STATE-OF-THE-ART CLINICAL ARTICLE

Anthrax

F. Marc LaForce

From the Department of Medicine, The Genesee Hospital, Rochester; and the University of Rochester School of Medicine and Dentistry, Rochester, New York.

Except for *Streptococcus pneumoniae*, no organism has contributed more to our understanding of basic infectious disease principles than *Bacillus anthracis*, the causative agent of anthrax. *B. anthracis* was the first clearly recognized bacterial pathogen when, in 1850, Rayer described filiform bodies in the blood of animals dying of anthrax [1]. In 1863, Davaine showed that anthrax could be transmitted to experimental animals by subcutaneous inoculation of infected blood [1]. The life cycle of the organism was unraveled by Koch, who recognized the importance of a dormant spore stage for perpetuation of the organism in soil. From these studies came the unimicrobial theory of infections, and Koch's postulates became part of the vocabulary of the experimental microbiologist. A few years later Pasteur successfully attenuated strains of *B. anthracis* and proved that these strains could protect sheep from fully virulent strains of the organism. Last, the recognition of woolsorters' disease (inhalation anthrax), an unusual but dangerous industrial infection, was an important stimulus to the development of the discipline of industrial hygiene and industrial microbiology.

Microbiology

B. anthracis is a sporulating gram-positive rod. The organism is nonmotile and grows well on blood agar plates. Individual colonies are sticky and stand up in stalagmite-like forms when they are lifted or touched with a bacteriologic loop. Susceptibility to a γ-bacteriophage can confirm the identity of the organism, and newly developed polymerase chain reaction techniques can identify as few as three spores of *B. anthracis* in a specimen. Serological diagnosis is possible with use of a sensitive and specific indirect microhemagglutination test. All virulent strains of the bacillus are pathogenic for mice.

The virulence of *B. anthracis* strains is due to the presence of a poly-D-glutamic acid capsule and the production of a three-component protein exotoxin that is made up of edema factor (EF), lethal factor (LF), and protective antigen (PA). The production of the toxin and the capsule is dependent on the presence of two plasmids: pXO1 (184.5 kbp [kilobase pair]) is required for the production of the three exotoxins, and pXO2 (95.3 kbp) contains the genes for synthesis of the poly-D-glutamic acid capsule. The poly-D-glutamic acid capsule is weakly antigenic and antiphagocytic. In 1884 Metchnikoff, who was working with virulent and attenuated strains of *B. anthracis* in Pasteur's laboratory, clearly described the inability of rabbit and guinea pig phagocytes to engulf virulent encapsulated anthrax bacilli; he also demonstrated that heat-attenuated strains of *B. anthracis* (used in vaccines) were readily phagocytized (figure 1) [2]. Contemporary studies of these attenuated strains have shown that they were unencapsulated and had been cured of the pXO2 plasmid.

EF is a calmodulin-dependent adenyl cyclase that causes edema when injected subcutaneously into experimental animals. LF is so called because it causes death through an unknown mechanism when injected into susceptible animals. Neither EF nor LF are toxic alone; they can produce their deleterious effects only when they are combined with PA, so named because of its use in protective anthrax vaccines.

Received 11 August 1994: revised 22 August 1994.
Reprints or correspondence: Dr. F. Marc LaForce, The Genesee Hospital,
224 Alexander Street, Rochester, New York 14607.

Clinical Infectious Diseases 1994;19:1009-14.

1058-4838/94/1906-0001$02.00

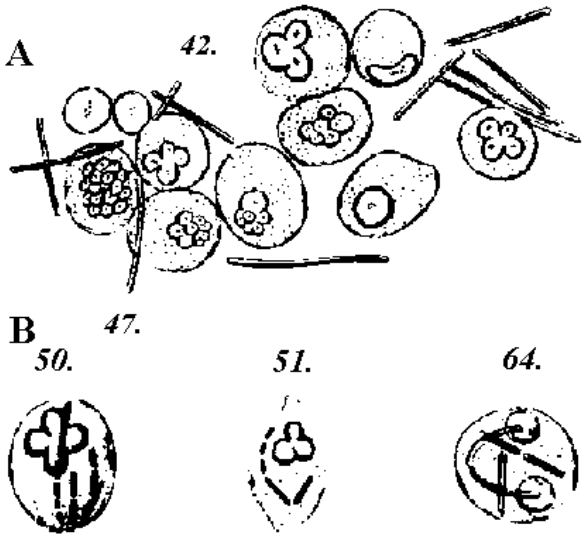

Figure 1. *A*. Failure of guinea pig phagocytes to internalize the virulent encapsulated strain $pXO2^+$ of *B. anthracis;* organisms are exclusively extracellular. *B*. Phagocytosis of unencapsulated attenuated strain $pXO2^-$ of *B. anthracis*; organisms are intracellular. (Both figures are reprinted with permission from [2]).

The anthrax exotoxin behaves somewhat like diphtheria, tetanus, and botulinum toxins and according to the A-B model first proposed by Gill [3]. The A component of the toxin is responsible for cell injury or death, while the B portion binds to the cell membrane and facilitates movement of the A component into the cytosol. Anthrax toxin is more complex in that it consists of the three components PA, EF, and LF. The proposed pathway whereby EF and LF are internalized by susceptible cells is as follows: PA functions like a bacterial toxin-B component and binds to a cell-surface receptor. After binding, a small 20 kD N-terminal piece is proteolytically cleaved from it. The larger remaining cell-bound PA piece (PA_{63}) now has an exposed binding site for either EF or LF, and the PA_{63}-EF or PA_{63}-LF can now undergo receptor-mediated endocytosis, whereby EF and LF are translocated to the cytosol. In planar phospholipid bilayers, PA_{63} forms cation selection channels, which suggests that cleavage of the PA_{20} piece allows for the insertion of the PA_{63} as a true membrane-bound protein with channel properties.

Epidemiology

The ecology and epidemiology of anthrax are summarized in figure 2. The organism has three distinct cycles that include multiplication of spores in the soil, animal infection, and human infection. *B. anthracis* can be part of normal soil flora and can undergo a burst of local multiplication that increases the number of organisms in the soil and enhances the risk of infection in grazing animals. The reasons for local proliferation of anthrax bacilli are still not well understood. The results of studies of agricultural outbreaks have suggested that conditions for multiplication become favorable when soil pH is >6.0, the soil is rich in organic matter, and there are major changes in the soil microenvironment such as those seen after abundant rainfall or a drought. Spores may also persist in the soil for long periods of time.

Perhaps the best-studied site of soil contamination with *B. anthracis* is the island of Gruinard, off the western coast of Scotland, which was the site of biological warfare experiments during World War II. These experiments showed that the spores could be explosively released and cause inhalation anthrax in experimental animals located downwind [4]. The trials were held in 1942 and 1943, and an estimated 4×10^{14} spores were dispersed on the island by explosive means. Annual tests for more than 20 years after the war showed that large numbers of fully virulent spores persisted in the soil. In 1986, after extensive microbiological sampling, the contaminated areas were disinfected with a mixture of formaldehyde and seawater. Resampling studies in 1987 showed that the soil no longer harbored *B. anthracis*, and the island returned to normal agricultural use. Surface decontamination, except in very unusual circumstances, is not practical, and epizootic anthrax will continue to occur in areas where the organism in highly endemic (e.g., Iran, Iraq, Turkey, Pakistan, and sub-Saharan Africa) and where the use of animal anthrax vaccine is not comprehensive.

Systemic anthrax is primarily a disease of herbivores, and humans become infected accidentally through contact with infected animals or their products. Anthrax is now rare in the United States; between 1984 and 1993, only three cases of cutaneous anthrax were reported to the Centers for Disease Control and Prevention. The last fatal case occurred in 1976, when a home craftsman died of inhalation anthrax after working with yarn imported from Pakistan. Cases of anthrax in the United States have been epidemiologically characterized as industrial or agricultural. In the 1950s and 1960s >80% of the cases that occurred in the United States

were industrial and were related to the manufacture of textiles for which imported fibers, particularly goat hair, were used [5]. The incidence of this infection has fallen dramatically because of decreased use of imported contaminated raw material and implementation of a highly successful immunization program among textile workers who are at risk. Despite the rarity of human cases, anthrax remains a potential threat because of continued outbreaks of animal anthrax and the importation of contaminated raw hair products.

Inhalation anthrax, or woolsorters' disease, develops following the inhalation of *B. anthracis* spores generated during the early stage of cleaning contaminated goat hair. The disease is largely of historical interest and has disappeared with the introduction of better industrial hygienic practices in the textile industry and the promotion of anthrax vaccine.

Pathogenesis

When the spores of virulent *B. anthracis* are introduced subcutaneously, they begin to multiply. The production of the antiphagocytic capsule facilitates local spread. Production of exotoxin occurs and results in extensive brawny edema and tissue necrosis. Airborne *B. anthracis* spores that are >5 μm in size pose no threat to the lung, as they are either physically trapped in the nasopharynx or are cleared by the mucociliary system. Spores between 2 and 5 μm in size are deposited on alveolar ducts or alveoli. They are phagocytized by alveolar macrophages and are transported to mediastinal lymph nodes where they multiply and cause hemorrhagic mediastinitis (but not true pneumonia). Bacteremia and meningitis are frequent complications.

Pharyngeal and gastrointestinal anthrax occur following ingestion of grossly contaminated and undercooked meat. Pharyngeal ulcers and brawny edema of the neck result from oropharyngeal multiplication of anthrax bacilli. Gastrointestinal anthrax occurs after intestinal absorption of anthrax bacilli and transport of the bacteria to the mesenteric lymph nodes, which is followed by the development of hemorrhagic adenitis, ascites, and septicemia.

Overwhelming infection due to *B. anthracis* results in uncontrolled intravascular multiplication of the organism and fatal toxemia characterized by hypotension and hemorrhage. For example, during the 12-hour period preceding death of guinea pigs infected with anthrax bacilli, the number of bacteria in the blood rises from 3 X 10^5/mL to 1 X 10^9/mL. If antibiotics are given after the intravascular bacterial count reaches 10^6/mL, experimental animals die despite a massive reduction in bacterial numbers. Furthermore, sterile blood from these guinea pigs produces a fatal toxemic syndrome when infused into healthy guinea pigs.

Clinical Manifestations

The clinical manifestations of cutaneous anthrax are striking (figure 3). The disease begins as a small, painless, but often pruritic, papule. The papule enlarges, develops vesicles, and within 2 days ulcerates to form an eschar. Gram stain of the vesicular fluid may show rare polymorphonuclear leukocytes and gram-positive rods. Cultures performed before antibiotic therapy is instituted are usually positive. The following clinical characteristics are strongly suggestive of cutaneous anthrax: the presence of edema out of proportion to the size of the lesion; lack of pain during the initial phases of the infection; and the rarity of polymorphonuclear leukocytes on gram stain. More than 90% of the lesions occur in exposed areas such as the face, neck, arms, or hands, and regional lymphadenopathy is common. The diagnosis, if considered, is relatively easy.

On the other hand, the early clinical diagnosis of inhalation anthrax is next to impossible. Initial symptoms mimic an influenza-like infection characterized by myalgias, fever, and malaise. Patients become dramatically sicker within 2-3 days, when severe dyspnea and hypoxemia develop. A key diagnostic finding on the chest roentgenogram is widening of the mediastinum (figure 4) [6]; pleural effusions are also common. Patients soon become hypotensive, and about one-half will also have hemorrhagic meningitis, as confirmed by results of gram staining and culture of the CSF. Inhalation anthrax is virtually always fatal.

Patients with pharyngeal anthrax present with fever, pharyngitis, and neck swelling. Characteristically, one or more eschars are seen on the pharynx. Throat cultures will be positive for *B. anthracis.* Patients with gastrointestinal anthrax present with severe abdominal pain and hemorrhagic ascites that develops rapidly. A gram stain of paracentesis fluid will be positive for gram-positive bacilli. Because both of these infections

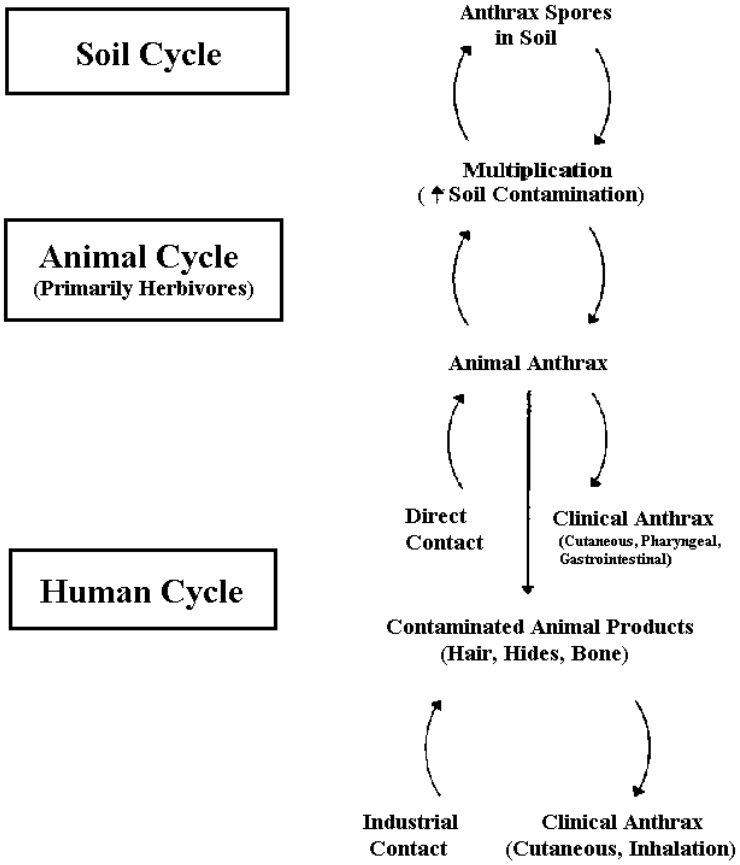

Figure 2. Cycles of *B. anthracis*. Anthrax is part of normal soil flora and under appropriate conditions can multiply. Grazing animals can become infected and further increase the soil contamination as infected secretions with sporulating *B. anthracis* are shed. Animals that are ill or dying of anthrax can infect humans who either slaughter them or consume contaminated meat. Raw materials such as hides, hair, or bones from animals that have died of anthrax are heavily contaminated with anthrax spores. These products can serve as a reservoir for human infection as they are used in a variety of manufacturing processes.

develop following the consumption of tainted meat, they can occur as familial clusters.

Table 1. Immunologic characteristics of virulence determinants of *B. anthracis*.

Factor	Immunologic activity
Capsule(poly-D-glutamate)	Weakly antigenic, nonimmunizing
EF	Antigenic, nonimmunizing
PA	Strongly immunogenic
LF	Antigenic, weakly nonimmunogenic
EF + PA	Strongly immunogenic
EF +LF	Weakly immunogenic
PA + LF	Immunogenic
EF + PA + LF	Immunogenic

NOTE. Adapted with permission from [8]. EF = edema factor; PA = protective antigen; LF = lethal factor.

Treatment and Prevention

Cutaneous anthrax responds well to treatment with penicillin G. In one study [7], 25 patients with cutaneous anthrax and positive initial cultures of blister fluid were given 2 million units of penicillin G intravenously; the blister fluid was cultured every hour, and 5 hours after the initiation of therapy all of the cultures were negative. A rare strain of *B. anthracis* with strong β-lactamase activity has been reported to be resistant to penicillin; most strains are resistant to cefuroxime. Ciprofloxacin, erythromycin, tetracycline, and chloramphenicol are alternative drugs for penicillin-sensitive patients. Even if the patient is promptly treated with antibiotics, cutaneous lesions will continue to progress through the eschar phase. There is no place for surgery in the management of cutaneous anthrax. Case fatality rates as high as 20% have been reported for untreated cases; however, with appropriate antibiotic therapy, fatalities are now unusual. Inhalation anthrax is always diagnosed late in its course, and antibiotic therapy, even when given in heroic doses, is unsuccessful. Gastrointestinal and meningeal anthrax are also serious infections that are associated with high mortality rates.

Prevention of anthrax largely depends on the use of vaccines since widespread decontamination of contaminated soil is impractical. The immunologic characteristics of potential components of an anthrax vaccine are summarized in table 1 [8].

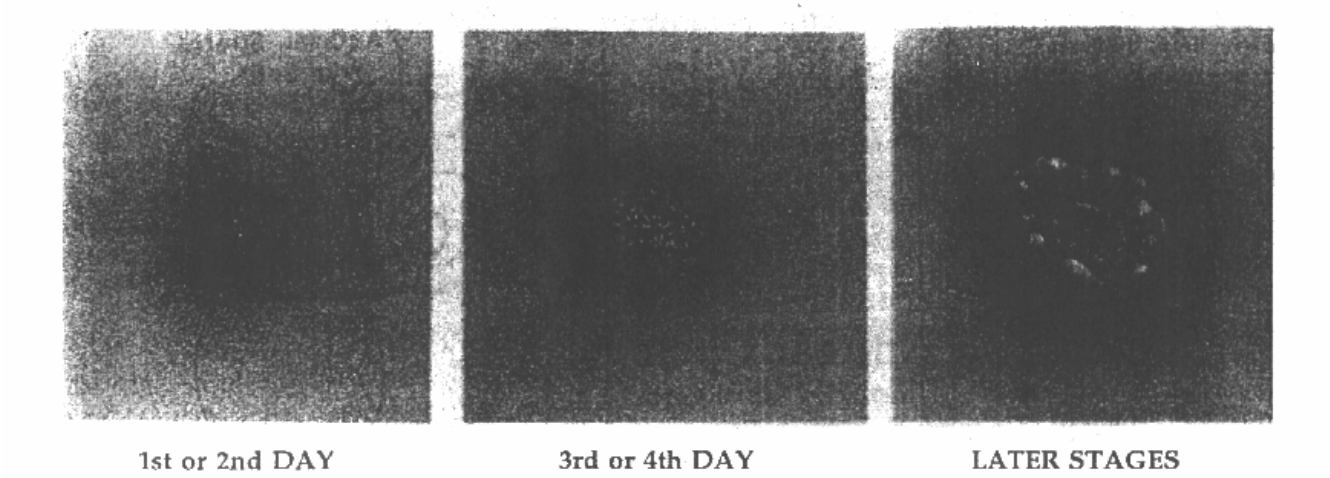

Figure 3. Evolution of cutaneous anthrax. Initial lesion begins as a pruritic papule that undergoes necrosis with the formation of an eschar and cutaneous vesicles.

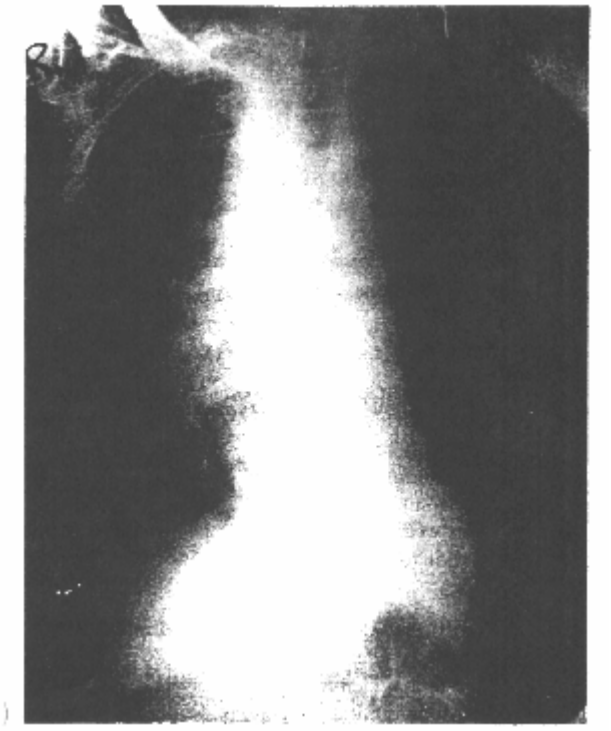

Figure 4. Chest roentgenogram showing a widened mediastinum due to hemorrhagic mediastinitis in a patient with a fatal case of inhalation anthrax. (Reprint with permission from [6]).

Animal studies have clearly shown that PA is the most significant immunogen of the three toxigenic components in protection against challenges with virulent strains of *B. anthracis* [9]. Nontoxigenic encapsulated strains ($pXO1^-$ and $pXO2^+$) and toxigenic nonencapsulated strains ($pXO1^-$ and $pXO2^-$) were not protective in guinea pigs, whereas toxigenic nonencapsulated ($pXO1^+$ and $pXO2^-$) strains conferred solid protection [9].

Sterne vaccine is a live, toxigenic, unencapsulated avirulent animal vaccine that is widely used. The main limitation of the Sterne vaccine is safety. Its use is sometimes associated with tissue necrosis at the site of inoculation, and there have been rare fatalities. Because of safety concerns, these spore vaccines have generally not been used for humans except in Russia, where a live spore vaccine has been developed for human use and is considered to be highly effective. A nonliving human anthrax vaccine, produced by the Michigan Department of Health, has been field tested and has been shown to be highly effective. The vaccine is an aluminum hydroxide-adsorbed cell-free filtrate of cultures of a strain of anthrax that produces PA in the relative absence of LF and EF. The vaccine, despite its proven efficacy, is not considered ideal because it must be given several times to insure protection, and local reactions have been reported. Third-generation anthrax vaccines are being developed and include highly purified PA vaccines and a strain of *Bacillus subtilis* that has been cloned with the PA gene. These vaccines are ready for clinical trial, but it is unclear whether funding will be available to perform these studies.

References

1. Wilson GS, Miles A. Anthrax. In: Topley and Wilson's principles of bacteriology, virology and immunity. 6th ed. Baltimore: Williams & Wilkins, 1975:2079.

2. Metchnikoff E. Concerning the relationship between phagocytes and anthrax bacilli. Rev Infect Dis 1984;6:761-70.
3. Gill DM. Seven toxic peptides that cross cell membranes. In: Jeljaszewica J, Wadstrom T, eds. Bacterial toxins and cell membranes. New York: Academic Press, 1978:291-332.
4. Manchee RJ, Broster MG, Stagg AJ, Hibbs SE, Patience B. Out of Gruinard Island. In: Turnbull PCB, ed. Proceedings on the International Workshop on Anthrax. Salisbury Medical Bulletin 1990;68(special suppl):1-105.
5. Brachman PS. Human anthrax in the United States. Antimicrob Agents Chemother 1965:111-4.
6. LaForce FM, Bumford F, Feeley J, Stokes S, Snow D. Epidemiological study of a fatal case of inhalation anthrax. Arch Environ Health 1969;18:798-805.
7. Ronaghy HA, Azadeh B, Kohout E, Dutz W. Penicillin therapy of human cutaneous anthrax. Curr Ther Res Clin Exp 1972;14:721-5.
8. Hambleton P, Turnbull PCB. Anthrax vaccine development: a continuous story. Adv Biotechnol Processes 1990;13:105-22.
9. Ivins BE, Welkos SL, Knudson GB, Little SF. Immunization against anthrax with aromatic compound-dependent (Aro^-) mutants of *Bacillus anthracis* and with recombinant strains of *Bacillus subtilis* that produce anthrax protective antigen. Infect Immun 1990;58:303-8.

Suggested Additional Reading

Finkelstein A. The channel formed in planar lipid bilayers by the protective antigen component of anthrax toxin. Toxicology 1994;87:29-41.
Gold H. Anthrax: a report of one hundred seventeen cases. Arch Intern Med 1955;96:387-96.
Plotkin SA, Brachman PS, Utell M, Bumford FH, Atchison MM. An epidemic of inhalation anthrax, the first in the twentieth century. Am J Med 1960;29:992-1001.
Turnbull PCB, ed. Proceedings of the international workshop on anthrax. Salisbury Medical Bulletin 1990;68(special suppl):1-105.
Turnbull PCB. Anthrax vaccines: past, present and future. Vaccine 1991;9:533-9.

OFFICE OF CONTINUING MEDICAL EDUCATION
UCLA SCHOOL OF MEDICINE

This test affords you the opportunity to assess your knowledge and understanding of the material presented in the preceding clinical article, "Anthrax," by F. Marc LaForce, and to earn continuing medical education (CME) credit.

The Office of Continuing Medical Education, UCLA School of Medicine, is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education for physicians. The Office of Continuing Medical Education, UCLA School of Medicine, certifies that this continuing medical education activity meets the criteria for 1 credit hour in Category I of the Physician's Recognition Award of the American Medical Association and the California Medical Association Certificate in Continuing Medical Education.

To earn credit, read the State-of-the-Art Clinical Article carefully and answer the following questions. Mark your answer by circling the correct responses on the answer card (usually found toward the front of the issue) and mail after affixing first class postage. To earn credit, a minimum score of 80% must be obtained.

Certificates of CME credit will be awarded on a per volume (biannual) basis. Each answer card must be submitted within 3 months of the date of issue.

This program is made possible by an educational grant from Roche Laboratories.

I. The virulence *of Bacillus anthracis* is dependent upon:
   A. pXOl plasmid
   B. pXO2 plasmid
   C. Both
   D. Neither
2. The protective antigen (PA) of *B. anthracis* has the following characteristics:
   A. Facilitates entry of EF into cytosol
   B. Facilitates entry of LF into cytosol
   C. Both
   D. Neither
3. The edema factor (EF) of ***B.*** *anthracis:*
   A. Is adenyl cyclase
   B. Depends on presence of pXO2 plasmid
   C. Both
   D. Neither
4. Antibiotics that are effective against *B. anthracis* include all of the following *except:*
   A. Chloramphenicol
   B. Erythromycin
   C. Cefuroxime
   D. Ciprofloxacin
   E. Ampicillin

5. Inhalation anthrax has been epidemiologically linked with:
   A. Imported bone meal used as a fertilizer
   B. Imported goat hair from Turkey
   C. Both
   D. Neither

6. The clinical characteristics of cutaneous anthrax include the following *except:*
   A. Case fatality rate of <l%
   B. Initial painful pruritic lesion
   C. Regional adenopathy
   D. Brawny extensive edema
   E. Evolution to an eschar despite administration of antibiotics

7. Characteristic radiological findings in cases of inhalation anthrax include:
   A. Mediastinal widening
   B. Lower lobe infiltrates
   C. Both
   D. Neither

8. The incidence of anthrax in the United States has decreased dramatically for all of the following reasons *except:*
   A. Availability of a human anthrax vaccine
   B. Disappearance of *B. anthracis* from the soil
   C. Improved industrial hygiene
   D. Availability of the Sterne vaccine for herbivores
   E. A decrease in the importation of contaminated raw material

9. Antibiotics have markedly improved the outcome of:
   A. Cutaneous anthrax
   B. Inhalation anthrax
   C. Both
   D. Neither

10. The cytological characteristics of vesicular fluid from patients with cutaneous anthrax include:
   A. Presence of gram-positive spores
   B. Presence of polymorphonuclear leukocytes
   C. Both
   D. Neither