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Notes

Gram Staining:

One of the most important and widely used differential staining techniques in microbiology is the Gram staining. This technique was introduced by Christian Gram in the year 1884. In this process, the fixed bacterial smear is subjected to the following staining reagents in the order listed- crystal violet, iodine solution, alcohol (decolorizing agent), and safranin or some other suitable counterstain. Bacteria stained by the Gram method fall into two groups: Gram positive bacteria which retain the crystal violet and hence appear deep violet in colour; and Gram-negative bacteria, which lose the crystal violet, are counterstained by the safranin, and hence appear red in colour.

Why does this procedure stain some bacteria purple-violet and others red?

This is due to the differences in the thickness and structure of cell walls between these two groups. The cell walls of Gram-negative bacteria are generally thinner than those of Gram-positive bacteria. Gram-negative bacteria contain a higher percentage of lipid than do Gram-positive bacteria. Experimental evidence suggests that during staining of Gram-negative bacteria the alcohol treatment extracts the lipid, which results in increased porosity or permeability of the cell wall. Thus, the crystal violet—iodine (CV-I) complex can be extracted and the Gram-negative organism is decolorized. These cells subsequently take on the colour of the safranin counterstain. The cell walls of Gram-positive bacteria, because of their different composition (lower lipid content), become dehydrated during treatment with

alcohol. The pore size decreases, permeability is reduced, and the CV-I complex cannot be extracted. Therefore, these cells remain purple violet. Another explanation, is based on permeability differences between the two groups of bacteria. In Gram-positive bacteria, the CV-I complex is trapped in the wall following ethanol treatment, which presumably causes a diminution in the diameter of the pores-in the cell-wall peptidoglycan. Walls of Gram-negative bacteria have a very much smaller amount of peptidoglycan, which is less extensively cross-linked than that in the walls, of Gram-positive bacteria. The pores in the peptidoglycan of Gram-negative bacteria remain sufficiently large even after ethanol treatment to allow the CV-I complex to be extracted. These two explanations are not mutually exclusive, and it is likely that both may contribute to the explanation of the mechanism of the Gram stain. Furthermore, if Gram-positive cells are treated with lysozyme (an enzyme) to remove the cell wall, the resulting structures, called protoplasts (cells lacking walls), will be stained by the CV-I complex. However, they are easily decolorized by alcohol. All this evidence points to the cell-wall structure of Gram-positive bacteria as the site of retention of the primary stain. Although Gram-negative organisms consistently fail to retain the primary crystal violet stain, Gram-positive organisms may sometimes show variations in this respect, i.e., a Gram-variable reaction. For example, old cultures of Gram-positive bacteria lose the ability to retain the crystal violet and hence will be stained by the safranin. Within some groups of bacteria, such as the archaeobacteria, some are Gram-positive and others Gram-negative; yet the cell wall structure and chemical composition of these bacteria is very different from that of other groups of Gram-positive and Gram-negative bacteria. Gram-positive bacteria differ from Gram-negative bacteria in other characteristics besides staining reaction. Gram-positive bacteria are usually more susceptible to penicillin and less susceptible to disintegration by mechanical treatment or exposure to some enzymes than Gram-negative bacteria. Gram-negative bacteria as a group are more susceptible to other antibiotics such as streptomycin. There are other differences between these two groups of bacteria. The Gram stain has its greatest use in characterizing bacteria. This staining technique is not generally applicable for other groups of microorganisms such as protozoa and fungi; however, yeasts consistently stain Gram-positive.