THE PRACTICAL APPLICATION OF B.C.G. VACCINE IN THE PROPHYLAXIS OF TUBERCULOUS INFECTION IN CHILDREN.*

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NEARLY twenty-six years have elapsed since Calmette and Guérin mentioned for the first time, in 1908, the striking experimental fact that a virulent strain of bovine tubercle bacillus cultivated through several generations on potatoes cooked in pure bile, with the addition of 5 per cent. of glycerin, had lost its virulence for cattle. Three milligrams of the original strain, injected intravenously, killed tuberculin-proof calves under 6 months of age, in from 4 to 6 weeks with the symptoms and lesions of acute miliary tuberculosis. The modified strain after having been transplanted thirty times at 25 days' intervals (therefore during a period of a little more than 2 years) on the bile-containing media, and injected at the same dose into the veins of young calves of the same age had no morbid effect at all. Subsequently, it was proved that it had also lost its virulence for monkeys, for rabbits, and guinea-pigs.

In 1913 Calmette and Guérin, having demonstrated that the new strain artificially produced by cultivation on bile, had permanently acquired its non-virulent properties, and did not loose them when cultivated again on ordinary glycerin-broth or glycerin-potato media, published a series of experiments showing that calves infected intravenously with the non-virulent bacillus became, after 4 or 5 weeks, extraordinarily resistant to inoculation with fatal doses of virulent tubercle bacilli. I will, for the sake of clearness and brevity, give one instance of those experiments :—

Eight nine months' old tuberculin-proof heifers received an intravenous injection of 1 mgrm., and after thirty days a second dose of 5 mgrms. of living bacilli modified by cultivation on bile. Thirty days after the second inoculation, each animal received an intravenous injection of 3 mgrms. of virulent bovine bacilli. A control heifer of the same age died 34 days after the virulent injection with generalised miliary tuberculosis. Seven out of the eight previously inoculated

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animals were killed in perfectly good condition after 1, 2, 3, 4, 8, 12 and 18 months respectively. None of them had the slightest tuberculous lesions. But the bronchial glands of each of them, ground in a mortar and injected into guinea-pigs, invariably infected them with tubercle.

Virulent tubercle bacilli had therefore been preserved alive in the bronchial glands of those heifers, as long as eighteen months. But they had been unable to produce any tuberculous lesion in the glands or elsewhere in the system of the previously inoculated animals.

Starting from those fundamental experiments, which have been abundantly repeated in France and in other countries, Calmette and Guérin pursued the most extensive and painstaking studies on the new non-virulent strain which is now universally known as B.C.G. (*Bacillus Calmette-Guérin*).

The protection conferred by B.C.G. to cattle against artificial or natural infection with virulent tubercle bacilli is now a well-ascertained fact; from researches conducted by Professor A. C. Rankin (of Alberta) under the auspices of the National Research Council of Canada, it seems that the protective influence of vaccination with B.C.G. does not last less than two years. Anthropoid apes submitted to vaccination experiments in the Pasteur Institute of Kindia (French Guinea) have been found protected for more than fifteen months. In guinea-pigs the protection is of shorter duration.

At the beginning of their studies, Calmette and Guérin believed that the B.C.G. strain was incapable of producing any tuberculous lesion in the animal, and it is true that, to the naked eye, no alterations are observed at the post-mortem of animals killed some time after intravenous injection. But in 1927 E. Coulaud, a co-worker of Calmette, showed that numerous microscopic miliary lesions of the follicular type can be seen ten days after the injection, in the lungs, in the liver, in the spleen. They never undergo caseous degeneration and heal spontaneously without leaving any scar. In rabbits and guinea-pigs killed eight or nine months after the inoculation there is a complete *restitutio ad integrum*.

Following vaccination with B.C.G., the animals are sensitive to tuberculin. Reinfected with virulent bacilli they react by Koch's phenomenon. Therefore cultivation on bile deprives tubercle bacilli of their virulence, but not of their antigenic properties.

Application to Human Beings. — Having thus satisfied themselves that the B.C.G. strain is innocuous for animals, and is able to immunise them against virulent tubercle bacilli, Calmette and Guérin, very hesitatingly at first, and later on with increasing confidence, decided to make use of their discovery for the benefit of infants exposed to tuberculous contacts.

The task which they then undertook was fraught with almost overwhelming difficulties. Whenever a new weapon against disease, after having successfully passed the trial of animal experiment, begins to be used practically on human beings, its universal acceptance by the medical profession as well as by the public is bound to be delayed by prolonged, seemingly unnecessary but unavoidable controversies. Physicians of my age well remember the severe criticisms encountered by such brilliant discoveries as diphtheria antitoxin or arsenobenzol. And yet the value of those *therapeutic* agents is so strikingly evident, that it seems impossible to doubt it. But the efficacy of a specific *preventive* agent is much more difficult to prove. There are still objectors against vaccination for small-pox, although Jenner's discovery is now nearly 150 years old. If the World War had not given us an unexpected opportunity for experimenting antityphoid vaccination on a gigantic scale, I doubt very much whether Sir Almroth Wright's wonderful life-saving invention would be as universally adopted as it is to-day.

Small-pox and typhoid fever are acute diseases, running a more or less fixed course. They are practically non-recurrent. They last not more than a few weeks. They are easily diagnosed. They are more epidemic than endemic. The beneficial influence of preventive measures directed against them does not require a great length of time to be manifested.

The case of tuberculosis is quite different. We know that among civilised people, tuberculous infection is almost universal and should by no means be regarded as synonymous with tuberculous disease which affects only a comparatively small minority of the people. Tuberculous infection, once acquired, is seemingly never eradicated. Tuberculous disease appears under the most varied aspects even in one and the same patient. It may last a few weeks and end with death or with recovery. It may last for years. It may recur after long periods of latency. Exposure to tuberculous infection is so

prevalent that hardly anybody escapes from it. But we know very little about the conditions which favour the evolution of infection into disease in some persons and prevents such evolution in other persons. Moreover, disease may develop many years after infection has taken place.

Evidently the prevention of this most protean disease is an exceedingly difficult problem in itself. But supposing it to be solved, it seems even more difficult to give an indisputable demonstration of its being really solved, before the lifetime of one human generation at least has elapsed. Furthermore, the lifelong experiment which is required cannot be made under laboratory conditions. It is affected with all the vicissitudes, uncertainties, and errors of the sociological experiment. It is influenced by psychological and even sentimental factors. It can be judged only by statistical criteria, and statistics are much more delicate and even dangerous to handle than are test-tubes or guinea-pigs.

There were many hypercritical people who shook their heads and declared that an accurate demonstration of the efficacy of antitubercle vaccination being so exceedingly difficult, it would be most unwise to give B.C.G. a trial. If Calmette and Guérin had listened to them, they would certainly have had a much quieter life. But they were not the kind of men who abandon a great endeavour because they have to face great obstacles. They were bound to make mistakes at the beginning. But mistakes can be corrected. The first duty is to begin.

Prevention must precede Infection.—Once infection has occurred, prevention is excluded. Therefore B.C.G. can be given only to people who have never been infected with tubercle bacilli. Fortunately von Pirquet has provided us with a very simple and harmless test of tuberculous infection. When Calmette and Guérin began their work it was commonly believed that almost all adults have a positive Pirquet reaction. The consequence was that only children having a negative Pirquet could be submitted to B.C.G. inoculation.

The Method of Inoculation.—What method of inoculation should be preferred ? Obviously the *subcutaneous* injection of B.C.G. is the surest way. But it seemed almost impossible to make it acceptable. In a certain proportion of cases it causes a harmless abscess at the site of inoculation, an abscess which may heal rapidly, but which may also last several weeks

and even months. A formidable obstacle indeed from the psychological point of view. Any non-tuberculous disease, whether slight or severe, occurring in vaccinated children would inevitably be attributed by parents—and also by medical men—to B.C.G.

Calmette, in order to avoid the deterring effect of the subcutaneous injection, advocated a method of administration which could produce no visible lesions and consequently would be accepted more easily by the people. He had persuaded himself that micro-organisms can penetrate easily through the mucous membrane of the small intestine of very young children into the lymphatic system without creating any lesion of the mucosa. In older children the intestinal wall is not so easily penetrated by the germs. Weigert, Disse, Behring and Römer, Ehrlich, Vaillard had formerly called attention to the fact that the epithelial cells of the intestinal mucosa are not fully differentiated in young mammals and are endowed with strong phagocytic properties facilitating the transfer of germs into the lymphatics and blood vessels. Calmette decided therefore to take advantage of those special conditions and to give the B.C.G. vaccine *by mouth*, and only to newborn infants. It is administered in three doses during the first ten days after birth. The procedure is perfectly harmless and never produces the slightest morbid symptom. For this reason it has been easily accepted in France and in several other countries. In France only, from July 1924 until March 1932, 423,321 infants have received B.C.G. by mouth. In 1932 the number of vaccinated children was 5699 in Belgium, 9129 in Brazil, 2166 in Montreal, more than 29,000 in Spain, 2910 in Greece, 614 in Holland, 6958 in Poland, more than 100,000 in Rumania, 5659 in the province of Norbotten (Sweden), 14,146 in Uruguay, the sum total being approximately 600,000. In all the just mentioned countries, the experiment has been conducted under the supervision of well-trained physicians and with great care.

Having figures of such importance at hand, one ought to be able to form an opinion regarding (1) the harmlessness of B.C.G. vaccination, (2) its efficacy.

Harmlessness of B.C.G. — Concerning the innocuity of B.C.G. administration to infants there is practically universal agreement. The experiments of Petroff and some other authors tending to demonstrate that isolated colonies of the

B.C.G. strain could occasionally recover virulent properties have been definitely proved to be vitiated by technical errors. It has been conclusively ascertained by the highest bacteriological authorities in Germany that the shocking Lübeck disaster was not due to B.C.G., but to virulent tubercle bacilli which had, through unaccountable negligence, been mixed with the B.C.G. culture in the laboratory. This most deplorable event became the occasion of exhaustive researches concerning the possibility of spontaneous variations of the B.C.G. resulting in its transformation into a virulent strain. A special expert commission was appointed by the Public Health Committee of the League of Nations in order to appreciate the result of these researches. They unanimously concluded that such a variation has never been obtained experimentally, and that not a single instance of its occurring spontaneously had ever been demonstrated.

Isolated cases of death from tuberculous meningitis or some other form of tuberculosis following B.C.G. administration are still published from time to time. But none of them can be regarded as conclusive. A thorough inquiry invariably either shows that there has been no post-mortem, or, if a post-mortem has taken place, that no tuberculous lesion could be found, and that death was caused by non-tuberculous disease. In some instances, a tuberculous meningitis has been discovered, but it was due to virulent human bacilli, whereas B.C.G. is originally a bovine tubercle bacillus.

If the newborn infant has a tuberculous mother, it is absolutely indispensable that it should immediately be separated from her or from any tubercle bacilli carrier living in the home. It should not be returned to the family until one month at least has elapsed since vaccination has been performed and it can reasonably be presumed that immunisation has taken place. Unobservance of this rule, emphatically insisted upon by Calmette and Guérin, exposes the child to infection by virulent bacilli before vaccination has produced immunity. Cases of tuberculosis occurring in vaccinated children who have thus been exposed during the non-immune period should evidently not be attributed to B.C.G.

Protection Conferred by B.C.G.—The harmlessness of B.C.G. vaccination being to-day practically unquestioned, we have now to discuss whether it really protects the children against virulent infection. It is indeed the essential problem

and by far the most difficult. Calmette's idea at first was that by vaccinating the greatest possible number of infants and comparing their death-rate from tuberculosis with the death-rate of non-vaccinated children exposed to contagion, it would be easy to form an opinion. As a matter of fact it cannot be denied that, in every country where B.C.G. has been experimented more or less extensively, the death-rate from tuberculosis has been found considerably lower among the vaccinated group than among the non-vaccinated. But the methods used for statistical computation are open to very serious criticism.

Large numbers do not facilitate an accurate and stringent control of the experimental conditions. The proportion of vaccinated children whose ultimate fate remains unknown is great. It is almost impossible to get reliable data concerning the amount of exposure to tuberculous infection to which all the vaccinated children were submitted. The proportion of post-mortems performed on deceased children is necessarily small. Moreover, the death-rate from tuberculosis among exposed non-vaccinated children has been very diversely estimated. When Calmette and Guérin began their work, it was generally admitted that primary infection in very young children is almost always fatal. We have learned since that it is not so. Many children recover from primary infection, even when exposure is more or less permanent. The fatalities are not therefore the only reliable test of infection causing disease. I do not propose to give a full account of the objections which have been opposed to Calmette's first statistics. Suffice it to say that they were to a great extent justified. They were not able to demonstrate that B.C.G. had no immunising power. But they showed that the statistical data could not carry sufficient evidence in favour of efficacious immunisation of children by B.C.G. The consequence was that Calmette and Guérin untiringly improved their control of vaccinated children and their statistical methods.

Another objection was raised concerning the *oral* administration of B.C.G., which makes it impossible to know to what extent the bacilli are really absorbed by the lymphatic vessels. A certain proportion of the vaccinal dose evidently travels the whole length of the intestinal tract and is finally eliminated with the stools. In some cases it may be the greater part of the dose, or even the whole dose. It seems likely that the

degree of immunisation is dependent on the number of bacilli absorbed. Animals inoculated *intravenously* or *subcutaneously* with B.C.G. always become allergic: they are sensitive to tuberculin. It is hardly possible to imagine an immunity which would not be manifested by allergy. Now it is a very striking fact that a large proportion of children having received B.C.G. by mouth do not develop sensitiveness to tuberculin before several months. In some of them the Pirquet reaction remains permanently negative. *It would appear therefore that the oral administration of B.C.G. does not regularly immunise all the children, and that it is an uncertain method of vaccination.*

Several workers have recently undertaken to test the efficacy of B.C.G. vaccination on infants by experiments conducted on a smaller scale, but under more stringent conditions, and giving due consideration to the criticisms which I have just mentioned. The best planned and, in my opinion, most important of those experiments we owe to Arvid Wallgren, of Göteborg (Sweden). I propose to give you a short account of his remarkable work, done under the exceptionally favourable conditions afforded to researches of that kind in the city of Göteborg.

In this sea harbour with a population of about 250,000 inhabitants, the Municipal Dispensary is so well organised that it controls practically every family, any member of which has an open pulmonary tuberculosis. Since 1928, every child considered by the Dispensary to be in danger of infection has been vaccinated with B.C.G., either at birth or, if at a later age, after his unsensitiveness to tuberculin had been duly ascertained.

In order to eliminate the causes of error due to vaccination by mouth, and the rather large and disagreeable abscesses sometimes observed after subcutaneous inoculation, Wallgren and his associates injected B.C.G. *intracutaneously*, thus ensuring that any eventual liquefaction of the focus of inoculation would be as small and superficial as possible. They observed that, with this method, tuberculin sensitiveness, manifested by a positive Pirquet, occurred in a more reliable and fairly constant manner than after peroral administration. The larger the dose of vaccine, the quicker does allergy occur. With a dose of from 0·5 to 1 mgrm., tuberculin sensitiveness is obtained after from one to three weeks, and with the dose

of 0·05 mgrm., which is used at present, after about seven weeks. In some cases injections had to be repeated until the child finally reacted.

No child was considered to be vaccinated, nor was it permitted to be exposed to any risk of infection, until it had reacted. To ensure this, all the children have been isolated in an institution or in a private home during the period of vaccination.

Up to the end of December 1933, the number of children vaccinated amounted to 355. For various reasons, among others the death of the source of infection, 123 of these children had not yet been exposed to tuberculous infection after vaccination. Three of these 123 children have died and all three of them have been examined post-mortem. The cause of the death was (1) congenital malformation of the heart with bronchopneumonia, (2) Pertussis-pneumonia, and (3) influenzal pneumonia.

Let us now consider the 230 children who, after having acquired allergy through B.C.G. vaccination, were exposed to tuberculous infection in their homes. For various reasons quite a number of them were kept away from their homes for a still longer period. Only 6 children were exposed to the risk of infection at the age of six weeks, 45 under the age of three months, and 79 under the age of nine months. The younger the child is exposed, the greater the risk of severe infection. The duration of exposure should also be considered. Nearly three-fourths of all children exposed during the first year of their lives have been exposed for a period of more than twelve months and some of them up to from six to seven years.

All vaccinated children have been examined clinically and roentgenologically at least once a year, and kept besides under the regular supervision of the Dispensary. Only one of the 230 exposed children exhibited, on X-ray examination, an infiltration of the hilus of a decided tuberculous character. The patient was a boy, whose father and a grown-up brother had open pulmonary tuberculosis. He had been vaccinated shortly after birth and was returned home, at the age of six months, after the completion of vaccination. After six months of exposure, he became ill with fever, and had symptoms of a respiratory disease. The roentgen examination showed density of the lower part of the right lung and of the hilus. Virulent bacilli were found in the fluid obtained by gastric

lavage. The tuberculous process presented by this boy was remarkably benign. He became afebrile after a week and his general condition was never affected. Although he passed through a whooping-cough during his stay at the hospital, he was discharged after five months. The densification of the lung and hilus disappeared rapidly. It is interesting to notice that the boy's sister, aged 6 years, who had not been vaccinated, was also infected at about the same time after a short visit at home. There resulted an extensive infiltration of the right hilus, for which she had to be nursed at the hospital a whole year.

In four cases the hilus pictures were slightly larger than normal, but in three of them the patient had had pertussis or pneumonia before examination, and none of the four children had exhibited any symptom suggestive of a manifest primary tuberculosis.

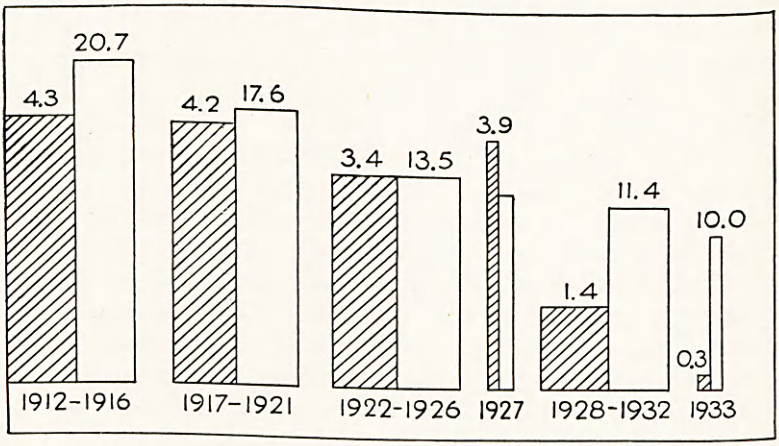
Out of the 230 children exposed, only 2 have died, one, after an exposure of one and three-fourths years, from epidemic meningitis, the other, after an exposure of six months, from acute pneumonia and diaphragmatic hernia. Both were examined post-mortem and no signs of tuberculosis were found.

It must be emphasised that not a single one of the vaccinated children missed the after examinations. Every one had been controlled, and there is no child in Wallgren's material whose fate is not known. Therefore the experiment is as nearly perfect as possible. All these 230 children have been vaccinated by the same method, insuring a complete incorporation of the vaccine. All of them have had a positive Pirquet after vaccination. All of them have been, once allergic, exposed for various lengths of time to virulent home infection. Out of these 230 children, one only exhibited a clinically and roentgenologically demonstrable infection with virulent tubercle bacilli and this infection was of a remarkably mild type.

Of course, there is no control material of unvaccinated children who have lived under the same conditions as the vaccinated ones, since practically all infants exposed to the risk of infection, but so far uninfected, have been vaccinated. Only those children who have been infected before the discovery of the source of infection could not be protected, and some of them have died from tuberculous disease.

But deaths from infantile tuberculosis occurring as a rule among those who are brought up in infected homes, the

attempt at the reduction of tuberculosis aimed at by the B.C.G. vaccinations must evidently have an effect on the total death-rate from tuberculosis in infancy in Göteborg. That this is really the case can be seen from the Chart, in which, arranged in five-year periods, the absolute number of deaths per thousand from tuberculosis among infants is shown. In the three five-year periods immediately preceding 1927, the death-rate was 4·3, 4·2 and 3·4 per thousand respectively. In 1927, which was a year of transition with but a relatively small number of vaccinated children, the mortality amounted to 3·9 per thousand. After 1928 it can be assumed that the principles advocated by Wallgren have been strictly applied.



The rate of mortality during this five-year period was only 1·4 per thousand; that is about 60 per cent. less than during the preceding period. In 1933, the first year in the next five-year period, the death-rate was only 0·3 per thousand.

It should be noted that the general tuberculosis death-rate, especially from pulmonary tuberculosis, has not shown the same downward tendency. It follows that the number of the sources of infection has not, broadly speaking, decreased in proportion to the decrease in the number of deaths from tuberculosis in infants.

I have quoted at some length from Wallgren's last paper *

* Arvid Wallgren, "Value of Calmette Vaccination in Prevention of Tuberculosis in Childhood," *The Journal of the American Medical Association*, ciii., 1341-1345, 3rd Nov. 1934.

concerning this admirable experiment, because it shows, most conclusively, in my opinion, that B.C.G. vaccination is really able to protect previously uninfected children against virulent infection. *The immunity conferred by B.C.G. is in all likelihood neither absolute nor lasting.* Therefore it is important that the children should be exposed to virulent infection, when the vaccinal immunity is at its height. The virulent bacilli are then absorbed and disposed of in the protected organism, without causing any damage, and the result of those repeated virulent reinfections, provided they are not too massive, is to transform the relative and short-lived artificial immunity produced by vaccination into a stronger and more lasting natural immunity.

Wallgren had been foremost among those who criticised Calmette's statistical methods. But instead of satisfying himself with negative criticism, he proceeded to constructive work and devised this most carefully planned experiment, from which all the causes of error which had handicapped Calmette's demonstration are eliminated, thus supporting with the most convincing proofs Calmette's contention that B.C.G. vaccination is a practically successful method for protecting children against tuberculous infection.

Before ending, I wish to mention also, in a few words, Heimbeck's experiments on tuberculin-negative probationary nurses at the Ulleval Nurses' School in Oslo. Nearly 50 per cent. of these students enter the school with a negative Pirquet, and since 1927 they are vaccinated with B.C.G., either subcutaneously or intracutaneously. Out of 136 vaccinated nurses only 3 (2·2 per cent.) became ill after having attended tuberculous patients in the course of their duty, while of the 34 unvaccinated, unaffected when they began their duty, not less than 14 (41·2 per cent.) fell ill with tuberculous disease.

We owe to Wallgren, Heimbeck, and other Scandinavian workers, such as Naeslund in Norbotten, considerable improvements in the study and technique of B.C.G. vaccination. It is to be hoped that their procedure will, as far as possible, be substituted everywhere for the less reliable peroral vaccination. The harmlessness of B.C.G. vaccination has been proved without doubt. Its effectiveness was still a matter for controversy. It is no more so. Calmette and Guérin's discovery must be regarded as the first successful attempt at specific prevention of tuberculous infection in human beings.

Demonstration. — *Dr Agnes R. MacGregor* gave a demonstration of whole lung sections illustrating pulmonary lesions in children. The specimens included examples of the " primary lung complex," localised tuberculous infiltrations, extensive tuberculous bronchopneumonia, and miliary tuberculosis ; also, for purposes of comparison, sections from cases of acute bronchopneumonia, and of chronic non-tuberculous pulmonary fibrosis and bronchiectasis.

Demonstration.—*Dr May H. Christison* showed photographs of colony variants of several species of *mycobacteria* grown on Petroff's gentian-violet-egg medium. Variants of *Bacillus Calmette-Guérin* (B.C.G.) included " S " and " R " forms described originally by Petroff, " S " and " I " forms described by Begbie, and a round smooth colony regarded as " S " by Krause, and " I " by Petroff. All the variants were derived from a single strain of B.C.G. and none exhibited the high degree of pathogenicity which has been ascribed to " S " variants by some investigators. Variants of the human and bovine types of *M. tuberculosis* were also shown.

The diversity of colony structure which may be exhibited by *mycobacteria* was well exemplified by photographs of the more rapidly growing *M. " lepræ "* (Brinckerhoff) and the Fish Tubercle Bacillus (Cobbett).