

# Lecture 4

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## I. Gram Stain

- A. Developed in 1884 by the Danish physician \_\_\_\_\_.
- B. Used to \_\_\_\_\_ between \_\_\_\_\_.
- C. The mechanism by which Gram-positive cell walls resist decolorization is not fully understood.

However, it is thought that:

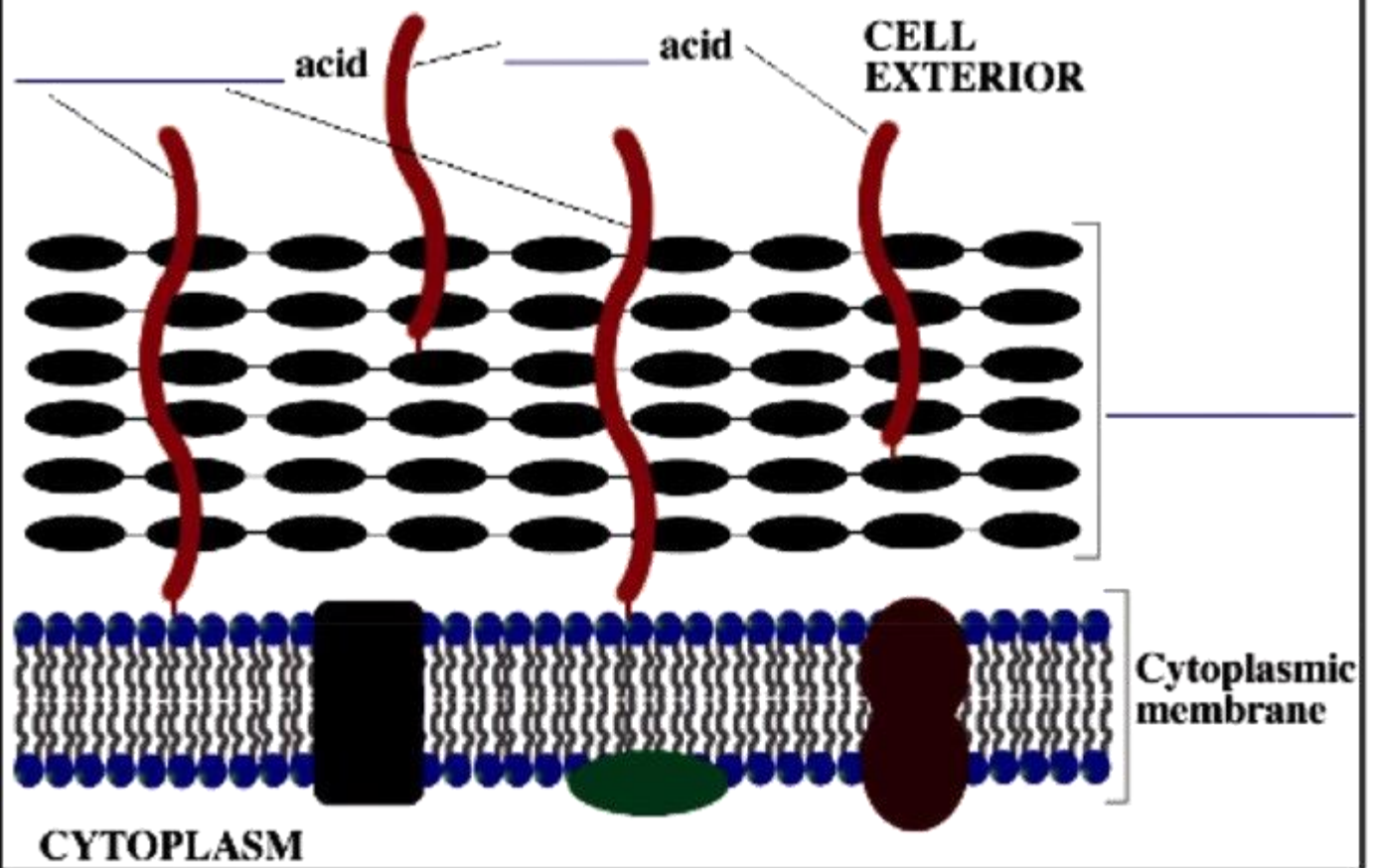
1. Gram-positive cell walls, upon alcohol treatment may become dehydrated and the \_\_\_\_\_ behind the thick peptidoglycan layer.
  2. In contrast, Gram-negative cell walls have \_\_\_\_\_ and it is thought that the alcohol may extract these lipids, making the \_\_\_\_\_.
  3. It is very important to perform the Gram stain on \_\_\_\_\_ because, as Gram-positive cells age, they may lose their ability to retain the crystal violet dye.
- D. This is one of the most important stains in microbiology as bacteria are often grouped by their Gram reaction.

## II. Acid-fast stain

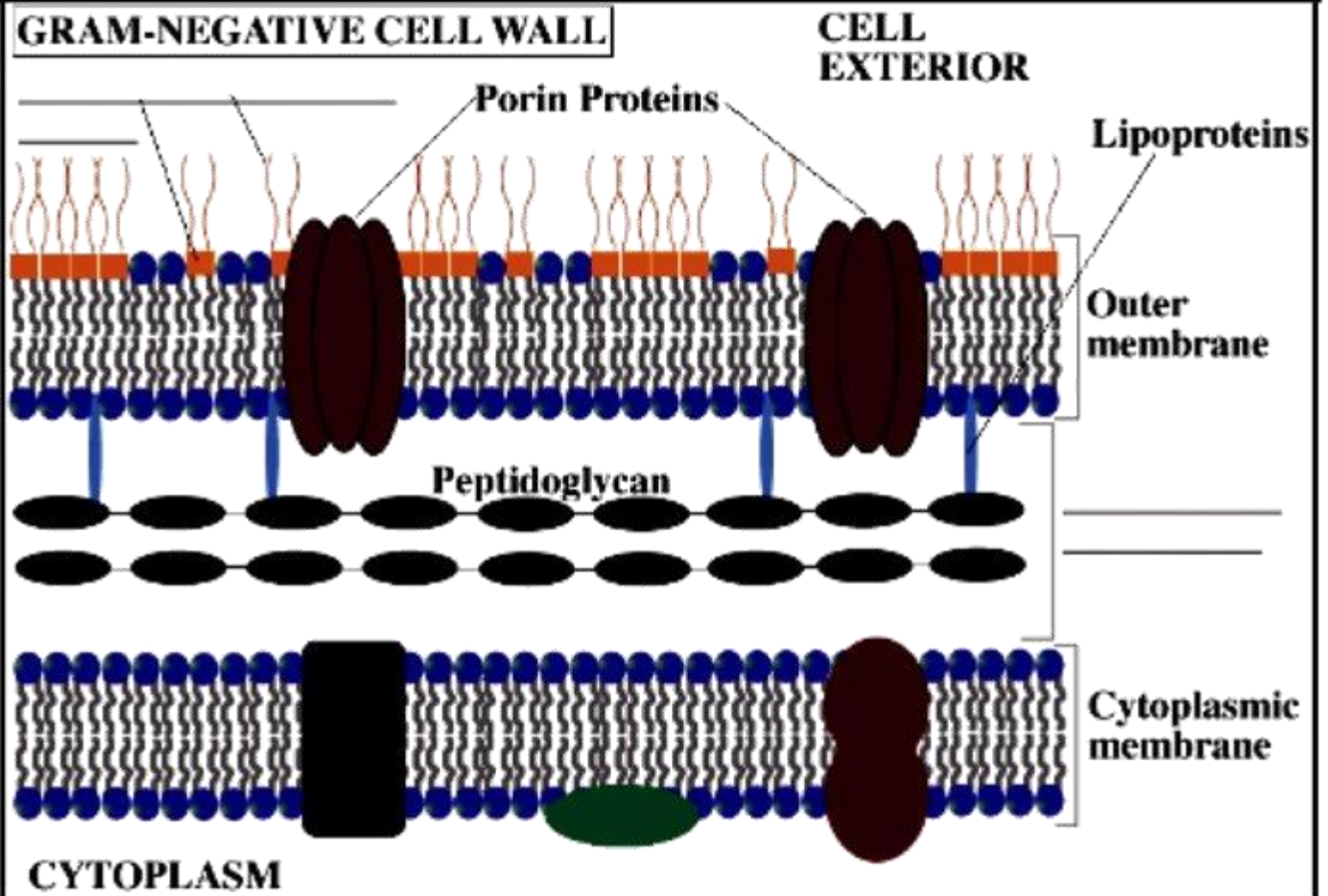
- A. Used to detect cells capable of \_\_\_\_\_.
  1. Cells that can retain this stain are termed \_\_\_\_\_.
  2. Acid-fast cells have a \_\_\_\_\_; specifically, they contain a great deal of a waxy lipid called \_\_\_\_\_.
- B. Acid-fast stains are important for identifying bacteria in the genus \_\_\_\_\_ (e.g. *Mycobacterium leprae* and *Mycobacterium tuberculosis*, which are the pathogens responsible for \_\_\_\_\_).
- C. The cell wall integrity is destroyed in dead cells, so it is important to perform this staining procedure on \_\_\_\_\_.

**See the next few pages for diagrams on how Gram stains and acid-fast stains really work.**

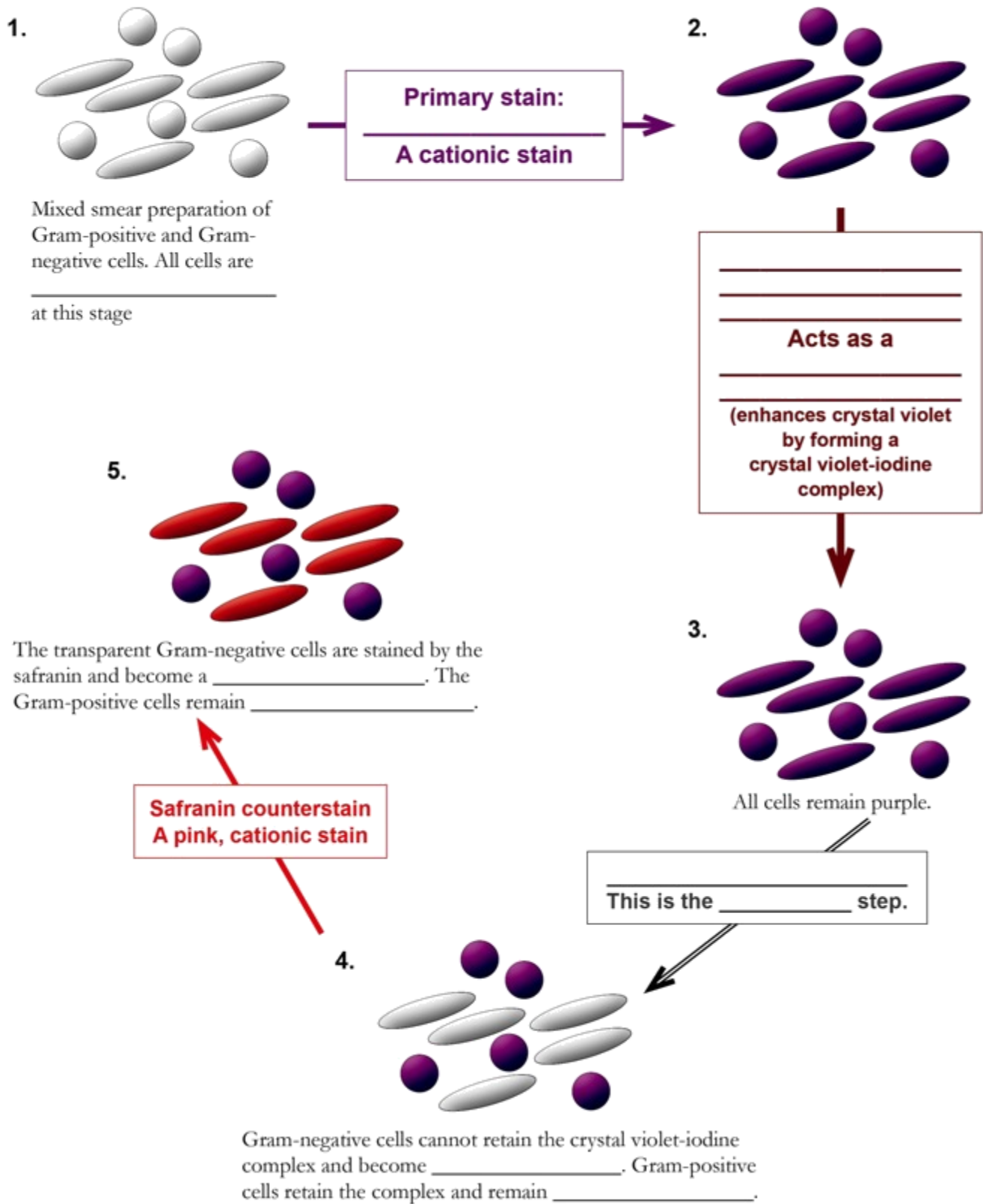
### GRAM-POSITIVE CELL WALL



### GRAM-NEGATIVE CELL WALL



# So, how does a Gram stain work?



## Gram stain table

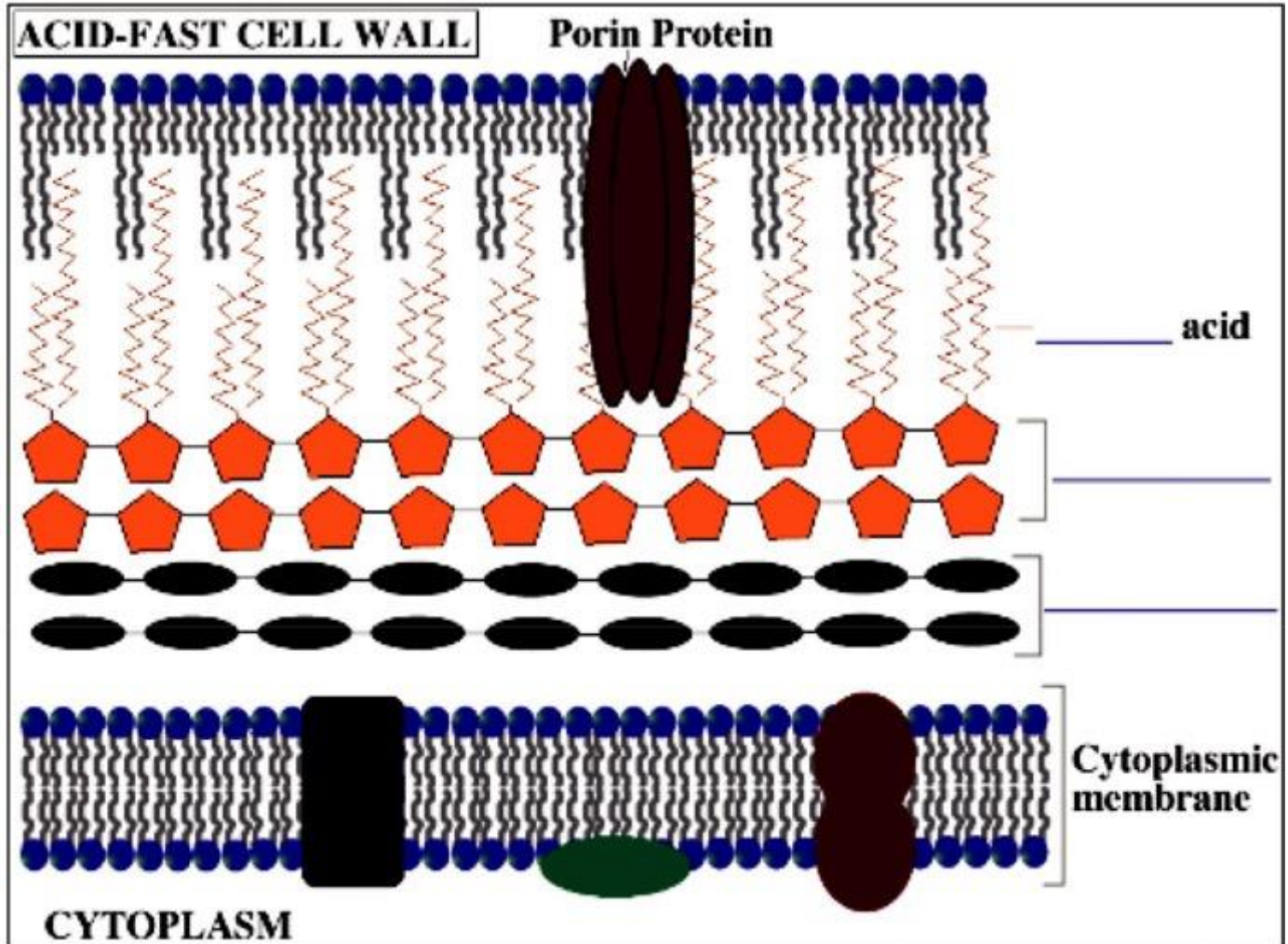
Procedure	Reagent	Color: Gram-positive	Color: Gram-negative
Fixed cells on slide		COLORLESS	COLORLESS
Primary stain	Crystal violet	PURPLE	PURPLE
Mordant	Iodine	PURPLE	PURPLE
Decolorizer	Alcohol	PURPLE	COLORLESS
Counterstain	Safranin	PURPLE	RED

Here's an easy way to remember the procedure for a Gram stain:

**C**ome **I**n **A**nd **S**tain  
**CRYSTAL** **VIOLET** **IODINE** **ALCOHOL** **SAFRANIN**

## Acid-fast stain table

Procedure	Reagent	Color: Acid-fast bacteria	Color: Nonacid-fast bacteria
Primary dye	Carbolfuchsin	RED	RED
Decolorizer	Acid-alcohol	RED	COLORLESS
Counterstain	Methylene blue	RED	BLUE



## So, how does an acid-fast stain work?

