FIVE YEARS' EXPERIENCE OF PROTECTIVE INOCULATION AGAINST TUBERCULOSIS IN CATTLE BY VON BEHRING'S METHOD.¹

By Dr STRELINGER.

THE general struggle in which we are engaged against that most widely distributed of all infectious diseases, namely, tuberculosis, renders it obligatory on practitioners to utilise every method by which the further spread of the disease can be checked. In this struggle we shall only attain success when human and veterinary practitioners work hand in hand, and, mutually assisting one another, turn to account in practice the experience successively attained as time passes.

That a general advance by both branches of medicine is essential to success follows naturally from the fact that the causes of human and animal tuberculosis are similar. Although the various types of tubercle bacilli may exhibit differences in their cultural properties and in their virulence, the pathological changes which they bring about in the human or animal organism are nevertheless more or less identical. Furthermore, both in the bodies of children and in those of adults, bovine bacilli have often been discovered. This fact has become of greater importance through the discovery of atypical forms of tubercle bacilli, for these atypical or so-called transition forms gradually accommodate themselves to the organism of the individual in whom they are found, the foreign tubercle bacilli gradually approaching in form and properties those bacilli which usually occur in that organism. In this way, perhaps, the varying degrees of virulence noted in different types of tubercle bacilli may be accounted for.

Infection with tubercle bacilli may occur in various ways. Infection through the digestive tract is certainly common, so that the greatest danger during early life is to be found in the ingestion of infected milk. If comparatively few cases of pronounced tuberculosis occur during the suckling period, whilst they increase as the years pass, the facts seem rather to support the view that tubercle bacilli received during the suckling period may long remain latent and only produce an outbreak of tuberculosis when special circumstances favour its development.

The important work of Pirquet regarding the so-called early diagnosis of tuberculosis renders important service in showing that tuberculous infection is in most cases to be referred to infection during the earliest years of life. The excessive sensitiveness of infected children to Pirquet's inoculation method also demonstrates in such cases that infection with tuberculosis has taken place even where under former conditions macroscopic or clinically detectable tuberculosis could not be demonstrated. In these cases early cultivation experiments and experiments on animals might explain the phenomena of excessive sensibility, and, simultaneously, the latency of the tubercle bacilli. Perhaps the granular unstainable form of

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tuberculous virus discovered by Von Much may contribute to the clearing up of this question.

In reference to the above, it is a pleasure for me to be able to record the results of the bovo-vaccin protective inoculations carried on during the last five and a half years.

We began our inoculations with the bovo-vaccin on the 15th September 1902, on the estates of H.R.H. Prince Ludwig of Bavaria, at Sárvár, Hungary. The managers of the estate, having been convinced of the value of the method, desired that the protective inoculation should gradually be carried out in all the farms of the estate. In conducting the inoculations, von Behring's directions were punctiliously observed.

The following observations were made during the progress of the vaccinations:—

In general, only two to three-weeks-old calves were inoculated, and such as had proved healthy on veterinary inspection. Three months after the first injection, the second was given. Before each inoculation the calves were kept under observation for one to two days and their temperatures taken. After inoculation the temperature observations were usually continued for eight to ten days. The animals were weighed approximately every month. The protective material was obtained exclusively from von Behring's factory at Marburg, on the Lahn. The instruments and everything used in the inoculations were sterilised immediately before use by boiling in 2 per cent. lysol solution in a specially constructed steriliser. During this sterilising process the inoculation material was rubbed down in suitable quantities in 1 per cent. sterile salt solution, so as to form a uniform emulsion. For the first inoculation one immunity unit, and for the second inoculation five immunity units, are employed. In general, the injection was made into the jugular vein of the left side. Before injecting the emulsion the seat of operation was washed with 2 per cent. warm lysol solution, and afterwards briskly rubbed with absolute alcohol.

All the animals inoculated in this way were submitted yearly to a veterinary clinical examination and to injection with tuberculin, but only such animals as had been inoculated a full year, measured from the time of the last injection, were submitted to the tuberculin test.

The yearly tuberculin test was invariably carried out in the following way:—

(1) The animal to be tested was kept in its usual stall for one to two days before the test, and its temperature was taken three times a day. The result of the temperature trials and the observations of the veterinary clinical examination were entered in the report of the tuberculin test.

(2) The injection itself was usually made during the evening. For animals up to two years old the dose was .3 cm., for older animals .5 cm. of tuberculin, subcutaneously injected. Seven to eight hours after injection the temperature was taken, and this was repeated six times at intervals of three hours.

(3) In judging of the results, the highest temperature before inoculation was taken. It should not be forgotten that the normal temperature of young undeveloped animals is usually 39.5° C.

(4) The results of the test have been divided into four classes, the animals being classified as follows :—

o. When the temperature remains practically normal after inoculation.

1. When the temperature does not rise more than 5° C.

2. When the temperature does not rise more than 1° C., and in young animals does not exceed 40° C.

3. Every animal in which the temperature rises above 40° C.

All animals comprised under classes 2 and 3 were regarded as having reacted.

At the end of 1907 and the beginning of 1908, 686 animals altogether were submitted to the tuberculin test. The oldest had been inoculated about the age of five and a half years. In the case of the youngest more than two years had elapsed since the protective inoculation.

A total number of 66 animals reacted, making the percentage of reactors equal 9.6 per cent.

Dividing the animals according to the age at which the inoculation was practised, the tuberculin test gave the following results :—

(1) Of 59 animals vaccinated before five and a half years old, 6 animals, or 10 per cent., reacted.

(2) Of 173 animals vaccinated before the age of four years, 24, or 13.8 per cent., reacted.

(3) Of 305 animals vaccinated before the age of three years, 22, or 7.2 per cent., reacted.

(4) Of 149 animals vaccinated before the age of two years, 14, or 9.4 per cent., reacted.

From these data it is clear that a large proportion of the oldest of the protected animals, some of them vaccinated five and a half years before, despite the numerous opportunities they had had of becoming infected, exhibited the desired powers of resistance,—a fact which seems to us all the more important inasmuch as we knew from our own experience that before the introduction of protective inoculation as many as 50 per cent. of two-years-old artificially-fed animals had reacted, and that this grave condition of affairs increased in proportion to the age of the animals.

In judging of the above results, we must bear in mind that the inoculated animals were transferred from one farm to the other without reference to danger of infection and without the slightest attempt to protect them from it, a fact which, from the farmer's point of view, is of great importance.

To attain such excellent results it is indispensable that in practising protective inoculation the directions laid down by von Behring should be scrupulously carried out.

To the above results of the tuberculin test, I will add certain other observations bearing on the question of protective inoculation.

In order to prevent repetitions, I must refer the reader to the reports which I published in the year 1905 at the International Veterinary Congress at Buda-Pesth, and will only repeat the most important of them at this point.

(1) One group of animals which had been protectively inoculated were slaughtered, and careful *post-mortem* examinations were made by skilled persons. Five animals slaughtered at varying periods after

the protective inoculation proved to be completely healthy. The sixth animal, which was born of a mother that had reacted strongly, showed a strong reaction after the first protective inoculation. Six months after the protective inoculation general swelling of the lymphatic glands occurred, and on *post-mortem* examination general subacute tuberculosis of the lymphatic glands was detected. The starting-point and the oldest centres were found in the peri-bronchial lymphatic glands, which had undergone serious changes, and these changes can with great probability be referred back to the time before the protective inoculation. It appears probable that in this case an existing latent tuberculosis had assumed an acute complexion as a result of the protective inoculation. This observation compels me once more to draw attention to the advice, that in carrying out protective inoculation every attempt should be made to choose only quite young and healthy animals.

(2) For the purposes of control two groups of animals were placed in one of the most markedly infected stables, and kept there for nine months along with the old stock. After the lapse of this time the animals which had been inoculated proved to be absolutely healthy. Of those not inoculated, however, although artificially reared, 50 per cent. reacted to tuberculin. As regards the later history of these two groups, I may state that at the last tuberculin test all the non-protected animals reacted, whilst of the protected group only one animal showed a reaction. This reacting animal was inoculated on the 15th September 1902, and, being one of the first animals operated on, received only one injection.

(3) To the above observations may be added a similar case noted more recently. For purely accidental reasons five animals of a particular group were not protected, whilst fifteen others were submitted to the regular process of inoculation. At the last tuberculin test the five non-protected animals all reacted, whilst of the fifteen inoculated animals none showed a reaction.

(4) For experimental purposes another group of animals was infected intravenously with highly virulent bovine cultures. Two animals of this group were more highly immunised. Three were submitted to the typical bovo-vaccination. The first two cows appear at the present time, five years after infection, completely healthy, and fail to react to tuberculin. Two of the animals immunised in the ordinary way, which were slaughtered a year after infection, were highly tuberculous. The third animal of this group was kept under observation for a further period. In the first and second years after infection it was not tested with tuberculin. During the last three years it has no longer reacted to tuberculin. It has appeared healthy on clinical examination, and has repeatedly had healthy calves.

(5) In one of our oldest and most severely infected byres twenty-one young cows were placed, partly for economic reasons, partly to discover how protected animals would behave when exposed to such grave danger of infection. On the occasion of the last tuberculin test two of these animals showed trifling reactions, but exhibited no clinical symptoms.

(6) On R. Sömjén's farm the calves were raised under perfectly natural conditions, being left in the infected stalls along with the

reacting mothers, by which the calves were fed. The only protection that was granted to these calves consisted in protectively inoculating them as early as possible. The results of the tuberculin test were just as favourable in the case of these animals as on other farms where the calves were artificially reared.

Having regard to these facts, which prove the good effects of bovo-vaccination in protecting animals against the danger of tuberculous infection, and which demonstrate that protective inoculation can be successfully carried out under the most ordinary economic conditions, it appears highly desirable that further attention should be directed to von Behring's method of inoculation by all interested persons.

Our experience shows that bovo-vaccination is capable of conferring marked protection against infection for a period of five and a half years (*see* above), the percentage of five-and-a-half-years-old animals protected by bovo-vaccination, and which reacted when afterwards submitted to tuberculin, only numbering 10 per cent., whilst before the introduction of protective inoculation 50 per cent. of two-years-old artificially reared animals reacted to tuberculin.

When we further consider that such good results can also be obtained on farms in which the inoculated calves are constantly kept together with highly tuberculous cows, it is plain that protective inoculation can be carried out with good results under ordinary agricultural conditions, and it seems justifiable to conclude that the power of resistance against tuberculous infection conferred on animals by bovo-vaccination, having already lasted for over five years, will probably prove to continue for some years longer.

Taking everything into consideration, I consider it absolutely superfluous to repeat the protective inoculation every year, as has recently been suggested. According to my view, inoculation should not be repeated even once, on account of the possibility of infection through the milk.

Instead of repeating the inoculation, I would recommend that, as far as economically possible, suitable hygienic measures should be adopted.

By Bang's method the byres can be rendered healthy, but they remain free from tuberculosis only so long as no tuberculous animals are introduced into them. Should breeding animals from such healthy byres be brought in contact with animals from other areas, they very soon contract tuberculosis. The great economic difficulty and the material sacrifice which Bang's method entails may well prove the ground why his method only makes such slow progress, and why the farmers in ever-increasing numbers show interest in Ostertag's method, the latter seeming simpler and more easily carried into practice.

Without going into details, I regard as the most important principles of Ostertag's method the systematically exact clinical examinations by which really tuberculous animals are to be sought out and removed, the artificial rearing of the calves, and the application of the tuberculin test only to the calves. The above-mentioned drawback of Bang's method, namely, the danger of animals from tubercle-free farms being infected if removed to other centres, is naturally also present under Ostertag's method, and it will be of great importance if one can not only protect the cattle from tuberculous infection,

but also confer on them an active resistance. The latter is attained by vaccination, and it is clear, from what has been said above, that a combination of Ostertag's method with von Behring's bovo-vaccination is the remedy sought for. I will endeavour in a few words to indicate the most important points about such a combined method:—

(1) The most important condition, in my estimation, is that the bovo-vaccination should be carried out with the most scrupulous care and exactly in accordance with von Behring's directions.

(2) The method of testing, by tuberculin, the adult animals protected by von Behring's method should be discontinued, and only employed in special cases. For it should be substituted exact clinical tests of the whole number of animals at fixed intervals.

(3) The tuberculin testing of the calves recommended by Ostertag can in the combined method be discontinued, inasmuch as the bovo-vaccin injections exhibit an action similar to tuberculin. (I may add that we have often noted that calves which react typically during the protective inoculation have later proved to be infected.)

(4) By combining the two methods, the artificial rearing of the calves, as recommended by Ostertag, could be discontinued, for our experiments have shown that calves develop just as well under ordinary agricultural conditions as those reared artificially, provided they have been protectively inoculated in early life. This appears to me an advantage of the combined method which should not be overlooked, because in agricultural practice artificial rearing is often difficult to carry out.

Having above sketched out the chief lines for practising a combination of both methods, I need only add, as a further important point, that the byres should be constructed with a careful regard to hygiene, and that the animals, so far as economic and atmospheric conditions permit, should be kept in the open air.

RED-WATER IN ENGLAND AND ITS CARRIERS.¹

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AFTER Smith and Kilborne, in 1888, had demonstrated the presence of hæmatozoa (the *pyrosoma bigeminum*) in the red blood corpuscles of cattle affected with Texas fever, which is a disease characterised by destruction of the red cells and frequently by the presence of a visible amount of the colouring matter of these cells in the urine, similar observations were made in all quarters of the globe in connection with the disease which in England we call red-water. Five years later, in 1893, the above authors showed that Texas fever could be produced in susceptible cattle by inoculating them with the blood of animals suffering from or which had recovered from an attack, and that the disease could be carried indirectly by ticks. These later observations have also been confirmed in various parts of the world, particularly in Australia and South Africa, and on the continent of Europe, and it is now generally accepted that wherever red-water is observed as a disease of cattle one may expect to find

¹ Read at Annual Meeting of the National Veterinary Association, Brighton, August 1908.