

Anthrax from 5000 BC to AD 2010

Peter C. B. Turnbull and Sean V. Shadomy

FROM ANCIENT TIMES TO THE 19TH CENTURY

Historical Names of Anthrax

Anthrax (Latin, a carbuncle) is derived from the Greek ἀνθραξ (*anthrax*) meaning coal and referring to the characteristic black eschar in human cutaneous anthrax. Other older names for the disease, such as “malignant pustule” or “black bane,” and names in other languages, such as charbon (French) and carbonchio (Italian), similarly reflect these features. Yet other names reflect other manifestations of the disease in humans and/or animals or its sources of infection, such as woolsorter’s/ragpicker’s/Bradford disease and the German equivalent Hadernkrankheit (rag disease), splenic fever, Milzbrand (German, meaning “spleen fire”), Siberian plague, Lodianna fever, and Pali plague in India, and many more. The many names in many languages reflect the historical and widespread recognition of the numerous features of anthrax before it was understood that they were all manifestations of a single etiological agent.

The earliest application of the name “anthrax” to the afflictions caused by *Bacillus anthracis* is uncertain. “Bloody murrain” was probably the most common term for the disease in animals in early English language texts, and carbuncle—or malignant carbuncle to distinguish it from other carbuncular manifestations—was the term used for the cutaneous infection in humans. From a book of 1766, Viljoen (1928) cites “Visit to your servant girl suffering from a considerable anthrax and found several furuncles on the back; cured same” but believes that “anthrax” at that time was a common term embracing any severe localized dermatitis and this was not a *B. anthracis* infection. According to Swiderski (2004), physicians attending George Washington diagnosed as “an anthrax” “a very large and painful tumor” which developed on his left thigh about 6 weeks after his inauguration as first president of the United States in 1789. However, that description and the description of “anthrax, or carbuncle” in the American edition of *The Surgeon’s Vade-Mecum* (1813) similarly appear unlikely to have been *B. anthracis* infections.

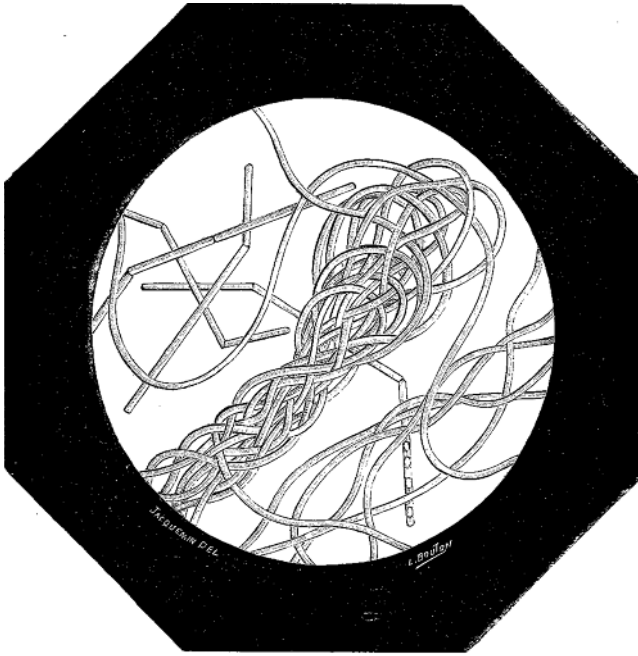


Figure 1.1 From a culture of anthrax blood in chicken broth at 24–48 h. (From Ch Chamberland's celebrated book *Charbon et Vaccination Charbonneuse d'après les travaux de Mr Pasteur*, 1883.)

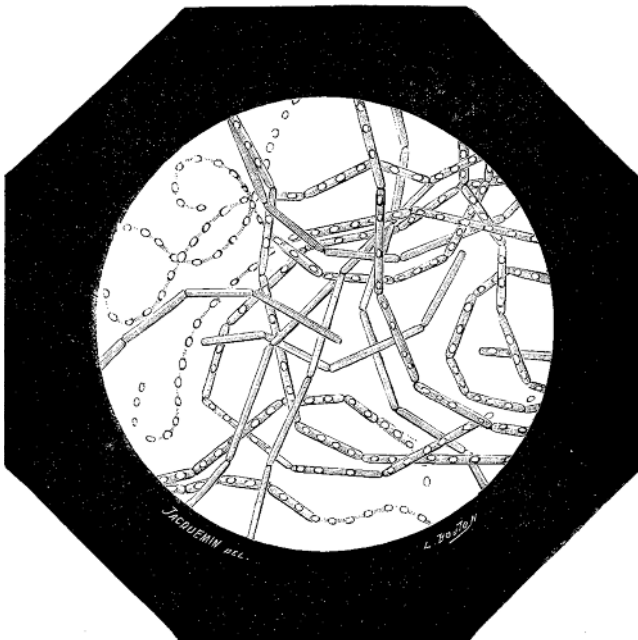


Figure 1.2 The same culture after several days, with spores now apparent. (From Ch Chamberland's celebrated book *Charbon et Vaccination Charbonneuse d'après les travaux de Mr Pasteur*, 1883.)

From Ancient Egypt to 1892

The allegedly long history of anthrax features in numerous papers and articles. Most scholarly among those readily accessible today are probably the papers of Klemm and Klemm (1959) and Blancou (2000) which, together, supply a brief but comprehensive review of earlier literature attesting to the historical familiarity with the disease through the ages. Klemm and Klemm (1959) suggest anthrax originated in early Egypt and Mesopotamia, where agriculture was established some 5000 years BC, and then address the feasibility of the frequently cited statement that the fifth plague of Egypt in the time of Moses (ca 1250 BC)—“a grievous murrain affecting cattle, horses, asses, camels, oxen and sheep”—was the earliest instance of systemic anthrax on record. As Ebedes (1981) points out, no other disease kills such a wide spectrum of species. Others also consider the sixth plague—boils breaking out in sores on man and beast—to have been cutaneous anthrax. Ebedes (1981) suggests these lesions affected only the Egyptians because only the Egyptians would have handled the carcasses of affected animals, the Israelites being forbidden to touch dead animals. Not everyone concurs with these hypotheses; Morens (2002), for example, considers the evidence that the fifth Pharaonic plague in the biblical book of Exodus was anthrax to be weak.

Klemm and Klemm (1959) and Blancou (2000) also summarize the evidence that early Greece was familiar with anthrax as depicted by Homer in his “Iliad” (ca 1230 BC), Hippocrates’ writings (ca 400 BC) and the plague of Athens in 430 BC (see also Morens and Littman, 1992), Aristotle in his *History of Animals* (ca 333 BC), Plutarch (ca AD 120), and Galen (ca AD 200); that it was described in Hindu literature of around 500 BC and that, based on the writings of Livius (ca 460 BC), Virgil (70 BC–90 BC), and Vegetius (ca AD 400), the Romans were well acquainted with it. Dirckx (1981) had no doubt that the Norican plague (now Bavaria) in Virgil’s third Georgic (ca 32 BC) was anthrax. Klemm and Klemm (1959) even suggest that anthrax may have contributed to events that led to the fall of Rome. According to Dong (1990), anthrax has featured in Chinese animal husbandry for millennia, being especially well described in the Jin and Sui dynasties (AD 500–600) by Ge Hong in his *Handbook of Prescriptions* and Ch’ao Yaun Fang in the *General Treatise on the Etiology and Symptomatology of Diseases*.

Klemm and Klemm (1959) believe that records of the occurrence of anthrax in post-Roman Europe begin with references to what is likely to have been this disease in the *Hippiatrika* (horse medicine), a tenth-century collection of veterinary writings, and the eleventh century *The Medicine of Quadrupeds* Blancou (2000) summarizes records of the death of a clan chief in Ireland in 1030 from what may well have been anthrax; of Arab authors in the twelfth and thirteenth centuries describing anthrax-like signs in cattle; the description in 1250 by German Emperor Frederick II’s chief veterinarian of what appears to have been anthrax in horses; a description by one Pietro di Crescenzi (Italy) of what is thought to have been anthrax in sheep in the late 1200s; and further descriptions of probable anthrax in animals and humans in 1316, 1523, 1673, and 1745. D. E. Salmon (after whom *Salmonella* species were named) informs us that “anthrax was frequently confounded with the rinderpest, but is described with sufficient precision to identify outbreaks of it in epizootic form in 996 A.D. and 1090 in France; in 1552 at Lucca, Italy; in 1617 at Naples, where numbers of human beings died from eating the flesh of animals affected with the disease” (Salmon, 1896). He further documents references to the disease in animals in Venice in 1598, extensive outbreaks in Germany, Hungary, and Poland in 1709–1714, its extensive spread in the early 1800s in Russia, Holland, and England, and again in Russia during the mid-1800s when, in 1864, “more than 10,000 horses and nearly

1000 persons perished from the disease.” In Novogrod (Russia), between 1867 and 1870, 528 people and 56,000 cattle died of anthrax (Klemm and Klemm, 1959; Koch, 1877). Some 60,000 people were reported to have died in the 1617 Naples epidemic, and 15,000 people allegedly died from anthrax in San Domingo (now Haiti) within 6 weeks in 1770 (Higgins, 1916; Morens, 2002).

In America, anthrax was first introduced into Louisiana at the time of French settlement in the early 1700s. Evidence is that it had spread to Kentucky by 1819 and to Philadelphia by 1836 reaching New York, New England, and California in the second half of the 1800s (Hanson, 1959; Klemm and Klemm, 1959). The appearance in 1819 of the disease in horses, cows, sheep, and humans in contact with infected animals was clearly documented by Kentucky physician Dr. J. Kercheval in 1824 (Hanson, 1959). Purdom (1954), presumably unacquainted with Kercheval’s report, claimed it was the “unenviable distinction” of Philadelphia to be the first city in the United States in which a recorded case of human anthrax was described in 1834 following skinning of cattle that had died of the disease. More human cases were reported in Louisiana in 1830, with further reports over “the next several years” of the disease in animals and humans in Texas, Wisconsin, New York, Mississippi, Vermont, Massachusetts, and California (Brachman, 1965).

In Africa, recently described as the “cradle of anthrax” (Smith et al., 1999), explorer Dr. Andrew Smith described the clinical form of anthrax in man and domestic animals in 1836, naming the disease “bloodzichte” or “quatsie.” That it was already endemic before the arrival of Europeans is clear from missionary Robert Moffat’s description in 1842 of what appears to have been anthrax as “... endemial, which assumes the form of a carbuncle, and carries off many cattle, and as the natives will on no account abstain from eating the dead meat ... always accompanied by considerable swelling attended with great stupor, though ... little pain.” The “horsesickness” described by famous explorer David Livingstone in 1857, also observed in zebras, was almost certainly anthrax (De Vos and Turnbull, 2004; Viljoen, 1928). It was clearly well recognized as a livestock disease by the 1870s (Gilfoyle, 2006) although still being confused with other diseases (Viljoen, 1928).

Clinical (as opposed to historical) descriptions of the cutaneous disease in humans were first given by Maret in 1752 and by Fournier in 1769, and Chabert gave a clear description of the disease in animals in 1780 (Wilson and Miles, 1964). Fournier recognized that anthrax could be transmitted to humans through the handling of animal hair and wools (Martin, 1975).

Order of Events in the Nineteenth Century

The causative agent of anthrax was established in the nineteenth century, but there are some discrepancies in the more readily accessible historical reviews as to the precise order of events and the appropriate credits. The reports and events in the first 75 years of the nineteenth century would clearly benefit from being carefully revisited by a proficient scholar with appropriate linguistic skills in at least French and German as well as English. Wilson and Miles (1964) and Blancou (2000) credit Barthélémy in 1823 as the first to demonstrate transmissibility by injecting a horse and a sheep with blood from a horse that had died of anthrax, this being repeated by Leuret in 1824. Contenders for being the first to associate anthrax with the presence of rod-shaped bodies in the blood of animals that had died from the disease are Brauell, Pollender, Davaine, and Rayer between 1855 and 1859, with Delafond apparently being the first to call these bacteria in 1860. Cohn (1872)

believed these to belong to the spore-forming *Bacillus* group and accordingly named them *B. anthracis*.

Davaine, in a series of papers in 1863–1864, showed how anthrax could affect a range of species, demonstrating that the disease could be transmitted to sheep, horses, cattle, guinea pigs, and mice by the subcutaneous inoculation of infected but not of normal blood. Finding this same bacillus in a malignant pustule in 1864, Davaine and Raimbert established the etiological connection between the disease in humans and animals for the first time (Wilson and Miles, 1964). Demonstration that infectivity was lost on passage of infective fluid through a clay filter was attributed to Tiegel and Klebs in 1864 by Wilson and Miles (1964), and to Davaine in 1873 by Klemm and Klemm. The first recorded observation of the intestinal form of anthrax was made by Wahl in 1861 and the first to recognize that woolsorter's disease was the inhalational manifestation of the disease was Bell in Bradford, England, in 1879 (Martin, 1975).

Generally undisputed is that the final proof of the bacterial cause was established by Robert Koch (1877), who detailed the sporulating characteristics of *B. anthracis*, the ability of the spores to survive long periods *in vitro* and to reproduce the disease after such periods when injected into animals. While giving credit to Koch for the observation of spore formation in the anthrax bacillus, Louis Pasteur pointed out in a communication to Koch in 1883 that 7 years previously he had noted the phenomenon in the microbe causing silkworm disease (might this have been *B. thuringiensis*?) and in consequence claimed priority for the discovery of this process (Mason, 1937). Certainly, Pasteur was working on anthrax in 1877 using his fermentation techniques to culture the bacterium from the blood and reproducing the disease with such a culture.

The observation by Koch in the 1870s that a piece of spleen from a mouse that had died of anthrax inserted under the skin on the back of a frog induced phagocytosis of the anthrax bacilli by frog leucocytes led to Metchnikoff's remarkable studies in the early 1880s on phagocytosis and what became known as opsonization in the blood or lymph of immunized animals (Metchnikoff, 1884). Metchnikoff also laid the foundation for studies on the pathogenesis of anthrax with early explanations for differing resistance in various species and apparently being the first to observe the capsule of *B. anthracis*—although he did not relate the capsule to resistance to phagocytosis.

Weichselbaum noted the methylene blue staining characteristic of the capsule in 1892 (M'Fadyean, 1904). M'Fadyean (1903) then immortalized this in the simple methylene blue capsule staining procedure which became, and remains today, the primary rapid diagnostic test for animals suspected of having died of anthrax.

Correct Diagnoses?

The many authors giving a brief history of anthrax in an introduction to a paper on some other aspect of the disease rarely raise the question as to whether the historical references were really anthrax. In the terminology prior to about 1850 for example, murraine (from Latin *morire*, to die), peste, and plague for animals, or peste, plague, and charbon for humans, were nonspecific and were applicable to other diseases such as rinderpest in animals, or *Yersinia pestis* plague, and smallpox in humans. In general, however, descriptions of sudden death in animals, frequently with more than one species involved, accompanied by hemorrhage from the orifices, gelatinous edema, and swollen lymph nodes or, in the case of humans, typical cutaneous lesions or deaths associated with animals that have died with the correct symptoms, can reasonably be accepted as having been anthrax.

Pasteur and the First Anthrax Vaccines

It is well-known that one of the most colorful parts of the history of anthrax is the story of Louis Pasteur's animal anthrax vaccine. This story, and how Pasteur's first report of successful protection of animals against deliberately induced disease in 1881 was actually preceded by the reports in 1880 of Greenfield in England and Chauveau and Touissant in France, has been covered in detail by this author elsewhere (Turnbull, 2010).

TWENTIETH-CENTURY ANTHRAX

Global Incidence

A wealth of information on the understanding of anthrax current at the outset of the 1900s lies in the three-volume (U.K.) Report of Departmental Committee on Anthrax (Report of the Departmental Committee, 1918). From this it is clear that, while animal and human case rates were beginning to be well recorded in the early years of the twentieth century in industrial countries such as Britain, Germany, and the United States, figures on the incidence of the disease in endemic countries were unavailable, despite efforts at determining these through both the British consuls and buyers of animal products in such countries, many of which were part of the then British Empire. It was well recognized, however, that, well into the century, anthrax was widely prevalent in many parts of eastern and southern Europe, central Asia, India, and much of Africa at least. For many of the countries in these regions, the knowledge was based on the high proportions of contaminated consignments among imports. Occasionally, figures are cited, some dramatic, such as >26,000 and >43,000 deaths among animals in Russia in 1913 and 1914, respectively (Eurich and Hewlett, 1930; Report of the Departmental Committee, 1918), 13,000 human cases, again in Russia, in 1924 (Pijper, 1926), and more than a million sheep dying of anthrax in Iran in 1945 (Report of the Committee of Inquiry on Anthrax, 1959).

Some of the best available data of the early 1900s are those for South Africa (Viljoen, 1928), where it rose from a disease of relative insignificance in 1905 to the most serious of the scheduled contagious livestock diseases by 1923. Viljoen (1928) attributes this largely to increased movement of animals and animal products with developing railway and road networks, the increase in stock ownership, careless owners, and failure of veterinary supervision to keep pace with these movements.

The association between the incidence in livestock in industrialized northern countries and importation of animal products became particularly apparent at the time of the two world wars. In Germany, 7181 cases in 1914 compared with just 743 in 1919 and 699 outbreaks in 1939 in the United Kingdom had fallen to 95 by 1946, rising again to 407 in 1951 and 1245 in 1956 with the reestablishment of importation of bone, meat, and blood meals (Wilson and Miles, 1964).

The First 30 Years

Isolation and Identification

The polychrome methylene blue capsule stain diagnostic test of M'Fadyean (1903) already referred to above, though simple, was probably the first highly significant milestone of the twentieth century. The role of the capsule in the virulence of anthrax came to light in the

early part of the century with Morihana in 1921 reporting the loss and reestablishment of virulence by cultures induced to lose and regain their capsules (Eurich and Hewlett, 1930).

Eurich and Hewlett's (1930) review of the literature at that time shows that it was also replete with a miscellany of seemingly bizarre observations. Papers in the 1920s variously reported "large spherical and lemon-shaped elements" in cultures on gelatin and glycerin agar, bent and hook-shaped bacilli in fish liver broth cultures, gram-negative coccobacillary forms, capsules of unusual size around bacilli grown on "brain agar" at 42–43°C, and formation of gonidia in the filaments of *B. anthracis* cells grown in broth cultures. Stable asporogenous mutants and rough and smooth colony types were also described, but it is hard to know if and how these corresponded to equivalents produced or recognized today.

Research at this time also focused on methods of isolation from both animal and environmental specimens and the best techniques for correct identification. The logic behind methods utilized today to isolate *B. anthracis* from environmental samples heavily loaded with other environmental *Bacillus* species is little changed from that in the 1920s, and it was already being appreciated that conventional biochemical and serological identification tests were unable to differentiate *B. anthracis* from closely related "anthracoid" *Bacillus* species. In the terminology of the time, "Serological races such as exist among pneumococci and meningococci" could not be identified. The one important serological test of the time, Ascoli's precipitin reaction of 1911 for determining whether animal products derived from animals that had died of anthrax, depended not on the specificity of the antiserum for *B. anthracis* but rather on the fact that *Bacillus* antigen in animal tissues could only be from *B. anthracis*. Seemingly, this test is still used in certain eastern European countries (WHO, 2008).

Immunity, Therapy, and Prophylaxis

It was well recognized at the outset of the 1900s that early diagnosis of cutaneous anthrax in humans was essential for successful treatment, which took the form of thermocautery, chemical caustics, surgical incision or excision, wet antiseptic dressings, or injection of antiseptic solutions, and which were increasingly supported and eventually wholly replaced by the administration of antiserum as the other treatments fell into disrepute as actually being dangerous in addition to being extremely painful and disfiguring (Regan, 1921; Report of the Departmental Committee, 1918). Regan (1921) gives the credit for the original production of suitable antiserum to Marchoux in France and Sclavo in Italy, both in 1895. It was equally well recognized that there was no means of diagnosing internal anthrax in the early stages, and claims of instances of recovery could not be supported by laboratory evidence; death was regarded as the almost invariable outcome. Joint problems, arthritis, and skin eruptions were adverse effects sometimes seen with the use of antiserum, but it was regarded as "not dangerous to life."

Natural immunity was also a topic of interest at this time. The existence of bactericidal ("anthracocidal") activities in the sera of some species were evidently known to Metchnikoff, Roux, and Marchoux in the 1890s but were subsequently shown not to be directly related to the relative susceptibility or resistance of a particular species to infection (Kolmer et al., 1920). Keller (cited without reference by Eurich and Hewlett, 1930) stated that in humans, anthracocidal activity appears in the serum in the seventh to ninth week of life. Penna et al. (1917) reported successful treatment of a large series of human anthrax cases by subcutaneous injection of normal bovine serum, and Zehetmayr (1922; cited by Eurich and Hewlett, 1930) later demonstrated that a subcutaneous injection of normal ox

serum exercised a protective effect against an injection of anthrax bacilli at the same site but not against bacilli injected at a distant site unless very large doses of serum are given. However, the efficacy of normal animal serum therapy could not be confirmed and was questioned by other investigators (Pijper, 1926; Regan, 1921).

While it was recognized that early diagnosis of signs of cutaneous anthrax in at-risk occupations was important for reducing mortality rates, it was admitted that it was impossible to diagnose internal anthrax before death, although it was believed that monitoring the opsonic index might be of value here (Report of the Departmental Committee, 1918).

As covered in a separate chapter on anthrax vaccine history (Turnbull, 2010), the possibility that acquired immunity to anthrax was the result of antibodies to a toxic “aggressin” dates back to 1889–1890. The aggressin and “protective antigen” in extracts of skin lesions and edematous fluids from animals with anthrax infections were further subjects of studies in the first decades of the 1900s (Bail, 1904; Bail and Weil, 1911; Salisbury, 1926; all cited by Lincoln and Fish, 1970). These studies laid the foundation for the later work leading to the human anthrax vaccines of today in the United States and in the United Kingdom.

Chemotherapy was also being trialed in the first decades of the 1900s. Eurich, following a recommendation by Ehrlich, tried using the arsenical salvarsan for the treatment of anthrax in 1910, adopting it formally in 1911, and later attesting to its success in his own and others’ hands (Eurich, 1933; Eurich and Hewlett, 1930). Also, based on Ehrlich’s studies on dyes as chemotherapeutic agents, argochrome (methylene-blue-silver) was used with apparent success in a small number of human cases, and acriflavine at a dose of 7 mg/kg in a 1% solution was used with apparent success in a number of sheep (Möslinger, 1924 and Burke and Rodier, 1923; both cited by Eurich and Hewlett, 1930). Especially noteworthy is that in his landmark paper on his discovery of penicillin, Fleming (1929) showed that *B. anthracis* was susceptible to penicillin—although it was another 15 years before it was used to treat the disease (see below).

Public Health Measures for Industrial Anthrax

J. H. Bell’s milestone discovery in 1879 in Bradford, England, that woolsorter’s disease was inhalation anthrax resulted over the next few years in numerous enquiries, codes of practice, and regulations in Britain for the wool and hair industries, first given legal force in 1897 and reinforced subsequently by the Wool, Goat and Camel Hair Processes Regulations 1905, the Horsehair Regulations 1907, the East India Wool Regulations 1908, the Anthrax Order 1910, the Anthrax Prevention Act 1919, the Anthrax Prevention Order (Shaving Brushes Order) 1920, the Hides and Skins Regulations of 1921, and so on. It was this dangerous aspect of industrial anthrax that led to the disease receiving rapidly increasing attention in the first decades of the twentieth century with the concerns of importing countries, such as the United States, Britain, and Germany, being transmitted to the countries in Africa, Asia, and the Middle East exporting contaminated products of animal origin.

The initial codes and regulations for the British wool and hair processing industries were based on the perceived role of dust in transmitting infection. When no obvious reduction in incidence accompanied dust suppression, removal of bloodstains from the material before further processing was recommended by the Bradford Anthrax Investigation Board in 1908. However, analysis by the Departmental Committee on Anthrax (Report of the Departmental Committee, 1918) revealed this also had had little impact on case rates, nor

did attempts at improving personal hygiene among the workforce. Furthermore, it proved impossible to implement reduction of the incidence of anthrax in the livestock of the exporting countries. The ultimate conclusion was that the problem was not one that could be addressed by further regulations at the factory level and that disinfection, carried out not by the factories themselves, but in dedicated government disinfection stations, was the way forward. From this arose the Duckering Process involving sequential treatment in baths of an alkaline soap solution and 2–2.5% formalin at 102–105°F followed by drying in hot air. The process was named after Elmhirst Duckering, a member of the Disinfection Sub-Committee, but should probably have been called the Delépine process after Professor Sheridan Delépine, Director of the Public Health Laboratory, Manchester, who designed and tested the procedure (Report of the Departmental Committee, 1918). Disinfection by this process was made a requirement for wool and hair of specified origins under the Anthrax Prevention Act 1919, and the further Orders of 1921 and 1935. The intention was for disinfection stations to be set up in the relevant exporting countries based on the British Government Wool Disinfection Station constructed in Liverpool, but in the end the Liverpool plant was the only one constructed. It operated for 50 years from 1921 to 1971. Interestingly enough, it was the conclusion of Parliamentary Committee of Inquiry in 1959 (Report of the Committee of Inquiry on Anthrax, 1959) that only a small part of the fall of incidence of anthrax in the wool and hair industries could be ascribed to the working of the Disinfection Station and that unspecified other factors had contributed to the reduction in cases.

Such a process was never implemented in the United States on account of both economic and political reasons. Economically, it was estimated that it would raise the cost of processed materials by 5 to 10 cents a pound; politically, it would result in loss of commerce to ports that did not have disinfecting stations (Steele, 1959). Consequently, control of industrial anthrax in the United States was dependent on mechanical and chemical approaches to factory hygiene.

Bioaggression

The early decades of the 1900s also saw the first use of the deliberate infection with anthrax as an act of aggression with, during World War I, Germany targeting horses being raised in neutral countries to supply to Germany's enemies (Wheelis, 1999). In the years immediately following World War I, little concern appears to have been attached to biological weaponry outside of France, and it was only at the urging of Poland that such weapons were included in the 1925 Geneva Protocol (Geissler and van Courtland Moon, 1999). France, recalling the arrest in 1917 of a German agent attempting to infect French cavalry horses with anthrax (and glanders) went through a period of intense biological weapon research in the period 1921–1926, which included *B. anthracis* among several other pathogens (Lepick, 1999). Then France joined other signatories of the 1925 Geneva Protocol and reduced the program to “technical monitoring.”

1930 to Sverdlovsk (1979)

The 1930s

With the 1925 Geneva Protocol in place, interest in anthrax fell back to being one of controlling the disease in livestock. Research in this decade was largely concerned with a search for an improvement on the Pasteur vaccine. As has been covered elsewhere

(Turnbull, 2010), the signal name of the decade was Max Sterne who, in 1937, formulated the livestock vaccine still in use in most countries of the world (WHO, 2008).

Also with a view to controlling the disease in livestock was some concern to understand how the disease was being transmitted and, therefore, how this could be prevented. Clark (1938) reasoned that most cases of bovine anthrax in South Africa were contracted by pica (chewing bones), rather than grazing, biting flies, or drinking contaminated water. The significant role of imported animal products continued to be monitored assiduously among trading countries. In the then Dutch East Indies, Kraneveld and Mansjoer (1939) and Kraneveld and Djaenoedin (1939, 1940) believed that biting flies (*Tabanus* spp.) played an important role in the spread of anthrax in that region and demonstrated this experimentally in horses and guinea pigs—further demonstrating that concurrent trypanosome infection in guinea pigs did not alter their sensitivity to anthrax infection. Kraneveld and Mansjoer (1939) also established that there was no multiplication phase in the tabanid vector.

The capsule of *B. anthracis* remained the subject of some interest at this time with credit going to Ivanovics and colleagues for its identification as a polypeptide of D-glutamic acid, also showing its poor immunogenicity (Ivanovics, 1939). An interesting observation of a quellung-type reaction on the non-polysaccharide capsule was made by Bodon and Tomsic (1934) but seemingly was never followed up for practical purposes or in relation to pathogenesis. Enhanced phagocytosis of capsulated anthrax bacilli by leucocytes hyper-immunized with capsulated bacilli was demonstrated by Boari (1938).

Chemotherapy also advanced in the 1930s. By 1930, antiserum treatment was the undisputed therapy of choice, but it required large (up to a liter) doses of hyperimmune animal serum administered intravenously with inevitable undesirable side effects. According to Lincoln et al. (1964), the cures effected in the 1920s and 1930s using antisera developed in asses, sheep, and oxen (seemingly not horses) exceeded those effected by antibiotics in the 1940s and 1950s. It is interesting to note that in Penna's 1917 report, the authors state that using bovine serum is preferable to equine serum due to the lower rate of serum sickness reactions seen with the bovine serum (Penna et al., 1917). As stated earlier, Eurich and others were vouching for the value of salvarsan in the early 1900s, and the early 1930s saw several favorable reports on the effectiveness of the less toxic neo-salvarsan (also developed in the laboratories of Eurich in 1912) although Gold (1942) did not find it of much value. Sulfa drugs then made their dramatic appearance in 1936, and Gold (1942), treating numerous cases from 1938 to 1941 with these, declared sulfathiazole the drug of choice as being the least toxic if not the most effective.

1940 to the 1972 Weapons Convention: Preparations for Biological Warfare

Germany had unconditionally ratified the 1925 Geneva Protocol in 1929 and, records showed subsequently, had adhered to this through World War II. However, British intelligence reports in the 1930s alleged that the Germans were developing delivery systems for anthrax spores within a substantial biological warfare (BW) program (Geissler, 1999) and accordingly established the Committee of Imperial Defence of a Bacteriological Warfare Subcommittee in 1936, which became the War Cabinet Biological Warfare Committee during the war (Carter and Pearson, 1999). Prior to the war, the Subcommittee was largely concerned with the availability of vaccines in the event of need with practical work only being authorized in 1940, 5 months after the war had begun. Most of the resulting work was with *B. anthracis*, with both offensive and defensive issues being addressed. On the offensive side, the logistics of delivery, ranging from large-scale production of spores through aerosol characteristics to dose determinations and delivery systems were

established, leading ultimately to the infamous experiments in 1942 and 1943 on Gruinard Island off the Scottish coast, the less well-known tests, also in 1942, on the beaches of Penclawdd on the Gower coast of Wales, the production and stockpiling of 5 million anti-livestock cattle cakes containing lethal doses of anthrax spores— 5×10^8 spores per cake—and at least one postwar set of sea trials (Hammond and Carter, 2001).

Neither the anti-human nor the anti-livestock devices were put to use, but the beneficial by-product of the U.K. war-stimulated activities, also begun in Canada and the United States in 1943 (Avery, 1999), was the resulting research which commenced after the war in these and other countries on the pathology and pathogenesis of anthrax. At this point the number of publications on anthrax, and these aspects in particular, burgeoned, and it is impossible to do justice here to all the researchers and their excellent papers that appeared from about 1946 onward; many, however, are reviewed by Lincoln and Fish (1970).

By far the greatest numbers of papers on these topics emerged from Camp (later Fort) Detrick in the United States and Porton Down in the United Kingdom. Events at the histopathological events following inhalation of anthrax spores were studied by Young et al. in 1946 in Camp Detrick and by Barnes in 1947 in Porton with both noting that germination and multiplication commenced, not in the lung itself, but in the lymph glands, and Cromartie et al. in 1947 in Detrick defined the histopathological events following cutaneous infection, comparing these events in susceptible and resistant animals.

Druett et al. (1953) in Porton demonstrated the loss of infectivity on inhalation of particles of $>5 \mu\text{m}$ carrying anthrax spores. Since naturally acquired anthrax only takes the inhalation form exceedingly rarely, the concern with potential bioaggression is seen in the emphasis on events following inhalation of spores.

Over the next two decades, a clear understanding of the nature of the toxin complex and the roles of the principal virulence factors of *B. anthracis* had been elucidated in the series of papers between 1953 and 1963 by Smith and colleagues at Porton under the overall heading “The Chemical Basis of the Virulence of *Bacillus anthracis*” together with a number of other supplementary papers and by large teams over the same time period in Detrick focusing extensively on the pathophysiological and neurological responses to anthrax infection and the toxin. The cause of death was established by Smith and colleagues in 1955 as a toxin (Keppie et al., 1955), and this team (Stanley and Smith, 1961) and Beall et al. (1962) in Detrick then established the three-component nature of this toxin. Progress on improvements in the purification of the three factors were reported by Fish et al. (1968), again in Detrick. A large proportion of the research of the period were concerned with the basis of immunity, the overall target being a vaccine for humans, and this is covered in detail elsewhere (Turnbull, 2010).

These research activities were brought to a focus point in the Conference on Progress in Understanding Anthrax convened by the Federation of American Societies for Experimental Biology, Bethesda, Maryland, in 1966 and the published proceedings in 1967. Curiously, that conference appeared to mark something of an end point to the almost frantic activity of the previous two decades with interest waning in the run up to, and after the 1972 Biological Weapons Convention—only to be rapidly rekindled by the Sverdlovsk incident in 1979 (Meselson et al., 1994).

Other Milestones of 1940 to Sverdlovsk

Highly significant among the early milestones of the period under consideration was the first use of penicillin to treat anthrax was in 1944 (Murphy et al., 1944), thus beginning

the long era thereafter when penicillin was the first drug of choice, replacing serum therapy and other chemotherapies in use at the time (WHO, 2008). A decade later, Henderson et al. (1956) showed the prolonged persistence of spores in the lungs of monkeys after inhalation exposure and, with penicillin as the antibiotic, formulated the approach recommended today of prolonged antibiotic administration with simultaneous vaccination in persons thought or known to have been exposed to aerosolized spores.

Recognition is also due to Brachman et al. (1962) and the Committee of Inquiry in Britain (Report of the Committee of Inquiry on Anthrax, 1959) for the in-depth understanding they supplied on the epidemiology of industrial and inhalation anthrax, Stein and Van Ness (1954) for their careful monitoring of veterinary cases and outbreaks and ecological insights (Van Ness, 1971), Knisely (1966) for the formulation of selective PLET medium which has remained the basis of detection of anthrax spores in environmental samples to the present day, and Shlyakhov et al. (1973) for the Anthraxin diagnostic skin test.

Also worthy of retrospective attention was the remarkable outbreak in humans and animals in Zimbabwe which began in 1978 and still had residual cases in 1982, with well over 10,000 human cases and countless cases in cattle and other livestock. Officially, this was put down to the breakdown in veterinary services, shortages of penicillin, and other consequences of a major insurgency occurring at the time, though opinions were expressed later that it may have resulted from deliberate release—an issue that will probably remain unresolved.

Sverdlovsk (1979) to the Anthrax Letters (2001) and Beyond

Resumption of research after the Sverdlovsk incident saw intensive and effective application of new techniques and technologies to the understanding of the pathogenesis of anthrax, with the infamous anthrax letters of late 2001 producing a second dramatic surge of research into numerous aspects of the disease. Once again, it is impossible in the space available here to give the individual credits that would ideally be desired to all the research groups and individuals behind the excellent work over the past three decades. However, it was a period when much of the work, together with the relevant credit, was well covered by periodic and readily available reviews and textbook chapters, and much of it will also form the introductory backgrounds of the chapters that follow this.

In summary, this period, which began with improved purification of the toxin components, then saw

- full structure-function characterization of the toxin components, the understanding of their “A-B” type of toxin action with the protective antigen (PA) as the binding unit and lethal and edema factors (LF, EF) as the active units, and, later, the nature of the binding site for PA, the relationships and interactions with other bacterial toxins and novel concepts for treatment of anthrax and other diseases based on the receptor-mediated endocytosis of the anthrax toxin,
- the elucidation in the early 1980s of EF as an adenylate cyclase and somewhat later the understanding of the action of LF as a protease acting on mitogen-activated protein kinase kinases at the end of the 1990s,
- elucidation of the plasmid-borne nature of the toxin and capsule genes in the early to mid-1980s, with sequences being established for the virulence factor genes in the late 1980s and early 1990s, and those of the plasmids pXO1 and pXO2 at the

end of the 1990s with finer analysis of regulatory control of these and of other important genes, such as germination operon genes, and the development of specific probes and polymerase chain reaction (PCR) primers for detection purposes,

- completion of the genome sequence by Read et al. (2003) with its profound consequences,
- steady progress in further understanding of the pathogenesis of anthrax and the nature of resistance and immunity to infection and toxin action commencing with the excellent work of Welkos and colleagues in the 1980s and focusing heavily on the role of macrophages in the 2000s,
- awareness and early characterization of other potential virulence factors, such as the S-layer, the exosporium, and anthrolysin O, and
- the breakthrough in molecular typing of *B. anthracis* for epidemiological and forensic purposes under the leadership of Keim, Jackson, and colleagues.

As in the two decades after World War II, the justification for much of the research from 1980 to the present day has been the need for an improved vaccine for administration to humans, again reviewed separately (Turnbull, 2010).

The period also saw the opportunity taken to apply the advancing technologies to the study of the natural disease in enzootic wildlife areas revealing valuable information about the natural ecology and epidemiology of the disease (WHO, 2008).

One particular sequel of significance to the anthrax letters has been a new look at approaches to diagnosis and treatment of anthrax, although this again focused heavily on inhalation anthrax as acquired by putative deliberate release scenarios.

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