THE ARTIFICIAL IMMUNISATION OF CATTLE AGAINST TUBERCULOSIS.¹

By LEONARD PEARSON and S. H. GILLILAND.

(From the Laboratory of the State Live Stock Sanitary
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EFFORTS to produce artificial immunity against tuberculosis have been conducted for a number of years and along many lines. In 1890 Robert Koch announced the discovery of tuberculin. It was claimed at that time that by the use of tuberculin resistance to tuberculosis could be increased in such a way as to assist in withstanding infection, and also that some infections already established might under the use of tuberculin be overcome. But extensive experimentation has shown that these effects are not sufficiently uniform, lasting, or powerful to be of distinct practical value.

Following this pioneer work of Koch came the work of many investigators with modified tuberculins and with extracts from tubercle bacilli made in a variety of ways. Repeated experiments with all these substances have shown that it has not been possible by their use to render animals immune to tuberculosis permanently, or to a practical degree. Experiments have been made by several investigators to test the immunising value of dead tubercle bacilli and of bacillary pulp. The results have been about the same as have followed the use of extracts from tubercle bacilli.

It became evident years ago that immunity against tuberculosis, to be of value, must confer protection against the organism of tuberculosis as well as against its toxins. There must be bacterial as well as toxic immunity. Efforts to immunise animals against living and virulent tubercle bacilli by inoculating them with living tubercle bacilli of low virulence were made as early as 1891 by Granchez and Ledoux-Lebard. In 1892 and 1893 Trudeau² found that by inoculating rabbits subcutaneously with living cultures of avian tubercle bacilli, he was able to increase their resistance to infection from mammalian tubercle bacilli of a culture known to be virulent for rabbits. De Schweinitz³ in 1894 discovered that it was possible to very greatly increase the resistance of guinea-pigs to inoculations of tuberculosis by inoculating them with attenuated tubercle bacilli of human origin; the process of attenuation consisting in prolonged cultivation (twenty generations) of glycerine beef broth of acid reaction. Tubercle bacilli of human origin grown in this way lost their virulence for guinea-pigs, but guinea-pigs inoculated with this non-virulent culture developed so much resistance to tuberculosis that when they were afterwards inoculated with tuberculous tissue from a cow they remained healthy, while other guinea-pigs inoculated with the same material from the cow died of tuberculosis in seven weeks. De Schweinitz injected very large quantities of human tubercle bacilli into cattle subcutaneously, intravenously, and intra-

¹ Reprinted from the "American Veterinary Review," September 1905.

² "New York Medical Journal," 23rd July 1893.

³ "Medical News," New York, 8th December 1894.

peritoneally. He found that by gradually increasing the dosage enormous quantities could be tolerated without injury. He administered as much as 500 cc. of a heavy suspension of tubercle bacilli at one time.

M'Fadyean¹ in 1901 and 1902 reported that he had found that the resistance of cattle to tuberculosis may be very greatly increased by the use of successive inoculations with tuberculous material or tubercle cultures of low virulence. In a paper of M'Fadyean's entitled "Further Experiments Regarding the Immunisation of Cattle Against Tuberculosis,"² the author states this conclusion:—

"It appears to be justifiable to conclude that, whatever may have been the degree of natural immunity possessed by these three experimental animals, it was much increased by the successive intravenous inoculations to which they were subjected. The immunity was not absolute, but it may be doubted whether a degree of resistance that would merit that term is obtainable by any method in cattle."

Von Behring announced in December in 1901 that he was engaged in studying the immunisation of cattle against tuberculosis, and he has since issued several reports upon his work. Von Behring has made a very large number of experiments in this field; he has endeavoured to produce immunity by the use of tuberculin, by the use of other tuberculosis toxins, by anti-toxins, by the use of dead tubercle bacilli, of tubercle bacilli weakened by chemical agents, and by the use of tubercle cultures of low virulence. A method for the vaccination of cattle against tuberculosis has been formulated by von Behring, and it is based on the use of tubercle bacilli of human origin that are non-virulent for cattle. In making this "vaccine" the tubercle bacilli are dried and ground to a powder. The vaccine material is sent out in this dried state. It is necessary to make a suspension of it in normal salt solution, whereupon it may be administered by intravenous injection. A number of investigators, as Lorenz, Schlegel, Eber, and Hutyra, have tested the resistance of cattle treated by von Behring or according to von Behring's method. It has been shown that the resistance of these animals to tuberculosis has, in most cases, been increased, and sometimes considerably so.

It is to be observed, however, that many of the cattle reported upon by the above authors were not vaccinated according to the method that von Behring now recommends, and as Eber says³ "... The tuberculin test is not a reliable means for determining the freedom from tuberculosis of an animal that has been treated with mild bovine or human tubercle bacilli unless a long time (generally more than half a year) has elapsed since the last administration of infectious material. ... Since neither of the treated cattle was vaccinated by the two vaccination methods with attenuated human tubercle bacilli according to the method now recommended by von Behring, the conclusions that are reached as a result of my investigations have only a relative bearing on an estimation of the value of the method now recommended by von Behring.

"The results of the investigations show, however, that it is possible to confer upon cattle by treating them with attenuated bovine or

¹ "Journal of Comparative Pathology and Therapeutics," June 1901, and March 1902.

² "Journal of Comparative Pathology and Therapeutics," March 1902.

³ "Zeitschrift für Tiermedizin," Band IX. Heft 3-4, 1905.

human tubercle bacilli a certain degree of resistance to artificial tuberculosis infection.

"Whether this resistance, as produced by the two-vaccination method, with attenuated human tubercle bacilli, that is now in use, furnishes protection against natural infection can only be determined by years of careful observation on the greatest possible number of animals immunised in this way and controls at the time of slaughter."

Hutyra¹ has reported some work conducted by him in 1903 and 1904 at the Royal Veterinary College of Hungary, wherein he tested the immunity of calves vaccinated according to von Behring's method, with material furnished by von Behring, and also with material prepared from cultures made by himself. Hutyra tested the resistance of his vaccinated animals by inoculating them with virulent bovine tubercle bacilli, and their resistance was compared to that of unvaccinated animals. He found that nearly all of the vaccinated animals had more resistance than the unvaccinated animals. One animal treated with von Behring's vaccine (No. 3) appeared to have received no immunity as a result of vaccination. Hutyra's own vaccine material from different sources appeared to give a noticeably higher degree of immunity than that obtained from von Behring's.

Recently, a large number of cattle, amounting to several thousands, have been vaccinated after the von Behring method in Germany, Austria, and Hungary. The process does not appear to be free from danger in every instance, as is shown by the reports of Marks, Casper, and others. It is as yet impossible to draw any conclusions whatever as to the efficacy of vaccinations from the results of this great number of vaccinations of cattle on farms. The only conclusion at which one may fairly arrive is that vaccination appears, in most cases, to be unattended by danger to the vaccinated animals. Whether immunity is conferred and, if so, whether it is sufficiently powerful or lasting for practical purposes, is not yet shown by the practical application of von Behring's method. Of the animals that have been vaccinated on farms and afterwards exposed to infection a number have been slaughtered, and some have been found to be afflicted with tuberculosis, while others have been found to be free from tuberculosis. The presence of lesions of tuberculosis is taken by some of von Behring's observers to mean that the animal was tubercular before it was vaccinated. Of course, this may be true. But it must be remembered that the absence of lesions of tuberculosis in vaccinated animals cannot fairly be taken to signify the existence of a serviceable degree of immunity, unless it is shown that the conditions under which these animals were kept were such as to lead to the infection of a considerable proportion of unvaccinated animals. And this evidence is in most cases lacking.

Klimmer has carried out some very useful investigations upon the immunisation of cattle against tuberculosis at the Royal Veterinary College at Dresden.² Klimmer protests against sending out vaccine material in the dry, powdered state, on account of the great danger to the operator who attempts, in the field, to prepare this material for use. Klimmer's practice is to prepare suspensions of tubercle bacilli in the laboratory, just as has been done for a number of years

¹ "Berträge zur Experimentellen Therapie," Heft 9, 1905.

² "Berliner Tierärztliche Wochenschrift," 5th July, 1905.

at the laboratory of the Pennsylvania State Live Stock Sanitary Board.

The vaccine material that has been prepared in Dresden appears to be harmless to the vaccinated animals, and indications thus far are that it confers a serviceable degree of immunity, but it is not yet possible to draw final conclusions from this work for the reason that the vaccinated animals are still living, and it is impossible to determine positively whether they are free from tuberculosis. Klimmer proposes the use of a vaccine material made of mammalian tubercle bacilli that have been rendered less virulent by passage through a cold-blooded animal. The advantage from such a culture would lie in the total absence of danger to the operator.

Work upon the vaccination of cattle against tuberculosis at the laboratory of the State Live Stock Sanitary Board of Pennsylvania commenced in the year 1900. It has, therefore, been underway for about five years. We were able to show three years ago¹ that it was possible to increase the resistance of animals to tuberculosis to a very high degree by treating them with several intravenous inoculations of non-virulent tubercle bacilli of human type. Two cattle thus treated were inoculated intratracheally with a suspension of bovine tubercle bacilli, and both of them wholly resisted the inoculation. The only lesions in either of these animals was a slight thickening upon the wall of the trachea at the place where the hypodermic needle was inserted. Two unvaccinated animals, inoculated at the same time, in the same way, with the same quantity of bovine tubercle bacilli, from the same culture, became extensively infected with tuberculosis, showing the lesions on the mucous membrane of the trachea and bronchi, in the lungs, and in the bronchial, mediastinal, and postpharyngeal lymphatic glands.

The immunity that was obtained in the case of these two animals resulted from the administration of seven intravenous injections of vaccine material during a period of ten weeks. The doses of tubercle bacilli ranged from 13 to 26 mg. While this experiment, and other similar experiments, were sufficient to lead us to the conclusion that animals may be rendered immune to tuberculosis, it was at the same time evident that a process depending upon the application of vaccine material seven different times, would be of comparatively small value in general practice. Therefore, we set ourselves to the study of the effects of other methods of vaccination and to the development of a simple and effective method. During the past three years we have attempted to determine the immunising effects of several strains of tubercle bacilli. We have administered vaccines in different doses, at long and short intervals, and with varying number of doses.

For these experiments we have been supplied by the State Live Stock Sanitary Board with a large number of animals. For two years we have had the use of a farm where about one hundred cattle have been kept for experimental purposes. We have also had the use of stables at the veterinary department of the university of Pennsylvania, where from twenty to twenty-five cattle have been kept, together with numerous goats and small laboratory animals. Through the use of the farm we have been enabled to keep a considerable number of cattle under practical farm conditions.

¹ "Philadelphia Medical Journal," 29th November 1902.

During the time that they have been under observation in our experiments, the resistance to tuberculosis of vaccinated animals has been tested through exposure to natural infection and by inoculation. When vaccinated animals have been exposed to infection and when they have been inoculated, unvaccinated, control animals have been equally exposed or inoculated. We regard this use of controls of the highest importance, because it is only in this way that a definite standard for comparison can be provided in order to measure the extent of resistance to a given amount of exposure. Moreover, it is only by the use of controls that one can ascertain whether exposure under natural conditions has been sufficient to lead to the infection of unvaccinated animals.

It is not enough to associate vaccinated cattle with tubercular cattle and then to conclude that the vaccinated cattle were immune because, when they were killed, they did not show lesions of tuberculosis. It might readily happen that the exposure was not sufficient to lead to the infection of unvaccinated cattle; but if, upon equal exposure, we find that the unvaccinated cattle are tuberculous, and the vaccinated cattle are free from tuberculosis, then we may safely and justly conclude that the vaccinated cattle received immunity from their vaccination. Our proof is based upon such clear evidence as this, frequently repeated.

Our experiments have shown that different strains of tubercle bacilli have different immunising values. Avian tubercle bacilli may produce an intoxication resulting in great emaciation, but avian cultures do not appear to confer upon animals so much immunity as results from the use of mammalian cultures that are non virulent for the animals upon which they are used. In order to produce artificial immunity in mammals it appears to be necessary to vaccinate with an attenuated mammalian culture.

We have not found any advantage either in respect to the degree of immunity or economy of time in the use of several vaccines made from cultures of different and progressive degrees of virulence.

The amount of immunity bears a rather definite proportion to the number of vaccinations and the amount of vaccine material used, provided however, that the animal be not "over vaccinated;" that is, that the vaccinations are not too close to one another, and that an excessive amount of vaccine is not administered. By over vaccination the resistance of an animal to tuberculosis may be reduced to a point below normal, or a fatal toxæmia may be caused.

Experiments have been made wherein animals vaccinated with different doses and different numbers of doses have been inoculated at the same time, and in the same way. While the resistance of all of the vaccinated animals to the inoculation has been much greater than the resistance of unvaccinated animals, it has been possible to observe a marked difference between animals vaccinated in different ways. For example, an animal vaccinated five times has more resistance than an animal vaccinated four times, and other conditions being equal an animal vaccinated four times has more resistance than an animal vaccinated three times.

From the practical standpoint, it is more important to ascertain the amount of vaccination that is necessary to protect animals under customary conditions of exposure and the shortest possible time

during which a sufficient degree of immunity may be conferred, than it is to determine how great a degree of immunity may be produced.

Since it has been necessary to try a great number of different methods of vaccination, and since the time required for an experiment of this sort is much greater than is usual in experiments conducted through laboratories of hygiene, it has been difficult and time consuming to accumulate evidence upon which to base a general method for the vaccination of cattle.

It is already clearly evident that different degrees of immunity, and serviceable degrees of immunity, may be obtained at will. Under natural conditions, animals differ in respect to their inherited or acquired resistance to tuberculosis. Conditions of exposure differ, being very much greater upon some farms and in some herds than in others. Therefore, it seems to be reasonable to conclude that animals will require more artificial immunity to tuberculosis under some conditions than under others. The case is similar to the protection of cattle against anthrax by vaccination. In Pennsylvania we have found by experience, extending over a series of years, that the immunity conferred by vaccinating with the first and second vaccine of Pasteur usually is sufficient. On a few farms, however, the exposure appears to be more intense, and it is found that on such farms it is necessary to use the third vaccine of Pasteur. Experience may show that it will be necessary to vaccinate different breeds of animals, and animals kept under different conditions, in slightly different ways.

The amount of immunity that is conferred by three vaccinations is rather high, and will probably be sufficient in most cases. Indeed, two vaccinations may supply a sufficient amount of immunity under some conditions. Under other conditions it may be necessary to vaccinate four times.

The subject of the duration of immunity is very important, and is one that can be solved only by observations upon a large series of vaccinated cattle, some of which may be killed and examined *post-mortem* from time to time during a term of years. Of course it is necessary that animals kept for this purpose shall constantly be exposed to infection and unvaccinated controls must be similarly exposed, and the *post-mortem* results must show that the amount of exposure was sufficient to cause the infection of the unvaccinated animals.

Our own experiments indicate that artificial immunity may endure at least two years, and there is every reason to expect that additional experiments will show that immunity will be of longer duration than this. Perhaps it may last throughout the entire life of the animal; but conclusions on this point are entirely premature.

The effect of tuberculosis vaccination upon cattle already infected with tuberculosis has also received our attention¹; it has been found that intravenous injections of tubercle bacilli of human type, non virulent for cattle, have a very marked influence not only in restraining the progress of existing lesions, but also in causing them to become encapsulated and to recede. In other words, such treatment appears

¹ "University of Pennsylvania Medical Bulletin," April, 1905. "American Veterinar Review," 1905.

to have a marked curative effect on some tuberculous animals. But this statement should be accompanied by a note of warning. We do not for a moment wish to imply that it is practicable, or even possible, at this time, to cure tuberculous cattle. The observations to be made in this direction are regarded by us as of importance chiefly as tending to throw additional light on the subject of immunity. It was shown that the treatment given had the same immunising effect upon infected animals as upon healthy animals. The effect of the immunisation in these cases was, first, to prevent the further dissemination of infection and, second, to cause the encapsulation and delimitation of the existing lesions.

It is probable that this method of treatment may prove to be of value with relation to young animals, or animals known to be but recently infected with tuberculosis. Such treatment will always have to be carried out with the most extreme care and under conditions that can be very fully controlled. It is not to be recommended for general practice.

As to the application of vaccination against tuberculosis; vaccination, properly applied, is effective, and we believe that it will prove to be of very great practical value. It is not possible on most American farms to establish a separate herd of infected cattle, and to keep the infected cattle as separate and distinct from the uninfected cattle as is necessary, if tuberculosis is to be treated according to the Bang system. The alternative, if tuberculosis is to be eradicated, is to destroy all of the animals that react to the tuberculin test. The latter plan involves a great deal of waste and loss, which, heretofore, has been unavoidable, and which has been amply justified because it has been less than that resulting from the continuation of the infection in the herd.

But it is important that a better, a less expensive, and, if possible, a more effective method shall be devised. It appears to be highly probable that vaccination may supply this method. It is admittedly impossible to test with tuberculin all of the tuberculous herds of a state. Therefore, it is not possible to discover and to either destroy or sequester all of the cattle that may be distributors of tuberculosis. Herds freed from tuberculosis by the use of the tuberculin test are always exposed to reinfection, and must be guarded by the double testing of all recruits and by systematic reinspections of the herd. All of this may be, and is, carried through successfully, and should be practised to a much greater extent than it is, unless an equally effective and cheaper method can be devised.

It will be a great step in advance to be able not only to discover that an animal is free from tuberculosis, but also to render that animal permanently immune to infection. It will be a great advantage to be able so to treat young cattle that they may resist tuberculosis even though they are continually exposed. The advantage to the breeder of being able not only to grow a herd free from tuberculosis, but immune to tuberculosis, will be very great. We believe that all of this may be looked forward to; but, until more experiments and observations are completed, vaccination should be applied only under such conditions as will permit exact observations to be made, and each vaccination must, for the present, be regarded as a scientific experiment.