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ARMY VETERINARY RESEARCH AS ILLUSTRATED BY STUDIES ON PATHOGENESIS OF ANTHRAX

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UNITED STATES ARMY BIOLOGICAL LABORATORIES FORT DETRICK

U.S. ARMY CHEMICAL-BIOLOGICAL-RADIOLOGICAL AGENCY
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Pathogenesis of Bacillus anthracis.
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ANIMAL RESEARCH

In conducting the research reported herein, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society of Medical Research.

ABSTRACT

Recent advances in the study and understanding of anthrax are depicted in a movie that involves a co-operative effort between veterinarians and biologists stationed at Fort Detrick.

The experimental work indicates that the spore is moved into the lungs by macrophages followed by germination, outgrowth, and production of toxin.

Evidence is presented that death of the host is due to a toxemia. Application to treatment and/or protection for the disease is also discussed.

My assignment is concerned with studies presently in progress on the pathogenesis of anthrax. These studies are presented as an illustration of the effort of a research team of which the Army Veterinarian is an integral member.

Anthrax has been known from antiquity. It was one of the seven plagues of Egypt. In the middle of the 19th century, anthrax also had the distinction of revealing for the first time the specific relationship of a microbe to an infectious disease.

Yet, when one looks at the research and knowledge of anthrax before 1955, we find little dependable knowledge as to the cause of death, effective therapy, and effective immunological procedures. Some progress has been made in these areas since 1955, and our broad study of the pathogenesis of anthrax was undertaken to understand the disease fully enough to allow us to develop treatment that will control anthrax late in the septicemic phase of this disease.

The film* we will view depicts a technique that was developed in order to answer the question of how an anthrax infection becomes established. Capt. Dean Hodges recently developed this technique at Fort Detrick while on active duty in the Army Veterinary Corps.

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* "Pathogenesis of Anthrax - by Cannulation of the Lymphatic System." Library No. RH-103. Tech. Releases, Tech. Information Division, Fort Detrick.

We see that anthrax is one of the few diseases in which the spore must be phagocytized in order for the infection to become established from the lung. The phagocyte picks up the spore as it would any particle and transports it to a lymph node where germination of the spore takes place. We have tested the lymph and blood repeatedly but have never encountered the spore, only the vegetative cell, thus supporting the observations of Ross.

Once infection of the lymph node is established, then the lymph becomes a source of bacilli continuously spilling organisms over into the blood stream. The lymph system serves as the primary route to the blood stream regardless of the site of infection (ID, IP, or aerosol). Now one has the right to ask how can you explain the fact that bacteremia occurs when both lymphatic ducts are cannulated and the lymph drained off. This question was answered by Malek et al. They demonstrated in sheep the development of secondary pathological lympho-venous communications in the popliteal node draining a site of cutaneous anthrax. They further demonstrated that the pathological communications through which organisms pass directly into the blood developed during the inflammatory process of the node.

In reviewing the literature on anthrax one finds the symptomatology broken down to three forms: peracute, acute, and localized.

1. The peracute, apoplectic, or fulminant forms are seen in cattle, sheep, and goats. These animals present a picture of cerebral apoplexy, and die, frequently without showing any previous evidence of illness.

2. The acute and subacute forms are most common in all speciessother than swine. The classical symptoms of this type are temperature, excitement, depression, stupor, spasms, respiratory or cardiac distress, convulsions, and bloody discharges, symptoms that are known to each of us.

3. A cutaneous or localized form occurs and is characterized by swelling and carbuncles in various parts of the body. In swine, anthrax usually is localized in the cervical lymph nodes where it results in swelling and hemorrhage. This form may go into the acute type or regress and recovery take place. In swine, death may occur from suffocation.

We believe a clear distinction must be made between resistance to establishment of the disease and to susceptibility to the toxin produced by the further development of the establishing organisms. Generalized anthrax is characterized by two stages, septicemia and toxemia. Each species of animals appears to have a characteristic rate of development' and final level of septicemia. We have observed the following constants.

Animal	Doubling Time, min	Terminal Concentrate, org/ml
Rat (NIH)	115	10^6
Rat (Fischer)	115	$10^{3.8}$
Mouse	45	$10^{6.9}$
Guinea pig	53	$10^{8.5}$
Monkey	65 (approx)	$10^{6.5}$ (approx)

The doubling rate of the septicemia stage in guinea pigs is not affected by any treatment we have tried. Treatments include immunization, egg-yolk treatment of spores (a treatment that increases virulence), virulence of strain, size of inoculum, and route of challenge. Only two treatments significantly change the terminal level of guinea pigs, immunization and strain of Bacillus anthracis. In the rat species different inbred strains have different terminal levels.

We conclude from several lines of evidence that the terminal level of organisms at death is directly related to that animal's sensitivity to toxin. This difference is shown by two strains of rats. The Fischer rat dies with a terminal concentration of 6310 organisms per milliliter of blood; the NIH black rat has 1,000,000 organisms per milliliter at death. The minimum amount of toxin required to kill a 250-gram animal is 21 units for the Fischer rat and 822 units for NIH rat. Thus, we see that the NIH rat has 160 times more organisms per milliliter of blood at death and is also 40 times more resistant to the toxin than is the Fischer rat. Thus it appears that, in hosts resistant to toxin, more organisms must be produced to yield a lethal quantity of toxin for that host than is needed for animals susceptible to toxin. Sterile anthrax toxin produced in vitro is in itself lethal for all species tested. Although there is individual and species variability, sterile, in vitro toxin and the bacterial disease both demonstrate an edema, cholinesterase inhibition, and death. The data presented in the film show also that degree of toxicity of in vivo toxin is proportional to the concentration of organisms in the body fluids. Specifically, the lymph of both monkeys showed parallel increase of both organisms and toxicity for rats.

In monkeys infected with anthrax it was demonstrated that cholinesterase activity was decreased about 24 to 48 hours after challenge with organisms. This was approximately 22 hours before death of the monkeys. Further tests with rabbits, using in vitro toxin, clearly demonstrated a downward trend in cholinesterase activity prior to death. Yet when atropine and 2-PAMCl (an oxime) were used to counteract toxin by protecting the animal against cholinesterase depletion, they were ineffective. We feel that the decrease in cholinesterase activity is due to interference with protein metabolism and, in itself, is not responsible for death.

Eckert, at the University of Cincinnati, has shown that in vitro toxin decreases the oxygen content of blood from 16 per cent (by volume) to less than two per cent at death, with an increase in carbon dioxide from 45 per cent (by volume) to 60 per cent in Fischer rats. Swedish workers have shown a similar phenomenon with rabbits infected with anthrax. There are outward symptoms of central nervous system involvement in some species receiving toxin produced in vitro. A spastic paralysis of the fore limbs in both the monkey and rabbit has been noted. The monkey shows this several hours before death, leading to a flaccid-type paralysis or extreme muscular weakness due to hypoxia. In the rabbit the spastic paralysis is evident just a few minutes before death. Hyperactivity is evident in most animals dying from toxin, as well as from the disease. In fact many animals show clinical improvement before death, with death occurring rapidly.

Both the sudden death syndrome and symptoms displayed by animals dying from either the disease or the toxin lead us to conclude that death probably is due to destruction of an area of the hypothalamus leading to a failure of the adrenal gland. This syndrome is also seen in other overwhelming bacterial infections, such as meningococcemia.

Immunization today uses two antigens, protective antigen, one of the three components of toxin, or spores of lowly virulent strains. Immunization increases the spore dose required to establish disease, extends the time to death, and lowers the terminal number of bacilli per milliliter of blood at death. Immunization may be largely overcome by parenteral infection of germinated spores, vegetative cells, or spores treated with egg yolk. This effect may result in a 10,000-times change in dose and is greatest with avirulent strains and resistant hosts. Septicemic growth in both immune and nonimmune hosts is at the same apparent generation rate of bacilli in vivo; however, death occurs with a lower terminal level in immune than in nonimmune hosts. These observations suggest that during the septicemia stage, host resistance factors are similar. These observations also suggest the following possibilities: (a) immunization is not toxin-neutralizing; (b) during the presepticemic stage, relatively greater amounts of toxin are produced and/or released in situ in immunized hosts than in nonimmunized hosts; and (c) increasing the host's immunity also increases the host's sensitivity to toxin. It appears probable from present data that our immunological procedures should be changed to include a toxoid composed of all three components of toxin.

On the basis of the knowledge that toxin affects the course of the disease and is responsible for the death of the host in anthrax, one can then envision the following therapy.

(a) Use of a bactericidal rather than a bacteriostatic antibiotic. Penicillin, streptomycin, and erythromycin or any combination of them should suffice for this phase.

(b) Use of an antitoxin antiserum to neutralize uncombined toxin. Use of an antitoxin is mandatory with systemic anthrax, but at this time we know of no domestic source of specific antiserum.

(c) Use of shock-combating agents, which would include fluids and/or blood, as well as drugs. Cortisone likely should be used as is indicated by the sudden-death syndrome.

(d) When feasible, oxygen should be employed in the treatment of humans and valuable animals.

The foregoing statements on therapy are based on incomplete experimentation. Specific treatment is now being developed experimentally and tested in our laboratories.

In summary, we have gone over the following points:

(a) Phagocytosis is necessary for infection.

(b) The lymph system carries the organisms to the blood stream.

(c) Present immunization primarily affects establishment of anthrax infection, not its course.

(d) Each species has its own terminal level at death.

(e) Toxin sensitivity and terminal levels are directly related.

(f) Toxin is present in monkeys dying of anthrax and increases during the course of disease.

(g) Cholinesterase is inhibited but not responsible for death.

(h) Central nervous system involvement is observed.

(i) Death is probably due to CNS-adrenal failure.

(j) A toxoid should be produced and included in our immunological procedures.

(k) Based on present knowledge, treatment should involve:

1. Bactericidal antibiotics

2. Antitoxin antiserum

3. Shock-combating agents and fluids

4. Oxygen ventilation

The Veterinary Corps is engaged in many and varied areas of the Army's research and development program. This presentation is only one small example of that work. A significant number of our personnel, roughly 25 per cent, are engaged in research as primary investigators or are used professionally in support of research programs.

The fields of investigation are as broad as the biomedical field itself, with areas of interest to any veterinarian. Histopathology, laboratory animal medicine, and radiobiology claim a large share of the assignments. There are also assignments in physiology, experimental surgery, bacteriology, and virology. At the present time, the Corps is sending officers to universities and army schools for advanced training and degrees in all of these fields. Any veterinarian can find challenges to his ability and professional knowledge in the Army Veterinary Corps today.