

AN IMPROVED STAINING METHOD FOR DEMONSTRATING BACTERIAL CAPSULES, WITH PARTICULAR REFERENCE TO PASTEURELLA¹

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Various techniques (see references) which have been used successfully in demonstrating bacterial capsules on other organisms were not found entirely satisfactory for showing the presence of a capsule on cells of *Pasteurella mastitidis*, an organism consistently found in mastitis of ewes (Marsh, 1932).

A modification of the Hiss (1905) method, which depends on the fixing action of phenol on the capsule, enabling the latter to be demonstrated in films of *P. mastitidis* and other gram-negative organisms, follows.

The amount of culture that can be picked up by a fine, straight platinum wire from a colony or an agar slant is suspended in a loopful of physiological saline containing 0.5 to 1 per cent phenol and 10 per cent blood serum, and spread into a thin film on a clean polished slide. To fix the dried film, the slide is dipped rapidly into methyl alcohol, drained, and flamed to burn off the excess alcohol. The preparation can then be stained for 30 seconds to 1 minute with any of the common bacterial stains, and washed with water. No particular advantage was found in washing the slide with 20 per cent copper sulphate solution, as in Hiss's method.

With crystal violet (prepared as in Hucker's modification of the gram stain) the organisms stain dark blue to black, whereas the background takes a light blue or purple stain and the capsule appears as a distinct colorless halo around the organism (figures 1, 2).

The capsules can also be demonstrated in a preparation stained by the gram method, using dilute carbol fuchsin as a counterstain, if the film is prepared and fixed as described above.

With cultures of *P. mastitidis*, it was found that the organisms from the iridescent peacock-blue (smooth) colonies were 90 to 100 per cent encapsulated, whereas those from the translucent grey to opaque (rough) colonies showed little or no encapsulation.

Capsules have also been demonstrated by this method in smooth cultures of *Pasteurella septica*, *Salmonella pullorum*, *Salmonella suispestifer*, *Escherichia coli*, *Eberthella typhosa*, *Shigella gallinarum*, and *Brucella abortus*.

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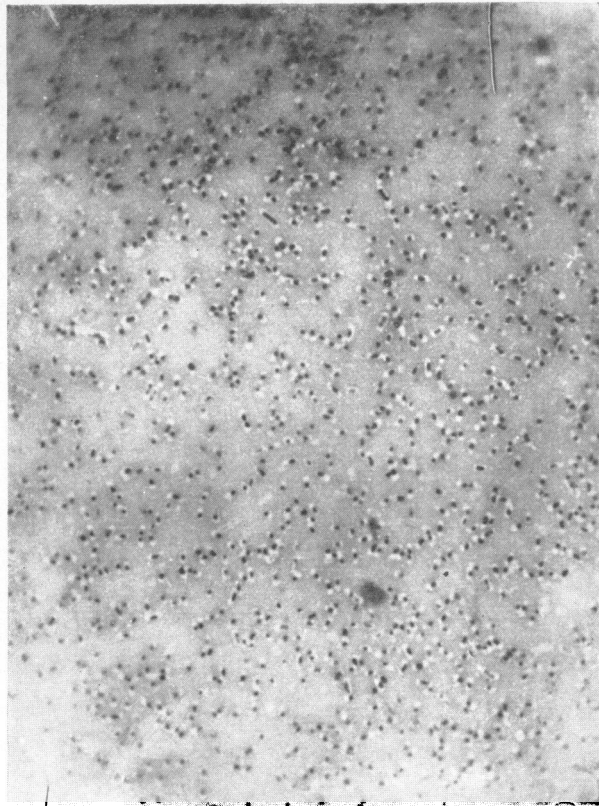


FIG. 1. PASTEURELLA MASTITIDIS STAINED WITH CRYSTAL VIOLET 30 SECONDS



FIG. 2. PASTEURELLA SEPTICA STAINED WITH CRYSTAL VIOLET 30 SECONDS

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