

# Anthrax

Anthrax  
"The Blackening of Blood"

## Attack of the anthrax "virus"



## Causative agent

✓ *Bacillus anthracis*

- Gram + bacteria
- Endospore forming
- Rod shaped
- Encapsulated

## *Bacillus anthracis* colony

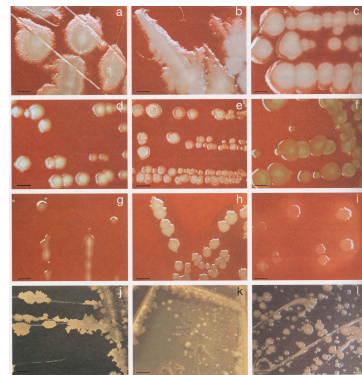
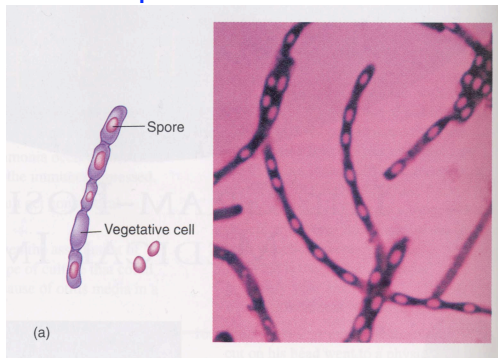
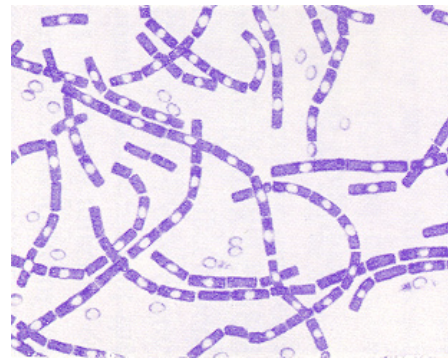


FIGURE 1. Colonies of endospore-forming bacteria on blood agar (a to k) and nutrient agar (l to O) after 24 to 36 h at 37°C. Bacteria from: (a) *B. anthracis*, (b) *B. cereus*, (c) *B. thuringiensis*, (d) *B. megaterium*, (e) *B. pumilus*, (f) *B. subtilis*, (g) *B. anthracis*, (h) *Bacillus* spores, (i) *Bacillus* lactisporus, (j) *Bacillus* pumilus, (k) *B. subtilis*, (l) *B. cereus*, (m) *B. thuringiensis*.

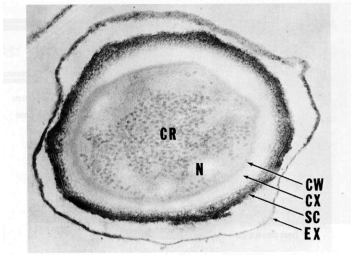
## Anthrax spores



## *Bacillus anthracis*

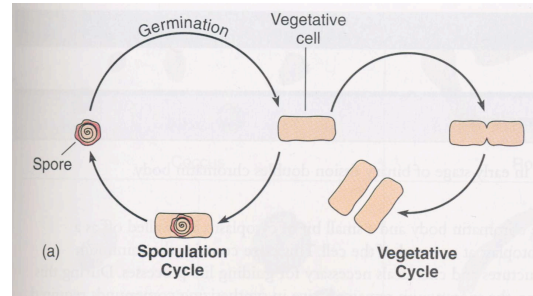


## Bacillus anthracis endospore



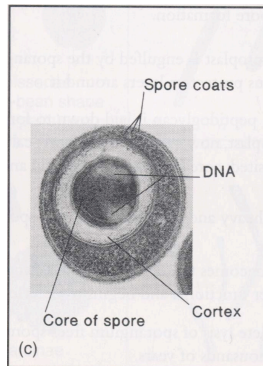
**Figure 3.45 Endospore Structure.** *Bacillus anthracis* endospore ( $\times 151,000$ ). Note the following structures: exosporium, EX; spore coat, SC; cortex, CX; core wall, CW; and the protoplast or core with its nucleoid, N, and ribosomes, CR.

## Sporulation



## Spores

Dipicolinic acid  
Low water  
Low metabolism



## Spores



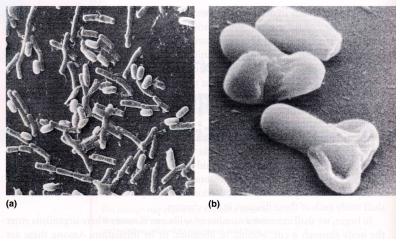
**Figure 11.17 Endospore-Formers.** (a) Endospores forming in the midportion of the cells of *Bacillus anthracis*. (b) Endospores forming at the ends of the cells in *Clostridium tetani*. Both of these species can cause fatal disease, but many other species of endospore-formers are harmless.

## Spore formation and germination

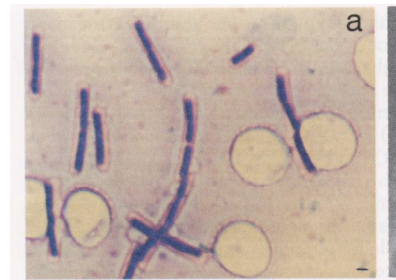
**FIGURE 9.1**

### *Bacillus anthracis*

*Bacillus anthracis* is the cause of anthrax. (a) Free spores and vegetative cells of *B. anthracis* visualized with the scanning electron microscope ( $\times 5,400$ ). Note the oval shape of the spores and the typical rod shape of the vegetative cell. (b) Anthrax spores in the process of germinating ( $\times 50,000$ ). The spore coat of the spore in the center of the photograph has divided and is beginning to separate. A vegetative cell may be seen emerging from the spore at the bottom.



## Capsule



Poly-D-glutamic acid

## Bacteria in Blood

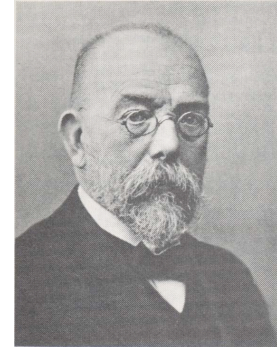
✓ 1863 & 1868 - C. J. Davaine

- Saw rod shape organisms in the blood of diseased animals, but not healthy ones
- Transmitted the disease by inoculating healthy animals with the blood of the diseased animals

## 1876 - Robert Koch

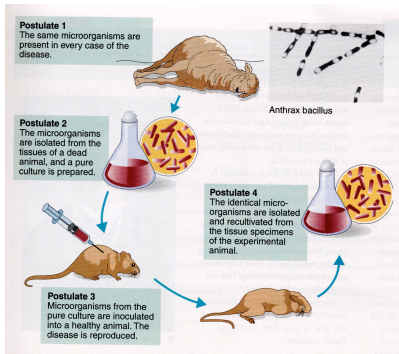
✓ First to isolate and identify *B. anthracis* as the cause of anthrax

- German Doctor
- Country practice
- Worked in his home
- Transmitted the disease through 20 mice



## Koch's Postulates

Postulates were originally outline by Jakob Henle in 1940

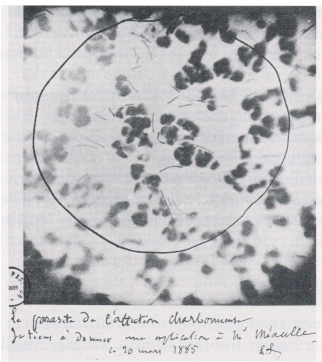


## Louis Pasteur

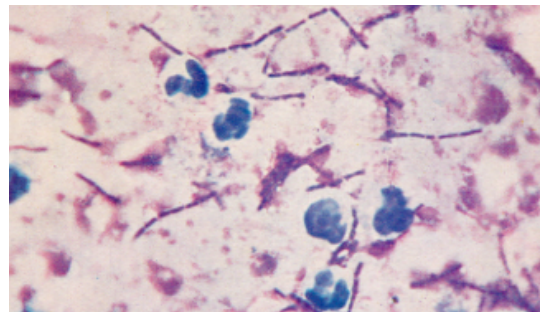


Figure 1.11  
Louis Pasteur (1822–1895), one of the founders of microbiology, is pictured here viewing a sample. Few microbiologists can match the scope and impact of his contributions to the science of microbiology.

## Louis Pasteur's original picture of *Bacillus anthracis*



## Tissue stain



## Host of *B. anthracis* (herbivores)

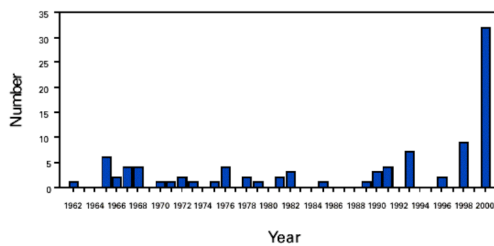
- ✓ Domestic cattle, sheep, goats
- ✓ Wild animals -- bison, antelope, zebra
- ✓ Normally found in soil
- ✓ Survives for decades >60 years

## Highly endemic countries

- ✓ Iran
- ✓ Sudan
- ✓ Iraq
- ✓ Turkey
- ✓ Most African countries
- ✓ Pakistan

## Anthrax quarantines in ND

FIGURE 1. Number of farms quarantined for anthrax — North Dakota, 1962–2000



## Forms of Anthrax

- ✓ Cutaneous anthrax
  - 90 - 95% of all cases
  - 20% lethal
- ✓ Gastrointestinal anthrax
  - 5% of all cases
  - 100% lethal
- ✓ Inhalation anthrax (Wool-sorters' disease)
  - 5% of all cases
  - 100% lethality within 3 days

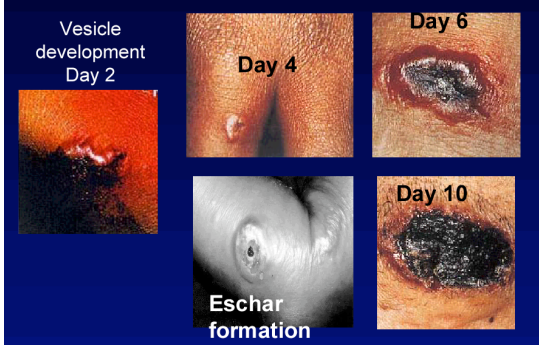
Cutaneous anthrax  
Break in the skin  
2-3 day incubation  
extensive edema  
secondary cellulitis  
meningitis

pimples  
vesicles  
ulcerates  
dries  
blackens  
no pain or pus



FIGURE 27.5 Cutaneous anthrax. A blackened ulcer at the site of infection.

## Acid fast and staining





### Gastrointestinal anthrax

- ✓ Cutaneous anthrax on the intestinal mucosa
- ✓ Eating infected meat
- ✓ Mild gastroenteritis
- ✓ Vomiting
- ✓ Fever
- ✓ Abdominal pain
- ✓ Bloody diarrhea

### Gastrointestinal anthrax

**MMWR Weekly** September 15, 2000 / 49(36):813-6

#### Human Ingestion of *Bacillus Anthracis*-Contaminated Meat --- Minnesota, August 2000

On August 25, 2000, the Minnesota Department of Health (MDH) was notified by the Minnesota Board of Animal Health (MBAH) of *Bacillus anthracis* isolated from a steer on a farm in Roseau County, Minnesota. The infected steer was one of five dead cattle found in a pasture on August 20. On the basis of phage typing of isolates cultured from tissues and blood samples by the North Dakota State University Veterinary Diagnostic Laboratory, *B. anthracis* was confirmed. This report describes the management of and public health response to human exposure to meat contaminated with anthrax.

On July 24, the farmer who owned the infected steer also had killed, gutted, and skinned a cow that was unable to rise. A local veterinarian approved the slaughter of the cow for consumption by the farmer's family. Immediately after slaughter, the farmer took the carcass (carcass X) to a custom meat-processing plant, on July 31 and August 1, carcass X was processed. Two family members ate hamburgers made from carcass X on August 15 and steaks on August 19, three other family members ate hamburgers on August 20. A sixth member prepared the meals and also may have eaten contaminated meat. All meat was reported to have been well cooked. To investigate the possibility that they had eaten contaminated meat, the family members were

### IV Drug users

**MMWR Weekly** June 09, 2000 / 49(22):489-492

#### Unexplained Illness and Death Among Injecting-Drug Users --- Glasgow, Scotland; Dublin, Ireland; and England, April-June 2000

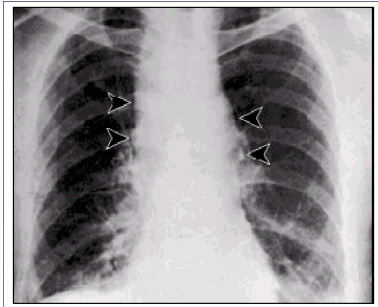
Since April 19, 2000, 30 injecting-drug users (IDUs) died or were hospitalized with unexplained severe illness in Glasgow, Scotland. Illness was characterized by extensive local inflammation (i.e., abscess, cellulitis, fasciitis, or myositis) at an injection site, and either 1) severe systemic toxicity (i.e., sustained systolic blood pressure <90 mm Hg despite fluid resuscitation and total peripheral white blood cell count [WBC] >30,000 cells/mm<sup>3</sup>), or 2) postmortem evidence of a diffuse toxic or infectious process including pleural effusions and soft tissue edema or necrosis in an IDU admitted to a hospital or found dead since April 1, 2000. As of June 5, in Glasgow, 16 (53%) of 30 IDUs evaluated had illnesses that met the case definition (Figure 1). In Dublin, eight (53%) of 15 IDUs, and in England, six (43%) of 14 IDUs had illnesses that met the case definition (Figure 1). Demographic information, peripheral WBC, and case-fatality among IDUs were similar in all three countries (Table 1). Most cases had progressive symptoms with a median of 3 days (range 0-14 days) between illness onset and hospitalization. Among persons who died while hospitalized, the median time from admission to death was 2 days (range 0-13 days). Pleural effusion and extensive edema at an injection site were prominent features at postmortem examination.

Cultures of blood and tissue yielded multiple organisms from several patients including group A streptococcus, *Staphylococcus*

### Inhalation anthrax

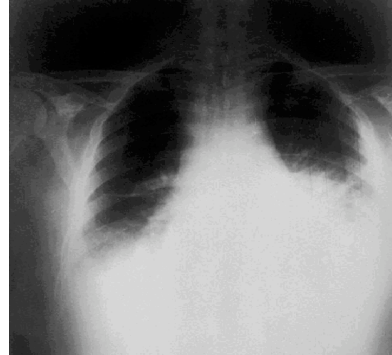
- ✓ Spore engulfed by macrophages
- ✓ Germinate in the lymphatic system
- ✓ fatal septicemia
- ✓ Mild
  - Fatigue
  - Malaise
  - Slight fever
- ✓ Cyanosis, disorientation, shock, coma, then death

## Beginnings of inhalation anthrax



JAMA 1999; 281:1735-1745

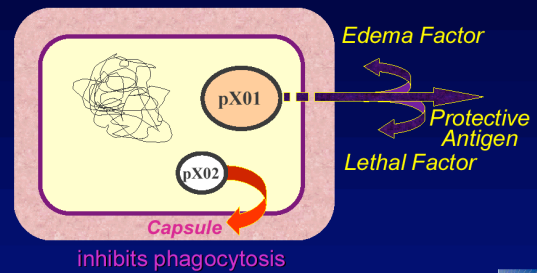
## Inhalation anthrax (near death)



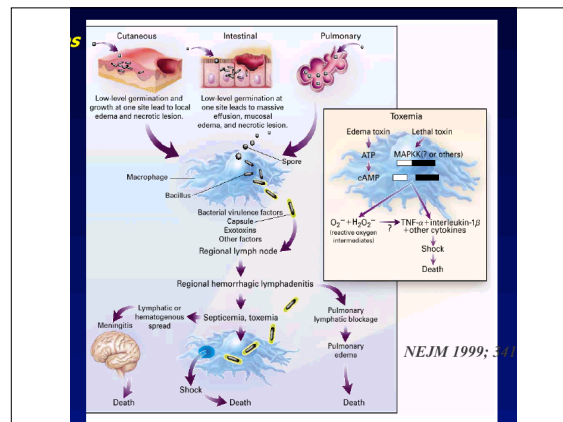
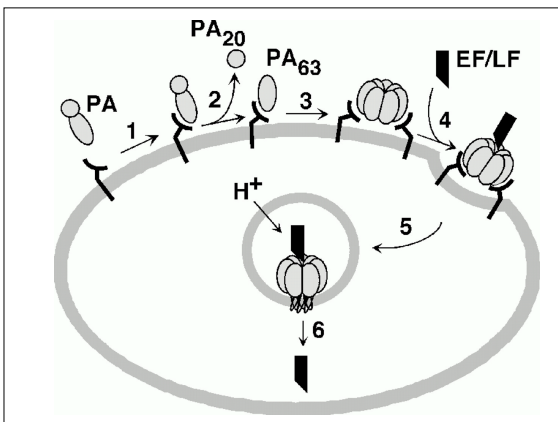
## Virulence factors

- ✓ Virulence is related to two plasmids
  - Plasmid 1 -- pX01 three genes - anthrax toxin
    - Protective antigen (PA)
      - ☛ Transports LF and EF into the cell
    - Lethal factor (LF)
      - ☛ Protease - cleaves the MAPK kinase, kills macrophages - leads to shock
    - Edema factor (EF)
      - ☛ Calmodulin-dependant adenylate cyclase - edema - imparment of neutrophils
  - Plasmid 2 -- pX02
    - capA, capB, capC genes
    - Code for capsule

## Bacillus anthracis: Virulence Factors

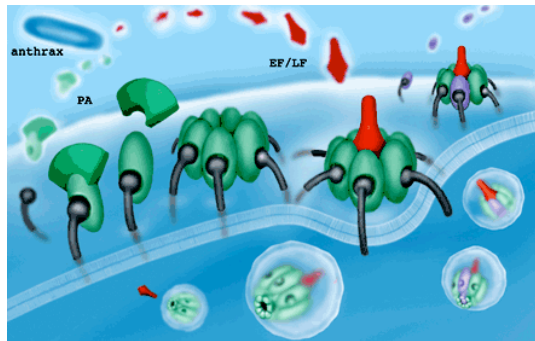


CDC

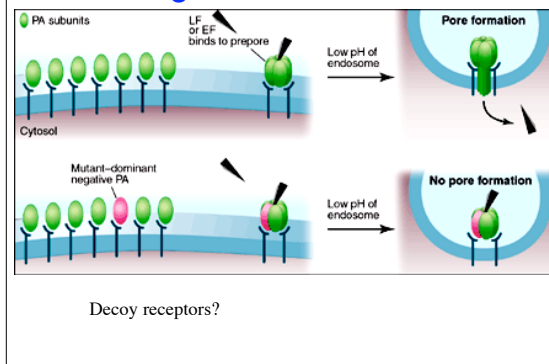


NEJM 1999; 341

## Anthrax toxin



## Preventing anthrax?



## Vaccines

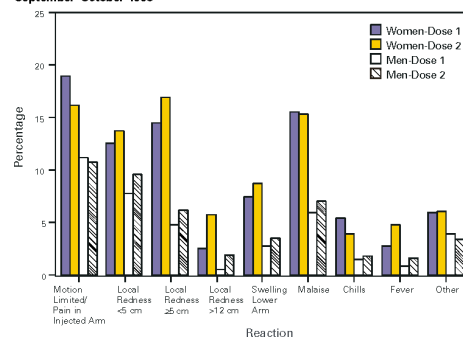
✓ First vaccine in the 1930s

- Livestock
  - Attenuated bacteria
  - Pasteur first to attenuate the organism
- Human
  - Cell culture extracts that contain the PA protein

✓ New? -- DNA vaccine with the PA and LF genes on a plasmid

## Vaccine side effects

FIGURE 1. Self-reported reactions to anthrax vaccine — United States Forces, Korea, September–October 1998



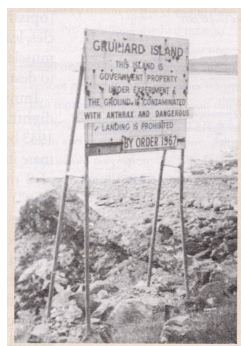
## Grainard Island

In 1941

60 sheep

1971 - still anthrax spores  
Spread by earthworms

1981 - notes led government officials to 10 lbs of soil  
1986 - the island was decontaminated  
1987 - sheep were on the island again



## Biological warfare

- ✓ Iraq, Russia, North Korea, and others
- ✓ 1979 - Former Soviet Union accidentally released spores
  - 79 cases of anthrax with 68 deaths
- ✓ 1990 -- Cult Aum Shinrikyo - 9 attempts of biological attacks

## Treatment

- ✓ Ciprofloxin
- ✓ Penicillin
- ✓ Gentamicin
- ✓ Erythromycin
- ✓ Chloramphenicol
- ✓ doxycycline

## Anthrax letter

