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Anthrax

■ Synonyms:	Woolsorter's disease (inhalational anthrax)
■ Etiology:	Inoculation or inhalation of spores of *Bacillus anthracis*, a gram-positive bacillus
■ Associations:	None
■ Clinical:	Cutaneous: papule, followed by vesicle, then ulcer with eschar at the site of inoculation; septicemia in approximately 20%
■ Histology:	Partial epidermal necrosis, dermal edema with fibrin, neutrophils, and abscess formation; positive gram stain expected
■ Evaluation:	Notifying local health department and laboratory of clinical suspicion, gram stain and culture of vesicle fluid or eschar base, skin biopsy with tissue culture
■ Treatment:	Ciprofloxacin or doxycycline
■ Prognosis:	99% survival for cutaneous disease if treated early, 20% survival for inhalational disease, and intermediate survival for gastrointestinal disease

Anthrax is an infection caused by the spore-forming bacterium, *Bacillus anthracis*. Infection occurs in mammals, particularly herbivores that ingest bacterial spores from soil. Human infection occurs from inhalation of spores, ingestion of animal meat contaminated with spores, or percutaneous inoculation of spores from exposure to infected animals or contaminated animal products. Anthrax has been an occupational disease of textile workers, farmers, butchers, veterinarians, and shepherds.

Anthrax is a historically important infection, thought to be the fifth and sixth plagues of ancient Egypt, brought by Moses. It was the cause of several disastrous animal plagues in Europe in the eighteenth and nineteenth centuries. In 1877, Robert Koch cultured *Bacillus anthracis*, the first proof of a microbial agent causing human disease (1). This discovery supported "germ theory" and gave birth to the science of modern microbiology. Subsequently, Pasteur and Greenfield successfully developed the first vaccine, composed of attenuated *B. anthracis* (2). Anthrax has been explored as an agent of biological warfare because of its exceptional virulence and capability to create an aerosol of odorless, invisible spores. Its spores could potentially be dispersed over densely populated areas, and generate disease in a multitude of people with high morbidity. This organism has gained notoriety more recently because of the anthrax attacks of 2001, in which anthrax spores were distributed by mail using the U.S. Postal Service, resulting in inhalational or cutaneous anthrax infection in 22 people.

Bacillus anthracis is a gram-positive, nonmotile, aerobic, spore-forming rod. The organism grows readily on standard culture media, especially sheep's blood agar, forming nonhemolytic irregular white-gray colonies, with tapered extensions (3). Gram stain of cultures reveals long chains of bacilli. Notifying the laboratory of clinical suspicion of *B. anthracis* is important because of the prevalence of the similar-appearing organism, *Bacillus cereus*, which is a frequent laboratory contaminant (4).

The infective unit of the organism is the endospore, which may reside in soil for decades. It is resistant to heat, ultraviolet and gamma irradiation, drying, and antimicrobial agents (5). Spores enter the body through broken skin, the lungs, or the gastrointestinal tract, and are engulfed by macrophages, and then transported to lymph nodes. Within the macrophage, they transform to the vegetative form and then multiply within the lymphatic system. They are eventually released in high concentrations into the bloodstream, resulting in sepsis. The

organism achieves its virulence by the production of three polypeptides and an antiphagocytic capsule, encoded on two plasmids, pXO1 and pXO2. Both plasmids are needed for the organism to achieve complete virulence. pXO1 encodes the three polypeptides, protective antigen (PA), lethal factor (LF), and edema factor (EF), which combine as binary toxins. PA combines with LF to form lethal toxin, and combines with EF to form edema toxin. The PA component of the toxin facilitates access to the host cell by its binding to cellular receptors. The complex is cleaved by the serine protease furin, causing oligomerization and subsequent transport of toxins into the cell. The lethal toxin is a zinc metalloproteinase that activates oxidative burst pathways, forming reactive oxygen intermediates. It also induces the production of tumor necrosis factor-α and interleukin-1β, which are involved in inducing septic shock. Plasmid pXO2 encodes three genes involved in production of the polyglutamic acid capsule that inhibits phagocytosis of the vegetative state of *B. anthracis* (4,6,7).

The three forms of anthrax infection in humans are cutaneous, inhalational, and gastrointestinal. The disease is spread to humans via contact with infected animals or contaminated animal products. Naturally occurring anthrax has been almost eradicated in the United States and in Western Europe due to the presence of long-standing vaccination programs for at-risk livestock, but is still relatively common in Asia Minor.

Cutaneous anthrax is by far the most common form of the disease. Infection usually occurs from contact with infected animals or animal products such as hides, wool, hair, or bones. The primary lesion is a papule, sometimes pruritic, occurring three to five days after inoculation. The face, neck, and extremities are most commonly affected (4). Within two days, vesiculation occurs, with central necrosis, and formation of a 1–3 cm painless ulcer and subsequent eschar (Figure 24.1) (3). The eschar accounts for the derivation of the organism's name from the Greek word *anthrakos*, meaning coal (1). Lymphadenopathy and lymphangitis may develop, with satellite hemorrhagic vesicles and edema. Inoculation sites on the extremities usually result in "malignant pustule" formation. More central sites tend to have prominent edema, resulting in the "malignant edema" presentation (3). The latter form is more likely to be complicated by airway obstruction, requiring concomitant use of systemic steroids and antibiotics. Secondary bacterial infection of the site is common, so that treatment with extended-spectrum antibiotics may be needed. While many cases of cutaneous anthrax are self-limited, antibiotic treatment is recommended because of the approximately 20% of cases in which disseminated infection associated with high mortality occurs. Case fatality rate is less than 1% in treated cases (8). Cutaneous lesions are reported to heal without scarring in the large majority of cases (4). However, in a recent series, considerable scarring requiring reconstructive surgery in 23% of cases was noted (3).

Inhalational anthrax is exceptionally uncommon, but was more prevalent in occupational settings as "woolsorter's disease" before adequate hygienic standards were employed. In contrast to inoculation anthrax, disease caused by inhalation of anthrax spores is usually fatal because the disease causes nonspecific influenza-like symptoms followed by rapid onset of septic shock and death. Important information regarding inhalational anthrax was yielded by an accidental release of anthrax

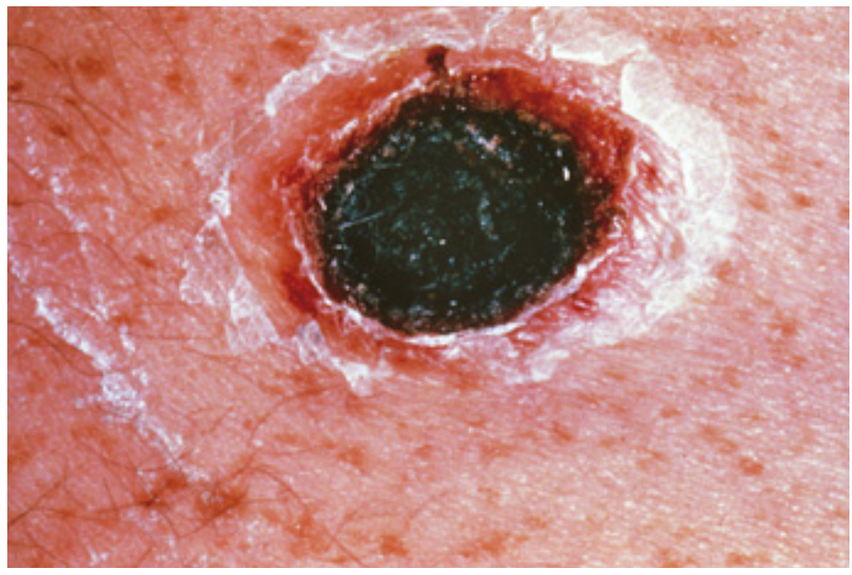


FIGURE 24.1. Cutaneous anthrax: ulcer, with central eschar, and surrounding erythema and edema.

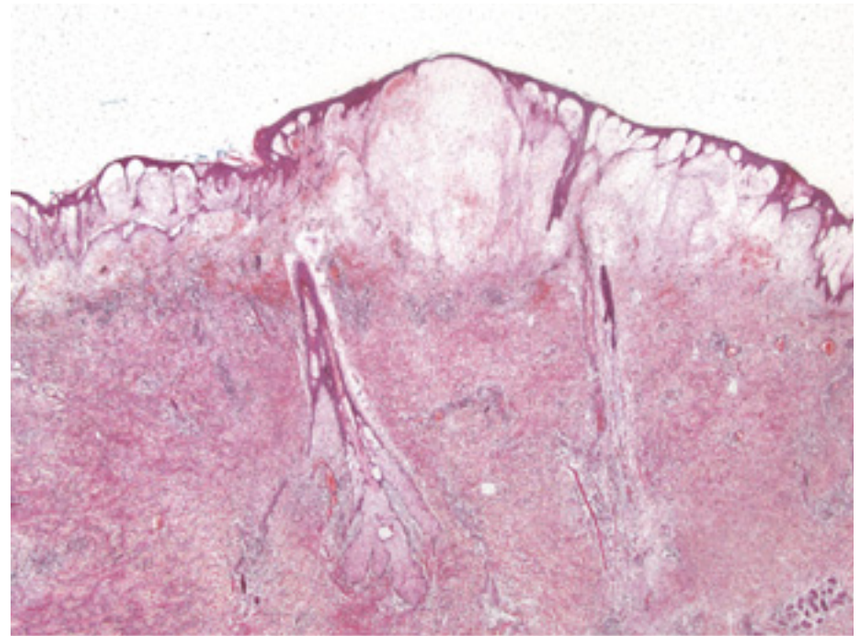
spores from a biological weapons facility in Sverdlovsk in 1979, in which 66 deaths occurred. The incubation time ranged from 2 to 43 days, with an average of about 10 days. The mortality rate was approximately 80% (9). Despite its rarity, inhalational anthrax was the mode of infection in 50% of the anthrax cases in the 2001 attacks, and would be expected to be the prevalent form of the disease in a biological weapons attack (10). In the setting of an anthrax outbreak, clinical features favoring inhalational anthrax over an influenza-like illness include presence of dyspnea, hypoxemia, chest pain, lack of sore throat or rhinorrhea, and presence of mediastinal widening, pulmonary infiltrate, or pleural effusion on chest radiography. Laboratory evaluation may reveal neutrophilia with bandemia and elevated liver enzyme tests (11).

Gastrointestinal tract and oropharyngeal anthrax are also uncommon. These infections result from ingestion of contaminated meat from infected animals. The incubation period is two to five days. Infection probably occurs in a manner similar to that in the skin, with ulcer formation, and bacterial proliferation in lymphatic tissues. Inoculation may occur at any point along the gastrointestinal tract, including the oropharynx. Symptoms and signs vary depending on the site of inoculation, and may include fever, abdominal pain, nausea, vomiting, dysphagia, constipation, diarrhea, melena, and ascites. Mortality is considerable and may be the result of sepsis or intestinal perforation (4).

Cutaneous, inhalational, and gastrointestinal anthrax may all be complicated by anthrax meningitis, a complication occurring during bacteremia. Central nervous system involvement portends a grave prognosis despite therapy (4).

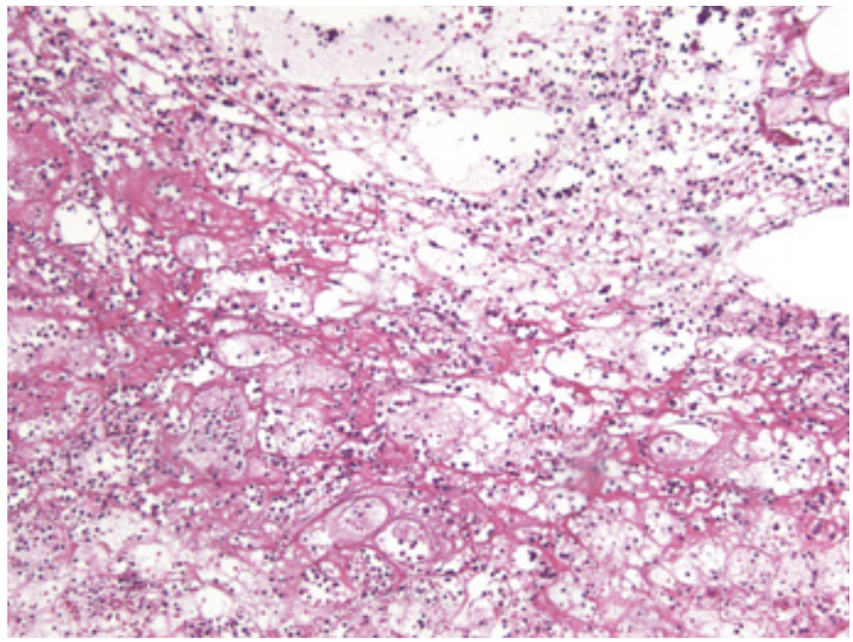
Diagnosis of anthrax may be made by blood culture in the setting of disseminated infection. Growth usually occurs in 6 to 24 hours (10). For cutaneous anthrax, vesicle fluid culture may grow the organism, and 64% of cases in one series had organisms identified by gram-stained smears of cutaneous lesion contents (3). The organism is unlikely to grow from skin or blood if the patient has received antibiotics prior to obtaining culture material (10). A new rapid diagnostic test for anthrax has recently been approved by the U.S. Food and Drug Administration that detects antibodies to anthrax-protective antigen in 45 minutes (12,13).

The differential diagnosis of cutaneous anthrax is considerable, and includes ecthyma, insect or arachnid bite reaction, tularemia, glanders, rickettsialpox, diphtheria, syphilitic chancre, ecthyma gangrenosum, and orf. In endemic or occupational settings, history of contact with animals or animal products is expected in cases of anthrax. Clinical features that may point to anthrax include relative lack of symptoms given impressive clinical findings, and prominent edema. Gram stain and culture of cutaneous vesicles or ulcers will yield the organism in the majority of cases. Skin biopsy specimens of *Bacillus anthracis* infection have prominent superficial dermal edema with variable epidermal necrosis, and an infiltrate of neutrophils throughout the dermis, with abscess formation. There is prominent dermal fibrin, with foci of vasculitis. Gram stain reveals gram-positive rods within the dermis (Figure 24.2A, 24.2B, and 24.2C) (14,15). Should there be



A

FIGURE 24.2. (A) Anthrax: mild epidermal hyperplasia and striking superficial dermal edema.



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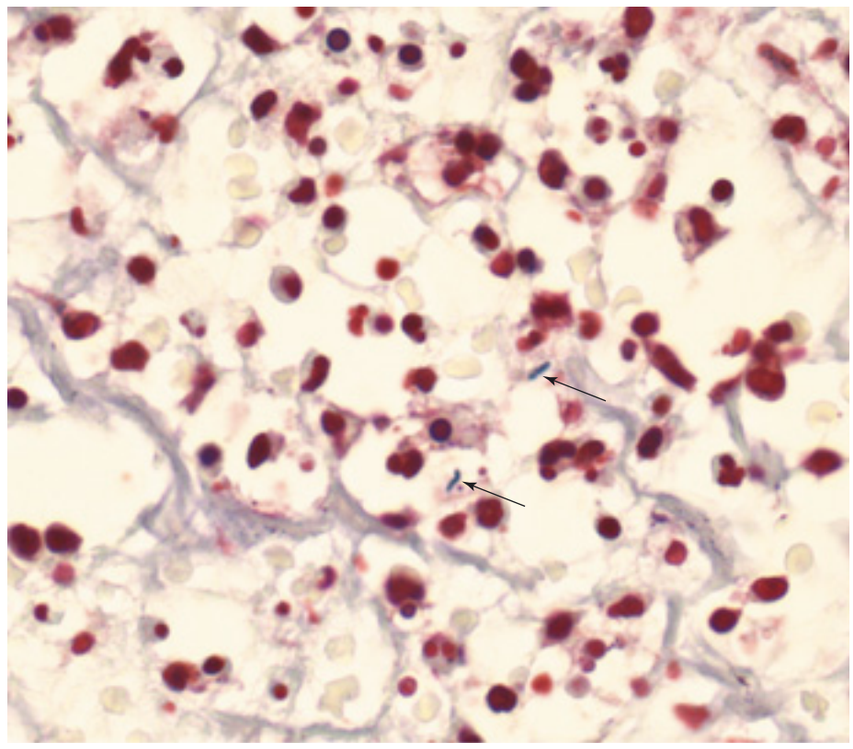

C

FIGURE 24.2. (B) Deep dermal and subcutaneous mixed inflammatory infiltrate of lymphocytes, neutrophils, and plasma cells, with edema and fibrin deposition. (C) Mixed inflammatory infiltrate with gram-positive bacilli (arrows). Courtesy of Eduardo Calonje, MD.

clinical suspicion of cutaneous anthrax, the following list of procedures should be employed:

Diagnostic Evaluation of Suspected Cutaneous Anthrax

1. Patient should be under contact precautions only, since the infective unit, the spore, is not shed.
2. Gram stain and culture of vesicle fluid, or unroofed eschar.
3. Two punch biopsy specimens, one for tissue culture, and one in formalin for routine processing.
4. Blood cultures if clinical suspicion of bacteremia is present.
5. Contact laboratory and local health department with clinical suspicion, bloodwork as recommended for serologic and diagnostic studies.

Diagnostic and management information on anthrax is available on the Internet from the U.S. Centers for Disease Control (16).

Cutaneous anthrax in endemic areas is treated successfully with penicillin, given intravenously in cases of "malignant edema" (3,17). However, treatment of cutaneous anthrax in the setting of a bioterrorism attack as recommended by the Working Group on Civilian Biodefense includes ciprofloxacin 500 mg p.o. twice daily for 60 days, or doxycycline 100 mg p.o. twice daily for 60 days. Complete treatment recommendations are delineated by the Working Group and U.S. Centers for Disease Control (10,16). The rationale for extended treatment is to cover the likelihood of concomitant inhalation of anthrax spores, which may incubate for close to 60 days before resulting in clinical infection. Penicillin was not recommended in this treatment protocol because isolates from the 2001 anthrax attacks showed an inducible β-lactamase that degrades the antibiotic. The clinical significance is uncertain, but suggests the potential for rapid onset of penicillin resistance (10). No resistance to fluoroquinolones has been identified, but *in vitro* high-level resistance can be induced by serial passage of *Bacillus anthracis* in media containing fluoroquinolones, suggesting that drug-resistant strains can be cultivated (18). No treatment other than antibiotics currently exists for anthrax. In a rodent anthrax model, infusion of antiprotective antigen antibodies with ciprofloxacin yielded greatly enhanced survival compared with ciprofloxacin alone, suggesting this as a potential therapeutic addition in humans (19).

Human anthrax vaccine is available as "anthrax vaccine adsorbed," a sterile filtrate of cultures of an attenuated unencapsulated, non-proteolytic strain of *B. anthracis*, containing predominantly protective antigen (2). Doses are given subcutaneously at 0, 2, and 4 weeks, with boosters given at 6, 12, and 18 months. Further boosters are recommended if continued exposure is a possibility. The vaccine has been widely used in military personnel and in some industries that have exposures to at-risk animals from endemic areas. Other immunizations are under investigation, including a transcutaneous system that has recombinant protective antigen of *Bacillus anthracis* and heat-labile toxin of *Escherichia coli* delivered by an adhesive patch. In a murine model, maximal immunity with protection against lethal doses of aerosolized anthrax spores was achieved with two doses, holding promise for future use in humans (20).

Naturally occurring cutaneous anthrax is a disease that has become almost nonexistent in the United States and Western Europe due to vaccination programs in at-risk animal reservoirs. However, the significance of this organism has been heightened recently because of its use in bioterrorism. The anthrax attacks of 2001 focus the potential for cutaneous presentations of this disease to be the first detectable manifestation of a terrorist biological weapons attack.

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