UNITED STATES GOVERNMENT

Memorandum

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
OFFICE OF THE SECRETARY

J. File

TO : Mr. Latker

DATE: January 17, 1968

FROM : Miss Bogosian MCB

SUBJECT : Significance of the Gram Staining Technique in Bacteria Classification

Certain differential stains such as the gram stain and the acid fast stain are of aid in classification. After staining by Gram's method some organisms are found to be violet and some red. All of the stainable spiral organisms, about 1/3 of the cocci and 1/2 of the bacilli, appear red.

The gram method of staining involves the following procedure: a thin film of organisms on a glass slide is fixed with heat, and then treated with gentian or crystal violet for one minute, rinsed quickly with water, and covered with Gram's iodine solution for one minute. Following another quick rinse in water the slide is decolorized with alcohol or a mixture of acetone and ether, washed thoroughly and counterstained with safranin for 10 seconds, washed with water and dried.

In the first step, all organisms are stained violet, and all assume a dirty bluish brown color after treatment with iodine. The iodides serve as a mordant to fix the violet dye in certain types of organisms. The safranin stains red those organisms which have been decolorized. The organisms which retain the violet dye are referred to as gram-positive and those which are decolorized and counterstained with the red dye are called gram-negative.

A positive explanation of this staining reaction, or a definite explanation of the different metabolic properties of the two classes of bacteria has not been found. Several facts are known from which partial theories have been derived. An intact cell wall is essential for retaining the crystal violet stain. Crushed cells, originally gram positive, stain gram negative after damage. Further, although lipids, polysaccharides, RNA and certain proteins can all retain the crystal violet-iodine-complex, these are not sufficiently different in number and amount to explain the binding of the blue stain by the gram positive organism. However, the predominance of mucopeptides in the gram positive and lipids in the gram negative might play an accessory role.

The crystal violet stains the cell wall as well as the protoplasm of both gram positive and gram negative bacilli. E. coli cells, which are gram negative, can actually bind more crystal violet per gram of cell weight than the typical gram positive bacilli. The organisms remain the same size after treatment with the iodine. With moderate washing the blue dye is washed from the cell wall but not from the protoplasm. The brief decolorization with alcohol removes the blue dye from the gram negative organism.

It is known that treatment with iodine produces a crystal violet-iodine-complex in both gram positive and gram negative organisms. Differential decolorization depends upon changes in the cell wall pores during the process of dehydration with alcohol. It is felt that the alcohol wash, after treatment with iodine, dissolves away much of the lipid from the cell wall of the gram negative bacteria, after which the crystal violet-iodine-complex easily escapes. On the other hand, it is thought that dehydration by alcohol of the cell wall of the gram positive organism reduces the size and the pores in the cell wall and makes decolorization more difficult.

The gram reaction is indicative of a more profound difference between gram positive and gram negative bacteria than simply their physio-chemical reaction to dyes, as shown by certain immuniological properties and variations in susceptibility to sulfonamides and antibiotics. Although a complete explanation for the difference in activity is not known, it is known that with the exception of the gram negative cocci, the sulfonamides are more effective in the treatment of infection caused by gram positive organisms. Further, with the exception of the gram negative cocci, penicillin is more restricted in its action against gram positive bacteria while streptomycin is in general more effective against gram negative bacilli. A complete explanation of the metabolic significance of the reaction of certain bacteria to the gram stain is not yet available.