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REPORT OF THE TECHNICAL
CONFERENCE FOR THE STUDY
OF VACCINATION AGAINST
TUBERCULOSIS BY MEANS
OF BCG

Held at the Pasteur Institute, Paris,
— October 15th-18th, 1928 —

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I.

REPORT ON THE TECHNICAL CONFERENCE FOR THE STUDY OF VACCINATION AGAINST TUBERCULOSIS BY MEANS OF BCG

The Health Committee, at its twelfth session, having considered the proposal received from Professor Calmette that it should examine the results of vaccination against tuberculosis by BCG, authorised its President to convene a few experts to establish and submit a plan of international studies on this question.

This Conference of Experts took place at the Pasteur Institute, Paris, from October 15th to October 18th, 1928.

The aim of this meeting was to establish the most appropriate methods to bring out the immediate and remote results of vaccination by BCG in laboratory animals, cattle and man.

The study of these various problems was undertaken by three Commissions, made up of bacteriologists, clinicians and veterinary surgeons. The questions of common interest to the three Commissions were considered in plenary meetings; other questions of special interest were discussed in sessions of the Commissions.

* * *

Dr. E. ROUX, Director of the Pasteur Institute, presided over the Conference, the Bureau of which also included:

Professor Th. MADSEN, Director of the State Serological Institute, Copenhagen, President of the Health Committee of the League of Nations.

Dr. L. RAJCHMAN, Medical Director of the Health Organisation of the League of Nations, Geneva. *Secretary* (ex officio).

The three Commissions were made up as follows:

1. *Bacteriological Commission.*

Professor BORDET, Director of the Pasteur Institute, Brussels (President);

Professor R. KRAUS, Director of the State Sero-therapeutical Institute, Vienna;

Professor NEUFELD, Director of the Robert Koch Institute, Berlin;

Dr. TZEKHNOVITZER, Sanitary and Bacteriological Institute of Ukraine, Kharkoff;

Honorary Secretary: Professor C. PRAUSNITZ, Director of the Hygiene Institute of the University, Breslau.

The Commission invited Professor J. CANTACUZENE, Director of the Institute of Experimental Medicine, Bucarest and Dr. A. REMLINGER, Director of the Pasteur Institute of Morocco, Tangier, to take part in its discussions.

Mr. S. A. PETROFF, of the Bacteriological Laboratory of the Trudeau Institute, Saranac Lake, N.Y., also presented the results of his experiments at one of the meetings.

2. *Clinical Commission.*

Professor Léon BERNARD, Professor of the "Clinique de la Tuberculose", Faculty of Medicine, Paris (President);
Dr. J. HEIMBECK, Physician at the Ullevål Hospital, Oslo;
Professor E. NOBEL, Pediatric Clinic of the University of Vienna;

Professor G. RONZONI, Director of the Institute for Tuberculosis, Milan;

Professor L. SAYÉ, Professor of the Faculty of Medicine, Director of the Service of Assistance to Tuberculosis Patients, Barcelona;

Professor A. SCHLOSSMANN, Director of the Pediatric Clinic, Dusseldorf.

Dr. VAN BENEDEN, of the Bacteriological Institute of the University of Liège, and Dr. WEILL-HALLÉ, Director of the Ecole de Puériculture, of the Faculty of Medicine, Paris, attended one meeting of this Commission and related the results of the vaccinations carried out by them.

3. *Veterinary Commission.*

Professor J. NOWAK, Director of the Institute of Research in Human and Veterinary Medicine, Cracow (President);

Professor A. ASCOLI, Istituto vaccinogeno anti-tuberculare, Milan;

Dr. H. BERGER, Chief Inspector of the Veterinary Services of the Netherlands, The Hague; assisted by Dr. FRENKEL, Veterinary Section of the State Hygienic Laboratory, Utrecht;

Professor VALLÉE, Director of the National Research Laboratory of the Ministry of Agriculture, Alfort (Seine);

Dr. ZELLER, Veterinary Section of the Reichsgesundheitsamt, Berlin.

Professor GERLACH, of the Federal Institute for Combating Cattle Disease, Mödling, near Vienna, also attended the meetings of this Commission.

Professor A. CALMETTE, Deputy-Director of the Pasteur Institute, Paris; and
M. C. GUÉRIN, Chef de Laboratoire at the Pasteur Institute, Paris, took part in the proceedings of the Conférence and of its Commissions.

Secretariat:

Dr. Y. BIRAUD, Health Section of the General Secretariat of the League of Nations, Geneva;
Dr. E. BURNET, Secretary of the Leprosy Commission of the Health Committee, Geneva;
Dr. G. ICHOK, Paris;
Dr. G. POIX, Paris.

* * *

The conclusions adopted by the Commissions of the Conference are the following:

1. CONCLUSIONS OF THE BACTERIOLOGICAL COMMISSION.

The bacteriologists attending the meetings of the Commission agreed unanimously on the basis of the experiments carried out, that BCG was a harmless vaccine (Ascoli, Berger, Bordet, Cantacuzène, Frenkel, Gerlach, Kraus, Neufeld, Nowak, Remlinger, Tzekhnovitzer, Vallée, Zeller).

Professor Nobel, however, held that, under exceptional conditions, BCG was capable of inducing fatal tuberculosis in laboratory animals.

The Commission considered that the mass of experiments on laboratory animals, published hitherto, showed clearly that BCG does not give rise to progressive tuberculosis (*tuberculose évolutive, progrediente Tuberkulose*).

The Commission took note of the researches undertaken at the institutes mentioned in Annex I.

* * *

In order to make these studies comparable and to allow conclusions to be drawn therefrom at a further meeting, the Commission suggested the desirability of solving the following problems (set forth in detail in Annex II):

1. Methods to be adopted in order to maintain unaltered the fixed properties recognised as characteristic of BCG.
2. Methods to be adopted for the study of the influence on BCG of passages through animals.

3. Methods to be adopted for immunising experiments; determination of doses of BCG and virulent bacilli to be used in them; adoption of strains of known virulence for the virulent inoculations.

4. Methods to be adopted in the studies on the variability and dissociability of BCG.

5. Methods to be adopted for the comparative study of the histological changes produced by inoculation of BCG and of virulent tuberculosis bacilli.

6. Necessity of entrusting the preparation of BCG (culture, make and distribution of vaccinal emulsions) to institutes of recognised scientific standing.

2. CONCLUSIONS OF THE CLINICAL COMMISSION.

1. It appears from the documents of which the Commission took cognisance :

1. That BCG, when administered *per os* to infants within ten days after birth, or hypodermically to older children and adults, was incapable of producing virulent tuberculosis lesions;

2. That, as regards the preimmunising properties of BCG against tuberculosis, a certain degree of immunity was induced by this vaccination.

* * *

Further research work upon vaccinated children, continuing for a more lengthy period and carried out in a uniform manner — and, in particular, a fuller knowledge of tuberculosis morbidity and mortality among individuals of varying age and environment — are necessary before the Commission is able to pass a final judgment on the value of anti-tuberculosis vaccination with BCG.

2. To this end the Commission requests the Committee of Expert Statisticians of the League of Nations Health Organisation to indicate the best methods of collecting and analysing data on the morbidity and mortality of infants, unvaccinated as well as vaccinated.

3. In order to obtain these data under strictly comparable conditions, the Commission has prepared a draft Individual Case Record (Annex III) designed for the use of the institutions entrusted with the studies mentioned above; this draft is to be submitted to the Committee of Statisticians for its opinion.

The Commission has also prepared a standard "Clinical Case Record" (Annex IV), to which is attached a schedule of

post-mortem examination, indicating the special information to be obtained from the autopsies for the purposes of these studies.

The Commission is of opinion that these studies should be entrusted to a limited number of dispensaries and hospital clinics. The data collected by these institutions according to the method which may be proposed by the Committee of Expert Statisticians, making use of the standard Clinical and post-mortem Case Records suggested, should be compiled and analysed by the Health Section of the League of Nations, with a view to their examination by the Conference at a future meeting¹.

The Commission requests the Health Committee to invite the co-operation of the institutions that are selected for these studies.

The Commission feels the more justified in recommending these further studies by reason of the conclusions adopted by the Veterinary and Bacteriological Commissions.

3. CONCLUSIONS OF THE VETERINARY COMMISSION CONCERNING THE PROPHYLAXIS OF BOVINE TUBERCULOSIS.

1. The mass of experimental data published and the *unanimous* opinion of practitioners who have used BCG upon bovidæ indicate that vaccination performed according to the technique advocated by Calmette and Guérin upon animals of this species is entirely *innocuous*.

2. The same experimental data and the observations made of the practical use of BCG upon bovidæ show conclusively that this strain of bacilli possesses *premunising properties* against both experimental and natural tuberculous infections.

These acknowledged premunising properties provide justification and encouragement for the extension of the experimental use of BCG in the prophylaxis of bovine tuberculosis.

The use of BCG should be continued in the various countries in accordance with the international experimental protocol which is attached, under the supervision of official veterinary services and competent bacteriological and pathological authorities, and in close touch with the Commission set up for the study of this problem by the Health Organisation of the League of Nations.

Wherever possible, the trials should be carried out strictly in accordance with the experimental rules (Protocol I, Annex V); in other cases, trials may be made more in conformity with the ordinary conditions of cattle-rearing, under continuous official supervision (Protocol II, Annex VI).

¹ Professor NEUFELD, Director of the Robert Koch Institute, Berlin, generously offered the hospitality of this Institute for the next Conference on BCG.

**ANNEXES TO THE RESOLUTIONS OF THE TECHNICAL
CONFERENCE FOR THE STUDY OF THE VACCINATION
AGAINST TUBERCULOSIS BY BCG**

Annex I.

RESEARCH INSTITUTES

- Austria:* State Sero-therapeutical Institute, Vienna.
Federal Institute for Combating Disease of
Cattle, Mödling, near Vienna.
Institute of Pathological Anatomy of the
University, Vienna.
- Belgium:* Pasteur Institute, Brussels.
Bacteriological Institutes of Liège, and Mons.
- Canada:* Laboratories working on behalf of the National
Research Council of Canada.
- Denmark:* State Serum Institute, Copenhagen.
- France:* Pasteur Institute, Paris.
National Research Laboratory of the Ministry
of Agriculture, Alfort (Seine).
- Germany:* Robert Koch Institute, Berlin.
Laboratories of Bacteriology and Veterinary
Medicine of the Reichsgesundheitsamt,
Berlin.
Institute of Experimental Pathology, Frank-
fort.
Pediatric Clinic of the Academy of Medicine,
Dusseldorf.
Institute for Tuberculosis Research (Professor
Brauer), Hamburg.
Hygiene Institute of the University, Breslau.
- Great Britain:* Laboratories working on behalf of the Medical
Research Council of Great Britain.
- Holland:* Veterinary Section of the State Hygienic Labor-
atory, Utrecht.
- Italy:* Istituto vaccinogeno antitubercolare, Milan.

<i>Morocco</i> :	Pasteur Institute of Morocco, Tangiers.
<i>Poland</i> :	Institute for the Experimental Study of Human and Veterinary Pathology, Cracow.
<i>Roumania</i> :	Serological Institute, Bucarest.
<i>United States of America</i> :	Laboratories of the New York City Department of Health, New York City.
<i>U.S.S.R.</i> :	State Bacteriological Institute, Kharkoff.

Annex II.

PLANS FOR FURTHER BACTERIOLOGICAL STUDIES
ON BCG

2. METHODS TO BE ADOPTED FOR THE STUDY OF THE
INFLUENCE ON BCG OF PASSAGES THROUGH
ANIMALS (Professor A. ASCOLI and Professor BORDET).

The experiments must apparently consist of :

(a) Injecting into new animals the inflammatory exudate taken at the site of inoculation after varying periods following the injection of BCG into these animals.

(b) Microscopic study and culture of this exudate ; search for BCG and other micro-organisms which might also be present. If BCG can be obtained from the culture of the exudate, its virulence must be determined as well as its biological properties in order to establish whether BCG can be considered as a definite type of organism with definitely fixed characters.

3. METHODS TO BE ADOPTED IN THE IMMUNISING EXPERIMENTS.

A. — *Experiments proposed by Professor Cantacuzène and Professor Vallée.*

The experimental animals selected will be guinea-pigs, rabbits, monkeys and bovines.

1. The doses of BCG will vary according to the animal species considered and the route of administration selected (oral, subcutaneous, intracardial and intravenous routes). The

doses are those prescribed by the authors of the methods, i.e.:

Guinea-pigs (8 to 30 days old) :

Subcutaneous : 10 injections of 2 mgr. of BCG at 48-hour intervals.

Oral route : 6 meals of 10 mgr. and 4 of 20 mgr. at 24-hour intervals.

If the intracardial route is preferred, 1 single injection of 2 to 5 mgr. of BCG.

Rabbits (15 to 20 days old) :

Oral route : 10 doses of 20 mgr. each at 24-hour intervals.

If the intravenous route is preferred, 1 single injection of 20 to 30 mgr. of BCG.

Monkeys (pithecians and chimpanzees) :

Subcutaneous route : 1 single inoculation of 50 mgr. of BCG suspended in 10 cc. of a fluid made up of one part of Sauton's solution to three parts of normal saline solution.

Oral route : 5 meals each of 50 mgr. of BCG within 8 or 10 days.

Bovines :

One single subcutaneous injection in the dewlap of 50 mgr. suspended in 10 cc. of a fluid made up of one part of Sauton's solution to three parts of normal saline solution.

2. If the Vallée strain of tubercle bacillus is used for the virulent test inoculations, the doses of this organism must be as follows :

Guinea-pigs :

Subcutaneous route : eye-instillation after 6 weeks of 1 drop of an emulsion containing 1 mgr. of bovine bacilli per cc.

Oral route : 2 infecting meals of 5 mgr. each at 24-hour intervals (3 months after vaccination).

Rabbits :

Intravenous route : 2 months after vaccination, inject 0.01 mgr. (or even 0.0001 mgr.).

Oral route : 3 months after vaccination, two infecting meals of 10 mgr. each at 24-hour intervals.

Monkeys

(pithecians and chimpanzees) :

Subcutaneous route : 1/100,000th of a mgr.

Oral route : 2 meals of 1 mgr. each at 48-hour intervals.

Bovines :

Intravenous inoculation: according to the animal's size, 3 to 5 mgr. of virulent bacilli (1 month at least after the premunisation).

If other strains than the Vallée strain are used, the doses given above must be considered as optimum.

B. — *Experiments proposed by Professor Neufeld.*

1. *Experiments of Parallel Vaccination in Bovines :*

(a) With BCG.

(b) With a culture of tubercle bacillus of human type and of normal virulence in the guinea-pig.

The vaccinations (premunisation) should be practised with the same technique for both vaccines, though with possibly different doses, either subcutaneously or intravenously.

The subcutaneous route would have the advantage of resembling more closely the vaccinations already made with BCG; the intravenous route (preferable in my opinion) would have the advantage of enjoying a more precise technique, dosage, etc., thanks to the great number of experiments (Behring, Koch) in which it was used and proved more efficacious.

Whatever route may be selected, it is absolutely necessary that in every case the test inoculation be carried out on the vaccinated animals and controls on the same day and with the same bacillary emulsion, *i.e.*, made up from the same culture tube and not only with the same strain.

This strain should be of a virulent bovine type; it should be injected intravenously in sufficient doses to kill all control animals in a few weeks.

N.B. — These experiments are not intended to show the immunising properties of BCG, which are already recognised, but to *measure quantitatively* the latter by means of a process recognised as capable of giving such a measure.

2. *Reproduction of the Monkey Experiments (Wilbert's and others) :*

Premunisation with BCG: (a) *per os*; (b) subcutaneously.

The virulent test inoculation might be done through any route, but at doses certainly fatal to the controls.

3. *Laboratory Experiments :*

Experiments resembling as closely as possible those published hitherto concerning human premunisation with BCG, *i.e.*, premunisation with smaller doses than those used in most of experiments published hitherto: (a) *per os*; (b) subcutaneously.

3. Have tubercle bacilli been demonstrated in this (or these) case(s) ?
In large numbers (mass infection) ?
In small numbers ?
In numbers unstated ?
4. Duration of the contact(s) with the tuberculous person (s) :
From the age of to the age of
Was the contact continuous or intermittent ?
5. If possible, state the clinical type of tuberculosis in the contact(s)
Date of death of the contact (if dead)

IV. *Prophylactic measures adopted :*

- A. Was the child "premunised" with BCG
or not

In case of premunition *by mouth :*

Who controlled the absorption of the vaccine doses (parents ; visiting nurse ; midwife ; doctor) ?

How many days old was the infant when given the :

First dose

Second dose

Third dose

In case of subcutaneous premunition :

How old was the child when inoculated ?
(if very young, state it in days)

After how many tuberculin tests ?

At what date were those tests performed ?

Which method was used for these tests
(intradermal or skin tests) ?

- B. Separation from the source of infection.

1. Was the child :

Isolated in hospital or in a special institution (state which) ?

Since when ? until when ? ..

2. Sent to a healthy environment :

Since when ? until when ? ..

3. Partially isolated in the family (precautions)

- C. Education of the family from the prophylactic point of view :

Frequency of the visits of the district nurses
..... every month

V. *Social Position:*

(Result of the social enquiry by the dispensary.)
In the dwelling: how many inhabitants?.....
 how many beds?

Has the child a bed or a cot for himself?.....
Does the child live in the same room as the tuberculous patient?.....

VI. *Feeding of the Child:*

Maternal breast feeding?..... up to?

Feeding by a wet nurse?..... up to?

Mixed feeding?..... from what age?

Artificial feeding?..... from what age?.....
 Is the feeding controlled by a welfare institution?
 Frequency of nurses' visits?.....
 Frequency of attendance at the clinic?.....

VII. *Clinical and pathological history of the child:*

Frequency of medical examinations.....
 every.....months.
 (a) Weight chart.....Weight every 3
 months.....
 (b) Pathological phenomena noted.....

VIII. *If the child died: Date of death.....*

Clinical diagnosis of the cause of death.....
Anamnesis and laboratory findings, if any.....
.....
.....
.....
Post-mortem findings (*cf.* Annex IV, Section II)...

2. SUPPLEMENTARY DATA WHICH CERTAIN INSTITUTIONS MAY BE IN A POSITION TO FURNISH.

IX. *Supplementary information on pregnancy and confinement:*

1. Condition of the mother during pregnancy; if tuberculous, at what stage of the disease?..
2. Prematuration?
3. Conditions of labour:
 Where did it take place?..... (at home, at the maternity hospital).
 Delivery attended by?..... (a doctor, a midwife, an unqualified person).

X. *Tuberculin tests (see note suggesting technique):*

Method used: Skin test (Pirquet)?..... Date of reading?

 Intradermal test (Mantoux)?.....

Tests performed by a physician?..... If not,
what other person?

Tests read by a physician?..... If not,
what other person?

XI. *X-Ray examinations* (see note suggesting technique):

Method used

Results of the reading (mediastinum, lungs, pleura):

First examination: date..... findings

Second examination: date..... findings

Third examination: date..... findings

Fourth examination: date..... findings

3. PROCEDURES SUGGESTED IN ORDER TO ATTAIN UNIFORMITY
AND MAKE THE RESULTS OF THE VARIOUS
EXAMINATIONS COMPARABLE.

(The procedures ought to be described in detail.)

1. *Tuberculin tests.*

Use preferably the intradermal test and indicate the doses of tuberculin employed.

If the intradermal test is not practicable, use the skin test and indicate the technique adopted.

Repeat the tests serially, at intervals of a fortnight or a month.

2. *X-Ray examinations.*

3. *Post-mortem examinations* (*cf.* Annex IV, Section II.)

Annex IV.

STANDARD CASE RECORD FOR A CHILD VACCINATED
AND DECEASED, PROPOSED BY PROFESSOR
LÉON BERNARD.

I. CLINICAL OBSERVATION.

1. *Birth.*

Date.

Full term or premature.

Weight.

2. *Tuberculous contact.*

Nature: father, mother or others.

Summary of the clinical history of the contact, especially findings of T.B.

Character of contact: precautions taken or not.

Housing conditions.

Supervision by doctors or nurses.

Length.

3. *Vaccination with BCG by mouth* (or by other routes).
State precisely how vaccination was performed.
At what age?
At what doses?
Was the child separated from tuberculous contacts immediately after vaccination?
For how long?
4. *Tuberculin tests.*
Method used.
Number of tests.
At what ages.
Results.
5. *Clinical and pathological history of the child.*
Note the apparent clinical cause(s) of death.
Age at death.

II. POST-MORTEM.

Macroscopic examination of the organs.

Viscera.

Tracheo-bronchial lymph-glands.

Mesenteric lymph-glands.

Note every alteration observed, tuberculous or otherwise.

1. If one finds one or several tuberculous lesions (caseous nodules or lymph-glands; other lesions), one must:

- (a) Examine smears of this lesion;
- (b) Make cultures from it and determine the type of the bacillus recovered (T.B. or BCG);
- (c) Inoculate the lesion to guinea-pigs (by subcutaneous and intravenous routes) and to rabbits (by intravenous route).

These animals, inoculated directly from the lesion(s):

- (1) Must be submitted to repeated intradermal tuberculin tests at varying intervals;
- (2) In dead or killed animals, the organs, and especially the tracheo-bronchial and mesenteric lymph-glands, must be examined macroscopically;
- (3) Smears of their lymph-glands must be submitted to a prolonged examination;
- (4) Cultures must be attempted from their lymph-glands and some of their viscera; the type of the bacilli recovered must be determined (T.B. or BCG);

- (5) Their organs must be inoculated to other animals (secondary inoculation). These organs will be the object of the same examinations as the former, as indicated in items (1), (2), (3), (4).
2. If no visible tuberculous lesion is found, one must ;
- (a) Make a histological examination of the lungs, the liver, the spleen and the tracheo-bronchial and mesenteric lymph-glands ;
 - (b) Bacilli must be looked for in smears of their organs ;
 - (c) A culture must be attempted from their organs and the type of the bacilli recovered must be determined as in paragraph 1 (b) ;
 - (d) Their organs must be inoculated to animals which will be submitted to the tests indicated in paragraph 1 (c).

Annex V.

PROTOCOL I.

VACCINATION OF CATTLE PERFORMED UNDER STRICT EXPERIMENTAL CONDITIONS.

1. The innocuity of the BCG for the bovine being recognised, the efficacy of this strain as an immunising agent against tuberculosis can be demonstrated in all strictness only after a prolonged period of observation : through controlled experiments involving an equal number of animals vaccinated and of controls living in the same infected environment.

2. For such experiments, farms containing at least twenty adult cows and a bull, and in which the normal practice consists in rearing calves with a view to the progressive replacement of the older animals, will be selected.

3. The experiment must be carried out in farms where at least 40 per cent of the cattle react positively to tuberculin at the beginning of the experiment.

4. These farms must of necessity be provided with a small isolation-byre carefully disinfected at the start and kept clean. The only animals to be admitted to this byre are the newborn calves which are to be vaccinated. They will be admitted *immediately after birth* and after the dressing of the umbilicus.

5. These calves will be reared *exclusively* by means of artificial feeding with boiled milk (with feeding-bottles or from pails), or preferably the milk of cows giving a negative tuber-

culin test. The calves must on no account be given the colostrum of the parent animal.

6. One-half only of the calves born, males and females, in equal numbers, must be vaccinated. The vaccination must be performed hypodermically within the first fifteen days of life, and repeated every year. Each vaccination will consist of the injection of an emulsion of 50 milligrammes of BCG in 10 cc. of a fluid composed of one part of Sauton's solution to three parts of physiological saline solution.

7. The other half of the calves born, males and females, in equal numbers, as far as possible, will be submitted *strictly* to the same conditions of life as the animals of the vaccinated group. They must, however, be isolated from the latter, in a special byre, until the day of the mixing of both lots in a common byre.

8. The pedigree of each of the calves in both lots must be carefully noted, so as to allow the tracing of tuberculosis among their parents.

One should endeavour to have in both lots an equal number of calves born of tuberculous cows.

9. When the immunity induced by vaccination may be considered as acquired by the animals, *i.e.*, thirty days at least after the inoculation, both the vaccinated and control calves will be left to live in common the normal life of the animals on the farm.

A permanent marking of the vaccinated, and a detailed description allowing their recognition when needed, must from that time be the only distinction between the animals of both lots.

10. In order to avoid any disturbance in the susceptibility of the animals of both groups, one should avoid all tuberculin tests among them before or after vaccination.

11. Post-mortem examinations alone, performed at the slaughter-house either at the end of the period of economic usefulness of the cattle, or after spontaneous death, or emergency slaughtering, will allow judgment to be passed on the comparative state of health of the animals vaccinated and of the controls.

The autopsy protocol must mention the conditions and lesions which might exist at the sites or the vaccine injections, in the prescapular, prepectoral, retropharyngeal, bronchial, mediastinal and mesenteric lymph-glands and the various organs.

If lesions are found which may be suspected of being of a tuberculous nature, their etiology must be determined by bacteriological and histological examinations.

12. The experiments must be continued under the same conditions for five consecutive years; conclusions may be drawn from them only when the sixth year has elapsed.

Annex VI.

PROTOCOL II.

PROCEDURE FOR THE PROPHYLAXIS OF TUBERCULOSIS IN CATTLE
ADAPTED TO THE IMMEDIATE NEEDS OF THE CATTLE-
BREEDERS AND TO BE CARRIED OUT UNDER THE
SUPERVISION OF THE VETERINARY SERVICES.

1. This method of prophylaxis is applicable to all cattle-farms infected with tuberculosis and in which local practice tends to the breeding of calves for the gradual replacement of older animals.

2. These farms must of necessity be provided with a small isolation-byre, carefully disinfected at the start and kept clean. The calves which are to be vaccinated will be placed in this isolation-byre *immediately after birth* and after the dressing of the umbilicus.

3. These calves will be reared *exclusively* by means of artificial feeding with boiled milk (with feeding-bottles or from pails) or milk from cows which do not react to tuberculin. The calves must on no account be given the colostrum of the parent animal.

4. The newborn calves, or at least the females intended for raising, will be vaccinated with BCG subcutaneously within the first fifteen days of their life.

5. The animals vaccinated will be *re-vaccinated every year*.

6. When the immunity induced by vaccination may be considered as established, *i.e.*, thirty days at least after vaccination, both the vaccinated and control calves will be left to live in common the normal life of the animals on the farm.

A permanent marking and a detailed description of the vaccinated animals allowing of their recognition will be made.

7. Post-mortem examinations alone, performed by a specialist or by the veterinary inspector of the slaughter-house at the end of the period of usefulness of the animal, or before that time after its death from natural causes or its emergency slaughter, may permit a judgment to be passed on the state of health of the animals vaccinated and conclusions to be drawn concerning the changes brought about by vaccination with BCG in the frequency of tuberculous infection of the animals on the farm.

The post-mortem findings must be set forth as indicated in paragraph 11 of Protocol I (Annex V).

8. The experiments thus conducted may be carried out for as long a period as may be necessary to base a reasoned opinion on this method of prophylaxis.

II.

PRELIMINARY STATEMENT ON THE METHOD OF PREVENTIVE VACCINATION FOR TUBERCULOSIS WITH BCG

(Calmette-Guérin Bacillus)

By Professor A. CALMETTE

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[Translation.]

A.

One of the most curious and unexpected facts recently revealed by experimental research is that, especially in urban areas, most human beings spontaneously vaccinate themselves in infancy, because they absorb, either in food, or through the various mucous membranes, or even in certain conditions through the skin, a few *tuberculosis bacilli*. These are so widely spread in our environment, and are so easily disseminated by the coughing or expectoration of consumptives, by dust, by flies, by unpasteurised or unboiled milk, and even by the intestinal excreta of subjects who are apparently healthy but have concealed tubercular lesions, that it is almost impossible to avoid them, and such precautions as may be taken with that object can only protect us in exceptional cases.

These very slight infections, however, caused by a few bacilli, produce no physiological disorder in the system. As a general rule, they merely sensitise it to tuberculin, and this sensitisation — which is inconstant — is sometimes transient and sometimes lasting. As a rule, these infections are readily tolerated, and, what is more surprising, they produce — provided always that no repetition occurs before a sufficient period has elapsed — a special state of immunity which makes other and more abundant doses of infective organisms harmless to the same subject.

This kind of immunity, represented by *resistance to re-infection*, is only found (as in syphilis and the piroplasmoses) if the organism harbours some representative of the specific parasite, and if these parasites are alive, but in small enough numbers and with a low enough virulence not to produce grave lesions or functional disorders fatal to life.

Except for a very short period, this immunity cannot be obtained by using bacilli killed by heat or by various physical or chemical agents, nor by substituting for these the varied substances that can be extracted from them.

It was for this reason that first Mafucci, in 1899, then MacFadyean, Pearson and Gilliland, and subsequently Behring with Römer and Ruppell in 1902, proposed to use for vaccinating cattle the human type of bacillus whose low virulence for the bovine species had been first demonstrated by Theobald Smith:

It will suffice to point out here that the principal reason why the large-scale experiments undertaken in various countries with a view to the practical application of the method of "bovo-vaccination", advocated by Behring, were not pursued was that it was found that animals inoculated with bacilli of the human type under the skin or in the veins emitted, intermittently and often over a long period, with their excreta or milk, germs which, not being always avirulent for the guinea-pig, might be dangerous to man and other sensitive animals.

In the hope of outflanking these obstacles, other experimenters endeavoured to vaccinate cattle with bacilli derived from birds (MacFadyean, Sheather, Edwards and Minett, 1913). In France, H. Vallée used a bacillus of equine origin which had little virulence for laboratory rodents. Gerald Webb and W. Williams, in the United States, and more recently Selter, at Bonn, did not hesitate to inoculate not merely animals but even children of various ages with very small doses of virulent bacilli of human origin.

It can hardly be imagined that the use of bacilli which are virulent for man or domestic animals, including poultry, will ever have the slightest chance of becoming a regular practice, because subjects thus *infected* and not *vaccinated* must inevitably remain under the continual threat of a possible generalisation of tuberculosis in consequence of the slow caseification of the tubercle.

As we had long been convinced of this truth, from 1908 onwards we endeavoured, in collaboration with C. Guérin, to obtain a strain of tuberculosis bacilli which would be genuinely and finally attenuated in Pasteur's sense, that is to say, made incapable of producing re-inoculable tuberculous lesions in the systems of the most sensitive animals.

The experimental researches we had till then been pursuing into tuberculous infection through the digestive tract had shown us that with pure bile it was possible to prepare very fine and stable emulsions of bacilli, and that these biliated emulsions were particularly favourable to intestinal absorption. In order to obtain cultures that could be more easily emulsified, we had the idea of inoculating the bacilli into media consisting primarily of *pure ox-bile*, and we found that, notwithstanding the strong alkalinity of these media, the culture developed with a very peculiar aspect, resembling, on potatoes cooked in 5% glycerinated ox-bile, cultures of *Bacillus mallei*.

Under the continued influence of the bile, the physico-chemical constitution of the cero-adipose envelope of the bacillus changes, as has been demonstrated by Albert Berthelot's analyses, and its pathogenic properties gradually alter, so that after thirty successive cultures on bile the attenuation of the virulence for the young bullock was already plain. After 230 cultures, the sub-culture taking place every 20 to 25 days (this took thirteen years in all), the bacillus had become incapable

of producing re-inoculable tuberculous lesions in the guinea-pig, even in such large doses as from 1 to 2 centigrammes, and the pathological alterations which it is capable of causing at such high doses disappear spontaneously in a few weeks or months, leaving no trace, even of sclerosis.

As the attempts we then made to restore its initial virulence to this hereditarily attenuated bacillus by successive passages through sensitive animals regularly failed, it seemed clear from our experiments that we were in possession of a strain of "vaccine-bacilli" which were apparently harmless to all animal species capable of contracting tuberculosis. This strain, which has since been studied by many research workers, is generally known as *BCG*.

I. EXPERIMENTAL PROOFS OF THE INNOCUOUSNESS OF *BCG* TO ALL ANIMAL SPECIES.

When, by subcutaneous, intraperitoneal, intravenous, intracocular, intraspinal or intracranial injection, we introduce up to one milligramme of *BCG* into the system of the guinea-pig or the rabbit, we do not produce in these animals any lesion presenting the characteristics of tuberculous lesions. Stronger doses (5, 10, 20 or more milligrammes), injected subcutaneously, cause the formation of a cold abscess, which, after the evacuation of its contents, is cured spontaneously without tumefaction of the neighbouring glands. In intraperitoneal or intravenous injections, these strong doses produce on the epiploon and the peritoneum, or in the viscera (lungs, liver, spleen), tuberculous nodules which, after a few weeks, or at most a few months, are re-absorbed and disappear, the animals having sustained no ill-effects.

If animals so inoculated are killed during the reactive period, when their organs contain small tubercles, and if these tubercles are pounded and emulsified and then re-inoculated into fresh animals, the latter remain completely unaffected, and do not contract tuberculosis.

Subcutaneous or intravascular inoculations of 50 or 100 milligrammes of finely emulsified *BCG* are readily tolerated by young or adult cattle which do not react to tuberculin. *BCG* is also harmless to monkeys of every species, including anthropoids, and to horses, goats, sheep, pigs, dogs, and poultry.

A healthy man can tolerate 1 milligramme injected intravenously, and tuberculous subjects themselves are not injuriously affected by subcutaneous injections of doses up to 1 centigramme. These injections merely give rise to cold abscesses at the site of inoculation, without tumefaction of the neighbouring lymphatic glands.

Doses of 1 centigramme administered *per os* and repeated three times at 48-hour intervals produce no physiological disturbance in young children or in young sucking animals.

All these facts, established by our experiments, have been confirmed by a large number of experimenters, including R. Kraus, Gerlach, H. Schrötter, Prausnitz, Kolle, Kirchner, Lange (of the Reichsgesundheitsamt), Aldershoff, Stanley Griffith, Lyle Cummings, O'Brien, William Park, A. Ascoli, Cantacuzène, Tzekhnovitzer and the Ukrainian Commission, Georges Blanc, Silberschmidt, Carlos Chagas, Arena, Martirene Fontecilla, Imamura and Takahashi, etc. Bruno Lange and K. Lydtin, at the Robert Koch Institute, have further demonstrated that BCG emulsions can be inhaled without ill-effects.

II. EXPERIMENTAL PROOFS OF THE EFFICACY OF BCG AS A PREVENTIVE OF VIRULENT TUBERCULOUS INFECTION.

The first demonstration of the protective effects of BCG against a virulent experimental infection was furnished by the experiments we published in 1913 and 1914 in the *Annales de l'Institut Pasteur*.¹

These experiments showed that young cattle which have been given BCG intravenously without ill-effects can, a month later, be tested with a dose of virulent bacilli which is fatal to controls in five or six weeks. Animals thus vaccinated and tested retain in their glands (principally the bronchial glands) living and virulent bacilli from the test for as long as 18 months, without these bacilli ever betraying their presence in the system by progressive tuberculous lesions.

Some of these bacilli are eliminated from the system, especially in the fæces. The remainder are tolerated and continue to be harmless as long as the immunity conferred by the living "vaccine-bacilli" persists.

In our experiments the duration of this protection from virulent experimental infections was at least 18 months in cattle. In laboratory rodents it did not exceed a few weeks. In the guinea-pig it quickly vanished, as was demonstrated by the experiments of A. Boquet and L. Nègre, and later by those of Remlinger and Bailly, which covered a great number of animals. On the other hand, it is marked and prolonged against natural contamination (through cohabitation with infectious cases) in such animals as the monkey, whose life is much longer, and this is certainly also the case in man. The experiments made by Wilbert on chimpanzees in Guinea are highly evidential in this respect. They have been confirmed by Kraus and Gerlach's more recent experiments.

Further evidence of the protective effects of BCG against the natural tuberculous infection is furnished by our experiments on close and prolonged cohabitation between vaccinated

¹ *Annales de l'Institut Pasteur*, Vol. 27, February 1913, and Vol. 28, April 1914.

and tuberculous cattle,¹ and by those carried out on farms by C. Guérin, A. Richart and M. Boissière², by A. Ascoli in Italy, more recently, in Brazil, by de Assis and Dupont, in Canada by the Edmonton Experiments Committee (Secretary : Dr. Rankin), etc.

The innocuousness of BCG to all animal species sensitive to the tuberculous infection, and its efficacy as a preventive of the natural or artificially produced infection, being thus demonstrated, the next step was to attempt to use it for the protection of man.

III. PREVENTIVE VACCINATION OR "PREMUNISATION" SHOULD AIM AT PROTECTING THE INFANT, IMMEDIATELY AFTER BIRTH, FROM THE NATURAL INFECTION.

Since the tuberculous infection is much more serious for the infant, particularly in the first few months of life, than for the older child or the adult, it is all the more necessary to endeavour to protect the infant *immediately after birth*, because at that period of its life it is completely dependent upon its mother and the persons who look after it. When these persons are germ-carriers, the risk of infection is so great that the infant scarcely ever escapes. It is for that reason that the tuberculosis death rate is so high during the first year among children born or brought up in infected surroundings. It is possible to question the figures which have been given by various authors and by ourselves on this subject, since in no country are there official statistics relating solely to *children of tuberculous parents*; but all phthisiologists agree that infants exposed to tuberculous infection in the family die in large numbers before reaching their second year, and that the forms of tuberculosis which develop during adolescence or adult life are, as a rule, the results of an infection contracted when very young, not from a few bacilli but from a larger number, the infection having remained latent or concealed until various circumstances arose favourable to it.

If vaccination to prevent tuberculosis is possible, therefore, it should be done to the infant *during the first few days after birth*, because it is only at that age that — apart from the relatively rare cases of infection *in utero* by the ultra-virus or by the tuberculosis virus — there is any hope that the subject will still be free from any pre-existing tuberculous infection; but as, on the other hand, this vaccination cannot be immediate, but takes some time to establish itself (about 25 days with BCG, according to experiments on animals), every effort should

¹ *Annales de l'Institut Pasteur*, Vol. 34, September 1920, page 553.

² *Annales de l'Institut Pasteur*, Vol. 41, March 1927.

be made during its inactive stage, by measures of separation or isolation, or at any rate by strict hygienic precautions, to prevent the young artificially premunised subjects from being exposed to virulent infections.

IV. POSSIBILITY OF VACCINATING NEWBORN INFANTS
per os ON ACCOUNT OF THE FACT THAT, DURING
THE FIRST FEW DAYS OF LIFE, THE INTESTINE IS
HIGHLY PERMEABLE BY BACTERIA.

Infants in the first few days after birth might, like calves, be vaccinated by the *subcutaneous injection* of a small quantity of fresh emulsion of BCG. On the other hand, apart from the fact that the families would probably object to such inoculations, they have this disadvantage, that they fairly frequently cause in the newborn child a cold abscess (though a harmless one), the spontaneous evacuation of which may be a nuisance.

For that reason, it has been found preferable to resort to vaccination *per os*, taking advantage of the fact that the intestinal mucosa of the infant less than two weeks old, like that of young sucking animals, is as yet composed only of incompletely differentiated cells, having a strong phagocytic power and capable of absorbing and conveying into the lymphatic and blood circulation the microbes introduced into the digestive tract. This phenomenon — first reported by C. Weigert — was demonstrated by Disse.¹ Later, A. Boquet, P. Nélis and L. van Boeckel, Römer and Behring, Ehrlich, Vaillard and, subsequently, Ramon and Grasset showed that not merely bacteria but even toxins can pass with the greatest ease through the intestinal mucosa of newborn animals, whereas this happens only irregularly and to a smaller extent in full-grown animals.

In recent publications, Pirquet and his pupils E. Nobel, Chiari and Solé have denied — though without offering any experimental proof of their contention — that newborn children can be vaccinated by this route. They do not believe that bacteria can be absorbed through the intestine.

It is easy, however, to establish the fact of such absorption, first, because, after 6 months or more, microbes are to be found in the system of vaccinated infants who died of diseases other than tuberculosis (Calmette, C. Debarge and R. Girod, Zeyland and Mrs. Piasecka-Zeyland), and secondly, because, as we have demonstrated with C. Guérin² in cattle and laboratory rodents, the bacilli thus absorbed are eliminated to a great extent through the biliary ducts and the intestine. An experiment which is

¹ *Deutsch. med. Woch.*, 1903. No. 1.

² *C. R. Acad. des Sc.*, 1909, Vol. 148, page 601, and *Annales de l'Institut Pasteur*, 1913, Vol. 27, page 162.

highly evidential in this respect was made at the Pasteur Institute by A. Boquet¹ with the Thimotee bacillus, which can be cultivated on the usual media. All that is necessary is to make guinea-pigs — even full-grown guinea-pigs — which have been fasting for 24 hours ingest a few centigrammes of this germ with bread-crumbs, then to kill them after variable periods and to seed the blood from the heart and the contents of the gall-bladder on glycerinated potato or on the Dorset-Petroff medium. We then find that the blood and bile thus taken 3 or 4 hours after the infected meal nearly always cause the development of pure colonies of Thimotee bacillus, whereas after a longer period the cultures are generally sterile.

Further proof of the absorption of tuberculosis bacilli through the intestine is furnished by the work of P. Nélis² and of this scientist with L. van Boekel. They demonstrated the rapid fixation of BCG in the lymphatic organs and the lungs, a few hours only after the absorption of a single meal of sterile food to which BCG had been added. This absorption is particularly intense and rapid in very young animals (sucking guinea-pigs and rabbits). P. Nélis also endeavoured to ascertain the exact moment of appearance and the duration of the intradermal reaction in the adult guinea-pig after ingestion of BCG. He found that sensitiveness to tuberculin appeared between the first and second month after three ingestions (at 48-hour intervals), and persisted for from 6 to 10 months, subsequently disappearing.

It is therefore beyond question that the administration of BCG *per os* is a process that can be perfectly well used with newborn infants, and, as we shall see later, its efficacy can no longer be disputed.

On the other hand, there is no denying that, for children more than a fortnight old, for adolescents, and for adults, only subcutaneous vaccination can be considered. We shall return to this point when we report the experiments already made on the subject.

V. FIRST EXPERIMENTS IN PREMUNISATION, 1921-22. — THEIR ENCOURAGING RESULTS MAKE THE PASTEUR INSTITUTE DECIDE TO EXTEND THE EXPERIMENT.

Having become convinced of the innocuousness of BCG injected into, or absorbed *per os*, even in strong doses, by a large number of animals of every species, we felt justified in sharing with our colleague, Dr. Weill-Hallé, Médecin des Hôpitaux de Paris and Director of the Child Welfare School of the Faculty of Medicine, the responsibility for an experiment which

¹ C.R. Soc. de Biol., 1927, Vol. 96, page 176.

² C.R. Soc. de Biol., 1927, November 26th.

he proposed we should attempt in July 1921 on an infant which was doomed to tuberculosis because it unavoidably lived with a consumptive grandmother — the mother, also consumptive, having died immediately after the child's birth. The child was made to ingest three doses of 2 milligrammes of fresh BCG culture in a teaspoonful of milk on the 3rd, 5th and 7th days respectively, making 6 milligrammes in all. No incident ensued. The child has developed on normal lines and has since remained completely free from tuberculosis.

Between July 1921 and July 1924, our collaborators B. Weill-Hallé, Turpin and Mlle. Coloni have thus, with our approval, vaccinated 317 children, whom they have been able to keep under regular observation; 67 of these children were born and reared in an infected environment, and the others in an apparently healthy one. The doses of BCG given to them were raised to a total of 3 centigrammes. Up to January 1927, only one death from tuberculosis was reported among these children, this being that of an infant 4 months old which had been nursed by its tuberculous mother for 3 months after its birth until the mother went to hospital, where she eventually died.

Apart from 14 of these children, who died of various diseases other than tuberculosis, they were all perfectly well developed, and had shown no sign of any functional disorder and no setback in their increase of weight.

In these circumstances, as it seemed perfectly clear that BCG was harmless to infants as to animals, young or full-grown, which are sensitive to tuberculosis, it was decided to pursue the experiment not only in the maternity wards of hospitals, but also among the patients of tuberculosis dispensaries and of such doctors as desired it.

Accordingly, from July 1st, 1924, the Pasteur Institute saw that it was able to respond to all requests, and a service for the preparation and free distribution of vaccine was organised to meet the needs of all France. At the same time, seed-cultures of BCG were placed at the disposal of all foreign laboratories.

As it appeared that the method was unquestionably harmless, the next necessity was to collect as much and as detailed information as possible in order to ascertain whether it was effective, as we had been convinced by the experiments on young cattle, and by those carried out on anthropoid apes in an infected environment at Kindia (French Guinea).

VI. TUBERCULOSIS DEATH RATE AND GENERAL DEATH RATE OF INFANTS BORN OR REARED IN AN INFECTED ENVIRONMENT.

To judge of the value of BCG as a preventive of tuberculosis, we shall obviously have to wait until we know more precisely the duration of the immunity conferred by the first vaccination after birth, and by any subsequent re-vaccinations.

We can at least, however, observe the influence of the vaccine on the decrease in mortality from tuberculosis and the general infant mortality, provided we have suitable data for comparison.

Such data cannot be found in the vital statistics regularly published by all civilised countries. The figures for tuberculosis mortality at different ages are of no use to us, partly because they are inaccurate owing to failures to notify the accurate cause of deaths, and partly because they cover the entire population and not exclusively those *born or reared in an infected environment*, with whom we are chiefly concerned.

Hospital statistics also are useless, because they concern only hospital patients, with the result that the proportion of deaths from tuberculosis at different ages is much higher there than in families where one or more members are tuberculous.

Our only hope, therefore, is to endeavour on our own account to obtain from tuberculosis dispensaries, which are now numerous in France and many other countries, information as to the subsequent history of children belonging to families under the supervision of these institutions, which are, as a rule, directed by phthisiological specialists and trained and enthusiastic visiting nurses.

In 1925 we undertook an enquiry among all the tuberculosis dispensaries then in existence, and we received 99 replies, which may be summarised as follows :

Out of 1,364 children born in 1922 of 1,362 tuberculous mothers (there were two pairs of twins), 327, or 24 per cent, succumbed to diseases presumed to be tuberculous *during the first year of life*.

This figure in itself was much lower than that (80 per cent) given by Léon Bernard, Robert Debré and Marcel Lelong for Parisian children born of tuberculous mothers, who could not be accepted by the "Placement Familial des Tout Petits". It was also much lower than the figures published by Professor Forssner of Stockholm (70 per cent), Professor J. Parisot of Nancy (53 per cent up to 2 years), and P. de Elizalde of Buenos Aires (79 per cent up to 1 year). On the other hand, it was slightly higher than the figure given by the Belgian Anti-Tuberculosis League (20 per cent), and much higher than that of R. Kjer-Petersen and J. Ostenfeld of Copenhagen (7.7 per cent) and than William Park's figure for the United States (7.6 per cent).

The investigation carried out in 204 antituberculosis dispensaries or social hygiene centres had the great advantage over the previous one of providing us with precise information concerning non-vaccinated as well as vaccinated children in families living under identical social conditions and environment, and looked after by the same staff of physicians and visiting nurses. This information is contained in the social enquiry cards of the institutions.

This material made it easy to compare accurately, not only the *mortality from tuberculosis* in the vaccinated and

non-vaccinated groups, but also their *general mortality*, the latter figures being beyond dispute, whatever defects the diagnoses of the causes of death may possess.

It is obvious that, if general mortality (from all causes) is markedly lower among the *vaccinated* than the *unvaccinated* in the population attending dispensaries, all other things being equal, it is because BCG vaccination succeeds in preserving children from infection in tuberculous families; it constitutes also the best clinical demonstration of the complete innocuousness of the method — a demonstration which amounts to experimental proof.

We shall first determine, from the answers given us by 204 dispensaries, what the *tuberculosis* and *general mortality* have been in 1925, 1926 and 1927 among unvaccinated children cared for by these institutions. These children numbered 4,854, 1,989 of whom were born of tuberculous mothers, while 2,865 were born and reared in contact with tuberculous persons.

Since the statistics of the results of vaccination, published hitherto by ourselves and other workers and clinicians in France and in other countries, were criticised by professional statisticians such as Professor Greenwood, in England, Rosenfeld and Götzl in Austria, we decided to make a new and comprehensive enquiry covering :

(1) All the tuberculosis dispensaries or social hygiene centres in France, controlled by the Ministry of Health and in operation for over 4 years (there were 204 such dispensaries on July 1st, 1928, and all of them kindly answered our questionnaire);

(2) All the physicians (972) who, between July 1st, 1924, and July 1st, 1927 — *i.e.*, for 4 years as a maximum and 1 year as a minimum — had vaccinated children born of tuberculous mothers or reared in notoriously tubercle-infected environment.

1. Children *not vaccinated with BCG*, born of tuberculous mothers, *not separated* : 1,980.

(a) Born in 1925	777
" 1926	694
" 1927	518
	<hr/>
Total	1,989

(b) Of these 1,989 children, the following numbers died of diseases *presumed to be tuberculous* at the ages of :

1 month to 6 months	119
6 months to 1 year	109
1 year to 4 years	130
	<hr/>

 Total 358

(c) Of these 1,989 children, the following numbers died of diseases *not presumed to be tuberculous* at the ages of :

1 month to 6 months	59
6 months to 1 year	32
1 year to 4 years	34

Total 125

Total deaths for this group	483
General death rate	24.3 %
Tuberculosis death rate	18.0 %

2. Children *not vaccinated with BCG*, born and reared in infected surroundings (excluding children of tuberculous mothers): 2,865.

(a) Born in 1925	1,097
" 1926	1,007
" 1927	761

Total 2,865

(b) Of these 2,865 children, the following numbers died of diseases *presumed to be tuberculous* at the ages of :

1 month to 6 months	112
6 months to 1 year	160
1 year to 4 years	122

Total 394

(c) Of these 2,865 children, the following numbers died of diseases *not presumed to be tuberculous* at the ages of :

1 month to 6 months	59
6 months to 1 year	46
1 year to 4 years	41

Total 146

Total deaths for this group	540
General death rate	18.5 %
Tuberculosis death rate	13.8 %

(It may be pointed out that for the whole population of France the general infant mortality was 8.3 deaths per 100 children *born alive* in 1927.)

Therefore, general mortality for the years 1925, 1926 and 1927 was 21.4 % for the whole group of 4,854 unvaccinated children born of tuberculous mothers or reared in a tuberculous environment. Their tuberculous mortality was 15.9 %.

This latter figure is markedly lower than the one we obtained in 1925 in an enquiry covering 99 dispensaries.

This mortality, however, is still very great and it is doubtful whether hygienic education of the families and stricter adherence to health rules can bring it lower than 7 %, a figure now reached by certain selected and supervised groups in Scandinavian countries and the United States.

We must now find out whether by the systematic use of BCG it is possible to obtain an important additional saving in human lives.

Observations on children of tuberculous families supervised by the same dispensaries and vaccinated in 1925, 1926 and 1927 will give the answer to this question.

VII. EXPERIENCE FROM 1924 TO 1928. — INFORMATION SUPPLIED BY TUBERCULOSIS DISPENSARIES AS TO THE NUMBER AND SUBSEQUENT MEDICAL HISTORY OF CHILDREN BORN OF TUBERCULOUS MOTHERS OR REARED IN INFECTED FAMILIES, VACCINATED WITH BCG AND AGED FROM 1 TO 4 YEARS ON JULY 1st, 1928.

These *vaccinated* children have been classified in exactly the same way as the *non-vaccinated*.

1. *Vaccinated children born of tuberculous mothers, not separated*: 879.

(a) Born in 1925	145
" 1926	311
" 1927	423 ¹
Total	<hr/> 879

(b) Of these 879 children, the following numbers died of diseases *presumed to be tuberculous* at the ages of :

1 month to 6 months	19
6 months to 1 year	13
1 year to 4 years	6
Total	<hr/> 38

(c) Of these 879 children, the following numbers died of diseases *not presumed to be tuberculous* at the ages of :

1 month to 6 months	73
6 months to 1 year	17
1 year to 4 years	13
Total	<hr/> 103

¹ The annual increase in the number of children vaccinated shows that vaccination is not merely accepted, but desired, by the families.

Total deaths for this group	141
General death rate	14.8 %
Tuberculosis death rate	4.3 %

2. Vaccinated children reared in infected surroundings (excluding children of tuberculous mothers): 1,489.

(a) Born in 1925	185
" 1926	477
" 1927	827
<hr/>	
Total	1,489

(b) Of these 1,489 children, the following numbers died of diseases presumed to be tuberculous at the ages of:

1 month to 6 months	12
6 months to 1 year	7
1 year to 4 years	11
<hr/>	
Total	30

(c) Of these 1,489 children, the following numbers died of diseases not presumed to be tuberculous at the ages of:

1 month to 6 months	58
6 months to 1 year	30
1 year to 4 years	16
<hr/>	
Total	104

Total deaths in this group	134
General death rate	8.9 %

(This is approximately the same as the general death rate under 1 year of age for France per 100 live-born children.)

Tuberculosis death rate	2.6 %
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It will be seen that, for vaccinated children born of tuberculous mothers reared by the latter or born and reared in infected surroundings, the general death rate is 11.8 %, whereas it is 21.4 % for unvaccinated children, and that for vaccinated children born and reared in infected surroundings the tuberculosis death rate is 3.4 %, as against 15.9 %, i.e., four times larger for the unvaccinated.

Even if there are errors of diagnosis in diseases not presumed to be tuberculous, nevertheless, it is clear that the death rate for vaccinated children is four times smaller than the rate for the unvaccinated, though they are living in the same conditions and under the same medical supervision.

Consequently, the value of vaccination for children under the age of 4 years cannot be disputed.

VIII. VACCINATION EXPERIMENTS MADE IN FRANCE IN 89 DEPARTMENTS.— INFORMATION SUPPLIED UP TO JULY 1st, 1928, AS TO THE SUBSEQUENT MEDICAL HISTORY OF 3,607 CHILDREN IN TUBERCULOUS SURROUNDINGS VACCINATED WITH BCG IN THESE 89 DEPARTMENTS BY 972 PHYSICIANS.

These 3,607 children *vaccinated at birth* may be classified as follows :

(a) According to age on July 1st, 1928 :

1 year	1,254
2 years	1,572
3 years	696
4 years	85
Total	3,607

(b) According to tuberculous contacts to which they have been exposed :

Tuberculous mother	1,212	=	33.6
Tuberculous father	1,150	=	31.9
Tuberculous father and mother	188	=	5.2
Tuberculous grandparents or collateral relatives	502	=	13.9
Tuberculous contact not specified	555	=	15.4
Total	3,607		

(c) Of these 3,607 children, the following numbers died of diseases *presumed to be tuberculous* (including all deaths diagnosed as from "meningitis", though we know that most of these deaths in very young children, particularly those under 6 months old, are due to the pneumococcus or to Pfeiffer's bacillus) at the ages of :

1 month to 6 months	29	} 54 = 1.5 % (including 5 under 3 months).
6 months to 1 year	25	
1 year to 4 years	12 = 0.3 %	
Total	66 = 1.8 %	

(d) Of these 3,607 children, the following numbers died of non-tuberculous diseases at the ages of :

1 month to		} 266 = 7.3 %
6 months . . .	176	
6 months to		} 90
1 year	90	
1 year to		} 63 = 1.7 %
4 years	63	

Total 329 = 9.1 %

The total number of deaths was therefore 395, i.e. :

General death rate from all causes : 10.9 %.

Death rate from diseases presumed to be tuberculous : 1.8 %.

It might be objected that possibly not all deaths of vaccinated children have been reported to us, and that the present number of vaccinated children in France (110,000 up to October 1st, 1928) is so large that there is a flagrant disproportion between the total number of children as to whom we have recent information and the number of vaccinated children in the whole country.

To this we reply : first, that there has been so much publicity through the medical Press in the medical world, and even through the general Press among the general public, particularly during the last few months, that every doctor who had observed some incident or had any deaths among children vaccinated by him has made them known to us ; so have the families themselves.

Moreover, it will be readily understood that we cannot ask directly for news of all the children vaccinated with BCG in France (110,000 up to October 1st, 1928) and in the French colonies (45,000 in Indo-China alone up to March 1st, 1928). All we could do was to write to doctors who had reported that they had vaccinated children born of tuberculous mothers, or reared in infected surroundings, and to all the tuberculosis dispensaries which had been in operation for at least three years.

We deliberately sought to place ourselves at the greatest possible disadvantage by applying our comparative investigation of the general death rate and the tuberculous death rate both to unvaccinated and vaccinated children of the same age and living in the same surroundings, these children being drawn exclusively from the patients of tuberculosis dispensaries.

Our feeling was that if we did this our figures and results would be beyond any possible dispute or question.

In order to check accurately these figures and results, we have carried out another enquiry dealing exclusively with children born of tuberculous mothers, not separated from the latter and vaccinated between July 1st, 1924, and July 1st, 1927, aged

now, therefore, 1 to 4 years, who lived in continual contact with their mothers from birth until death or until October 1st, 1928.

We did not include in this study the infants whose tuberculous mothers died within a few weeks or months of birth, thus removing the possibilities of maternal contagion before the end of the first year.

The number of children regularly observed under those conditions was 814. Of these, 20 (2.4 %) died of diseases presumed to be tuberculous (under this heading we include all deaths ascribed to "meningitis"), even though 6 of them occurred before the age of 4 months, and were not likely therefore to be tuberculous.

Sixty-five (8 %) died of various non-tuberculous diseases.

In this special group of children, therefore, who remained in constant contact with their tuberculous mothers, general mortality (from all causes) was 10.4 % between 0 and 4 years, that is to say, scarcely higher than the general mortality of infants between 0 and 4 years in the whole of France (11.1 % in 1926, and 9.27 per hundred live-births in the first year of life in 1926).

During the same period, the 204 dispensaries and anti-tuberculosis centres which answered our questionnaire had registered *1,989 children born of tuberculous mothers who were neither separated from them nor vaccinated*. The tuberculosis mortality of these infants was 18.0 %, instead of 2.4 %, and their general mortality 24.3 %, instead of *10.4 % among the vaccinated*.

The premunising value of BCG is therefore clearly proved in this class of children who have so few chances of escaping maternal infection.

It is nearly certain that the results of vaccination would be better still were it possible to remove the children — as we can easily do for calves — from the source of virulent infection, for about a month after birth, *i.e.*, the period required for the establishment of tuberculous immunity as shown by the experiments.

It is also possible that the method may be perfected and made more precise, especially in the method of administration of BCG to children.

The reduction of the general infant mortality brought about by BCG vaccination is always obvious when nearly all children born in a city or a district are vaccinated, whenever children of apparently tubercle-free families or infected families. This is the case in certain parts of Roumania.

As instance of this fact, we shall describe what was done among the population of Ligny-en-Barrois, a small town of 5,000 inhabitants in the Department of Meuse.

Vaccination was adopted as a routine procedure on July 9th, 1926. From July 9th, 1926, to September 24th, 1928, 237 children were born at Ligny; 210 were vaccinated with BCG, 27 were not.

Of the 210 vaccinated, 5 died from various non-tuberculous diseases. Of the 27 unvaccinated, 5 died also from various non-tuberculous diseases.

The general mortality of the 27 unvaccinated was therefore 18.5 %, as against 2.3 % among 210 vaccinated.¹

What arguments can stand against such a fact ?

IX. RESULTS OF POST-MORTEM EXAMINATION OF CHILDREN WHO DIED OF DISEASES NOT PRESUMED TO BE TUBERCULOUS.

In France, only a small number of post-mortems have been made on children vaccinated with BCG who died of diseases *not presumed to be tuberculous* (for instance, by Léon Bernard at the Hôpital Laennec (7 autopsies)). One was made by us at the Pasteur Hospital on a heredo-syphilitic child which was vaccinated and separated from its mother at birth, and died at the age of 6 months, presenting no tuberculous lesions; in its mediastinal and mesenteric glands we found intact BCG which stained with Ziehl solution. Inoculation of these glands and of the pounded spleen of the child into a series of guinea-pigs produced no effect.

Two other post-mortems, equally negative, of vaccinated children who died of gastro-enteritis at five and six months respectively have been reported to us.

A larger number have been made abroad, particularly in the Ukraine by Iakhnis, in New York by William Park, and in Madrid by Bravo y Friar and Munejero. At Poznan (Poland), Dr. Zeyland and Mme. Piasecka-Zeyland have performed many autopsies recorded in the *Annales de l'Institut Pasteur* (December 1928, supplement).

In no case was it possible to discover any suspicious lesion in vaccinated children who died of non-tuberculous diseases, or to isolate virulent bacilli in children who had not been exposed to tuberculous contacts.

X. THE TUBERCULIN REACTION IN CHILDREN VACCINATED *per os*.

B. Weill-Hallé and Turpin, J. Parisot and H. Saleur have found that the tuberculin skin-reaction became positive within from 1 to 6 months in about one-third of the children vaccinated with BCG. H. Buschmann,² at Bleialf (Eifel), obtained approximately the same figures, but they are higher for children who

¹ *Note by the Editor.* — If these figures are corrected and account is taken of the fact that all early deaths (occurring before the age of vaccination) are, of course, included in the mortality of the unvaccinated group, the rates for both groups are still significantly different: 13.7% and 2.38%, a ratio nearly 6 to 1.

² *Monatsschrift für Kinderheilkunde*, Bd. 37, 1928, page 393.

have been vaccinated and exposed to infectious contacts than for children reared in apparently healthy surroundings. This means that in children reared in infected surroundings a virulent infection frequently intervenes, but remains harmless, thanks to the protective vaccination.

Pirquet and Nobel emitted the hypothesis that those of the children who do not react to tuberculin, although they have ingested BCG, are not really vaccinated at all. In support of this opinion they offer no proofs, but they maintain it because they are convinced that anybody who is to resist tuberculous re-infections must be *allergic, i.e.,* sensitive to tuberculin.

Experiments on animals, however, and also the results of vaccinations, of children born of tuberculous mothers and separated from them at birth, or reared in infected surroundings, run counter to this view.

Let us first consider what happens in the case of cattle.

We inoculated 20 milligrammes of BCG intravenously into eight young bullocks which did not react to tuberculin, and a dose of 50 milligrammes of the same BCG subcutaneously into eight other bovines of the same age, which equally did not react.

The animals in these two batches were then subjected to the tuberculin test after 2 months and after 6 months. We found that after 2 months only four of the eight beasts in the first batch (intravenous inoculation) had become allergic, and after 6 months none of them was allergic, while the eight animals in the second batch (subcutaneous inoculation) were allergic after 2 months and still allergic after 6 months.

After 12 months only one reacted positively to the intravenous test. This means that *intravenous* inoculation, which disperses the BCG bacilli throughout the system, producing (at all events with this dose of 20 milligrammes *very finely emulsified*) only minute tuberculous lesions which are rapidly cured and re-absorbed, confers only a transient allergy, whereas *subcutaneous* inoculation, which creates a large local lesion persisting for some considerable time, causes a tuberculin sensitisation of much longer duration.

This notwithstanding, all our animals retained their immunity, and resisted, 15 to 18 months after vaccination, the intravenous inoculation of a dose of virulent tubercle bacilli which was fatal to controls in 5 or 6 weeks.

It is clear, therefore, that subjects vaccinated with BCG retain the benefits conferred by vaccination long after they have lost the power to react to tuberculin.

Nor is it necessary that a subject should have already manifested sensitiveness to tuberculin for him to be able to resist virulent infections. Thus, in the adult guinea-pigs vaccinated by Nélis¹ by ingestion of a strong dose of BCG (0.30 gramme in three doses of 0.10 gramme at 48-hour intervals),

¹ Soc. de Biol., November 26th, 1927.

the intracutaneous reaction was, in general, positive only after 2 months, and became negative after from 6 to 10 months. It was between the 25th and 35th days after absorbing the BCG *per os* that these animals presented the maximum resistance to virulent infections likewise produced *per os* or by ocular instillation.

In young guinea-pigs, as Freund¹ first, and then J. Valtis,² found, both after vaccination and after virulent inoculations, the tuberculin reactions are irregular or absent, even when the animals have already tuberculous lesions; and J. Valtis has recently found that it is the same in young children. These, even if born of tuberculous mothers and harbouring ultravirus in their system, sometimes remain for a long period insensitive to tuberculin.

Lastly, we know that the bacillary proteins, bacilli of aviary tuberculosis killed by heat, and paratuberculous bacilli sensitise animals to tuberculin for a varying period of time *without conferring any immunity on them.*

Accordingly, immunity, that is to say, resistance to bacillary infections, and hypersensitiveness to tuberculin are two distinct and independent conditions in organisms infected with Koch's bacillus.

The reason why sensitiveness to tuberculin does not always appear, or is ordinarily belated, in vaccinated children is that BCG (except when absorbed in heavy doses) does not as a rule produce *follicular lesions* (characterised by the formation of giant cells) in the lymphatic organs. If it does produce such lesions — which is the case in reacting subjects — these promptly and spontaneously disappear, as may readily be seen in experimental animals, and, in that case, the subject remains allergic so long as they are not entirely cured, or if he chances to be infected by virulent bacilli from his environment; but his health does not suffer in any way.

This is shown quite clearly by clinical observation of our vaccinated subjects in tuberculous surroundings.

Léon Bernard has recorded particulars of children born of tuberculous mothers and *separated from them from birth*, vaccinated with BCG, then submitted to a tuberculin skin-test nearly every month for 2 years.

These children, though removed from all contact with tuberculous persons, showed the allergic reaction in the proportion of 50 % after 1 ½ to 12 months; generally after 3 months.

We have never considered that tuberculin allergy was useless to the individuals vaccinated with BCG, though certain writers have misquoted us in this connection (Bessau in Leipzig, Moro in Heidelberg, Selter in Bonn, Degkwitz in Greifswald). We have only stated that a subject could be immune, that is to say,

¹ *Journal of Immunology*, 1927, Vol. XIII, page 285.

² *Soc. de Biol.*, July 21st, 1928, page 554.

resistant to tuberculous infections, without presenting tuberculin allergy; and we have proved it experimentally.

We consider it necessary to bring about as promptly as possible the allergic state in the subjects vaccinated, because *possible allergic reactions* (and especially the intradermal test, which is more sensitive and reliable than the skin-test) *enable us to state with certainty that the vaccinated subject is really harbouring germs — whether vaccine bacilli or virulent bacilli.*

That is why we are considering the use of subcutaneous injections of BCG in the vaccination of individuals who are past the age when oral vaccination is practicable, *i.e.*, over 10 days old.

We also think that, in future, the *subcutaneous* route must be used for the re-vaccination after one, two or three years of children who, having been vaccinated at birth, do not react, or no longer react, to tuberculin; for this absence of reaction after the first year is a warning that they have lost, or very soon will lose, the protection conferred by their previous vaccination.

We shall return to this subject a little later.

XI. OBJECTION CONCERNING POSSIBLE RECOVERY OF VIRULENCE.

It is common knowledge that there are some viruses, spontaneously or artificially attenuated, which can recover their initial virulence by growing in the system of animals, at first very sensitive or weakened, and then increasingly resistant or vigorous.

May not the case be the same with BCG, and is there not reason to fear that this bacillus, which is at present devoid of its original virulence, may gain it after a more or less prolonged sojourn in an organism as sensitive as that of the young child?

We asked ourselves that question long ago, of course, and it will be believed that, if we consented to test the preventive action of BCG against tuberculosis on infants, it was not until we had acquired the completest conviction that this “vaccine-bacillus” is and remains harmless, not only to the child, but to all sensitive animals.

Many experimenters have endeavoured, like ourselves, to restore its virulence to BCG; R. Kraus and Gerlach at Vienna, Bruno Lange at Berlin, Stanley Griffith at Cambridge, William Park in New York, Aldershoff at Utrecht, A. Ascoli at Milan, Kirchner at Hamburg, Tzekhnovitzer and the Ukrainian Commission at Kharkoff, Elbert, Gelberg and Zonkerman at Minsk, Nasta and Catzap in Bucarest, G. Blanc in Athens, C. Chagas in Rio de Janeiro, etc., have never succeeded in doing so. Tzekhnovitzer’s experiments have been especially numerous and prolonged. He has tried to make BCG virulent by injecting it into organisms (guinea-pigs or monkeys) poisoned, for example,

by small doses of diphtheria toxin, or by increasing doses of tuberculin, or by a vitamin-free diet; and into animals previously infected with other germs, such as the streptococcus; and by successive passages from animal to animal; all these methods failed; so did the partial or complete exclusion of the reticulo-endothelial system (removal of the spleen) (Tzekhnovitzer, Korshun, Elbert, etc.).

None of these methods has been successful, so that R. Kraus was able to state, and rightly, that BCG was really a strain of Koch's bacillus *finally and hereditarily attenuated*, like Pasteur's anthrax vaccines.

It is only S. A. Petroff, of the Trudeau Laboratory, at Saranac Lake (N. Y.), who, notwithstanding the conclusions of all the bacteriologists throughout the world, claims to have succeeded in restoring the virulence of BCG by inoculating it by successive passages in the testicle of the guinea-pig. On the other hand, neither Cantacuzène nor Bruno Lange nor R. Kraus, nor ourselves, nor any of those who have taken the trouble to repeat this experiment, have obtained the same results. It is therefore to be supposed that S. A. Petroff must have used a culture containing, perhaps, some virulent bacillus of human origin. This is the more probable because he says that he isolated from his culture two kinds of colony—"R" non-virulent, and "S" virulent, for the guinea-pig, but of very little virulence to the rabbit. All the experimenters, however, who have studied the BCG culture coming directly from our laboratories have satisfied themselves that it is never capable of communicating a re-inoculable tuberculosis to the guinea-pig; it is, in fact, of purely bovine origin. It is therefore obvious that S. A. Petroff's S colony is derived from a virulent human bacillus accidentally introduced into his culture.

In fact, S. A. Petroff told us in our own laboratory that he found only one "S" (virulent) colony in about 50,000 "R" (non-virulent) colonies. If this were so, 1 milligramme of BCG, which contains 40 millions of bacilli, should include 800 virulent germs. As 10 virulent bacilli are sufficient to induce fatal tuberculosis in guinea-pigs, all guinea-pigs which receive subcutaneously 1 milligramme of BCG should die of tuberculosis; in fact, however, it is easy to observe that such a dose of BCG is not only never fatal, but not even pathogenic.

There is no justification for the supposition that such a recovery of virulence can take place in the system of vaccinated children, particularly as BCG is of *bovine* origin, and therefore is naturally of low virulence for man.

We have already alluded to the researches of Zeyland and Mme. Piasecka-Zeyland with cultures isolated from mesenteric lymph-glands of children vaccinated who died from non-tuberculous diseases.

J. Parisot, L. Fernier and H. Saleur inoculated guinea-pigs with the pus of cold abscesses caused by the subcutaneous

inoculation of BCG to 11 individuals of various ages. They never could produce the slightest tuberculous lesion in the inoculated animals. This pus did contain acid-fast bacilli, but even after remaining 4 months in the human body the latter proved completely avirulent.

Some of our clinical colleagues have put forward the hypothesis that possibly, much later on, after some years, BCG, having long remained a harmless parasite, may again become capable of causing tuberculous lesions. This apprehension also is quite unjustified, for our experiments on animals show that the "vaccine-bacilli" — like the virulent bacilli themselves in many cases — are gradually eliminated and disappear, leaving no trace, and in that case the immunity to tuberculosis, *i.e.*, the resistance to re-infections, gradually declines, so that the organism recovers its capacity for contracting tuberculosis under the same conditions as an organism which has never previously contracted an infection.

It is possible that, in the future, some laboratory device, different from any that has yet been tried, may succeed in restoring the virulence of BCG and making it capable of conveying tuberculosis to experimental animals; but what we can say with certainty is that, in the present state of our knowledge, no clinical observation and no laboratory experiment confirmed by several workers either proves or justifies the fear that BCG may be a source of danger to the subjects vaccinated.

XII. EFFECT OF BCG VACCINATION ON THE GENERAL INFANTILE MORTALITY.

It is surprising to observe that, wherever BCG vaccination is practised among children living in an apparently healthy environment, as well as among those whose families are tuberculous, the general mortality (from all causes) decreases progressively until it reaches two-thirds or even one-half of the mortality observed previous to the use of BCG, and still prevailing in neighbouring localities.

This fact is apparent in the statistics of Roumania. It is also a clear consequence of the vaccination campaign, already referred to, in the small town of Ligny-en-Barrois (Meuse). It is noticeable also in other more important towns, such as La Rochelle, where, since March 1927 until July 1928, 52 out of 72 infants born each month (832 out of 1,156) were vaccinated. While the mortality of the non-vaccinated was during these 16 months 9.9 %, it was only 3.8 % among the vaccinated.

What are the causes of this difference between the general death rates of the vaccinated and non-vaccinated, which clearly exceeds the difference in mortality from tuberculosis directly ascribable to vaccination? Perhaps it may be ascribed to a stimulation by BCG of the lymphocyte and lymph-gland

defences, since the BCG absorbed circulates in the lymphatic system. Whatever the explanation, the fact remains. It is reported in the answers to our investigation questionnaire by many physicians, who wonder why, during epidemics of measles, whooping-cough or influenza, the vaccinated children show a stronger resistance and die less than the non-vaccinated, while their resistance is also higher to the gastro-intestinal infections so common in infants. This is an additional proof, and a particularly strong one, not only of the protective efficacy of BCG against those infections the main or the secondary agent of which is the tubercle bacillus, but also of its complete innocuousness, for, if BCG were capable of producing toxic effects or severe infections, the general death rate of the children vaccinated would be increased instead of being decreased, as it so obviously is.

The use of the BCG method of vaccination against tuberculosis may, therefore — as Jules Renault advised it, basing his judgment on his own clinical experience at the Hôpital St. Louis — be extended to all children, whether born in healthy, suspicious or infected environments, without fear of the slightest incident.

XIII. EXPERIMENTS ON PREVENTIVE VACCINATION BY SUBCUTANEOUS INOCULATION OF BCG INTO CHILDREN NOT PREMUNISED AT BIRTH AND INTO ADULTS.

Even when we began our experiments on the vaccination of newborn infants *per os*, we thought that it would surely be possible to use BCG to premunise subjects of all ages who do not react to tuberculin, and who therefore stand some chance of being not yet, or no longer, infected by virulent bacilli.

We therefore applied to the Health Departments of those of our colonies in which tuberculosis is still uncommon, and from which contingents of native troops are recruited to serve in certain home garrisons.

Thanks to the very kind co-operation we have received, it was possible to make an initial experiment, beginning in 1925, on some hundreds of young soldiers in West Africa (Senegal) and Madagascar.

It is common knowledge that the African native, particularly if he lives in a district remote from the coast and has had but little contact with Europeans, is sensitive to tuberculosis. When transported to the European continent, the natives very quickly contract severe forms of tuberculosis, which develop rapidly, closely resembling those observed in infants. They behave like organisms free from all previous immunising infection. The obvious thing to do, therefore, was to try to protect them, not by *ingestion* of BCG — whose efficiency seemed

likely to be too irregular, because the intestine of the adolescent or adult has no longer more than a very doubtful and limited capacity to absorb bacteria — but by *subcutaneous inoculation* of a dose which we set at *one-tenth or one-twentieth of a milligramme*, because this dose is as near as possible to the dose which no longer produces a cold abscess at the site of inoculation.

The experiment has constantly developed, and at the end of 1927 an official circular was issued ordering its extension to as many as possible of the young negro soldiers serving in France.

We cannot yet give the results, but we are already in a position to say that none of the Senegalese or Malagasy (about 200 of each race) who have been vaccinated and distributed among the French garrisons has contracted tuberculosis.

Parallel with this experiment, Weill-Hallé and Turpin in Paris, J. Parisot at Nancy, and Wallgren at Gothenburg (Sweden), were undertaking a series of experiments in the vaccination of children and adolescents of various ages by subcutaneous injections of varying doses of BCG. The results are plainly interesting and encouraging.

The same applies to the large-scale experiments in vaccination of adult subjects which have been reported in recent publications¹ by J. Heimbeck of Oslo, and to those which have been carried on for nearly two years past by Scheel in rural districts of Norway.

We are not yet quite certain of the proper dose of BCG to be injected subcutaneously at different ages. So far we have tried to avoid causing the formation of cold abscesses, and have accordingly injected only a very small dose of bacilli. J. Heimbeck and Scheel use 0.02 or 0.05 milligramme. Weill-Hallé injects 0.05 milligramme even in very young children, and we have advised, for the experiments now projected on Bantu negroes in South Africa, that the dose of 0.05 milligramme should be adhered to, since it scarcely ever causes an abscess and is sufficient to produce tuberculin allergy after from 4 to 8 weeks. It might even be possible to reduce the dose further, down to 0.01 milligramme as J. Parisot and H. Saleur are doing in Nancy.

The most important thing, however, to prevent the formation of abscesses, while at the same time providing adequate pre-munition, is that the emulsions injected should be *thoroughly homogenous, very fine, and should contain no clumps or glass particles.*

The preparation of these emulsions requires particular care, a special equipment (balls of rustless steel and not glass, flat jars made of "Pyrex" and not of glass), a diluent of constant pH (Sauton's synthetic medium of pH = 7.2, in a 1-in-4 dilution with sterile distilled water). Further, it is essential that before

¹ *Annales de l'Institut Pasteur*, 1928, Vol. 42, pages 170 and 956.

injecting BCG the vaccinator should satisfy himself, by *two intracutaneous tuberculin tests* (preferable to the skin test, which is less sensitive and less reliable), that the subject to be vaccinated is not in a state of allergy, otherwise he will certainly develop an abscess, as an expression of *Koch's phenomenon*.

We are indeed satisfied that, even when injected in large doses (up to 10 milligrammes) into tuberculous subjects, BCG is harmless, as witness the numerous experiments of Sörgo at Vienna, those of H. Maendl and O. Lichtwitz at Grimmenstein (Lower Austria), and those carried out by us in collaboration with L. Guinard; but if vaccination were extended to subjects already reacting to tuberculin, that is to say, infected in varying degrees with virulent bacilli, it would be ineffectual. Its failures would then be interpreted in a bad light, and the confidence which doctors and the public may have in its correct use would be lost.

It is for that reason that, before advocating the extension of the method, we prefer to wait until its technique is more fixed, and we do not contemplate that it will be extended except with the assistance of phthisiologists or doctors familiar with the handling of tuberculin; whereas the vaccination of newborn infants *per os* can, as a rule, be satisfactorily carried out without preliminary training — as the wide experience gained in France demonstrates — by medical practitioners, midwives, and visiting nurses from tuberculosis dispensaries.

B.

WORK ON BCG AND VACCINATION OF NEWBORN
INFANTS IN VARIOUS COUNTRIES.

(October 1928.)

The bibliography of BCG is already considerable (more than 1,000 notes or papers). In the following pages we shall merely mention the principal experimental work and, as regards the vaccination of children in the various countries, we shall merely give, by countries, summaries of the notes and observations which have been forwarded to us.

I. ARGENTINE REPUBLIC.

In the Argentine Republic, the introduction of BCG for the preventive vaccination of newborn infants has been undertaken by Dr. Andrés R. Arena, Director of the La Plata Health Services. It has now been regularly used since 1925 on all children born of tuberculous mothers in the model maternity ward of the Tornu Hospital. By the end of August 1928, 251 infants had been vaccinated. Their tuberculosis mortality (meningitis) was barely 1.1% and the general mortality 21.0%. No ill-effects were observed, and the development of the premised infants is absolutely normal.

Other vaccinations, numbering 715, until September 1928, were carried out in tuberculous families by several physicians in the province of Buenos Aires, without any incident being observed.

In addition, animal experiments were performed in a laboratory of the Ministry of Agriculture by Drs. Santiago S. Quiroya, Abel Rottgardt and Raphael Scisso, first on guinea-pigs, then on young pigs. They showed the complete innocuousness of BCG for these animals, and trials on a larger scale are being carried on in order to find out whether the method is practicable for the prevention of tuberculosis in pigs as the preliminary experiments tend to indicate.

II. AUSTRALIA.

Mr. Harold Woodruff, Director of the Melbourne Veterinary School, has carried out vaccination experiments with BCG, a culture of which was supplied to him by the Pasteur Institute, but we have received no recent report on the subject.

III. AUSTRIA.

R. Kraus and Gerlach, of Vienna, have, during the last three years, published some very important papers on BCG experimental work. After numerous experiments, the results

of which had appeared to him at first to be contrary to ours, he satisfied himself that BCG can determine the formation of tuberculous cells in the animal, and that consequently it cannot be said to be free from all *pathogenic* properties; whilst, on the other hand, it is certainly not *nosogenic*, which means to say that it is incapable of causing progressive tuberculous lesions. In his view, the innocuity of BCG is obvious, and, in this respect, his opinion is diametrically opposed to that published by Chiari, E. Nobel and A. Solé of Pirquet's School of Pediatrics. It should, moreover, be recognised that the experiments described by the latter are anything but evidential.

Working with Gerlach, R. Kraus carried out inoculation experiments with virulent tuberculosis, by scarification on the superciliary arch of monkeys vaccinated by BCG and of unvaccinated monkeys, using the technique which R. Kraus had previously devised, jointly with Grosz and Volk, to show the effects of re-infection on animals vaccinated with a benignant primo-infection.

These experiments proved that, whereas tuberculosis becomes generalised in unvaccinated monkeys, it remains localised and not very aggressive in vaccinated monkeys.

R. Kraus has asked the health authorities to allow Calmette's method of tuberculosis prevention to be used under their supervision in trials on children, and in large-scale experiments on cattle.

IV. BELGIUM.

As early as 1924, Professor Malvoz and his assistant, Dr. J. van Beneden, organised a service for the preparation and distribution of BCG at the Microbiological Institute of Liège University, and urged medical practitioners to use it. Their propaganda was so effective that the number of children vaccinated in the provinces of Liège, East Flanders and Luxemburg has rapidly increased.

In 1924, the number of children vaccinated was 38. It rose to 147 in 1925, to 383 in 1926, to 867 in 1927, and during the first seven months of 1928 the figure of 1,200 has already been reached. "Up to the present, there has been no sign of trouble", writes Dr. J. van Beneden, in a letter dated September 1st. "We have a few thoroughly typical histories of deeply infected families, with a record of babies dying one after another from tuberculous meningitis up to the moment that the vaccine was administered... Everywhere we meet with nothing but praise and encouragement."

Dr. Herman, of the Mons Institute of Hygiene, is also preparing BCG for the western provinces of Belgium.

V. BRAZIL.

Preliminary experiments carried out at the Oswaldo Cruz Institute in Rio de Janeiro proved the innocuousness of the BCG culture to guinea-pigs and rabbits, even through intracerebral inoculation in the latter, of the enormous dose of 30 milligrammes. The Director of the Institute, Professor Carlos Chagas, after reaching an understanding with the Vital Brazil Institute in Nictheroy and the Brazilian League against Tuberculosis, decided therefore in April 1927 to increase the use of BCG vaccination, which had been practised on an experimental scale since 1925. Until June 30th, 1928, 656 children, the majority of whom were in contact with tuberculous patients, were vaccinated. Their development was quite satisfactory and no untoward incidents occurred.

On the other hand, from April 1926 to April 1928, A. de Assis, with Professor O. Dupont, of the Graduate School of Agriculture and Veterinary Medicine, vaccinated 45 calves in a herd highly infected with bovine tuberculosis (80 %). These calves remained in strict and continuous co-habitation with tuberculous animals. They were re-vaccinated after one to two years — each time with doses of from 50 to 100 milligrammes of BCG. The vaccinated calves grew normally and at the end of the second year none of them showed the slightest sign of tuberculous infection, though they had been exposed to a mass and continuous contamination and though the controls, when slaughtered, showed without exception characteristic tuberculous lesions.

It was decided to continue the use of BCG for the prophylaxis of tuberculosis, both in infants and bovines.

VI. BULGARIA.

The BCG vaccine has been prepared and distributed by the Sofia Institute of Hygiene (Professor T. Petroff) since October 1926, at the request of the Directorate of Public Health, and after a favourable opinion had been given by the General Medical Council.

In 1927, 352 children were vaccinated, of whom 16 were living in contact with a tuberculous mother, 11 with a tuberculous father, 3 with a tuberculous mother and father, and 18 with tuberculous collateral relatives; 176 others were living in an environment in which tuberculosis was rife. A single child, which was in contact with a consumptive father and mother and which had been vaccinated too late (11th to 15th day), died at three months from catarrhal — probably tuberculous — broncho-pneumonia, but a post-mortem could not be performed.

Of the other children vaccinated, 12 died of non-tuberculous diseases. The general death rate was thus 5.8 per cent and the tuberculosis death rate 0.48 per cent among the vaccinated children. " Convinced as I am of the efficacy of the vaccine ", writes Professor T. Petroff, " I regard it as the most reliable means of preventing tuberculosis in this country, especially at the present time when we are faced with such bad social and economic conditions and a lack of hygienic education ".

VII. CANADA.

The School of Applied Social Hygiene of the University of Montreal (Director, Dr. A. Baudouin) undertakes the preparation of BCG and its distribution and administration by its visiting nurses after consultation with the medical practitioners. From June 23rd, 1926, to June 30th, 1928, 615 children were vaccinated, 113 of these being more than 12 months old by the latter date; 53 are living in contact with germ-carriers. Up to the present, 27 deaths in all have been recorded, all of them due to non-tuberculous diseases. The general death rate is thus 4.3 per cent and the tuberculosis death rate *nil* among the vaccinated children.

A Committee of Tuberculosis Research was set up in the province of Alberta in order to try the method of BCG vaccination on newborn calves. The Committee is presided over by Mr. Bow, Minister of Health. It includes all scientific authorities of the university and province. Its Rapporteur is Professor Allan C. Rankin. The Committee published a preliminary report on its researches begun in 1925. According to this report, not only did BCG prove innocuous and incapable of inducing progressive tuberculous lesions, but its immunising properties were demonstrated particularly through an experiment on 23 calves, of which 16 were vaccinated and 7 used as controls. The vaccinated calves were first fed with pasteurised milk only for two months, then brought into contact with a tuberculous herd and fed with milk containing bacilli. One year later they were slaughtered and none of them showed any trace of tuberculous lesions, although all controls, with one exception, proved severely infected.

The trials have now been largely extended to suit the practical conditions of cattle-raising.

VIII. CHILE.

BCG is prepared for the vaccination of calves by the laboratory of M. Descazeaux and for the vaccination of children by Dr. M. A. Sepulveda at the Husmul Hospital.

From the beginning of 1927 to January 1st, 1928, 600 children were vaccinated, 80 of them in Dr. Monckeberg's clinic and

120 in. that of Dr. O. Salas. The majority are children of tuberculous parents.

" We are in a position to state ", writes Professor Fontecilla, " that the method is absolutely harmless. None of the vaccinated children has shown the slightest pathological reaction or any suspicions of tuberculosis symptoms. "

IX. CUBA.

Dr. Oscar Jaime, Professor at the Faculty of Medicine at Havana and Director of the Anti-Tuberculosis League Dispensary, has started a vigorous propaganda campaign in favour of the use of BCG, which is prepared by the Laboratory of the League and by the National Laboratory of Cuba. The vaccination of newborn infants of tuberculous parents was first performed at the Municipal Hospital, in the last months of 1927.

X. CZECHOSLOVAKIA.

The National Veterinary Institute of Brno has undertaken the work of preparing the BCG vaccine for the prevention of tuberculosis in cattle. One of the members of this Institute, Dr. R. Harnach, has given special attention to the vaccination of poultry with a strain of aviary BCG which the Pasteur Institute supplied to him. In a first note, published in the *Annales de l'Institut Pasteur*, he reported the highly encouraging results which he was able to obtain, although the strain used was not yet sufficiently attenuated (105 passages on bile). He found that chickens, and especially chicks, can be vaccinated with complete success, through the digestive tract, against infection by heavy doses of virulent culture also introduced through the digestive tract.

XI. FRENCH WEST AFRICA AND NORTH AFRICA.

Since 1925, the Dakar Pasteur Institute has introduced the use of BCG as a general practice in the Dakar and St. Louis maternity hospitals. Nearly 2,000 children of the black race and 80 children of the white race, born in these two towns, have been vaccinated without the slightest trouble occurring.

In Algeria, since the end of 1924, Dr. Edmond Sergent and Mlle. Rougebief have been preparing BCG at the Algiers Pasteur Institute and supplying it free of charge to all medical practitioners or midwives who care to use it. By October 1st, 1928, 2,963 newborn infants had been preimmunised. Out of this number, 1,399 had been vaccinated within one to three years and 124 of them were born of tuberculous mothers or had been brought up in an infected environment. Only one of them died

from a presumably tuberculous infection (diarrhoea persisting for a month, with terminal meningeal symptoms).

The general mortality of the vaccinated during the first year was 7.0 %.

In Morocco, the bacteriological laboratory under the direction of Dr. Hornus at Rabat has, for some months past, been in a position to meet all the requests for BCG which are made to it. Dr. Lapin is now engaged in organising distribution centres at Casablanca, Mazagan and Fez, and Mr. Balozet, who is in charge of the laboratory work of the agricultural department, is carrying on interesting researches on the vaccination of young pigs.

At the Tangiers Pasteur Institute, Dr. Remlinger prepares BCG and has done a great deal of experimental work which bears witness to its absolute innocuousness, not only to laboratory animals but also to man. Thus, having had the opportunity of observing a child of 10 years who was suffering from leonine leprosy, and who had come from Iquitos (South America), he attempted — though, it should be said, without any very considerable therapeutic result — to treat it with subcutaneous injections of BCG in gradually increasing doses.

From September 1927 to March 1928, the child in question thus received without any ill-effects an aggregate dose of 2 grammes of BCG, in nineteen injections under the skin of the thigh, the doses per injection ranging from 1 centigramme to 1 ½ centigrammes. Each injection produced a slight thermal reaction with lassitude and fever lasting from 24 to 36 hours, and then gave rise to an abscess which was accompanied by no adenitis and which, after opening of its own accord, quickly healed. It is impossible not to regard this as the plainest of proofs of the complete harmlessness of BCG.

In Tunis, a BCG vaccination service for children has just been started by Dr. Charles Nicolle.

XII. GERMANY.

A large number of papers on experimental work have been published in Germany during the last two years, particularly by Bruno Lange (Robert Koch Institute), Selter (Bonn) and O. Kirchner (Hamburg), Kolle and Gersheimer (Frankfort-on-Main).

Only a few experimental vaccinations of children have been carried out up till now. From 1925 to the end of July 1927, H. Buschmann,¹ at Bleialf, in the Eifel, vaccinated 89 children of tuberculous families without any ill-effects. The children have thriven. None has died. Of these children, 30 were divided

¹ Monatschrift für Kinderheilkunde, 1928, vol. 37, page 393.

into two groups; 15 were removed from the tuberculous environment at birth, and brought back into contact with tuberculous germ-carriers in their families at the end of the first year. The other 15 remained in the tuberculous environment without anything being done to prevent contagion.

The mortality from various diseases was not higher among the vaccinated children than among the unvaccinated. Two cases of bronchial pneumonia among vaccinated children were completely cured.

H. Buschmann infers from this that vaccination is completely harmless. In his view, there is nothing to suggest that the BCG can undergo a reversion to virulence. It proves effective as a protection against natural infection. The method is simple and inexpensive.

From the experimental work which he has carried out alone or jointly with K. Lydtin on guinea-pigs and rabbits, Bruno Lange, of the Robert Koch Institute, has been led to the conclusion that BCG has completely lost its virulence and cannot recover it by passages through animals. The guinea-pig is able to tolerate large doses of culture injected by the parenteral route without any progressive tuberculous process being engendered. Small foci occur in the viscera, as was noted by Kraus, Korschun, Selter and Blumenberg; but the infection does not develop. Nor does it develop after inhalation or ingestion of BCG. The small foci heal and disappear. If a specimen is taken from these in guinea-pigs or rabbits, and inoculated into healthy animals, no recovery of virulence is observed.

B. Lange's research work has also shown him that guinea-pigs inoculated with BCG acquire a much higher degree of sensitiveness to tuberculin than those which have been given dead bacilli, a result which is contrary to those obtained by Bessau.

Using the method of ingestion, B. Lange was unable, even with enormous doses of dead bacilli rising to 1 gramme, to render 18 guinea-pigs sensitive to tuberculin, whereas with BCG 8 animals out of 10 reacted. Ten guinea-pigs which had inhaled large quantities of dead bacilli were not rendered sensitive, whereas, with BCG, 5 animals out of 8 became allergic.

By preventive inoculation it is possible, as pointed out by Calmette, to confer on guinea-pigs a certain power of resistance to virulent infection; but the effects of protective inoculation only last for a certain time. This power of resistance is exerted in a highly effective manner against infection by strains of low virulence, but it is also noticeable in the case of highly virulent strains, whether the infection by such strains is introduced subcutaneously in small doses, or by inhalation through the natural channels.

Generally speaking, B. Lange confirms A. Calmette's results in regard to the innocuousness and immunising properties of his culture. Recently, he attempted to repeat S. A. Petroff's experiments, but was unable to secure the same results.

In his very interesting work, O. Kirchner endeavoured to increase the virulence of BCG by successive passages and re-inoculations on the cornea of the rabbit. He succeeded in carrying out three series of five to six passages, but no more. He comes to the conclusion that BCG really is a bacillus in which the attenuation of virulence has been fixed.

At Frankfort-on-Main, Kolle and Igersheimer have experimented with the inoculation of virulent bovine bacilli into the anterior chamber of the eye of rabbits vaccinated with BCG by the intravenous route. They found that in the vaccinated animals the lesion of the iris remains local, whereas in the unvaccinated it quickly results in the generalisation of the tuberculous process.

XIII. GREAT BRITAIN.

Up to the present, the public health services have taken no decision regarding the preparation and use of BCG. A few vaccinations have been carried out privately in London, Glasgow, Leeds, Manchester, Cardiff and a few other places, with doses of vaccine procured from the Pasteur Institute of Paris, and sent by air to tuberculous families at the request of medical practitioners. None of these families has had cause to regret it, for no ill-effects have been reported to us.

Experimental research work has been undertaken by Stanley Griffith at Cambridge, by S. Lyle Cummins at Cardiff, by R. A. O'Brien, C. C. Okell and H. J. Parish at the Wellcome Physiological Research Laboratories. They all confirm the innocuousness of BCG to experimental animals.

XIV. GREECE.

On April 14th, 1925, the Greek Red Cross started a service for the vaccination of newborn infants against tuberculosis, first in the maternity ward of the Athens Refugee Hospital and then in the dispensaries. This service, under the direction of Mlle. H. Vassilopoulo, had, by the beginning of 1928, premunised 699 infants. The vaccine is prepared by the Athens Pasteur Institute (Director, Dr. Georges Blanc).

Of these infants, 74 remained in contact with tuberculous parents, whilst 625 were living in uninfected surroundings or were not in contact with any known source of infection; 37 were born of tuberculous mothers.

The tuberculosis death rate of these infants was 0.8 per cent. (one single death from a threpsia and broncho-pneumonia possibly tuberculous), while the tuberculous proportional mortality in Athens among children unvaccinated, living in tuberculous contact and supervised by the Red Cross visiting nurses is 12 per cent.

XV. HUNGARY.

Professor Koranyi and Professor Bokay have for some months past been organising the preventive vaccination of infants at the Maternity Clinic of Budapest University. The vaccine is prepared by Professor Hugo Preisz.

XVI. INDO-CHINA.

From 1925 to January 1st, 1928, the Saigon Pasteur Institute reported that 48,000 children had been vaccinated by the Saigon and Cholon maternity services, 17,000 being dealt with in 1927. At Hanoi, where BCG is also prepared by the local Pasteur Institute, 6,500 infants were vaccinated in 1927.

Many of these infants are supervised by the dispensaries attached to the maternity clinics. No ill-effects have been observed. Vaccination is readily accepted and even desired by the native families. The medical profession and the public regard it as entirely harmless.

XVII. ITALY.

Extensive experiments with BCG on a large number of animals (cattle and goats), and subsequently on children, were undertaken at the end of 1925 by Professor Alberto Ascoli at Milan and in the whole of Northern Italy; and, somewhat later (beginning of 1926), at Bologna by Professor Ottolenghi.

In July 1928 Professor Ascoli published statistics¹ relating to 222 infants vaccinated in Lombardy and Venetia and in Tuscany, 22 of them between the ages of 2 and 3 years; 122 had tuberculous mothers, 50 tuberculous fathers, and 20 tuberculous mothers and fathers.

Among these vaccinated children the *general death rate* was 9.9 per cent, the *tuberculosis death rate* 0.4 per cent (a single death, probably from tuberculosis, but not verified).

XVIII. JAPAN.

BCG is being studied at Professor Sata's laboratory and at the tuberculosis clinic of the Osaka School of Medicine by Dr. Satake, Dr. A. Imamura and Dr. M. Takahashi, who have already published a summary of their first research work.²

¹ *La vaccinazione antitubercolare con bacilli vivi negli animali e nell' uomo.* Milano, 1928.

² *Annales de l'Institut Pasteur*, December, 1927, pages 1330-1334.

Their experiments showed that a dose of 0.01 milligramme of BCG is sufficient to render guinea-pigs sensitive to tuberculin and to produce local (cutaneous) immunity.

Doses below 0.01 milligramme are ineffectual.

They also showed that, by a suitable dose of BCG, guinea-pigs can be immunised against intestinal infection by the human type of virulent tuberculosis bacillus; and the immunity is evident, even though the intradermal reaction should be negative in these animals.

On June 18th, 1928, Professor Sata informed us that he had completely satisfied himself of the absolute harmlessness of BCG for laboratory animals.

XIX. LATVIA.

Professor Kirchenstein, of Riga, has been preparing BCG since February 1925. By December 31st, 1926, 28 infants, whose ages now range from 1 year and 8 months to 3½ years, had been vaccinated; 3 died of non-tuberculous diseases. The general death rate of these vaccinated children is 11.5 per cent. Their tuberculous death rate is *nil*.

XX. MAURITIUS.

In Mauritius there is a laboratory under the direction of two ex-students of the Pasteur Institute, Dr. Barbeau and Dr. Maya, who have been devoting their efforts since 1925 to spreading the practice of BCG vaccination. On December 31st, 1927, 2,400 newborn infants had been premunised, that is:

In 1925 (four months)	62
In 1926	792
In 1927	1,546

At the time of drafting the last report (March 31st, 1928), information had been obtained on 787 infants vaccinated by 27 medical practitioners; 32 had died of non-tuberculous diseases. Their *general death rate* was only 4 per cent, whereas the general death rate in the Island among non-vaccinated children of the same age was 11.9 per cent during the period September 1st, 1925, to March 31st, 1927.

Of the vaccinated children, 28 remained in contact with their germ-carrying parents. All of these developed normally in spite of very unsatisfactory conditions of hygiene and housing.

From July 16th, 1926, to December 31st, 1927, 419 calves were vaccinated by M. Lionnet, veterinary surgeon attached to the Agricultural Service in the Island. These animals were in no way affected in their growth.

XXI. NETHERLANDS.

Dr. Heijnsius van den Berg, President of the Amsterdam Anti-Tuberculosis Society, had, from January 1926 to the end of 1927, personally vaccinated 80 newborn infants, all of whom were living in tuberculous surroundings and all of whom developed normally. These children proved resistant to the common childhood ailments — measles, whooping-cough, etc. Only one death from a presumably tuberculous disease occurred in a child of 18 months. Another of the vaccinated children died of hereditary syphilis.

Fifty children living under similar conditions were used as controls. At the end of 18 months, 12 had contracted tuberculosis (24 per cent) and 9 (18 per cent) had died. In addition to this, one of the controls had died of pneumonia. As all these children died in hospital, a post-mortem was performed in every case.

Dr. Heijnsius van den Berg reports a particularly interesting observation of a vaccinated child, 1 year and 8 months old, whose mother was seriously ill with pulmonary tuberculosis; this child remained perfectly healthy, whereas a small cousin, 1 year and 10 months old, who had not been vaccinated, contracted tuberculosis from the same source of infection. It may be assumed that the small cousin could not have been infected from any other source; she regularly visited her sick aunt and often shared her bed.

Professors Aldershoff (of Utrecht) and van Loghem (of Amsterdam) have experimented separately with BCG. They came to the conclusion that BCG is harmless, that it does not produce re-inoculable tuberculous lesions in animals, and that its virulence was not modified even after a series of 21 cultures on potatoes in contact with unheated glycerinated horse serum.

XXII. NORWAY.

Highly important experiments were started at the beginning of 1926 by J. Heimbeck and O. Scheel with the object of applying the method of BCG vaccination to adolescents or adults who did not react to tuberculin and were hence presumably not yet infected by the tuberculosis bacillus. These experiments are being carried on, first at the Ullevål Hospital Nursing School, Oslo, on 89 nurses, and, secondly, with the inhabitants of Oslo and three rural districts. At the moment, they cover 1,393 persons of various ages who received doses ranging from 0.05 milligrammes to 0.01 milligrammes of BCG. Dr. Heimbeck has already published two papers on these experiments.¹ He also

¹ *Annales de l'Institut Pasteur*, 1928, February, page 170; and August, page 956.

reported on his own vaccinations and on those carried out by Dr. Scheel in document C.H./BCG/6. The results are highly encouraging and agree with those obtained by B. Weill-Hallé in Paris, J. Parisot at Nancy and Wallgren at Gothenburg.

XXIII. POLAND.

Since February 1926, the Directorate-General of Health and the Union of Polish Anti-Tuberculosis Organisations have been using the method of vaccination with BCG; the vaccine is prepared at the State Hygienic and Serotherapeutic Institute. A special vaccination committee is presided over by Professor Michalowicz, director of a clinic for children's diseases at Warsaw.

Previous to June 1st, 1928, 349 children had been vaccinated at Warsaw, 601 at Poznan. The vaccinated children, several of whom are over two years, all develop normally and show so far no sign of tuberculous infection. Beside Warsaw and Poznan, Wilno, Czestochowa, Lodz, Suprasl and Pabjanice have organised BCG vaccination services.

Highly interesting research work has been carried out at Poznan by Dr. and Mme. Zeyland, with the object of detecting BCG elements in the organs of children vaccinated at birth *per os* who have died of various diseases. These scientists have been able to perform more than 34 post-mortems, and, except for one infant which was not isolated after vaccination and which lived in tuberculous surroundings, none showed any tubercular lesions.

In three cases it was possible to recover BCG cultures, one of these cases being that of a child which died of erysipelas at the age of 3 months. Guinea-pigs inoculated with these cultures remained unaffected; which proves that the BCG had not increased in virulence by remaining in the body of the children; which also proves that BCG is absorbed through the intestinal mucosa.

A paper by the same authors¹ showed that BCG is harmless, when used under the conditions prescribed for anti-tuberculosis vaccination, and that necrotic lesions produced in animals by the inoculation of heavy doses of culture are due to the fact of the bacilli being then concentrated in clumps in the tissues. These lesions, which are not inoculable in series, are identical with those produced by injections of BCG or virulent bacilli killed by heat.

XXIV. ROUMANIA.

Experiments in the vaccination of newborn infants have been carried out on a large scale in Roumania since the end of 1926, under the direction of Professor J. Cantacuzène. At the

¹ *Annales de l'Institut Pasteur*, June 1928, page 652; and December 1928 (supplement).

moment, there are two centres for the preparation of the vaccine, one at the Bucharest Institute of Serotherapy, the other at the Jassy Institute of Hygiene, the latter under the direction of Professor Ciuca.

By the end of March 1928, two years after the first trials, the number of children vaccinated was 9,274, 6,083 of whom were in Bucharest, representing 64.9 per cent of births; 413 were over a year old.

At Craiova, 695 out of 970 infants born were vaccinated, the remaining 275 being left as unvaccinated controls. The general death rate was 26.4 per cent among the unvaccinated and 6.4 per cent among the vaccinated.

At Jassy, the general death rate has during the last three years fluctuated between 14.95 and 15.30 per cent among unvaccinated children below 1 year of age. In 1927, it was only 5 per cent among the vaccinated children.

The tuberculosis death rate was practically nil — 1 death at Bucharest out of 5,767 vaccinated children and another at Craiova out of 695 vaccinated children. These two children belonged to tuberculous families.

By way of conclusion to his report, which was submitted in April 1928 to the Office international d'Hygiène publique, Professor Cantacuzène writes: "The efficacy of anti-tuberculosis vaccination by BCG seems plain, and it is entirely harmless."

Dr. Nasta and Dr. Catzap have made experimental attempts to increase the virulence of BCG by injecting it subcutaneously in doses of 10 milligrammes to guinea-pigs and subsequently treating the animals by repeated injections of tuberculin. All of these gave a definite reaction to this substance, no sign of activation of the infection being noticeable in the guinea-pigs. So far as they were concerned, the BCG remained harmless.

XXV. RUSSIA (UNION OF SOCIALIST SOVIET REPUBLICS).

Numerous important experiments have been carried out at the suggestion of the late Professor Tarassewitch in several laboratories and hygiene organisations of Russia. Prominent among them is the Ukrainian Commission, set up at Kharkoff for the study of BCG, which has published a whole series of papers on the experiments which have been carried out uninterruptedly for the last three years, both by the vaccination of young cattle and by that of newborn infants. The first three reports of this Commission appeared in the *Annales de l'Institut Pasteur* in 1927 and 1928. (See also document C.H., BCG., 3).

The members of the Ukrainian Commission shared out the work among themselves, and have studied the question of BCG in all its aspects, from the experimental, clinical and pathological point of view.

The experimental reports are drawn up by Professor Tzekhnovitzer, who confirms the complete absence of pathogenicity in BCG and the permanence of the characteristics of this strain. All the attempts to render BCG virulent failed. With that object in view, all sorts of laboratory devices were tried: inoculation of animals weakened by avitaminosis, or poisoned, either by repeated doses of tuberculin or by toxins such as diphtheria toxin, or infected with pathogenic organisms such as streptococcus.

It was also found that the BCG excreted during the lactational period through the mammary glands of animals which had been vaccinated when young was never virulent. Passages of culture through the testicles of guinea-pigs did not increase its virulence, a result which is contrary to that reported by S. A. Petroff, of Saranac Lake (N. Y.), and it was not possible to isolate virulent and non-virulent colonies from the culture.

Dr. Iakhnis, who was especially entrusted with the testing of BCG through vaccination of children living in tuberculous surroundings, has, from September 1925 to August 1st, 1928, vaccinated 695 children, whom he has been able to keep under regular observation. Of these, 290 are now between 2 and 3 years old and 202 between 1 year and 2 years old.

The *general death rate* among the vaccinated children up to the age of 1 year was 5.2 per cent, and 2.2 per cent between the ages of 1 and 2. That of the *unvaccinated* controls who were living, like the vaccinated children, in infected surroundings was 8.3 per cent up to the age of 1 year and 4 per cent between the ages of 1 and 2.

The *tuberculosis mortality* among vaccinated children living in infected families was 2.5 per cent up to the age of 1 year and *nil* between the ages of 1 and 2. Among the unvaccinated children living under the same conditions it was 16.9 per cent up to the age of 1 year and 9.8 per cent between the ages of 1 and 2.

The observation of children premunised with BCG and kept away from any contact with tuberculous persons confirms the absolute harmlessness of BCG.

In three years, the Ukrainian Commission has experimented on a large number of animals: 700 guinea-pigs, 300 rabbits, 50 head of cattle, 5 goats, 2 foals and 6 monkeys. It has also carried out clinical and anatomical-pathological observations on 800 newborn infants, and was able to perform 50 post-mortems on children who had been vaccinated and had died of various diseases, carefully examining their organs and inoculating from them in case of doubt. Such inoculations never produced tuberculosis in experimental animals.

Other researches have been carried out in various laboratories at Moscow, Kiev and Leningrad. In general, they have led to the same conclusions.

XXVI. KINGDOM OF THE SERBS, CROATS AND SLOVENES.

Two centres for the preparation and distribution of BCG vaccine are now in existence, one at Belgrade (Institute of Hygiene, Professor Rancovitz) and one at Dubrovnik (Dr. Igor N. Asherhov). During 1927, and until September 1928, 321 infants had been vaccinated either in the maternity wards of the National Hospital or at the obstetrical clinic in Belgrade. Some other vaccinations were done in the Department of Kragujevaç. All the children vaccinated were living in infected surroundings. After a year the mortality from tuberculosis was nil. In a Belgrade orphanage, Professor Radoslavievitch vaccinated subcutaneously 40 children between the ages of 6 to 17 with doses of BCG ranging from 0.25 mgr. to 0.1 mgr. Nearly all of them had a local abscess without complications. Their state of health remained excellent.

XXVII. SPAIN.

BCG is prepared at Barcelona in the Municipal Laboratory, at Madrid in the Alfonso XIII Institute, and since 1926 in the Llorente Institute (Dr. Megias).

In January 1928, we received, from Madrid, a report of the Director of the Llorente Institute giving an account of the vaccinations performed by a number of clinicians, pediatricians and accoucheurs. In the foundlings asylum, La Inclusa, in Madrid, 358 newborn infants were vaccinated, 880 other infants being left unvaccinated for purposes of comparison. By the end of the first year, the tuberculosis death rate among the vaccinated infants was 1.1 per cent. Among the unvaccinated infants it amounted to 3.8 % per cent, all cases being verified by post-mortem.

At the Alfonso XIII Institute, Dr. Partearroyo undertook experimental work to see whether he could succeed in increasing the virulence of BCG in laboratory animals. He did not succeed.

Professor Sayé has been following up the cases of the 277 children vaccinated during the last four years at Barcelona, together with 285 unvaccinated infants living in similar tuberculous surroundings. The results of his observations are published in document C.H./BCG./12.

XXVIII. SWEDEN.

Several laboratories have undertaken the preparation of BCG: at Stockholm, that of Professor Jundell's paediatric service; at Gothenburg, that of Professor Wallgren's paediatric service; at Upsala, that of Dr. Carl Naeslund.

As far back as 1925, Dr. G. Krikork had carried out BCG vaccinations on children born of tuberculous women who were under treatment at the Falkenberg sanatorium. In a letter

dated March 14th, 1928, he wrote to say that he had by then vaccinated 16 children, and added: "none has died, and it seems that those who contend that, other conditions being equal, the vaccinated children develop better than the others are right".

Dr. Carl Naeslund, of Upsala, informed us that in agreement with the National Swedish Antituberculosis League, an extensive trial of BCG had begun in August 1927 in the Province of Norrbotten, in which tuberculosis is particularly rife. In September 1928, 960 infants had already been vaccinated without ill-effect and with satisfactory results. Until now none died from tuberculosis.

At Stockholm, Professors Jundell and Forssner vaccinated, to begin with (up to November 15th, 1927), 120 calves, which were then placed in infected cowsheds, together with an equal number of control calves. These experiments are being continued and have now been extended to a larger number of animals. At the same time, the vaccination of children of tuberculous mothers is being proceeded with in the paediatric clinics.

From Falun, Dr. Malström wrote as follows, on December 23rd, 1927: "I have enquired into the death rate in the Province of Carelia among children who have been born and reared in tuberculous surroundings, have not been vaccinated, but are under the supervision of our dispensaries. Of 156 of such children, 18, or 11.5 per cent, died before the end of the first year, 7 of them, or 4.5 per cent, of diseases definitely ascertained to be tuberculous. Between the ages of 1 and 2, the total death rate is 6.3 per cent, 5.5 per cent representing deaths from tuberculous diseases. Between August 1925 and December 1927, I vaccinated 25 children all of them living in contact with tuberculous persons. None has died of tuberculosis; 1 only died of broncho-pneumonia; 20 remained throughout in tuberculous surroundings."

At Gothenburg, Professor Arvid Wallgren has, since the beginning of 1927, been carrying out trial vaccinations in his paediatric clinic by subcutaneous injections into children of various ages who have been reared in tuberculous surroundings but do not yet react to tuberculin. After vaccination, he keeps them in his hospital, away from virulent infection, until the tuberculin allergy has become established, i.e., for a period of from 3 to 8 weeks, and then sends them back into the tuberculous environment. All these children, 33 in number (October 1928), show no trace of tuberculosis and none has died. The experiments are being continued.

XXIX. SWITZERLAND.

BCG is prepared at Zurich by Professor Silberschmidt and at Berne by Professor Sobernheim. The number of newborn infants vaccinated up to now in the various cantons is little more than 100.

Experiments on animals have confirmed the fact that the culture is not virulent, notwithstanding the contrary opinion advanced by Professor Galli Valerio, of Lausanne, which is based on the observation of two guinea-pigs which he inoculated with BCG. He states that one died after 16 days, the other after 70 days, with definite tuberculous lesions. The tuberculous products obtained from these two guinea-pigs, when inoculated into two others, also killed them, without any trace of bacilli being found in their organs. Professor Galli Valerio ascribes the death of the last two guinea-pigs to poisoning by the products of bacilli which had undergone lysis.

In a note published by the *Revue médicale de la Suisse romande* (XLVII, 1927, December 25th, page 1011), R. Girod and C. Debarge gave an account of their observation and post-mortem examination of a premature infant which was vaccinated with BCG and died from gastro-enteritis when 25 days old. Its mother was suffering from definitely diagnosed tuberculosis. In the marrow of one of the vertebræ of this child, a few acid-resisting bacilli were found, but there was no tuberculous lesion either in the marrow of the vertebræ or in any other organ. This corroborates the opinion of Professor Askanazy, of Geneva, who considers that the bone marrow acts as a blood-filter and it shows that BCG absorbed *per os* penetrated through the mucosa of the intestine and spread in the lymphatic organs.

XXX. UNITED STATES OF AMERICA.

During the last eighteen months, experiments have been carried out on a limited scale and have been followed with the greatest care by William H. Park, Director of the New York City Public Health Department Laboratories. In a letter dated August 24th, 1928, he gives us the following information.

There are now 118 vaccinated children and 289 who are kept as unvaccinated controls. The general death rate among the controls is 14 per cent, the tuberculosis death rate being 7.2 per cent.

Among the vaccinated infants, 16 are more than 12 months old; 17 are living in families in which there are cases of open tuberculosis; and 45 are in contact with cases of closed tuberculosis.

Of the vaccinated children, 7 died of non-tuberculous diseases, 1 of broncho-pneumonia when a fortnight old, 1 at 4 months from causes which could not be detected by post-mortem, 2 at 5 months from enteritis, 1 of malformation of the heart when a fortnight old, 1 of otitis at 3 months, one of congenital debility when a fortnight old (premature).

It was possible to perform a post-mortem on 4 of these children, and in no case was any trace of tuberculosis found.

One vaccinated child only died at the age of 4 months of military tuberculosis. The mother had died — also of military tuberculosis — immediately after confinement, and the child had not been in contact with her. This was obviously a case of hereditary tuberculosis. The *tuberculosis death rate* among the *vaccinated children*, counting in the above-mentioned case, is 0.8 per cent and their *general death rate* 6.7 per cent.

Other vaccinations were carried out in New York at the Post-graduate Medical School and Hospital by Dr. Adelaide B. Bayliss. Unfortunately, the reports of these, which were very favourable, have been destroyed by a fire.

The experimental research work undertaken by Professor Maximow¹ at the University of Chicago is particularly interesting. The object has been to study how cultures in the lymphatic tissue of the rabbit behave towards BCG and towards virulent bovine bacilli. It has shown that, whereas the virulent bacilli have a toxic effect on the phagocytes, even at a certain distance, BCG is entirely innocuous to them, even to the lymphoid elements, which are the most sensitive.

XXXI. URUGUAY.

In November 1927, at Montevideo, a model anti-tuberculosis dispensary, founded and directed by Dr. José Martirené, Head of the Public Welfare Department, was solemnly inaugurated. This dispensary comprises a laboratory which undertakes the preparation of BCG and its distribution throughout the country.

In 1925, Dr. Moreau introduced the method of vaccination into Uruguay, since when large numbers of children have been preimmunised.

The supervision of the children vaccinated is entrusted to carefully chosen persons and to visiting nurses.

Up to October 1928, 2,690 infants were vaccinated, 1,524 of that number in Montevideo; 39 died; autopsies were performed on 27 cases. None showed tuberculous or suspicious lesions and no death was ascribable to tuberculous diseases.

All the survivors are in good health; their size and weight are normal. None show any sign of tuberculous infection. No opposition to vaccination was encountered from the families. The children vaccinated in contact with their tuberculous parents (61, of whom 12 were born from tuberculous mothers) are in perfect health. Two died from influenzal bronchitis.

The vaccinated children born of healthy parents have developed normally; none have shown any symptom of tuberculosis.

¹ *Annales de l'Institut Pasteur*, 1928, March, page 225.

None of the guinea-pigs inoculated in a bacteriological control of BCG showed any sign of tuberculosis.

* * *

Such is the information which the Pasteur Institute has been able to collect up to September 1st, 1928, on the use of BCG, either in experiments or for the prevention of human tuberculosis, in various countries other than France.

III.

NOTE ON THE INNOCUITY OF THE BCG VACCINE TO CATTLE

By C. GUÉRIN, (*Pasteur Institute, Paris*)

(September 1928)

We may recall that the experimental use of the BCG vaccine on cattle involves one inoculation, under the skin of the dewlap, of 50 milligrammes of bacilli in a very fine suspension, in a 1-in-4 dilution of 10 c.c. of Sauton's medium, this operation being carried out during the first 15 days of life.

The inoculation is followed within 48 hours by the appearance of a soft local oedema, which rapidly densifies and after some 10 days presents the appearance of a nodule as large as a pigeon's or hen's egg, hard, painless and persisting for a period varying from three months to a year or more. This nodule, which is accompanied by a positive reaction to tuberculin from the 20th day after vaccination, has little tendency to form an abscess; in about 1 per cent of cases, however, it becomes the seat of a cold abscess, which may open spontaneously, but as a general rule forms an obscurely fluctuating tumour, which may attain the volume of a large orange and contains a white, creamy, thick pus. A puncture with the thermocautery releases this pus, and, in either case, this local lesion, which has no effect on the animal's general health, cures itself without treatment.

As early as September 1924, we asked veterinary surgeons practising in agricultural districts to make experiments in their rural practices in order to ascertain how cattle react to the subcutaneous inoculation of BCG at first vaccinations and at successive re-vaccinations.

More than 150 practitioners responded to our appeal. In June 1928 we sent to 97 of them, who had made vaccination experiments in 1924, 1925 and 1926, the questionnaire reproduced below.

Up to September 1st, 1928, we had received 49 replies. The total number of beasts (vaccinated and re-vaccinated) experimented upon and observed by our correspondents was 2,315 head.

PASTEUR INSTITUTE, PARIS

**QUESTIONNAIRE CONCERNING EXPERIMENTS ON THE
VACCINATION OF CATTLE AGAINST TUBERCULOSIS BY BCG.**

M....., Veterinary Surgeon at

- Date of beginning of experiments : Month.....Year.....
Was vaccination carried out on farms infected with tuberculosis ?
.....
Were calves born in infected byres effectually isolated immediately
after birth ?
Were they immediately fed with boiled milk or milk from non-
tuberculous cows ?
Were the calves vaccinated within the first 15 days of life ?.....
Have you observed any accident consequent upon vaccination ?
.....
Has the growth of the vaccinated animals been normal ?.....
Give the total number of animals vaccinated and re-vaccinated
on the farms on which you worked.....
Has annual re-vaccination been regularly carried out ?.....
Give the number of vaccinated animals which died from causes other
than tuberculosis, or were slaughtered owing to accidental causes.....
Were tuberculosis lesions revealed by the post-mortem, if any ?
.....
Give the number of vaccinated animals slaughtered for butcher's
meat..... At what average age were they slaughtered ?
.....
Were cases of tuberculosis found among those animals on veterinary
inspection ?
If so, what was the usual form of the lesions : glandular or visceral ?
.....
Any personal observations as to the innocuity of vaccination and
the changes in health conditions on infected farms since vaccination
has been introduced :

All these beasts were born in byres infected with tuberculosis, and were kept there after birth. Consequently, vaccination always took place in infected surroundings. Indeed, at the beginning of these experiments, we did not feel that it was absolutely necessary to subject calves born in infected byres to the initial prophylactic measures which subsequent experiments have shown to be indispensable, namely, *the effective and immediate isolation of calves born in infected surroundings, and their feeding with bacteria-free milk during the month following, i.e., until the protection conferred by vaccination may be regarded as established.* The only point in which this neglect might be important was the estimate of the efficacy of vaccination — a point with which we did not propose to deal.

To the questions: "Have you observed any accident consequent upon vaccination, and has the growth of the vaccinated animals been normal?", the 49 practitioners are unanimous in their reply.

In their replies, variously worded ("*No accident*", "*Absolute innocuousness*", "*Nothing abnormal*", "*Perfectly healthy*", "*Quite harmless*", "*No danger whatever*"), they all state that the subcutaneous inoculation of BCG, in this dose of more than 2,000 million living bacilli, has never set up a pathogenic lymphatic and glandular infection, the external symptoms of which could not have failed to attract their attention.

It might, however, be objected that, although the subcutaneous inoculation of BCG may produce no progressive lesions taking rise at the site of injection, it may be setting up a general infection of the lymphatic system, ultimately giving rise to visceral lesions which the practitioner could not detect. Our reply to this consists in the negative post-mortem examination of 30 head of cattle aged 3 and 4 years, vaccinated and re-vaccinated each year with a dose of 2,000 million bacilli on each occasion, in whom no tuberculous lesion has been found.¹

This disposes of the hypothesis of the possible recovery, after a long interval, of some degree of virulence by BCG in the living organism. It must not be forgotten that BCG is a bacillus of *bovine* origin; and if it is incapable of recovering its original virulence in its original environment — cattle — *a fortiori* it will be still less capable of doing so in man, a species for which the bovine bacillus is already highly differentiated.

* * *

¹ *Annales de l'Institut Pasteur*, March 1927. . . .

We give below a list of the 49 veterinary surgeons who have replied to our questionnaire.

They have vaccinated and re-vaccinated a total of 2,315 head of cattle. Of this number, 103 died from causes other than tuberculosis, most dying in the first few months of life from early gastro-intestinal disorders; and 121, having outlived their economic usefulness, were slaughtered. Among these latter, post-mortem examinations revealed in 8 animals undoubted tuberculous lesions. At the beginning of this note, we stated that all the animals vaccinated with BCG were born in infected byres, and were kept there during the early weeks of life, while neither of the regular prophylactic measures (isolation and use of a bacillus-free food for a month) was taken.

These 8 head of cattle, belonging to different farms, were as follows :

1. A heifer not vaccinated until the age of 1 year, *i.e.*, not in the conditions prescribed by us (small lesions of a mesenteric gland).

2. A heifer slaughtered at the age of 5 months (tuberculosis of the retro-pharyngeal glands); a note on this case was published in the *Recueil d'Alfort* (May 1927). The dam of this animal, suffering from *tuberculous mammitis*, suckled her calf from birth.

3. A bullock, 1 year old (slaughtered); generalised tuberculosis. It had been vaccinated at the age of 4 weeks — too late, in view of the thoroughly infected nature of his surroundings.

4. A heifer, 1 year old (small lesion in a bronchial gland).

5. A heifer, 15 months old (tuberculosis of the retro-pharyngeal glands).

6. A bullock, 6 months old (tuberculosis of the retro-pharyngeal glands).

7. A bull, 2 years old (lungs confiscated owing to tuberculosis).

8. A heifer, 1 year old (generalised tuberculosis); this animal had been bought when a fortnight old, and came from an unknown farm. It had been vaccinated after the end of the prescribed period, *i.e.*, too late.

VETERINARY SURGEONS	LOCALITY	ANIMALS VACCINATED AND REVACCINATED	REMARKS
Fénelon	La Ferté-s.-Jouarre	149	No accident
Viaut	Fécamp	18	No accident
Derré	Reugny	7	No accident
Laroche	Pionsat	12	Absolute innocuousness
Vidal	Toulouse	12	Absolutely no danger
Hautceur	Cambrai	180	Absolute innocuousness
Sonnet & Lancelot	Eu	1	No accident
Malou	Argenton-s.-Creuse	137	Perfect innocuousness
Ruchon	Bantignies	10	Nothing abnormal
Rombert	Meun	6	Perfectly healthy
Magallon	Grenoble	9	No accident
Delabraze	Neuville-aux-Bois	22	Perfectly healthy
Cayasse	Pont-Faverger	33	Nothing abnormal
Brugge	Lannoy	65	Quite harmless
Didelin	Oloron	35	No accident
Leblond	Aubigny-au-Bac	10	Nothing abnormal
Charbonnel	Challans	8	Perfectly healthy
Brinet	Magny-en-Vexin	300	Absolute innocuousness
Dumolin	Dunkirk	13	Nothing abnormal
Paillère	Langeac	6	No accident
Ducoux	Bourges	113	Innocuousness unquestionable
Montois	Tourcoing	4	Growth normal
Bax	Domart-en-Ponthieu	44	All in perfect health
Duez	Maing	20	No accident!
Rivière	Fives	60	No danger whatever
Fichelle	Canteleu-Lille	10	Nothing abnormal
Salomé	Douai	12	Growth normal
Arkwright	Villecresnes	3	Splendid animals
Quinot	Brienon	20	No accident
Sigbert Jerlow	Falkenberg	138	Innocuousness certain
Durocher	Voulx	12	No accident
Labeyrie	St.-Jean-de-Luz	8	Growth normal
Jouanne	Laon	70	No accident
Valen	Orgères-en-Beauce	14	No accident
Granmont	Estissac	81	Perfectly healthy
Rousseau	Le Quesnoy	248	Nothing abnormal
Dischamps	Billom	29	Vaccination harmless
Roger & Ricaud	Gournay	45	Absolute innocuousness
Mondine	Outarville	15	Growth perfect
Dauvois	Grigneville	45	No accident
Lefebvre	Wasquehal	34	Innocuousness certain
Desliens	Châtillon-s.-Seine	45	Innocuousness certain
Poutrain	Valenciennes	50	Never any accident
Sabourin	Podensac	3	Nothing abnormal
Nicodème	Vervins	24	Absolute innocuity
Duvaux	Neufchâteau	25	No danger whatever
Richard & Bois-sière	Gruville	110	Absolute innocuousness
	Total...	2,315	

VETERINARY SURGEONS	DEATHS OTHER THAN FROM TUBERCULOSIS	SENT FOR SLAUGHTER	FOUND TUBERCULOUS	REMARKS
Fénelon Derré	8	24 1	0 1	Heifer, not vaccinated till 1 year old. Small lesion in a mesenteric gland.
Hautœur	5	1	1	Heifer, slaughtered at 5 months. Tuberculosis of the retro-pharyngeal glands, discussed in a note in the <i>Recueil d'Alfort</i> (May 1927).
Malou Brugge Brinet	2 1 10	1 35	0 1	Bullock, 1 year old. Generalised tuberculosis. Vaccinated when 4 weeks old.
Dumolin Pallère Ducloux Bax Rivière	2 1 2 3	1 3 1	0 0 1	Heifer, 1 year old. Small lesion in a bronchial gland.
Fichelle Salomé Quinot	4 1 2	1 1	0 1	Tuberculosis of the retro pharyngeal glands (15 months).
Jerlow	8	2	1	Tuberculosis of the retro-pharyngeal glands (6 months).
Lubeyrie Valen Rousseau	1 6 17	3 6 10	0 0 1	Pulmonary tuberculosis. Only the lungs were confiscated (bull, aged 2 years).
Dischamps Roger & Ricard Dauvois Lefebvre	4 3 1 3	1	1	Generalised tuberculosis. The animal was bought when a fortnight old from an unknown farm, and was vaccinated too late (slaughtered when 1 year old).
Desliens Richart & Bois- sière	3 22	30	0	
	103	121	8	

INSTRUCTIONS

For Experiments on Anti-tuberculosis Vaccination of Cattle by means of BCG.

I. Principle of Vaccination. — The vaccine consists of living tuberculosis bacilli of bovine origin, rendered completely harmless to animals of every species, including man (BCG Vaccine). When inoculated under the skin of a horned beast free from tuberculosis, it causes the formation of a small lesion as large as a pigeon's or hen's egg, painless, and with no tendency to pus formation. The animal thus vaccinated reacts to the tuberculin test for several months; the positive reaction, together with the vaccinal lesion disappears generally between the tenth and the fifteenth month after vaccination. Throughout this period, the animal remains premunised against tuberculous infections.

II. Animals for which it is intended. — The vaccination is intended for:

(1) Calves born on farms infected with tuberculosis, infection for them being rapid and inevitable;

(2) Calves which, although born on farms free of tuberculosis, may be exposed to occasional contaminations.

In either case, all calves born on the farm, whatever is to be their subsequent history, should be vaccinated as soon as possible after birth, and in no case more than a fortnight later. On infected farms, it is further necessary to withdraw these calves, immediately after birth and during the succeeding month, from any possible tuberculous contamination in their environment or diet (isolation, boiled milk, milk from non-tuberculous cows).

III. Form and Method of Despatch of the Vaccine. — The vaccine is sent in separate doses, each sufficient for one animal. It is put up in ampoules sealed over the flame, each containing 10 c.c. of emulsion. Before uncorking for use, the ampoules should be thoroughly shaken up. — The vaccine should be used as soon as possible after delivery, and in any case within ten days.

IV. Method of Inoculation. — The 10 c.c. syringe is first boiled and fitted with a sufficiently long needle, and then filled by direct suction from the tube containing the vaccine. The needle should be pushed

deep into the conjunctive tissue of the dewlap, towards the uppermost third of it. The vaccinated animals require no special after-care, but they should not be subjected to tuberculin tests, even the intracutaneous.

V. Re-vaccination. — As in the case of other active vaccinations (anthrax, swine erysipelas), re-vaccination should be carried out every year, in accordance with the above instructions.

The vaccine for cattle tuberculosis is issued free to veterinary surgeons only, who are responsible for its use.

Application for these vaccines should be made to LABPASTEUR,
35, Rue Dutot, Paris (XV^e).

The vaccines will be despatched on TUESDAYS and FRIDAYS.

IV.

CRITICAL STUDY OF THE LITERATURE ON BCG AND VACCINATION AGAINST TUBERCULOSIS THROUGH CALMETTE'S METHOD

By Y. BIRAUD, M. Sc., M. D., C. P. H.,

Member of the Health Section of the League of Nations Secretariat

INTRODUCTORY REMARKS.

The rapid growth of an abundant literature in all languages induced by the work of Calmette and his co-workers on vaccination against tuberculosis by means of BCG makes it difficult for a review of this literature to be absolutely complete. With a few exceptions, the present study is confined to the articles published before August 1928.

We had also to limit ourselves to the articles relating original experiments or theories on the problem of BCG and its application, thus excluding the articles of mere didactic value, whatever their merits might be.

On the other hand, the alphabetical list of authors which accompanies the present study includes references, not only to articles bearing directly on BCG, but also to facts and theories connected with it: the problems of immunity to tuberculosis, tuberculin allergy and mortality of infants in tuberculous environment.

We do not pretend to describe fully the researches of Calmette, which are summarised in the December (1928) number of the *Annales de l'Institut Pasteur* and also appeared in book form. We shall therefore only sketch them briefly and describe chiefly the researches carried out along the same lines by scientists other than Calmette; their results, and their apparent or real discrepancies, the objections of fact, method or theory brought against the themes of Calmette and his co-workers; since articles of a controversial nature are bound to be scattered in the medical Press of the various countries and therefore less accessible to the reader. The fact that we must devote particular attention to the points in controversy might create a misleading impression on the reader, who would neglect Calmette's original publications.

We intend to deal in succession with the innocuousness of BCG and its premunising efficacy.

Part I.

STUDIES ON THE INNOCUOUSNESS OF BCG.

1. EXPERIMENTS OF CALMETTE AND HIS CO-WORKERS.

In 1908, Calmette discovered the possibility of cultivating a bacillus of bovine type on potatoes cooked in pure ox-bile, glycerinated at 5 % and kept at 38° C. in an excess of bile.

As early as 1909 (43), he showed that, by successive cultures on this medium, the bovine strain gradually lost its virulence and became innocuous for bovines, then monkeys and finally for laboratory rodents.

(a) *Bovines.*

Six-months-old calves which died in 4 to 6 weeks of acute miliary tuberculosis after intravenous inoculation with a dose of 3 mg., the original strain, could tolerate 1 to 5 mg. without ill-effect. Moreover, animals thus inoculated had acquired strong resistance to experimental infection after 30 days.

Calmette based his method of "premunition" against tuberculosis on this resistance acquired by means of impregnation with live bacilli — a fact which, moreover, agrees with the theory on which he based his experiments from 1906 onwards, and with the experiments carried out later by RÖMER (244-245), KRAUS, GROSZ and VOLK on the prophylactic effect of infection with small doses of bacilli.

Calmette, when trying to produce immunity in bovines, found that the latter could without ill-effect support large repeated doses (up to 200 mg.) of BCG inoculated intravenously (45).

The prophylactic results obtained will be explained later. The histo-pathological effects of *subcutaneous* inoculation with BCG depend upon the dose injected, and are as follows : A dose of 2 mg. produces no local reaction at any time, does not produce tuberculin allergy, nor, indeed, resistance to virulent infection. A dose of 50 to 100 mg. produces an almost immediate reaction — an oedematous mass slightly smaller than an egg, which becomes hard and mobile. A soft swelling again appears from the 13th to the 18th day and then disappears. The tumour, which still remains perceptible after 6 months, gradually disappears between the 10th and the 18th month. It shows no tendency to form an abscess nor to burst outwards. In 75 % of the cases, inoculation is not accompanied by any general disorders. In 25 % of cases, there is a rise of temperature from 38.6° to 40.5° about the 15th day, lasting for 5 to 6 days, after which all becomes normal.

Post-mortem examinations of bovines at varying periods after inoculation with BCG show the absence of tuberculous lesions in the various organs: only the presence of BCG in a saprophytic state in the lymph glands.

(b) *Effects of BCG on Laboratory Rodents.*

The extreme susceptibility of these animals to tuberculous infection makes them especially suitable for research work on the harmfulness of BCG.

Intravenous inoculation of the rabbit and hypodermic inoculation of the guinea-pig with the millionth part of a milligramme of tuberculosis bacilli (*i.e.*, 40 live bacilli) are known to be enough to cause them to develop fatal tuberculosis in 5 to 8 months, and larger doses will produce death within a shorter period.

A. *Effects of BCG Inoculation on the Guinea-pig.*

In the *guinea-pig*, inoculation with BCG produces the following reactions (58):

Doses of $\frac{1}{2}$ to 1 mg., slight local swelling, which disappears in a fortnight.

Doses of 3 to 10 mg., oedema, a small abscess opening towards the 10th day and healing in 2 to 4 weeks; slight local adenitis. The animal remains healthy and infection does not become general.

Doses of 1 cg. to 1 gr., local lesion as above and tuberculous follicles disseminated in the various organs, disappearing in a few weeks without leaving scarred lesions. These tubercles can never be re-inoculated. No fatal tuberculous infection nor even alteration of the general health; only a slight lessening of resistance to pasteurelloses and other secondary infections (*infections de sortie*).

Injected intraperitoneally. — Over 3 mg., acute reaction of the mesentery — yellowish nodules containing bacilli and polymorphonuclear cells in a network of connective tissue. These nodules, when re-inoculated, do not produce tuberculous lesions nor do they affect the health of the inoculated animal, and disappear completely in 3 to 5 months.

By intracardiac injection. — 1 to 10 mg. in suspension in 0.5 to 1 c.c. of liquid produce no ill-effects. After a fortnight, general gland swelling sets in suddenly, which lasts for 10 days and then disappears without leaving any traces.

More than 10 mg. tuberculous granulations appear in the viscera, but there is no tendency to caseation or to sclerosis, and the cure is *complete*.

Per os. — The absorption of BCG is irregular in full-grown animals, but better in animals only a few days old.

B. *Effects of BCG Inoculation on the Rabbit.*

The rabbit tolerates the hypodermic inoculation of BCG (1 to 5 mg.) as readily as the guinea-pig, but reacts much more strongly than the former to intravenous injection; 20 mg. and over, injected by this means, *may* induce pneumonia, causing death in 2 to 3 weeks. Generally, however, this dose causes the formation in the intestines of macroscopic tubercles, which disappear in 5 to 6 months. Moreover, these tubercles are not capable of producing tuberculous lesions when re-inoculated into fresh animals, which is a proof of the non-virulence of BCG.

Histological Lesions caused by Intravenous Inoculation of BCG in the Rabbit.

These lesions have been very carefully studied by COULAUD (83).

After doses of :

5 to 10 mg., discrete lesions.

15 mg. (600 million bacilli) also cause :

In the Lung :

After 24 hours, lesions of the alveoli, slight cedema of the septa.

After 9 to 10 days, the lungs are riddled with follicles : epithelioid cells embedded or not in lymphocytes, follicles occasionally larger than alveoli — a very few giant cells, microscopic leucocytic embolisms, acid-fast bacilli rare.

From 10 to 20 days, swelling (without lesions) of the peribronchial lymphoid nodules.

From 20 to 40 days, intensification of the above process.

From 40 to 100 days, regression of the lesions ; gradual reduction of the follicles, occasional sub-cortical desquamative alveolitis.

100 to 140 days, gradual reduction of the follicles, regression of the peribronchial infiltration.

200 days, no trace of the preceding lesions ; *normal* aspect of the lungs, no sclerosis.

At no time were patches of caseous degeneration observed.

In the Liver :

First 2 to 3 days, microembolism.

7 to 9th day, follicles, a few giant cells.

20th day, follicles composed entirely of epithelioid cells ; a few large mononuclear cells.

60th day, the majority of the follicles have disappeared ; numerous giant cells.

150th day, no trace *whatever* of the lesions.

In the Spleen :

12 to 14th day, numerous epithelioid cells in the sinuses.

60th day, sinuses full of epithelioid and giant cells.

120th day, disappearance of epithelioid cells.

200th day, normal spleen.

The *kidneys* and *other organs* remained normal throughout.

Hence, after transient miliary tuberculosis, there is complete *restitutio ad integrum*, without caseation at any stage.

(c) *Effects of BCG on Monkeys.*

WILBERT (310) has conducted experiments at the Pasteur Institute in Kindia on monkeys not previously exposed to spontaneous tuberculous infection, from which it is difficult to protect those living in menageries or laboratories in Europe.

He found that chimpanzees and pithecians suffered no ill-effects from the absorption *per os* of 5 doses of 50 mg. of BCG administered every other day with food, or from the hypodermic injection of 50 mg.

The latter induces transient gland swelling and a local cold abscess, which bursts, discharges and heals without complications. There are no other tuberculous lesions. The annual repetition of inoculation produces no ill-effects.

(d) *Vaccination of Human Beings.*

Vaccination per os.

Since 1921, close upon 110,000 vaccinations have been effected *per os* on newborn babies in France and almost as many in the French colonies and abroad, the infant receiving during the first 10 days of its life (*i.e.*, the period when the intestine is most easily permeated) 3 doses of 1 cg. of BCG each, at 48 hours' interval (*i.e.*, a total of 3 cg. or 1,200 million bacilli).

These doses produce no general reaction or digestive disturbance even in premature infants. This has been without exception the experience of hundreds of doctors who have performed the vaccinations.

The vaccinated infants, although a large number of them are in contact with tuberculous cases and the majority belong to the poorer classes, show a normal weight curve. The number of deaths due to respiratory troubles is below the average and the total mortality during the first year is equal to or below (8.9 %, 7.6 % and 6.6 %) (52 and 202) that of the general infant population (8.7 %).

Mortality was much lower still in the 2nd and 3rd years.

Subcutaneous Vaccination.

WEILL-HALLÉ and TURPIN (302-308) have vaccinated infants subcutaneously. A dose of 1/15th mg. did not cause the formation of cold abscesses such as result from larger doses (as in the guinea-pig).

Neither does 1 cg. produce a cold abscess in adults, provided always that the subject is not already tuberculised (and in an allergic state).

Several hundred vaccinations were performed without accident on coloured soldiers showing negative tuberculin reactions.

On the whole, the conclusions of Calmette and his collaborators on the subject of the non-pathogenicity of BCG may be summarised as follows :

BCG is a non-virulent strain, *i.e.*, not capable of producing tuberculous disease nor generalised progressive tuberculosis.

The lesions it may produce on inoculation are generally localised. They never pass through a stage of caseation; in fact, they have a tendency to spontaneous regression and *restitutio ad integrum*.

Further, these lesions cannot be re-inoculated in series into susceptible animals.

2. VIRULENCE OF BCG FOR LABORATORY RODENTS.

(a) Before dealing with experiments in confirmation of results previously obtained, a clear definition should be given of what various writers understand by the term virulence or avirulence of a strain of bacilli and of BCG in particular.

Calmette considers a strain " avirulent " when it does not produce tuberculosis with a tendency to spread, fatal and capable of re-inoculation. Others, on the contrary, use the term exclusively to connote a strain which produces *no lesion*, even of a benign nature, and consider any other strain as " virulent " or " pathogenic ". The lack of a uniform definition has given rise to a discussion between French and German writers which has had the effect of greatly obscuring the issues as regards the pathogenicity or innocuousness of BCG.

Experiments regarding the innocuousness of BCG for laboratory rodents have been made by a large number of investigators. A still greater number have made experiments on the immunisation of these rodents by means of BCG against inoculations of virulent tuberculosis bacilli, considering the innocuousness of BCG an established fact.

The majority of investigators have confined themselves to inoculating guinea-pigs and rabbits with BCG in varying doses and by different routes. Others have attempted to lower the resistance of laboratory rodents by various methods in order to discover whether BCG infection could spread and become general in a debilitated organism.

Others have attempted to increase the original virulence of the BCG strain by successive passages.

The following investigators have confirmed Calmette's conclusions with regard to the innocuousness of BCG :

A. DE ASSIS, at the Institut Vital in Brazil (16), has observed in the guinea-pig only local lesions, which healed spontaneously and were not capable of inoculation in series. He, too, noted a certain decrease of resistance to intercurrent infections in laboratory rodents after the use of strong doses of BCG.

G. BLANC (33), at Athens, has attempted to increase the virulence of BCG in rabbits by intra-conjunctival injections of the germ, and by re-inoculation in series of material from the abscess thus produced.

Seven passages failed to render the BCG capable of producing clinical tuberculosis among guinea-pigs and rabbits.

HEYMANS (130), operating on 150 guinea-pigs and rabbits with intramuscular doses of BCG up to an amount of 50 mg., observed in them only primary lesions at the site of inoculation and local gland reaction without loss of weight, in spite of subsequent inoculations with virulent bacilli.

IGERSHEIMER also concluded from his intra-ocular inoculations of guinea-pigs that BCG is innocuous.

KIRCHNER (140), taking advantage of the susceptibility of rabbits to infection by corneal injection, attempted by the injection of 0.00001 mg. of bacillary emulsion to increase the virulence of a strain of BCG by passages. Although he effected 4, 5 and 6 passages in 3 parallel series, he did not succeed in modifying the virulence.

R. KRAUS (148-163), who was one of the first to draw attention to the pathological lesions produced by BCG inoculations, considers that BCG is to a certain extent virulent, as it produces local lesions, but that it is not "nosogenic", as it does not produce clinical disease. It is true that in excessive doses it does produce tuberculous lesions, but these are benign in character and tend to heal spontaneously. A dose of 20 cg., which is a huge one for a guinea-pig, very rarely produces generalised lesions. Kraus has not succeeded in increasing the virulence of BCG by passages.

B. LANGE (171-173), inoculating guinea-pigs and rabbits parenterally with large doses of BCG, only succeeded in producing local foci which healed spontaneously, but no progressive tuberculosis. He could only produce extensive lesions by means of very heavy doses injected intravenously, and did not succeed in infecting the animals with the disease by inhalation or ingestion. He effected prolonged and repeated passages through animals, without, however, increasing the virulence of BCG.

LEURET AND CAUSSIMON obtained similar results in guinea-pigs by subcutaneous (181), intraperitoneal and intravenous (182) inoculation. Their attempts at re-inoculation were unsuccessful.

METALNIKOV AND SEKRETEVA (200) devoted particular attention to the study of the phagocytosis of BCG injected subcutaneously and demonstrated the preponderant action of large phagocytic wandering cells.

MOURIQUAND, BERTOYE AND SEDALLIAN (205) confirmed the total disappearance after 2 years of all lesions due to inoculation in the guinea-pig.

NASTA AND CATZAP (209), at Bucharest, endeavoured to discover whether BCG could not tuberculise guinea-pigs if the latter, after intraperitoneal inoculation of 10 mg., were rendered susceptible to infection by tuberculin injections. These experiments were repeated 5 times on 20 guinea-pigs, which were killed at regular intervals; the animals showed no generalisation of the tuberculous process.

OKELL AND PARISH (218) found that their guinea-pigs were not rendered tuberculous by the very heavy dose of 20 mg. 5 times repeated.

RANCOVITZ (239), at Belgrade, only found local lesions and gland reaction without general tuberculosis in several hundred guinea-pigs and rabbits he inoculated. He noted pulmonary necroses following upon embolism after the intravenous injection of heavy doses. He considers that the accidents caused by BCG must be attributed to the fact that large quantities are collected at one point rather than to its natural virulence. He was unable to obtain cultures or to re-inoculate animals from BCG lesions.

REMLINGER AND BAILLY, at Tangier (240) (241), consider BCG practically innocuous for the guinea-pig, after having inoculated 200 of these animals by every possible route and in doses rising from 10 mg. to 50 cg. and even 1 gr. (214). Guinea-pigs which had received large repeated doses of BCG (up to 90 cg.) showed no unfavourable symptoms and were still alive after 10 months.

Comparing (241) the *general* mortality of guinea-pigs inoculated with BCG (84/306) with that of the controls (37/129), they found the latter somewhat higher (28.6 %, as compared with 27.4 %). In their opinion, the decreased resistance to intercurrent diseases in consequence of BCG inoculation is negligible.

ROLLÉ (247) observed in guinea-pigs, rabbits and dogs lesions only at the site of inoculation and of the local glands; these only produced a local abscess on re-inoculation. Doses of less than 10 mg. did not produce any lesion. Pus from a local

abscess produced by 50 mg. of old culture (16 weeks) could not be re-inoculated nor subcultured.

C. SCHLOSSMANN, at Tartu (Estonia), has studied the tuberculine-producing properties of BCG in guinea-pigs and concluded that it is innocuous.

SILBERSCHMIDT, at Zurich (276), has not observed a single case of the development of tuberculosis in his numerous experiments on rabbits and guinea-pigs inoculated *per os* or parenterally with BCG.

SUAREZ (282-283), studying the pathogenicity of BCG in 120 guinea-pigs, confirmed the relative innocuousness of this bacillus in hypodermic doses of 1 to 100 mg. On making post-mortem examinations, he found, in addition to small local follicular lesions, as described by Calmette, general and fatal lesions of pseudo-tuberculosis caused by the bacillus of Vignat and Malassez.

Of 40 guinea-pigs inoculated intraperitoneally with 5 to 10 mg. of BCG by KRAUS, 18 survived at 4 months. Autopsy showed no macroscopic lesions; only small follicles with transparent contents enclosing BCG bacilli were found on histological examination. The other guinea-pigs had died of pseudo-tuberculosis, which had also caused the death of 9 guinea-pigs out of 18 inoculated by Kraus with 5, 10 and 15 mg. of BCG. No lesions were found on post-mortem examination.

SUAREZ *never* observed caseation, but he found regression of the follicular lesions, which were thus distinct from ordinary tubercles. He was unable to re-inoculate in a series the organs of guinea-pigs impregnated with BCG. He considers that, for innocuity tests, 50 mg. is a heavy dose for a guinea-pig, as one-millionth of a milligramme of virulent bovine bacilli normally suffices to kill the animal. On the other hand, the 15 mg. of BCG which a rabbit can tolerate represent 150,000 to 500,000 fatal doses of bovine bacilli of normal virulence.

TZEKHOVITZER (288-289) (see also document C.H./BCG/3) and the other members of the Ukraine BCG Commission (KOCHKINE, GOLDENBERG, KHMOLNITSKY, AGALLI, OBUKHOVSKI, JACKHNIS, etc.), operating on several hundred animals, only found local lesions with regressive tendency in guinea-pigs, even when these were inoculated with heavy doses, and when the vitality of the animals was lowered by lack of vitamins or by the injection of diphtheritic toxin or of cultures of streptococci or by repeated injections of tuberculin. They were unable to produce progressive tuberculosis by re-inoculation from the lesions.

J. ZEYLAND AND Mme. PIASECKA-ZEYLAND (311), at Poznan, continued Coulaud's experiments on *intravenous* inoculation of the rabbit with doses of 15 to 20 mg. Like the latter, they found

benign miliary lesions, *i.e.*, follicles containing bacilli and epithelial cells, occasionally giant cells regressing towards the 60th day without caseation in a single instance. Intra-arterial and intracardiac inoculations produced similar results.

These investigators never observed necrotic lesions when the method of inoculation permitted the bacillus emulsion to disperse completely either through the entire organism (injection through the carotid artery) or through a particular organ (long after intravenous injection).

Intrapleural and intrarenal inoculations produced necrotic lesions identical with those caused by inoculation at the same site with *dead BCG*.

The latter, when injected intravenously into the rabbit, may produce necrotic and follicular miliary lesions. Hypodermically, it may give rise to typical granuloma.

They therefore consider that BCG is avirulent, but that it has a certain degree of toxicity (endotoxin), to which they attribute the necrotic lesions which are sometimes observed. LUBARSCH indeed had shown that similar lesions were produced by the Timothy grass bacillus, which everyone is agreed in considering avirulent.

ZOZAYA, at Mexico (312), also found [that BCG is not capable of producing tuberculosis.

GERLACH, at Vienna (103-104), who introduced several different strains of BCG into more than 50 guinea-pigs and rabbits hypodermically, intracardially, intraperitoneally and *per os*, found lesions in animals killed 4 weeks after inoculation. These lesions had disappeared 5 ½ months after hypodermic inoculation. Macro- and microscopically, they resembled tuberculous lesions, just as stained BCG resembles the Koch bacillus.

These lesions, which are more or less localised, cannot be re-inoculated unless they are fresh and contain numerous bacilli. They tend to regress and disappear in course of time, and heal, even if the infecting dose was large (104).

Gerlach therefore considers that BCG cannot be considered as "avirulent" in the strict sense of the word, but that its inherent virulence is necessary if it is to possess immunising properties.

KUHN (166), at Buenos Aires, tracing the evolution of lesions produced by BCG inoculations in the guinea-pig, has broadly confirmed Calmette's experiments with regard to the regression of BCG lesions after the 2nd month and their disappearance after 6 months. A considerable number of his animals, however, especially those which received heavy doses, died, showing acute abscesses in addition to specific BCG lesions. These must apparently be attributed to secondary infection.

MAXIMOW, at Chicago (198), studied the innocuousness of BCG by his own method of a hanging drop of culture from lymph-gland tissue.

When such tissue cultures are inoculated with tuberculosis bacilli of normal bovine type, the latter is seen to have a toxic action at a distance on the phagocytes and gradually causes the cultivated tissue to degenerate and perish. This is not the case with BCG, which continues to develop but without having any prejudicial effect upon the development of the polyblasts.

BCG taken from tissue cultures and inoculated into the guinea-pig was proved not to have recovered its virulence.

TZEKHOVITZER and his collaborators (289) continued and confirmed these experiments.

Other authors have reported different results or have deduced different conclusions from their experiments :

BOCCHINI (35), at Perugia, injected 30 mg. of BCG intravenously into rabbits (*i.e.*, a dose of double the amount considered innocuous by Calmette). He killed 4 on the 20th and 40th days. Post-mortem examination revealed small nodules on their lungs, which the microscope showed to be lesions of desquamative broncho-alveolitis, with degeneration of the cells, and acid-fast bacilli (similar to those already described by Coulaud). The animals which were not killed survived. The writer admits that, even if BCG injected intravenously may not be altogether innocuous, the harm caused by small doses administered *per os* must be negligible.

LIGNIÈRES (184) found that the attenuation of virulence of BCG is fixed, and that the germ, after remaining for a year in calves or guinea-pigs, does not cease to be "absolutely innocuous" for vaccinated guinea-pigs, and remains "incapable of producing tuberculosis" by hypodermic or intraperitoneal injection. He does not consider, however, that he can commit himself as regards its absolute innocuity, because one guinea-pig which died 25 months after inoculation still showed a small nodule containing BCG on the omentum, and this in spite of the fact that the guinea-pig showed no other tuberculous lesion and that the cultures from the blood and organs of the animal remained sterile. Lignières, on the basis of this finding, seems to indicate that BCG may possibly be noxious for certain particularly susceptible subjects.

Other experiments, carried out in September 1926 and in 1927, led him to conclude (186, p. 7) that: "(1) BCG is absolutely incapable of causing lesions of progressive tuberculosis; (2) that by passage through susceptible animals it does not recover the virulence of the normal Koch bacillus".

In the same report, the writer stated that he could not consider as innocuous an organism which invades the whole lymphatic system in the way that BCG does.

NOBEL (214, 215, 216) inoculated the peritoneum of 24 guinea-pigs with BCG. Three died spontaneously of "tuberculosis", 2 of intercurrent diseases, 19 were killed at various intervals.

The writer attributes to tuberculosis the death of animals dying 15 to 20 days after inoculation with 5 mg. of BCG, "showing the most severe tuberculous lesions".

Nobel was able to cultivate BCG taken at autopsy from 3 guinea-pigs, and to inoculate it into other animals. He considers the BCG strain as "virulent", although the animals vaccinated by him survived longer than the controls after subsequent inoculation with bovine bacillus of normal virulence.

CHIARI (75,76) made pathological examinations of guinea-pigs inoculated at the Children's Polyclinic in Vienna (CHIARI, NOBEL, SOLÉ) (77,78). He describes lesions of the omentum and liver visible the 4th week after intraperitoneal inoculation which, after 8 weeks, differed from those produced by the Koch bacillus by the absence of coagulation necrosis. More often there is destruction of the tissue with exudate as indicated by the presence of leucocytes in addition to neoplasia (epithelioid and giant cells). He points out the resemblance of the BCG lesions to those described by STERNBERG, caused by inoculation with tuberculous bacilli killed by heat.

KORSHUN (144,145,146) and his collaborators at the Metchnikoff Institute, Moscow, found that, after parenteral injection of BCG in the guinea-pig, there is a rise in temperature, generally in two stages: the first sets in from the 2nd to the 4th day and lasts from 1 day to 5 days; the 2nd sets in between the 9th and 15th day and lasts from 7 to 14 days, after which the temperature becomes normal and the weight, which had practically remained stationary, begins again to increase. Korshun points out that intracardiac injections in the guinea-pig more often produce specific "tuberculous" lesions than hypodermic or intraperitoneal injections. The lesions are as a rule purely local; sometimes they are found in organs remote from the site of injection. They are definitely circumscribed, heal by fibrotic transformation and do not affect the health of the animals. These guinea-pigs were kept under observation for 10 months and none died of tuberculosis.

The investigator in two cases succeeded in producing local lesions with sclerotic evolution by the inoculation of a BCG tubercle. The same result was obtained with cultures of the same origin. He considers that these facts in no way argue against the use of BCG for the vaccination of human beings.

DWYKOFF AND MASOUROWSKI (95) are of the same opinion.

GALLI-VALERIO (101), at Lausanne, expresses doubts as to the innocuousness of BCG, basing his opinion upon an experiment made only on 2 guinea-pigs, which died 16 and 70 days after inoculation. The re-inoculation of their lesions into fresh subjects also caused death, but BCG was only found at the site of inoculation.

SELTNER AND BLUMENBERG (273), using doses of 5, 10 and 30 mg. of BCG subcutaneously, observed, as Calmette had done, that the nodule of inoculation was sometimes ulcerated, and that there was glandular swelling from the 25th to the 60th day. They also observed in certain cases small foci of intraglandular caseation, which were, however, regressive, and under the microscope appeared very different from the caseation caused by the Koch bacillus. Follicular hyperplasia appeared in proportion to the doses injected.

Caseum from the lymph glands inoculated into 9 guinea-pigs caused adenitis in 1 and nothing in the remaining 8.

These investigators consider that BCG certainly produces tuberculous infection, but that it heals "too easily" to afford lasting immunity.

UHLENHUTH (291), reviewing various works on the innocuousness of BCG, draws attention to some of the differences existing between those of Gerlach, Kraus and Korshun and those of Calmette. Although he considers BCG practically "apathogenic", he thinks that the possibility of reversion to virulence *in vivo* cannot be excluded.

PETROFF, BRANCH, STEENKEN, at Trudeau (N. Y.) (226-227), have succeeded in dissociating strains of BCG by culture on Petroff medium with passage through the testicles of guinea-pigs. Certain colonies, which they call "R" (rough), show a corrugated surface, are waxy in appearance, do not penetrate the medium and are difficult to emulsify.

The "S" (smooth) colonies, on the contrary, are more filamentous, irregular, penetrating, easy to emulsify, and do not agglutinate like the others when the pH of the medium reaches 7.2.

Inoculated into the *guinea-pig*, the "R" strain produces local typical BCG lesions without generalisation, by all methods of inoculation.

The "S" strain, on the other hand, causes death from generalised tuberculosis in 34 days when injected subcutaneously, 17 and 26 days intracardially, 75 days when injected into the testicles, 42 to 91 days into the eye, and 9 to 19 days into the spinal fluid.

In the *rabbit*, the "R" and "S" strains only caused local lesions when inoculated intra-ocularly or into the left ventricle, or into the spinal fluid.

The testicles of 4 guinea-pigs were inoculated with BCG (1.25 to 2.5 mg. of dry BCG). After 1 to 2 weeks they were ground and inoculated into the testicles of fresh animals. Generalised lymphatic tuberculosis ensued in one case only. This strain, when recovered and re-inoculated subcutaneously, caused caseous lesions in the rabbit similar to those of the "S" strain. There were no deaths.

TZEKHOVITZER (document C.H./BCG/3, p. 4) took up Petroff's dissociation experiments. He also succeeded in

isolating colonies of a morphological character similar to the "R" and "S" colonies obtained by the latter. The emulsion of these colonies, when inoculated intratesticularly, intracardially or hypodermically into guinea-pigs, caused no clinical lesions, and on autopsy no general tuberculosis was found.

Moreover, the "R" and "S" colonies, separately cultivated, had lost their morphological differences after the 2nd or 3rd subculture.

SCHURMANS VAN STECKHOVEN, at Amsterdam (266-268), in his thesis, described lesions he found in a large proportion of guinea-pigs inoculated with BCG. Histologically these were tubercles, and the author concluded that BCG is "tuberculo-genous" even in a small dose.

Schurmans, moreover, did not succeed in re-inoculating these "tuberculous" lesions.

Conclusions.

Hence it would appear that, in the opinion of the large majority of investigators, BCG can be administered in considerable doses to the guinea-pig *per os*, intraperitoneally, subcutaneously and intramuscularly without causing a definite disease or a general tuberculous process; when inoculated intracardially the same applies, provided that the BCG emulsion inoculated is fine, homogenous and free from lumps (capable of producing embolic lesions, with rapid death or visceral necrotic lesions which heal with difficulty on account of thrombosis and the quantity of endotoxins given off at the same point).

The same remarks apply to the intravenous inoculation of rabbits.

The majority of discordant opinions are due to the acceptance of the appearance, a few weeks after inoculation, of lesions which are macro- and microscopically similar to typical Koch bacillus tubercles as a criterion of "pathogenicity", of "tuberculo-genicity", or of "virulence".

3. VIRULENCE OF BCG FOR GOATS.

The innocuousness of BCG for goats has not been particularly studied, but it has been proved incidentally in the course of research work on the premunisation of that animal by ASCOLI (9, 10), who introduced BCG *per os* and subcutaneously, and by GERLACH (104) and OBUKHOVSKY (document C.H./BCG/3, p. 10), who inoculated subcutaneously and intravenously (25 mg.).

4. VIRULENCE OF BCG FOR MONKEYS.

S. GRIFFITH (100), at Cambridge, after inoculating monkeys subcutaneously and *per os*, is of the opinion that BCG is innocuous when thus administered.

TZEKHOVITZER (document C.H./BCG/3), at Kharkoff, operated on a few *Macacus rhesus* and *cynomolgus* which had already been used for a year for experiments of another kind; like WILBERT, he inoculated them with 50 mg. of BCG, and apparently observed no signs of trouble before inoculating them with bacillus Vallée $\frac{1}{2}$, 7 and 13 $\frac{1}{2}$ months afterwards.

A. SCHLOSSMANN (document C.H./BCG/4) inoculated some *Macacus rhesus* intravenously and subcutaneously with doses of 15, 30 and 50 mg., but as these animals were kept in contact with others suffering from acute pulmonary tuberculosis, his experiments throw no light on the question of innocuousness. It is worthy of note, however, that, in spite of infectious contact, 2 monkeys inoculated respectively with 15 and 30 mg. intravenously, 2 with 30 mg. intravenously and subcutaneously, 2 with 50 mg. subcutaneously remained healthy and showed no signs of tuberculous lesions upon post-mortem examination.

5. VIRULENCE OF BCG FOR BOVINES.

The result of Calmette's and Guérin's researches into the action of BCG in bovines has already been stated. The latter has described his last observations and the position in regard to vaccination in France in a memorandum annexed hereto (p. 66).

In Italy, more than 1,000 vaccinations of bovines were carried out between 1925 and 1928 by GENTILI, under the direction of ASCOLI (10), in the provinces of Pavia and Sondria. Their observations agree with those of Guérin. They noticed a transient rise of temperature upon re-vaccination. The autopsies carried out by these authors showed no tuberculous lesions attributable to BCG. They once succeeded in recovering BCG in pure culture at the inoculation focus after 11 months; it had not recovered its virulence.

Under the direction of BERGER (document C.H./BCG/2), more than 1,000 calves have been vaccinated since 1925 in the Netherlands. The observations made upon them and the 130 autopsies carried out by FRENKEL in no case revealed tuberculous lesions which these investigators felt justified in attributing to BCG.

GUÉRIN, RICHART AND BOISSIÈRE (116) have since 1921 vaccinated 88 calves a few weeks after birth at an infected stock farm. In spite of the fact that the animals were fed with their mothers' milk *unboiled* and were not segregated, their health did not suffer in any way and no tuberculous lesion was found in the 30 post-mortem examinations which were made. The slaughtered animals yielded a good quantity of meat, which is a proof of sound health.

GEROSA (105), after many vaccinations, concluded that BCG is absolutely innocuous.

GERLACH (102, 103, 104), after investigations made in collaboration with KRAUS (161) on the innocuousness of BCG for calves, instituted a vaccination campaign in infected byres.

HEYMANS, in Belgium (131 and 132), studied the period of survival of BCG in the organs of bovines as compared with that of the tuberculosis bacillus of human type.

JUNDELL inoculated 2 calves intravenously with virulent Koch bacillus 4 and 8 months after they had been premunised with BCG. Infecting doses of 5 and 2 ½ mg. had no ill-effects on the animals, whilst 1 mg. of the same strain killed the control in 1 month.

In addition, the writer vaccinated 120 calves in 1927, keeping them under observation and in contact with infection together with 120 controls. In December 1927, there was no sign of tuberculosis in the vaccinated animals. (Communicated to Professor Calmette, December 7th, 1927.)

B. LANGE AND LYDTIN (172), in the course of experiments in premunisation, injected bovines with doses of 160 mg. of BCG subcutaneously or 20 mg. intravenously. There were no ill-effects during the 6 months of observation previous to experimental infection with virulent bacilli.

LIGNIÈRES (183) injects calves with 50 mg. and finds that this does not affect their health. After autopsy, inoculations from various organs and even from the contents of the original nodule of inoculation did not produce tuberculosis in guinea-pigs. Among bovines, this writer does not know of a single case in which BCG could be proved to have produced even the slightest ill-effects.

MELLO (199), at Turin, working with doses of 50 mg. injected subcutaneously, also concluded that BCG was innocuous for bovines.

OBUKHOVSKY AND PACHKOWSKY (217), at Kharkoff, devoted special attention to the elimination of BCG from the milk of cows after introduction into the dewlap of a dose of from 50 to 100 mg. After a period of incubation, the elimination of bacilli in the milk — where they are, indeed, difficult to discover — continued up to 2 ½ to 3 months. The inoculated cows, and also guinea-pigs and rabbits inoculated with milk containing BCG, remained perfectly healthy. A post-mortem examination of the latter showed no tuberculous lesion.

Comparative experiments in the inoculation of cows with Koch bacilli, on the contrary, produced tuberculosis in guinea-pigs inoculated with the infected milk.

SILBERSCHMIDT (276) describes the results of 300 vaccinations of calves inoculated by BÄR in the Canton Vaud. In 3 post-mortem examinations out of 6 in which tuberculosis bacilli were looked for, they were found, but the 11 autopsies made did not reveal a single case of progressive tuberculosis.

SCHROEDER, of the Bureau of Animal Industry at Washington, convinced of the non-virulence of BCG, had by the time of his death 1,000 calves vaccinated.

TZEKHOVITZER (288), together with AGALLI, GRIGOROVITCH, OBOUKHOVSKY and STEPANIENKO, studied the non-virulence of BCG in young bovines and horses. Thirteen bovines weighing from 50 to 204 kg. were injected with 80 to 200 mg. of BCG subcutaneously; 2 others with 100 to 200 mg. intravenously. While under observation for 6, 7 and 8 ½ months before inoculation with virulent bacilli, they showed no signs of ill-health and their weight increased from 18 to 180 kg. Two post-mortem examinations carried out at 6 and 8 ½ months showed the specific BCG granuloma at the site of inoculation and slight hyperplasia of the lymphatic, bronchial and mesenteric glands without histological lesions. There were no macro- or microscopic tubercles.

In order to discover whether any biological modification of BCG had supervened, guinea-pigs and rabbits were inoculated with cultures taken from the organs of the above bovines, from the granuloma and from the mesenteric and peribronchial glands. No trace of tuberculosis was found 3 to 4 months afterwards.

Two *colts* inoculated with 200 mg. of BCG, one intravenously, the other subcutaneously, remained in perfect health. Their weights increased respectively from 88 to 244 kg. in 12 months and from 140 to 326 kg. in 13 months.

The members of the Ukraine Commission therefore concluded that BCG was perfectly innocuous for bovines and for laboratory rodents. The same investigators (document C.H./BCG/3) also kept 400 vaccinated bovines under observation for 2 or 3 years without noting any untoward effects.

UHLENRUTH (294), of Freiburg-in-Breisgau, also concluded that BCG was innocuous for bovines.

E. A. WATSON (301), at Toronto, alone appears to have obtained different results from the use of BCG in bovines. In one series of experiments, no animal showed signs of tuberculosis, while in another all showed progressive lesions. The animals in both series were in contact with infected bovines. In this case the probability cannot be dismissed that the BCG culture was either accidentally contaminated or that the bovines must have been infected before immunity could be established, since observations on thousands of bovines in France, Italy, Russia, Austria, etc., gave similar results and led to the conclusion that BCG was innocuous for bovines. As BCG is precisely of bovine origin, its innocuousness is the more significant.

* * *

In the preceding pages mention has been made of various attempts at enhancing the virulence of BCG by passages or by

the artificial lowering of the general resistance of inoculated animals. They have all led to the conclusion that BCG is a true fixed virus which does not, under any circumstances, again become capable of producing tuberculous disease or progressive tuberculosis in inoculated animals. [UKRAINE COMMISSION (289), (document C.H./BCG/3), KIRCHNER (140), KRAUS (152), LANGE (173), LANGE AND LYDTIN (171), NASTA AND CATZAP (209)].

6. VIRULENCE OF BCG FOR MAN.

Before proceeding with this subject, all the terms of the problem should be carefully stated. The problem is as follows: Is BCG in immunising doses capable of adversely affecting the health of the subjects vaccinated?

The point is not whether BCG can be administered in any dose to any subject.

BCG injected hypodermically in Tuberculous Subjects.

CALMETTE had noted (58) the formation of a cold abscess after subcutaneous injection of BCG in tuberculised individuals, the true Koch phenomenon. Although these abscesses normally discharge and heal, he did not consider it desirable to administer BCG to individuals in whom tuberculosis was such as to produce positive allergical reactions.

The symptoms of reaction in tuberculous subjects no doubt vary according to the dose of BCG inoculated and the method of inoculation. Some investigators have tried to use them for *curative purposes*.

SORGO (277), in 88 cases of *pulmonary tuberculosis*, injected 600,000 to 2,400,000 BCG subcutaneously in from 1 to 6 doses. The period of observation is still too short (3 to 7 months) for any definite judgment to be formed of the results obtained.

VOLK (298) experimented with BCG in cases of *lupus*.

Similar attempts to cure *leprosy* have been made by PONS and CHASTEL (234), SOUCHARD (278), NOVAES and ROW. None of these observed any serious complications; only transient rises of temperature after subcutaneous injection, and small local abscesses.

LAVALL AND POMARET (167), on the other hand, observed one case of *leprosy* in which death ensued quickly from tuberculous cachexia, after 4 subcutaneous injections of 10 mg. of BCG each. Injections in heavy doses had produced local abscesses and prolonged febrile reaction.

BCG injected hypodermically in Non-tuberculised Subjects.

Premunisation by hypodermic injection has been effected in several hundred cases under careful medical supervision in France amongst recruits from Africa and Madagascar, with negative tuberculin reaction and without adverse results. It has been and still is practised among the civilian native population (ROUSSEAU 252).

Administered in doses of 0.01 mg. (i.e., 400,000 bacilli) subcutaneously in the acromial region, BCG produces no cold abscess. When 3.5 mg. are administered, an abscess forms 15 to 20 days after injection, but without local and general reaction and heals rapidly after or without lancing (58, p. 213) (experiments on 140 Malagasies kept under observation for 18 months).

In the case of adults with negative tuberculin reaction, HEIMBECK (126), at Oslo, injects 0.05 mg. to avoid the cold abscess, which is troublesome when the dose is increased to 0.2 mg. He experimented on more than 500 subjects, 44 of whom were nurses and were kept under particularly close observation. No accidents were recorded in the course of a whole year.

BCG injected hypodermically in Infants.

WEILL-HALLÉ (305-308), operating on infants from a few weeks to a few months old, gradually reduced the dose injected subcutaneously from 1 mg. to 0.25 mg., then to 0.06 mg. (i.e., 2,500,000 bacilli), in order to lessen the local reaction (small abscess). The injection produces no general symptoms and no alteration in the weight curve. About the 3rd week, a small subcutaneous nodule appears, while the reaction of the lymph glands is slight or nil. About the 35th to 40th day a small cold abscess forms, which discharges about the 60th day and lasts from 2 to 3 months; the fistula disappears spontaneously.

Among 16 infants vaccinated, 3 deaths occurred (at 2, 13 and 16 months), which cannot definitely be ascribed to non-tuberculous causes owing to lack of information. It should be pointed out, however, that they ensued in the case of infants who were in contact for 3 weeks, 2 months and 3 months with tuberculous parents before vaccination (308).

BCG administered to Infants per os.

Reference has already been made at the beginning of this report to the absence of symptoms of reaction and disturbance of general health in the many thousands of children vaccinated *per os* with 30 mg. administered in 3 doses.

The material in hand derived from the observation of vaccinated infants gives us particulars regarding the aggregate results of artificial inoculation with BCG and of frequent accidental infection with virulent tuberculosis bacilli.

In order to judge of the innocuousness of BCG in infants, the vaccine must be administered *per os* or subcutaneously to infants with healthy mothers, living in no danger of contact with tuberculous cases. Post-mortem examinations should be made and should include histological examinations and the inoculation of guinea-pigs.

Conclusions as to the Innocuousness of BCG for Various Animal Species.

Conclusions concerning the innocuousness of BCG for laboratory rodents under certain conditions of dosage and preparation of the bacillus emulsion apparently apply also to other animals. Experiments made on *goats* show that these animals can take doses of 25 mg. intravenously or hypodermically without ill-effect. Experiments on *monkeys* show that the *Macacus* tolerate 50 mg. injected subcutaneously.

Finally, thousands of vaccinations were performed on *bovines* and tens of thousands on *human beings* by many veterinary surgeons and physicians without accidents.

We feel ourselves therefore justified in saying that writers who have used BCG either experimentally or in veterinary and human practice are almost unanimous in recognising the innocuousness of the doses prescribed by Calmette and his co-workers, and that the attempts at increasing the virulence of BCG have failed.

Part II.

PREMUNISING EFFICACY OF BCG

It has been pointed out in the first part of this study that although, in the various animal species, BCG is quite incapable of producing a progressive generalised and fatal tuberculosis, it is likely to cause local lesions by its mechanical action and the concentration of its endotoxin in a certain spot. We are therefore able to deduce the dose of BCG which can be readily tolerated by each animal species according to the various routes by which the bacillus may be introduced.

The study of the premunising efficacy of BCG is still more complicated, for the immunity acquired will depend, not only on the quantity of BCG introduced and the route used, but also

on the quantity of virulent bacilli introduced for the experimental infection, the method of their introduction, the interval separating the inoculations with BCG from those with the virulent strain, and, finally, the degree of virulence of the strain for the species of animal under consideration.

We think it essential to point out that "absolute" acquired immunity, resistance *in abstracto*, so to speak, does not exist in practice (PETROFF, 199).

One cannot reasonably hope to obtain from a para-specific immunity-infection, such as that produced by impregnation with BCG, an unlimited resistance to any dose of virulent bacilli.

On what criterion are we to base our judgment concerning the premunising efficacy of BCG? Should it be on the resistance of premunised animals and human beings to the infections to which they are exposed in normal conditions of life in contagious surroundings? Or should we base our opinion on resistance to a large number of lethal doses of virulent bacilli?

We believe that the first of these criteria is the most important one and that, in the study of the resistance to test inoculations, the positive findings alone have a real significance.

In the course of the experiments, we must therefore note what doses of virulent bacilli BCG prevents from developing into progressive tuberculosis, and also to what extent BCG may retard this development when these doses are exceeded.

In the case of an animal inoculated with known doses, we have two criteria for determining the efficacy of premunition: on the one hand, the prolongation of the life of vaccinated animals as compared with that of controls after inoculation with powerful doses of virulent bacilli (relative resistance); on the other hand, absolute resistance when smaller doses (the size of which it is important to determine) do not lead to the development of progressive tuberculosis.

Only when we have grasped these notions of relativity, can we usefully begin to study the experiments made with regard to the efficacy of BCG.

1. PREMUNISING EFFICACY OF BCG AND ITS LIMITS IN LABORATORY RODENTS.

CALMETTE (58, p. 111) has not succeeded in producing lasting or complete immunity in these animals, but only in prolonging their lives in comparison with controls. He administered 2 to 5 mg. intravenously to *guinea-pigs* of 150 to 200 g.; 50 days later, they received 0.5 mg. of virulent bacilli (fine emulsion: 1 cg. per c.c.). The infection remained localised during *three to five months* in the glands of the neck, after which protection ceased and tuberculosis became general.

As a rule, subcutaneous or intraperitoneal preventive inoculations have little effect on the guinea-pig.

Calmette administered *per os*, with a pipette, 6 meals of 10 mg. emulsified BCG, and then 4 others of 20 mg. at 24-hour intervals, to 36 *young guinea-pigs* (8 to 30 days old). Three months afterwards, two infecting meals of 5 mg. each (ditto to 6 controls). The 6 controls died of generalised tuberculosis at periods varying between 70 and 110 days later. Thirteen vaccinated guinea-pigs, *i.e.*, one-third, died of pasteurellosis with adenitis and a few splanchnic tubercles before the 110th day.

Sixteen others died between the 110th and the 150th day. All had mesenteric adenitis, 4 with a few small tubercles on the spleen, 3 with generalised tuberculosis of the lungs.

Seven survivors were killed on the 200th day. One had generalised tuberculosis, 4 had a few small visceral tubercles, 2 had mesenteric adenitis *only*.

In short, there was protective action — inasmuch as two-thirds of the vaccinated animals survived the controls — but the protection was ultimately inadequate. It is true that the infecting dose of 10 mg. of virulent bovine bacilli was considerable.

In *rabbits*, Calmette finds that subcutaneous injections of BCG afford no appreciable protection against virulent intravenous inoculation with 0.01 mg. of bovine bacillus. *Intravenous* injections of from 20 to 30 mg. afford protection for about 6 months against a virulent inoculation (which kills controls in 50 to 75 days), after which the lesions pursue their fatal course.

"Absorption of BCG *per os* several times, on the other hand, is capable of conferring on *young* rabbits a considerable power of resistance to mass infections artificially produced through the same channel" (46, p. 115).

Twelve *young* rabbits from 15 to 20 days old received 10 meals of 20 mg. of BCG each; then, 3 months later, 2 infecting meals of 10 mg. of virulent bovine bacillus; 3 controls also received these meals.

The controls were killed:

On the 70th day, mesenteric adenitis, a few caseous granulations on the intestine.

On the 120th day, adenitis plus tubercles disseminated over the lung.

On the 130th day, ditto, plus large caseous foci on the lungs and numerous tubercles on the small intestines.

Six vaccinated animals in excellent condition were killed:

On the 150th day, 2 had sclerous mesenteric adenitis and a few granulations on the intestine; 3 ditto, plus a small tubercle on one lung; 1 with small tubercles on both lungs.

Six vaccinated animals in perfect condition were killed:

On the 180th day, all had *sclerous* mesenteric adenitis; 3 had no other lesions; 3 had a few tubercles on the lungs.

Bocchini (35) inoculated 16 rabbits, which had previously received 50 mg. of BCG intravenously, with 0.5 mg. of virulent bovine bacillus by the same route; at the same time, 6 controls were inoculated. All the rabbits died within 3 months; the vaccinated animals showed, on microscopic examination, epithelial desquamation of the pulmonary bronchioles and alveoli, with giant cells; liver and spleen hypertrophied, with lymphocytic infiltration and small foci of necrosis. This author concludes from his experiment that BCG is not harmless, and did not in this case prevent a virulent infection.

Chiari (76) administered 10 and 5 mg. of BCG to a guinea-pig, and then a month later inoculated it with 1 mg. of virulent bovine bacillus. The animal died at the end of 8 months. The autopsy revealed marked tuberculous lesions of the lung and spleen, and a characteristic hepatic cirrhosis. The same results were discovered when the virulent inoculation was made with the human-type of bacillus.

Dwykoff and Masourowski (95) vaccinated 17 guinea-pigs *per os* and subcutaneously with doses of 0.015 to 0.045 mg. and 0.0001 to 0.05 mg. respectively. All died within periods of from 3 weeks to 3 months, except 4 which survived for 4, 5 and 7 ½ months. All showed tuberculous lesions with a sclerous tendency, marked in proportion to the duration of survival. According to these authors, the tendency to sclerosis explains the prolongation of resistance and life in the vaccinated animals, Dwykoff and Masourowski also observed that guinea-pigs inoculated successively with two virulent strains, the second more virulent than the first, always showed extensive lesions.

Harnach (24), at Alfort, has carried out experiments in the protection of *birds* from tuberculosis with BCG of an avian type obtained by 105 passages on biliated glycerinated potato. He administered the BCG *per os* to 2-day-old chicks and intramuscularly to hens.

The vaccinated fowls effectively resisted a digestive infection of 2 mg. of virulent avian tuberculosis bacillus; they were also unaffected by cohabitation with tuberculous birds, but their immunity did not avail against 1 mg. of virulent culture inoculated into the veins. In this case, the vaccinated birds showed at autopsy between the 7th and 9th weeks tubercles of varying size; the controls died within 7 weeks. Harnach concludes from this that the protection is satisfactory against a tuberculous infection through the natural channels.

Heymans (130) premunised his guinea-pigs by intramuscular injection of BCG (1 to 50 mg.); he inoculated them by the same route with a 1% emulsion of tuberculous gland containing bovine bacillus, the virulence having been maintained by passages through rabbits; this strain killed non-premunised guinea-

pigs in 30 to 40 days. Weaker emulsions at 1/1,000, 1/10,000, 1/100,000 still killed the animals, but took longer to do so.

All the vaccinated animals died, but they survived after inoculation twice as long as the controls, though infected with equal doses (60 to 70 days instead of 30 to 40; 90 to 120 days instead of 50 to 60).

Vaccinated guinea-pigs and rabbits cohabiting with animals suffering from open tuberculosis (tuberculous ulcer) contracted the disease with fatal results.

IMAMURA AND TAKAHASHI, at Osaka (136), observed that in guinea-pigs inoculated with BCG the lesions caused by inoculation (0.01 mg.) with virulent Koch's bacillus developed earlier, but showed a clearer tendency to heal than in the controls; in the latter, lymphatic lesions were also more marked.

They do not note any protection of guinea-pigs from virulent infection with doses of living BCG less than 0.001 mg., nor with dead BCG.

They hold that a dose of 0.01 mg. of BCG has a definite immunising effect.

LANGE AND LYDTIN (171) conclude from their experiments on guinea-pigs that BCG does protect these animals to a certain extent from virulent infection, but only for a limited time; its protective power is very strong against strains of low virulence. It is also definite against highly virulent strains in small doses given intracutaneously or by inhalation.

On several occasions vaccination *per os* has completely failed (though it is true that the subjects were adult guinea-pigs), and the authors regard the parenteral route as preferable in the guinea-pig.

In the rabbit, however, the intravenous route gives better results.

Basing their conclusions on their experiments on animals, Lange and Lydtin feel unable to recommend any wide extension of the practice of vaccination with BCG.

NOBEL (216) has endeavoured to preimmunise guinea-pigs by administering BCG intraperitoneally; he obtained no effective protection by this method against a virulent inoculation, but merely secured in most cases a prolongation of life as compared with the controls.

OKELL AND PARISH (208), inoculating preimmunised guinea-pigs with virulent tuberculosis bacillus of the human or bovine type, noted that the guinea-pigs in question tended to survive the controls: the smaller the dose of virulent bacillus, the longer the period of survival. They obtained complete protection in only 27% of cases.

The vaccinated guinea-pigs in their series either showed no sign of infection or showed a generalised tuberculosis similar to that of the controls.

These authors do not consider their results to be appreciably better than those obtained by former methods with attenuated living bacilli, bacillary emulsion (BE), or dead bacilli.

PETROFF AND BRANCH (227) have endeavoured to ascertain the effect of premunition in 38 guinea-pigs.

I. Eight new-born guinea-pigs received 20 mg. of BCG *per os* 4 times; then, 1½ months later, 3 drops of virulent bacillus emulsion at 100 million per c.c. by ocular instillation.

After 8 months, of those vaccinated, 3 were still alive; 5 had died between the 130th and 231st day, 3 of tuberculosis;

After 8 months, of the controls, 5 were still alive; 2 were dead of tuberculosis.

II. Eight new-born guinea-pigs received 8 mg. of BCG in 4 subcutaneous injections; infection by inhalation of a virulent human strain

After 8 months, of those vaccinated, 1 was still alive; 7 were dead, 4 of generalised tuberculosis.

After 8 months, of the 9 controls, 6 were dead of generalised tuberculosis.

III. Ten adult guinea-pigs received 12 mg. of BCG in 4 subcutaneous injections, and then, 1 month later, 500 virulent human bacilli subcutaneously.

After 1 year, of the 10 vaccinated, 3 were still alive; 7 were dead, 5 of generalised tuberculosis (after surviving 279 days).

IV. Twelve adult guinea-pigs received 12 mg. of BCG in the peritoneum in 4 injections, and 1 month later subcutaneous inoculation with 500 virulent human bacilli.

After 1 year, of the 12 vaccinated, 4 were still alive; 8 were dead, 3 of generalised tuberculosis (after surviving 123, 238 and 279 days respectively).

After 1 year, of the 10 controls, 1 was still alive; 9 were dead, 6 of generalised tuberculosis (1 survived for 351 days); 2 others were tuberculosis.

From these experiments Petroff and Branch conclude that laboratory rodents are not protected by BCG.

RANCOVITZ (239) has, on the contrary, observed that premunised guinea-pigs tolerated the injection of homogenised tuberculous sputum, whereas controls died in 2 months.

ROLLÉ (247) notes the difference in the protection conferred on guinea-pigs by old and new BCG cultures. In guinea-pigs receiving old cultures, no inoculation abscess formed, and they died at the same time as the controls, 56 to 60 days after the subcutaneous inoculation of 0.5 mg. of virulent Koch's bacillus.

Those premunised with 3- or 4-week-old cultures showed the local abscess, but died after 115 to 154 days (after the inoculation ulcer had healed).

The same author administered 20 mg. of virulent bacilli (10 mg. intravenously and 10 mg. intramuscularly) to a premunised rabbit. The control died on the 80th day; the vaccinated rabbit was killed on the 216th day and showed a slight inoculation abscess (sterile) and characteristic follicles in the lung.

SATAKE, at Osaka (255), introduced by injection into the stomach of guinea-pigs weighing 200 g. 1.5 mg. of BCG. After a month, he inoculated them with virulent human-type bacillus by the same route. In those which had received 5 mg. of BCG, he did not find any tubercles on post-mortem examination. He concluded that BCG produced immunity to the human bacillus, in spite of the inconstancy of the tuberculin reactions observed.

SELTNER AND BLUMENBERG (273) premunised 50 guinea-pigs by subcutaneous injections of 5, 10 or 30 mg. of BCG. After 44 days, half this number received, by the same route but on the other side of the abdomen, 1/1,000th part of a milligramme of virulent bovine bacillus.

The controls killed between the 74th and 110th days all showed caseated nodules at the site of inoculation, caseated glands, and extensive tuberculosis of the splanchnic organs. In the vaccinated animals, the inoculation nodules were *not* ulcerated; these did not progress, but even receded in 150 days. The glands were hypertrophied. There were more or less marked tuberculous lesions in the liver and lung, but far less marked than in the controls. The BCG had therefore a distinct immunising effect. In no case, however, did it suffice completely to protect the vaccinated animals from re-infection. In all these animals, tuberculous lesions were detected at the site of inoculation and in the corresponding glands. In 6 cases, the infection had not gone beyond the lymphatic glands. Selter and Blumenberg consider that the infection by BCG is cured too rapidly to produce lasting immunity. In their opinion, frequent re-vaccination is necessary to maintain immunity.

TZEKHOVITZER AND KOCHKINE (288, 289, and document C.H./BCG/3), at Kharkoff, have carried out a great many premunising experiments on guinea-pigs and rabbits by various routes. Their first results may be summarised as follows (288):

1. The absorption of BCG *per os* by young guinea-pigs against inoculation *per os* with virulent human or bovine bacilli only prolonged the lives of the vaccinated animals as compared with the controls.

2. It was ineffective against a virulent subcutaneous inoculation.

As regards rabbits, these authors have noted great irregularities in the lesions produced by virulent bacilli in the vaccinated animals.

Further experiments (289) gave rather more favourable results : a subcutaneous injection of 25 mg. of BCG into 3 guinea-pigs was followed after 1½ months by the absorption of 10 mg. of Vallée's bacillus *per os*.

Three months after this, 2 controls showed generalised tuberculosis on post-mortem examination, while the third had a discrete tuberculosis.

The vaccinated animals killed after 4½ months showed only very slight lesions.

BUKHOVSKY (289) caused 14 3-week-old rabbits to absorb daily meals of BCG, 8 of 20 mg. and 2 of 24 mg. (= 208 mg.). Two months later, together with 10 controls, they received *per os* 2 doses of 5 mg. of virulent Vallée's bacillus at 24 hours' interval.

Of the 10 controls examined after death, from 2 to 7 months after inoculation, 7 were tuberculous; 1 had a histologically verified tuberculosis of the kidney; 2 were healthy.

Of the 14 vaccinated animals, two showed tuberculous lesions, 12 were quite sound.

This very favourable result may be due to the fact that the rabbits used were very young.

TZEKHOVITZER (document C.H./BCG/3) gives an account of his experiments on subcutaneous vaccination against infection by inhalation of a virulent strain (Vallée). The doses of BCG employed were 5 mg. Infection was brought about after 35 days by means of a mechanical inhaler with a 1/100,000 bacillary emulsion. The autopsies conducted shortly after infection showed, in general, tuberculous lesions, in a more advanced stage in the controls than in the vaccinated animals. From all the experiments conducted on rodents by members of the Ukrainian BCG Commission, TZEKHOVITZER (document C.H./BCG/3) concludes that the vaccination of these animals has given favourable results *in comparison* with the controls, and that the results seem to depend on the age of the animals, the method of administering the vaccine, and the infecting dose.

ZOZAYA (312), in Mexico, has also only been able to note a prolongation of life, as compared with the controls, in vaccinated rodents, in which the generalisation of tuberculosis is delayed to a certain extent.

* * *

In short, apart from a few isolated satisfactory results, almost all the experiments in vaccinating laboratory rodents

with BCG have, like Calmette's experiments, proved unsatisfactory as regards absolute protection; in most cases, vaccination has only retarded the fatal development of the tuberculosis inoculated.

2. PREMUNISING EFFICACY OF BCG FOR GOATS AND PIGS.

ASCOLI (9) has verified the protection conferred on goats by the ingestion of BCG *per os* and its subcutaneous injection against intravenous inoculation with virulent bovine bacilli. Subcutaneous premunisation has given appreciably better results; it is only fair to say that the goats were more than 10 days old.

Ascoli has, however, experimented on pigs under 10 days old, and has compared in their case the degree of protection afforded by buccal and subcutaneous premunisation respectively. Premunisation is equally effective under either method against small doses of virulent bovine bacilli. Against large doses the protection conferred by injection is, in his opinion, much greater.

3. PREMUNISING EFFICACY OF BCG IN MONKEYS.

It should be remembered that, after CALMETTE'S first experiments on *Macacus sinicus* and cynomolgus (310) with doses varying from 1 to 5 mg. administered parenterally, WILBERT re-examined the question of the innocuousness and efficacy of BCG in these animals in Guinea, where there was less likelihood of their having been accidentally contaminated with tuberculosis than in European laboratories. He satisfied himself that the virulent bacilli at his disposal ("Ratti HR" human and "Vallée" bovine BV) were fatal to monkeys. Chimpanzees and pitheciens died in from 3 to 6 months with doses of 1/100,000th of a milligramme (400 bacilli) subcutaneously or 2 doses of 1/1,000th of a milligramme *per os*; larger doses accelerated the fatal development of the infection.

Sixteen vaccinations were carried out, as follows:

4 by injection of 3 meals of 50 mg. of BCG;

12 by a single hypodermic injection of 50 mg.

Twenty apes were infected:

10 by 2 injections of 0.001 mg. each of virulent HR or BV strain;

10 by single subcutaneous injection of 0.00001 mg. of BV or HR.

There were 20 controls.

Infected animals, vaccinated animals and controls were placed in each cage.

Of the 19 vaccinated animals (between September 1923 and February 1925), which had all lived in cohabitation with

infected pitheciens and healthy controls, on June 15th, 1926 :

6 were still in good health ;

11 had died — 3 of malaria, 3 of trauma, 2 of pasteurellosis, 1 of bacillary dysentery, 1 of cerebral haemorrhage. None of these showed any tuberculous lesions on post-mortem examination.

Of the 20 *infected* monkeys :

19 had died of generalised tuberculosis ;

1 of intestinal obstruction 8 days after inoculation.

Of the 20 *controls* (contact infection) :

19 had died of tuberculosis (1 after 2 months, 6 after 3 months, 12 between 4 and 5 months, and only 1 after 9 months) ;

1 died of pasteurellosis after 15 days.

From these experiments we must conclude that BCG is effective as a premunising agent for more than a year against infection through contact with several tuberculous animals. It was possible to maintain immunity by further absorptions of BCG without any ill-effects.

Stanley GRIFFITH (114), working in England on *Macacus rhesus*, was not able to obtain similar favourable results. He prepared his animals by injection or ingestion of BCG.

Ocular instillation of 0.01 mg. of bovine bacillus caused the death by tuberculosis of a vaccinated animal. The same occurred in the case of the cohabitation of a vaccinated animal with a tuberculous monkey. About three-fifths of the vaccinated animals died of tuberculosis, as against two-thirds of the controls. He therefore naturally concludes that BCG is ineffective as a premunising agent.

A. SCHLOSSMANN (document C.H./BCG/4), at Düsseldorf, has also conducted experiments with a view to protecting monkeys (*Macacus rhesus*) from infection when living with infected monkeys.

Eight artificially infected monkeys were used as infective agents against 11 vaccinated monkeys and 20 controls ; 5 vaccinated monkeys and 5 controls became tuberculous, while 6 in each group remained healthy.

The 11 vaccinated animals received the following doses of BCG :

1, intravenous, 15 mg., remained healthy ;

1, intravenous and subcutaneous, 15 mg., developed tuberculosis ;

1, intravenous, 30 mg., remained healthy ;

4, intravenous and subcutaneous, 30 mg., 2 contracted tuberculosis, 2 remained healthy ;

4, subcutaneous, 50 mg., 2 tuberculous ; 2 healthy.

There is therefore no appreciable difference between the results for the vaccinated monkeys and the controls.

On the other hand, Schlossmann has noted that, whereas all the monkeys housed in cages in the open air survived in good health, those whose cages were indoors all died.

TZEKHNIVITZER and his collaborators (document C.H./BCG/3) vaccinated subcutaneously with a dose of 50 mg. 3 *Macacus* of 4,140 to 6,600 gms. after verifying the fact that they gave a negative tuberculin reaction. These animals and 2 controls were inoculated with 0.0001 mg. of Vallée bacilli respectively after 1½ months, 7 months and 13½ months, as well as 2 controls (3,400 to 7,120 gms).

One control died after 1½ months; the vaccinated monkeys died of generalised tuberculosis after 5½ months.

The last control was killed after 5½ months and was found to have contracted a pronounced tuberculosis.

The monkeys in question had already been used for measles and scarlet-fever experiments; they never reacted to tuberculin even after virulent inoculation.

4. PREMUNISING EFFICACY OF BCG IN CATTLE.

It may be desirable to recapitulate the essential data of the main experiments conducted by CALMETTE and GUÉRIN on the vaccination of cattle.

I. So far back as 1911 (45), Calmette had proved that cattle which had received in their veins up to 200 mg. of BCG could readily tolerate the intravenous inoculation of virulent bacilli.

II. His first systematic attempts to vaccinate against artificial infection go back to 1913 (46, 47).

Eight heifers, 9 months old, received intravenously, at one-month intervals, 1 and then 5 mg. of BCG (at its 34th passage on bile); then 3 mg. of virulent bovine strain (S. Nocard), which was also given to a control.

The latter died of acute tuberculosis on the 34th day, whereas the vaccinated animals remained in good health. They were killed at various times between the 1st and 18th months, and were found to be free from tuberculosis (although their glands, inoculated into guinea-pigs, infected the latter with tuberculosis).

III. The second series of experiments (1919-24) (51) was concerned with *subcutaneous* vaccination, which other experiments had shown to be preferable to intravenous vaccination.

The immunising doses employed were 50 mg. in a single injection for 6 heifers and 100 mg. for 6 others. Eight controls were placed among the vaccinated animals. The virulent inoculations were made every three months after premunisation: they were given in doses of 5 mg. of Vallée

strain intravenously. In all the vaccinated animals there was a violent thermic reaction for 24 hours, followed by return to health. In the controls, the fever made its appearance on the 13th or 14th day only, and continued until death, which occurred between the 30th and 45th days. Two controls, still alive but emaciated and ill, were slaughtered on the 60th day: they also had miliary tuberculosis.

The vaccinated animals killed 2, 3, 8, 11 and 12 months after the test inoculation did not show any tuberculosis. The glands of those killed 11 and 12 months after inoculation were no more virulent for the guinea-pig (like those of the animals (58) vaccinated intravenously) (Exp. II).

IV. The first experiments in the protection of cattle against contact infection were unfortunately brought to a premature close by the war (58).

Vaccinations were made intravenously, and were repeated once or twice in the same manner. At the end of the observations, 32 months after the beginning:

Of 4 controls, 3 showed pulmonary tuberculous foci; 1 was unscathed, none had shown any clinical symptoms.

Of the 3 animals which had received only one pre-munising injection, 2 showed a few tubercles in the lungs and in the mediastinal and mesenteric glands.

The other 4 vaccinated animals were free from tuberculosis.

V. We would merely remind the reader of GUÉRIN, RICHART AND BOISSIÈRE's experiment (116) on protection against natural infection, which succeeded, by simple parenteral vaccination of newborn calves and their annual re-vaccination between the years 1921 and 1926, in eliminating tuberculosis from an infected byre. Of the 110 cattle thus vaccinated, 30 were slaughtered on account of old age and post-mortem examination did not reveal any tuberculous lesions.

ASCOLI (10, 13) has conducted several similar experiments on the pre-munising efficacy of BCG in cattle in the neighbourhood of Milan.

At *Stradella*, 7 heifers vaccinated hypodermically and 3 controls were inoculated with the same doses. An epidemic of septic pneumonia which broke out in the byre made it impossible to follow the tuberculosis infection up to its natural termination. The post-mortem examinations showed tubercular lesions in the lungs of the controls only. Two vaccinated heifers survived.

At *Cascinazza*, 2 calves vaccinated in February 1925 and re-vaccinated in February 1926 were inoculated intravenously in April 1926, as also were 2 controls, with tuberculosis virus isolated from an ox of the same stock. The controls sickened

and died 27 and 30 days after the test inoculation; the vaccinated animals survived in good health and gained weight normally.

LANGE AND LYDTIN (172) have carried out the following experiments:

I. Intravenous inoculation of 2 mg. of Vallée's strain into calves which had received 4 months previously:

1, 20 mg. of BCG intravenously: slaughtered after 75 days; no tuberculosis.

4, 160 mg. of BCG subcutaneously: 1 died of miliary tuberculosis on the 56th day; 3 survivors were slaughtered on the 76th day, microscopic tubercles (BCG lesions?).

4 controls: 2 died on the 10th and 25th days; 2 slaughtered, showing small pulmonary lesions and microscopic tubercles.

II. Intravenous inoculation of 20 mg. of Vallée's strain 6½ months after premunisation with 100 mg. of BCG subcutaneously.

2 vaccinated animals, 2 controls: all died in about 20 days.

The Vallée strain used killed a control in 31 days at 5 mg. and in 38 days at 0.5 mg.

The authors conclude that the intravenous is preferable to the subcutaneous route, which they consider ineffective from the point of view of premunisation. It is unfortunate that the animals experimented upon were slaughtered so soon (after 75 days); this, in the first experiment, made it impossible to see the ultimate effect of vaccination and of the virulent infection in the controls. As, moreover, there is no indication regarding the results of inoculation of the lesions into guinea-pigs, it is difficult to gather whether the phenomena observed in the vaccinated animals were BCG lesions which had not yet receded, or actual tuberculous lesions in course of development.

In the second experiment, the dose of 20 mg. of virulent bacilli by the intravenous route would seem to be capable of overcoming any resistance. (It is equal to 40 times the dose which was fatal in 38 days.)

LIGNIÈRES has verified the protection conferred by BCG on calves against infection by cohabitation (183). Studying the relations between tuberculosis immunity and allergy (185), he points out that calves which received 50 mg. of BCG subcutaneously resisted the test infection. With a dose of 6 mg. of BCG they did not resist, and their tuberculin reaction was irregular. With a dose of 2 mg., there was neither resistance nor allergy.

MELLO (199) vaccinated newborn calves subcutaneously with 50 mg. of BCG. Ten days later he inoculated one of them intravenously with 3 mg. of virulent bovine bacilli, which

produces fever in vaccinated animals, but mere uneasiness in controls. A post-mortem examination showed discrete tuberculous lesions, a few caseous nodules, and mesenteric adenitis. Another which received the same infecting dose but 30 days after premunisation, did not show any microscopic lesions or tuberculosis bacilli.

The control showed generalised tuberculosis.

Other experiments on 23 calves led the author to conclude that BCG given to calves very shortly after birth confers complete resistance to small doses of virulent bacilli and to attenuated tuberculosis bacilli; that in the case of infecting inoculations in huge doses it is not always possible to confer complete immunity; and that the effect of premunisation in these cases is to limit the lesions and lengthen the survival period.

Mello's experiments show how necessary it is to wait, not 10 days, but 1 month, before making the test inoculation after vaccination, in order that the vaccination may have time to act.

TZEKHOVITZER, KRAFT-PALEI, ELENIEVSKI AND PETROFF reported in 1927 (288) the following experiments :

Thirteen cattle received doses of 83, 166 and, in 2 cases, 100 and 300 mg. of BCG subcutaneously, and then, after 7 or 8 months, 5 mg. of the Vallée strain.

All survived in good health without fever and fattened satisfactorily, except one, which died from another cause (the post-mortem revealed no tuberculous lesions 1 ½ months after inoculation).

The 3 controls died in less than a month and a-half of miliary tuberculosis.

Some of the vaccinated animals showed a very small number of tubercles in certain organs. Histologically, they were still circumscribed by connective tissue, from the surrounding tissue, and were quite different from the tubercles observed in the control animals.

Of the 2 animals vaccinated intravenously with 100 and 200 mg. of BCG respectively, the former, in excellent health when slaughtered, showed a small caseous nodule of indeterminate character in the left lung. The other had no trace of tuberculosis.

These authors conclude that there is a clear resistance to virulent intravenous infection.

The more recent experiments of ОВУКHOVSKI (document C.H./BCG/3) give similar favourable results.

I. Six cattle which had received first 100 mg. of BCG subcutaneously and then 5 mg. of Vallée bacilli remained *outwardly in good condition*. When slaughtered, they showed at the post-mortem :

- 1, a specific induration of the right lung;
- 4, an engorgement of the mediastinal glands;
- 1, nothing.

Seven controls, all clinically tuberculous, showing at the post-mortem tubercles evolving in the lungs and bronchial glands.

II. Four cattle premunised with 100 mg. of BCG received, with 1 control, after 16 to 18 months, 10 mg. of Vallée bacilli.

The control died in 25 days of miliary tuberculosis;
1 vaccinated animal died in 27 days of miliary tuberculosis;

3 vaccinated animals survived in good health.

* * *

We have already referred, in the chapter concerning the innocuousness of BCG, to the experiments conducted by WATSON (301), who, in a first series, found no tuberculous lesions in any of the vaccinated animals exposed to contact by cohabitation, whereas, in the second series, all the vaccinated animals and only one of the controls died of tuberculosis, which would suggest that the culture was not pure.

Experiments conducted on cattle with BCG, and thousands of vaccinations carried out on cattle in various countries, clearly prove the innocuousness of BCG. Its efficacy against contact tuberculous infection, experimentally proved by CALMETTE, GUÉRIN and their collaborators, in doses of 50 to 100 mg. administered subcutaneously, has been confirmed by LIGNIÈRES. Vaccinations carried out under the direction of ASCOLI, BERGER and OBUKHOVSKI in Italy, Holland and the Ukraine, not to mention those made by GUÉRIN in France, seem to confirm the efficacy of the product in this dose and administered by this route. This will be definitely proved when animals isolated and fed on sterilised milk during the first month have been observed over a sufficient period of time, and been finally examined post-mortem.

Protection against test intravenous inoculation by subcutaneous premunisation with doses of 50 mg. of BCG, obtained by Calmette against a dose of 3 mg. (Nocard strain) and 5 mg. (Vallée strain), has also been obtained against doses of 3 mg. (Vallée strain) by MELLO (199), 2 mg. of the same strain by LANGE and LYDTIN, in 3 cases out of 4, by TZEKHOVITZER against 5 mg., and by OBUKHOVSKI even against 10 mg.

It has been proved by MELLO, on the other hand, that a virulent inoculation given in these same doses too soon after vaccination is fatal, as is also the heavy dose of 20 mg. (LANGE AND LYDTIN).

It should not be forgotten that 0.5 mg. of Vallée strain can cause the death of controls in less than 40 days.

5. PREMUNISING EFFICACY OF BCG IN MAN.

A. Vaccination of Infants per os.

1. The first vaccinations of human beings with BCG were carried out by WEILL-HALLÉ AND TURPIN (302, 303, 305, 307) from July 1921 onwards by ingestion. They established the complete tolerance of the intestine to 3 successive doses of 10 mg. of BCG, or 1,200,000,000 bacilli each. Of 120 patients vaccinated in 1921, 80 were found to be in good health in 1928; 24 were living in an infected environment.

2. From 1922 to the end of 1925, WEILL-HALLÉ AND TURPIN vaccinated 317 infants, 57 of whom were living in an infected environment. By January 1st, 1927, only 1 death from tuberculosis had occurred in this group.

The increase in weight of the vaccinated infants was normal.

3. After July 1924, French medical practitioners and dispensaries began to vaccinate with BCG furnished by the Pasteur Institute. The Institute supplied the vaccine free of charge and, by sending questionnaires to the medical practitioners, obtained a succession of reports as to the condition of the various vaccinated infants which enabled the observation cards to be kept up to date.

In 1927, the material thus collected was embodied by MOINE (202) in a report dealing with 981 infants in tuberculous surroundings, vaccinated between July 1st, 1924, and February 1st, 1926. This author gave the following death rates among children vaccinated one or two years previously :

	Number of deaths	Number of infants	General mortality %	Mortality from tuberculosis %
Infants in contact with tuberculous mother	17	303	6.3	0.7
Infants in contact with tuberculous father	15	288	5.69	1.14
Infants in contact with tuberculous father and mother ...	4	35	12.9	0
Infants in contact with tuberculous collateral relatives ..	4	86	4.97	1.22
Infants in contact with unspecified tuberculous persons ..	39	257	16.5	0.5
Infants born of tuberculous mothers separated at birth..	1	12	8.3	8.3

4. BIRAUD (32) studied the observation records of 1,050 vaccinated infants living in contact with tuberculous persons. He computed the following mortality rates, using the life-table method :

		Mortality in the first year		Mortality in the second year	
		General %	From tuberculosis %	General %	From tuberculosis
Vaccinated infants living in contact with tuberculous persons.....	1.050	7.6	1.55	1.88	0
Vaccinated infants living in contact with tuberculous mothers.....	487	6.6	2.46	1.04	0

5. OTT (64, 65) gives the following figures for vaccinated infants in the Department of Seine Inférieure on January 1st, 1927 :

Infants vaccinated between July 1st, 1924, and January 1st, 1927 : 228

Number whose whereabouts could be traced on the latter date : 224.

Infants living in contact with tuberculous persons; number having : died 24 out of 181.

Infants not living in contact with any person known to be tuberculous; number having died : 8 out of 43.

Six deaths had been attributed to tuberculosis.

6. MORLOT (58) recorded the following figures on January 1st, 1927, at the Havre Dispensary : 4 deaths among 44 vaccinated infants; none attributed to tuberculous disease.

7. CALMETTE has also published (55) in summarised form the observation records of the vaccinated infants whose deaths were attributed to tuberculosis : the ætiology of the tuberculous infection remained obscure in one case only; in the others, the source of infection was evident and the disease might perfectly well have been contracted during the period preceding the establishment of immunity.

8. Later publications by CALMETTE (64, 65, 66) contain more recent figures with regard to the same data, the percentages calculated being even lower¹.

Calmette draws a favourable conclusion as to the efficacy of BCG vaccination *per os* by comparing the general death rate and the mortality from tuberculosis among the vaccinated, first with the infantile mortality in the general population [8.15% (Moine) or 7.6% (Biraud), as against 3.7%] and then with the corresponding figures for such infants living in a tuberculous

¹ Professor Calmette also publishes in the *Annales de l'Institut Pasteur*, December 1923, a summary compiled from the particulars of infants living in contact with tuberculous persons which were obtained by sending questionnaires to French medical practitioners and dispensaries in July 1928.

environment, in different towns and at different periods. These figures vary considerably in relation to each other according to the period, the social environment and the degree of infectiousness of the contact concerned.

50.5 % (WEINBERG): mortality during the first year of life among children of tuberculous mothers, at Stuttgart, 1873-1889.

50.28 % (STRAUSS): mortality during the first year of life in poor tuberculous families in Paris, 1906-1911.

52.6 % (GUILLON): mortality during the first year of life among patients attending the anti-tuberculosis dispensaries in the Department of the Seine, 1925.

80.0 % (BERNARD, DEBRÉ, LELONG): infants of tuberculous mothers, not separated, Paris, 1925.

20.0 % (FALLOISE): mortality from tuberculosis during the first year of life among infants born of tuberculous mothers.

24.0 % (CALMETTE): mortality from tuberculosis during the first year of life among children of tuberculous mothers attending the French dispensaries between 1922 and 1925.

Having regard to these figures, Calmette is of opinion that the infant mortality rate among infants living in contact with tuberculous persons in *France* is at least 25 % and considers that the reduction from 25 % to 7-8 % should be attributed to the effects of vaccination.

* * *

A large number of workers have published figures of the death rate among infants vaccinated by them. Of these, we can only give a brief extract. It must be admitted that the majority of these statistics are only provisional and incomplete, and that many of them afford an indication rather than proof of the premunising action of BCG.

In *Algeria*, ROUGEBIEF (250) reported 623 vaccinations carried out in 1925 and 1926. Of 60 infants kept under observation for over a year (22 living in tuberculous surroundings), 12 died, but none of tuberculosis.

In *Belgium*, MALVOZ AND VAN BENEDEN (21, 22, 196) reported 386 vaccinations in 1924, 1925 and 1926. An investigation was made in 1926 concerning:

23 infants vaccinated more than 2 years previously:
16 followed up (10 in contact with tuberculous persons);

2 deaths not due to tuberculosis.

115 infants vaccinated from 12 to 24 months previously:
53 followed up:

10 not in contact with tuberculous persons; 5 deaths not due to tuberculosis;

34 in contact: 1 death from tuberculosis (after whooping-cough); 1 death not due to tuberculosis.

BUSCHMANN (42) vaccinated 89 infants between 1925 and July 1927, 30 of whom were kept under observation for 2 years. He performed Pirquet skin-tests in series and found repeated variations in the reaction of the same individuals to this test. He noted a higher proportion of positive skin reactions among infants living in contact with tuberculous persons (20 to 26%) than among the others (13.3% at 4 to 6 months). He believes that protection is afforded against natural infection.

In *Bulgaria*, PETROFF AND MAYANOFF have prepared BCG at Sofia and have supervised vaccination. For the period October 1926 to December 15th, 1927, particulars are as follows:

352 vaccinations were carried out; 224 of the vaccinated children were re-inspected in 1927; 13 deaths.

Of 185 not living in contact with tuberculous persons: number living, 176; number having died of non-tuberculous disease, 9.

Of 52 living in contact with tuberculous persons: number living, 48; number having died, 4, including 1 death from tuberculous broncho-pneumonia at 10 months (tuberculous father and mother); number having died from other diseases, 3.

In *Canada*, 381 vaccinations were carried out in 1926 and 1927, under the supervision of the School of Social Hygiene at the University of Montreal in that city:

Among 234 vaccinees, kept under observation, 9 deaths had occurred on December 31st, 1927, none being due to tuberculosis.

Of 15 vaccinees, kept under observation and living in contact with tuberculous persons, 14 were living; 1 had died of capillary bronchitis.

In *Spain*, over 2,000 infants have been vaccinated at Madrid, Seville, San Sebastian, Gijon and Barcelona.

The following results were obtained at La Inclusa, Madrid (foundling institute), by BRAVO Y FRIAR AND MUNEYERRO:

Vaccinated in 1927: 358 infants, 880 controls. *Post-mortem examination was carried out in the case of every death.*

Of the 358 vaccinated, 250 were living, 158 died. The post-mortems showed that only 4 deaths were due to tuberculosis, this being 1.1 death from tuberculosis per 100 vaccinees.

Of the 880 controls, 287 died: 34 from tuberculosis = 3.8%. 593 were living.

The heavy general death rate in both groups is explained by the special conditions under which they are recruited: illegitimate and deserted children; artificial feeding and infection which plays havoc in a community of infants.

From November 1924 to August 1928, SAYÉ and his co-workers DOMINGO and MIRALBELL (256, 257, 258) (document C.H./BCG/12) vaccinated 442 infants, 277 of whom were followed up and carefully watched.

Among the 218 living in a tuberculous environment, there occurred: 48 deaths (18.34 %), including: 5 from tuberculosis, or 2.29 %; 6 from presumed tuberculosis, or 2.75 %; 29 from other diseases, or 13.30 %.

Among the 59 living in a healthy environment, there were: 11 deaths, or 18.64 % (none from tuberculosis).

These death rates relate to the total deaths during the 1st, 2nd and 3rd years and the first 10 months of the 4th year.

The total tuberculosis mortality during that period was: $11/218 = 5.04\%$; and $50/285 = 17.54\%$ (in an unvaccinated group of controls in the same environment).

Infants living and *healthy*: vaccinated, $169/218 = 76.14\%$; controls, $44/101 = 43.56\%$.

Details of these observations, together with a more complete analysis, have been published in document C.H./BCG/12.

In the *United States*, William PARK (222) vaccinated 50 infants: 4 deaths, none due to tuberculosis. Among 100 controls: 7 deaths from tuberculosis.

In *France*, ARMAND-DELILLE (3) has reported the following results of vaccinations carried out in Finistère:

Among 303 vaccinees in contact with tuberculous mothers: general mortality, 6.3 %; mortality from tuberculosis, 0.7 %.

Among 288 vaccinees in contact with tuberculous fathers: general mortality, 5.6 %; mortality from tuberculosis, 1.14 %.

BRAULT AND ROCHARD (40) vaccinated 220 infants; 200 were kept under observation; many in a suspect environment; 9 exposed to heavy infection: 5 deaths, of which 1 due to tuberculosis.

WEILL-HALLÉ (305) also carried out vaccinations *per os* in Paris.

Of 469 vaccinees, 317 were kept under observation.

Among 236 of these, living in a healthy environment, there were 7 deaths; among 67 living in a tuberculous environment, there were 7 deaths, including 1 or 2 from tuberculosis.

Total number of deaths from various causes among 469 vaccinees: 33, or 7.1 %.

The 33 deaths can be classified as follows :

6 early deaths during the first 2 weeks, coinciding with small hospital epidemics ;

16 deaths occurring later from non-tuberculous diseases ;

10 due to undetermined causes ;

1 definitely ascertained to be due to tuberculosis, at the age of 4 months : infant fed by tuberculous mother.

In *Greece*, LAMPADARIOS (168) reported 699 vaccinations carried out from April 1925 to April 1928 :

540 were followed up, including 74 in a tuberculous environment :

Among 466 under observation in an uninfected environment : 31 deaths not due to tuberculosis ; 1 death from tuberculosis (doubtful).

Among 74 under observation in an infected environment : 1 death not due to tuberculosis, and a death from tuberculosis.

In the *Netherlands*, Heijnsius VAN DEN BERG (133) carried out vaccinations at Amsterdam on a limited group maintained under particularly careful observation ; moreover, the infants were kept in hospital during the first weeks of life, thus enabling the BCG to act under ideal conditions.

80 vaccinated, 22 of them in the manner prescribed by Calmette : 1 death from tuberculosis at the age of 5 months, or 6 % ; 1 death from syphilis. The remaining 20 were living at 18 months.

50 controls : 12 contracted tuberculosis during the first 18 months ; 9 deaths (18 %) ; 1 death from pneumonia. The post-mortem examinations confirmed the diagnosis.

Heijnsius van den Berg concluded that BCG is effective as a prophylactic, although tuberculin allergy did appear only in the case of the infants living in contact with tuberculous persons.

In *Italy*, A. ASCOLI (13 and 14) carried out 222 vaccinations from 1925 to June 1928 at the Anti-Tuberculosis Vaccine Institute at Milan on infants living in a tuberculous environment, only 21 deaths occurring between the ages of 16 days and 11 months (all in children of tuberculous parents) ; only 1 case possibly attributable to tuberculosis after whooping-cough.

In *Mauritius*, the BCG vaccine prepared by BARBEAU AND MAYA was used by 36 medical practitioners, who carried out 2,400 vaccinations between September 1925 and December 31st, 1927.

Among 787 vaccinees in respect of whom the questionnaires were completed, the general mortality was 4 %, no death being attributed to tuberculosis.

The mortality in the island among unvaccinated infants of the same age (0 to 19 months) was 11.9 %.

Of 28 vaccinated who lived in contact with tuberculous persons: 4 died from non-tuberculous diseases; 24 are normal. (Communicated by Dr. Maya.)

In *Roumania*, a widespread movement in favour of vaccination has been started under the guidance of CANTACUZÈNE (71), particularly in the towns. At Bucharest, 65 % of the newborn babies are vaccinated.

CANTACUZÈNE AND MIHAIEȘTI (72) report that the number of infants vaccinated rose from 938 in January 1927 to 2,274 in March 1928, of whom over 600 were in Bucharest.

Vaccination appears to be accompanied by a considerable decrease not only in the mortality from tuberculosis but also in the general death rate.

At Bucharest, in 1926-27, 238 deaths occurred among 5,767 vaccinated infants (4 %), of which 1 only was due to tuberculosis, whilst the infant mortality among the unvaccinated amounted to 22.1 % in 1927.

Of 101 newborn babies living in an infected environment, only 1 died.

At *Craiova*, the general mortality among the vaccinated in 8 months was 6.4 %, as against 26.4 % among the unvaccinated.

At *Jassy*, it was 5 %, as against 15 %.

These figures are very suggestive, although the percentages calculated in respect of the vaccinated do not correspond to real infant-mortality rates, as the observations did not always extend over a complete year.

In *Sweden*, at Falun, between August 1926 and December 1927, MALMSTRÖM (195) vaccinated 25 infants, all of tuberculous parents, 3 being separated at birth.

In December 1927, 24 were in good health; 1 death (premature child, died at 4 months from ordinary bronchopneumonia).

Enquiries made by this worker in the same province (Dalecarlia) among the tuberculous families supervised by the dispensaries showed that the mortality during the first year was 11.5 % (18/156) and the infantile mortality from tuberculosis 4.5 % (7/156).

The total mortality between the ages of 1 and 2 in this environment is 6.3 %, and the mortality from tuberculosis, 5.5 %. There is thus an appreciable difference between the vaccinated and unvaccinated.

In *Ukraine*, between November 1925 and August 1927, JAKHNIS (138) vaccinated 472 infants, 404 of whom were

kept under observation. On April 15th, 1927, their situation was as follows :

	Living	Dead
Not in contact with tuberculous mothers	156	19
In contact with suspects	112	6
In contact with persons with active tuberculosis	95	—
In contact with persons with open tuberculosis	41	1 (pulm. tbc.)
	404	26

Jakhnis noted that the percentage of tuberculin reactions was higher in proportion as the risks from tuberculous infection were greater. He finds these results encouraging, but is of opinion that a local comparative survey of the mortality of unvaccinated infants in a tuberculous environment is necessary.

With certain exceptions, such as the figures prepared by Heijnsius van den Berg, Malmström, Sayé and a few others, the statistics relative to vaccination *per os*, a brief extract from which has been given above, are not sufficiently accurate or complete to constitute experimental proof, and merely afford an indication as to the probable innocuousness and value of the Calmette method.

Criticism of Calmette's Statistics.

Since Calmette has resorted to statistics as a means of proving the efficacy of the BCG method, his statistical reports have naturally been subjected to careful scrutiny and criticisms.

These criticisms have come from GREENWOOD (110, 111, 113) (London), and ROSENFELD (248) (Vienna), specialists in medical statistics, and a pediatrician, WALLGREN (299) (Göteborg). Other workers, moreover, have reproduced these criticisms in the medical press.

Some of the criticisms advanced are applicable not only to Calmette's statistics, but also to those prepared by other authors. We reproduce them in some detail, statistics being a subject which does not lend itself to summarised or general treatment. Moreover, by pointing out the defects of this method, we hope to secure their avoidance in future work.

The criticisms made are of two very different kinds : some relate to the methods used in collating the data concerning vaccinees and to the computation of the death rates, others to the selection made by Calmette in the statistical data concerning unvaccinated children (for purposes of comparison with the mortality of the vaccinated).

The defects of statistical method are of somewhat different kinds in Calmette's successive papers on the results of vaccination in adults :

1921 to 1926, *Annales de l'Institut Pasteur*, February 1926 (55) ;

June 1st, 1924, to January 1st, 1927, *ibid.*, March 1927 (60) ;

June 1st, 1924, to December 1st, 1927, *ibid.*, January 1928 (64).

The criticisms can be summarised as follows :

1. The information collected refers only to one section of the vaccinated, and involuntary selection may therefore have occurred, since the group concerning which the information is available may not give a true picture of the whole (248).

2. All the percentages that have been calculated do not represent real annual mortality rates, since they are based on the deaths of infants who had not completed their first year (55 and 64), and among whom a further number of deaths, increasing the percentage quoted, was obviously bound to occur before the completion of that period. This is a considerable cause of error, and its correction should roughly double the rates published. GREENWOOD (113), WALLGREN (299), ROSENFELD (248).

BIRAUD (32) and MOINE (202), who have avoided this error, have arrived respectively at general mortality rates of 7.6 % and 8.9 % for vaccinated infants living in contact with tuberculous persons (first year), working to some extent on the same observation records as those used by Calmette, who computed the rate at 3.1 % (64).

3. The mortality percentages in the case of the vaccinated are artificially decreased by the fact that the category " vaccinees " does not include : (1) infants dying during the very first days of life ; (2) some infants born prematurely, or in a weak condition, and not fit to be vaccinated.

The deaths of these infants affect the general infant-mortality rate and not that of the vaccinated, which is therefore decreased to that extent (248).

(During 1921-25, the mortality within the first four days alone amounted in France to 14.9 % of the deaths under 1 year, or 1.38 deaths per 100 births.)

4. The death rates from *tuberculosis* computed in the case of the vaccinated are misleading, for, in view of the difficulties of clinical diagnosis and the absence of post-mortem examinations, a large number of deaths attributed to causes other than tuberculosis (athrepsia, broncho-pneumonia, congenital debility, etc.) must in reality be due to that disease (248).

The chief criticisms referring to the *comparison* of the mortality of the vaccinated with that of the non-vaccinated are as follows :

1. The 1926-27 mortality rates for the first year of life among infants living in contact with tuberculous persons have wrongly been compared with :

(a) The mortality rates of unvaccinated infants living in a tuberculous environment, in a different country, at a period long past (1873-1889), seeing that infant mortality from tuberculosis has decreased since that date by nearly two-thirds in the country from which the statistics were drawn (Stuttgart);

(b) With the tuberculosis mortality rates of unvaccinated infants *born of tuberculous mothers* under the care of dispensaries (24 %).

In the series under consideration, the disease in the mothers was sufficiently severe to cause 54 % of them to die in less than three years.

For the comparison to be valid, the vaccinated would also have had to be in contact with their *tuberculous mothers* and the disease would have had to be as severe in the latter as in the other group (299).

Moreover, a part of the vaccinees belong to a higher social grade and so have normally a lower infant mortality rate than the unvaccinated infants of the class attending the dispensaries (248).

2. Calmette's comparison of mortality rates for vaccinated infants with proportional rates of mortality from tuberculosis (Seine-Inférieure), especially with the proportional mortality in hospitals, cannot be justified, as it may lead to mistakes in interpretation.

The same applies to the mortality rates in hospitals, which are always higher than those of the general population (248).

3. The comparison between the mortality from tuberculosis among the vaccinated and the unvaccinated is only allowable when the diagnoses have been made with the same accuracy in both groups (GÖTZL, 108).

4. GREENWOOD (113) points out certain errors of quotation in Calmette's paper as regards Weinberg's figures, which refer to the general death rate and not to mortality from tuberculosis, and, as regards those of RÖEPKE, which are based on 36 cases only, not 78.

Certain authors, KJER-PETERSEN and OSTENFELD (141), GREENWOOD (110, 113) and GÖTZL (108), have drawn attention to the low infant mortality among the dispensary patients at Copenhagen and Vienna (general mortality 7.7 % and 7 % respectively, and tuberculosis mortality 4.9 % and 3.4 %).

Indeed, the infant mortality at Vienna is lower among the population attending dispensaries (7%) than among the population as a whole. These figures are a proof of the prophylactic and educational efficacy of the dispensaries. Those who quote these figures incline naturally to the belief that the work of the dispensaries and their influence on mortality must be the same in France as in the towns mentioned above, and, consequently, that the death rate, even among the *unvaccinated* infants of dispensary patients, should not be appreciably higher than that of the general population.

The survey of the records of observations collected by the Pasteur Institute (32) which we made personally in 1926 led us to conclude, at the time, that the general mortality among vaccinated infants, whether living in contact with tuberculous persons or not, was appreciably lower than that of the general infant population in France. An examination of the various statistics, since compiled by other authors in France and abroad, gives the same impression in spite of defects of some of these compilations.

* * *

A different set of facts has been used to throw doubt on the innocuousness of BCG. We refer to observations relative to vaccinated infants who have presumably contracted or died of tuberculosis.

BAIGUE (18) reported to the Besançon Medical Society 3 deaths of vaccinated newborn babies from undetermined causes. The only post-mortem examination carried out, however, proved the absence of tuberculosis.

CHENARD AND FERRIER (74) reported a fatal case of suppurating adenitis in an infant vaccinated with BCG (Vitré) and the infection of her unvaccinated sister. This case was studied by LIGNIÈRES (188). It may be stated briefly as follows:

Denise D., born August 22nd, 1926, developed a swelling of the neck in March 1927, which became ulcerated: diarrhoea, temporary improvement and later an abscess on the buttock, cachexia, and death on November 12th, 1927 (15 months).

The pus in the sores contained an acid-fast bacillus, strepto- and staphylococci; the stools contained an acid-fast bacillus, termed "para-tuberculous" by the Pasteur Institute.

The sister of the above, born on August 15th, 1927, unvaccinated, developed at the age of 6 months, after 3 months' contact with the above, open adenitis with acid-fast bacilli which were identical with the Koch bacillus, but which, according to Lignières, behave like BCG inoculated guinea-pigs, and are not human bacilli (no tuberculation, no passage).

The sanitary conditions were poor and the child not well cared for, but the parents and cattle on the farm were healthy.

Lignières inferred from this that a fatal infection had been engendered by the BCG, "which would thus seem to be pathogenic under special conditions in the case of particularly susceptible subjects".

Mmes. GIROD AND DEBARGE (107) describe the case of an infant born prematurely in the Geneva Lying-in Hospital of a mother suffering from closed lymph-gland tuberculosis.

Weight at birth, 2,200 g.; vaccination on 3rd, 5th and 7th day *per os*; digestive disorders after separation from the mother on the 12th day persisting in spite of treatment; on the 24th day fever, diarrhoea, weight, 1,880 g.; death on the following evening.

Post-mortem examination: lungs normal; no glandular enlargement; small lenticular ulcers measuring from 2 to 3 mm. on the mucosa of the small intestine; other organs negative.

Histological examination of the various organs: negative like the Ziehl-stained slides, excepting a vertebra which showed acid-fast bacilli on the smears and in section.

Nothing to suggest that the ulcers were tuberculous (no bacilli, no tubercles in the mucous membrane, no swelling of local glands).

The author considers that the absence of any anatomical lesion shows that ingestion of the BCG does not provoke any tuberculous lesion even in a weakly infant.

TAILLENS (284) reports the case of a vaccinated infant at Lausanne who died of tuberculous meningitis at the age of 6 months.

"Father suffering from pulmonary tuberculosis; contact from time to time; three brothers and sisters died of 'bronchitis', 'broncho-pneumonia' and meningitis at the ages of 13, 3 and 4 months respectively.

"Fed at the breast until the age of 4½ months. The child was normal until then. At 5½ months: cough, arrested development. At the hospital: intradermal reaction positive; shadow of the hilus clear, rising to the right apex; fever round about 38°C. Torpor, cyanosis, Kernig's sign, lymphocytosis of cerebro-spinal fluid (200 lymphocytes per c.c.).

"No post-mortem examination. Clinical diagnosis: meningeal and pulmonary tuberculosis."

Tailens is uncertain whether this should be considered a failure of the method or a case of BCG tuberculisaton.

Now, the vaccination was only carried out on the 9th, 11th, 13th and 15th days, that is, after the time-limit laid down by Calmette, and the infant was not kept away from contact

with the father, which would appear to have been sufficient to produce fatal infection in the previous children.

NOBÉCOURT (212, 213) saw in his clinic at Paris 10 infants vaccinated with BCG. Five showed a positive tuberculin reaction, 4 had "tuberculous" lesions (3 cases of tracheo-bronchial glandular enlargement, 2 being slight), 1 case of mediastinal reaction with cutaneous and osseous foci of tuberculosis. This author wonders at what moment the infection took place — before or after the 4th week? He is quite prepared to believe that it was after.

In his opinion, these facts do not prove the danger or inefficacy of BCG, but the necessity of preventing contact with persons suffering from tuberculosis by removing the child at birth.

* * *

B. *Subcutaneous Vaccination of Human Beings.*

We have already alluded to the subcutaneous vaccinations carried out by WEILL-HALLÉ and other authors with a view to extending the advantages of premunisation to non-tuberculous persons who have reached an age at which the permeability of the mucous membrane of the digestive tract is no longer sufficient for the absorption of vaccine administered *per os*.

Certain Scandinavian workers have obtained significant results with this method.

HEIMBECK (125) (document C.H./BCG/6), noting the "havoc" which tuberculosis had been causing for some years among nurse probationers at the Ullevål Hospital at Oslo who began their service with a negative tuberculin reaction, vaccinated, in 1927, 44 newly enrolled nurses who showed a negative Pirquet reaction; 12 nurses refused to be vaccinated. These 44 nurses remained free from tuberculosis, while, of the 12 unvaccinated, 4 developed active tuberculosis (2 pulmonary tuberculosis, 2 erythema nodosum). The number of cases of tuberculosis which had occurred during the previous years among the nurses with a negative Pirquet reaction was as follows: 5/51 in 1924; 8/72 in 1925; 11/62 in 1926.

This observation by Heimbeck amounts to a real experiment. It should be noted that, after the first vaccination with hypodermic BCG, all the nurses showed a positive tuberculin reaction and that this also occurred in 160 out of 163 adults vaccinated outside the hospital, who were examined for allergy 2 to 4 months after vaccination. Moreover, these positive reactions persisted (one for more than 20 months).

WALLGREN (299) vaccinated 25 infants living in an infectious environment at Gothenburg with doses ranging from 0.01 mg. to 0.5 mg. In order to avoid infection during the first days, he kept the patients in hospital from 3 to 4 weeks after vaccination. The infants were kept under very careful observation, with repeated X-ray examinations. No case of clinical tuberculosis occurred. No death took place.

PARISOT AND SALEUR (196), at Nancy, carried out hypodermic vaccinations with a single dose of 0.01 mg. (400,000 bacilli) on 269 children of ages ranging from 1 to 16 years: 139 were in contact, or in danger of coming into contact, with tuberculous persons; 130 had no direct contact with tuberculous persons. These vaccinations were preceded by 2 tuberculin tests, clinical and X-ray examinations.

Local reactions were observed in 28 cases out of 269, especially at the beginning, when doses of 0.25 mg. were used: a few nodules. In 9 cases only (3%), these softened and burst outwards, after which they slowly healed of their own accord. No general symptom was observed, even among children living in contact with tuberculous persons and those previously vaccinated *per os*.

These investigators therefore arrive at the conclusion that the subcutaneous method is innocuous and consider it of great value in many cases where administration *per os* is impossible.

We merely mention in passing the subcutaneous vaccinations carried out in colonial environment by GIRARD (106), LEGENDRE, MATHIS, ROUSSEAU (252), GROSFILLEZ (115), N. BERNARD (29), etc.

6. TUBERCULIN REACTIONS OF VACCINEES.

Leaving entirely aside the question of the relationship between immunity to tuberculosis and tuberculin allergy, we can briefly summarise the facts ascertained in regard to the latter in vaccinated animals.

BORREL, BOEZ AND COULON (38) proved that BCG was capable of producing tuberculin, and, by comparative experiments with other bovine and human tuberculosis strains, they showed that there was no parallelism between the tuberculin-producing properties of these strains and their virulence.

BOQUET AND NÈGRE (36) showed that a single infection with 1 mg. of BCG was sufficient to produce a positive intradermal tuberculin reaction in a guinea-pig. In rabbits that had been given from 0.01 mg. to 0.001 mg. of BCG, a febrile reaction of 1° C. was observed after intravenous injection of 0.04 c.c. of crude tuberculin.

CALMETTE AND GUÉRIN (58, p. 78) noted that cattle reacted to tuberculin for a longer time after subcutaneous inoculation with BCG than after intravenous inoculation.

These workers consider that "sensitisation to tuberculin persists so long as the symbiosis of the tuberculosis bacillus continues with cellular elements which are not fixed by nature but have become so".

In their opinion, the tuberculin reaction "reveals the existence in the organism of an active or latent, newly constituted or old-standing focus of the symbiosis of a virulent or non-virulent bacillus with some lymphatic, macrophage or giant cell".

COULAUD (82) noted that rabbits inoculated intravenously reacted rapidly to tuberculin, whereas when they only developed discrete lesions after the ingestion of BCG they did not react, whatever the test dose might be.

IMAMURA AND TAKAHASHI (136) noted that allergy occurred in guinea-pigs vaccinated parenterally with 0.01 mg. of BCG. The heavier the dose injected, the more intense was the allergy. It was found possible to obtain allergy in certain cases with killed BCG.

KORSHUN and his co-workers (145) observed a positive intradermal reaction in the case of guinea-pigs 1 month after inoculation with BCG. This reaction persisted for several months (more than 7), diminishing progressively.

Bruno LANGE (173) obtained an allergic reaction by inoculating guinea-pigs intravenously (100% of the cases and, *per os*, 8 guinea-pigs out of 10).

On the other hand, he was unable to sensitise any one of 18 of these animals killed with bacilli administered *per os* in doses up to 1 g.

This investigator is of opinion that tuberculin sensitiveness and immunity are not necessarily connected.

LIGNIÈRES (185) noted allergic reaction in the case of cattle premunished with subcutaneous doses of 50 to 100 mg.; after 3 or 4 months, the febrile reaction to the tuberculin injection may disappear, but the local reactions — among others, the ophthalmic reaction — may persist. Later, until the 12th month, alternations of positive and negative reactions are recorded. This investigator considers that the protection only lasts as long as the allergy.

The dose of 6 mg. of BCG produces variable results as regards both allergy and immunity; the 2 mg. dose gives negative results in both cases. According to Lignières, 10 mg. of BCG given to newborn guinea-pigs *per os* in 4 doses does not cause allergy in more than half the cases. From this he

infers that, when the reaction is negative, the BCG has not been absorbed.

NÉLIS (211) noted the appearance of positive tuberculin reaction in guinea-pigs from 1 to 2 months after absorption *per os* of 36 cg. of BCG. It lasted from 6 to 10 months.

It was observed to appear earlier and to last longer when the ingested doses of BCG were heavier.

NOBEL (210) holds, with PIRQUET (229), LANGER (174) and the majority of the German workers, that immunity is proportionate to the tuberculin allergy, and that preimmunisation necessitates a positive allergic reaction; he is of opinion that a mild re-infection of a vaccinated subject should cause allergy.

SATAKE (255) has obtained variable tuberculin reactions after introduction of 1 to 5 mg. of BCG through the stomach of guinea-pigs. He noted resistance to inoculations of virulent human bacillus in cases giving a negative tuberculin reaction.

UHLENHUTH (291-293) considers that the tuberculin reaction gives no indication of the degree of immunity conferred by a vaccine. Like CALMETTE AND SELTER, he believes that calves possessing a high degree of immunity can react negatively to tuberculin, whilst, on the other hand, guinea-pigs preimmunised with BCG, and showing a very strong allergic reaction, do not resist virulent infection.

DOLD (91) made similar observations in the case of animals injected with killed bacilli.

In the case of infants vaccinated *per os*, WEILL-HALLÉ noted an appreciable difference between the number in a healthy environment and those in a tuberculous environment who reacted positively to tuberculin.

His percentages month by month are as follows :

Months	Infected environment %	Healthy environment %
3	11.1	2.5
6	16.6	4.1
9	25.9	5.8
12	26.6	7.6
15	44	7.4
18	50	12.9
24	60	28

After hypodermic vaccination, however, the reaction is invariably positive.

We have seen that the Scandinavian investigators obtained nearly 100 % positive tuberculin reactions in the case of subjects vaccinated hypodermically.

* * *

The foregoing proves that parenteral inoculation of BCG in animals as well as in human beings causes sensitisation to tuberculin after a certain lapse of time, which varies according to the dose and the method of introduction. On the other hand, the administration of BCG *per os* only produces this hypersensitiveness in an irregular manner.

In these circumstances, it was fairly natural for the majority of PIRQUET's followers to conclude that the allergy of subjects vaccinated *per os* is due to the non-absorption of BCG by the intestinal mucous membrane, and hence to regard this method of vaccination as prophylactically unreliable.

CALMETTE replies to this argument by showing the absence of correlation between immunity and allergy (in the case of cattle) and the priority of the one to the other in the case of guinea-pigs. He points out, in particular, that the presence of a small number of bacilli in the organism may produce either no allergic reaction or a very delayed reaction. In Calmette's opinion, the tuberculin reaction, which is more frequent among vaccinees living in contact with tuberculous persons, is due to the fact that the virulent bacilli which they receive produce follicular lesions or tubercles which cause hypersensitiveness to tuberculin, although the immunity acquired prevents the tubercles from developing and causing general infection.

Calmette, moreover, quotes his own earlier research work into the penetration of tuberculosis bacilli through the intestinal mucous membrane of young kids; that of RÖHMER, BEHRING and EHRLICH into the penetrability of the mucosa by toxins, experiments which were recently repeated and confirmed by RAMON and NATTAN-LARRIER.

We do not claim in this report to decide the important question of immunity to tuberculosis and of tuberculin allergy. We would merely observe that, leaving theoretical discussion aside, penetration of BCG can be demonstrated by direct and indirect proofs.

Among the former, we put the observation of slight and transient polyglandular enlargement and mediastinal gland reaction which follows upon the administration of BCG *per os* to newborn babies. This reaction was studied thoroughly by SAYÉ (document C.H./BCG/12, and also discussed by ASCOLI (13).

These observations might with advantage be extended in the future in order to throw light on the relations existing between the 'cryptic bacillary impregnation and the hypersensitiveness to tuberculin.

The detection of glandular hypertrophy and of the presence of BCG at the post-mortem examination of infants vaccinated *per os* who have died from intercurrent causes (64) is also a proof of penetration.

Leaving aside tuberculin reactions when positive in a healthy environment, we shall rank as merely indirect proof the same conditions observed in animals, and in particular the glandular hypertrophy, which can be easily felt *in vivo* through the abdominal wall in a guinea-pig premunised *per os*.

Indirect proof of a different kind, but of still greater significance, is furnished by the acquired resistance displayed by subjects vaccinated *per os*, as shown by certain statistics in which the morbidity and mortality from tuberculosis is particularly low in spite of unfavourable sanitary conditions and an infectious environment.

Part 3.

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V.

**LIST OF THE DOCUMENTS PREPARED FOR THE
CONFERENCE ON BCG**

- C.H./BCG/ 1. Professor PRAUSNITZ. — *Memorandum on Preventive Vaccination against Tuberculosis by means of BCG.* (F. and E.)¹
- C.H./BCG/ 2. Dr. H.C.L.E. BERGER. — *Vaccination Experiments on Bovines by means of BCG in the Netherlands.* (F. and E.)
- C.H./BCG/ 3. Professor TZEKHOVITZER. — *New Experiments on the BCG Antituberculosis Vaccine carried out by the Ukraine BCG Commission.* (F. and E.)
- C.H./BCG/ 4. Professor A. SCHLOSSMANN. — *Attempts at Vaccination of Monkeys against Tuberculosis.* (F.)
- C.H./BCG/ 6. Drs. J. HEIMBECK AND SCHEEL. — *Hypodermic Vaccinations of Adults carried out in Norway.* (F. and E.)
- C.H./BCG/ 5. HEALTH SECTION. — *List of References to the Literature on BCG.* (F.)
- C.H./BCG/ 7. HEALTH SECTION. — *Note on the Innocuousness of BCG.* (F.)
- C.H./BCG/ 8. HEALTH SECTION. — *Note on the Premunising Efficacy of BCG in Laboratory Animals and Cattle.* (F.)
- C.H./BCG/ 9. HEALTH SECTION. — *Note on the Premunising Efficacy of BCG in Man.* (F.)
- C.H./BCG/10. Professor R. KRAUS. — *Summary of the Results of Experiments on Vaccination by means of BCG.* (F.)
- C.H./BCG/11. Professor C. RONZONI. — *Present Status of Premunisation with BCG in Italy.* (F.)
- C.H./BCG/12. Professor L. SAYÉ. — *Observations of Infants vaccinated in Barcelona.* (F.)
- C.H./BCG/13. Professor LÉON BERNARD. — *Observations of Infants vaccinated with BCG (with Autopsy Findings).* (F.)
- C.H./BCG/14. Professor A. SCHLOSSMANN. — *Researches on BCG carried out in German-speaking Countries.* (F.)
- Professor Alberto ASCOLI. — *Vaccination against Tuberculosis by means of Live Bacilli in Animals and in Man.* (Milan, 1928.) (F. and Italian.)

¹ F. and E. stands for French and English texts ; and F. for French text.

- C.H. 745 (I) Professor A. CALMETTE. — *Preliminary Statement on the Method of Preventive Vaccination against Tuberculosis by means of BCG.* (F.)
- C.H. 745 (II) C. GUÉRIN. — *Note on the Innocuousness of BCG Vaccine in Cattle.* (F.)
- C.H. 745 (III) Dr. Y. BIRAUD. — *Critical Study of the Literature on BCG and Vaccination against Tuberculosis with Calmette's Method.* (F.)