A REVIEW OF RECENT LITERATURE ON VACCINATION AGAINST TUBERCULOSIS

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The first attempt of vaccination against tuberculosis was made by Cavagnis in the year 1886. He injected increasing doses of sputum of tubercular patients, previously mixed with a diluted solution of carbolic acid.

Grandcher (1889) and Ledoux followed by vaccinating rabbits, but they failed and gradually changed this method for intravenous inoculations of cultures that had been previously attenuated by age in artificial media, substituting them for more virulent strains. The rabbits so treated were more resistent to infection than their controls, but sooner or later they acquired the disease.

Other authors, Richet, Hericourt (1890), Dixon (1889), Turdeau (1892) and Schwernertz (1894) carried on vaccination with human strains previously destroyed by heat or with cultures of avian origin which were gradually substituted for strains of human origin.

Behring in 1902 published his method of "Jennerization" whereby he injected calves intravenously with a dose of from four to twenty mg. of dried cultures of Bac. Tuberculosis previously desiccated in a vacuum and whose virulence had been considerably lowered. Since that time this method has been the origin of numerous experiments and consequently it has been especially applied to cattle by Hutyra, Thomassen, Rosignol, Wallee, Belfanti, Stazzi, Mullie and others. It seems perfectly proven that this method of vaccination offers an appreciable resistance in infection, whether natural or artificial, but lasting possibly one year. All virulent strains inoculated after vaccination seem to invade the bronchial and mediastinal lymph glands where they remain, until the resistance thus created by vaccination disappears and then the organism is rapidly invaded.

Koch, Schurtz, Hutyra and Weber tried intravenous inoculations of virulent strains in calves, but they soon found that all the organs were virulent to guinea-pigs.

Vallee made experiments with strains of equine origin that were obtained by Borrel and that were avirulent for guinea-pigs, but his results were similar to those of Behring.

Theobald Smith tried vaccination with bovine strains that were

previously inactivated at 60° C, and Levy used cultures macerated in glycerine or in concentrated solutions of galactose.

All these methods showed poor results and were substituted by using dead cultures treated by ether, chloral, iodine, the hypochlorites, sodium chloride and radiations of the spectroscope, etc.

Great expectation was aroused by the work of Friedman, de Moeller and Klimer who used human and bovine strains that had previously been passed through cold-blooded animals, but it was soon demonstrated that bovine or human strains do not multiply in cold-blooded animals and that their virulence is not altered.

All these experiments made the investigators believe that conditions must be made as similar as possible to those prevailing in human tuberculosis and that strains of human or bovine tuberculosis could be used in which modifications in order to produce a low virulence should be reduced to a minimum. Dead bacilli and those whose protoplasm suffers considerable alterations have a very low vaccinating power and are practically worthless.

So far the employment of preventive vaccines in animals free from tuberculosis has no other object than to place them in the same condition as tubercular patients to reinfection. The only appreciable benefit is to protect them from acute forms of tuberculosis during a short period of time following vaccination.

Recently, there has been a considerable amount of work done to obtain a successful vaccine. Dr. Jaime Ferrán of Barcelona, Spain, has elaborated a new theory whereby he explains that the tubercle bacilli is developed from a saprophyte. He claims that the tubercle bacilli is only one of the forms of the causative agent of tuberculosis and that environment is what makes the saprophyte go through various transformations which he designates as Alpha, Beta, Gama and Epsilum and then gets the acid-fast characteristics becoming what is known by us as the tubercle Bacilli. He has developed a vaccine made of various strains of saprophytes isolated from man and other mammals which he cultured. The killed cultures are inoculated subcutaneously or orally and are used both to prevent and treat the disease.

Shiga, a Japanese investigator, has also prepared a vaccine consisting of human strains that have been attenuated by successive cultures through glycerine broth to which progressive doses of Triploflavine are added. He also uses an immune serum prepared from calves that have been cured by this vaccine. His serum vaccine is used both as prophylactic and therapeutic treatment.

Calmette, of the Pasteur Institute has prepared a vaccine which has created a great deal of expectation and comment. After the

work of many years he has developed a culture which holds its acid-fast characteristics, but is avirulent.

Since the work of Marfan in 1886 who observed that patients who had tubercular lymph glands became more resistant to other tubercular reinfections than those who were exposed to contagion for the first time, Calmette and Guerrin decided to experiment in young calves that were infected through the digestive tract, coming to the following conclusions:

1. Animals that have been injected with virulent cultures of Bacillus Tuberculosis contract the disease. They may develop a glandular or pulmonary tuberculosis or both. They react to tuberculine for two months and they may be cured.

2. Those animals that have been cured after inoculation are no more susceptible to infection for a certain period of time even if they are given virulent cultures of the bacillus, therefore they shall be considered as vaccinated. On the other hand, animals that have been inoculated two or three times repeatedly at short intervals of time never get cured, their lesions get worse going rapidly into caseation.

In this way they interpret the cicatrical tubercular lesions found at autopsy, that is, those patients have been cured after inoculation, while those that remain tubercular have received several inoculations at successive short periods so that their lesions have undergone caseation.

3. A small contamination of slight intensity determines a benign infection that confers a certain degree of immunity to successive infection, while successive contamination with massive doses produces a very advanced tuberculosis which is usually fatal.

Since all the methods of vaccination so far have failed, Calmette and Guerrin with his coworkers have tried to impregnate the lymphatic system as quickly as possible with living cultures of tubercle bacilli that have been made virulent.

They are opposed to the use of virulent strains as (used by Wells and Williams in the United States and Selter in Germany) since it is possible to produce advanced lesions in weak subjects.

They have developed a culture of Bacillus Tuberculosis of bovine origin that is avirulent for all animals and has fixed hereditary and cultural characteristics. After many experiments they found that a virulent organism that has been passed thirty times (one every twenty-five days) through potato slants in five per cent glycerinated ox bile became avirulent for calves and guinea-pigs. This organism was passed two hundred and thirty times through the same media in a period of thirteen years and then when injected in

massive doses intraperitoneally or intravenously into rabbits and guinea-pigs the bacilli were perfectly harmless. Moreover, they produced an antigen capable of developing numerous antibodies in the inoculated animal. In the usual culture-media it looks like the Bacillus of Koch and is an active producer of tuberculin.

This organism once inoculated invades the lymphatic system where it remains, producing a marked resistance to infection. This resistance has been called by Calmette "state of premunition" and the organism is designated as the B. C. G. in honor of Drs. Calmette and Guerrin.

In further experiments these investigators have demonstrated that bacteria passed with great facility through the mucous membrane of the intestines in the first ten days of life and that as the person or animal grows older the chances are very much lessened. Disse, thinks that this is due to the absence of "Protoplasmic cells" in the intestines of the new born.

In adults the B. C. G. is capable of going through the walls of the intestines, but with great difficulty and in very small numbers.

Vaccinations made in calves and monkeys with B. C. G. orally, makes them immune to artificial inoculations for eighteen months. Revaccinations have been made in these animals in order to reinforce the "premunition" with very good results. From these experiments they concluded that revaccinations can be made at the age of one year and three years respectively so as to reinforce the resistance to infection originally created by the first vaccine.

Vaccinations are also administered orally to infants. The doses of B. C. one centigram each are given at forty-eight hour intervals, in warm milk, immediately before breakfast. Infants vaccinated in this way are made immune for more than four years as demonstrated by Weill, Halle, and Turpin (1922). None of the children vaccinated have contracted tuberculosis although the majority of them have lived subject to constant exposure. Calmette concludes that with his vaccine he can protect infants until they are five years old precisely in the most dangerous period of their life when they are in contact with their tubercular parents.

These satisfactory results established a definite orientation as the first five years of life is the period of possible exposure to massive infection.

Revaccinations should be performed and are only recommended in children who are in contact with tubercular families. In perfectly healthy surroundings the revaccinations are believed to be unnecessary.

Several objections have been made to Calmette's method of vacci-

nation, which he has tried to answer. German investigators claim that children thus vaccinated do not react to tuberculin, but Professor Calmette believes that this is a question of time as after the second year of age the vaccinated infants usually react. Furthermore he claims that this reaction is not necessary to prove immunization, for in animals that have been vaccinated and reacted to tuberculin this property gradually disappears and the animals later become immune to artificial inoculation.

Another objection is the possibility of the B. C. G. becoming virulent when once installed in the lymphatic system. Calmette answers these objections by saying: "In autopsies made in vaccinated children who have died of some other disease the B. C. G. has been isolated in pure culture from the mesenteric glands and these pure cultures were perfectly avirulent for guinea-pigs and other laboratory animals."

Several investigators who inoculated rabbits with massive doses of B. C. G. intravenously, found that a follicular lesion was formed in all the organs and concluded that this was a form of tuberculosis produced by massive doses of the organism. Calmette and Guerrin took the matter up more carefully and were able to produce the same follicular lesions by introducing from twenty to thirty centigrams of dry B. C. G. cultures intravenously into rabbits; but the organs of these rabbits were perfectly avirulent to guinea-pigs and furthermore the lesions were found to heal and disappear completely three months after inoculation.

One of the most serious objections made is that vaccinated infants die later from tuberculosis, but he claims that these cases fortunately have not been many and are cases of children infected in utero through placental inoculation.

The eighth of August, 1927, Dr. Pedro Gutiérrez Igaravídez received a culture planted on July 2nd in the Pasteur Institute; this culture was brought to our laboratory on August 10th and replanted by Dr. Salvador Giuliani on August 20th in suitable media. On August 24th a new batch was inoculated from the original culture. From August 26th to September 12th the tubes were incubated at 38° C. and at this date the cultures were identified as pure cultures of B. C. G. At present Dr. Giuliani has five generations of the original culture and has performed inoculations in experimental animals.

The B. C. G. grows in the ordinary culture media for Bacillus Tuberculosis, but it grows especially well on potato slants with glycerinated five-per-cent veal bullion Ph7 o 7.2. The potatoes must be previously submerged for some hours in water containing 10 grams of sodium carbonate per liter. These potatoes are dried in

SOME OF CALMETTE'S OBSERVATIONS ON INFANTS THAT HAVE BEEN IN DIRECT CONTACT WITH TUBERCULAR PATIENTS AFTER VACCINATION

Patient's initials	Date of birth	Date of vaccination	In contact with	Condition at one year of age	Additional data
X.	November 19, 1925..	The 3rd, 5th and 7th day after birth	Tubercular grandmother	In good health.....	Has had strong attack of measles and recovered in good condition.
J. H. M.	April 3, 1925.....	The 3rd, 5th and 7th day after birth	Tubercular father..	In good health.....	Has had grippe, bronchopneumonia and meningitis and recovered without showing signs of tuberculosis.
M.	September 19, 1925	The 3rd, 5th and 7th day after birth	Tubercular mother, sister died of tuberculosis	In good health.....	Had measles at 15 months of age and is in apparent good health.
Th. H...	December 8, 1925...	The 3rd, 5th and 7th day after birth	Lives with tubercular mother	In good health.....	In good health.
Dup. ...	July 16, 1925.....	The 3rd, 5th and 7th day after birth	Born from tubercular mother who died March 1926	In good health.....	At 18 months of age in perfect health.
L.	November 6, 1925..	The 3rd, 5th and 7th day after birth	Lives in tubercular family, three infants died before of tubercular meningitis	In good health.....	In perfectly good health at fourteen months of age.
A. F....	July 8, 1925	The 3rd, 5th and 7th day after birth	Mother died tubercular and father advanced tubercular	In good health.....	Apparently healthy. Is sixteen months old.
R..	September 24, 1925	The 3rd, 5th and 7th day after birth	Mother tubercular, died	In good health....	Had pneumonia in October, 1926. Is well at 18 months of age.
A. T....	March 14, 1925	The 3rd, 5th and 7th day after birth	Tubercular mother and two tubercular uncles	Good health, vaccinated at one year of age	In excellent condition at two years of age.
J. K.....	July 31, 1925, premature birth	The 3rd, 5th and 7th day after birth	Tubercular father..	Good health, vaccinated at one year	Good health at eighteen months
M. D ...	May 19, 1925..	The 3rd, 5th and 7th day after birth	Mother tubercular. The 1st four infants died of tubercular, meningitis	In good health.....	In perfect health.
A. G....	March 13, 1925.....	The 3rd, 5th and 7th day after birth	Mother died of pulmonary tuberculosis on April 1926	In good health, revaccinated the 1st year	In very good health at 21 months of age.

filter paper and fixed in Roux tubes that have been previously filled with the glycerinated broth. The whole tube is then sterilized at a temperature of 120°C for thirty minutes and then incubated for three successive days to prove sterilization.

The inoculation is made with platinum spatula, disseminating well the inoculating sediment on the surface of the potato slants. The tubes are closed and incubated at 38°C.

Usually after the twentieth day the growth is abundant enough to prepare emulsions, but it is desirable to wait until the twenty-fifth day to get the largest possible number of living organisms.

It is important to see that the B. C. G., keeps all its essential cultural characteristics, so after ten cultures through glycerinated media, two or three cultures should be planted in ox-bile media which is prepared as follows:

Place in a large vessel the contents of several ox gall-bladders, sterilize at 120°C. for thirty minutes and keep at room temperature for three weeks. By this time a large sediment of biliary pigment is formed at the bottom of the vessel and this should be separated by filtration. Divide the bile into two equal portions. Into one of these portions submerge the potato fragments and add five per cent of glycerine; then submerge the whole vessel in a water-bath at 75° C, for three hours. The second portion of bile with five-per-cent glycerine is added and is distributed through the Roux tubes up to the constriction. At the end of three hours the potato fragments are taken out of the bile and wiped in filter paper in order to remove the excess of bile. Now they are ready to be fixed into the Roux tubes. All the tubes are sterilized at 120°C for thirty minutes.

B. C. G. grows distinctly on this media and from the tenth to the thirtieth day all the surface is covered by a grayish green film that becomes thicker gradually. After the twenty-fifth day the growth gets thicker taking a bright-coffee color, looking very much like an old culture of bacillus Mallei. The bacilli cultivated in this way are granular, thinner and longer than those cultivated in ordinary media, but they have the same acid-fast characteristics.

After three or four cultures in bile media they are taken back to glycerinated potato slants and look again like the cultures of Tuberculosis Bacillus. From the glycerinated media the cultures can be passed to liquid or solid media without acquiring virulence.

BIBLIOGRAPHY

Calmette—Annales de L'institut Pasteur 1911.
F. Klempeer Zeit f. Klein met. 1905.
A. Gebert y P. Caort—Bacteroterapia, Vacunación y Sueroterapia, segunda edición Salvat y Co., Barcelona.

Gutiérrez Igaravídez P. La profilaxis Vaccinal de la Tuberculosis según el método Calmette-Guerrin. Bull. P. R. M. A. No. 157–158 July and Sept. 1927.
Marfan—Arch. Gen. de Medecine Vol. 1886 p. 423 et 573.
H. Valle et Rossignot Bull. de la Soc. de Med. Veter. Prat. 15 mars 1906 p. 42.
C. Guerrin Annal. de L'institut Pasteur 20 aout. 1906 p. 609.
Romer-Stizungster O, aerztl. Veens zu marbug 19 mai 1908.
R. Krauss et Wolk Centr. f. Bakl, 42–1908 et 47—1910.
Webb & Williams J. A. M. A. 57–1911 p. 1431.
Selts Dents Med. Woch 51, 1925 p. 933.
Disse Berl. klin Woch. 1–1903.
Calmette, Guerrin et Delearde. Acad. des. Sc. mai 1906.
E. Prospert Soc. de Biol. 19 julliet 1924.
J. Valtis Acad. des Sc. 181–15 oct. '25.
Arloing et Dufout-Acad. des Sc. 181–9 nov. 9—25.
Couvelaire A acad. des Sc. 183 nov. 26.

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SOMMAIRE DU. NO. 3