A CONTRIBUTION TO THE STUDY OF DELAYED OR "LATENT" TUBERCULOUS INFECTION.

By S. DELEPINE,

M.B., M.SC.,

Professor of Public Health and Bacteriology and Director of the Public Health
Laboratory, University of Manchester.

IN the course of experiments made with the view of ascertaining the effect which the action of various agents had upon the virulence¹ of tuberculous products of human and bovine sources, one is sometimes able to observe evidence of a reduction in the pathogenic power of bacilli, and this reduction may be sufficient to render the cobaye capable of resisting bacillary invasion much in the same way as the meriones resists the action of bacilli of normal pathogenic power.²

The author has shown that for the purpose of estimating the effect which various agents have upon the virulence of a given strain of tubercle bacilli, the cobaye is eminently suitable, because this animal reacts readily and in a very uniform manner to tubercle bacilli obtainable from human, canine, bovine, equine, porcine and other mammalian tuberculous lesions, and in a certain proportion of cases can also be infected with avian tuberculous products.

Subcutaneous inoculation on the inner aspect of the hind leg at the level of the femoro-tibial articulation with a pure culture or matter containing an extremely small number of bacilli produces in the cobaye typical lesions, the extent and distribution of which are of great value in determining the number and virulence of the bacilli. Subcutaneous inoculations in other parts of the body, or intraperitoneal injections, yield much less comparable results. By using this method the author was able to observe that the virulence of bacilli obtained at different times from sources of a similar kind was capable of considerable variation.

Thus a certain strain of tubercle bacilli, obtained from a human lesion and exhibiting normal virulence so long as it was cultivated on blood serum at the optimum temperature, produced only slight and

¹ The word "virulence" is here used in its most general sense, including all that renders the organism capable of producing irritative and toxic lesions and of multiplying and spreading in its host.

² See Annual Report of the Medical Officer to the Local Government Board, 1908-09, pp. 344-9, with communication by S. Delépine, "On the Prevalence and Source of Tubercle Bacilli in Cow's Milk," in which there are references to previous papers published since 1893.

slow-spreading lesions after being kept absolutely dry in the dark for two months. After desiccation for a period of over four months, the same bacillus was incapable of producing any infective lesion. Other cultures were affected in the same way, but some were able to resist desiccation for longer periods. Pathological products such as tuberculous sputa, pus, milk are affected in a similar way by drying.

The action of radiant sunlight is so rapidly lethal that it is not easy to regulate the exposure so as to determine accurately the reduction in the virulence of the bacilli.

A reduction of virulence was also observed in the case of tuberculous milk kept at a low temperature (below 6° C.) in the dark for a considerable time. This milk, which had been collected with aseptic precautions and direct from a tuberculous udder in a sterilized vessel, contained a large number of virulent tubercle bacilli and a few other bacteria. When fresh it produced extensive uncomplicated tuberculosis in cobayes inoculated subcutaneously with it. The lesions were as usual extensive in all the lymphatic ganglia connected with the seat of inoculation three weeks after the inoculation. After this milk had been kept for 490 days,¹ as stated above, it was still capable of producing tuberculosis, but the mode of spread of the infection was abnormal. In the subcutaneous tissue and subjacent muscles in the neighbourhood of the seat of inoculation on the inner aspect of the leg, a large number of small isolated or confluent tubercles formed during the first month, and it was only towards the end of the first month that the inguinal glands began to enlarge. When the cobayes were killed, three months after inoculation, it was found that the tissues on the inner aspect of the thigh and the abdominal walls (including the peritoneum) in the corresponding inguinal region were the seat of a very large number of small fibro-caseous tubercles, almost confluent; the lymphatic glands behind the diaphragm were not more affected than is generally found to be the case three or four weeks after inoculation, but the liver, spleen, the lungs and most of the lymphatics in front of the diaphragm were the seat of extensive tuberculous lesions.

There appeared, therefore, to have been during the first month a tendency to a localization of the infective process, but afterwards the infective organisms which had been able to pass beyond the locality first affected must have regained their virulence and power to multiply, for the organs situated at a distance from the seat of inoculation showed lesions resembling closely those observed in the course of normal infection.

¹ The same milk was tested again after being kept four and a half years, when it was found that the bacilli were all dead, or at any rate incapable of producing tuberculosis. Cobayes inoculated with large quantities of this milk remained well for over one year after inoculation, and all their organs were found healthy *post-mortem*.

Similar effects were observed in the case of tuberculous cultures and tuberculous milk which had been heated for a definite length of time up to temperatures approaching lethal temperatures.

While conducting in 1899 a series of experiments on the sterilization of milk artificially infected with tubercle bacilli of human and of bovine origin, the writer observed that cobayes inoculated with milk which had been submitted to certain temperatures for a certain length of time remained apparently free from infection for several weeks, but that ultimately they became tuberculous. This delayed infection was observed when the milk had been heated to:

65° C.	for 180 minutes (3 hours).	
70° C.	" 45 "	
74° C.	" 30 "	
76° C.	" 10 "	
85° C.	" 15 "	(In this case there was a delay of two months.)

Heating was conducted with very great care, as if the object had been to ascertain the coagulation temperature of a proteid. (This fact is of some importance in view of the statements which have been made as to the rapid lethal effect of a temperature of 85° C.) These experiments were repeated in 1911 with naturally infected milk obtained from the tuberculous udder of a cow. This milk was highly pathogenic when fresh, 0.2 gramme of it being capable of producing in 15 days a tuberculous ulcer at the seat of inoculation, well-marked tuberculosis of the popliteal, superficial, and deep inguinal, sublumbal and renal lymphatic ganglia corresponding to the leg inoculated, and distinct tuberculous lesions of the retrohepatic, bronchial, suprascapular ganglia, and of the spleen, liver, and lungs.

Some of that milk was divided into two parts, one of which was kept for control experiments and the other heated in a steam-jacketed copper pan, provided with a stirrer by means of which the milk was kept in constant motion, the temperature of the fluid was recorded by means of a well tested and controlled recording thermometer during the experiment. In the course of eight minutes the temperature rose from 58° C. to 78° C., and was for four minutes above 68° C. A sample of that milk was collected as soon as the temperature had reached 78° C. The same milk was heated for ten minutes more, the temperature rising gradually to 92° C. when a second sample was taken.

Three sets of cobayes were inoculated subcutaneously with the original milk and with the same milk after it had been heated to 78° C. and 92° C. respectively. The quantity of untreated milk inoculated was 1 c.c.; the quantity of treated milk was very much larger, the sediment of 40 c.c. obtained by centrifugalization being used. The animals inoculated with these samples were examined frequently during

life and the lesions recognizable by inspection and palpation noted. After the death of the animals they were dissected carefully and the various organs examined for tubercle bacilli, and it was found, *firstly*, that, during life, the cobayes which had been inoculated with milk which had not been heated had at the seat of inoculation a well-marked lesion beginning to ulcerate at the end of two weeks, and that the inguinal glands close to the seat of inoculation were distinctly enlarged and indurated. The cobayes which had been inoculated in the same way with heated milk showed, at the end of *ten weeks*, no ulceration, and no local lesion recognizable by palpation. *Secondly*, that the cobayes inoculated with untreated milk died within six weeks, and that at the time of their death they were affected not only with extensive tuberculous lesions at the seat of inoculation, and of the lymphatics connected with the local lesions, but also of most of the lymphatic glands of the body, in addition to which the spleen, liver, and lungs were extensively affected.

On the other hand the cobayes which had been inoculated with the heated milk were found, on being killed after eleven weeks, to be affected quite differently. In these, besides there being no local ulcer, there was either no visible local subcutaneous lesion, or only a very slight increase of fibrous tissue. There was also very slight enlargement of the popliteal and inguinal glands nearest the seat of inoculation, and only a few tubercle bacilli could be revealed in these. Other abdominal lymphatic ganglia, as well as the spleen, liver, and lungs, were the seat of extensive tuberculous lesions containing many tubercle bacilli.

Another group of experiments was conducted in which thin layers of milk were heated and evaporated at the same time. The temperature of the milk was raised from about 12°C. to 98°C. in less than three minutes, the material not being kept at the higher temperature for more than three seconds.

The results of inoculation were practically the same as in the previous experiments. A cobaye, killed five weeks after inoculation, *i.e.*, at a time when some indication of inguinal swelling had begun to be noticeable, was found to have very slightly enlarged popliteal inguinal and sublumbar lymphatic ganglia, tubercle bacilli being demonstrable in each. There was no sign of any lesion at the seat of inoculation. The lesions were similar to those observable from eight to ten days after inoculation with unheated milk.

The two last sets of experiments may be summed up as follows:

1. Cobayes inoculated with untreated tuberculous products rich in bacilli developed at the seat of inoculation extensive tuberculous lesions two weeks.
2. Cobayes of the same size and kept under identical conditions as the last, after being inoculated with a much greater quantity of

"LATENT" TUBERCULOUS INFECTION III

the same tuberculous products previously heated as stated above, developed very slight lesions at the seat of inoculation in the course of eleven weeks.

3. The viscera and the majority of the lymphatic ganglia of the cobayes inoculated with the untreated tuberculous products were the seat of extensive tuberculous lesions six weeks after inoculation.

4. In the cobayes inoculated with the heated tuberculous products, the viscera and lymphatic ganglia were in several cases affected nearly to the same extent as those of the cobayes inoculated with the untreated material, but this result was obtained after eleven weeks only.

5. From these results it would appear that most of the heated bacilli were at first barely capable of overcoming the resistance offered to them by the phagocytes in the region first infected, but that some bacilli had escaped and had been carried away probably by some of the migratory cells. These bacilli had been able to multiply and to regain their pathogenic power.

6. The facts observed seem also to indicate that the recuperation of this pathogenic power became more marked as the distance from the seat of inoculation increased, for, while the local lesions remained insignificant, those of distant organs were practically as extensive as those found in the animals inoculated with untreated milk.

7. The relation between the extent of the tuberculous lesions and the distance from the seat of inoculation indicates that the bacilli which had successfully overcome the various obstacles offered to their progress had gradually become not only more numerous but also more virulent. This suggests strongly a process of *reinforcement of virulence by relays*, similar to that observed in the production of a "Virus fixe" by passage through a series of animals according to Pasteur's method.

In the experiments referred to in this paper, the period of latency did not exceed three or four weeks, but it was sufficiently long to reveal an aspect of infection processes which must be of great importance, more especially in relation to latent infections.

The fact that tubercle bacilli were still living and capable of a modified pathogenic action after being kept for nearly 500 days in a natural tuberculous product (milk), supports the view that under certain circumstances tubercle bacilli are still infective after remaining dormant for a considerable period of time.