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A NOTE ON THE STAINING OF BACTERIAL CAPSULES.

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Möller (1951) discussed the merits of various methods of demonstrating bacterial capsules. He rightly emphasized that "relief" methods of staining (nigrosin, india ink) were unsuitable for morphological studies, and that retraction of the background stain from the bacterial surface could give rise to artefacts closely simulating capsules. Möller suggested that "positive" methods of capsule-staining, such as Hiss's or Muir's, were preferable to "relief" methods, but did not give constant results. He therefore proposed a modification of Hiss's method which was claimed to give clear and regular staining. Essentially, this method consisted in suspending the bacteria on a slide in a drop of serum-glucose solution, fixing with lead acetate-formalin fixative, staining with crystal violet solution, and washing with saturated copper sulphate.

An objection to this method is that the film of serum in which the bacteria are dried tends to shrink from the bacterial surface (or the surfaces of other small particles) and to produce haloes, more or less intensely delineated by stain, which simulate capsules. This is, in fact, a form of "relief" staining in which dyed albuminous material replaces nigrosin or india ink as a background stain.

At this laboratory, a modification of Muir's method has been devised for investigating *Brucella* capsules. This gives regular staining, and a clear and often brilliant differentiation between capsule and soma. The background remains clean.

The primary stain was strong Ziehl-Neelsen (2 per cent. basic fuchsin) to which CTAB (cetyltrimethyl ammonium bromide) was added to make a 0·4 per cent. solution. Ziehl-Neelsen stain with more than 1 per cent. basic fuchsin tended to weaken on standing because of precipitation of the dye. It was therefore found advisable to mix 9 parts of Ziehl-Neelsen (1 per cent basic fuchsin) with 1 part of a 10 per cent. alcoholic solution of basic fuchsin, and not to keep this for more than a week. The counter stain was 0·1 per cent. methylene blue diluted in 10 per cent. Sörensens phosphate buffer at pH 7·4.

The following procedure was suitable for staining *Brucella* capsules:—

Fix by heat.	
Unheated strong Ziehl-Neelsen-CTAB solution	30 sec.
96 per cent. alcohol (without intermediate rinsing)	1 min.
Rinse and apply Muir's mordant	1 min.
Rinse and apply 96 per cent. alcohol	1 min.
Rinse and stain with 0·1 per cent. alkaline methylene blue	5 sec.

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It is perhaps necessary to emphasize that the age and stage of growth of the organisms have an important influence on the avidity of the different bacterial structures for various dyes. The times of application of the carbol-fuchsin stain and of the alcohol in particular should be adjusted in accordance with the results of a preliminary trial.

Moreover, the capsules of various bacteria differ widely in their chemical constitution, so that no one procedure will give equally brilliant staining with all bacteria. A preliminary test, hwever, quickly indicates the modification necessary to give good results.

If the primary stain is used too dilute, the cell membrane may be understained and can be easily decolorized. It will then, after counter staining, resemble a capsule. This is most likely to occur when the cytoplasmic body has retracted from the cell membrane as a result of certain fixing and mordanting procedures. If strong carbol-fuchsin is used, the cell membrane stains deeply and withstands considerably more decolorizing than the capsules.

SUMMARY.

The efficiency of Muir's capsule stain could be much increased by adding a surface-active agent (CTAB) to the primary carbol-fuchsin stain, and by using 0·1 per cent. alkaline methylene blue as a counter stain. Heating of the stain was not necessary.

REFERENCE.

MÖLLER, O. (1951). A new method for staining bacterial capsules. *Acta Path. et Microbiol. Scand.*, Vol. 28, No. 2, pp. 127-131.