HORSESHOE CRABS: PREHISTORIC PARAMEDICS

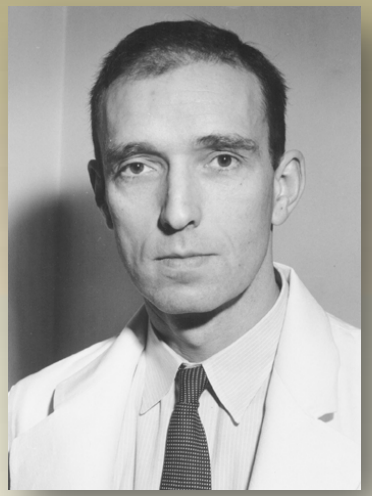
How the horseshoe crab transformed the science of medical safety

Supplemental Presentation
High School—Grades 9-12

Schoolyard Films, Inc.

It all started with a Bang

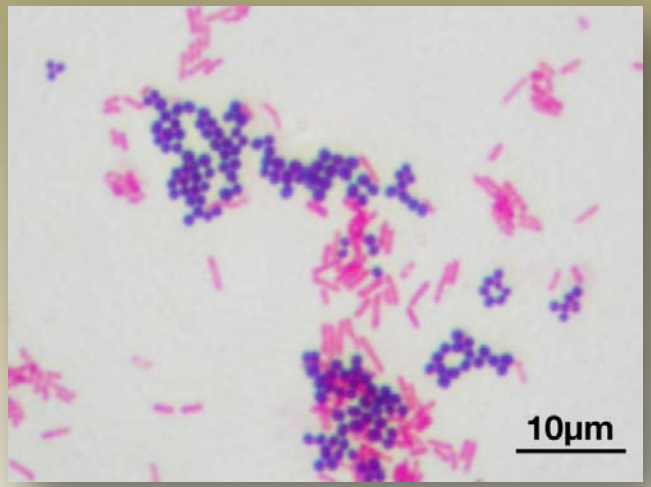
- Dr. Fred Bang studied horseshoe crabs at Johns Hopkins University.
- Dr. Bang noticed something strange about a dying horseshoe crab – it's blood had turned to a gel-like substance!
- Dr. Bang investigated further and determined that infection from **bacteria** caused the blood to clot.
- He teamed up with Dr. Jack Levin, a hematologist, to study this phenomenon further.



Dr. Frederick B. Bang
(*Courtesy The Alan Mason Chesney Medical Archives of The Johns Hopkins Medical Institutions)*

But first--some background on bacteria

- Bacteria are grouped into two major categories
  - **gram-negative**
  - **gram-positive**
- "Gram" comes from a cell staining technique developed by Dr. Hans Christian Gram.



- Gram-positive (+) bacteria stain purple with this technique. Gram-negative (-) do not.
- Gram-negative bacteria have an outer cell membrane, which prevents the cell from staining.

The Good and the Bad

- Gram-negative bacteria are everywhere.
  - Air
  - Water
  - Your intestines!

- How bacteria can be helpful:
  - Fixing nitrogen (*Nitrosomonas europaea*)
  - Making vinegar (*Acetobacter aceti*)
  - Cleaning up chemical spills (*Pseudomonas putida*)
  - Digestion (*Escherichia coli*)

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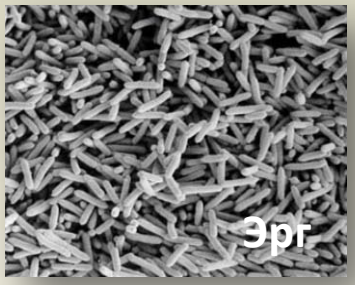

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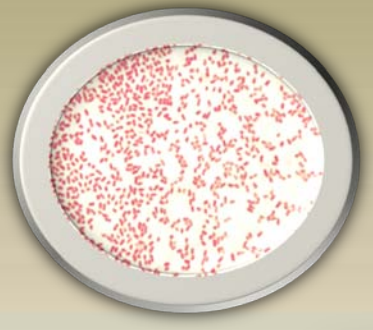
The Good and the Bad



- How bacteria can be harmful:
  - Food poisoning (*Salmonella enterica*)
  - Meningitis (*Neisseria meningitides*)
  - Pneumonia (*Streptococcus pneumoniae*)

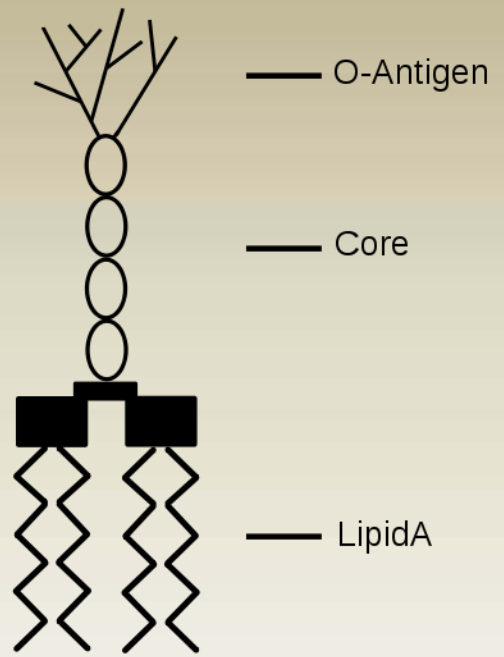
Preventing Infections

- Our skin, gastrointestinal tract, liver, and mucus membranes usually protect us from harmful gram-negative bacteria.
- If these bacteria enter our bloodstream directly, they can cause severe infections.
- Bacteria produce **endotoxins**, which cause fevers.

What's an endotoxin?

- Endotoxins are toxic compounds formed in the outer cell membrane of gram-negative bacteria.
- Endotoxins are a type of **pyrogen.**
- Pyrogens cause severe fevers in mammals.
- "Pyro"means fire or heat.

Endotoxin Schematic



LIPOPOLYSACCHARIDE

Pyrogen-free, please

- All injectable medicines, vaccines, and surgical equipment must be certified as **“pyrogen-free.”**

- Sterilization with heat may kill bacteria, but it doesn't remove the endotoxins produced by bacteria.

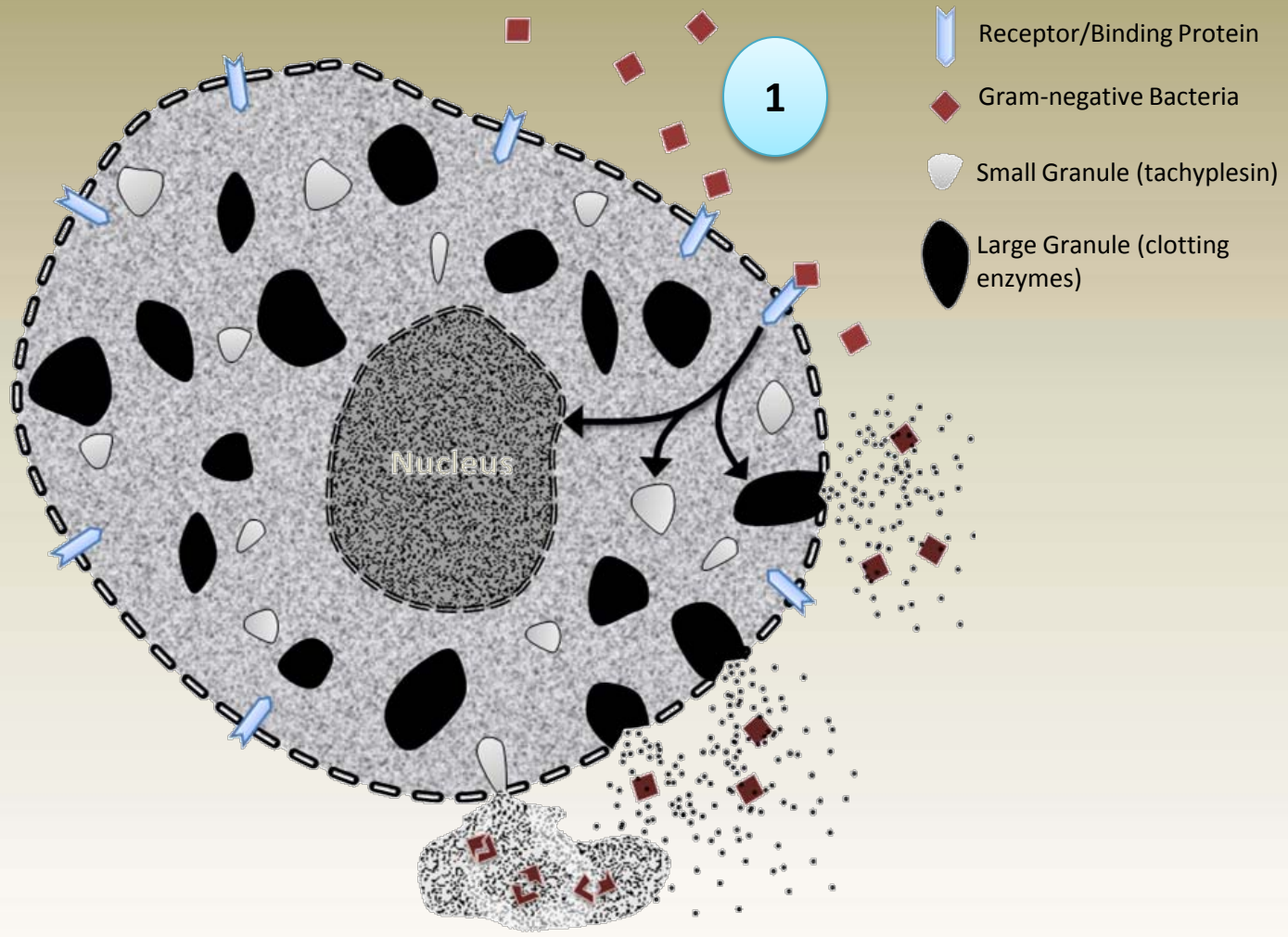
- How can we be sure our medicines are safe?

Back to Bang and Levin

- **Dr. Bang** and **Dr. Levin** continued their study of the horseshoe crab immune system.
- They discovered that **amoebocytes** in horseshoe crab blood act like a primitive immune system.
- Dr. Bang realized that amoeboyctes could be used to test drugs for the presence of endotoxins.
- Up until then, rabbits were used to see if medicines where contaminated.

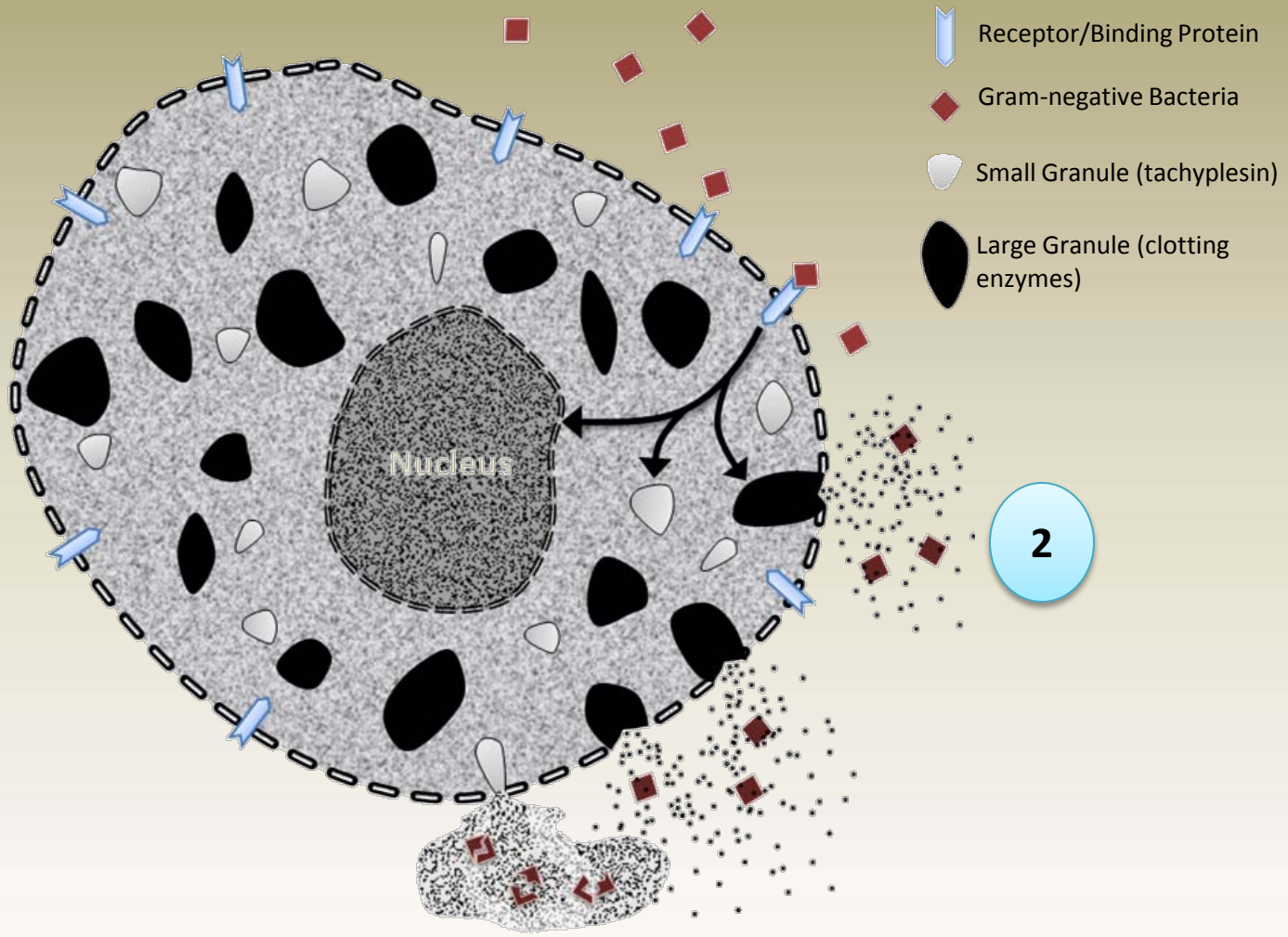
Amoebocytes at Work

1—Receptors on the plasma membrane of the amoebocyte detect the presence of endotoxins from gram-negative bacteria. This results in cellular reactions, which direct large and small granules to the plasma membrane.



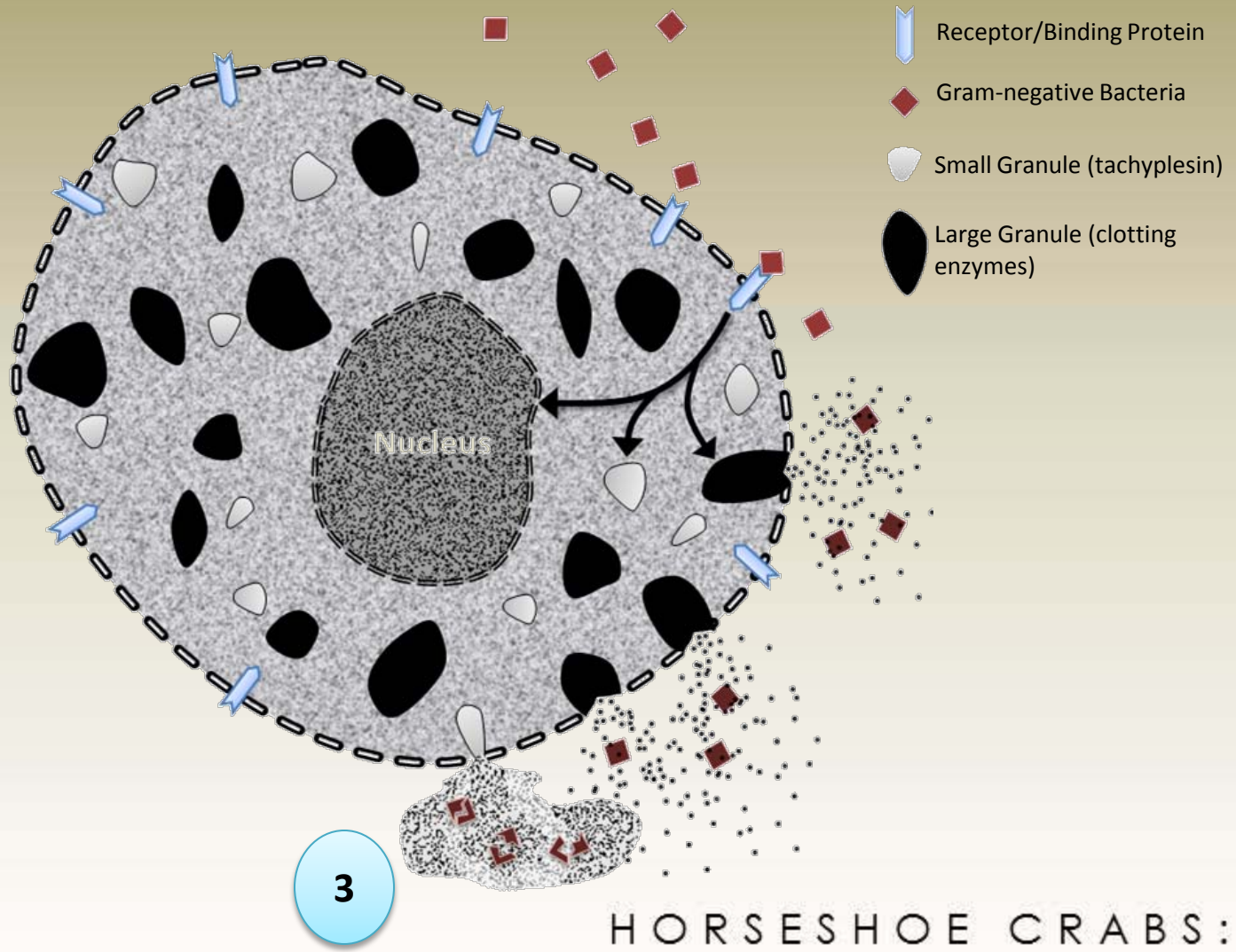
Amoebocytes at Work

2—Clotting enzymes and coagulating proteins are released from the large granules into the surrounding plasma. This activates the clotting mechanism and a gel-like clot forms around the invading microbes.



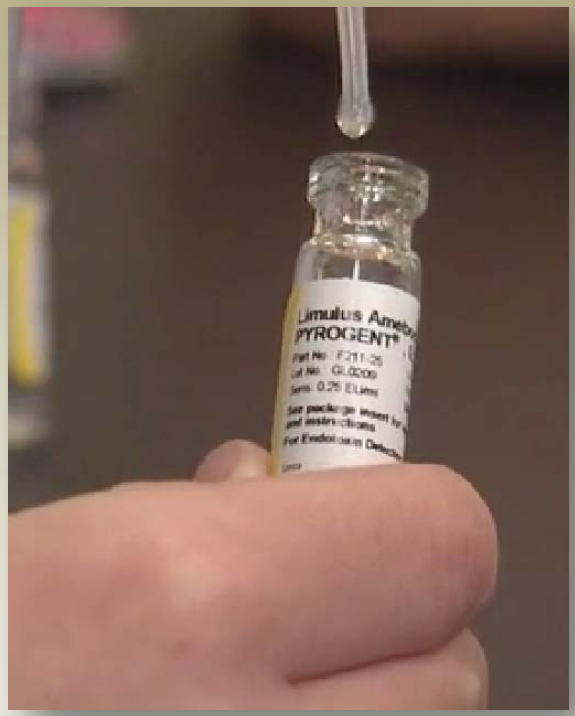
Amoebocytes at Work

3—Anti-microbial compounds, such as tachyplesin, are released from the small granules resulting in the destruction of the bacteria.



The LAL Test

- The **gel-clot reaction** of the amoebocyte immune response is the basis for the LAL test.
  - **L**= Limulus, the genus of the Atlantic horseshoe crab
    A= amoebocyte
  - **L**= lysate, the cell contents extracted from amoebocytes after they are lysed (broken down)
- After Bang and Levin published their findings, other researchers investigated and refined the LAL technology.
- Today, the LAL test is an industry standard for detecting endotoxins.
- Many lives have been saved by this scientific discovery.



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