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Bacillus Calmette-Guérin (B.C.G.)

Animal Experimentation and Prophylactic Immunization of Children

An Analysis and Critical Review

S. A. PETROFF, Ph. D., and ARNOLD BRANCH, M. D.

Research and Clinical Laboratory of the Trudeau Sanatorium, Trudeau, N.Y.

THE feverish enthusiasm manifested in the wholesale antituberculosis vaccination in European countries, and mainly in France, has accomplished one very good purpose. It has administered a stimulus to the stagnant question of prophylactic immunization.

The literature on B.C.G. vaccination is rapidly accumulating. Nearly 100,000 infants have been treated by this method. It is surprising that only a small number of them have come up for a statistical study. The favorable results reported have been gained largely from impressions and not from facts. On account of the many inquiries which have come to our attention the present review has been written.

The gradual decline year after year in the mortality from tuberculosis as shown by reliable statistics, is the best index that the campaign adopted years ago is sound and effective. This campaign has also influenced the death rate due to other causes, with the exception of heart disease and cancer. Education, sociological activities and various health measures stimulated by prosperity have played an important part in the improvement of living conditions and in the prolongation of life. However, this decline of mortality from tuberculosis has in no way influenced the investigator to discontinue his search for a method which can be safely used for vaccinating children against tuberculosis.

Attempts at prophylactic immunization against tuberculosis date from the time of the discovery of the microörganism. Koch, Pasteur, Trudeau, Dixon, Maragliano, Pearson and Gilliland, Theobold Smith, Calmette, Römer, Baldwin, Webb and Williams and many others have tried to devise some method which could be used safely for this

purpose. Many methods have been studied and recommended. The methods which most demand our attention today are three: Vaccination with (1) virulent living, (2) avirulent living, and (3) heat-killed virulent tubercle bacilli. In the following pages the discussion will be limited to the method advocated by Calmette and his coworkers, which consists of vaccination with avirulent living organisms.

PREMISES ON WHICH CALMETTE'S METHOD OF VACCINATION ARE BASED

The prevalence of tuberculous infection among children in the civilized world, as demonstrated by the tuberculin skin test, varies considerably. Hamburger and Monti [1] in Vienna reported 94 per cent positive reactions in children at the age of 12 years. In rural districts in Germany, Römer [2] found 66 per cent of the children to be infected at the age of 11. Very similar findings have been reported by S. Mueller [3] in Berlin, Moro [4] in Munich, Petruschy [5] in Dantzig and Northmann [6] in Dusseldorf. In Paris 35 per cent of apparently healthy children up to the age of 5 give a positive tuberculin reaction. Arnfinsen [7] studying 8000 children of various ages observed a positive reaction in 33 to 38 per cent. In the United States the incidence of infection detectable by the tuberculin reaction is not so great as that reported in European countries. Veeder and Johnston [8] in St. Louis observed a positive tuberculin reaction in 44 per cent of children from the ages of 10 to 14. Some children with clinical disease were included in this group. Slater [9] studied two groups of children in Minnesota: The first with no history of exposure reacted positively in 10 per cent, and the second who were living in tuberculous surroundings reacted positively in 80 per cent. Myers and Magiera [10] reported 41 per cent in a study of 2000 children in Minneapolis. Still,[11] in a group of 58 children with no history of tuberculosis and living in the lower East Side of New York City, observed only 9.8 per cent positive Pirquet tuberculin reactions.

The foregoing figures reveal the fact that by the time children reach the age of puberty, from 40 to 90 per cent may have become infected, the social and economic conditions mostly influencing the percentage of infection.

Let us for a moment examine the statistics of early infant infection which have stimulated the present activities for prophylactic immunization. McLean and Jeidell [12] examining 2000 infants under 1 year of age found a positive infection in 7.35 per cent, while in a second group of 512 children of 3 years of age it reaches 32.5 per cent. Spolverini [13] found 7 per cent positive reactions in a study of 900 infants of the same age, Sander [14] 6 per cent up to 2 years, and Armstrong [15] 15 per cent for the same period. In New York City, Drolet's

studies [16] reveal that from 10 to 12 per cent of all children become infected with tubercle bacilli before they reach 1 year of age. Taking the birth rate of 1923 when 130,000 infants were born, he calculates that in all probability 13,000 of these children became infected during the first year of life.

If we now turn our attention to the study of possible infection in children brought up in a family with a history of tuberculosis, the occurrence of the positive tuberculin reaction is materially increased. Harms and Seitz [17] studying 129 infants under 1 year of age reported 60 per cent positive tuberculin reactions, Röpke [18] 67.5 per cent and Barchetti [19] 73 per cent. The mortality from tuberculosis in the first year of life, after infection has occurred, appears to be very high. Brown [20] reports 70 per cent, Hemplemann [21] 78 per cent, Davis [22] 45 per cent, Reuben and Smith [23] 68 per cent, Lemaire [24] 57 per cent, Harms and Seitz [17] 23.9 per cent, Lozano [25] 26 per cent, and Wahlquist and Myers [26] 8.4 per cent. Petersen and Ostenfeld of Denmark, as quoted by the *British Medical Journal* [27] give only 6.2 per 1,000, while the same authors quoted by Calmette [28] give 7.7 per 100.

From these brief statistics it seems that infants are born with no appreciable resistance to an excessive infection. If they are born in families in which the tubercle bacilli are disseminated continuously, infection will probably take place almost immediately. Excessive infection takes place more often from contact with a tuberculous mother, and in the majority of instances a progressive disease is the result, which may later lead to meningitis or miliary tuberculosis. On the other hand, if the infant comes in contact with only a few organisms and at proper intervals, it may escape serious consequences.

Under ordinary circumstances, and not in tuberculous surroundings, without doubt children occasionally pick up a few organisms which find their way to the lymph nodes, setting up a small, non-progressive focus which according to the majority of investigators is responsible for a degree of resistance to progressive disease present in the human race. Statistics show that the percentage of positive tuberculin skin reactions increases with age, and conversely the mortality from tuberculosis diminishes as the child becomes older. It seems then, according to Calmette,[29] that in humans as in animals, an infection of mild nature is very desirable. The excessive infections must be avoided and the intervals well regulated. The organism used for producing mild infection should be of low virulence and the vaccination must be carried out in infants which have not been infected. The vaccinnation must be repeated from time to time in order to supply the lymph nodes with living organisms.

Calmette [20] is convinced that most of the infection in children takes place by the digestive route, for the reason that the intestinal mucosa of the infant during the first 10 days absorbs the organism much more readily than at any other time during life. If, according to this author, the infection takes place through the intestinal tract, then the same route must be used for vaccination. For this reason and also because of its simplicity, all Calmette's vaccinations have been carried on by feeding the organism to new-born babies. Two factors enter into the discussion when the method of vaccination is based on the use of living microörganisms: (1) Is it dangerous? (2) Is it effective?

I. PATHOGENICITY OF BACILLUS CALMETTE-GUÉRIN (B.C.G.)

The organism was isolated by Calmette and his associates in 1908. It was a bovine organism originally of moderate virulence for guinea pigs, rabbits and cattle, but after continuous cultivation on glycerinated potato bile medium it has lost its power of producing generalized tuberculosis, not only in cattle but also in small laboratory animals. Even in 1913 we find some reference that after 30 to 34 passages on this medium the organism had lost some of its pathogenicity. The authors claim that the environment and the medium have played a great part in the change. In an earlier publication Calmette and his coworkers claimed that the organism did not form tubercles or set up a tuberculin hypersensitiveness. Later they amended this statement in answering some of the objections made on the pathogenicity of this organism. We shall briefly review some of Calmette's experiments.[30]

When the organism was injected into the cellular tissue of the dewlap of newly born calves, it did not disseminate as in the case of a virulent organism, but remained localized at the site of inoculation for a year. Abscesses aspirated revealed acid-fast rods, which were not capable of infecting guinea pigs on subinoculation.

Subcutaneous inoculations of 3 mg. in guinea pigs and rabbits produced very slight, localized lesions in the form of small nodules which disappeared after 2 to 3 weeks. When 5 to 10 mg. were inoculated, edema developed on the following day. Within 10 to 12 days a small abscess appeared at the point of inoculation, breaking externally and suppurating from 2 to 4 weeks and then cicatrizing. The regional lymph nodes became enlarged but not caseous and soon they subsided to normal size. Excessive doses from 100 to 150 mg. after 2 to 4 weeks produced small granular follicles in the spleen, which disappeared in the course of 2 months. When suspected tissues were triturated and inoculated into a second series of animals no progressive disease was noted. However, very often when guinea pigs were inoculated with

doses of 50 mg. or more, many became emaciated and died. Many tuberculous-like changes were present in the viscera but these authors claim that the changes were due to pseudotuberculosis, from which *Bacillus pseudotuberculosis rodentium* was isolated.

Intraperitoneal inoculations of small doses were very well tolerated. Three mg. produced nodular reactions in the omentum. Later these nodules increased in size and persisted for 6 months and then completely disappeared.

Intracardiac and intravenous inoculations of 1 to 10 mg. had no ill effects in guinea pigs and rabbits. In about 15 days the lymph nodes at the groins became swollen and remained so for 10 days. This swelling completely disappeared in a few months. In the animals killed at the height of this reaction, the liver, spleen and lungs usually showed follicular lesions which were visible to the naked eye.

Administration per os was well tolerated, even in doses up to 100 mg. After 2 to 3 weeks following the feeding there was a general glandular enlargement, especially in the mesenteric lymph nodes. If the animals were killed during this period, small follicular lesions were found in the viscera and the tracheo-bronchial lymph nodes contained many acid-fast bacilli. All these lesions showed no tendency to progress, and gradually disappeared.

Direct intrapulmonary inoculations of 10 mg. of B.C.G. in half of the animals formed small, deep-seated abscesses. These abscesses were well borne by the animal and never culminated in death unless a superinfection of Pasteurella organisms occurred. In animals which survived 8 months the abscesses containing many acid-fast organisms were still present. In no instance were these authors able to transfer the suspected tuberculous lesions into a second series of animals, nor to increase the virulence of the organism.

Remlinger and Bailly,[31] Kühn,[32] Tzekhnovitzer,[33] Gentili, Gerosa, Mangiarotti, Nai, Setti, Zotini and Bassi,[34] Okell and Parish,[35] and others have confirmed Calmette's observations. However, there is a group of investigators including R. Kraus,[36] Selter and Blumenberg,[37] Gerlach,[38] Nobel,[39] Chiari, Nobel and Sole,[40] who have been able to produce lesions in guinea pigs and in some instances progressive disease leading to the death of the animal.

The organism is non-pathogenic for monkeys according to Wilbert,[41] who inoculated a number of chimpanzees, baboons and other types of monkeys. All tolerated very well even 100 mg., per os or intravenously. Subcutaneous inoculation produced slight edema with persistence of painless induration. Animals dying from intercurrent disease showed no evidence of tuberculosis.

OCCURRENCE OF TUBERCULIN REACTION IN ANIMAL EXPERIMENTATION

The development of the tuberculin reaction depends on the mode of inoculation. By ingestion a small number of animals become allergic. The same has been the result by the intravenous route. Nélis [42] states that tuberculin skin hypersensitiveness appears in adult guinea pigs between the 1st and 2d months and lasts from 6 to 10 months after ingestion of B.C.G.

Tzekhnovitzer [33] claims that guinea pigs become hypersensitive to tuberculin after treatment with B.C.G., the highest intensity occurring from 2 to 4 months. Seventy per cent of those infected orally and 45 per cent of those infected by the subcutaneous route react. The intraperitoneal and intracutaneous inoculations apparently do not sensitize animals to tuberculin. Rabbits do not react very readily to tuberculin after treatment with B.C.G. organisms. The ophthalmic reaction, however, is positive in 100 per cent of all animals.

Bruno Lange [43] obtained positive reactions to tuberculin in 100 per cent of guinea pigs inoculated intravenously, in 80 per cent of those inoculated orally and in 62 per cent after inhalation.

In regard to phagocytosis, Metalnikov and Secreteva [44] noticed that the cellular reaction is very similar to that observed in animals infected with virulent organisms.

II. IMMUNITY IN ANIMALS VACCINATED WITH B.C.G.

Calmette [29] and his coworkers claim that intravenous inoculation of 25 to 30 mg. of the organism in a fine suspension will protect rabbits against 0.001 mg. bovine bacilli which kills controls in 75 days. This resistance began to diminish in about 6 months, after which the lesions due to the virulent bovine infection progressed very rapidly, causing the death of the animal. Subcutaneous and intraperitoneal sensitization was not very effective. Rabbits of 15 to 20 days of age when sensitized by the buccal route by making them ingest organisms dropped with a pipette, resisted subsequent infection of a virulent organism very much like those vaccinated intravenously. If they were inoculated 3 months after the B.C.G. vaccination with a virulent bovine culture, the sensitized animals outlived the controls by 6 months and at autopsy only discrete lesions could be seen.

Guinea pigs were not so suitable for immunization for the reason that they are extremely susceptible to intercurrent infection. The adult guinea pig, following a single intracardiac inoculation of 5 to 10 mg. or by two subcutaneous inoculations of 50 mg. at 2 months-interval, shows considerable resistance to an infecting dose of 0.001 mg. Or still better,[45] if very young guinea pigs of 8 to 20 days of age are

given ten feedings of 100 to 150 mg. of the organism with a pipette at 20 hours-intervals, they will resist 0.001 mg. of a virulent culture. The infecting dose may be given either by intraocular instillation of a drop of fine emulsion or may be fed to young guinea pigs. In both instances, according to these authors, the results are very satisfactory.

Guérin, Richart and Bossière [46] studied a large number of cattle on a farm where the latter were raised for the production of butter. On this farm in 1915, in a herd of 67 head, 47 per cent reacted positively to the tuberculin test. Year after year the reactors were slaughtered. This procedure was not effective in completely eradicating tuberculosis from the farm. In 1919, 38 per cent were still positive to the tuberculin test. In 1920, the number of reactors was increased to 41.7 per cent. Vaccination in the new-born cattle started January 1, 1921. No special precaution, according to the authors, was taken to eliminate the possibility of contamination. In 1922, one year after the vaccination, 20 cattle gave a definitely positive and 9 a very suspicious tuberculin reaction, or a total of 45 per cent of 64 head. Many of those animals were vaccinated and revaccinated. In 1923, there remained 26 of the 1919-20-year animals, all giving a positive tuberculin reaction. In 1924 only 5 reactors were allowed to live with the young vaccinated animals. In 1925, only 2 remained of the original 1919 animals, 3 having been killed. In 1 of the 3 reactors killed, no tuberculosis could be found, in the 2d there were only few tubercles in the lungs, and in the 3rd animal there was an old calcified lesion in the lungs. In 1926, 7 years from the beginning of the experiment, there were still 2 of the infected animals which were reported in the 1919 experiment and which gave a positive reaction to tuberculin.

In the meantime, the second generation of these vaccinated animals were revaccinated and the vaccination repeated each following year. At the time of their report, the group of vaccinated animals consisted of 58 cattle living in contaminated surroundings. There is no record of how many of the vaccinated cattle became infected, as the tuberculin test was not done. These authors omitted the tuberculin test on Calmette's suggestion, as he believes that it is of doubtful diagnostic value, giving no information as far as exogenous infection is concerned. Furthermore, if in the vaccinated cattle an implantation of a virulent organism has taken place, setting up only a benign tuberculosis, tuberculin administered may bring about a violent allergic reaction, disseminating the virulent organisms. In such an event a progressive disease may follow. The validity of the claims for establishing protection in the vaccinated animals was based on autopsies done on a few cattle which have died from intercurrent disease.

Tzekhnovitzer [88] concluded that B.C.G. given orally in young guinea pigs does not prevent oral infection with virulent organisms. The animals may live a little longer than controls but the lesions are very similar to those seen in the control animals. Rabbits vaccinated subcutaneously lived longer than the controls and the lesions were not of the progressive type. In cattle after intravenous immunization with B.C.G. followed by 5 mg. of virulent bovine culture, the controls died in 6 weeks of miliary tuberculosis, while the sensitized animals survived and appeared apparently normal. Two, killed after 3 months, showed nodular tuberculosis. Two, killed 6 months after the virulent inoculation, showed subpleural tubercles, calcified bronchial and mesenteric lymph nodes and a few tubercles in the lung. Another animal killed in the 9th month showed a number of tubercles in the lungs. The last animal killed in the 10th month showed a caseous mass in the left lung. All were apparently in good health when killed.

Watson,[47] in a limited number of cattle vaccinated with B.C.G., observed no conclusive evidence of protection. The animals were vaccinated with B.C.G. in the usual way, were kept away from contaminated surroundings for a short period, and then put in with a tuberculous herd, exposing them to natural infection. The animals in the group were killed at various intervals from 2 to 24 months. All were free from tuberculosis.

A second series of animals were treated approximately the same as were the preceding series, kept under observation for 15 months and then killed. Progressive tuberculosis was found in every one of these vaccinated animals. The discrepancy in the two series is explained by the author in that the source of infection was different although the vaccinations were carried out exactly alike.

In a series of 4 unvaccinated new-born cattle which were put in contaminated surroundings and killed some 15 to 20 months later, only 1 of the animals had demonstrable tuberculosis. The author concludes that a large series must be employed and that animals with no natural resistance must be chosen for experiments of this nature. He calls attention to the possibility that the virulence of the organism which causes the infection in a herd may fluctuate from time to time. Tuberculosis may spread very slowly in one herd and very rapidly in another. This variation may be due to the natural resistance which some of the herds possess, the organism causing no clinical manifestation even with its continuous presence. Gradually the animals become resistant to this particular organism. However, as soon as a new organism is introduced into the herd, the occurrence of the disease is much more marked than previously.

Wilbert [24] reported a study of 15 chimpanzees and 59 Pithecians in East Africa, which if it can be repeated will strengthen the status of the B.C.G. vaccination. Monkeys of all ages were vaccinated either by a single subcutaneous inoculation of 50 mg. or by five ingestions, each consisting of 15 mg. at intervals of 8 to 10 days, and all showed resistance when exposed to natural contact infection. Some of the monkeys have been under observation since 1924 and were still living and in good health at the time of writing. The life of controls and of infected animals has always been very short. Autopsies on many of the vaccinated chimpanzees which have died from intercurrent diseases showed no tubercular lesion. This author concludes that B.C.G. can establish a definite protection in monkeys.

PREPARATION OF VACCINE AND METHOD APPLIED IN INFANTS

The organism ordinarily is cultivated on glycerin potato bile medium. When a large quantity is desired, especially in the vaccine preparation, it is advisable to cultivate it on a synthetic medium known as "Sauton's." Surface inoculation is made by floating the seed and an abundant growth accumulates in a short time. Only cultures of 3 weeks' development are recommended by the author for the preparation of the vaccine. The fluid medium is removed from this 3 weeks' growth, and a number of sterile glass beads are introduced. The flask is fastened to a shaking machine and after 15 minutes of vigorous shaking it is diluted with a mixture containing 1 per cent dextrose and 4 per cent glycerol in water. The whole mass is well mixed and allowed to stand until the clumps settle. The supernatant suspension of living organisms is then diluted so that 1 c.c. represents 5 mg. moist weight of the bacilli. Two c.c. of this suspension are mixed with milk and fed to infants. The feedings are made on the 3rd, 5th and 7th days after birth. Each feeding represents approximately 400,000,000 of living organisms.

The history of the vaccination of infants with B.C.G. as a prophylactic measure dates from July, 1921, when Turpin and Weill-Hallé fed some infants at the Maternity Hospital in Paris. For the following 2 years the vaccination was practically neglected. It was not taken up seriously until July 1, 1924. In a recent article Calmette [28] and his coworkers tabulate the results from the 3 years' vaccination of 52,772 children, which cover a period from July, 1924, to December 1, 1927. Eight hundred and forty infants were vaccinated in 1924; 4,336 in 1925; 14,654 in 1926; and 32,942 in 1927. Sixty-two hundred and nineteen of the vaccinated were born in tuberculous families, 5,749 of whom have been under observation for 1 to 3½ years. Of these, 3,808

were vaccinated less than 1 year, 118 of whom died, making a general mortality of 3.1 per cent and a tuberculous mortality of 34 infants or 0.9 per cent. Tuberculous meningitis is given as the cause of death in 30 cases or 88.4 per cent of the tuberculous deaths. The remaining deaths were due to some other form of tuberculosis. Of the 84 infants in this group who died from other causes than tuberculosis, 29 or 34.5 per cent died from gastroenteritis.

The remaining 1,941 of these 5,749 infants represent a group from 1 year to 3½ years of age. Twenty-one deaths occurred in this group, or a general mortality of 1.2 per cent, 4 of whom, 0.2 per cent, died from tuberculosis.

In a third group of 917 children from 2 to 3½ years of age there was no tuberculous mortality. It is not quite clear if the third group is included in the second group.

Perhaps the best controlled study on infant vaccination is that made by Weill-Hallé and Turpin.[48] Their statistics consist of 469 cases vaccinated with B.C.G., 92 of whom they lost track of and could not include in their study. Another group of 60 was excluded because the duration of time after the vaccination was too short. Of the whole group they analyzed only 317 cases which were well followed with the intracutaneous skin test. Death occurred in 14 of these 317 infants. Two hundred and thirty-six were from healthy surroundings and 67 from tuberculous environment. The 14 deaths were equally divided between the two groups, 7 in the first and 7 in the second. The skin test was done approximately every 3 months, and the results are tabulated in the following table:

	Intracutaneous Skin Test	
	Infants Vaccinated, Living in Tuberculous Surroundings	Infants Vaccinated, Living in Healthy Surroundings
Age in Months	Tuberculin Skin Test Positive in Per Cent	Tuberculin Skin Test Positive in Per Cent
3	11.1	2.5
6	16.6	4.1
9	25.9	5.8
12	26.6	7.6
15	44.4	7.4
18	50.0	12.1
24	60.0	28.0

A glance at this table shows that the tuberculin skin reaction was positive more frequently in the group of children who were in tuberculous surroundings than in the group in which apparently there was no source of infection. The tuberculin skin reaction does not give us any information, especially in the group vaccinated and living in healthy surroundings, as to whether the infection was due to B.C.G. or to an exogenous source. Sixty per cent of children at the age of 2

years living in contaminated surroundings have reacted positively to tuberculin. This probably means that a large percentage of infection in this group was of exogenous character. It does not vary in any way from the group of children who have never been vaccinated and have lived in tuberculous surroundings. It seems that in spite of the vaccination with B.C.G. and the sociological measures, the implantation with virulent tubercle bacilli has taken place.

Selter,[49] analyzing these figures, offers the same criticism. It would be interesting for this author to follow not only the children brought up in surroundings where the possibility of infection is great, but also in the second group which give no history of infection.

Sayé, Domingo and Miralbell,[50] using Calmette's method, vaccinated 203 children with B.C.G. Six deaths from tuberculosis occurred among the vaccinated, or approximately 3 per cent. A number of the infants died from gastroenteritis but unfortunately no record is given as to the nature of this gastroenteritis.

Moine [51] in a study of 882 infants reports a mortality from tuberculosis in only 0.8 per cent. Biraud [52] reports 2.46 per cent mortality from tuberculosis in a group of 1872 vaccinated infants. Ott [53] gives a mortality of 1.9 per cent from tuberculosis in 157 infants. Rougebief,[54] following 60 cases of 623 vaccinated infants in Algeria gives no mortality from tuberculosis. Blanc,[55] in Greece, reports no mortality from tuberculosis in 136 infants vaccinated. Cantacuzène [56] reports no tuberculosis mortality in 578 infants. Keller [57] also reports good results but he believes that living B.C.G. vaccination should be controlled with killed organisms. Bernard [58] had no accident in 20,000 children vaccinated in Indo-China. Malvoz and Van Beneden,[59] in Belgium, report only 1 death from tuberculosis in a group of 374 vaccinated infants, and Iakhnis,[60] of Lithuania, studying 472 vaccinated infants, reports that he obtained ten times more positive tuberculin reactions in the vaccinated children than in the unvaccinated. He has noticed no ill effects from the vaccination.

THE AUTHORS' STUDY ON THE BIOLOGICAL CHARACTERISTICS OF B.C.G. CULTURAL CHARACTERISTICS

Our study was based on cultures obtained from three different sources, one from Dr. Watson of Ottawa, Canada, another brought to us by Dr. Lawrason Brown from the Pasteur Institute, and the third was sent direct to us by Prof. Calmette.

The organism grows well on glycerinated bile potato medium. The single colonies are perfectly round and considerably moist. The growth appears from 15 days on, gradually increasing in size. In time the

glistening surface dries out, assuming a mat appearance. They are very easily emulsified and the suspension obtained is very similar to that obtained from the avian tubercle bacillus. The organism grows well on the surface of practically all fluid media used for the cultivation of the tubercle bacillus. Sauton's medium, recommended by Calmette, is very suitable for obtaining a large amount of growth. The filtrate after the 3rd week develops very strong tuberculin. Guinea pigs inoculated with the organisms develop skin hypersensitiveness in about 15 days.

The first culture studied was that obtained from Dr. Watson. Eight guinea pigs were inoculated subcutaneously, the amount varying from 2 to 16 mg. Two guinea pigs received 2 mg.; 2, 4mg.; 2, 8 mg.; and 2, 16 mg. Sixty-one days after the inoculation 1 animal from each group was killed and tuberculosis of the viscera was present. The other 4 animals were allowed to live. Five months from the beginning of the experiment, 1 of the animals which was inoculated with 4 mg. died and the cause of death was recorded as generalized tuberculosis. In the 6th month the animal receiving 16 mg. also died from generalized tuberculosis. At the 7th month the 2 remaining animals, i.e., 1 receiving 2 mg. and 1, 8 mg., were killed. At autopsy no evidence of tuberculosis could be found. At that time we could not explain the discrepancy observed in these animals, 2 having died from tuberculosis, while the other 2 living 7 months were free from tuberculosis. The experiment was suspended for a short time until we obtained another culture.

With the second culture an attempt was made to increase the virulence by rapid animal passage. The technic consisted in inoculating from 1 to 2½ mg. into the right testicle of 2 guinea pigs. After 7 to 14 days the infected testicles were removed aseptically under anaesthesia. The tissues were triturated and reinoculated into the right testicle of 2 other animals. Four such series were done. Three gave negative results after the 3rd, 4th and 5th passages. There was only localized tuberculosis which eventually healed. In one series, however, on killing the animal of the second passage, there was generalized tuberculosis of the viscera, and the spleen was large and nodular. The lymph nodes were very caseous. This caseous material was inoculated in the other guinea pigs and invariably generalized tuberculosis developed and death occurred.

During the cultivation of B.C.G. on fluid media a difference in the character of the growths appeared in some of the flasks. Some were veil-like with small islands of dense growth scattered throughout, while in others the growth was heavy and of uniform structure. This imme-

diately suggested to us the possibility of variants similar to those observed in the reproduction of other acid-fast organisms, and if B.C.G. could be dissociated, at least we could explain some of the discrepancies observed in animal inoculations.

A small amount of the third culture received directly from Calmette was triturated in salt solution with a pH of 7.8, filtered through two layers of Whatman paper No. 5 and 5 drops of the filtrate smeared over the surface of gentian-violet plates. After 6 weeks there appeared two distinct types of colonies. One, which predominated, was waxy, with smooth wrinkles, the folds of which extended from the center to the periphery. The outline was clear-cut, round, raised, and did not extend into the medium. This colony was very difficult to emulsify in salt solution of pH 7.2. We shall call this the "R" colony. The other colony, which we shall refer to as "S," was small, in irregular wrinkles on the surface and was not as smooth and waxy as the "R" colony. The outline was irregular and at times extended into the medium. This colony was much more readily emulsified.

On synthetic fluid media the "R" colony grows in the form of small islands or peninsulas and the growth is readily broken up and lifted with the loop. On the same media the "S" colony grows more tenaciously and in a solid mass very much like a thin veil. In attempting to remove a small amount of the surface growth, practically the whole surface is lifted. Both of these colonies produce tuberculin. Inoculated into guinea pigs, both set up a hypersensitive state to tuberculin. The complement fixing and precipitin antibodies can be demonstrated in rabbits following intravenous inoculations.

A comprehensive study of the biology of the "R" and "S" colonies, with special reference to virulence and immunity, will appear in the near future. All the work so far points out that the two colonies dissociated from the original Calmette strain behave differently, not only in regard to cultural characteristics, but also in virulence for guinea pigs and rabbits. The "R" colony always produces some tubercles which in time heal, while the "S" colony invariably produces progressive disease terminating in the death of the guinea pig. The chemistry of the two colonies is also different.

IMMUNITY EXPERIMENT

The value of B.C.G. vaccination was tried in a limited number of animals and the experiment is not yet completed. The experiment consisted of 38 vaccinated and 26 control guinea pigs. They were divided into four groups.

Group I consisted of 8 very young guinea pigs not older than 36

hours. They were fed with an emulsion of B.C.G. bacillus, four doses of 0.8 mg. being given, a total amount of 3.2 mg. dry weight or approximately 20 mg. moist weight as used by the French investigators. Four and one-half months later 3 drops of a suspension of living virulent culture consisting of 100,000,000 organisms per c.c. were instilled in the eye. Seven controls were infected by the same method. Eight months after the instillation with living tubercle bacilli, in the vaccinated animals, 3 are still living and 5 have died between the 130th and 231st days. Three unquestionably died of tuberculosis. One died from pneumonia complicated with tuberculosis, and in the other which died from a chronic otitis the cervical lymph nodes were enlarged and contained acid-fast organisms. Of the control animals 5 are living and 2 have died. Death in 1 of the animals was due to tuberculosis and in the other to chronic otitis with some tuberculous involvement.

The second group consisted of 8 sensitized animals and 9 controls. In this group also very young guinea pigs not older than 36 hours were used. They were inoculated subcutaneously with a suspension of B.C.G. of 0.25 mg. every 5th day. Four doses were given, a total amount of 1 mg. dry weight or 8 mg. moist weight. The vaccinated and the control animals were then infected by the inhalation method with H 37. At the end of the 8th month, 1 of the vaccinated animals is living and 7 are dead. Four died of extensive tuberculosis, 1 of cellulitis with some tubercular lesions and in 1 the cause of death was undetermined but there were tubercle bacilli at the site of inoculation. The 7th animal died of postpartum peritonitis with considerable amount of tuberculous involvement. Six control animals died of generalized tuberculosis.

The third group consisted of 10 full grown guinea pigs. Four inoculations were made subcutaneously from 2 to 3 days apart. A total amount of 12 mg. of moist weight of the organisms was given. The infecting dose was given subcutaneously a month later, consisting of 500 organisms of H 37. One year later 3 of the animals are still living and 7 are dead. Five which lived more than 279 days died of generalized tuberculosis. One living 98 days died with chronic sinusitis with no macroscopic evidence of tuberculosis. In another animal which also died with sinusitis on the 102nd day there was tuberculosis in the spleen and lymph nodes.

The fourth group consisted of 12 adult guinea pigs. Vaccinations were made intraperitoneally and four doses were given between 2 and 3 days interval, or a total amount of 12 mg. moist weight. A month later they were inoculated subcutaneously with 500 virulent H 37 organisms. One year later 4 of these 12 animals are still living and 8

have died from various causes. Two died shortly after the inoculation and have been excluded from this group as death occurred too soon after infection. One died on the 79th day from gastroenteritis with no evidence of tuberculosis. Another died on the 95th day with chronic otitis and no tuberculosis. Three others died on the 123rd, 238th and 279th days of generalized tuberculosis. In 1 animal dying on the 264th day the cause of death was due to strangulated gut and tuberculosis was present in the spleen and lymph nodes.

Ten controls were used for Groups III and IV. They were inoculated also with 500 tubercle bacilli of the same origin. A year later 1 is living and 9 are dead. One died on the 97th day with chronic otitis with demonstrable tuberculosis in the spleen, one on the 100th day of peritonitis and no tuberculosis, and 1 on the 251st day of pneumonia with tuberculosis of the spleen. The remaining 6 guinea pigs died of generalized tuberculosis, some living as long as 351 days.

DISCUSSION

In the preceding pages an attempt has been made to bring together some of the outstanding features concerning the method of B.C.G. vaccination. The organism in question was isolated some fifteen years ago or more from a heifer. At the time of isolation it was pathogenic for cattle, rabbits and guinea pigs. By cultivating it in glycerinated ox bile potato during this period, it has almost completely lost its virulence, but has maintained the property of forming localized tubercles, of producing tuberculin, of rendering inoculated animals skin hypersensitive, of producing the formation of specific antibodies and according to the sponsors of producing an immunity against virulent reinfection.

According to the majority of workers, B.C.G. is innocuous for small laboratory animals, lesions once established having a tendency to heal. The experiments of Gerlach,[38] Nobel,[39] Kraus,[36] Selter,[37] and others are not, however, in agreement as to the innocuousness of the organism. Some of them have been able to establish progressive disease in guinea pigs, eventually leading to death, and Nobel and Gerlach were able to pass the lesions from animal to animal. Korschun [61] has been able to increase the virulence of B.C.G. in animals by treating them first with diphtheria toxin.

In two instances we have been able to demonstrate generalized tuberculosis by direct inoculation and in one other instance tuberculosis was established after the second animal passage. In defending their position Calmette and his collaborators state that any positive tuberculosis in animals when inoculated with B.C.G. is due to a con-

tamination with a virulent tubercle bacillus. Accusing any trained worker of carelessness in contaminating cultures is not a justifiable criticism.

An explanation of these discrepancies reported on the virulence of this organism is offered by the recent work on dissociation which has been observed in the majority of bacterial species.

Recently we [62] have observed that any culture of tubercle bacillus may contain two types of colonies which can be differentiated by their cultural characteristics and virulence for animals. We have found two types of colonies present in a culture of B.C.G., one "R" colony (rough) * which is non virulent, and the other "S" (smooth) * which is virulent for guinea pigs. The "R" colony grows very well on glycerin bile potato and Sauton's media, while the "S" does not produce any visible growth in bile but grows in Sauton's.† The latter, however, does not die in the bile medium. Scrapings made from the surface of the inoculated culture 1 and 2 months after incubation invariably produce progressive tuberculosis in guinea pigs, involving the lymph nodes and the viscera. The lesions are similar to those seen in guinea pigs inoculated with bovine tubercle bacilli. These scrapings will also yield a profuse growth after cultivation on suitable egg media. From the lesions of this "S" colony we believe that B.C.G. has still the ear mark of a bovine organism but lacks the power of producing progressive tuberculous lesions in rabbits. By continuous subculturing on potato bile media, Calmette has gradually eliminated most of the "S" colony, allowing the "R" to predominate. The virulent "S" colony, however, has never been completely eliminated, and although present in very small numbers, under favorable conditions it may increase in number to such an extent that reversibility of virulence may take place. An observation of this nature is not uncommon with any other bacteria. It cannot be thought that the tubercle bacillus is an exception.

The animal experiments submitted as the basis of vaccination with B.C.G. are unsatisfactory. There is, at most, only weak evidence to show that this organism establishes any appreciable degree of protection in small laboratory animals. The only evidence being that some of the vaccinated animals outlived the controls. Selter [37] believes that some degree of protection can be established, but this is due to the slight degree of virulence still present in the organism. We are inclined to believe that the protection conferred by B.C.G. is very slight and not greater than that obtained with heat-killed organisms where there

* The nomenclature is arbitrary. They are both rough but have a different structure.
† In a recent paper we have been misquoted by Calmette.[28]

can be no question of danger. The life cycle of the tubercle bacillus is not completely understood and especially of the B.C.G. We consider its use, at least for the present, should be deferred. After the accumulation of more experimental data we may be brought to modify our position.

We cannot completely ignore the autopsy findings of Taillens,[63] Girod and Debarge [64] in infants who had been vaccinated, nor the tuberculin reaction observed by Weill-Hallé,[48] where there was evidence that B.C.G. produces some tuberculous changes in vaccinated infants living in healthy surroundings. The mere fact that 28 per cent of infants vaccinated and living in healthy surroundings reacted to the intracutaneous tuberculin skin test forces us to such a conclusion. It will be interesting to know if any of these infants develop clinical tuberculosis later in life. Of course if such occurrence takes place, there are no means for determining whether the disease is due to B.C.G. or to some exogenous infection.

Further, in regard to the immunity in cattle, the results reported by Guérin, etc., have not been universally accepted by men who have studied this problem in cattle. Watson,[47] of Ottawa, states that tuberculosis may spread very slowly in one herd and again very rapidly in others and he believes the resistance existing in the former is due to the persistence of the tubercle bacillus in the animal body for many years, causing no clinical disease under normal conditions. If, however, some of the infected but not ill animals were put in with a stock free from tuberculosis, they may infect fatally many cattle of the second herd, the original infected lot outliving the second lot. This author also believes that very little could be gained by vaccinating calves, and even if the vaccinated animals were more resistant to superinfection than the unvaccinated, the former could not completely resist an implantation of a virulent organism. After such implantation takes place, the organism in all probability will be localized in the lymph nodes and remain there for a long time doing no damage, but after years of strenuous milk production the animal's resistance may diminish to a point where disease begins to develop.

Calmette's original cattle experiments in 1913 [65] are also open to criticism. He only uses 1 control in each group with 7 or 8 vaccinated animals. The statement is made that 3 mg. of the Vallée bovine strain invariably killed the control in 5 weeks of generalized tuberculosis, while the vaccinated continued in good health. Watson, however, using the same Vallée strain observed that some control calves which had been inoculated intravenously with 250 mg. of the organism and killed 11 months later showed only lymphatic tuberculosis. Did Calmette

use identical suspensions in his control in each experiment, or did he assume from previous experiments that the Vallée strain always killed in this dose? We now know that unless we cultivate our human H 37 and bovine B 1 tubercle bacilli on suitable media the virulence diminishes. For this reason when testing the resistance of vaccinated animals the same number of controls must always be used.

Uhlenhuth[40] is inclined to believe that the cattle experiments reported by Calmette where infection was by contact were not carried out as they should have been. The heads of the animals were turned toward the wall, thus preventing the droplet infection. At least the certainty of natural infection was lessened.

We suggest that the success reported by Guérin in cattle experiments was not due to the protection established by vaccination with B.C.G., but to the elimination of the contaminators on the one hand, and to the development of natural resistance on the other. This point seems obvious in the animals killed 7 years after they have reacted positively to tuberculin, the autopsies revealing only few small lesions.

The favorable reports coming from Europe on the vaccination of new-born babies are so amplified that they have reached the point of distortion. Careful analysis of the statistics submitted reveal one outstanding fault, viz., that not a single group of vaccinations has been controlled. We have failed so far to find comparable figures which may lead us to some conclusion. Mortality rates from tuberculosis in infants, in one instance, are quoted as far back as 1889. Have not the conditions and our knowledge of sociological measures been improved in recent years so as to bring the mortality to a lower level? Calmette claims that in France he has reduced the mortality from tuberculosis in the first year of life from 25 per cent before the vaccination to 0.9 per cent after the vaccination. Does this 25 per cent mortality in infants in tuberculous families refer to the group of known infected as detected by the positive tuberculin reaction, or does it represent a percentage from the general mortality? It is not quite clear in our minds.

It must be remembered that most of the vaccinated children born of tuberculous mothers were removed from contact for at least 4 months, thus decreasing the possibility of an infection in this early period of life. This isolation gives the infants an opportunity to adjust the normal mechanism of defense against any infection. No infants in France without vaccination have been submitted to a similar preventive measure early in life. How then can we compare the two groups, the vaccinated and the unvaccinated? Two factors are involved in these studies, vaccination and hygienic preventive measure.

Further, how can we determine the cause of death in infants from tuberculosis without post-mortem examination? There are other forms of tuberculosis in infants besides tuberculous meningitis which are not easily diagnosed. Calmette presents only 3 autopsies, involving a study of several hundred deaths. Many of the vaccinated infants have died of gastroenteritis; in fact, over 30 per cent of the deaths occurring in the vaccinated infants not due to tuberculosis have been recorded as gastroenteritis. Could it not be possible that in some this gastroenteritis was of a tuberculous nature? While it is probable that the organism, by feeding, may find its way into the mesenteric lymph nodes and then be distributed throughout the body, it is conceivable that when such large doses are administered at birth the mucosa may become involved directly. The innocuousness of the organism has been based on the observation that no fatalities have been reported after oral vaccination. Fortunately, contrary to Calmette, the experimental evidence is against any great danger of infection taking place by this route. Findel,[66] Reichenbach,[67] Selter,[68] Brown, Petroff and Pesquera [69] could not readily infect guinea pigs by feeding. The only misgiving we have is that some virulent type (S) may be carried along with the non-virulent.

Weill-Hallé observed only 2 per cent positive tuberculin reactions up to 3 months in a group of infants vaccinated by the oral route and living in healthy surroundings. When the vaccine was administered subcutaneously 100 per cent of the infants reacted intracutaneously to tuberculin. Is this not sufficient proof that very few of the organisms pass through the intestines of the infants?

In explaining the 0.9 per cent mortality from tuberculosis in infants vaccinated with B.C.G., Calmette [28] and Surrez [70] do not, as a rule, attribute it to an infection of exogenous character subsequent to vaccination. They profess to believe that tuberculosis occurred in these infants usually as a result of fetal infection with the ultra-microscopic filtrable form of the tubercle bacillus which traversed the placenta, a theory which is still a speculation. In our laboratory we have not yet confirmed the existence of a Berkefeld filtrable form of the tubercle bacillus.

We are further told not to worry about the B.C.G. because it is a bovine organism which is not virulent for human beings; but is it not a false security to depend upon its presence for protection without any knowledge of what immunity, if any, it confers? Infection with the bovine tubercle bacillus in infancy is today a rare occurrence; but what becomes of this organism after puberty in those that have been infected? Does the bovine organism transmute into the human

tubercle bacillus after inhabiting the body for a long time? All evidence up to the present is against such transmutation, but we must not be too dogmatic in completely ignoring its possibility.

The differentiation of the human and bovine cultures has been based on the ability of the organism to produce progressive disease in a rabbit. If a culture inoculated in dosage of 0.01 mg. infected the rabbit and death followed in 60 days, then the organism was bovine, and if it did not, it was human. This holds true probably with the two extreme types; but how about the intermediate ones? If B.C.G. was a bovine tubercle bacillus at the time of isolation, it must possess all conventional biological characteristics, i.e., it must be virulent for rabbits, cattle and guinea pigs and give the alkaline curve on broth as described by Theobald Smith. The dissociated "S" virulent colony of this organism infects only guinea pigs at present with a marked lymphatic involvement comparable to many other bovine tubercle bacilli, is no longer pathogenic in small dosage for rabbits, and the acid base curve is that seen in the human organism. In other words, the organism has some human and some bovine characteristics. Mudd [11] also points out that B.C.G. behaves like the human strains studied in his interfacial experiments.

The main object of the present campaign in prophylactic immunization has been to reduce the mortality from tuberculosis during the first year of life. Vaccination with living organisms has been suggested and very extensively used. There is evidence that the general mortality has been reduced and there are indications that some of the infants have been infected, while others, regardless of the prophylactic measures and vaccination, have been reinfected with small numbers of virulent organisms.

It seems advisable and a much safer procedure not to introduce another living organism into the body but rather, by hygienic measures, to eliminate as much as possible the danger of excessive infection from the new-born babies. Such preventive measures are effective, as was pointed out by Grancher some twenty-five years ago, and more recently, Debré and Lelong [12] and others have been able to prevent infection in infants by removing them from their tuberculous mothers. König,[10] studying the mortality in Prussia, states that in 1925 the general mortality among 246,488 infants under sanitary supervision was reduced from 12 to 5.5 per cent. Among them there were 188 male infants living in tuberculous surroundings of whom only 6.3 per cent reacted positively to tuberculin and 140 females of whom only 5.7 per cent reacted. These remarkable figures have been obtained not by B.C.G. vaccination but by educational and preventive measures.

In the United States and Canada where the campaign against tuberculosis is made very effective by the use of various sociological and health measures, mortality and infection in childhood have decreased to a very low level and we cannot see why such a prophylactic measure as advocated by the French investigators should be introduced at present. We may recall that Friedmann's vaccine which was condemned in 1912 was also a living organism, and the potential virulence of it was probably of lower degree than B.C.G. We believe that watchful waiting is the best position at present in reference to vaccination of infants. We cannot completely ignore the possibility of a prophylactic vaccination method and the one advocated demands our careful consideration. The careless use of poor statistics obtained from a study of human beings is going to lead us nowhere.* Therefore, we strongly advocate experiments in cattle on a large scale in order to confirm or refute Calmette's claims.

* The reader is referred to a comprehensive analysis of Professor Calmette's statistics by Dr. M. Greenwood in the *British Medical Journal* of May 12, 1928.

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Institute for the Protection of Child Health in Soviet Russia

BY government decree there was recently established in Moscow the Government Scientific Institute for the Care of Child Health. The institute, which is doing theoretical as well as practical work, has the following purposes:

1. Study of the physical and psychological nature of normal and abnormal children between the ages of 4 and 17 years.

2. Study of methods for the prevention and treatment of physical and mental disorders in children of these ages.

3. Training of workers for the care of the health of children and young people.

The institute consists of the following four divisions: division of the normal child, division of the physically abnormal child, division of mental and nervous disorders, and the statistical division. A separate section will be devoted to the training of physicians as specialists in child health work.

The following auxiliary agencies are connected with the institute: an experimental dietetic dining room; a clinic for normal children, one for physically defective and one for mentally defective children; a sanatorium, a playground conducted on the principles of physical therapy; a diagnostic clinic, and a museum. The institute is coöperating with other organizations interested in child health.—*Voprosi Zdravoo Khranenia,* Moscow, 1:74, 1928; 4: 81. 1928.