A Cell Wall Stain for *Escherichia coli*

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The common staining procedures used in bacteriology do not stain the cell wall. This has been explained on the basis of a low affinity of the wall for dyes; i.e., the dye may pass through the wall but is not retained within it. During the course of trying to develop a stain to differentiate the internal structures of bacteria, a technique was developed to stain the wall.

Preparation of the Stain

An aqueous solution of crystal violet is treated with a solution of potassium hydroxide until all the dye is precipitated. The precipitate is filtered off and thoroughly washed with water. It is then brought into solution by adding an equivalent amount of silicic acid and water sufficient for a one per cent solution of the dye. The mixture is agitated at intervals and allowed to stand until it goes into solution. After 48 hours the solution had a *p*H of about 2.2.

Method

The organisms are smeared in a 1% solution of Metanil yellow (*p*H 9.5) and allowed to air dry. The smear is covered for 2-3 minutes with the crystal violet solution described above. The stain is washed off with water and the smear mordanted in a .5 per cent solution of mercuric chloride for about 10 seconds. The smear is washed with water and allowed to dry thoroughly. The slide is then placed on a 50° C warm plate for 10-20 seconds before a thin film of alkaline nigrosin is spread over the smear. The nigrosin film may be spread by a small loop or glass slide. Upon drying, the slide is ready for observation.

The walls of all cells are not stained. However, by searching the smear, cells having the wall well stained will be found. In thick smears there is some differentiation in color of the cytoplasmic area of the short and long rods (snakes). In some fields, the cytoplasmic area of the short rods will be violet and that of the snakes green in color. The walls of the snakes are usually more deeply stained.