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On the recognition and therapy of Simian woolsorter's disease*

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In the Brown Lectures of 1880 and 1881 Prof. W. S. Greenfield (1880, 1881), reported the anthrax bacillus to be the aetiological agent of woolsorter's disease and presented what remains today the classical description of the clinical manifestations and morbid anatomy of respiratory anthrax in man. Though at present infrequent, woolsorter's disease still, in the era of antibiotics, is associated with a high mortality rate. Experimental evaluation of therapeutic approaches to woolsorter's disease, as distinguished from cutaneous anthrax, has been limited since the mediastinal cellulitis and intrathoracic lymphadenitis characteristic of the human disease have not been observed in experimental respiratory anthrax of lower animals. For example, guinea-pigs, sheep and rhesus monkeys develop a septicaemia, and such lesions as are found at post-mortem examination are secondary to this septicaemia. Recent observations (Gochenour, Gleiser & Tigertt, 1962) of the clinical course and morbid anatomy in rhesus monkeys receiving early, inadequate penicillin prophylaxis following inhalation of spores of *Bacillus anthracis* indicated that some animals so modified had a febrile course and exhibited extensive intrathoracic lymph node and mediastinal involvement. Such animals closely simulate the pattern of woolsorter's disease as originally described so clearly by Greenfield and confirmed by others. These animals have served as a model for examination of therapy with penicillin and with tetracycline.

MATERIALS AND METHODS

The anthrax spore suspension and respiratory exposure were as previously described by Gochenour and his associates. Thirty-eight young adult *Macaca mulatta*, weighing from 2·3 to 3·1 kg., were exposed to inhaled doses of spores ranging from 7000 to 1,016,000 with a mean of 621,000. Beginning 24 hr. after respiratory exposure all received 150,000 units of procaine penicillin G intramuscularly once daily for 5 days. Conventional (65 kV, 30 MA.) and supervoltage (1 MeV., 3 MA.) chest roentgenograms were obtained on all animals before respiratory exposure and at 12 hr. intervals after discontinuance of penicillin. In

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addition, rectal temperatures and clinical evaluation were accomplished at 6 or 8 hr. intervals. In some animals blood cultures were obtained at the time the second course of treatment was to begin.

In these studies limited animal-holding facilities and the extent of observations upon individual animals necessitated four separate exposures and study periods. Since presented doses of spores and test subjects and their responses appeared comparable they have been considered together.

Upon clear evidence of chest involvement therapy was instituted (exclusive of controls) either with penicillin (300,000 units procaine penicillin G intramuscularly at 8 or at 12 hr. intervals for 7 days) or tetracycline (100 mg. intramuscularly every 6 hr. for 5 days then every 12 hr. for an additional 5 days; two monkeys received 100 mg. intramuscularly and 125 mg. intragastrically followed by 125 mg. intragastrically every 8 hr. for 7 days). Aside from considerable myositis due to intramuscular tetracycline, the factor causing the change to intragastric administration, therapy *per se* was uncomplicated.

Complete post-mortem examinations were performed on all animals. Those surviving the acute phase of the study were killed between 55 and 84 days following exposure.

RESULTS

Early penicillin prophylaxis. As previously reported (Gochenour *et al.* 1962) the prophylactic regimen of penicillin employed was inadequate. Of the thirty-eight monkeys one remained well throughout and four experienced transient fever following cessation of the initial course of penicillin but recovered spontaneously. One monkey died from non-specific pneumonitis 3 days after cessation of penicillin. One animal died of anthrax septicaemia 48 hr. after exposure; this animal had received but a single dose of penicillin. Five other monkeys died 3–9 days following cessation of penicillin without having developed abnormal chest X-rays; death was due to anthrax septicaemia, meningitis, or both. These animals did not exhibit significant mediastinal or pulmonary involvement at autopsy, thus confirming clinical and radiographic impressions. One monkey died of anthrax involvement of the mediastinum 19 days after the initial course of penicillin; the last chest roentgenogram was obtained 4 days earlier and was normal.

Radiographic evidence of involvement of pulmonary and mediastinal structures was present in 25 (66 %) monkeys. The characteristic lesion of primary interest, mediastinitis, was manifested by a smooth, diffuse widening of the superior mediastinum (Pl. 1). Such a finding, with or without pulmonary lesions, was detected in nineteen animals. In six monkeys the only radiographic lesions were localized pneumonic infiltrates in the lower lobes (Pl. 2). Histopathologically these latter lesions correspond to the superimposition of anthrax bronchiolitis upon pre-existing lesions caused by the lung mite, *Pneumonyssus simicola* (Berdjis, Gleiser, Hartman, Kuehne & Gochenour, 1962). Since the process in such monkeys differs from that of woolsorter's disease, they are considered separately.

Mediastinitis was detected by X-ray as early as 3 and as late as 14 days (mean = 6 days) following cessation of the 'prophylactic' course of penicillin. Mediastinal

changes were first detected upon supervoltage films in fourteen (74 %) of the nineteen animals which developed mediastinitis; in no case was the conventional film positive before the 1 MeV. film. The usefulness of the supervoltage technique was further emphasized in that the conventional films of twelve of these remained equivocal or negative. Of course, therapy (see below) undoubtedly halted the progression of lesions. In the case of monkeys with pulmonary parenchymal lesions the two film techniques appeared equally applicable.

Typically, fever preceded recognizable roentgen changes by a few hours to several days; three of the monkeys with chest lesions remained afebrile throughout and in eight animals chest lesions were detected prior to or coincident with the onset of fever. It is of practical importance that in only half the monkeys were blood cultures positive at the time chest lesions were first detected by X-ray. Otherwise there were no significant manifestations of illness in untreated monkeys until 12–24 hr. before death. At that time the apparently benign course of illness gave way to severe prostration and shock; tachypnea and cyanosis were common. The mean duration of life in untreated animals, after X-rays were positive, was 2 days (range = $\frac{1}{2}$–4$\frac{1}{2}$ days). The seeming diphasic course of this disease in monkeys, i.e. an apparently 'benign' febrile illness which suddenly turns into a fulminant terminal phase, parallels that of woolsorter's disease in man, further validating the experimental model.

At post-mortem examination untreated animals with clinical and radiographic signs of mediastinal involvement exhibited a gelatinous haemorrhagic mediastinitis (Pl. 3). There were, in addition, evidences of anthrax septicaemia. Pulmonary parenchymal lesions were variable accompaniments of mediastinitis. Those monkeys dying with primary pulmonary lesions exhibited the anthrax bronchiolitis previously mentioned in addition to evidences of septicaemia. In no case was significant mediastinitis detected in the absence of prior recognition of mediastinal involvement by chest X-rays.

Early antibiotic therapy. Since chest roentgenograms reveal lesions during the initial 'benign' phase of anthrax mediastinitis in monkeys and in man (Plotkin, Brackman, Utell, Bumford & Atchison, 1960) and such lesions are reasonably distinctive in appearance, they may serve as invaluable aids to early diagnosis. Accordingly X-ray detection of significant chest lesions was taken as the indication for institution of therapy in these studies. Penicillin, distinct from the earlier 5-day course of 'prophylaxis', was administered to monkeys with mediastinitis. Defervescence was prompt, within 48 hr., and chest films returned to normal in 1–7 days (mean = 3$\frac{1}{2}$ days). Two treated monkeys, however, ultimately died of anthrax. One expired with mediastinitis, septicaemia and meningitis 5 days after institution of penicillin therapy; of particular interest was the recovery at autopsy of *penicillin resistant B. anthracis* from this animal's spleen. The organisms recovered produce penicillinase and are resistant to penicillin in concentrations of 100 units per ml. The other death, due to meningitis, occurred 25 days after completion of an apparently successful course of therapy. Whether or not this death could have been prevented by administration of protective antigen at the time of illness is a matter of speculation; available evidence suggests it could

(Henderson, Peacock & Belton, 1956). In all, then, penicillin therapy instituted at the time of X-ray diagnosis of anthrax mediastinitis was successful in eight of ten monkeys. Such results differ from those in untreated animals wherein but 1 of 6 survived ($\chi^2 = 3{\cdot}81$;* $0{\cdot}06 > P > 0{\cdot}05$). The one untreated survivor had slight but definite mediastinal widening on 1 MeV. films over a 3-day period beginning the 12th post-exposure day; this monkey remained afebrile throughout.

Two of the three monkeys with mediastinitis treated with tetracycline survived. Defervescence and return of X-rays to normal appeared similar to that resulting from penicillin. The other died 4 days following initiation of therapy with tetracycline; post-mortem examination revealed haemorrhagic mediastinitis, lymphadenitis and evidence of septicaemia.

Of the six monkeys exhibiting only pulmonary lesions by X-ray, three were treated with tetracycline and survived. The three untreated animals died of pneumonia and septicaemia. X-rays of treated animals cleared in 2–7 days.

It is noteworthy that cultures of lung taken from *all* survivors of the study when they were killed, 55–84 days after exposure, were positive for *B. anthracis*. In none of these, however, was penicillin resistance detected. This evidence of prolonged spore retention, as well as that of others (Henderson *et al.* 1956), together with the late death of a 'successfully' treated monkey emphasizes the importance of an adequate antigenic experience in protection from inhalation anthrax as enunciated by Henderson and his co-workers, and by Gochenour *et al.* (1962).

DISCUSSION

Animal studies (Keppie, Smith & Harris-Smith, 1955) clearly indicate that to be successful antibiotic therapy of anthrax infections must be instituted during the initial phases of illness. Recent case reports of inhalation anthrax (Plotkin *et al.* 1960) suggest this to be true for man as well. Later in the illness antibiotic treatment, even though effective in reducing bacterial numbers, does not prevent death. The relatively non-specific initial symptoms of human inhalation anthrax renders early, and for that matter *ante mortem*, diagnosis difficult. As a result most patients have remained untreated until late in the illness; consequently death has been the rule.

The experimental model of woolsorter's disease in rhesus monkeys utilized here suggests that antibiotic therapy with either penicillin or tetracycline, begun at a time when distinct chest roentgen changes can be recognized, is efficacious. Such roentgen evidence appeared early in the course of illness and preceded the late fulminant course by 12–90 hr. Extrapolation from an animal model to human disease will always be limited to the extent to which the model parallels the disease in man. The model utilized herein simulated human inhalation anthrax to a degree unknown in other systems. Hence, the observation that chest roentogenographic changes permit recognition of the illness at a time when antibiotic therapy is effective seems particularly pertinent. Individuals potentially exposed to *B. anthracis* who experience respiratory symptoms deserve early and repeated, care-

* χ^2 test with correction for continuity.

fully obtained chest X-rays. An alertness to the possibility of woolsorter's disease and recognition of distinctive radiographic changes leading immediately to vigorous antibiotic treatment may make possible the control of this otherwise fatal illness.

Numbers of animals are inadequate to compare the efficacy of the two antibiotics, penicillin and tetracycline. One death occurred with each antibiotic several days after institution of therapy. In the case of the monkey dying while receiving penicillin, anthrax bacilli resistant to that antibiotic were recovered. This observation suggests that if penicillin were chosen as the therapeutic agent it should be supplemented with a second antibiotic; streptomycin, shown to be effective in the studies on guinea-pigs conducted by Keppie and his associates might be suitable. Alternatively, a broad spectrum antibiotic might be employed as the supplemental drug either initially or added subsequently if 'antagonism' is a concern. Since the mechanism of penicillin resistance of the organism encountered here was penicillinase production, the recently introduced penicillinase-resistant penicillins should be considered. A broad spectrum drug alone would appear efficacious though perhaps suboptimal, based on these limited studies.

Uniform presence of spores for many weeks in surviving animals and the late death emphasize the need for adequate antigenic stimulation in animals exposed to anthrax aerosols. In practice, all treated individuals should probably receive protective antigen after recovery in order to assure such stimulation.

The results of the present study suggest that supervoltage diagnostic film techniques are advantageous for the earliest detection of mediastinal soft tissue changes. Where appropriate equipment and personnel are at hand such techniques are to be recommended for suspected mediastinal infection. Such a recommendation does not imply, however, that conventional radiographic techniques do not detect the mediastinal lesions such as those of woolsorter's disease early enough for effective therapy.

SUMMARY

1. A model of anthrax infection in rhesus monkeys closely simulating human inhalation anthrax has been employed to study the course of the illness and methods of recognition and therapy.

2. Roentgenographic evidence of mediastinitis was detected early in the illness and served as a suitable indication for starting treatment.

3. Therapy with either penicillin or tetracycline appeared efficacious when started at the time chest roentgenograms first evidenced disease.

4. Isolation of penicillin-resistant *B. anthracis* from an animal dying during treatment with penicillin is reported.

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EXPLANATION OF PLATES

PLATE 1

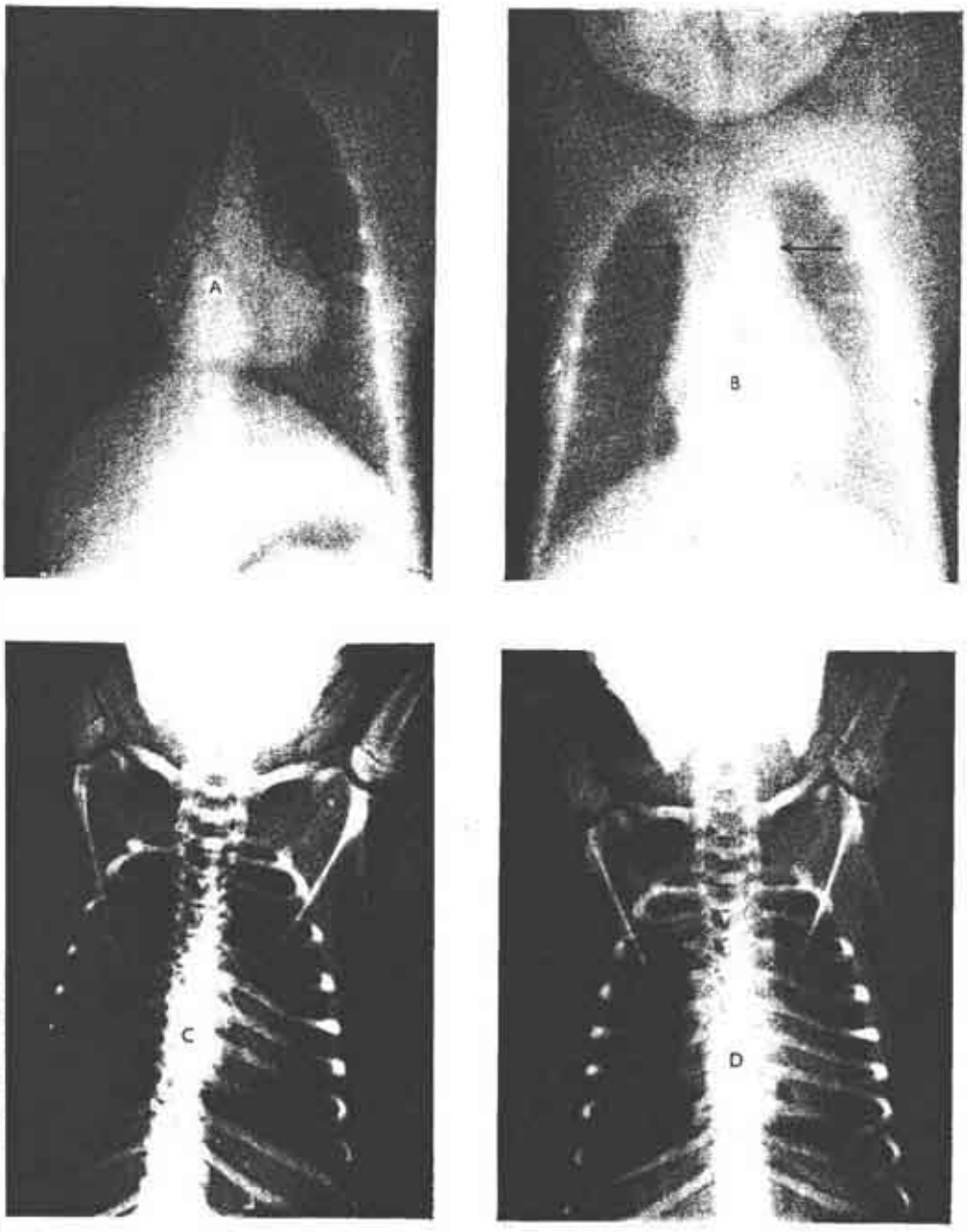
Radiographic appearance of Simian woolsorter's disease. A and C are normal. B and D reveal marked widening of the superior mediastinum. A and B are supervoltage films (1 MeV.); C and D are corresponding films obtained by conventional technique. Note the superior definition of soft tissues achieved with the supervoltage technique. (U.S. Army photograph.)

PLATE 2

Pneumonic lesion due to *B. anthracis*. A is normal, B reveals an infiltrate adjacent to the right cardiac border. (U.S. Army photograph.)

PLATE 3

Post-mortem specimen of a monkey displaying marked mediastinitis. See Pl. 1 for radiographs of this animal. (U.S. Army photograph.)



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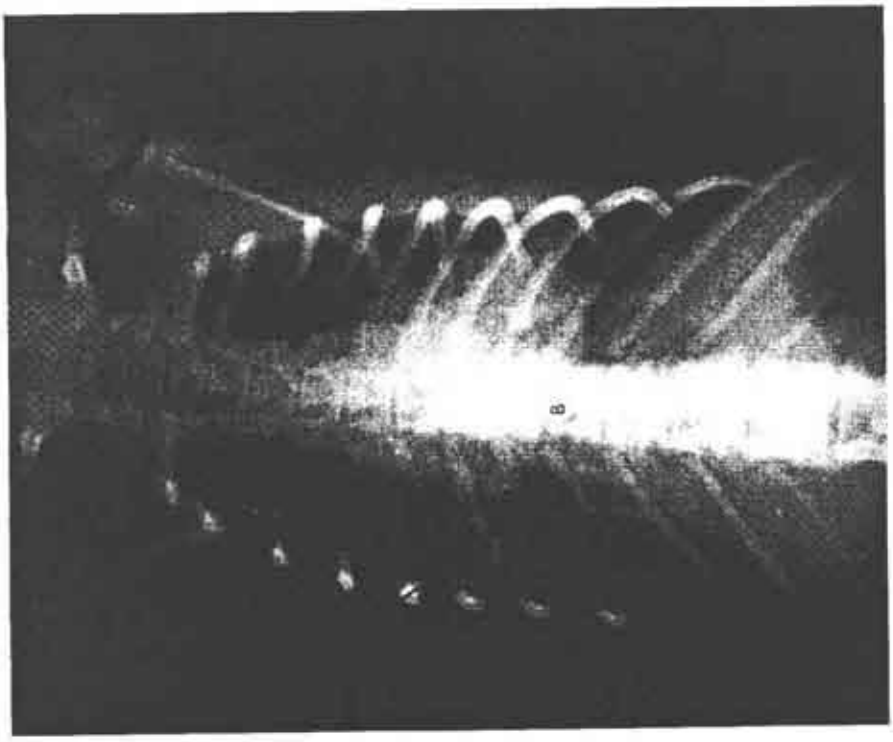
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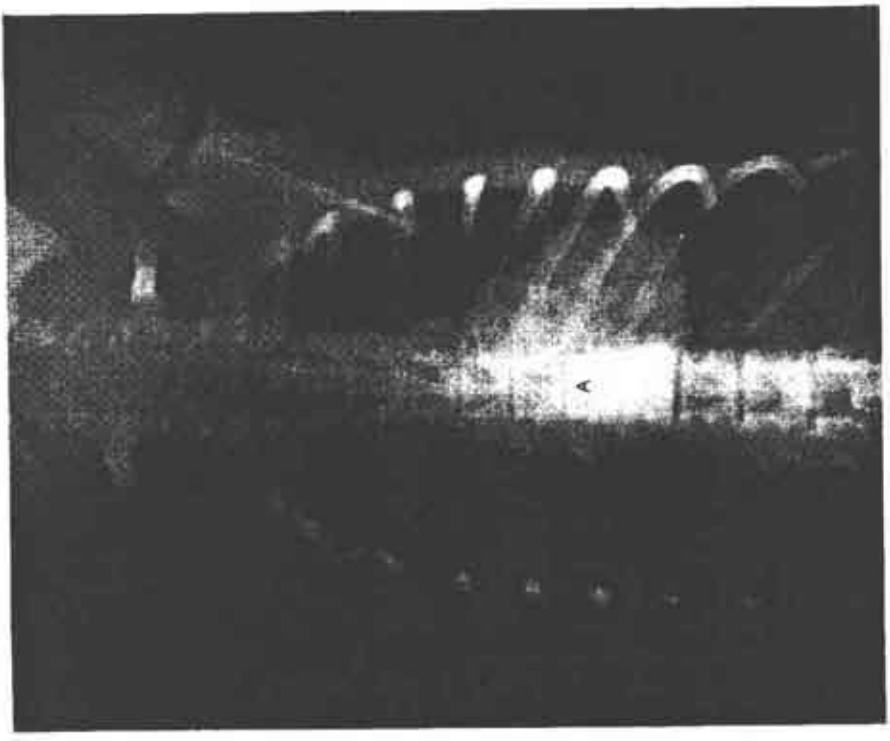
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