard has been fitted once since 1956. However, on my arrival in Karachi in 1960 the appearance of the ceiling in the animal quarters was nearly the same as that described in my report 1956.[16] During my stay a new ceiling of plates of asbestos was put up (Sugg. B, p. 28),[16] and the grooves between the asbestos sheets were covered with wooden mouldings. Moreover, it has been ensured that no gaps are left between the ceiling and the walls and between the walls and the roof, so that no squirrels or rats can enter the rooms from the loft. The roof, which during my stay in 1960 was not waterproof, has been repaired and wooden windows with glass panes have been fitted in the two walls facing south and north. Arrangements will be made to install more tight fitting between the window frames and the walls and to affix an iron net to the iron bars surrounding the yard (Sugg. A, p. 28).[16]

The old cages have been replaced by new ones and the boxes stored on top of the ceiling in yard I have been removed (Sugg. C and G, p. 28).[16]

There are still no satisfactory facilities for washing hands, for sterilizing instruments, trays, buckets, and cages; or for aseptic autopsy, etc. Inoculation, and sometimes also autopsy of animals is carried out in the sterilization room R, where also used syringes, forceps and scissors are boiled. Trays used for autopsy of animals are sterilized by soaking them with lysol. Dead animals are burned without previous autoclaving. Only two metal trays are available.

The BCG Laboratory itself breeds its own guinea pigs. A stock of albino guinea pigs for breeding purposes was supplied from Statens Seruminstitut, Copenhagen, several years ago. However, due to infections, the hard climate, etc., it has proved difficult to obtain a sufficient number of suitable animals for the control of the current batches of vaccine. The animals are fed with grain, bran, carrots, and lucerne, and look nice and healthy. The animals for breeding are kept in the compartments, whereas the test animals are placed in metal cages each housing two guinea pigs.

4. SPECIAL PRECAUTIONS AGAINST CONTAMINATION

Medical examination of staff members before appointment and the current Tuberculosis control of them are carried out as described previously by me (p. 5).[16] The possible occurrence of open tuberculosis in the family of the closest contacts of the staff members is not investigated. However, I was informed that the previous Director of the Laboratory, Dr M. Hasan, taking into account that four staff members of the BCG Laboratory have been dismissed from the BCG department in the past due to infection with tuberculosis, is considering arranging for regular examination every three months of the members of the family of each BCG staff member.[6]

All staff members in the BCG Production Section work with BCG exclusively, except the Chief of the Laboratory, who also prepares the dilutions of tuberculin, and the two laboratory attendants who are partly employed in the Tuberculin Dilution Section as mentioned in the attached list of personnel.

The staff members in the BCG Production Section usually wear gowns supplied by the Laboratory (Sugg. G4, p. 27);[16] however, trousers are not changed. When working in the rooms K, L, M, and N the staff put on special sandals which are placed on the shelves in front of door 14, as mentioned in Section II A. Before entering the sterile rooms for work, the personnel do not take a shower nor put on clean underwear. (Sugg. A, p. 28).[16] When working in the sterile section, the personnel use special sandals and sterilized caps, blouses, masks and trousers. New sets have been procured, though not in adequate numbers.

The day before being used, the floors of the rooms of the sterile section are swept, using special brushes for these rooms; the floors are then rubbed with lysol and the sterilamps are lit for 20-30 minutes. Next morning, tables, incubators, refrigerators and cupboards are washed with lysol and ultraviolet light is put on for 30 minutes. The floors of the rooms outside the sterile section are swept early every morning and then rubbed with lysol; later the walls and the furniture are washed with lysol.

The sterile section is locked by means of the door leading from K to F. The keys are in the custody of the Director of the Laboratory and the senior technician. The incubators and refrigerators in the sterile rooms are not fitted with locks. No visitors are allowed in the sterile rooms.

5. PREPARATION OF MEDIA

The BCG Laboratory prepares the various media to be used for growing the cultures of BCG, for preparing BCG vaccine and dilutions of tuberculin, and for controlling the sterility of the above cultures and preparations. The media are prepared according to the prescriptions used by the Statens Seruminstitut, Copenhagen. During my stay I was present during the preparation of one batch of Löwenstein medium which was done correctly, and the product looked satisfactory. Unfortunately the different batches of Löwenstein medium are not kept separately. Therefore tubes from different batches are probably used simultaneously.

The pH of the Sauton medium before autoclaving is 7.4 and after sterilization 7.2, measured colorimetrically by a comparator. The pH of the diluted Sauton medium is not measured.

6. TECHNIQUE OF (A) SUBCULTURE OF STRAINS AND (B) PREPARATION OF VACCINE

A. The Danish strain 956 obtained from the Statens Seruminstitute, Copenhagen, in 1951, has since then been maintained uninterruptedly. The freeze-dried strain 1102, received from the Institut Pasteur, Paris, has also been maintained regularly.

The strains are maintained at 37°C on bile potato medium, transfer of 14-day-old culture on this medium being made every 14 days on to fresh bile potato medium. At the same time transfer of 14-day-old bile potato culture is made on to Sauton potato medium. Fourteen days later a piece of the pellicle on the Sauton medium in the bottom of the tube containing the Sauton potato medium is transferred to flasks with Sauton medium (S_1); after 10-11 days, subcultivation from the film is made on to flasks with fresh Sauton medium (S_2) to be used for vaccine preparation 10-11 days later.

Three parallel strains, viz. two descendants from strain 956 and one descendant from 1102, are maintained regularly every week, a set of S_2 cultures of each of the three strains being available each week for preparation of vaccine. Establishment of S_2 cultures is made by transfer of culture from one mother flask only on to 12 flasks of fresh Sauton medium. The maintenance of strains and preparation of cultures are always performed by the Chief of the Laboratory. According to the records and from my personal observation, the growth on the potato media and in the culture flasks is generally satisfactory, though variations in the intensity of growth may occur. Since August 1959 the pH of the Sauton medium in a sample of the S_1 culture-flasks is measured colorimetrically; the values range from 7.3 to 7.8.

B. Since I left Karachi on 7 June 1956 and up to 21 April 1959, when batch 259 was prepared, a total of seven batches only (batches 252-258) have been produced. Since then vaccine has been prepared more regularly once or twice a week, and up to 31 March 1960 a total of 45 batches have been produced: batches 259-302. (For 301 two batches were prepared, but from the same culture). All these vaccines, except batches 279 and 301A, were prepared by the Chief of the Laboratory. Strain 956 was used for preparing 29 batches and strain 1102 for 16 batches. The age of the S_2 culture used for vaccine preparation was nine days for one batch (302), 12 days for three batches (259, 271, 275), 14 days for three batches (260, 261 and 269) and 10-11 days for the remaining 38 batches (see Table 1). The technique (except for

batches 301-302) was as follows: the contents of three or four flasks of S_2 culture were harvested and pressed, using a Birkhaug apparatus (ITC model). The weight of the weighing beaker including the pressed culture mass was then determined. The culture was transferred to a large flask of the Fernbach type containing 2.5 kg stainless steel balls (diameter 4 mm). The amount of culture transferred for homogenization varied from batch to batch ranging from 5333 to 1[illegible] 782 g., on an average 9434 g per batch (see Table 1). Homogenization of the culture was made first by shaking the homogenization flask by hand rather violently (about 40-50 revolutions per minute), then by rotating the flask in a grinding mill (ITC type) at a speed of 20-25 r.p.m., the rotation axle deviating about 25° from the horizontal. After the first comminution of the culture 20 ml of diluted Sauton medium was added to the homogenization flask and a second comminution made. Finally, sufficient diluted Sauton medium was added to obtain a stock suspension of 40 mg/ml. From batch 301, prepared 18 March 1960 and onwards, the preparation technique used at the Statens Seruminstitut, Copenhagen, has been adopted by the Karachi Laboratory. Table 1 shows that the duration of the homogenization period for the different batches has varied between 2 1/2 and 5 1/2 minutes.

Previously the yield of the culture in the culture flasks used for vaccine preparation was not determined. The pH of the Sauton medium in these flasks is measured using colorimetric procedures; the values range from 7.0 to 7.9.

The vaccine for intradermal use (concentration 0.75 mg/ml) is prepared by adding 19.0 ml of the stock suspension to an Erlenmeyer flask with 1000 ml diluted Sauton medium. A sample of the final vaccine is transferred to a test tube for macroscopic inspection of the homogeneity of the vaccine and for testing it for sterility.

The vaccine is released for being filled off after 48 hours' observation of the sterility tests. It is filled off by hand into 10 cc ampoules of clear glass, using a large flask with an outlet at the bottom. Ordinary Bunsen burners are used for sealing the ampoules. A filling team consisting of one filler and three sealers is able to fill off 2.5 to 3.0 litres vaccine per hour.

7. CONTROL TESTS

A. Sterility tests

As regards the type of media used for the sterility tests and the steps during maintenance and production of culture and the vaccine being controlled, reference should be made to my report, 1956, Section VI (a).[16] It should be added that the Director of the Laboratory does not remember having observed any contamination of the control media after storage of these at 37°C for 48 hours before use. The Sauton and potato media are indirectly tested for sterility before use: the tap water in a test tube affixed to each flask of Sauton medium during sterilization is afterwards tested for sterility. All the tests seem to be adequate. However, no special medium is used for testing for fungi. The results of the sterility tests are carefully recorded.

Of the 44 batches (in fact 45 batches were prepared, but the vaccines 301A and B were prepared using the same cultures) of vaccine prepared during the period 21 April 1959 to 31 March 1960, four proved to be secondarily contaminated, i.e. a contamination rate of 9.1 per cent. Table 2 shows the site and cause of contamination for the four batches. It should be noted here that the mother cultures for the cultures used for preparing the vaccines were all proved to be sterile. None of the above four batches was tested for viability and potency.

B. Tests for viability

This test has been resumed, starting with batch 259 prepared on 21 April 1959 (Sugg. C 1, p. 31).[16] The last batch tested for viability before the test was resumed was batch 246, prepared 17 February 1956.

An estimate of the number of viable bacillary particles in the batches is obtained by counting the colonies formed on the surface of the tubes of Löwenstein medium inoculated with 0.1 ml of vaccine. Two doses of BCG have been used for viability tests, viz. 2 1/2 x 10^{-6} mg and 1/2 x 10^{-6} mg. Two series, each of ten Löwenstein tubes, are inoculated with vaccine, each tube in the one series being seeded with the first-mentioned dose and each tube in the other series with the second dose. No randomization of the tubes has been made and the tubes have never been coded. After seeding the tubes are placed horizontally for 24-48 hours at room temperature

the stoppers are then sealed with paraffin and the tubes incubated at 37°C. Colony counts are made quite regularly after two, three, four and five weeks' incubation and possibly also after still longer periods.

From vaccine batch 300 and onwards three different doses: 8×10^{6}, 2×10^{-6} and $1/2 \times 10^{-6}$ mg are used for seeding the Löwenstein tubes, randomization procedures and unbiased readings being employed. (See later Section VIII).

The testing of the vaccines and the reading of the colonies is made either by the Director of the Laboratory or by one or the other of the two technicians. Unfortunately the name(s) of the tester(s) and reader(s) for the various batches of vaccine is (are) not indicated in the records.

During my stay in Karachi the colony counts were tabulated.

Of the 45 batches of vaccine prepared from 21 April 1959 to 31 March 1960, 32 were tested for viability. However, no results are available for batch 282, as the medium used for the viability test proved to be contaminated. Strain 956 was used for preparing 20 and strain 1102 for the remaining 11 batches of the 31 vaccines from which results of viability tests are available.

Table 1 shows the mean number of colonies per tube of Löwenstein medium for each series of inoculated tubes after incubation of these at 37° for three, four and five weeks. The estimated number of viable units given in the table is based on the mean number after five weeks' incubation of the tubes. The number of tubes used for estimating the mean number of colonies per tube in each series is only indicated (in brackets) for the colony counts after five weeks' incubation. The mode of distribution of the colonies in each series of tubes and the ratio between the mean number of colonies for the two doses are also based upon the counts at five weeks' incubation.

Table 3 shows the interval between the dates of preparing and testing the vaccine. From both Table 1 and 3 it would appear that this interval has varied considerably, viz. from 2-3 days up to 20 days.

Determination of the viability of the vaccines gave the following results: Usually growth could not be demonstrated until after three weeks' incubation of the inoculated Löwenstein tubes at 37°C. The colony counts were in general considerably

higher after four weeks' incubation than after three weeks. After five weeks' incubation there was generally a slight further increase, though for a few batches a rather high increase (see Table 4). The lag phase for the Pakistan vaccine thus seemed to be somewhat longer than what is generally found with the Danish vaccine (when tested in Copenhagen).

Great variations in colony counts were found from batch to batch (evaluation based on the mean number of colonies after five weeks' incubation of the tubes) ranging from 0.71 - > 98.8 colonies per tube for the dose $2\frac{1}{2} \times 10^{-6}$ mg and from 0.4 - 40.1 colonies per tube for the dose $\frac{1}{2} \times 10^{-6}$ mg: a variation of more than 100 times.

Batches 259-274 were prepared during the hottest season of the year. Eight of these 16 batches (seven prepared from strain 956 and one from strain 1102) were tested for viability, viz. at 2-3 days after their preparation. The number of viable units per ml for these batches ranged from 0.6 - 5 millions, on an average 2.74 million per batch (based upon the inoculation dose $\frac{1}{2} \times 10^{-6}$ mg). Batches 275-302 were prepared during the cooler season of the year; 24 of these 29 vaccines (14 prepared from strain 956 and ten from strain 1102) were tested for viability, 2-3 days after preparation for 11 of the batches and at a later time for the remaining vaccines, (one batch, vaccine 282, prepared from strain 956, must be excluded from the material due to secondary contamination of the tubes of Löwenstein medium), and showed a content of viable units ranging from 1.05-60 millions per ml, on an average 11.12 millions per batch (based on inoculation dose $\frac{1}{2} \times 10^{-6}$ mg except for batch 293, for which only the dose $2\frac{1}{2} \times 10^{-6}$ mg was used). Eight of the batches (six prepared from strain 956 and two from strain 1102), distributed at random among the 23 batches, had a viability within the range of that for the above eight batches prepared in the hottest season of the year. However, it should be noted that the five batches (284, 288, 292, 294 and 296) with the lowest viability (1.05, 1.95, 1.2, 2.1 and 3.45 mill. viable units per ml, all prepared from strain 956) were not tested until 16, 9, 5, 9 and 5 days respectively after their preparation, a delay which may account to some extent for their low viability.

Apparently in this material there is thus a tendency for the "summer" vaccines to show a lower viability than the "winter" vaccines; the reason for this tendency is not known (possible strain differences may be of significance). However, it must be admitted that the material is too limited and uncertain to permit definite conclusions to be drawn.

Evaluation of the relationship between the number of colonies developed after inoculation of the two doses $2\frac{1}{2} \times 10^{-6}$ mg and $\frac{1}{2} \times 10^{-6}$ mg gave the following results: of the 31 batches for which colony counts were available, five batches (nos. 272, 276, 293, 300 and 302) must be excluded from the material, as there was contamination and the tubes inoculated with the dose $2\frac{1}{2} \times 10^{-6}$ mg (vaccines 272 and 276 respectively) could not be read, and due to the fact that this dose was the only one used for batch 293, whereas the dose $\frac{1}{2} \times 10^{-6}$ mg only was used for the batches 300 and 302. For the remaining 26 batches, the relationship between the colony counts for the two doses varied from batch to batch.

For the majority of the batches, the number of viable units per ml evaluated on the basis of the counts at five weeks' incubation was larger to varying extents for the inoculation dose $\frac{1}{2} \times 10^{-6}$ mg than for the five times stronger dose. In six cases, however, (batches 259, 269, 285, 289, 292 and 298) the number of viable units was slightly larger for the dose $2\frac{1}{2} \times 10^{-6}$ mg.

It is quite obvious that an increase of the colony counts directly proportional to the increased dose of inoculation could not always be expected, since a certain degree of variation will always occur. If one considers an increase of the colony counts of 3.8-6 times to be "normal" when the dose of inoculation is raised five times, the relation between the counts for the two test doses employed could be classified as "normal" for 13 of the batches. For 11 batches the mean number of colonies for the dose $2\frac{1}{2} \times 10^{-6}$ mg was only 1.7 - 2.9 times higher than the mean number for the five times lower dose. For batch 284 the mean number of colonies was the same for both doses and for batch 287 the mean number of colonies for the dose $\frac{1}{2} \times 10^{-6}$ mg was 2.4 times higher than for the dose $2\ 1/2 \times 10^{-6}$ mg.

The mode of distribution of the colonies between the single tubes in each series of Löwenstein tubes inoculated with the same dose, i.e. the variation in the number of colonies from tube to tube within each series varied greatly. For the

batches mentioned above (272, 276, 293, 300 and 302) which were tested with only one dose, the mode of distribution was "normal". Among the 26 batches, tested with two doses, "normal" mode of distribution was obtained in both series of Löwenstein tubes for 17 batches, poor distribution was found in both series of tubes for three batches, and for six batches "normal" mode of distribution was demonstrated in one of the two series and poor mode in the other. Table 4 shows the poor mode of distribution for the nine batches mentioned.

An interesting phenomenon was the sudden occurrence at the four or five weeks' reading of innumberable colonies on the surface in 1-4 tubes in either or both series of Löwenstein tubes which had shown "normal" mode at the previous readings. I had the opportunity of inspecting the tubes for batches 288, 289 and 290, and no doubt the reason for the sudden occurrence of innumerable colonies was the spreading of the condensed water from the bottom of the tubes over the whole surface of the medium. When making inquiries into the matter, I learned that it was the habit of the person reading the colonies to place the tubes horizontally on the table during the reading. The similar phenomenon observed for batches 271, 281 and 286 could presumably be explained in the same way (the relevant tubes had been discarded before my arrival in Karachi). The tubes with innumerable colonies were, of course, excluded when the estimate of the mean number of colonies per series of tubes was made.

C. Innocuity test

For this test 1 ml of a 5 mg/ml vaccine prepared from the final stock suspension of each batch being tested for potency (see next paragraph) is inoculated intraperitoneally into one coloured guinea pig, and until batch 277, prepared 14 November 1959, a similar dose was also inoculated subcutaneously into one further guinea pig. The animals are not tested with tuberculin before vaccination; they are weighed once a week until six weeks after inoculation and then once every month.

The records show that all the animals had gained weight during that period. Usually the animals were killed and autopsied after two months; a few had died spontaneously, mostly due to pneumonia. In no case had signs of the possible presence of virulent tubercle bacilli been demonstrated.

All animals tested for innocuity since September 1959 had been kept for the purpose of being autopsied by me. On 21 March 1960 nine of these animals were killed and then examined by me: no signs of progressive TB were found. Due to the very poor and rather risky conditions under which autopsy is made in the Karachi Laboratory, I refrained from examining more animals. On account of the very few suitable animals available for the Laboratory, the test for the innocuity of the culture on Sauton potato medium which is destined to be the mother culture of a batch of vaccine is done only occasionally.

D. Potency test

This test[8] was resumed by the laboratory simultaneously with the test for viability, i.e. from batch 259 of 21 April 1959 (Sugg. B, p. 31).[16] For the test four injections of 0.1 ml of vaccine of 1/1 (0.75 mg/ml), 1/10, 1/100 and 1/1000 strength are given intracutaneously to 1-2 male albino guinea pigs. No randomization of the animals is made, and the animals are not tested with tuberculin before vaccination, and they are not weighed after vaccination. The sites of injection are inspected twice weekly up to 42 days after inoculation. On each weekday after inoculation the hairs of the abdomen are clipped before reading the nodules. The size of the nodules appearing is not measured with a millimeter ruler, but the approximate size is indicated in the protocols by drawing the outline of the nodules. The occurrence of necrosis is also recorded. Vaccination of the animals and the reading of the reactions are most often done by the Chief of the Department, if not, then by the senior technician. Arrangements for ensuring unbiased readings are not made. The protocols do not give any information as regards the person(s) who tested the vaccine or read the reactions.

All the 32 vaccines tested for viability were tested for potency except five (batches 269, 283, 284, 285 and 287); in addition batch 274, not tested for viability, was tested for potency. It will be seen from Table 1 that for 19 batches the dates of testing the vaccine for viability and potency were the same. For eight batches (vaccines 264, 282, 286, 288, 289, 290, 291 and 293), however, the tests for potency were carried out 2, 1, 2, 3, 3, 10, 10 and 11 days respectively later than the test for viability. On making inquiries into this matter, I learned

that the 10-fold serial dilutions of vaccine to be used for the viability and potency tests had been prepared simultaneously. In the cases where the animals were inoculated at a later date than the date where the vaccines were tested for viability, the serial dilutions for vaccinating the animals had been stored in a refrigerator.

For 13 of the batches two animals and for 15 of the batches only one animal were used for the test (see Table 5). The weight of the animals (see Table 5) at the time of testing varied from 340-720 g, the average weight being 574.9 g (the animals for batch 302 are not included in this estimate).

Already on the first day of reading the reactions in the animals (2-6 days after inoculation) nodules had developed at the four sites of injection for all guinea-pigs; however, the further development of the nodules varied.

The evaluation of the potency in guinea-pigs is based upon the records of the development and size of the nodules at the four sites of inoculation and a possible resulting necrosis of these as shown in Table 5.

During the period 11-15 September 1959 eight of the 14 animals, vaccinated with seven different batches, died of pneumonia.

The 15 batches tested for potency in one animal only (vaccines within the batch numbers 281-299) must be excluded from the evaluation due to the rather great variations between guinea-pigs as regards their way of reacting. The results from testing each batch of vaccine for potency in one animal only do not allow for any estimation to be made. Also three of the 13 batches tested in two animals, i.e. 271, 272 and 302, must be excluded as the animals vaccinated with batches 271 and 272 died from pneumonia within 25 days, 14 and 17 days after vaccination, respectively, and unfortunately I am not in possession of the results of testing batch 302.

The remaining ten vaccines have been divided arbitrarily into three categories as regards potency: strong, moderately strong and weak. Four vaccines were strong, two moderately strong and four were weak. The <u>weak</u> vaccines (259, 262, 263 and 264) were prepared during the summer and showed low viability; of the two <u>moderately strong</u> vaccines, both showing low viability, one (261) was prepared during the summer, and the other (279) during the winter. The remaining four <u>strong</u> vaccines were prepared during the winter. The viability of one of these (274) was not examined; the

three other vaccines (276, 277 and 300) showed relatively high viability (for batch 277 an exceptionally large amount of culture was used for vaccine preparation).

It must be admitted that due to the very limited number of animals used for the test, the lack of exact measurement of the size of the nodules, the varying length of the period between the dates of preparation and testing the vaccines and between the dates of testing the vaccines for viability and potency, it is difficult to form an exact impression of the potency of the Pakistan vaccine.

E. Test for Tuberculin sensitivity

The guinea pigs used for the potency test of the 14 vaccines within batch nos. 259-286 (except vaccine 271 and 272) were tuberculin tested at from 45-101 days after vaccination, on an average 59.8 days, by injecting 20 TU of PPD RT23 in buffered Tween 80 solution intracutaneously in the flank; the area had been depilated seven hours in advance. At the reading of the reactions 24, 48, 72 and 96 hours later, the transverse and longitudinal diameter of the redness was measured with a millimeter ruler.

F. Determination of homogeneity

As already mentioned in Section VI, paragraph B, the homogeneity of each batch of vaccine is roughly estimated by visual inspection of the freshly prepared vaccine. In addition the homogeneity of the vaccine tested for viability is examined by microscopical examination of slides stained by the Ziehl-Neelsen method.

Slides of the majority of the vaccines tested for viability during the last year were kept and were examined by me. In general, more than half of the bacillary units in the vaccines were separate bacilli, the remaining units consisted mainly of smaller clumps, sometimes also of some bigger and even some very big clumps. Variation between the vaccines as regards their dispersion seemed to occur. However, it must be admitted that the test is very rough and it was difficult to form an exact opinion of the dispersion of a given vaccine, as the distribution of the different bacillary units varied from one visual field to the other. On this account, I shall refrain from drawing any conclusion from the results of the tests for homogeneity as regards the influence the dispersion of the vaccines may have had on the number of viable units demonstrated in the vaccines.

G. Determination of moisture content in the pressed culture

The culture mass used for preparing the vaccine is dried in a desiccator for one week with silicagel as desiccant. Results are available for 16 of the 44 batches made during the last year (see Table 1). The water content ranged from 64.2 per cent. to 73.2 per cent. with an average of 68.5 per cent.

H. Tightness of sealed ampoules

The sealed ampoules are tested for possible leakage at a pressure of about 210 mm. The rate of leakage is about 5 per cent.

I. Test for transmission of light

No such test for controlling the concentration of the Pakistan vaccine is carried out.[9]

J. Testing in children for potency of each batch of vaccine has not yet been done regularly.[12,16,23]

8. SPECIAL INVESTIGATIONS

A. During my stay in Karachi I had the opportunity of examining under microscope the germinating rate of the freshly prepared Pakistan batches of vaccine Nos. 300 and 302, when inoculated on to Dubos' solid agar medium (plates of this medium taken with me from Copenhagen).[24,25] The investigations showed that the speed and mode of multiplication of the bacillary units of these two Pakistan vaccines was very good and corresponded to what is generally found for freshly prepared Danish routine vaccine (when tested in Copenhagen).[20]

B. In Section VII, paragraph B a report is given of the great variations observed as regards the viability of successive batches of Pakistan vaccine. The factors responsible for these variations are discussed in Section IX. In order to avoid such great variations in future, I tried my best during my stay in Karachi to persuade the Director of the Laboratory and his assistants both to introduce standard techniques for preparing and controlling the Pakistan vaccine and to employ randomizing procedures when selecting animals, tubes of Löwenstein medium, etc., for the tests and to ensure unbiased readings of the results of testing.[20] I also trained them in using the above

techniques and procedures. Thus the testing of Pakistan vaccines 300 and 302 for viability was made under my supervision using randomization and coding of the tubes of Löwenstein medium to be inoculated. In order to obtain readable number of colonies and thereby adequate counts, three doses of both vaccines were inoculated.[20] Batch 302 was tested on two different preparations of Löwenstein medium in order to reveal possible differences between them as regards their quality as a breeding soil.[20] The results given in Table 6 show that a satisfactory ratio between the colony counts for the three different doses of vaccine inoculated, and a "normal" mode of distribution of the colonies in the tubes in each series of the seeded tubes of Löwenstein medium was obtained, and that the quality of the two preparations of Löwenstein medium appeared to be the same.

C. Nutrient plates were exposed directly for one hour to the air blown in by each of the three air conditioning plants in rooms C-E, and plates were also left open for one hour on the working benches in the same laboratories. The ultraviolet lamps had been lit for one hour in advance and the air conditioning system had been in function simultaneously. During the exposure of the nutrient plates, no work was being carried out in the sterile rooms. After exposure the plates were incubated at 37°C. Only a few colonies appeared on some of the exposed plates.[16]

D. On several occasions during my stay in Pakistan, unbiased readings of the same tubes of Löwenstein medium inoculated with different batches of vaccine were done independently by the Director of the Laboratory, the senior technician and me. Only small variations were revealed between the number of colonies counted by us.

E. On several occasions plans for establishing a new air conditioning system in the BCG Laboratory were discussed in detail by Drs Hasan, Shafique Ahmad and me, sometimes when representatives from Ali Automobiles Limited, Karachi, were also present. On my departure from Karachi there was general agreement between us to accept the plan for setting up a new ventilation plant, as worked out by Ali Automobiles at the request of UNICEF, as the best solution of the problem.

9. DISCUSSION

After the cessation of the Second World War several large BCG mass-vaccination campaigns were started both in and outside Europe, at the beginning under the auspices of the Scandinavian Red Cross and Relief Organizations, later conducted by the International Tuberculosis Campaign, and finally directed and sponsored by WHO-UNICEF. The BCG vaccine used for these campaigns was primarily supplied by a few production centres, viz. the Pasteur Institute in Paris, the BCG Laboratory in Gothenburg, Sweden, and the State Serum Institute in Copenhagen. However, it was the policy of the above organizations to establish new BCG laboratories in the various countries where mass vaccination campaigns were about to be started or were already in progress and to educate a number of bacteriologists in the technique of preparing and controlling BCG vaccine for the purpose of commencing large scale production of vaccine in the newly erected laboratories or in laboratories already existing. The reason for this policy was partly (a) financial, because the vaccine could then be supplied at the expense of the respective governments and the expenditures for the long and costly transportation of the vaccine by air from the above-mentioned three laboratories could be saved, and (b) practical, as the lifetime of the liquid BCG vaccine is rather limited and with the shorter distance between the production centre and the place where the vaccine was to be used it might be easier to ensure supply of vaccine in its freshest state when prepared in the respective countries. Also in case of emergency, war for example, it would be a great advantage to be able to draw on locally produced vaccine.

One of the biggest BCG laboratories established under the above scheme was the BCG Production Laboratory in Karachi.[10,1]

Quite a number of BCG laboratories erected during the first decade after the Second World War have met with a series of difficulties of various types from time to time, with the result that some laboratories have had to be closed down for shorter and longer periods, and unfortunately the Karachi Laboratory has shared the disastrous fate of these.

Due to various factors the current batches of vaccine prepared by the laboratories all over the world have previously varied very much as regards viability.[16,18,19,11] In addition large differences have been demonstrated in past years between the biological efficacy of vaccines prepared by different production centres.[4] From the point of view of comparison of the results of vaccination from one country to another, this is a very unfortunate matter.

It should be noted that on the whole it is now possible to standardize the technique of preparing liquid BCG vaccine and the methods for its control;[13,14,17,20] if a laboratory does not succeed in preparing "standard" vaccine, it might be better to obtain vaccine prepared from another production centre which is successful in this respect.

On account of the unsatisfactory results of vaccination with vaccines produced from different sources, as mentioned above, the opinion is more and more gaining ground that further BCG laboratories should not be established, and that it might even be considered reducing the number of the existing production centres; instead a few large laboratories should be maintained for the purpose of covering the demands of large areas for vaccine. In this way a supply of "standard" vaccine could be ensured, which would mean great benefit, both for the results of immunization and for the comparison of the results of vaccination. In particular, this advantage would hold good when suitable freeze-dried vaccine will be available in the probably not too distant future. However, WHO has not yet recommended the introduction of freeze-dried BCG vaccine for general use.

It might be appropriate to make clear briefly what are the main tasks of a BCG production centre. These are as follows:

1. To supply a "sterile" (non-secondarily-contaminated) vaccine, prepared from a potent strain, of as high viability as possible, and in such a concentration - with due respect to avoiding a too high complication rate - that the highest and longest lasting allergy (and thereby presumably also immunity) is obtained in the vaccinated individuals.

2. To supply vaccine of "standard" (uniform) quality, i.e. with as little variation from batch to batch as possible.

3. To be able at any time to meet the demands for vaccine from the field workers.

4. To keep itself informed of improvements and new aspects in this particular field of work, and

5. To develop better methods for production and control, and possibly carry out research.

The fulfilment of the above claims requires satisfactory and safe working conditions in the laboratory, the full activity, skill, interest, energy and high sense of responsibility on the part of the Director and deputy director of the Laboratory, a sufficiently large, well-educated and reliable staff, strict discipline and the loyal support of the authorities.

Let us then consider the situation in Pakistan. From the previous sections of this report, it would appear that a considerable improvement has taken place in the BCG production laboratory in Karachi since my visit in 1956, and the majority of the main demands for reducing the risk of contamination of the BCG cultures and vaccine have been met. Thus all precautions against the presence of white ants in the building have been taken, and actually at the moment it is not possible to demonstrate signs of fresh attacks from these creatures. A series of alterations in the premises for the purpose of preventing gross contamination of the air have been made; on the whole, sufficient electrical power is now available and a sufficient quantity of water is also supplied. As a result, the vaccines (with some few exceptions) have proved to be sterile. Better working conditions have been procured, the rooms in the laboratory have been repainted and look much nicer than previously, and the conditions in the animal quarters are much better. I have also been given to understand that dismissal of such staff members who prove to be disobedient, unreliable, lazy and non-qualified by the Director of the Laboratory is now possible, and my impression of the present staff is favourable. Finally, some tests used previously for controlling the vaccine have been resumed and new ones adopted.

However, the laboratory still suffers from some important deficiencies. As already pointed out in my report 1956 (page 25)[16] it must be admitted that the position of the BCG production centre in Karachi is most unfortunate. In addition the building will probably never become ideal for its present purpose. Still a number of alterations

of the building will have to be made, and a new air-conditioning system and better facilities for sterilization of media and utensils and for inoculation and autopsy of animals will have to be established before mass production of vaccine can be resumed. This means a heavy expenditure on the part of the Pakistan Government and perhaps also UNICEF. It should also be borne in mind that constant maintenance of the building and the animal quarters is needed which presumably will be rather costly. The question is therefore now: what can be done?

On the assumption that on certain conditions (see later) WHO will approve the present laboratory, I think three alternatives should be taken into consideration:

(a) Apparently it is the intention of the Government of Pakistan to move governmental offices, institutions, laboratories, etc., to Islamabad near Rawalpindi some time in the future, and this may also apply to the BCG laboratory. It might therefore be worth while to arrange for the establishment, either immediately or at a later date, of a quite new BCG laboratory in Islamabad to which the equipment of the present laboratory could be moved in due course. In this way further investment of money into an unsatisfactory institution could be avoided, much better and safer working conditions could be provided, and meanwhile the BCG cultures and the staff (or some staff members only) could still be maintained. Supply of vaccine could be obtained in the meantime from another laboratory at the expense of the Pakistan Government or perhaps be delivered by UNICEF.

(b) The present laboratory should be made ready for mass production and function either preliminarily until a new laboratory has been established in Islamabad or permanently if no new laboratory is set up.

(c) The present laboratory should be closed down and production of BCG vaccine in Pakistan given up completely. Vaccine from abroad would then have to be imported.

In short, what is to be decided is whether mass production of BCG vaccine in Pakistan is to be resumed and continued, and if so, where the vaccine should be prepared. For the solving of the problem, I would like to refer to my statement above regarding the tendency to centralize the preparation of BCG vaccine. It must be emphasized that it is of course completely in order for a big country like Pakistan with its more than 90 million inhabitants to have its own BCG production centre. I also feel sure that if

either a new laboratory were erected in Islamabad or if the working conditions of the present Laboratory were arranged as suggested in this report and the premises be maintained properly; if standardized techniques for production and control of vaccine are introduced, and if the Director of the Laboratory maintains strict discipline and arranges the work as suggested here, a good vaccine product will result. However, in addition to the technical and practical side of the problem, its economical aspects must also be considered. Will it pay to maintain such a laboratory - in particular the present one - if a good vaccine can be obtained from other sources at reasonable prices, particularly if in the not too distant future freeze-dried BCG vaccine from "central" laboratories might be available and perhaps be supplied at reduced rates or even free of charge through UNICEF? It might therefore be appropriate to make a thorouth estimate in Karachi of all expenses in connection with the maintenance of a laboratory for the purpose of producing 30 litres vaccine per week. Such an estimate should be based upon the expenditures in connexion with either the reconstruction of the present laboratory, including the procurement of a new air-conditioning system, autoclaves, etc., or the setting up of quite a new laboratory in Islamabad, the control tests of the vaccine, research work, possibly the establishment of a plant for preparing freeze-dried vaccine, and the extensive investigations thus implied.

The problem of the future supply of BCG vaccine for Pakistan is, of course, a matter to be decided upon by the Pakistan Government, probably in consultation with UNICEF. The three alternatives mentioned above were presented to the Director-General of Health, Brigadier Sharif, at a meeting on 28 March 1960, where the situation of the BCG Laboratory in Karachi was discussed in detail. On that occasion, and also on my departure from Karachi, in May, I was under the impression that the Director-General of Health was in favour of alternative No. 2. Accordingly, I gave him to understand that I would advise UNICEF to make arrangements for establishing a new air conditioning system and a new and bigger autoclave and hot air sterilizer of the "through-the-wall" type, provided that the Pakistan Government would arrange for carrying out the alterations suggested in this report and would be willing to guarantee good maintenance of the laboratory in future.

In the period between my last conversation with the Director-General of Health in May 1960 and the submittal of this report the views of the Pakistan Government and UNICEF regarding the above problem may have changed. The suggestions made in Section X

of this report regarding the alterations of the premises and animal quarters and regarding the technical equipment and the conclusions drawn, are based on the state of affairs as of May 1960.

At its Third Session in London, 2-7 May 1949, the WHO Expert Committee on Biological Standardization set up "REQUIREMENTS FOR LABORATORIES ENGAGED IN THE PREPARATION OF BCG VACCINE FOR THE UNICEF VACCINATION CAMPAIGN".[23] It is stated in the first paragraph of these requirements: "In the present state of our knowledge it is unfortunately unavoidable that vaccination with BCG should have to be performed on human subjects before all the necessary precautionary testing of the vaccine has been completed. This position is unsatisfactory, and a reliable drying technique, including a sufficiently accurate estimate of the viable bacilli together with their properties in the dried product is an urgent necessity". It is indeed very unfortunate that a test for the viability of the liquid BCG vaccine is not directly included in the control tests prescribed in the WHO Requirements, the more so as the Expert Committee in its above statement emphazises the importance of the viability of the dry BCG vaccine. In this connexion it is of interest to note that the examination for the number of viable BCG bacilli in the liquid vaccine is one of "the tests of the vaccine which can and should be made in the laboratory" as stated in 1949 by the International Tuberculosis Campaign (J. Holm).[7]

During the last decade the producers of BCG all over the world have paid more and more attention to the problem of the viability of the vaccine, and the superiority of the live vaccine over the killed vaccine as regards allergizing and immunizing capacity has been demonstrated clearly.[4,21,22]

As a result, tests for viability of the current batches of liquid vaccine are carried out regularly by no doubt the majority of the BCG production laboratories and are now regarded as indispensable for the evaluation of the quality of the vaccine. It is therefore quite obvious that when nowadays an assessment of the BCG production centre has to be undertaken, an evaluation of the different properties of the vaccine product including also its viability should be made, although the test for viability is only hinted at in the WHO Requirements. It is only to be hoped that the WHO Requirements will be revised as soon as possible and a test for viability directly included as essential.

A test for viability could be made in different ways:

(i) by inoculating graded doses of the vaccine either:

(a) on to solid medium, e.g. Löwenstein medium, for a subsequent counting of the colonies appearing after incubation of the medium at 37°C, or

(b) into tubes with fluid medium for determining the highest dilution of vaccine still giving growth;

(ii) by studying by microscopy the multiplication of the BCG bacilli when inoculated on to Dubos' solid agar medium,[24,5] and

(iii) by determining the oxygen-absorbing capacity of the vaccine in the Warburg apparatus.

Some laboratories are using all three tests for viability, some only two. However, the majority only use one test, generally the enumeration of colonies.

Already during my visit to the Karachi Laboratory in 1956, it was demonstrated that the colony counts on Löwenstein medium for the current batches of Pakistan vaccine showed very great variations.[16] Similar observations were later made by me when visiting other production centres, and apparently this is a feature recurring constantly.[18,19,11] Also during my visit to Karachi in 1960 great variations between the batches as regards their viability were observed.

The reason for these variations may be actual differences in the viability of the vaccine (due to variations in the age and quality of the cultures used for preparing the vaccines (unfortunately the yield of culture in the culture-flasks has only been measured for batches 300-302), the type of strain, the amount of culture homogenized, the homogenization procedure, the storage conditions of the vaccine before testing (duration and temperature of storage)), differences in the degree of dispersion of the bacilli, in the testing technique and the quality of Löwenstein medium, inadequate sealing of the stoppers in the tubes of Löwenstein medium, differences in the temperature in the incubator where the inoculated tubes of Löwenstein medium are kept, in the technique of reading the colonies (different readers), etc. Apparently "summer" vaccines tended to have a lower viability than "winter" vaccines. Batch 269, prepared 16 July 1959 from 14-day-old culture showed the lowest viability.

Concerning the varying relationship from batch to batch between the colony counts obtained after inoculating two different doses of the same vaccine on Löwenstein medium, some failure in the testing technique (e.g. shaking of the test tubes), inadequate sealing of the Löwenstein tubes (resulting in a dessication of the medium), possibly inferior quality of the Löwenstein medium, secondary infection of the tubes or some failure in the reading technique may have been the cause. In this connexion, it should again be noted (as stated in Section VII, paragraph B) that the cause of the sudden occurrence of from many up to innumerable colonies in some tubes, which at previous readings showed few colonies only, was found, in the majority of the cases, to be due to the spreading of the condensed water over the surface of the medium during the reading of the colonies.

From the above it would appear that the variations in viability between the batches of Pakistan vaccine are much too big, and it is thus very difficult to get a true picture of the viability, as many factors may influence the colony count. It must be borne in mind that for the last two batches tested, viz. 300 and 302, both of which showed a high germination rate of the bacilli similar to that of Danish vaccine and which were tested for viability on Löwenstein medium using randomization and coding of the tubes of Löwenstein medium, standardized testing techniques and unbiased readings, satisfactory results were obtained, with a good relationship between the colony counts for the different inoculation doses and "normal" distribution of the colonies on the tubes in each series of seeded tubes.

The two other tests for controlling the viability of the vaccines, viz. the determination of the germination rate during microscopy of the bacilli and the oxygen-absorbing capacity are both very useful for obtaining quick information (for the latter even quantitative) regarding the viability before the vaccine is issued for use. It is therefore worth while to consider including these tests in the regular control of the vaccine.

It has been mentioned before (see Section VII B) that the interval between the dates of preparing and testing the vaccines has varied considerably. However, the keeping properties of the Pakistan vaccine have never been examined. It is therefore quite obvious that the various tests for viability, potency, innocuity, etc., of the vaccine should be made simultaneously and in the freshest state of the vaccine when also

issued for use in humans. Only in this way is it possible to correlate the results of the laboratory tests of a given vaccine mutually and to compare them with those obtained with the same batch in the field, and to make comparison between successive batches.

The problem of the keeping properties of the BCG vaccine deserves great considerat: For the Danish vaccine these are well known from extensive field and laboratory investigations.[4,14,15,17,18] Rather strong BCG-induced allergy can be obtained in the Pakistan population as shown in children vaccinated in 1956 under controlled conditions with two batches of Danish vaccine (vaccines 1187 and 1188) stores for 28-47 days;[2] "an even stronger allergy might have been obtained with fresher vaccine". However, "assessment in 1956 of mass vaccinations in Pakistan revealed both intermediate and low levels of post-vaccination allergy"[2] and this applied both to children vaccinated between September 1953 and February 1955 with six various lots of Karachi vaccine as well as to those vaccinated in July 1955 with one lot of Danish vaccine (batch 1159). In this connexion it should be noted that the above three Danish vaccines, batches 1159, 1187 and 1188, when controlled in freshly prepared state in Copenhagen, showed nearly the same high viability, viz. a content of 23.8, 32.2 and 26.2 million viable bacillary units per mg and thus did not differ from what is generally found as regards viability for Danish vaccine. It is regretted that it is not possible at present to see the results of the viability tests of the six lots of Karachi vaccine mentioned. The low allergenic potency of the seven vaccines reported on may be due to a possible reduction in their viability possibly caused by inadequate storage of the vaccine before use (exposure to intense heat and light). It would therefore be of great importance to examine the keeping qualities of the Karachi vaccine (when satisfactory results from testing a series of successive vaccines for viability are available), both when stored at different temperatures for different periods of time and when exposed to light at varying lengths of time.

10. RECOMMENDATIONS

1. Personnel

The post as assistant chief should be filled (Sugg. B, p. 25).[23,16] It might be a good idea to have the person in question trained in a laboratory abroad in order to learn the latest methods for preparation and control of the vaccine.

The personnel should be kept in constant training.

2. Laboratories

A. The premises

1. A new air conditioning plant should be installed for the supply of the sterile section with climatized absolutely sterile air drawn in for filtration from outside and possibly also partly from inside, and blown into the rooms from above, thus causing a positive pressure. The air should be cooled down to obtain a working temperature inside the laboratories of not more than 24°C even when three or four Bunsen burners are burning for many hours. The air should be changed five to seven times per hour and an even distribution of the air being blown in should be ensured in order to avoid the causing of any draught. The air should leave the section through the anterooms, the hatches, and possibly through outlets at floor level. The filters should be fitted so that changing and reloading are carried out outside the sterile section. Great care should be taken not to leave any gaps between filter and frame. The sterilizing effect of the air conditioning system and the function of the system as a whole should be controlled regularly. (Sugg. C, p. 25).[16]

2. The vacuum testing, labelling and packing of the vaccine ampoules should be moved from room N to room K, for which purpose roller blinds of black light-proof oil cloth might be fitted in front of the window in room K. All sterilization procedures should then be moved from room R to room N. A big cupboard-shaped, through-the-wall-type autoclave and a big hot air sterilizer of the same type should be procured and be fitted in the doorway opening to room O (unless this doorway has to be used as emergency exit) and in the window gap of the neighbouring eastern wall of room N. The doors of the apparatus should be on a level with the wall and the "body" of the apparatus thus be placed in room O and on the back verandah. Airtight fitting between the apparatus and the wall should be ensured to prevent dust from entering the Laboratory from outside by that way. The doors of the apparatus should be insulated in order to reduce the heat given off from the apparatus. The apparatus should be operated from inside in room N. The small upright circular autoclave, left idle since the establishment of the Laboratory, and the present small hot air sterilizer should be placed along the eastern wall of room N to be used for sterilization of occasional smaller quantities. If sufficient space is available, also the distillation apparatus could be placed along this wall. If possible, arrangements should be made for removing the air from

the big autoclave by vacuum before sterilization. A vapour hood furnished with an exhaust fan or a suction system should be installed above the sterilizing apparatus. Cooling of the room should be made by moving one of the present air-conditioning apparatus from the sterile section to this room. The sterilization apparatus should be furnished with thermographs, external thermometers, manometers and manographs (autoclaves) and should be able to work automatically on time. Maximum thermometers should be placed in the autoclaves and hot air sterilizers during sterilization, and until thermographs have been installed, reading of the temperature in the hot air sterilizers (as shown by the external thermometers) should be taken and recorded every half hour. The sterilizing effect of autoclaves and hot air sterilizers should be controlled at regular intervals[3] (Sugg. D and E, p. 26).[16]

3. Preparation of the media should be moved from room K to room N. Possibly the filling off of vaccine should be moved from room E to room C and the maintenance of the strains and culture from room C to room E.

4. Room A should be used as changing room for the personnel. A sufficient number of separate cupboards with locks and a wash basin should be installed there. A new ante-room should be made in front of room F; room F should be turned into a bathroom with shower (possibly screened by a thin wall), floor-outlet, electric water-heater, etc. (Sugg. L, p. 27).[16] Room X might be used as office for the deputy director. If so, better electric light and a fan should be provided and a writing desk installed.

5. The same precautions to prevent further infestation of the rooms by white ants as suggested in 1956 (Sugg. F, p. 26)[16] should be taken. Wooden windows and doors should possibly be replaced by steel windows and doors.

6. The roof of the building should be made waterproof and be regularly controlled for tightness; also tight fitting between the roof and the walls should be ensured to prevent squirrels and rats from entering the loft. The fitting between the ceilings and the walls in the sterile section and between the ceilings and the wooden mouldings covering the grooves between the sheets of compressed hardboard forming the lower part of the double layered ceiling should be made airtight in order to maintain a positive air pressure inside the rooms when the air-conditioning system is in function. Possibly the ceiling should be insulated. The ceiling in the tuberculin section needs to be repaired. (Sugg. H, p. 27)[16]

7. All precautions should be taken to prevent dust, sand and bacteria from entering into the Laboratory from outside (Sugg. G, p. 27).[16] All doors should be kept closed; the wooden doors 2 and 8 should be supplied with thresholds, and doors 5 and 6 should be closed or bricked up. The wall erected in the corridor of the tuberculin dilution section should be replaced by a wall separating completely the three rooms and room Z from the rest of the building.

8. Alternating current should be used for all lighting of the rooms and all electrical apparatus. Better electric light (fluorescent light) should be provided above the window table and the wall table in room K. A sufficient number of tubes of fluorescent ordinary and ultraviolet light (Sugg. I and K, p. 27)[16] and a table lamp for counting colonies should be fitted.

9. The panes of the hatches between rooms C and K and between rooms E and N should be painted black (Sugg. J, p. 27).[16]

10. Suggestions for a more practical placing of incubators inside the sterile section have been made to the Director of the Laboratory (Sugg. Q, p. 28).[16] For practical reasons, the media inoculated for the sterility control and the seeded Löwenstein tubes should be kept in an incubator placed outside the sterile section.

11. Repainting of the Laboratory is needed in some places, for example, where peeling off of the paint of the ceiling has taken place and where repairs have been undertaken.

B. Equipment

The Laboratory should always have sufficient technical equipment available in order to be able at any time to meet the demands from outside for vaccine and tuberculin. The three pieces of apparatus which are out of function should be repaired (see section II B).

The following supplementary technical equipment is required:

One large cupboard-shaped, through-the-wall-type autoclave to be heated electrically. This should be fitted with thermograph, manograph, external thermometer and manometer, and be able to work automatically on time; the air inside the autoclave should be removed by suction before sterilization is started.

One large through-the-wall-type hot air sterilizer, fitted with thermograph and external thermometer which is able to work automatically on time.

One electric pH-meter.

Thermographs for the two autoclaves previously procured for the Laboratory, for the two hot air sterilizers (one in the BCG production section and one in the tuberculin dilution section), for four incubators and two refrigerators, if at all possible to furnish these items with such measuring instruments.

Extra sterilamps with accessories.

Extra metal trays for dissection of animals.

Maximum thermometers for autoclaves and hot air sterilizers.

Maximum and minimum thermometers for incubators and refrigerators.

One micrometer.

Adequate burners for sealing ampoules, possibly in conjunction with a compressor.

Possibly extra refrigerators.

One electric hair clipper with spare parts.

3. The Animal Quarters

1. To prevent wild squirrels and rats from roaming in yard 1, an iron net should be affixed to the iron bars surrounding the yard (Sugg. A, p. 28).[16] The roof and the walls should be constantly controlled for tightness; it should be particularly ensured that there are no gaps in the fittings between the ceiling and the walls, between the walls and roof, and in the walls above the ceiling.

2. A greater number of suitable animals should be made available (Sugg. E, p. 28).[16] The high death rate among the animals during winter should be prevented; a veterinarian might be consulted in this respect.

3. Room R (see sketch of laboratories) should be used as inoculation and autopsy room and satisfactory facilities provided for washing hands and for sterilizing instruments (Sugg. F, p. 28).[16] The big autoclave which has been idle since the establishment of the laboratory should be erected here to be used for sterilizing used trays, buckets and other infectious material.

4. Special Precautions against Contamination

(1) Consideration should be given to controlling the family contacts of each staff member for tuberculosis every three months (suggestion made by Dr M. Hasan).[6]

(2) Outdoor clothes should be exchanged for laboratory garments (clean gowns and trousers, to be supplied by the Laboratory) and these garments should not be worn outside the Laboratory (Sugg. G 4, p. 27).[16]

(3) Before entering the sterile rooms for work, the personnel should take a shower and wash their whole body thoroughly with soap. They should then put on clean underwear (to be supplied by the Laboratory) and sterile jackets, trousers, caps and masks. An adequate supply of these garments should be ensured (Sugg. A, p. 28).[16]

(4) Instead of being swept, the floors in the sterile section should be soaked with soap and water and possibly then rubbed with lysol. Walls, working benches and furniture should also be cleaned in the same way at the same time as the floors (the day before being used for sterile work), and during the cleaning process the air conditioning system should be allowed to work in order to remove dust. The sterilamps should possibly be left burning overnight. (Sugg. B, p. 28)[16]

(5) The floors in the rooms outside the sterile section should be washed instead of swept in order to avoid whirling up the dust.

5. Preparation of Media

(1) An electrical pH-meter should be provided.

(2) The pH of a sample of each preparation of the diluted Sauton medium should always be measured after sterilization.

(3) Each batch of Löwenstein medium should be given a number. The tubes of each batch should be kept in separate wire baskets and each basket should be furnished with a label indicating the number of and date of preparation of the batch of Löwenstein medium. (This suggestion has been accepted by the Director of the Laboratory)

6. Technique of (A) Subculture of Strains and (B) Preparation of Vaccine

(A) 1. Only one strain (956, found to be sterile when examined in Copenhagen 1956) should be used for vaccine production. Another strain (1102) may be maintained as reserve.

2. Reference should here be made to suggestions paragraph A 2-3, p. 29 in my report 1956,[16] which also apply to this report.

(B) 1. The temperature in the laboratory should be measured at the beginning of, during and after the completion of the vaccine preparation and the results recorded.

2. Instead of preparing a single large quantity of stock suspension (40 mg/ml) of vaccine once or twice a week several smaller stock suspensions (the number depending upon the amount of vaccine required) should be prepared separately once a week only using separate equipment and diluent for each stock suspension. Reference should here be made to the suggestion, paragraph B 2, page 30, report 1956,[16] which also applies to this report.

3. The method of production should be standardized in order to obtain uniformity of the quality of the current batches.[13,14,15,16,17,18,19] Cultures of uniform intensity of growth should be selected for preparing the vaccines; almost the same amount of pressed culture should be used for homogenization of each stock suspension; the homogenization technique should also be uniform, and among others shaking of the homogenization flasks by hand should be avoided and be replaced by grinding in a machine.

4. The yield of growth in the culture flasks used for preparation of vaccine should be measured as an indication of the intensity of the growth. (Sugg. B 1, p. 29)[16,18]

5. The vaccine should be issued in red or amber coloured ampoules. (Sugg. B 5, p. 30)[16,14,15,17,19]

6. To speed up the procedure of sealing the ampoules, use might be made of burners of the type employed by the BCG Laboratory in Madras, India, where petroleum gas is also used. The present gas pressure might also be increased and possibly the gas be mixed with oxygen.

7. The filled off vaccine could be released for issue if the contents of the samples of ampoules have proved sterile after 24-48 hours' incubation at 37°C of the inoculated control media.

It should be mentioned that the Karachi Laboratory has already adopted the alterations suggested in this section, except the two next last recommendations.

7. Control Tests

A. Sterility tests

1. Reference should here be made to the suggestions paragraph A 1 and 2, page 31 report 1956,[16] which also apply to this report.

2. Samples of tubes of each batch of Löwenstein medium should be placed at 37°C for ten days before the batch is used for the viability test.

B. Tests for viability

1. In order to avoid similar great variations between the batches as regards their contents of viable units as observed for previous batches, the technique of carrying out the test for viability on Löwenstein medium should be standardized on the following lines:

(a) The test should be carried out on all batches at the time when the vaccine is ready for issue. At least two ampoules of vaccine, selected at random from the batch, should be tested for viability.

(b) As long as the range of variation between the current vaccines regarding their viability when tested in freshly prepared state is not known; in order to get readable colony counts the following three different doses of vaccine should be used for inoculation of the Löwenstein tubes: 8×10^{-6} mg, 2×10^{-6} mg and $1/2 \times 10^{-6}$ mg (see sections 8 and 9). If after some months the range of variation between the batches proves to be rather low, the highest or lowest dose for inoculation may be omitted.

(c) Only one or two staff members (in the latter case after it has been proved repeatedly that approximately similar results are obtained when testing the same vaccine independently) should perform the test.[13,14,20]

(d) In order to be able to compare the results of testing the viability of current batches of vaccine, the same vaccine should be inoculated on to tubes of two successive batches of Löwenstein medium[20] (see sections 8 and 9). Two to three different suitable batches of Löwenstein medium, prepared according to a standardized prescription, should therefore always be available for the test.

(e) The tubes of Löwenstein medium to be seeded should be selected at random from the total batch of medium, and each tube should be labelled with a code number according to a random plan of coding.[20]

(f) After being seeded the position of the tubes should be corrected so that the inoculum is evenly distributed on the surface of each tube.[20] The cotton wool stoppers of the tubes should be sealed not later than 24 hours after seeding the tubes; these should then be placed in incubator at 37°C. Adequate sealing of stoppers should be ensured.

(g) Reading of colonies should be carried out at three, four, five and six weeks after inoculation of the tubes.

(h) Only one or two readers (in the latter case after having proved repeatedly that approximately similar results are obtained, when reading the same tubes with colonies independently) should make the reading. Unbiased reading of the colonies should be ensured. The reader should not know the results of previous readings. The records should therefore be in the custody of the Director of the Laboratory (Sugg. D, p. 31).[16,20] Sufficiently strong electric light should be available for reading the colonies; the tubes should be kept upright during the reading to prevent a spreading of possible condensed water over the surface of the medium.

(i) After completion of the final reading of colonies the results should be tabulated and evaluated.

It should be noted that the suggestions mentioned above for the viability test on Löwenstein medium as a whole have been accepted by the Director of the Laboratory.

2. In order to obtain a preliminary impression of the viability of the vaccine before being issued for use, the germination rate of the bacilli in each batch might be examined by microscopical examination of their growth on Dubos' solid agar medium (Sugg. C 2, p. 31).[16,24,5] The examination of the vaccine for its oxygen-absorbing capacity in Warburg's apparatus supplies quick, quantitative information as regards viability and might therefore be adopted by the BCG laboratory.

C. Innocuity test

1. Two guinea pigs should be inoculated subcutaneously with 5 mg of each batch of vaccine at the same time as the vaccine is being tested for viability. One of the animals should be killed at six weeks, the other one at one year after inoculation, and both should be autopsied.[23]

2. Two guinea-pigs should be inoculated subcutaneously or intraperitoneally with 5 or 10 mg of the culture on Sauton potato medium destined to be the mother culture of the vaccine. They should be killed and autopsied 3-4 weeks later, i.e. just before the time when the vaccine prepared from the above culture is being released for issue.[23]

D. Potency test

1. Each batch of vaccine should be tested for potency[8] at the same time as it is tested for viability. At least two and preferably 4-6 male albino guinea-pigs selected at random and weighing from 350-450 g should be used for testing each batch.[20]

2. One or two staff members only should inject and read the animals, and it should be ensured that approximately equal results are obtained by the two readers reading the same animals independently.[20] Reading of the local reaction after vaccination should be started at one week after injection and then made two to three times weekly up to six weeks after vaccination. The size of the transverse and longitudinal diameter of the appearing nodules should be measured with a millimeter ruler once a week and recorded. The time of occurrence of necrosis should be recorded. To ensure unbiased reading of the animals, they should be read in random order and the results of reading should be dictated to a secretary (Sugg. D, p. 31).[16,20]

F. Determination of homogeneity

The slides should be kept for at least one year after testing the vaccine.

I. Test for transmission of light

It might be useful to introduce this test, using a spectrophotometer.[9]

J. Testing in children for potency

When the vaccine is to be used in humans again each batch of vaccine should be tested in children (Sugg. E, p. 31).[16,23,12]

Other Recommendations

A. At intervals samples of vaccine might be sent for examination and evaluation to another laboratory approved by WHO.[18,11,19]

B. Examination should be made of the keeping properties of the Pakistan vaccine when stored at different temperatures for varying periods of time or when exposed to sun and daylight.[4,14,15,17,18,19]

C. The staff should be trained in undertaking mass production of vaccine, and satisfactory results from testing a number of these vaccines for sterility and viability should be available before mass production of vaccine for use in humans should be started.

D. Careful records should be made of all work carried out in the Laboratory, particularly concerning any deviation from the normal procedure (page 35, line 11 from bottom).[16,18,19]

CONCLUSIONS

From the survey of the state of the BCG Laboratory in Karachi as given in this report, it would appear that some essential problems have still not been solved: the vacancy of the post as Deputy Chief of the Laboratory; the installation of a new air-conditioning system; the conditions under which sterilization of media and utensils is carried out; the defective state of the roof of the building; the alterations and improvements suggested for the building and animal house; the lack of a sufficient number of suitable guinea-pigs; the unsatisfactory and unsafe working conditions when autopsy of animals has to be carried out. In addition, very great variations have been demonstrated between the batches of vaccine as regards their viability, which makes it difficult to form an impression of the quality of the Karachi vaccine. On this account, I am not able to recommend the approval of the Laboratory at the moment. However, I cannot conclude this report without expressing my deep disappointment, because the Laboratory will still have to be closed down until the suggestions made in this report have been followed and until the conditions have been found satisfactory as the result of a probable further visit of a WHO consultant.

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LIST OF PERSONNEL EMPLOYED IN THE BCG PRODUCTION CENTRE, KARACHI

A. Section for Vaccine Preparation

Post	Personnel
Chief	Dr Shafique Ahmad Appointed May 1958
Deputy Chief	Post vacant
1 Senior technician	Mr Abdur Rashid Appointed December 1951 Education: Matriculation and two years' intermediate science
1 Technician	Mr Hamid Hussain Appointed January 1952 Education: Non-matriculated
4 Laboratory assistants	Mr Abdur Rehman Khan Appointed July 1951 Education: Matriculation
	Mr Mohammad Wazir Appointed December 1953 Education: Non-matriculated
	Mr Abul Qasim Mian Appointed June 1957 Education: Matriculation
	1 post vacant
1 Media maker	Mr Abdur Razzaque Appointed December 1951 Education: Non-matriculated
3 Laboratory attendants	Mr Hafiz Mohammed Sharif Ullah Appointed October 1951 Education: Non-matriculated
	Mr Abdur Rahman Bhatti Appointed January 1954 Education: Non-matriculated
	Mr Hilal Ahmad Appointed July 1954 Education: Non-matriculated
1 Animal attendant	Mr Nanoo Masih Appointed December 1951 Education: Non-matriculated

2 Sweepers	Mr Ichchi Appointed December 1951
	Mr Sadiq Masih Appointed February 1959
3 Secretaries	Mr Hamid Ullah Khan (Head Clerk) Appointed November 1953 Education: Matriculation
	Mr Abdus Sattar Mian Appointed February 1956 Education: Matriculation
	1 post vacant
1 Messenger	Mr Saifur Rahman Appointed March 1957 Education: Non-matriculated
2 Night watchmen	Mr Ghulam Mohammad Khan Appointed December 1953
	Mr Allah Dad Appointed August 1956
1 Driver	Mr Faiz Mohammad Appointed October 1953 Education: Non-matriculated
1 Gardener	Mr Rehan Shah Appointed June 1956
23 Total	

B. Section for Preparation of Dilutions of Tuberculin

This section is situated in the western part of the building housing the BCG Department, and the following staff members are employed here:

1 Technician	Mr Mokaddes Hussain Appointed March 1953 Education: Matriculation and two years' intermediate science
1 Technical attendant	Mr Abdus Sattar Khan Appointed January 1952 Education: Non-matriculated

Two laboratory attendants of the BCG Production section, Messrs. Hilal Ahmad and Abdur Rahman Bhatti, are to a certain extent also employed in the Tuberculin Dilution Section.

Table 1

EXTRACTS FROM PROTOCOLS OF BCG PRODUCTION LABORATORY, KARACHI, CONCERNING BCG VACCINES 259-302

Batch No.	Date of prepar.	Dates of testing for viabil.	Dates of testing for potency	Age in days of culture used for prepar.	Strain used for vaccine prepar.	Amount in g of cult. homog.	Techn. of homog.	Rate of moisture in semidry cult. (%)	Mean No. of colonies per tube of Löwenstein inoculated with 2½x10^{-6} mg BCG. Tubes incub. at 37°C for: 3 weeks	4 weeks	5 weeks	½x10^{-6} mg BC[G] Tubes incub. at 3[7°C for:] 3 weeks	4 weeks	
259	21/4 59	23/4 59	23/4 59	12(3)	956	7.293	H1+M2+M1		4.5	6.7	7.1(10)	0.0	1.3	
260*	4/5 -			14(4)	956	12.984	H1+M2½+M1	68.3						
261	11/5 -	13/5 -	13/5 -	14(4)	258	9.851	H1+M2½+M1		2.6	4.2	5.8(9)	0.7	1.6	
262	18/5 -	20/5 -	20/5 -	11(4)	956	9.460	H1+M2+M1	72.3	3.6	4.9	6.3(9)	0.5	1.2	
263	28/5 -	31/5 -	31/5 -	10(4)	956	7.175	H1+M2+M1	69.0	4.4	4.4	4.4(10)	1.0	1.3	
264	4/6 -	6/6 -	8/6 -	10(4)	956	12.185	H1+M2½+M1		0.0	2.9	3.2(9)	0.4	1.7	
265*	11/6 -			10(4)	956	5.694	H¼+M1½+M1	64.9						
266*	25/6 -			10(4)	956	15.032	H1+M2½+M1							
267*	2/7 -			10(4)	952	6.881	H1+M2+M1							
268*	9/7 -			10(4)	956	8.470	H1+M2+M1	70.7						
269°	16/7 -	18/7 -		14(4)	956	11.457	H1+M2+M1	67.6	0.9	2.5	2.4(7)	0.0	0.4	
270*	27/7 -			11(4)	956	9.119	H1+M2+M1	68.5						
271	18/8 -	20/8 -	20/8 -	12(4)	956	10.168	H1½+M2½+M1		4.3	12.0	9.5(6)	1.1	3.9	
272	27/8 -	29/8 -	29/8 -	10(4)	1102	8.237	H1+M2+M1			contaminated		1.1	1.4	
273*	3/9 -			10(4)	1102	9.922	H1+M2+M1							
274**	8/10 -		10/10 -	10(4)	1102	10.155	H1+M2¼+M1							
275*	3/11 -			12(4)	956	7.896	H½+M2+M1							
276	9/11 -	14/11 -	14/11 -	11(4)	956	11.302	H1+M2½+M1			non-readable			10.3	1
277	12/11 -	14/11 -	14/11 -	10(4)	1102	17.782	H1½+M3+M1			35.6	>36.3(9)		9.3	
278*	19/11 -			10(4)	1102	8.592	H1+M2+M1							
279	26/11 -	30/11 -	30/11 -	10(3)	1102	9.221	H1+M2+M1		8.6	11.1	11.7(10)	0.7	3.0	
280*	30/11 -			11(3)	956	7.613	H1+M2+M1							
281	3/12 -	5/12 -	5/12 -	10(3)	1102	9.693	H1+M2+M1		11.7	12.2	12.4(10)	2.2	2.9	
282	7/12 -	9/12 -	10/12 -	11(4)	956	5.450	H1+M1½+M1			contaminated			contaminated	
283°	10/12 -	30/12 -		10(3)	1102	7.181	H1+M2+M1		0.0	5.5	9.8(10)	0.0	1.3	
284°	14/12 -	30/12 -		11(4)	956	5.688	H1½+M1+M1		0.0	0.7	0.7(10)	0.0	0.7	
285°	21/12 -	30/12 -		11(4)	956	9.121	H1+M1½+M1		0.0	2.6	21.3(10)	0.0	0.4	
286	28/12 -	30/12 -	1/1 60	11(4)	956	8.946	H1+M1½+M1		10.2	17.7	15.6(8)	1.9	8.3	
287°	4/1 60	6/1 60		11(4)	956	9.294	H1+M1½+M1		4.0	6.3	6.5(10)	9.3	15.4	1
288	11/1 -	20/1 -	23/1 -	11(4)	956	9.020	H1+M1½+M1½		4.3	3.7	3.6(9)	0.7	1.3	
289	18/1 -	20/1 -	23/1 -	11(3)	956	9.255	H1+M1½+M1½		9.6	14.7	13.3(7)	1.3	1.1	
290	28/1 -	3/2 -	13/2 -	10(4)	1102	9.345	H1+M1½+M1½		52.3	>57.5	>68.4(8)	19.6	27.9	2
291	1/2 -	3/2 -	13/2 -	11(4)	956	10.438	H1½+M1½+M1		64.3	78.6	>98.8(9)	30.5	38.9	4
292	8/2 -	13/2 -	13/2 -	11(4)	956	11.623	H½+M1+M1		3.0		4.2(10)	0.5		
293	11/2 -	13/2 -	24/2 -	10(3)	1102	10.156	H1+M1+M1½		21.9		23.3(10)			
294	15/2 -	24/2 -	24/2 -	11(4)	956	11.832	H1+M1½+M1	70.0	2.1	3.7(10)		1.2	1.4(10)	
295	18/2 -	24/2 -	24/2 -	10(4)	1102	10.130	H½+M2+M1	67.0	30.7	22.6(10)		5.2	5.9(10)	
296	22/2 -	27/2 -	27/2 -	11(3)	956	11.914	H1+M2+M1	72.0	4.8	9.8	10.6(10)	0.3	2.1	
297	25/2 -	27/2 -	27/2 -	10(3)	1102	9.321	H1+M2+M1	66.6	12.7	18.1	22.3(10)	0.3	3.6	
298	29/2 -	5/3 -	5/3 -	11(3)	956	9.256	H½+M2+M1	64.2	26.2	36.6	32.7(9)	5.0	6.3	
299	3/3 -	5/3 -	5/3 -	10(4)	1102	8.752	H½+M1½+M1	68.8	9.0	12.5	12.5(10)	3.7	5.9	
300	10/3 -	12/3 -	12/3 -	10(4)	1102	11.270	H1+M2+M1	73.2					5.3	
301*A B	18/3 -			11(3) (3)	1102	7.656 5.333	M3+M1+M1+M½ M3+M1+M1+M½	67.6						
302	31/3 -	2/4 -	2/4 -	9(3)	956	7.379	M3+M1+M1+M½	65.8				3.1	4.4	

* not tested for viability and potency.

° not tested for potency.

** tested for potency but not for viability.

The figures in brackets in the column "Age in days of culture" indicate the number of culture flasks used for vaccine preparation.

The figures in brackets in the 2 columns "Mean No. of colonies at 5 weeks' incubation" indicate the number of tubes used for estimating the mean number of colonies.

> : one or more tubes in the series showing innumerable colonies.

H : homogenization by hand shaking.
M : homogenization in a grinding mill.
} The figures following H or M indicate the period of homogenization in minu[tes]

medium °C for: 5 weeks	Estimated No. of viable bacillary units in millions per ml vaccine for the seeding doses: $2\frac{1}{2} \times 10^{-6}$ mg	$\frac{1}{2} \times 10^{-6}$ mg	Ratio between mean No. of colonies per $2\frac{1}{2} \times 10^{-6}$ mg and per $\frac{1}{2} \times 10^{-6}$ mg	Mode of distribution of colonies in each series of Löwenstein tubes seeded with $2\frac{1}{2} \times 10^{-6}$ mg	$\frac{1}{2} \times 10^{-6}$ mg	Potency in guinea pigs (nodule-producing capacity) after intradermal inoculation with 4 decreasing doses of vaccine
1.3(9)	2.13	1.95	5.5	normal	normal	weak
3.0(9)	1.74	4.50	1.9	normal	normal	moderately strong
1.5(10)	1.89	2.25	4.2	normal	normal	weak
1.6(7)	1.32	2.40	2.5	bad	normal	weak
1.9(9)	0.96	2.85	1.7	normal	normal	weak
0.4(9)	0.72	0.60	6.0	normal	normal	
3.3(9)	2.85	4.95	2.9	bad	bad	animals died from pneumonia
1.6(7)	contaminated	2.40			normal	1 animal died from pneumonia
						strong
2.7(10)	non readable	19.05			normal	strong
9.3(10)	>10.89	13.95	3.9	normal	normal	strong
3.1(10)	3.51	4.65	3.8	normal	normal	moderately strong
3.0(9)	3.72	4.50	4.1	normal	bad	
	contaminated	contaminated				
3.9(10)	2.94	5.85	2.5	bad	normal	
0.7(10)	0.21	1.05	1.0	normal	normal	
4.2(10)	6.39	6.30	5.1	bad	normal	
5.8(9)	4.68	8.70	2.7	bad	bad	
5.8(9)	1.95	23.70	0.4	normal	normal	
1.3(10)	1.08	1.95	2.8	bad	normal	
2.2(8)	3.99	3.30	6.0	bad	bad	
8.1(7)	>20.52	42.15	2.4	normal	bad	
0.1(10)	>29.64	60.15	2.5	normal	normal	
0.8(10)	1.26	1.20	5.3	normal	normal	
	6.99			normal		
	1.11	2.10	2.6	normal	normal	
	6.78	8.85	3.8	normal	normal	
2.3(10)	3.18	3.45	4.6	normal	normal	
4.5(10)	6.69	6.75	5.0	normal	normal	
6.0(9)	9.81	9.00	5.5	normal	normal	
5.3(7)	3.75	7.95	2.4	normal	normal	
5.1(10)		7.65			normal	strong
4.6(10)		6.90			normal	

Table 2

SITE OF CONTAMINATION FOR THE FOUR CONTAMINATED PAKISTAN BCG VACCINES
PREPARED 1959-60

Batch no. of vaccine	Date of preparation	Strain used for vaccine preparation	Site of contamination			Cause of contamination
			Culture flasks	Stock suspension	Diluted vaccine	
267	2. 7.59	956	sterile (4)	contaminated	contaminated	Fungi
273	3. 9.59	1 102	1 cont. (4)	contaminated		Staphylococci
275	3.11.59	956	1 cont. (4)	contaminated	contaminated	Fungi
301^{A}_{B}	18.3.60	1 102	1 cont. (6)	contaminated	contaminated	Sarcina

The figures in brackets indicate the number of flasks with BCG culture used for preparing the vaccines

Table 3

INTERVAL BETWEEN THE DATES OF PREPARATION AND TESTING FOR 31 PAKISTAN BCG VACCINES, PREPARED 1959-60

Interval in days between dates of preparation and testing	Number of batches	Batch No.
2-3	19	
4	1	279
5	4	276 292 296 298
6	2	290 295
9	3	285 288 294
16	1	284
20	1	283

Table 4.

9 BATCHES OF PAKISTAN B.C.G.VACCINE PREPARED 1959-60, SHOWING BAD MODE OF DISTRIBUTION OF COLONIES IN TUBES OF LÖWENSTEIN MEDIUM.

Batch no.	Number of colonies developed in series of 10 tubes of Löwenstein medium, each tube inoculated with: $2\frac{1}{2} \times 10^{-6}$ mg B.C.G. — Tubes no. 1	2	3	4	5	6	7	8	9	10	Total no.of col.	Mean no.of col.	Incubation period at 37° C. in weeks	$\frac{1}{2} \times 10^{-6}$ mg B.C.G. — Tubes no. 1	2	3	4	5	6	7	8	9	10	Total no.of col.	Mean no.of col.	Incubation period at 37° C. in weeks
263	0	0	0	17	0	0	5	0	22	0	44	4.4	5													
271	5	0	0	4	3	14	2	2	10	3	43	4.3	3	0	0	1	1	1	4	1	1	0	2	11	1.1	3
	5	0	0	5	19	29	2	17	25	18	120	12.0	4	0	0	1	2	16	17	0	1	0	2	39	3.9	4
	5	0	0	5	∞*	∞*	2	∞*	∞*	45	57	9.5	5	0	0	1	2	24	∞*	0	1	0	2	30	3.3	5
281														1	0	3	2	1	4	9	1	0	1	22	2.2	3
														4	1	3	2	2	4	9	1	0	∞*	26	2.9	4
														4	1	3	3	2	4	9	1	0	∞*	27	3.0	5
283	3	0	0	0	4	0	0	38*	1	9	55	5.5	4													
	4	17	11	7	2	0	0	38*	1	18	98	9.8	5													
285	0	0	0	0	0	0	0	0	0	0	0	0.0	3													
	0	0	0	0	0	0	0	0	8	18	26	2.6	4													
	13	25	15	0*	0*	16	25	0*	27	28	149	21.3	5													
286														4	0	0	0	0	3	0	0	12	0	19	1.9	3
	9	9	19	12	13	9	28	32	24	22	177	17.7	4	10	6	0	3	0	14	16	2	17	6	83	8.3	4
	15	12	13	18	19	15	∞*	∞*	10	23	125	15.6	5	5	6	0	8	10	5	∞	2	8	8	52	5.8	5
288	1	7	1	4	1	4	5	2	12	6	43	4.3	3													
	1	7	1	4	1	6	5	2	∞*	6	33	3.7	4													
289	8	8	∞*	13	∞*	15	21	16	∞*	12	93	13.3	5	0	1	1	1	44*	∞*	2	2	2	0	9	2.2	5
290														30	25	∞*	33	∞*	13	dry	35	26	35	197	28.1	5

* Abnormal number of colonies, not used for estimating the mean number except for Batch 283.

Table 5

EVALUATION OF THE POTENCY (NODULE-PRODUCING CAPACITY IN GUINEA-PIGS) OF 28 PAKISTAN BCG VACCINES PREPARED 1959-60

Each Animal Inoculated Intradermally with 4 Different Doses of Vaccine

Batch No. of vaccine	Date of testing	Guinea-pig No.	Interval in days between vaccination and time of appearance of necrosis of nodules — Dose of vaccination: 0.1 ml of 1/1 ccms.	1/10 ccms.	1/100 ccms.	1/1000 ccms.	Remarks	Estimated degree of potency
259	23/4-59	704 (590)	15	15	-	-	died 14/9 pneum.	weak
		705 (580)	15	-	-	-		
261	13/5 -	710 (700)	16	28	-	-		moderately strong
		711 (720)	20	28	-	-		
262	20/5 -	712 (700)	-	-	-	-		weak
		713 (650)	21	-	-	-	died 14/9 pneum.	
263	31/5 -	720 (690)	13	-	-	-		weak
		721 (700)	16	-	-	-	died 11/9 pneum.	
264	8/6 -	726 (680)	16	-	-	-		weak
		727 (690)	11	-	-	-	died 15/9 pneum.	
271	20/8 -	730 (690)	11	-	-	-	died 14/9 pneum.	
		731 (700)	11	25	-	-	died 14/9 pneum.	
272	29/8 -	736 (690)	13	-	-	-	died 15/9 pneum.	
		737 (700)	13	-	-	-	died 12/9 pneum.	
274*	1o/10 -	944 (450)	10	14	10	14		strong
		945 (450)	7	10	14	14	died 16/2-60 ?	
276	14/11 -	950 (550)	3	7	14	18		strong
		951 (530)	3	7	14	-		
277	14/11 -	954 (560)	3	7	10	10		strong
		955 (550)	3	7	10	10		
279	30/11 -	960 (510)	16	16	-	-		moderately strong
		961 (520)	16	19	-	-		
281	5/12 -	964 (550)	19	23	-	-		

282**	10/12 -	966 (600)	4	-	-	-		
286	1/1-60	970 (400)	13	13	-	-		
288	23/1 -	972 (350)	14	14	-	-		
289	23/1 -	974 (340)	25	-	-	-		
290	13/2 -	976 (550)	6	10	-	-		
291	13/2 -	978 (450)	17	-	-	-		
292	13/2 -	980 (520)	10	17	-	-		
293	24/2 -	982 (590)	6	14	31	-	Exam. not completed	
294	24/2 -	986 (410)	14	27	-	-	"	
295	24/2 -	984 (400)	14	-	-	-	"	
296	27/2 -	988 (630)	11	21	28	-	"	
297	27/2 -	990 (500)	11	14	-	-	"	
298	5/3 -	995 (590)	11	11	-	-	"	
299	5/3 -	997 (650)	11	21	14	-	"	
300	12/3 -	840 (600) 842 (690)	10 7	21 10	- -	- -	"	strong
302	2/4	? ?	Results not available					?

The figures in brackets indicate the weight in grams of the animals at the time of inoculation.

- = no occurrence of necrosis.

*** Vaccine not tested for viability.**

**** Tubes of Löwenstein** medium contaminated.

ble 6

PAKISTAN BCG VACCINES 300 AND 302

ium 10^{-6}mg BCG ub.at 37°C for		Estimated No. of viable bacillary units in millions per ml vaccine for the seeding doses in mg			Ratio between mean no. of colonies per		Mode of distribution of colonies in each series of Löwenstein tubes seeded with		
weeks	5 weeks	8 x 10^{-6}	2 x 10^{-6}	$\frac{1}{2}$ x 10^{-6}	8x10^{-6}mg and 2x10^{-6}mg	2x10^{-6}mg and $\frac{1}{2}$x10^{-6}mg	8x10^{-6}mg	2x10^{-6}mg	$\frac{1}{2}$x10^{-6}mg
5.3	5.1(10)	7.30	10.13	7.65	2.9	5.3	normal	normal	normal
3.0	2.9(9)	4.50	5.33	4.35	3.4	4.9	normal	normal	normal
4.4	4.6(10)	5.58	5.73	6.90	3.9	3.3	normal	normal	normal

ating the mean no. of colonies per dose of vaccine inoculated.

Ta

RESULTS OF VIABILITY TESTS FOR

Batch No.	Date of prepar.	Date of testing	Date of prepar. of Löwenstein medium	Mean No. of colonies per tube of Löwenstein med inoculated with							
				8×10^{-6} mg BCG			2×10^{-6} mg BCG			$\frac{1}{2}$ x	
				Tubes incub. at 37°C for			Tubes incub. at 37°C for			Tubes inc	
				3 weeks	4 weeks	5 weeks	3 weeks	4 weeks	5 weeks	3 weeks	4
300	10/3-60	12/3-60	unknown	87.4	82.6	77.9(9)	27.8	27.0	27.0(10)	5.3	
302	31/3-60	2/4-60	3/3-60	33.6	49.1	48.0(8)	10.7	14.9	14.2(8)	1.5	
			19/3 -	50.2	59.4	59.6(10)	11.6	14.8	15.3(10)	3.1	

The figures in brackets indicate the number of tubes used for estim

PLAN OF BCG PRODUCTION LABORATORY, KARACHI, PAKISTAN

2

Z Y X W A B C D E

1 12 14 F G 13 0 3

K 15 N

? ? V T S H I II L M

10 8 6 5

11 9 7 4

U R PP P

Door

Window

Hatch

Veranda

The "sterile" section is heavily outlined.
See text for lettering of rooms and door numbers.

W N E S

Animal Quarters