A Note on the M’Fadyean Staining Reaction for Anthrax Bacilli

BY

MAJOR J. D. E. HOLMES, M.A., D.Sc., M.R.C.V.S.

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MAJOR J. D. E. HOLMES, M.A., D.Sc., M.R.C.V.S.
Imperial Bacteriologist.

In the Journal of Tropical Veterinary Science, Vol. IV, No. 1, pp. 68—70, Mr. Mitter of the Bengal Veterinary College drew attention to a supposed difference between the staining reactions of anthrax bacilli in that Province of India and in the Continent of Europe. In brief, he was unable to obtain the violet staining reaction which M’Fadyean described as being diagnostic of anthrax bacilli when an anthrax film is treated with a 1 per cent. methylene blue solution. In 1903, in the Journal of Comparative Pathology and Therapeutics, M’Fadyean published a paper in which he fully describes the method of obtaining the violet reaction, and remarks on its value as a diagnostic agent both in fresh and in putrid anthrax blood. There are many points of technique on which the author lays special stress and which should be carefully noted by everyone wishing to obtain similar results.

The reaction is obtainable only in preparations of anthrax from blood and other body fluids, not in artificial cultures of the bacilli. Schäffer of Berlin, while confirming M’Fadyean observations, found that when anthrax was grown on blood serum, on which medium the bacilli form capsules, the red staining of the capsules could be observed. The violet staining is constant in anthrax of any of the domesticated animals as well as in the mouse, guinea pig and rabbit. It is obtainable immediately after death, and it may still be detectable when all the anthrax bacilli have undergone dissolution in the unopened carcase. The reaction is diagnostic of anthrax. In a well prepared film of anthrax, the color reaction is so marked that in most instances it is possible to detect the trace of red or purple by naked eye inspection, especially when it is held up to the light.

To prepare the film, a smear preparation is made in the ordinary method. The film should not be very thin, for then the fixation is liable to be too complete. For the same reason, the film should be spread on a slide, and not on a cover glass. After drying at ordinary tempera-
ture, the slide is fixed, by lowering it, film side upwards, into the flame of a Bunsen burner, for a second. Repeat this three times, or until the under surface of the glass is a little too hot to be borne by the skin of the palm of the hand. Particular attention should be paid to the process of fixation, as the violet reaction is obtained only when the preparation is imperfectly fixed.

M’Fadyean draws special attention to this. “It is important to notice that what might be thought trivial departures from the directions given above will entirely prevent one from obtaining the reaction. For instance, if the film be fixed with sublimate formalin or osmic acid solutions, no trace of the violet reaction will be obtained. Failure will also occur if, in fixing the film by dry heat, the temperature is allowed to rise as high as 150°C. 100°C is sufficient, and it should not be allowed to rise much above that. Again, the film ought not to be very thin. Partly for this reason, and partly because of over-heating in the Bunsen flame, such thin films as are obtained by pressing two cover glasses together, generally fail to show the violet reaction. As an alternative method of fixation, the slides or cover glasses carrying the dried films may be immersed for a few minutes in absolute alcohol or methylated spirit. The stain must not be applied to the hot slide, or heated until the steam begins to rise, as is recommended for some methods of staining, nor must the preparation be washed in alcohol after staining.”

It is also necessary to be careful that the stain is lightly washed off and that no pressure is used when drying the film between absorbent paper. The violet stained amorphous granules of the capsule, being imperfectly fixed, are easily removed from the film, and rough handling in washing or drying may cause an apparent failure to obtain the reaction. As regards the stain, M’Fadyean was, at first, of opinion that it was necessary to use a 1 per cent. solution of methylene blue which had been in stock for some time and thus acquired a polychromatic quality, or that it was necessary to add a ½ per cent. bicarbonate of soda to a freshly prepared 1 per cent. solution of Grubler’s powder. Subsequently, it was found that freshly prepared, pure medicinal methylene blue gave the reaction, without the addition of bicarbonate of soda.

For some years past I have made use of the violet reaction in the diagnosis of anthrax, and have never failed to obtain a positive result, in anthrax blood, when the above directions have been carefully adhered to. I have used it in summer and winter temperature, both on the hills and on the plains in India, and found no variation.
A. Staining reaction of bacilli almost completely fixed.
B. Staining reaction of bacilli incompletely fixed.
C. Staining reaction of bacilli and amorphous granules of the capsules when fixation has been very slight.
I am therefore inclined to believe that Mr. Mitter's failure to obtain the violet reaction in anthrax blood is due to some defect in technique. He mentions that his films were fixed by "passing three times through the flame of a Bunsen burner." This would certainly cause complete fixation and a negative result. The slide should be lowered, film side up, on to the top of the flame, for a second, so that the flame should not reach the film side, but simply heat the under surface of the slide. It will generally be found that the reaction varies, in different parts of the film, in accordance with the degree of fixation. In the illustration attached, (A) shows where almost complete fixation has occurred. Consequently, the capsule of the bacilli appears as a thin violet stained line. In (B), fixation was incomplete, and the bacilli are surrounded by a thick, disintegrating violet capsule. In (C), the fixation has been very slight and the violet stained capsules are more swollen and disintegrated, and amorphous granules of the completely disintegrated capsules are seen lying apart from the bacilli.

There is no reason to suppose that the anthrax bacilli in India present any variation in virulence, staining reactions, or other biological characters, from those possessed by anthrax organisms met with in Europe or other countries.

In Europe, inoculated anthrax proves fatal to sheep, guinea pigs, rabbits, horses and cattle. In India, inoculated anthrax is equally fatal to these species of animals with the exception of cattle. When Indian cattle are inoculated with anthrax, they show a high temperature reaction, with slight local swelling at seat of inoculation, for three to four days—and recover. Very few cases have a fatal termination. Recently, through the courtesy of Sir John M'Fadyean, I obtained a culture of anthrax from his laboratory, with the object of comparing the virulence of the English and the Indian strain, in different species of animals in India. Both strains showed identical degrees of virulence. Sheep, guinea pigs and rabbits were inoculated with a like amount of a 48 hours broth culture. The mortality, and the period from time of inoculation to death, were in each species very similar. In cattle inoculated from the English strain the reaction was identical with that from the Indian virus. No mortality among cattle resulted.
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