PATHOGENESIS OF INHALATION ANTHRAX

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In the late 19th century pulmonary anthrax, as it was then called, was prominent in England and in Germany as an occupational hazard of woolsorters or ragpickers. Excellent clinical histories and gross pathological observations are included in the writings of Spear (13), Greenfield (9), and Eppinger (6). Two theories were propounded rather early to account for the pathogenesis of this disease. The first of these, strongly favored by Eppinger, suggested that the inhaled spores were phagocytosed and transported from the alveoli to other sites where germination occurred with the subsequent development of a septicemia. The other theory, which was espoused as late as 1924 by Fraenkel (7), held that the primary lesion was an erosion of the tracheobronchial tree usually occurring near the point of bifurcation. The erosion was thought to have the characteristics of the localized skin lesions seen in man, namely, a neutrophilic cellular response, massive edema, hemorrhage, and central ulceration. It was thought that a pneumonia due to anthrax bacilli developed secondarily to this initial point of entry. Septicemia was then believed to occur late and perhaps incidentally.

In recent years pulmonary or inhalation anthrax has become relatively rare due to improved hygienic measures in hair-processing plants. Although contaminated goat hair is known to be imported and processed in this country, most of the manifestations of *Bacillus anthracis* have been of the cutaneous variety and have occasioned numerous reports in the literature, most prominent of which is that of Gold (8). It therefore aroused considerable interest when in the fall of 1957 an outbreak of anthrax of the inhalation type occurred in millworkers in Manchester, N. H. This outbreak over a 10-week period resulted in the death of four individuals; three autopsies were performed and the cause of death was proved to be disseminated anthrax. The findings in these cases, coupled with three other cases occurring recently but with little or no proximity to an industrial source of the disease, have provided an opportunity to compare them to experimental results in animals with a view to resolving the apparently divergent views of pathogenesis.

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Studies in Experimental Animals

Buchner (4), in 1888 infected rabbits, guinea pigs, and mice with anthrax by exposing them to clouds of spores. He killed animals at different intervals after exposure and cut sections of the lungs in an attempt to find the site at which germination of the spores took place. He was unable to find vegetative forms in the walls of the alveoli except in one alveolus of one case. Young, Zelle, and Lincoln (15) exposed monkeys, guinea pigs, mice, dogs, and rats to clouds of anthrax spores generated by nebulizers in a modified autoclave through which the cloud moved continuously. Their observations indicated that involvement of the pulmonary tissues was secondary to the effect of the systemic disease which followed inhalation of anthrax spores. They were unable to determine the original site of invasion of the spores and the method by which they were transported but indicated that the true invasion of the host occurred through the lymphatic system. Barnes (3) repeated in part the experiments of Young et al. (15) in rabbits, guinea pigs, and mice and reached a similar conclusion. At examination postmortem he described a varying amount of edema of the mediastinum but could find no specific pulmonary lesions either grossly or microscopically. Druett, Henderson, Packman, and Peacock (5) exposed guinea pigs and monkeys to clouds of homogeneous particles containing anthrax spores. Infection of the aninals occurred most effectively following inhalation of clouds containing single spores (approximately 1 μ in diameter). Particles of a diameter up to 5 μ were only slightly less effective, but with particles larger than this critical figure the infectivity fell off remarkably rapidly. In the case of guinea pigs, clouds of particles of single spores were about 17 times as infective as clouds of 12-μ

particles and for the monkey, the ratio was 14:1. Druett and colleagues, on the basis of their experiments, were reluctant to conclude at what site invasion of the body took place. Ross (11) instilled liquid suspensions of anthrax spores intratracheally in guinea pigs and examined the tissues from these animals at intervals beginning immediately thereafter and extending for a period of 25½ hr. By virtue of a heavy inoculum of spores and a differential staining technique, she was able to follow the progress of the spores. Her observations indicate that spores were phagocytosed usually within a matter of minutes by macrophages present in the alveoli and transported into the draining lymphatics. In the sinusoids of the lymph nodes, proliferation of the spores occurred with the formation of vegetative forms, some of which were found within the phagocytes themselves. When the vegetative forms escaped from the phagocytic cell, however, multiplication occurred very rapidly and bacilli in chains were frequently seen 19 hr after injection although some resting spores were still found in phagocytes in the lung alveoli. Following the multiplication of the bacillary form, invasion of the efferent lymphatic vessels occurred and dissemination followed. Polymorphonuclear leukocytes present are stated by Ross to take no part in the reaction of the tissue to the invading bacilli. These findings by Ross tend to confirm the views of Barnes and others that no true respiratory infection is produced, at least under laboratory conditions.

Albrink and Goodlow (1) have reported the experiments performed at Fort Detrick with chimpanzees. These animals were exposed by a face mask to clouds containing anthrax spores in small particle sizes. The animals were carefully observed with essentially daily blood cultures, physical examinations, and temperatures. In the first group, two animals were exposed to a dose of 35,000 spores in serial fashion within ½ hr of each other. One animal exhibited a positive blood culture on the second day which was maintained during the subsequent 2 days and then sporadically until the 11th day, at which time and subsequently no organisms could be demonstrated in the blood. The second animal developed a positive blood culture on the third postexposure day which was maintained sporadically through the 11th day, after which no organisms could be demonstrated. During this period, the animals appeared completely healthy and did not exhibit any deviation from what was considered to be their normal temperature.

A second group of two animals was exposed to doses of 40,000 and 65,000 spores. The first animal developed a bacteremia on the second postexposure day and continued to increase the number of organisms in a sample of blood until the sixth postexposure day, when he died. The second animal developed a positive blood culture on the first postexposure day which continued to increase until he too died on the sixth postexposure day. These animals appeared normal until approximately 4 hr before death in one animal and 7 hr before death in the other. The second animal was examined 2 hr before death and was found to have a subnormal temperature, to be very lethargic, and hard to arouse. He appeared cyanotic with a rapid and weak pulse; his respirations were shallow and labored and he had dullness to percussion of his chest.

The original two animals were again exposed to doses of approximately 100,000 spores 6 weeks after their blood cultures had become negative following the first exposure. Positive cultures on both animals were found on the fourth and fifth day postexposure. In one animal, further blood cultures were negative and he maintained an excellent state of health. The second animal had increasing numbers of bacteria in his blood and died on the eighth postexposure day.

Careful examination of the three animals that died revealed no breaks or ulcerations of the mucosa of the respiratory tract. The mucous membranes of the trachea and bronchi appeared markedly edematous in all three animals and hemorrhagic in the first two. The pulmonary parenchyma showed signs only of edema and hemorrhage and no pneumonia was seen either grossly or microscopically. An acute hemorrhagic mediastinitis characterized by gelatinous edema of the adipose tissues was marked in these three animals. The architecture of tracheobronchial lymph nodes and others in the mediastinum was obliterated by hemorrhage. There was a polymorphonuclear leukocytic exudate and extensive hemorrhage which extended beyond the capsule of the lymph nodes to involve the adjacent fibroadipose tissue. Histological examination of the tracheal and bronchial walls disclosed a varying pattern of involvement of the lamina propria which in some regions exhibited intense edema,

in others edema and hemorrhage, and in still others an early neutrophilic accumulation associated with the edema. Infiltration of the mucosa itself was seen in a few foci but the epithelium was intact throughout. Sections of all tissues revealed variable numbers of anthrax bacilli in the blood vessels supplying these organs. No evidence of a hemorrhagic meningitis was present. The spleens in all animals were large, congested, and soft. Although gelatinous edema of the perirenal fibro-adipose tissue was observed in two of the three animals, the kidneys showed no signs of the infective process other than the bacteria seen in blood vessels on microscopic sections. Of especial significance is the absence in these animals of any signs of acute tubular necrosis (lower nephron nephrosis) which was described by Ross (10) in guinea pigs dying from the effects of bacteremia due to *Bacillus anthracis*.

Description of Recent Human Cases

Manchester epidemic. The pathological findings in the three fatal cases that were autopsied during the Manchester, N. H., epidemic have been documented in detail by Albrink, Brooks, Biron, and Kopel (2). Two of the three cases showed a massive hemorrhagic mediastinitis which was comparable to that described in the chimpanzees. One of these and the third case exhibited hemorrhagic meningitis. In all cases the mucous membranes of the respiratory tract were completely devoid of erosion or ulceration save for one minor bronchus which appeared to have a microscopic pustule associated with cocci rather than with anthrax bacilli. Changes in lymph nodes, mediastinal tissues, and pulmonary parenchyma were comparable to those from the chimpanzees.

Other human cases. Brachman, Pagano, and Albrink (3*a*) have recently studied two fatal cases of inhalation anthrax occurring in Philadelphia and characterized by rather remote histories of exposure. One of these cases resembles those previously described in the pathological findings. The other case is of particular interest because it occurred in an individual with Boeck's sarcoid of 2½ years' duration. This 28-year-old Negro man died after a very short acute illness characterized by profound dyspnea, hemoptysis, and chest pain. At autopsy he was found to have diffuse involvement with sarcoidosis of heart, lungs, spleen, liver, lymph nodes, and kidneys. No ulcerations were found in the mucous membranes of the trachea or bronchi. The lungs were markedly heavy with a diffuse nodularity of all lobes and what appeared to be a consolidation of the right hilum. On microscopic examination the lungs were extensively involved with chronic granulomata composed of numerous multinucleated giant cells, large epithelioid cells, and occasional Schauman bodies. No necrosis was evident in the granulomata. In sections from the region of the right hilum, there was an acute necrotizing pneumonia. In some foci, numerous anthrax bacilli were scattered about the region of the acute pneumonitis. It is of interest that the pneumonic process was essentially devoid of any hemorrhagic elements. The lung elsewhere showed considerable edema. The architecture of the lymph nodes of hilar and mediastinal regions was almost completely obliterated by dense collagenous scars and accumulations of mononuclear cells. Multinucleated giant cells were present but in much smaller numbers than in the sections of the lung. In the sinusoids there were collections of polymorphonuclear leukocytes and short chains of gram-positive bacilli resembling anthrax bacilli. These organisms were also seen in blood vessels of other organs.

A further case of comparable interest is that of a 53-year-old white man who had worked as an electrician with only casual contact of anthrax organisms but who developed the inhalation form of the disease and died within approximately 4½ days after first onset of symptoms (W. D. Tigertt, *personal communication*). At autopsy he manifested an extensive hemorrhagic mediastinitis with massive destruction of tracheobronchial lymph nodes. No ulceration or erosion of the tracheobronchial tree could be discovered. On microscopic examination, it was found that the lamina propria of trachea and bronchi was hemorrhagic in some foci, edematous in others, and infiltrated by neutrophiles in still other locations. A small nodule was found in the right middle lobe which grossly appeared hemorrhagic and on microscopic examination showed the presence of necrosis and an acute purulent pneumonitis. Very few organisms were found and none grew on culture, except from one tracheobronchial lymph node where they were also demonstrable microscopically. This result apparently was due to the treatment which the patient had received in his period of hospitalization. The small hemorrhagic nodule in the right

middle lobe is of particular significance because in one series of tests a trace of beryllium was found in this region; in retrospect, chest X-ray films indicated that there had been a slight density present here prior to the time of the fatal illness. Of further significance was the finding of an acute esophagitis in close association with involved lymph nodes of the mediastinum.

Comparison of Disease in Humans and Experimental Animals

From the foregoing summary of the pathological changes encountered in experimental animals, with particular emphasis on those observed in the chimpanzee, and in the cases of the naturally occurring disease in humans, it seems readily apparent that qualitatively, at least, the morphological expressions of this disease are comparable. There was in most cases a hemorrhagic mediastinitis associated with the massive destruction of tracheobronchial lymph nodes. Careful examination of the mucous membranes of the tracheobronchial tree have been carried out in the chimpanzees and in most of the human cases and have disclosed no ulcer, signs of erosion, or grossly observable pustule comparable to that described by some of the earlier workers and believed by some to be the primary lesion of inhalation anthrax. Rather, changes can be demonstrated in the lamina propria of the tracheobronchial mucosa of both animals and humans; these are characterized by moderate edema, mild hemorrhage, and, in some foci, accumulations of polymorphonuclear leukocytes. This reaction is similar to that found in the adjacent, massively involved mediastinum but is less extensive and seems to be an earlier stage in the disease process. Therefore, it is highly probable that the large mucosal lesions described by Fraenkel (7) occurred after involvement of mediastinal tissues and were a result of that involvement rather than a precursor. The fact that the only significant pneumonia demonstrable occurred in two humans with altered pulmonary tissue indicates clearly that anthrax pneumonia per se does not exist in the previously normal individual and suggests that it develops only when the normal mechanisms of clearance have undergone marked functional alteration by pre-existing pathology. All of the observations recorded here fit in with the statements made by Ross (11) regarding the phagocytosis of spores in the alveoli and their transport to draining lymph nodes and subsequent germination. The principal lesions of inhalation anthrax then are an acute hemorrhagic mediastinitis, a secondary septicemia with anthrax bacilli, and the widespread toxic effects associated with that septicemia.

It appears from the two cases of the men who developed pneumonitis that the morphological expression of the disease may be modified by involvement of the lymphatic system. Thus, the patient who had the extensive sarcoidosis in the lungs and lymph nodes presented a substantial pneumonia which may have been caused by the anthrax bacilli. The rather small focus of pneumonia present in the right middle lobe of the other individual, if it were due to the anthrax bacilli, might have developed because the normal pathway of exit of the phagocytosed spores was blocked or distorted due to a pre-existing lesion. This avenue of approach could be pursued experimentally by study of the disease in animals with established unilateral pneumoconiosis.

Cause of Death in Inhalation Anthrax

For many years it was considered that death from anthrax resulted from the thrombosis of many small blood vessels due principally to massive numbers of anthrax bacilli. Studies of a wide variety of animals which have had experimentally induced or naturally occurring disease do not lend credence to this view, however, and the mechanism of death must be sought elsewhere.

In animals and humans with inhalation anthrax which have developed a hemorrhagic mediastinitis, it is obvious that there would be a cause of both respiratory and cardiac embarrassment by hemorrhage into the already involved mediastinal lymph nodes. This could lead to the development of a vicious circle with increased anoxia leading to increased permeability, thus greater swelling, thus more involvement of venae cavae due to increased pressure of surrounding mediastinal structures. Involvement of autonomic nerves should also be borne in mind, for there could be malfunction due either to the increase in pressure or to local chemical effects of necrotic tissue. Therefore, the possibility cannot be denied that in some cases the mediastinitis may be the precipitating cause of death. The fact remains, however, that animals and some humans die of the effects of disseminated anthrax without

the development of a mediastinitis. This is particularly true in subjects who received their exposure by an extrapulmonary route.

That the anthrax bacillus is capable of elaborating a toxin has been well established. This was originally shown in guinea pigs by Smith, Keppe, and Stanley (12). Since that time, the isolation and partial purification of the toxin has been accomplished by Thorne, Molnar, and Strange (14). Unfortunately, insufficient experimentation on animals has been performed to permit other than speculation as to the site of action of this substance and whether it functions during the course of naturally occurring disease in all species of animals. Nevertheless, the morphological observations which have been made by a variety of observers in a large number of animal species indicate that the alterations seen can best be explained on the basis of a toxic action. The target cells appear to be those of the reticulo-endothelial system, although it must be emphasized that this may simply be a matter of the time of exposure of these cells rather than a selective action. If one inspects lymph nodes which appear grossly normal but are in the vicinity of other hemorrhagic nodes, one can find some loss of cells lining the sinusoids. When hemorrhagic nodes are examined, the cells which remain are large, somewhat rounded, and have nuclei with rather densely basophilic staining properties. Varying degrees of mitotic activity may be observed in some of these hemorrhagic nodes. It appears, therefore, as if there were a direct toxic action on the cells of the reticulo-endothelial system of the lymph nodes by the anthrax bacilli, or by a product which they have elaborated. This then results in necrosis of the lymph node and involves the surrounding tissue as well. Perhaps, because of the necrosis in the lymph node, there is elicited a marked polymorphonuclear leukocytic response so that in some foci, either in the lymph nodes or in the immediately adjacent tissues, there occurs a purulent cellular response. The manifestations of edema and hemorrhage, seen elsewhere as in the lung, are highly suggestive of the effect of an increased permeability of vascular endothelium. This increased permeability may be found in the absence of a mediastinitis, as in animals receiving an intracutaneous injection of spores, and therefore must be ascribed to the effect of some substance rather than a direct mechanical effect. It is unlikely that this could be due simply to the breakdown products of the destroyed lymph node, although this must remain a possibility. The fact that death may occur in animals and man after sterilization of the blood and after toxin can no longer be found in the blood stream suggests that there may be an irreversible linkage with the toxin and the cells of the reticulo-endothelial system.

The possibility that there might be a direct toxic effect on cardiac muscle or on cells of the central nervous system has not been excluded, although no evidence for this has as yet been suggested in any of the studies either on experimental animals or of naturally occurring disease in humans. It is perhaps significant that in none of the chimpanzees or in the human cases that have been cited here has there been any evidence of the lower nephron nephrosis described by the British workers in guinea pigs. The reason for this difference is not clear.

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