"BACILLUS ANTHRACOIDES."

A STUDY OF ITS BIOLOGICAL CHARACTERS AND RELATIONSHIPS AND ITS PATHOGENIC PROPERTIES UNDER EXPERIMENTAL CONDITIONS.

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Introduction.

It has long been recognised that within the group of Gram-positive aerobic sporing bacilli there occur saprophytic organisms which simulate the anthrax bacillus closely, both in their morphological and cultural characters, for example, in the specially characteristic appearance of surface colonies on culture medium. Organisms of this type have been described in bacteriological literature as "anthrax-like" bacilli, *B. anthracis similis* (McFarland, 1898), *B. pseudo-anthracis* (Burri, 1894; Hartleb and Stutzer, 1897), *B. anthracoides* (Hüppe and Wood, 1889; Bainbridge, 1903; Ponder, 1912; and others), and the biological relationship of such organisms to *B. anthracis* and other members of the group is of some interest and practical importance. In the routine examination of pathological and other material for *B. anthracis*, such organisms may be encountered and inoculation tests in animals may be considered necessary to ensure their differentiation from the anthrax bacillus. These organisms, however, have not been systematically studied and there is some confusion in the literature regarding their various characters and relationships. Further reference will be made to this later.

The attention of the writer was first drawn to these organisms in the examination of shaving brushes from a consignment which had been reported to be contaminated with *B. anthracis*. These brushes were found to contain large numbers of organisms presenting the general morphological and cultural characters of the anthrax bacillus, their colonies, for example, being very similar, though the subsequent biological tests and inoculation experiments in animals differentiated them clearly from this organism. The inoculation tests proved that they were not devoid of pathogenic properties and at first raised the question as to whether they were attenuated forms of *B. anthracis*. The pathogenicity of this type of organism under experimental conditions seemed therefore not only of considerable biological interest but also of practical importance in view of its apparent relationship to *B. anthracis*, and the initial observations thus led to a biological and experimental study of various strains from different sources and a further consideration of their relationship both to the anthrax bacillus and other members of the group

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of Gram-positive aerobic sporing bacilli. For convenience, such organisms biologically resembling *B. anthracis* will be designated *B. anthracoides*. In regard to the finding of this organism in shaving brushes, it may be quoted here that Page (1909), while examining a large number of samples of bristles and horse hair for anthrax bacilli, frequently encountered "anthrax-like" bacilli with colonies closely resembling those of *B. anthracis*, and recognised three types, one of which corresponded with the *B. anthracoides* described by Bainbridge.

Apart from *B. anthracis*, the well-known representatives of the group to which *B. anthracoides* belongs, *e.g. B. subtilis*, *B. mesentericus*, *B. megatherium*, *B. vulgatus*, have generally been regarded as non-pathogenic both under natural and experimental conditions. It will be shown that, in contrast with these organisms, *B. anthracoides* possesses pathogenic properties. Attention, however, has been drawn by various workers to the presence of organisms described as *B. subtilis* in certain pathological conditions, *e.g.* infection of the eye, especially where there has been a penetrating wound of the globe. The presence of *B. subtilis* in eye infections is discussed by Axenfeld (1908), who also reviews the literature. Gourfein (1904) found bacilli of this group so frequently present in the conjunctiva that he spoke of a "Subtilis conjunctivitis." Michalski (1904) also reported a house epidemic of acute conjunctivitis which he attributed to "*B. conjunctivitidis subtiliformis*"; this organism, he stated, resembled in many cases *B. subtilis* and in others *B. megatherium*. Baenziger and Silberschmidt (1902) have reported two cases of purulent iridocyclitis, the causal organism of which was stated to be the *B. subtilis*. Stregulina (1906) carried out many experiments with a view to obtaining pathogenic strains of *B. subtilis* from soil. Out of 25 strains obtained from this source, 16 were virulent to guinea-pigs and three produced a typical panophthalmitis similar to that described by Silberschmidt (1903). Sheen and Klein (1915) described an infected wound of the finger from which an "anthrax-like" bacillus was isolated. This organism was found to be pathogenic to mice and it was concluded that it belonged to the class of organisms which have been described and termed "Anthracoid." More recently, Sweany and Pinner (1925) have reported the presence of a pathogenic *B. subtilis* which was isolated *post mortem* from the heart blood of a tuberculous patient, and quote Stueber (1921) as describing a case of acute haemorrhagic panophthalmia which was at first diagnosed bacteriologically as an anthrax infection. This is of special interest in view of the close resemblance in morphological and cultural appearances to *B. anthracis* exhibited by certain members of the subtilis group, *e.g. B. anthracoides* referred to above. These authors also quote Kelemen (1924) as describing a case of sepsis and pneumonia which was caused by *B. subtilis*. Bais (1927) has recorded an instance where *B. subtilis* was isolated from the blood of a Javanese coolie on two separate occasions. This patient suffered from a continued fever with severe chills and a painful cough. On examination his right lung showed signs of disseminated infiltration

which later went on to cavitation of the lung. There was no malarial parasite demonstrable in the blood and on autopsy there was no evidence of a tubercular infection. Kayser (1902) and others have found *B. subtilis* pathogenic when injected into the anterior chamber of the eye in rabbits, but they found that death resulted only after the intraperitoneal injection of large doses of the organism. Other pathogenic effects have been attributed to the *B. subtilis*. Seitz (1913) reported that he had isolated *B. subtilis* from the faeces of a patient with acute enteritis and that mice died after the injection of living or dead bacilli of this strain. In addition, he found that when cultures were introduced by the mouth, these animals developed an acute enteritis and the bacillus was recovered from the spleen and heart blood. Much confusion exists in the literature regarding this group of organisms—their classification and nomenclature—and is due to many earlier workers assuming that the organisms of this group which they met with, whether non-pathogenic or feebly pathogenic, were either *B. subtilis* or *B. megatherium*. They omitted, however, to give a detailed account of the morphological and cultural characteristics of the organisms they describe, and it seems possible that the organisms reported as being pathogenic *B. subtilis* have in reality been species other than the classical *B. subtilis* (Ehrenberg). The designation "*B. subtilis*" is still frequently used to designate various representatives of the group.

Preliminary observations on strains of *B. anthracoides* isolated from shaving brushes.

In the initial investigation, five strains were isolated in pure culture from different shaving brushes. These were recognised in the primary cultures from the brushes by the very close similarity of their colonies to those of *B. anthracis*, which consisted of an opaque centre of a pearl-grey colour and a less opaque periphery with an irregular wavy margin. The colonies had a ground-glass appearance and differed from those of *B. anthracis* only in the rather greater density of their centres and in their size, being slightly larger than those of *B. anthracis*.

The morphological characters of these organisms were as follows: large, Gram-positive bacilli, about the same average size as the anthrax bacillus, occurring singly, in pairs and in chains which were rather short. The ends of the bacilli were "square cut," but were not so definitely rectangular as *B. anthracis*; spores mostly central, though in many individuals, slightly eccentric, approximately of the same cross diameter as the bacilli. One-sixth of an agar slope culture of each of these strains was injected subcutaneously in mice. One strain proved lethal in 24 hours. On autopsy there was some degree of subcutaneous oedema of a gelatinous nature present at the site of inoculation: this, however, was localised and did not involve a wide area as in an infection with *B. anthracis*. The spleen was somewhat enlarged, soft, and congested, and there was some exudate present in the peritoneal cavity. In addition, the peritoneal surface of the bowel, especially of the small

intestine, showed marked congestion. Films from the local lesion and the peritoneal exudate showed the bacilli in large numbers. Bacilli were also present in films of the heart blood, and while tending to occur singly and in pairs, showed a few short chains. When stained by the polychrome methylene blue method of McFadyean, there was no evidence of capsule formation. A smear from the spleen showed fairly numerous bacilli but again no capsule was demonstrable. The organism was recovered in culture from the heart blood, spleen and local lesion. A 24 hours' agar slope culture of the original strain was also injected intraperitoneally into a guinea-pig, which survived. Further inoculation tests were then carried out with the other four strains, one-sixth of an agar slope culture being injected intraperitoneally in mice. On the day following the inoculation, 0·2 c.c. of sterile saline was injected with a syringe into the peritoneum of each mouse and immediately aspirated and plated on agar. Typical colonies were obtained, and from these agar slopes were inoculated and again one-sixth of an agar slope culture was injected intraperitoneally into mice. On the following day the mice injected with three of the strains died. The autopsy in each case showed similar features to those already described, although there was very little subcutaneous oedema and the bacilli were not very numerous in the heart blood. The bacilli were recovered in culture from the heart blood of each mouse. This procedure was again repeated with the remaining strain and this time the mouse died in 24 hours, the features at autopsy being identical with those already described. In this manner five strains were obtained from shaving brushes, which proved virulent to mice either immediately after isolation or after one or two passages.

A further batch of shaving brushes from the same consignment was examined later, and of seven brushes, each from a separate box, four yielded growths of similar organisms of the *B. anthracoides* type.

THE BIOLOGY OF *B. anthracoides*.

An attempt was now made to obtain some information as regards the natural habitat of *B. anthracoides*. It should be noted that organisms of this type have been isolated from earth (Silberschmidt, 1903; Stregulina, 1906) and water (Zikes, 1903), as well as from materials commonly examined for the presence of *B. anthracis*. An idea of the nature of material from which *B. anthracoides* was isolated and the number of strains obtained, is given in Table I.

It must be stated that in the examination of the materials detailed below, many organisms of the *B. subtilis* group were encountered, but only those strains which showed "anthrax-like" colonies on agar and which were pathogenic to mice were considered to be *B. anthracoides*. All of the 25 strains of *B. anthracoides* obtained from the above sources show a close similarity in their biological characters and each strain is pathogenic to mice, lethal effects being obtained on subcutaneous injection of one-fifth of an agar slope culture.

Table I.

	No. of specimens examined	Material	No. of strains of *B. anthracoides* obtained
1	13	Shaving brushes	9
2	6	Dogs' hair	4
3	6	Rabbit fur	2
4	6	Guinea-pig hair	2
5	6	Earth	1
6	6	Water	0
7	6	Dust	2
8	6	Air	0
9	3	Sheep's wool	3
10	2	Oil cake	2
	Total 60		Total 25

Morphology.

Films from agar cultures show large, straight sporing bacilli, rectangular in shape with square ends, although some exhibit slight rounding of the ends. Chains are seen but there is no great tendency to form long chains. Most of the spores are central but many occupy a slightly eccentric position and are approximately of the same cross diameter as the bacilli. The bacilli are motile with peritrichous flagella, but the motility is moderate and can only be seen as a rule in young cultures where the bacilli occur singly or in pairs; in this respect they differ entirely from *B. anthracis*. The staining reaction is Gram-positive and in films of heart blood or spleen smears there is no evidence of the presence of a capsule when stained with polychrome methylene blue, contrasting again with *B. anthracis*. In blood films and spleen smears the bacilli show a greater resemblance to *B. anthracis*; the ends are more square and there is a greater tendency to chain formation.

Resistance of Spores.

The spores possess considerable resistance to heat, being killed only after 15 minutes' exposure in a steam steriliser at 100° C.

Cultural Characters.

The organism grows well on ordinary media, growth taking place quickly at 37° C. and rather slowly at room temperature.

Surface Colonies on Agar Plates. Single colonies are fairly large (7–8 mm. diameter) and of a pearl-grey to greyish-white colour, and are slightly moist and shining. They are slightly raised, with an opaque centre and a more transparent irregular margin, and on the whole present a ground-glass appearance. They adhere slightly to the medium. When examined under the low power, the appearance of the margin of the colony is identical with that of a colony of *B. anthracis*. Single colonies on agar are indistinguishable from those of *B. anthracis*.

Single Stroke Culture on Agar. There is a luxuriant greyish-white growth along the line of inoculation, opaque at the centre but becoming more transparent at the edge, which is fluffy and irregular in contour and has a

ground-glass appearance. The growth is moist and shining, and although slightly adherent to the medium, can be easily emulsified.

Stab Culture in Agar. Growth takes place along the needle track with very slight rounded lateral outgrowths, the growth being more dense in the upper part of the medium.

In *Bouillon* a uniform turbidity is produced with, at first, a delicate pellicle which, however, is not permanent. Flakes of growth form which float in the medium and later sediment to the bottom of the tube.

Stab Culture in Gelatin (15 *per cent.*). Liquefaction takes place, starting at the surface on the fifth day and spreading downwards along the line of inoculation in a funnel-shaped manner. Lateral outgrowths are present and are more marked in the upper part of the needle track but are not so pronounced as the spiking of *B. anthracis*. Liquefaction takes place more quickly than in the case of *B. anthracis*.

Colonies on Gelatin Plates. Single colonies are greyish-white, have a woolly fringed appearance and lie in a cup-shaped area of liquefaction.

On *Potato* a moist, creamy-white growth occurs which later becomes reddish-brown in colour. With some of the strains the colour tends to assume a dirty greyish-brown appearance. Kohler (1921), in examining 27 strains of "anthrax-like" bacilli, differentiated 8 types. Of these, Type 5 gave a citron-yellow colour on potato, while Type 8 gave a reddish growth.

In *Litmus Milk* there is a slight acidity at first, with later coagulation of the milk. Still later, digestion takes place.

On *Blood Agar*: a single colony on blood agar after 24 hours' incubation shows haemolysis beneath and extending slightly beyond the margin of the colony. In 48 hours the area of haemolysis has increased in size and surrounds the colony, diffusing into the medium. Jarmai (1913), Hallermann (1925), and Hutyra and Marek (1926) state that this feature can be used as an aid to the differentiation between anthrax and anthrax-like bacilli.

On *Solid Serum* the medium rapidly becomes liquefied, starting on the second day and becoming complete in 6 days.

Biochemical Reactions.

B. anthracoides produces acid in glucose, saccharose, maltose, dextrin, salicin and glycerin, but not in lactose or mannite. There is no gas formed. There is no hydrolysis of starch.

I append a brief summary of the descriptions of organisms of this type isolated by some of the earlier workers, and note their observations.

B. Anthracoides—Hüppe and Wood, 1889.

Habitat. Isolated from earth and water.

Morphology. Bacilli of same size as *B. anthracis*. Ends more definitely rounded; endospores present. Non-motile. Grows well at room temperature.

Gelatin. White felted woolly maze. Stab culture liquefies like Anthrax bacillus.

Potato. White dry growth but not like *B. mycoides.*

Litmus milk. Reaction alkaline; milk coagulated.

Bouillon. No pellicle—woolly masses under the surface.

Pathogenicity. Not virulent to white mice. Large doses in guinea-pigs produced local inflammatory reaction.

B. pseudoanthracis—Burri, 1894.

Habitat. Isolated from "American meat powder."

Morphology. Generally in fairly long chains. Spore formation takes place in 24 hours. Motile but movement rather slow and only seen in short chains and single bacilli.

Gelatin plates. Irregular, roundish, greenish-yellow colonies; with lens shows convoluted thread-like appearance. Later, irregular star-shaped colony surrounded by thickish fluid.

Gelatin stab. Liquefaction starting in funnel-shaped manner in 2 days—complete in 4–6 days.

Single stroke on agar. Greyish growth, irregular at edge. Does not spread over whole slope. Growth soft and easily broken up.

Potato. Greyish-white growth which becomes moist and shining but not folded.

Bouillon. Turbidity, later pellicle which increases in thickness in a few days and broth becomes clear. If tube shaken, pellicle breaks off and falls to bottom but another forms in 24 hours.

Milk. In 2–3 days, coagulation—no acidity.

Pathogenicity. Not virulent to white mice.

B. Anthracis similis—McFarland, 1898.

Habitat. Isolated from pus.

Morphology. Large rectangular bacillus, ends slightly rounded. Tends to form long chains in which ends of bacilli are flattened. Spore formation takes place in 24 hours. Non-motile.

Gelatin stab culture. Growth identical with that of *B. anthracis.*

Single stroke on agar. Growth similar to Anthrax, forming a continuous growth, greyish-white in colour, feathery at the edges.

Single colonies on agar. Large, flat, translucent, and fluffy at the edges. Filaments form parallel wavy bundles at edge of colony like Anthrax bacillus.

Potato. Luxuriant, dry, whitish growth. When old, looks somewhat scaly.

Bouillon. Pellicle is formed but sediments in a few days.

Pathogenicity. No effect in white mice or guinea-pigs.

B. Anthracoides—Bainbridge, 1903.

Habitat. Isolated from Chinese horse hair.

Morphology. Closely resembles *B. anthracis.* Ends slightly rounded and forms short chains. Spores central. Motile.

Gelatin stab culture. Liquefaction begins early and is complete in 48 hours.

Single colonies on agar. Can hardly be distinguished from colonies of *B. anthracis.* Colonies have opaque centre with more translucent fluffy rim. Wavy felted appearance of margin.

Single stroke on agar. Continuous growth with finely granular appearance and fluffy edges, surface moist. After 48 hours' growth becomes pitted.

Bouillon. Slight turbidity, with white, slightly stringy growth which sinks to the bottom.

Milk. Acidified, coagulated and finally digested.

Pathogenicity. Virulent to mice, but not to guinea-pigs.

It is apparent that although the strains of "Anthrax-like" bacilli described by these earlier workers bear a close resemblance to each other and to *B. anthracis* and the *B. anthracoides* described in this paper, especially in the

appearance of their colonies on agar and gelatin, there exists a certain amount of variation in the characters described. For example, Hüppe and Wood, and McFarland found that their strains were non-motile and in addition the features exhibited by these strains, when grown on potato, in milk and in bouillon, vary considerably from those described by Burri and by Bainbridge. The *B. anthracoides* described earlier in this paper corresponds more closely to that of Bainbridge than to the strains reported by other workers, and these two organisms show a close similarity in their various features (more especially in regard to pathogenicity), contrasting with the strains of Hüppe and Wood, Burri, and McFarland.

PATHOGENICITY TO MICE AND PASSAGE EXPERIMENTS.

Following the observation that these organisms were virulent to mice and especially since they produced a condition of gelatinous oedema of the subcutaneous tissue at the site of inoculation which was very similar to the inflammatory oedema produced by the anthrax bacillus, an attempt was made to increase their virulence by passage through mice. The culture used was one that had been isolated from dog's hair, and the dose employed was one-fifth of a 24 hours' agar slope culture which was injected subcutaneously.

The mouse used in the first passage died in 24 hours and showed the *post mortem* features already described. The bacilli were recovered in pure culture from the heart blood, subcultured on agar and inoculated into a second mouse, which also died in 24 hours and presented a similar *post mortem* picture. The *post mortem* appearance of the third mouse was similar to the two previous ones, but in addition the subcutaneous oedema was more gelatinous and extended over a greater area. There was also a greater tendency to chain formation of the bacilli in the heart blood. In the fourth and fifth passages the subcutaneous gelatinous oedema was again well marked and the bacilli were more numerous in the heart blood and spleen smears. In the sixth passage the whole of the subcutaneous tissue was very oedematous and the pleural and peritoneal cavities contained an excess of fluid. Bacilli were numerous in the heart blood and spleen smears. The mouse inoculated for the seventh passage died 4 hours after the injection and on autopsy showed only very slight subcutaneous oedema and the bacilli were rather scanty in the heart blood and spleen smears. The passage was repeated and this time the mouse died in 24 hours. There was marked gelatinous oedema of the subcutaneous tissue which was somewhat haemorrhagic. The spleen was slightly enlarged, soft and congested, and there was an excess of fluid in the pleural and peritoneal cavities. Bacilli were numerous in the heart blood and spleen smears.

In the eighth passage the animal survived. It was repeated without a lethal effect. The passage was again repeated and this time the animal died in 24 hours. The subcutaneous oedema was not so pronounced and the bacilli were less numerous in heart blood and spleen smears.

In the ninth passage the mouse survived. At this stage, from the results

obtained in passing this organism in mice, it was inferred that individual mice vary in their powers of resistance to the organism. This was demonstrated by injecting two animals of equal weight subcutaneously with the same dose of cultures as in the following experiments:

A. Culture before passage:
   Mouse (1) died in 24 hours.
   Mouse (2) survived.

B. Culture after sixth passage:
   Mouse (1) died in 24 hours.
   Mouse (2) survived, but died later (third day).

The appearance on autopsy of mouse (1), Experiment A, was as described above, with well-marked subcutaneous gelatinous oedema, but in the case of mouse (1), Experiment B, there was very little subcutaneous oedema and the bacilli were not very numerous in the heart blood and spleen smears. In Mouse (2), Experiment B, the oedema was still less pronounced and the bacilli were very scanty in the heart blood and spleen.

It appears then that the virulence of this organism can be increased on passage to a certain degree, as evidenced by the more pronounced subcutaneous oedema present on autopsy and the greater number of bacilli in the heart blood and spleen smears. The extent, however, to which the virulence can be raised seems to be limited, and it evidently cannot be raised to a sufficient degree to produce a pathogenic effect in specially resistant animals. Silberschmidt (1903) encountered this variation in the susceptibility of individual mice to similar organisms isolated by him from two cases of panophthalmitis.

Pathogenicity to guinea-pigs.

A culture of the strain used for the passage experiments in mice was injected intraperitoneally into a guinea-pig without ill effects, the dose being one-fifth of an agar slope culture. The bacilli were recovered by injection of sterile saline into the peritoneum and plating the aspirated fluid. A second guinea-pig was then inoculated intraperitoneally, the dose this time consisting of the whole agar slope culture. This animal died in 24 hours.

Post mortem there was no subcutaneous oedema seen but there was an excess of blood-stained exudate present in the peritoneal cavity. The intestines, especially the small intestine, showed marked congestion and haemorrhage, while the spleen was only slightly enlarged and congested. The bacilli were present in films of the heart blood and spleen but were not numerous. The bacilli were recovered from the heart blood and peritoneal exudate, and an agar slope culture was now injected subcutaneously into a guinea-pig, which died in 24 hours. As before, there was no marked local reaction at the site of inoculation. There was a large quantity of blood-stained exudate present in the peritoneal cavity and the small intestine showed very intense congestion with numerous petechial haemorrhages. The spleen was not enlarged and was

only very slightly congested. The bacilli, with some enterococci, were present in large numbers in the peritoneal exudate but were still very scanty in the film of heart blood from which, however, they were recovered on culture.

An attempt was made to increase the virulence of the bacilli by passage through guinea-pigs. This, however, was not successful and the passage did not raise the virulence of the organism to any considerable extent.

It is interesting to note the marked enterotropism which occurred not only on intraperitoneal inoculation of the guinea-pigs but also when the bacilli were injected subcutaneously. Silberschmidt noted this effect after intraperitoneal inoculation of his two strains, but stated that guinea-pigs withstood subcutaneous injection of much larger doses than were lethal when injected intraperitoneally. The dosage he employed was a whole agar slope culture for intraperitoneal injection, but he was unable to kill guinea-pigs by the subcutaneous route and found that injection of the bacilli by this route only produced a localised abscess. Abscess formation at the site of inoculation in animals which survived the injection of these bacilli has not been observed, and in the case of guinea-pigs which died there was little local inflammatory reaction.

Bullock and Cramer (1919) have used the injection of ionisable calcium salts along with non-pathogenic cultures of *B. welchii* and *Vibrion septique* as a means of producing "kataphylaxis" or "défense rupture" with subsequent exaltation of the virulence of these organisms; the salts used were either calcium chloride or calcium nitrate. Experiments were carried out to ascertain if this method would increase the pathogenicity for guinea-pigs of *B. anthracoides*. The minimal lethal dose of calcium chloride was first ascertained, and a sublethal dose was injected along with a sublethal dose of the organism. The guinea-pig, however, survived the injection, and the experiment, when repeated, gave similar results.

The dose was then increased until an amount was given which was just lethal, and this was injected into a guinea-pig along with a sublethal dose of calcium chloride with a view to ascertaining whether a more pronounced septicaemia would result. The *post mortem* features, however, were in no way different from those already described, and it is noteworthy that although the bacilli were recovered on culture from the heart blood and spleen, they were only scanty in the spleen smear and were not observed in the films of heart blood.

The injection of a lethal dose of calcium chloride along with a lethal dose of *B. anthracoides* caused no difference in the *post mortem* findings.

An attempt was made to increase the virulence of the organism by injecting it into mice along with emulsions of other organisms that had been killed by heat. For this purpose killed cultures of *Staphylococci*, *B. coli* and *B. proteus* were used along with a sublethal dose of *B. anthracoides*. The mice survived the injections except where the bacilli had been injected along with a killed culture of *B. proteus*, when the mouse died in 24 hours, with the usual

post mortem findings. Owing to the varying susceptibility of mice to injections of *B. anthracoides*, this was not regarded as conclusive evidence of an increase of virulence and the experiment was repeated, using guinea-pigs. With guinea-pigs, however, a sublethal dose of the organism when injected along with a killed agar slope culture of *B. proteus* failed to produce any effect. Similarly, no increase of virulence was obtained on injecting the organism along with a filtered culture of *B. proteus*.

Feeding experiments were carried out with a view to repeating the observations of Seitz (1913) who reproduced an enteritis in mice by feeding them with a pathogenic *B. subtilis* isolated from a case of acute enteritis. Mice were therefore fed with a culture which had previously been ascertained to be virulent on subcutaneous injection. Throughout the experiment none of the mice under observation showed any ill effects, although the bacilli were recovered from their excreta.

It is of interest to note that injections of *B. anthracoides* killed by exposure to 100° C. for 15 minutes produced no ill effects in mice. Similarly the injection of the sterile filtrate of a 24 hours' broth culture proved non-toxic.

Animal experiments with related organisms.

For purposes of comparison the pathogenicity of various organisms of the Subtilis group was ascertained. In all, 49 strains have been tested. Certain of these were standard cultures:

From the National Collection of Type Cultures (one strain of each):

B. subtilis—Hay.
B. mesentericus—Jordan Lloyd's type B I.
B. megatherium—Lister Institute.
B. mycoides—C.R. II.

From this laboratory:

B. subtilis (three strains).
B. mesentericus (one strain).
B. megatherium (one strain).
B. mycoides (one strain).

All of the standard strains proved harmless to guinea-pigs and when injected subcutaneously into mice proved to be non-pathogenic even if the entire growth from a 24 hours' agar slope culture was inoculated.

In addition to these strains of the better known members of the group of Gram-positive aerobic sporing bacilli, thirty-nine strains were isolated which differed biologically from them and from *B. anthracoides*. These were also found to have no pathogenic effects even when very large doses were injected into mice, with the exception of one strain which had the following characters.

This strain was isolated from a specimen of catgut.

Single colony on agar. Colony moderately large—9–10 mm. in diameter—and of a pearl-grey colour, opaque from the centre just to the margin where there is a narrow transparent edge. The margin of the colony is slightly irregular.

Single stroke culture on agar. Growth is greyish-white in colour, slightly dry with a tendency to irregular lateral outgrowths especially at the bottom of the tube. The growth is fairly adherent to the medium.

Stab culture in agar. Growth takes place along the inoculation stab, with fine rounded outgrowths especially at the top of the medium.

Bouillon. Growth confined to a thick pellicle on the surface.

Single colony on gelatin. Colony of a yellowish-grey colour; granular, with slight hairlike outgrowths.

Stab culture in gelatin. Growth takes place along the line of inoculation with short lateral outgrowths in the upper part of the medium. Liquefaction is saccate and starts on the sixth day.

Potato. Growth dry and white in colour and has an appearance as of coiled white threads after 24 hours' incubation. Later, the colour becomes brownish grey.

Litmus milk. Slight acidity; no clot or digestion.

Blood agar. Marked haemolysis in 24 hours.

Solid serum. Liquefaction starting in 48 hours and complete in 4 days.

Biochemical reactions. Acid in glucose, saccharose, maltose, mannite, salicin, dextrin, and glycerol—not in lactose. No hydrolysis of starch.

Pathogenicity. When a large dose (*i.e.* a whole agar slope culture) is inoculated subcutaneously into a mouse, the animal dies in 24 hours. On autopsy there is only a slight inflammatory reaction at the site of inoculation and the spleen is slightly congested and not enlarged. Bacilli are present but are rather scanty in films of heart blood and spleen smears.

The identification of the pathogenic strain described above presents some difficulties, for while the organism corresponds in most details to *B. subtilis*, the appearance of colonies on agar resembles those of the *B. megatherium*. It should also be noted that the appearance of the growth of this strain on potato differs entirely from that of any of the classical types of the group.

BIOLOGICAL RELATIONS OF *B. anthracoides* TO OTHER MEMBERS OF THE GROUP OF GRAM-POSITIVE AEROBIC SPORING BACILLI.

Before discussing the position of *B. anthracoides* in the group of Gram-positive aerobic sporing bacilli, it is useful to compare some of its morphological and cultural features with those of the well-known members of this group.

The most important organism of this group for which *B. anthracoides* may be mistaken is *B. anthracis*, owing to the similarities of some of their morphological and cultural characters already described. These organisms differ in the following respects: *B. anthracoides* is motile, flagellate and possesses no capsule and, furthermore, it can be differentiated from *B. anthracis* when grown in bouillon and on potato, and by the haemolysis it produces when grown on blood agar. It is necessary, however, when examining *B. anthracoides* for motility to use cultures that are very young, since older ones may contain a few single motile bacilli which can well be overlooked. Similarly the pellicle which *B. anthracoides* forms on broth is fragile, and if not examined early enough may appear as flakes floating in the broth or may have deposited.

B. anthracoides has distinct haemolytic powers as contrasted with

B. anthracis, which shows very little or no haemolysis. Hallermann, and Hutyra and Marek lay stress on this as an aid in distinguishing anthrax from "anthrax-like" bacilli. Jarmai (1913) states that "anthrax-like" bacilli can be differentiated from *B. anthracis* more easily and quickly by their haemolytic properties, the presence of a zone of haemolysis round a colony on blood media indicating an "anthrax-like" organism, while the absence of haemolysis indicates a pathogenic *B. anthracis*.

These characters are sufficiently definite to differentiate *B. anthracis* and *B. anthracoides* without having to resort to animal inoculation tests. If inoculation tests are made with *B. anthracoides* in *large doses*, mistakes may arise through confusing it with virulent *B. anthracis*. On the other hand, if small doses are used, animals will tolerate the *B. anthracoides* while *B. anthracis* produces typical pathological effects. Even in the case of animals dying after injection with *B. anthracoides*, the relatively small numbers of bacilli in the heart blood and spleen, the slight tendency to chain formation and the absence of capsule differentiate this organism from *B. anthracis*.

In the differentiation of "anthrax-like" bacilli and *B. anthracis*, Jarmai considers the haemolytic properties and the absence of a capsule of the anthrax-like bacilli to be most important. Lehmann and Neumann (1927) consider that the best differentiation is the active motility of the "anthrax-like" bacilli, but are uncertain whether pathogenic strains of such bacilli might not in reality be motile strains of *B. anthracis*. According to Pokschischewsky (quoted by Lehmann and Neumann), anthrax-like bacilli give a positive but weak Ascoli reaction and are pathogenic to mice and guinea-pigs.

A satisfactory comparison of the *B. anthracoides* with the *B. subtilis* described by Ehrenberg (1838) and later by Cohn (1875) cannot be made as these writers based their observations almost entirely on the morphological features of the organism. It is interesting, however, to note that Chester (1904) in his review of the *Bacillus subtilis* group of bacteria paid particular attention to the morphological characters of this group, and stated that "the essential features of the present system of classification of the *B. subtilis* group are morphologic rather than cultural."

For comparative purposes reference has been made to the following authorities for descriptions of the better known members of the group: *B. subtilis* Ehrenberg (see Migula, 1900), *B. mesentericus fuscus* (see Flügge, 1886), *B. mesentericus vulgatus* (see Flügge, 1886), *B. ellenbachiensis* Stutzer, 1898, *B. megatherium* de Bary (Lehmann and Neumann, 1927). The characters of *B. mycoides*, *e.g.* the feathery rhizoid colonies, are sufficiently definite to allow of this species being sharply differentiated from other closely related organisms.

It is obvious from a comparison of the characters of *B. anthracoides* and *B. subtilis* that these organisms show a great dissimilarity in many of their features, *e.g.* colony appearances, growth on potato medium, and, in addition, the former does not correspond in detail with any of the other well-known members of the group, which according to Bergey (1923) includes over seventy

species. None of these correspond to the pathogenic strains recorded in this paper, which, however, in general characters resemble the *B. anthracoides* of Bainbridge and the "anthrax-like" bacilli described by Page.

In addition there occur frequently other Gram-positive aerobic sporing bacilli whose characters are not so well known, and it is often a matter of great difficulty to identify such organisms with classical types. This is exemplified in the case of the pathogenic strain described on p. 316. Various classifications of the group have been made, and that of Ford (1927) is particularly interesting in that he divides these organisms into two subgroups, one of which is pathogenic and includes *B. anthracis* Koch, *B. anthracoides* Hüppe and Wood, *B. aerobius sepis* Legros and Lecène, and *B. piliformis* Tyzzer.

It is unfortunate that most of the earlier workers, in describing "pathogenic *B. subtilis*," have failed to give a complete account of the morphology and cultural characters of the organisms they isolated. Certain of the biological features recorded, although now insufficient for satisfactory identification, indicate that the organisms described did not belong to this species. It would appear, therefore, that the claims of earlier workers regarding the pathogenicity of this species are not justified. Similarly, it is evident that the designation "*B. subtilis*" has been loosely used, and that until there is a satisfactory classification of the members of the group many of the organisms encountered cannot be accurately identified.

The pathogenic strains recorded in this paper differ in many respects from the classical *B. subtilis* as described by Migula, and it seems justifiable to place such organisms in a separate species to which is applied the designation *B. anthracoides* as used by Bainbridge and others.

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CONCLUSIONS.

1. There occurs in nature an organism belonging to the group of Gram-positive aerobic sporing bacilli which closely resembles *B. anthracis*, especially in its cultural characters.

2. These organisms occur commonly in materials that are frequently examined for the presence of *B. anthracis*.

3. The *B. anthracoides* is pathogenic to guinea-pigs and mice under experimental conditions, and would appear to occupy a position between the virulent *B. anthracis* and the non-pathogenic members of the group of aerobic sporing bacilli, *e.g. B. subtilis*, *B. mesentericus*.

4. Subcutaneous injection of cultures of *B. anthracoides* produces a local inflammation with gelatinous oedema and a fatal septicaemia.

5. Only large doses of living organisms are lethal and attempts to increase the virulence of this organism by various methods have not proved successful.

6. Individual animals vary considerably in their resistance to the organism.

7. With the exception of *B. anthracis*, the *B. anthracoides* contrasts with the other members of the group in its pathogenic properties under experimental conditions. Twenty-five strains of this organism have been isolated, each of which possesses pathogenic properties. The pathogenicity of 49 strains of other representatives of the group has been tested, and only one of these was found to have lethal effects.

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