STUDIES IN THE GROUP OF AEROBIC SPORING BACILLI - WITH PARTICULAR REFERENCE TO BACILLUS ANTHRACOIDES AND ANTI-ANTHRAX IMMUNITY

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GENERAL INTRODUCTION

The group of gram-positive, aerobic, sporing bacilli comprises a very large number of organisms which resemble each other closely in their morpho-:logical characters but differ in cultural appear-:ances and pathogenic properties. Of this group the anthrax bacillus was the first to be recognised and has been most thoroughly studied on account of its important pathological relationships in man and animals. The early recognition of this organism was possible owing to its relatively large size and its ability to grow readily on artificial media outside the body, and in fact, the discovery of the bacillus and the study of its morphological and biological characters constituted the starting point of critical bacteriological investigation. The anthrax bacillus was first observed by Pollender as early as 1859 in blood obtained from the "enlarged and pulpy" spleens of cattle that had died of the disease, and later by Davaine (1863) who applied to it the name Bacillus anthracis. At this period much attention was being paid to the occurrence of anthrax with a view to ascertaining the nature and means of spread of the disease. The causal relation of Bacillus anthracis to the disease was not, however, clearly established until the work of Koch was completed in 1876. Koch noted the appearance of division of the bacilli in the blood and deduced that multiplication took place in /

in the tissues; he similarly observed division tak-:ing place outside the body and in addition noticed spore formation. He was able to isolate the bacillus in pure culture and by inoculating animals with such cultures to reproduce the disease. These observations were shortly afterwards confirmed by Pasteur (1878). At this time the conditions which brought about the spread of the disease were still unknown: Pasteur as a result of his experiment concluded that infection might arise from dead anthrax animals which had been buried in the ground, and with this hypothesis in mind directed his studies to discovering the behaviour of anthrax bacilli in soil. He found that when the organisms were mixed with soil they multiplied and then formed spores which were present and capable of producing a fatal infection even after some months had elapsed. At the same time he buried a sheep that had died of anthrax in the garden of a farm, and found that the bacilli remained alive and virulent in the ground two years later. In this experiment it was noted that a large number of the bacilli were found in the upper layers of the earth, and in addition to the natural contamination from the excreta of diseased animals, Pasteur surmised that this was due to worms carrying them from the deeper layers to the surface. In this way Pasteur was able to show that the carcass of an animal dead of anthrax may infect the soil, and that /

that the organisms present in the deeper layers of the earth can gain access to the surface and contaminate any pasturage growing in the vicinity. He demonstrated also, that infection could take place in animals by the oral administration of the organism along with sharp bristles which injured the mucous membrane. Since the original observations of Koch and Pasteur, anthrax infections as they occur in man and animals have been thoroughly investigated, until at the present day our knowledge of the etiology and pathology of the disease and of the biological characters of B. anthracis is fairly complete.

Anthrax is an acute infectious disease occurring epizootically in sheep and cattle, producing in these animals a rapidly fatal septicaemia with enlargement of the spleen and the characteristic multiplication of the bacilli in the blood. Geographically and zoologically it is one of the most widespread of infectious diseases and indeed is world-wide in its distribution; it occurs frequently in Europe and Asia though less commonly in Britain and America. Local epizootics of the disease have occurred occasionally in this country, but as a rule only a few sporadic cases are seen. The disease in the human subject is not common, and does not occur as a natural infection from man to man. It may, however, be communicated to man directly or indirectly from animals /

animals and is therefore most frequently seen in those whose occupations expose them to such contact. In this way three forms of the disease are met with: (1) as a skin infection in the form of a malignant pustule (2) as an infection of the respiratory organs in Woolsorter's disease and (3) only very occasionally as an infection through the alimentary canal.

The variation in virulence of B. anthracis for different species of animals is well known. Some of these e.g. cattle, sheep, guinea-pigs, mice, etc., are very susceptible to the disease while others such as rabbits, horses, and deer are less so. Certain animals on the contrary possess a natural immunity; this is specially seen in Algerian sheep, white rats, fowls and to a lesser extent in cats and dogs. Lower vertebrates such as fish and reptiles also possess a natural immunity to the infection. The human subject appears to occupy an intermediate position in the scale of susceptibility to anthrax, the disease only occurring in those in close contact with affected animals or contaminated articles and tending to remain as a localised infection.

In a similar way it is found that various members of the group of gram-positive, aerobic, sporing bacilli have different pathogenic effects in animals. It has generally been regarded that of this group only B. anthracis possesses pathogenic properties, and that the other species are saprophytic. There have been /

been recorded, however, instances where such apparently saprophytic organisms as "B. subtilis" have been responsible for infection in the human subject. This seems to have occurred most frequently in eye infections, although septicaemic conditions have occasionally been reported. This has been confirmed experimentally in animals, and it will be shown later that there exists in this group an organism, e.g. B. anthracoides, which closely resembles B. anthracis in its morphology and cultural characters, and is not devoid of virulence. If animal inoculation tests be carried out with this organism using large doses of culture, lethal effects can be demonstrated in mice and guinea-pigs. At the same time it is a remarkable fact that in this biological group B. anthracis alone, in the course of natural evolution should have been selected out with such highly specialised parasitic properties that it produces, when infection occurs, a virulent disease with, as a rule, fatal results in susceptible animals, while the other members of the species are either saprophytic or only very weakly pathogenic. Following Bail's (1910) method of classification of bacteria according to their power of producing aggressin, the group might be classified as follows:-

(1) true parasites which always produce aggressin e.g. B. anthracis; (2) "half-parasites" whose aggressin producing power is variable, e.g. B. anthracoides and other /

other weakly pathogenic species; (3) saprophytes e.g. B. subtilis (Ehrenberg), B. mesentericus, B. megatherium, B. mycoides, etc. The occasional record of instances in which "B. subtilis" has exhibited pathogenic effects would indicate that this organism might be classified as a half-parasite, but it should be noted that in such reports the identity of the so-called pathogenic "B. subtilis" was not clearly established owing to the omission in the published papers of important details of the biological characters of the organism. It must also be recognised that the designation "B. subtilis" has been loosely used to include various representatives of the species, and has not always been restricted to a single organism. This has occurred more particularly where observations have been based on the morphology and pathogenic properties of a member of this group without special attention being paid to its biological characters. It must be assumed therefore that the organisms of this group recorded as being pathogenic have not been satisfactorily identified as "Bacillus subtilis" although reported as such, and that the classical B. subtilis (Ehrenberg) must at present be regarded as a true saprophyte incapable of producing pathogenic effects in man or animals.

Like B. anthracis the saprophytic members of the group have a world-wide distribution in nature and can be found almost everywhere. Numerous organisms of this /

this species can be isolated from earth, water, vege-:table matter, human and animal excreta, dust and even from the air, and in addition to well-known organisms such as B. subtilis, B. megatherium, etc. many other bacilli are frequently encountered whose biological characters do not coincide with those of any classical strain, and it is often very difficult to identify them accurately. This will be referred to again.

In the study of this group of organisms attention need not be paid to the morphological and biological characters of B. anthracis as these are already sufficiently well known. Reference will be made later to the effect of the organism on certain tissues, and recent advances in relation to the artificial immunisa-:tion of animals will be discussed. It is necessary, however, to give a detailed account of the half-para-:sites i.e. the members of the group which are weakly pathogenic, and in the first section a description will be given of organisms which closely resemble B. anthracis, and a study will be made of their biological characters and relationships and their pathogenic properties under experimental conditions.

SECTION I A.

BACILLUS ANTHRACOIDES AND OTHER ORGANISMS CLOSELY RE-:SEMBLING THE ANTHRAX BACILLUS IN BIOLOGICAL CHARACTERS.

INTRODUCTION

It has long been recognised that within the group of Gram-positive aerobic sporing bacilli there /

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 bacilli presenting the general morphological and cultural characters of the /

the anthrax bacillus, their colonies, for example, being very similar to those of B. anthracis, though the subsequent biological tests and inoculation experiments in animals differentiated them clearly from this organism. The inoculation tests elicited the fact 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 re-:lationship 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 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 very closely resembling those of B. anthracis, and recognised three types, one of which corresponded with /

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 be regarded as non-pathogenic both under natural and experimental conditions. It will be shown how, in contrast with these, B. anthracoides possesses pathogenic properties when tested under experimental conditions. 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 gives a review of 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 a large number of experiments with /

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 to belong 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 haemorr-:hagic 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 /

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 intra-:peritoneal 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. A great deal of confusion exists in the literature regarding this group of organisms - their classification and nomenclature - and is due to the fact that many of the earlier workers assumed 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 described and it /

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 six shaving brushes were examined from a consignment reported to be contaminated with B. anthracis. The method employed was as follows :- the ends of the hairs of the brushes were cut off into a sterile mortar and were emulsified with saline. The emulsion was centrifuged and agar plates were inoculated from the deposit which was then injected into guinea-pigs. All these animals survived the injection but from five of the brushes organisms which closely resembled B. anthracis were isolated in pure culture. These were recognised in the primary cultures 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 /

larger than the colonies 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 were, in the majority of individuals, placed centrally, though in many, slightly eccentric spores were seen. The spores were approximately of the same cross diameter as the bacilli, (see fig. 10). 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, (see fig. 4). 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 /

methylene blue method of McFadyean, there was no evidence of capsule formation, (see figs. 1 & 2). A smear from the spleen showed fairly numerous bacilli but again no capsule was demonstrable, (see fig. 5). 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 1/6th 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 /

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 the 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 the B. anthracoides was isolated and the number of strains obtained, is given in the following table :-

	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

It must be stated that in the examination of the materials detailed above, 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 1/5th of an agar slope culture.

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. The majority of the spores are situated centrally 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, (see fig. 9) but the motility is not of a very active nature and can only be seen as a rule in young cultures where the bacilli occur singly or in pairs, and 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 /

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. The colonies 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. In fact, the resemblance of single colonies on agar to those of B. anthracis is so great that it is impossible

to /

to distinguish them.

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%). Liquefaction takes place, starting at the surface on the 5th 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 /

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 2nd day and becoming complete in 6 days.

Biochemical Reactions.

B. anthracoides produces acid in Glucose, Saccharose, Maltose, Dextrin, Salicin and Glycerin, but /

but not in Lactose or Mannite. There is no gas formed. There is no hydrolysis of starch.

It might be of interest to give a brief summary of the descriptions of organisms of this type isolated by some of the earlier workers, and to 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 /

yellow colonies; with lens shows convoluted thread-like appearance. Later, irregular star-shaped col-:ony 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 /

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 section, especially in the appearance of their colonies on agar and gelatin, there exists a /

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 to a considerable extent from those described by Burri and by Bainbridge. The B. anthracoides described above corresponds more closely to the organism described by 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 1/5th of a 24 hours' agar slope culture which was injected subcutaneously.

The mouse used in the first passage died in 24 hours /

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, (see fig. 3). 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 /

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 on this occasion 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 6th passage - Mouse (1) Died in 24 hours
Mouse (2) Survived, but died later (3rd 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 /

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 1/5th 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 intraperiton-:eally, 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 /

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 /

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"defense 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 B. anthracoides. The guinea-pig, however, survived the injection and the experiment, when repeated, gave similar results.

The /

The dose of B. anthracoides 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.

Bail (1910) has shown that if a non-lethal dose of a virulent organism be injected into a suitable animal along with the aggressin of that organism, the initial non-lethal dose becomes fatal. Following this observation experiments were carried out with anthrax "aggressin" which was obtained in the following manner :- An emulsion of B. anthracis in saline was made so as to be just faintly turbid and 0·5 c.c. were injected intraperitoneally into a guinea-pig which died in three days. The animal on autopsy showed lesions typical of an anthrax infection, and the peritoneal cavity was washed out with saline and the washings collected. The spleen

was removed and ground up in saline and this emulsion and the peritoneal washings were centrifuged at high speed for 1½ hours. The supernatent fluids from the spleen emulsion and peritoneal washings were then removed and each divided into three parts. To one part of each was added (1) phenol to a concentration of 0·5% (2) acriflavine 1 in 1000 and (3) chloroform 2%. These were allowed to stand for three days at the end of which time cultures were inoculated to test the sterility. It should be noted that it was found impossible to prepare an anthrax "aggressin" with toluol as a sterile preparation could not be obtained. The anthrax "aggressin" was now inoculated into guinea-pigs along with a dose of B. anthracoides which was just sub-lethal. The animals, however, survived the injection and showed no sign of illness. Similarly the injection of the peritoneal washings of a guinea-pig which died after an intraperitoneal inoculation of B. anthracoides along with a sub-lethal dose of B. anthracoides failed to have any effect on a fresh guinea-pig. In this experiment it was not thought necessary to sterilise the peritoneal washings which were merely centrifuged to remove the excess of bacilli. It would appear then that the injection of a sub-lethal dose of B. anthracoides along with a B. anthracis "aggressin" or an analogous preparation from the homologous organism does /

does not increase the virulence of the bacillus.

An attempt was made to increase the virulence of the B. anthracoides by injecting them 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 when the bacilli had been injected along with a killed culture of B. proteus; in this case 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 B. anthracoides, 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 a filtered culture of B. proteus along with B. anthracoides.

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 /

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 /

members of the group of Gram-positive aerobic sporing bacilli, thirty-nine strains were isolated which differed biologically from them and from the 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 hair-like 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 6th day.

Potato /

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 the B. anthra-:coides in the group of Gram-positive aerobic sporing bacilli, it is useful to compare and contrast some of the morphological and cultural features of the B. anthracoides with those of the well-known members of this group.

The most important organism of this group for which the B. anthracoides may be mistaken is the B. anthracis, owing to the similarities of some of their morphological and cultural characters. There are, however, differences in the characters of these two organisms, the most important being the motility with the possession of flagella and absence of capsule of the B. anthracoides. In addition, the appearance of cultures of the B. anthracoides when grown in bouillon and on potato, and the haemolysis when grown on blood agar are of assistance in making a differentiation from B. anthracis. These features, however, present some difficulties: for example, when examining for motility it is necessary to use a very young culture since in older cultures there may be very few single bacilli present and consequently the motility is not likely to be seen. Similarly the pellicle which forms on a broth culture of B. anthracoides is fragile and quickly becomes detached and broken off and if not examined early enough may appear /

appear as flakes of growth floating in the broth or as a sediment at the bottom of the tube.

Haemolysis appears to be more constant, the B. anthracoides having marked haemolytic powers as contrasted with B. anthracis, which shows very little or no haemolysis. Hallerman, and Hutyra and Marek lay stress on this fact as an aid to the differential diagnosis between anthrax and "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 with large doses of culture are carried out, the B. anthracoides might be mistaken for 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 infection 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 /

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, short descriptions will now be given of the more important morphological and cultural /

cultural features of some of the better known members of the subtilis group, these being taken, when possible, from the observations of the original workers. In the case of B. subtilis, the description given is taken from Migula, and B. megatherium from Lehmann and Neumann, as these were considered to be the best descriptions given by earlier writers.

B. SUBTILIS EHRENBERG (Migula, System der Bakterien, 1900)

Fairly large, Gram-positive bacillus, rounded at the ends and with central or subterminal spores, (see fig. 12). Motile, with peritrichous flagella. Optimum temperature 25-35. Aerobic.

Gelatin Plate Culture. The colonies appear first as small round white points lying in cup-like depressions. The centres of the colonies appear as greyish-white to yellow discs which are irregularly granular, with striae joining them to the periphery, the edge being either teased out or very finely toothed. With low magnification, colonies consist of a central area appearing as a mass of fine chains, the edge of which is characterised by innumerable fine hair-like tendrils.

Stab Culture in Gelatin. A white line of growth occurs with very fine lateral outgrowths. Liquefaction takes place in a funnel shaped manner and a membrane forms on the surface.

Agar /

Agar Slope Culture. A culture on sloped agar gives a thick grey growth.

On Potato a very luxuriant thick greyish-white growth occurs, covering the whole surface of the potato which later becomes very wrinkled.

B. MESENTERICUS FUSCUS (Flügge, Die Mikroorganismen, 1886).

Habitat. Hay dust, air, potatoes.

Morphology. Small, short, slender bacillus with rounded ends having a tendency to form roundish spores. Often occurs in pairs or short chains of about four bacilli. Bacillus is very motile and has peritrichous flagella.

Gelatin Plate Culture. Round, whitish colonies appear with sharp edges. Later, when liquefaction commences delicate outgrowths occur and the colonies become yellowish brown in colour and have a finely granular appearance.

Stab Culture in Gelatin. A whitish growth occurs along the needle track. Liquefaction starts in a funnel-shaped manner and is complete in 4-6 days.

On Potato a thick yellowish growth appears, spreading quickly over the whole surface of the potato. Later, the surface becomes wrinkled and brown in colour.

B. MESENTERICUS VULGATUS (Flügge, Die Mikroorganismen, 1886).

Often incorrectly designated "potato bacillus".

Morphology /

Morphology. Thick, fat bacillus often occurring in chains. Spores roundish to oval. Motile, with peritrichous flagella.

Gelatin Plate Culture. Colonies are bluish white in colour and are almost transparent; later, the centre becomes more opaque and white in colour and is sunk in fluid gelatin. With low magnification young colonies are seen to consist of definitely granular discs with rough edges.

Stab Culture in Gelatin. Liquefaction takes place in a funnel-shaped manner.

On Potato growth takes place as a very wrinkled thick white skin which quickly grows over the entire surface of the medium and is adherent to the potato.

Milk is coagulated and has alkaline reaction.

B. ELLENBACKIENSIS STUTZER (C. f. Bakt., II Abt. IV, 1898, 31).

Bacilli occur mainly in long chains but some single organisms are motile.

Culture on Gelatin Plates. Young colonies are whitish and appear like fine grains of sand. Older colonies have an irregular, roundish form and consist of a thick, white central area with an ill-defined edge composed of short teased-out filaments. Liquefaction occurs in 48 hours and colonies lie in crateriform depressions.

Culture on Peptone Agar (weakly alkaline).

There is no growth anaerobically.

Surface /

Surface Colonies. After 24 hours at room temperature there appear greyish-white, round, oily, shining colonies with sharp edges. When magnified, the colonies appear granular and later filaments develop round the colonies. The edges have the same appearances as anthrax colonies.

In Broth, which is slightly alkaline, a pellicle forms which, when broken off, sinks to the bottom of the tube.

On Potato (slightly alkaline), a yellowish grey thick growth occurs and is raised with slightly indented edges.

B. MEGATHERIUM DE BARY (Lehmann and Neumann, 1927, p. 610).

Morphology. Fairly large, thick, Gram-positive rods; ends not rounded; often occurring in long chains, (see fig. 13). States that organism becomes smaller after prolonged cultivation. Rather slowly motile by means of peritrichous flagella. Aerobic.

Gelatin Plate Culture. Surface colonies, when magnified, show a compact centre with, at the periphery, a row of rather long, very fine delicate hairs. Colonies resemble those of B. subtilis and B. mesentericus.

Stab Culture in Gelatin. Tube or sack-shaped liquefaction takes place along the stab, and liquid gelatin is turbid.

Colonies on Agar. White to greyish-white, slightly /

slightly elevated, moistly shining discs, with a delicate, extremely transparent zone. Later become opaque and yellowish brown in colour.

Agar Stroke Culture. As B. subtilis.

Bouillon. Moderately cloudy, often with pellicle formation.

Potato. Very similar to B. subtilis but colour more yellow and also has mealy appearance.

(see fig. 14)

A description of B. mycoides is not given as its characters are sufficiently definite (e.g. the feathery rhizoid colony) to enable an accurate identification to be made of this organism.

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 in 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 B. anthracoides recorded in this section, which, however, in general characters resembles the B. anthracoides of Bainbridge and the "anthrax-like" bacilli described by Page.

In addition to these there occur frequently other Gram-positive aerobic sporing bacilli whose characters are not so well known and it is often a matter /

matter of great difficulty to identify such organisms with classical types. This is exemplified in the case of the pathogenic strain described on p. 34. Various classifications of the group have been made, and that of Ford (1927) is particularly interesting in that he divides these organisms into two sub-groups, one of which is pathogenic and includes B. anthracis Koch, B. anthracoides Hüppe and Wood, B. aerobius sepis Legros and Lecene, 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 B. subtilis 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 above differ in many respects from the classical B. subtilis as described by Migula and it seems justifiable

to /

to place such organisms in a separate species to which is applied the designation B. anthracoides as used by Bainbridge and others.

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 Bacillus 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 repre-:sentatives of the group has been tested and only one of these was found to have lethal effects.

SECTION I B.

BACILLUS SUBTILIS

The great majority of the members of the group of Gram-positive aerobic sporing bacilli are incapable of producing pathogenic effects in man or animals, and exist essentially as saprophytes. These saprophytic organisms are ubiquitous and have, as has already been stated, a wide distribution in nature. They can frequently be isolated from earth and vegetable matter and, occurring as they do in large numbers in dust and air, are occasionally met with as laboratory contaminants. It is possible therefore that members of this group which have been found present on culture media inoculated with pathological exudates may have been regarded merely as contaminants which have gained access to the media at the time of inoculation, and been discarded sithout further investigation. At the same time it should be remembered that whereas organisms of this type can be found frequently in human and animal excreta, they are rarely seen in direct microscopical examination of pus or other pathological exudates. It has been stated that they may occur in chronic sinuses (Zinsser, 1927) but probably only when these are neglected and grossly contaminated with dirt, and such organisms when isolated prove to be harmless to laboratory animals even when injected in very large doses /

doses. It must be assumed, therefore, that the saprophytic members of this group have no relation to the etiology or progress of diseased conditions and when present indicate only that some degree of extraneous contamination has occurred. Such contaminating organisms have generally been referred to as "B. subtilis", without, as a rule, any reference being made to their biological characters, and in this way the term "B. subtilis" has been indiscriminately used to denote many members of the group and has not been reserved for a single classical type. The descriptions of B. subtilis recorded by Ehrenberg (1838) and later by Cohn (1875) do not include the cultural characters of the organism and although they are referred to as classical strains a standard type of B. subtilis possessing definite cultural characters with which other members of the group can be compared does not appear to have been universally recognised. The B. subtilis described by Migula (1900) has been contrasted earlier (p. 39) with B. anthracoides and it is interesting to compare now this organism with strains described by other authors. For this purpose short abstracts will be given of some of the cultural characters of organisms described as B. subtilis by Bergey and by Ford and in addition a description will be given from the writer's own observations of a strain of B. subtilis obtained from /

from the National Collection of Type Cultures.

B. subtilis (Ehrenberg) Cohn - quoted from Bergey.

Colonies on gelatin

Circular, whitish, entire, becoming creamy-white, spreading filamentous.

Gelatin Stab

Whitish surface growth, liquefaction stratiform.

Colonies on agar

Spreading, greyish, amoeboid with crenate margin.

Agar slope

Thin greyish-white, membranous, glistening, spreading, adherent.

Broth

Turbid with fragile pellicle and greyish sediment.

Potato

Luxuriant, warty, grey, becoming pink with vesicles over surface.

B. subtilis (Ehrenberg) Cohn - quoted from Ford.

Colonies on agar

Surface colonies weakly refractive, spreading concentrically or in amoeboid fashion from small dense nuclei; under the low power edges may be complete or finely crenate. If water of condensation be present one or two colonies frequently overgrow the entire plate. The colonies are usually membran-:ous, dry, hard, and glassy and can be separated from /

from the agar only with difficulty.

Agar slope

Weakly refractive, glassy, membranous growth along the line of inoculation but a spreading, dry, membranous growth on the surface of the agar extending to the wall of the tube.

Colonies on gelatin

Surface colonies round, homogeneous, spreading, thin and granular. Under low power are granular.

Gelatin stab

Slow growth along line of inoculation and rather slow cup-shaped surface liquefaction with scum production.

Broth

Single isolated pellicles appear on the surface in 24 hours. In 48 hours these unite to form a thin branching scum which gradually becomes more dense and tough. Medium grows turbid in first 24 hours, but later clears. Scum is precipitated as a whole in about ten days. This manner of scum formation is characteristic of B. subtilis.

Potato

Growth on potato characteristic. It is luxuriant and warty, having the appearance of many large and small dewdrops scattered along the line of inoculation. In 48 hours a pink pigment collects on top of this growth and persists. In older cultures a decided rose-red line in the substance of the potato marks the limit of the growth. In ten days the vesicles /

vesicles dry down and only a reddish-brown dry growth remains on the discoloured medium. Later the growth is moist and sticky.

B. subtilis Hay Lister Institute

Colonies on agar

Colonies are small, round, opaque to the periphery and show no irregularity of the margin.

Agar slope

Greyish-white growth which is moist and shining and slightly adherent to the medium.

Stab culture in agar

Growth limited to the upper part of the line of inoculation. Surface growth as on agar slope.

Colonies on gelatin

Colonies circular, greyish white in colour. Under low power edge shows fine hairlike outgrowths.

Stab culture in gelatin

Liquefaction stratiform. Very slow growth along line of inoculation; no lateral outgrowths.

Bouillon

Turbidity with rather thin pellicle; slight deposit also present.

Potato

Smooth, moist, yellowish-brown growth which later becomes a darker brown colour; growth not wrinkled.

It is obvious that the three strains of B. subtilis /

subtilis described above vary considerably from that recorded by Migula. For example the manner of liquefaction and the absence of lateral out-:growths in the stab cultures in gelatin together with the appearance of the growth on potato of these organisms contrast with the characters of Migula's strain. There is a close similarity, however, in the characters of the organisms described by Bergey and by Ford.

This variation of the descriptions by different authors of members of this group is not confined to B. subtilis but applies also to such organisms as B. megatherium, B. mesentericus, etc., and in consequence, the identification of new strains presents great difficulties. Such variation may have been due to the dissociation of organisms into "rough" and "smooth" types as observed by Soule (1928) in the case of B. subtilis. He obtained this dissociation by growth of the organism in large volumes of broth and noted a "rough" type which had a thin flat colony with a tendency of the outer fringe of growth to curl under and resemble the Medusa head appearance shown by colonies of B. anthracis while the "smooth" colony was smaller, whiter and more compact and glistening. Gratia (1924) obtained "rough" and "smooth" colonies of B. anthracis which were reversible if freshly isolated but not when the type was kept constant for

a /

a long time. He also found that the "smooth" type was less virulent than the "rough" and that rabbits could be immunised to anthrax by intravenous injection of the former followed by the latter type of colony.

In view of this possibility standard strains of B. subtilis, B. megatherium, B. mesentericus, B. anthracoides and a virulent strain of B. anthracis, which had been freshly recovered from a guinea-pig, were inoculated in large volumes (1 litre) of broth, incubated and plated daily on agar. At the same time agar plates were inoculated every 24 hours from the above strains which were kept on solid media. In no case was there any variation into "rough" and "smooth" colonies and the type of colony of each strain employed remained constant throughout the experiment. Dissociation of the organisms of this group is therefore not always easily obtained and will not serve to explain the discrepances in the descriptions by different authors of the same strain of organism.

In this way it seems that the identification of the saprophytic members of this group must remain uncertain until standard descriptions of classical types are universally recognised and a standard classification adopted.

SECTION II

IMMUNITY TO ANTHRAX.

ATTENUATION OF VIRULENCE OF BACILLUS ANTHRACIS.

The first important observation in relation to the production of immunity to anthrax infection was made by Pasteur (1883) who noted that cattle which survived experimental inoculation with anthrax bacilli were subsequently resistant to a second infection. Following the method of attenuation of virulence discovered by him while working with chicken cholera, Pasteur directed his attention to obtaining attenuated cultures of B. anthracis which would produce a benign form of the disease in animals and protect them against a subsequent fatal infection. He found that the continued growth of the bacilli at a temperature of 42° to 43°C. caused them to lose their ability to form spores, and that in addition their virulence became gradually less, so that after twenty-four days' exposure to this temperature they were unable to kill such susceptible animals as sheep, rabbits, or guinea-pigs. Cultures which had received such treatment constituted Pasteur's "premier vaccin", and the injection of these protected against the inoculation of the "deuxieme vaccin" which was obtained by growing the organism at 42° to 43° for twelve days only. In this way Pasteur was able to demonstrate that the subcutaneous

inoculation /

inoculation of these vaccines protected animals against the injection of a dose of virulent anthrax bacilli which would otherwise have been fatal. The efficacy of this method of vaccination was established by a large number of experiments and since then it has been frequently used in sheep and cattle as a preventive measure against natural infection, but although it has reduced the incidence of the disease in these animals, deaths have occurred occasionally either during or immediately after vaccination.

Other methods have been employed to attenuate cultures of B. anthracis, for example by growing the organism in media which inhibits luxuriant growth. Recently Schilling (1926) has reported that anthrax bacilli can be attenuated by growing them for six weeks on agar containing an excess of sodium chloride.

To test this method the following experiment was carried out. Three cultures of B. anthracis of known virulence were grown on salt agar; the quantity of sodium chloride used and the amount of growth obtained is indicated in the following table.

Culture	Percentage of Sodium Chloride					
	5%	6%	7%	8%	9%	10%
B. anthracis (Glasgow strain)	++	++	+	±	±	-
B. anthracis ("McC.")	++	++	+	±	±	-
B. anthracis (Paddington)	++	++	+	±	±	-

++ = moderate growth
+ = growth limited - rather scanty
± = growth very scanty
- = no growth.

In the above experiment the optimum amount of sodium chloride required to restrict growth was found to be 7%, and the cultures were grown on such media, subinoculations being made on to fresh salt-agar each week. At the end of six weeks it was found that the virulence of the organism was not reduced and in fact, after three months growth on this medium there was only a very slight decrease in the virulence of the cultures.

The examination of films of the organism grown on the above media showed that a great alteration in morphological characters had taken place, many bizarre involution forms being present, (see fig. 15). These consisted mainly of large pear-shaped or globular bodies with also elongated and irregular forms which stained rather faintly and showed a close similarity to the involution forms of B. pestis when grown on salt-agar.

Anthrax bacilli were also grown on agar containing 0·1 per cent. phenol without any alteration of their virulence.

It is evident, therefore, that the method devised by Pasteur of attenuation by growth at 42°-43°C. is, at present, the only satisfactory procedure for obtaining vaccines for purposes of immunisation.

The subcutaneous inoculation of sheep and cattle with vaccines of attenuated cultures has now been in use for /

for a considerable time and undoubtedly has diminished the mortality of these animals from natural infection besides being applied in the immunisation of animals for the production of prophylactic antisera, but at the same time it should be recognised that the immunity acquired may pass off in a certain proportion of cases in a comparatively short time, and that fatalities may occur during the process of immunisation. It is interesting to note that Koch (1882) found that although sheep immunised against anthrax by subcutaneous injection were protected against artificial subcutaneous inoculation of anthrax, they were less thoroughly protected against the ingestion of anthrax spores which is, at least, one of the ordinary methods of natural infection.

THE PATHOGENIC ACTION OF BACILLUS ANTHRACIS

The manner in which the anthrax bacillus acts in the tissues and produces its pathogenic effects is still not fully understood, and although a toxic action takes place, as is indicated by the extensive inflammatory oedema of the subcutaneous tissue and the general toxaemia, it has been found impossible to obtain toxins from the organism when grown in artificial culture media. It has been known for some time that the bacilli are extremely scanty in the oedematous exudate of an animal infected with anthrax /

anthrax, and that few are present in the skin even at the site of inoculation. This has been confirmed by Rivalier (1924) who states that "in no instance are bacteria found free in the epidermis or in the dermis of an animal dead of an anthrax septicaemia. When the surface of the skin is inoculated, microscopic examination reveals no bacteria, even when the skin is excised at the height of the local reaction." Filtrates of bouillon cultures and even dead bacilli produce very little effect in animals, and it has been suggested that the toxic action may be due to the formation in the tissues of toxins which are not produced in artificial culture media. Bail (1910) considers that such toxins are of the nature of aggressins and states that the protective properties of an anti-anthrax serum is due to its containing anti-aggressins.

BESREDKA'S PRINCIPLE OF LOCAL IMMUNISATION

The classical method of immunisation by the subcutaneous injection of anthrax vaccines can be carried out successfully in the case of larger susceptible animals, but it has been found difficult and often fails absolutely in small laboratory animals such as rabbits and guinea-pigs. Besredka (1921) has drawn attention to the manner in which the skin reacts to Pasteur's anthrax vaccines, and has /

has succeeded in immunising rabbits and guinea-pigs by cutaneous inoculation of attenuated cultures, followed by virulent anthrax bacilli. He states that the immunity produced in guinea-pigs by such a procedure is due not to the presence of antibodies in the blood, but to a local action of the organism in the skin, and that the serum from an animal immunised in this manner does not protect another against the subcutaneous injection of the second vaccine or a small quantity of virulent bacilli.

Besredka is of the opinion that since some particularly vulnerable portal of entry is essential for infection, ~~that~~ protection against invasion can only be obtained by the local immunisation of the surface tissue which constitutes the specific portal of entry, irrespective of any generalised reaction of the other cells of the body as evidenced by the presence of antibodies in the serum. Further, he affirms that when such a specific area is immunised the body as a whole is protected against subsequent infection. Following this principle he has directed his attention to the experimental production of immunity against various infections by the vaccination of the tissue at the portal of entry of the disease in question. In this way he has found it possible to produce a local immunity of the intestine against paratyphoid bacilli by the oral administration of the living organism

along /

along with ox bile; of the respiratory tract against B. diphtheriae by the intratracheal injection of killed cultures of the organism; and of the skin against B. anthracis by the intracutaneous injection of anthrax vaccines. Should his views be confirmed it would indicate that in future all attempts to create artificial immunity should be carried out by the vaccination of the tissue or organ "specific" for each disease. The experiments recorded in this section have been undertaken in order to confirm if possible this theory of local immunity in anthrax infections.

THE QUESTION OF CUTANEOUS INFECTION BY BACILLUS ANTHRACIS.

Besredka believes that in the guinea-pig the anthrax bacillus has a particular affinity for the skin in which it multiplies and secretes its toxin, and that in other tissues the organism is harmless. This theory of the action of the anthrax bacillus has been confirmed by Balteanu (1922) who found that animals injected subcutaneously, intravenously, or intraperitoneally with virulent anthrax bacilli did not die if precautions were taken to avoid injury and infection of the skin at the time of inoculation.

The following experiment was undertaken to test this claim. Two rabbits were inoculated intravenously with an emulsion of B. anthracis through the marginal vein of the ear. The site of inoculation was cauterised thoroughly /

thoroughly by the application of a red hot solder bolt to the site of inoculation of one animal to prevent infection the skin but not in the other rabbit which served as a control. Both rabbits died within three days. On autopsy there was very little noticeable to the naked eye apart from congestion of the internal organs; gelatinous oedema was present only in a small area around the site of inoculation in the ear of the control animal. The organism was recovered from the heart-blood and spleen of each animal.

The experiment was repeated and a minimal amount of inoculum used but the animals died as before.

Beltrami and Romat (1923) obtained similar results and Besredka believes that this was due to the insufficient cauterisation of the skin. He points out that after cauterisation of the ear the organism has ample time to return to the point of inoculation and so infect the skin. It should be noted, however, that there was no evidence in the experiments recorded above of any inflammatory oedema of the subcutaneous tissue at the site of inoculation in those rabbits in which cauterisation of the ear had been carried out, as would be expected had infection of the skin occurred.

That infection does not take place through the conjunctival sac has been demonstrated by Aitoff (1922) who was unable to infect laboratory animals by /

by the instillation into the conjunctiva of a dense emulsion of bacilli from agar cultures. None of the animals showed the slightest reaction although the organism was found in the conjunctival sac on the following day and in some cases even after seven days had elapsed.

It has also been shown by Besredka (1920) and by Brocq-Rousseu and Urbain (1924) that very large numbers of anthrax bacilli can be injected intratracheally in animals without producing ill effects if precautions are taken to avoid infecting the skin.

Boquet (1924) points out that infection following the ingestion of B. anthracis does not occur as long as the mucosa of the alimentary tract remains intact, but that infection takes place when the organism is injected into the submucous tissue of the mouth or into the tongue. It is of interest to note that Pasteur was able to infect animals by the oral administration of anthrax bacilli only when some sharp material such as bristles, which injured the mucosa, were mixed with the food. Sobernheim (1913) states that the ingestion of spores is responsible for the infection of cattle through the intestine, but that considerable numbers of them are necessary for infection to take place. More recently Sanarelli (1924) has shown that while the vegetative forms of anthrax bacilli are destroyed by the gastric juice /

juice of rabbits and guinea-pigs, spores may invade the blood either from the intestine or the lungs and may remain ingested by phagocytes for a number of days without injuring the animal. If, however, the resistance of the animal is lowered by increased temperature or by the injection of a cytolytic substance, such as lactic acid, which destroys phagocytes without harming the organism, the spores which are liberated, rapidly germinate and produce a fatal septicaemia. This work has been confirmed by Basset (1925) who found that anthrax spores were much less pathogenic than vegetative cultures of the bacilli.

Another interesting observation was made by Boquet who obtained positive blood cultures from six out of ten guinea-pigs which had previously ingested anthrax bacilli, but he found that after bleeding the animals by cardiac puncture they all died of a typical anthrax infection. This he considered was due to the traumatism of the skin since the characteristic oedema was found at the point where the needle penetrated the skin and control animals that were not bled survived. Boquet also showed that guinea-pigs which had ingested bacilli, died of anthrax if the skin was injured by shaving, irritation or contusion whereas control animals in which the skin was intact survived.

The following experiment confirms to a certain extent /

extent these observations. Six guinea-pigs were deprived of food for 24 hours and were then fed daily with bran in which an emulsion of anthrax bacilli was mixed. After ten days two of the animals died while the rest remained apparently healthy. On autopsy only slight pathological changes were noted but the organism was isolated in pure culture from the heart-blood in each case. The surviving guinea-pigs were still fed with anthrax bacilli and a week later a small quantity of blood was withdrawn from each animal by cardiac puncture and inoculated into tubes of bouillon. Before carrying out this procedure the hair of the chest wall of the guinea-pigs was removed by depilation and the surface of the skin sterilised with spirit and iodine, and the animals were placed in fresh cages to avoid any extraneous contamination. The cultures in each case yielded growths of B. anthracis. Each of these guinea-pigs died within four days after cardiac puncture. On autopsy two of them showed pathological lesions typical of an anthrax infection. The characteristic oedema was present in the subcutaneous tissues but it was not localised or specially marked at the point where the needle penetrated the skin during the heart puncture. The other two animals showed no obvious lesions.

This experiment is of great interest and would indicate that, as the result of the ingestion by guinea-pigs /

guinea-pigs of sporing cultures of anthrax bacilli, the organisms reach the blood stream and may circulate in the blood without producing definite pathological effects. According to Sanarelli (1924) they are carried in the spore phase by phagocytes, and an analogous phenomenon has been recognised in the case of the tetanus bacillus by Vaillard and Vincent (1891-92) Francis (1914) and others. The death of the guinea-pigs after heart-puncture seems therefore more likely to be due to some factor related to this experimental procedure, possibly traumatic in nature, which favours the rapid germination of the spores present in the circulating blood rather than to the contamination and infection of the subcutaneous tissues by the withdrawal of the needle used in the course of heart puncture.

The importance of the skin in anthrax infection has also been noted by Plotz (1924) who demonstrated that laboratory animals survived the subcutaneous injection of anthrax bacilli if care were taken not to infect the skin at the time of inoculation. The method he employed was to insert 1 to 3 c.c. of virulent bacilli in capsules of collodion or glass or in capillary tubes in the subcutaneous tissue, and after the skin had completely cicatrized to liberate the organisms by breaking the capsules.

Beltrami and Romat (1923) were unable to obtain /

obtain such results in similar experiments, but Besredka is of the opinion that the failure of their experiments was due to insufficient cicatrization of the skin.

Many other workers deny that the skin is the only organ sensitive to anthrax and have been able to produce lethal effects in animals by injecting the organism into various situations in the body even when precautions were taken not to infect the skin. It has been shown by Cernaianu and Suhatzeanu (1924) that rabbits do not survive the intravenous or intraperitoneal injection of the organism or inoculation into the brain, liver or muscles even when precautions are taken to avoid infecting the skin. On autopsy bacilli were obtained from the blood of such animals but they were unable to find any oedema of the skin or subcutaneous tissue, indicating that contamination of these tissues had not occurred at the time of inoculation. Combiesco (1923) confirms the work of Besredka and Balteanu but makes the reservation that the inoculation subcutaneously, intravenously, or intraperitoneally of anthrax bacilli does not produce the disease in rabbits or guinea-pigs as long as the quantity injected does not exceed a certain limit. He affirms that when the dose injected is very large the animals die in spite of all precautions to avoid infecting the skin. Combiesco doubts if the skin is really a sensitive /

sensitive organ and considers it to have a purely mechanical action and that in this situation phagocytosis occurs more slowly than in other parts of the body, and in this way allows the bacilli to become encapsulated. He states also that phagocytosis of the bacilli does not take place when they have formed capsules, and that the death of an animal after the injection of a large number of bacilli is due to the inability of the leucocytes to ingest all the organisms before capsule formation occurs. Similar results were obtained by Gratia (1924) who found that the intravenous injection of anthrax blood containing capsulated bacilli killed rabbits even although careful precautions were taken to avoid infecting the skin.

Wollman (1925) is of the opinion that the ease with which capsule formation takes place in the skin does not by itself explain the importance of skin infection. He states that the number of bacilli deposited in the skin when contamination takes place after an intraperitoneal or intravenous injection is very much smaller than a minimal lethal dose of the organism and is therefore incapable of causing death. He attributes the fatal infection in such cases to the combined action of the extra-cutaneous organism and those deposited in the skin. Wollman (1925) thinks that the action of contamination of the skin in the process of anthrax infection is probably due to /

to the abundant production, in the area of the skin infected, of substances favouring infection (Bail's aggressins) and that the superiority of cuti-vaccination over other methods of immunisation might be due in part to the particularly favourable conditions that it creates for the production or absorption of such substances and in consequence to the establishing of an anti-aggressin immunity.

IMMUNISATION OF LABORATORY ANIMALS BY CUTANEOUS INOCULATION OF ANTHRAX VACCINES.

As the result of his theory that infection takes place only through the skin, Besredka believes that immunity to anthrax can only occur as the result of cutaneous immunisation. He has succeeded in immunising guinea-pigs and rabbits either by applying vaccines to a scarified area of skin or by injecting them intracutaneously. Plotz found that rabbits vaccinated by the intracutaneous method obtained such a high degree of resistance that they withstood the injection of five hundred times the lethal dose of anthrax bacilli, whereas those vaccinated subcutaneously obtained only a very slight immunity or none at all. Similarly Brocq-Rousseu and Urbain (1924) have demonstrated that guinea-pigs which survived the intratracheal injection of large doses of anthrax bacilli, when subsequently inoculated intracutaneously or subcutaneously did not withstand even a minimal lethal /

lethal dose of the organism. They found, however, that animals vaccinated by the cutaneous method survived the injection into the lungs, liver, kidneys or peritoneal cavity of very virulent anthrax bacilli, indicating that a solid immunity had been acquired by the previous vaccination. In addition it has been shown by Besredka and Trevise (1922) that immunised guinea-pigs withstand the inoculation of anthrax blood as well as cultures of virulent bacilli.

It should be noted that other methods of vaccination have been successful. Cordier (1925) was unable to vaccinate animals successfully by the intracutaneous method, but succeeded when the vaccines were injected intratesticularly or into the medullary cavities in bones. Gratia (1924) found "rough" and "smooth" types of colonies of B. anthracis and was able to immunise rabbits successfully by the intravenous injection of the "smooth" type followed later by the "rough" type. That cutaneous immunisation is successful in larger animals has been shown by Brocq-Rousseu and Urbain (1923) who immunised horses by this method. They were unable to find any agglutination, deviation of complement or precipitation reactions with serum obtained from immunised horses, and found that such immune serum did not protect guinea-pigs from small doses of the second anthrax vaccine. Monod and Velu (1925) have recorded /

recorded 21,640 intradermal vaccinations which were carried out by veterinary workers in Morocco. Of these 14,405 were performed on cattle, 2,520 on sheep, 4,640 on pigs and 75 on horses and in each case vaccination was carried out with one single intracutaneous injection. The results obtained were very satisfactory as the animals vaccinated were exposed without harm in a badly infected area.

EXPERIMENTS IN GUINEA-PIGS.

In view of the results obtained by the authors quoted above, it is of interest to record the following experiments in laboratory animals. The vaccines of B. anthracis which were used had been attenuated by Pasteur's method and were obtained from Professor A. Besredka; the technique employed was as described in his original paper.

Six guinea-pigs were inoculated cutaneously by shaving an area of skin on the abdomen 2 by 4 inches so that the surface appeared raw and red, and a swab soaked in the first vaccine was rubbed thoroughly over the scarified area. In 24 hours the inoculated surface was intensely red and showed dark necrotic patches which coalesced together, so that in 48 hours a necrotic scab had formed over the abdomen. The inflammatory reaction was limited to the site of inoculation and in two days time the redness was not so marked and the scab was starting to separate from /

from the abdominal wall. In the next four days the skin gradually returned to normal but three of the guinea-pigs appeared very thin and wasted. The second vaccine was applied by scarification of the healed area in a similar manner to that described above. The inflammatory reaction which was present in 24 hours was more severe than after the first vaccine, two of the guinea-pigs dying in 48 hours, and the rest four days after the application of the second vaccine. Control animals inoculated with the second vaccine only died in 48 hours. On autopsy the animals that died in 48 hours and the control animals showed the typical pathological lesions of an anthrax infection. In the other animals the subcutaneous tissue showed only slight inflammatory changes and there was no gelatinous oedema present. Congestion of the internal organs was present and was most marked in the small intestine especially in the control animals which also showed a sero-sanguinous exudate in the peritoneal cavity. Bacilli were numerous in films of heart-blood and in spleen smears, and the McFadyean reaction was well marked. The organism was recovered on culture of the heart-blood of each animal.

Since the method of inoculation used in the above experiment did not permit an exact estimate of the amount of vaccine administered, a further series of six guinea-pigs were inoculated intracutaneously /

intracutaneously with 1·0 c.c. of the first vaccine. Following the injection there was some swelling, redness and induration at the site of inoculation in each animal, but the local reaction was not severe and there was no necrosis of the skin. Three of the guinea-pigs died in four days time and showed typical pathological changes on autopsy. The surviving guinea-pigs were inoculated intracutaneously with 0·2 c.c. of the second vaccine but all died within three days and showed similar post-mortem findings.

Another series of six guinea-pigs was inoculated starting with smaller quantities, but although they survived the intracutaneous injection of 0·25 c.c. and 0·5 c.c. of the first vaccine they all died after receiving 0·1 c.c. of the second vaccine.

The results detailed above indicate that the cutaneous method of immunising guinea-pigs with anthrax vaccines may be quite unsuccessful, and that although many survived the first vaccine, all the animals died when only small quantities of the second vaccine were introduced into the skin.

EXPERIMENTS IN RABBITS

The cutaneous immunisation of rabbits although not entirely successful has given more satisfactory results. Out of eight rabbits inoculated in this manner six survived while two died during the process /

process of immunisation, the first, four days after receiving 1·0 c.c. of a 48 hours broth culture of virulent bacilli, and the second three days after the injection of the "second vaccine". A possible explanation of the death of these two animals is that a small quantity of inoculum may have been injected into the subcutaneous tissue as well as into the skin, although care was taken to avoid this occurring when the rabbits were inoculated.

The surviving rabbits, with one exception, received numerous injections of virulent anthrax bacilli without any ill effects. The detailed histories of these animals are as follows :-

Rabbit I

18/11/27	0·2 c.c. "Vaccine I" injected intra-:cutaneously.
20/11/27	Slight inflammatory reaction at site of inoculation with some induration of skin.
22/11/27	Skin normal
23/11/27	1·0 c.c. "Vaccine I" injected intra-:cutaneously.
25/11/27	Only slight inflammatory reaction of skin, no induration.
27/11/27	Skin normal.
28/11/27	0·2 c.c. "Vaccine II" injected intra-:cutaneously.
30/11/27	Slight inflammation and induration of skin.
2/12/27	Skin normal
3/12/27	1·0 c.c. "Vaccine II" injected intra-:cutaneously.
4/12/27	Swelling, redness and induration of skin.

9/12/27 /

9/12/27 Skin normal

10/12/27 0·25 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.

23/12/27 0·5 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.

30/12/27 1·0 c.c. B. anthracis 48 hours' broth culture injected subcutaneously.

12/1/28 1·0 c.c. B. anthracis 48 hours' broth culture injected intravenously. Localised reaction at site of inoculation in ear - redness, swelling and oedema.

27/1/28 Ear normal

15/2/28 2·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously and 2·0 c.c. intraperitoneally.

5/3/28 1·0 c.c. B. anthracis 48 hours' broth culture injected intraperitoneally.

23/3/28 1·0 c.c. B. anthracis 48 hours' broth culture injected intravenously.

2/4/28 1·0 c.c. B. anthracis 48 hours' broth culture injected intraperitoneally.

2/5/28 1·0 c.c. B. anthracis 48 hours' broth culture injected intravenously and 1·0 c.c. intraperitoneally.

12/5/28 1·0 c.c. B. anthracis 48 hours' broth culture injected intravenously and 1·0 c.c. intraperitoneally.

18/5/28 0·5 c.c. agar slope emulsion (opacity No. 6 Brown's tubes) injected intravenously and 0·5 c.c. injected intraperitoneally.

24/5/28 1·0 c.c. agar slope emulsion (opacity No. 8 Brown's tubes) injected intravenously.

26/5/28 Rabbit died.

On autopsy there was no gelatinous oedema of the subcutaneous tissues, and apart from some congestion of the spleen and intestine there was no obvious pathological lesion. The organism was recovered /

recovered in large numbers from the spleen and heart blood. It should be noted that on the 24/5/28 the same quantity of an emulsion of anthrax bacilli was injected into Rabbit 7, but in spite of having withstood a greater number of injections of B. anthracis, Rabbit I which had previously appeared quite healthy, died. The explanation of the death of this animal probably lies in the fact that the immunity produced by this method of immunisation while sufficient to protect it from moderate doses of B. anthracis does not avail against an excessively large dose of the organism.

Rabbit 2.

25/1/28	1·0 c.c. "Vaccine I" injected intra-:cutaneously.
27/1/28	Swelling and redness with some induration at site of inoculation.
1/2/28	Skin normal. 0·5 c.c. "Vaccine II" injected intracutaneously.
3/2/28	Redness and induration of skin.
5/2/28	Skin normal
10/2/28	1·0 c.c. "Vaccine II" injected intracutaneously.
12/2/28	Inflammatory reaction at site of inoculation.
14/2/28	Skin normal.
15/2/28	0·25 c.c. of a 48 hours' broth culture of a virulent strain of B. anthracis injected intracutaneously.
18/2/28	Slight inflammatory reaction at site of inoculation.
25/2/28	Skin normal
5/3/28	/

5/3/28 1·0 c.c. 48 hours' broth culture B. anthracis injected intracutaneously.

7/3/28 Only very slight inflammatory reaction at site of inoculation.

7/3/28 Rabbit died.

On autopsy localised gelatinous oedema was present in subcutaneous tissues at site of inoculation. Internal organs deeply congested and B. anthracis was recovered from the local lesion, spleen and heart-blood.

Rabbit 3.

25/1/28 1·0 c.c. "Vaccine I" injected intra-:cutaneously.

27/1/28 Swelling, redness and induration of site of inoculation.

28/1/28 Skin at site of inoculation becoming necrotic.

5/2/28 Necrosis still present.

7/2/28 Necrotic scab separating from skin.

10/2/28 Skin practically normal. 0·5 c.c. "Vaccine II" injected intracutaneously.

12/2/28 Redness and induration of skin at site of inoculation.

14/2/28 Skin normal

15/2/28 1·0 c.c. "Vaccine II" injected intra-:cutaneously.

18/2/28 Slight inflammatory reaction at site of inoculation.

25/2/28 Skin normal.

5/3/28 0·25 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.

14/3/28 0·5 c.c. " intracutaneously.

23/3/28 1·0 c.c. " intracutaneously.

29/3/28 /

29/3/28 1·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.

11/4/28 2·0 c.c. " intracutaneously.

2/5/28 2·0 c.c. " intraperitoneally.

12/5/28 1·0 c.c. B. anthracis 48 hours' broth culture injected intravenously and 2·0 c.c. injected intraperitoneally.

21/5/28 Rabbit exsanguinated by heart puncture and serum preserved.

Rabbit 4.

6/2/28 1·0 c.c. "Vaccine I" inoculated intracutaneously.

8/2/28 Slight redness and induration of skin at site of inoculation. No necrosis.

10/2/28 Skin normal. 0·5 c.c. "Vaccine II" injected intracutaneously.

12/2/28 Slight redness and induration of skin at site of inoculation.

14/2/28 Skin normal

15/2/28 1·0 c.c. "Vaccine II" injected intracutaneously.

18/2/28 Rabbit died - result of autopsy similar to Rabbit 2.

Rabbits 5 and 6.

11/2/28 1·0 c.c. "Vaccine I" injected intracutaneously.

12/2/28 Redness and induration of skin at site of inoculation.

14/2/28 Skin normal

15/2/28 0·5 c.c. "Vaccine II"

18/2/28 Redness and induration of skin

25/2/28 Skin normal

5/3/28 1·0 c.c. "Vaccine II" injected intracutaneously.

14/3/28 0·25 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.

23/3/28 /

23/3/28 0·5 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.

29/3/28 1·0 c.c. "

2/4/28 1·0 c.c. "

11/4/28 1·0 c.c. "

2/5/28 1·0 c.c. "

12/5/28 2·0 c.c. "

21/5/28 Rabbits exsanguinated by heart puncture and serum preserved.

Rabbit 7.

10/2/28 to 23/3/28. Intracutaneous injections of "Vaccines I and II".

29/3/28 0·25 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.

2/4/28 0·5 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.

11/4/28 1·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.

18/4/28 1·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.

2/5/28 1·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.

12/5/28 1·0 c.c. B. anthracis 48 hours' broth culture injected intravenously and 2 c.c. intraperitoneally.

18/5/28 0·5 c.c. of an emulsion of a 24 hours' agar slope culture of B. anthracis (opacity No. 6 Brown's tubes) injected intravenously and 0·5 c.c. injected intraperitoneally.

24/5/28 1·0 c.c. emulsion from agar slope culture (opacity No. 8 Brown's tubes) injected intravenously.

31/5/28 Rabbit exsanguinated by heart puncture and serum preserved.

Rabbit 8.

10/2/28 to 23/3/28. Intracutaneous injections of "Vaccines I and II".

28/3/28 /

28/3/28 0·25 c.c. of a 48 hours' broth culture of a virulent strain of B. anthracis injected intracutaneously.

2/4/28 0·5 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.

11/4/28 1·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.

20/4/28 1·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.

2/5/28 1·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.

7/5/28 1·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.

11/5/28 Rabbit exsanguinated by heart puncture and serum preserved.

IMMUNISATION OF RABBITS BY SUBCUTANEOUS INJECTIONS OF ANTHRAX VACCINES.

It has been found impossible to immunise rabbits by the subcutaneous injection of anthrax vaccines as is shown in the following experiment :-

Rabbit 9.

10/2/28 1·0 c.c. "Vaccine I" injected subcutaneously.

12/2/28 Localised swelling at site of inoculation.

14/2/28 Rabbit normal.

17/2/28 0·5 c.c. "Vaccine II" injected subcutaneously.

19/2/28 Rabbit died. Post-mortem findings typical of anthrax infection.

Rabbit 10.

10/2/28 1·0 c.c. "Vaccine I" injected subcutaneously.

12/2/28 Localised swelling at site of inoculation.

14/2/28 Rabbit normal

17/2/28 0·5 c.c. "Vaccine II" injected subcutaneously.

19/2/28 /

19/2/28 Localised swelling.

23/2/28 Normal

25/2/28 1·0 c.c. "Vaccine II" injected subcutaneously.

26/2/28 Rabbit died. Post-mortem findings typical.

Four other rabbits were treated in this manner, three of which died after the injection of 0·5 c.c. of the "second vaccine" while the fourth died after the injection of 1·0 c.c. of this vaccine, as in the case of Rabbit 10.

The mechanism of the production of immunity after cutaneous vaccination presents many difficulties. Besredka believes that the skin is the only organ that is sensitive to anthrax infection and that as soon as this tissue is protected the body as a whole is immune. He states that the serum of an animal cutaneously immunised has no protective properties, and when injected into a guinea-pig along with a lethal dose of B. anthracis does not prevent the death of the animal. This has been confirmed by Brocq-Rousseu and Urbain (1923) who found that such serum as well as having no protective properties did not contain any complement-deviating antibodies, agglutinins or precipitins. On the other hand Gratia (1924) found agglutinins in the serum of immunised animals in dilutions of 1:10,000 and states that protective substances can be demonstrated when the serum is injected along with the /

the bacilli in guinea-pigs and not separately as in the experiments of Besredka.

Protective experiments were carried out with serum of rabbits immunised solely by the cutaneous method, and with the serum of Rabbit 7 which had received injections of anthrax bacilli intravenously and intraperitoneally as well as by the cutaneous route. The method employed was to inject a fixed quantity of serum (1·0 c.c.) along with a fixed quantity (0·5 c.c.) of decimal dilutions of an emulsion of B. anthracis into mice. With each experiment a control series of mice were inoculated with 0·5 c.c. of different dilutions of B. anthracis only.

Injection of Graduated Doses of an Emulsion of B. anthracis (No. 8 Brown's tubes) in Mice along with Serum of Rabbit 8 (cutaneously immunised).

	B. anthracis emulsion only.	B. anthracis emulsion and 1·0 c.c. serum.
(1)	0·5 c.c. emulsion diluted 1:1000. Died 30/5/28	0·5 c.c. emulsion + 1·0 c.c. serum. Died 30/5/28
(2)	0·5 c.c. emulsion diluted 1:10,000. Died 31/5/28	0·5 c.c. emulsion + 1·0 c.c. serum. Died 31/5/28
(3)	0·5 c.c. emulsion diluted 1:100,000. Died 31/5/28	0·5 c.c. emulsion + 1·0 c.c. serum. Died 31/5/28
(4)	0·5 c.c. emulsion diluted 1:1,000,000. Died 31/5/28	0·5 c.c. emulsion + 1·0 c.c. serum. Died 31/5/28
(5)	0·5 c.c. emulsion diluted 1:10,000,000.Died 31/5/28	0·5 c.c. emulsion + 1·0 c.c. serum. Died 31/5/28
(6)	0·5 c.c. emulsion diluted 1:100,000,000.Died 31/5/28	0·5 c.c. emulsion + 1·0 c.c. serum. Died 1/6/28
(7)	0·5 c.c. emulsion diluted 1:1,000,000,000 Died 31/5/28.	0·5 c.c. emulsion + 1·0 c.c. serum. Died 31/5/28
(8)	/	

	B. anthracis emulsion only	B. anthracis emulsion and 1·0 c.c. serum.
(8)	0·5 c.c. emulsion diluted 1:10,000,000,000. Died 31/5/28	0·5 c.c. emulsion + 1·0 c.c. serum. Died 1/6/28
(9)	0·5 c.c. emulsion diluted 1:100,000,000,000. Died 31/5/28	0·5 c.c. emulsion + 1·0 c.c. serum. Died 1/6/28
(10)	0·5 c.c. emulsion diluted 1:1000,000,000,000 Died 31/5/28	0·5 c.c. emulsion + 1·0 c.c. serum. Died 31/5/28
(11)	0·5 c.c. emulsion diluted 1:10,000,000,000,000. Died 31/5/28.	0·5 c.c. emulsion + 1·0 c.c. serum. Died 1/6/28
(12)	Serum control 1·0 c.c. serum only. Survived.	

None of the mice which received the serum outlived the corresponding control animal for any length of time and on autopsy all showed pathological changes typical of anthrax infection.

The experiment detailed above was repeated with the serum of Rabbit 7, which had been immunised by intravenous and intraperitoneal injections as well as cutaneously, and also with Sclavo's anti-anthrax serum with similar negative results in each case.

DISCUSSION

Anti- anthrax immunity presents problems of great complexity. In the first place we still lack definite knowledge regarding the offensive mechanism of the organism. No toxic substances have been demonstrated in culture yet anthrax is a disease with undoubted manifestations of profound toxaemia.

It /

It has therefore been assumed that the anthrax bacillus only produces its specific toxin when growing in the tissues. Bail (1910) considers these to be analogous to aggressins and has claimed that the action of an anti-anthrax serum is due to anti-aggressins i.e. an antitoxic agent. There is no evidence that anti-anthrax immunity is due to anti-:bacterial substances and Sobernheim (1910) and others have failed to detect any bactericidal action in immune sera. Sclavo's serum has been found to contain a precipitin, as evidenced by Ascoli's reaction, but this is certainly not a protective anti-substance.

Besredka believes that a local cutaneous immun-:ity is the essential feature of resistance to anthrax and maintains that this can be brought about by cutaneous vaccination of animals with anthrax vaccines. Such a method produces an immunity of the skin, and as, according to his view, the other tissues are naturally resistant the animal thus acquires a general immunity.

The manner in which such local immunity is produced has not, however, been satisfactorily explained. His theory is that the immunity developed in this way is due to the remarkable susceptibility of the skin in which a "reaction takes place between the receptive cells of the reticulo-endothelial layer and the anthrax·bacillus." Besredka holds that /

that the bacillus has a great affinity for these receptive cells, and that this is followed by a "reaction which liberates a new substance, a product of secretion or disintegration of the organism." When this substance is present in sufficient quantity it produces a negative chemotaxis which repels the leucocytes and the bacillus is free to multiply. If a slightly virulent organism is employed, such as the "first vaccine" possessing only a feeble affinity for the receptive cells, the new product which he has postulated appears only in small quantities insufficient to repel the leucocytes, and phagocytosis takes place. He considers that the phagocytic action of the leucocytes is purely mechanical and that the results of infection depend on the reaction between the receptive cells of the skin and the organism with the formation of a negatively chemotactic substance. He states that the survival of animals after the intraperitoneal or intravenous injection of anthrax bacilli, without contamination of the skin, is due to the complete phagocytosis of the bacilli which in the absence of negative chemotaxis are eliminated like any other foreign body.

Other observers hold that skin susceptibility to anthrax is not specific, and have shown that any area with relatively slight blood supply, such as the brain, testicle or muscle, is just as susceptible as the skin /

skin. In such areas there is a relative absence of protective cells and fluids which allows the organism to modify and adapt itself either by capsule or aggressin formation and so invade the rest of the body. With this view the writer is inclined to agree. This theory will not, however, explain the reaction which takes place when animals are cutaneously immunised with anthrax vaccines, although it would account for the susceptibility of the skin to anthrax infection.

There seems little doubt that the cutaneous immunisation of animals with anthrax vaccines as advocated by Besredka has given successful results especially when applied to the larger susceptible animals, and that the immunity produced is superior to that obtained by subcutaneous inoculation. Further, cutaneous vaccination has proved a safer method of immunisation since fatalities are much less frequent than when the vaccines are administered by the subcutaneous route.

In the series of experiments on guinea-pigs recorded in this section it was found impossible to immunise cutaneously these animals either by intracutaneous injections or by the application of vaccines to a scarified area of skin. This appears to be due to the extreme susceptibility of these animals since they do not survive even when the vaccines are administered in very small quantities.

The /

The method has been more successful in rabbits which attained a remarkable degree of immunity as evidenced by their tolerance of numerous injections of virulent anthrax bacilli, but the immunity produced, even in rabbits, cannot be regarded as absolute since one animal died after the intravenous injection of a massive dose of the organism. The experimental infection produced in this rabbit was, however, many times greater than could occur under natural conditions. By analogy sheep and cattle immunised in this way would be adequately protected against epizootic anthrax. On the other hand none of the rabbits which were being immunised by the subcutaneous route survived the inoculation of the vaccines, and in this way it can be said that cutaneous immunisation offers a method that is more likely to be successful in animals which are markedly susceptible to the disease.

Anti-anthrax serum has been employed therapeutically for some years, and has been found to have preventive and curative properties which, however, cannot be attributed to the presence of any demonstrable antibodies. It has been shown above that neither commercial anti-anthrax serum or serum obtained from rabbits immunised by different methods protect mice from injections of very small quantities of anthrax bacilli. This may be due to the extreme susceptibility of mice to the infection /

infection as in the case of guinea-pigs, in view of the proved clinical value of the serum in the treatment of anthrax in the human subject, and such negative results with these animals may not be of definite significance. It should be noted that mice and guinea-pigs, as contrasted with man and the less susceptible animals, do not possess in the slightest degree any natural immunity, and it is possible that the therapeutic action of Sclavo's serum may depend on a reinforcement of a natural immunity present in the tissues. In the absence of such natural immunity the serum would exert no protective properties (v. infra). In this connection, however, the observations of Kraus and Beltrami (1921) are of special importance. They have claimed that normal ox serum has equal effects with immune anthrax serum in experimental anthrax in rabbits, which could not be due to any natural protective substances since the ox itself is naturally highly susceptible to the disease. The effect of the serum might therefore be entirely non-specific and depend on a non-specific reaction due to the injection of an alien protein.

It seems probable that the skin is not only one of the most susceptible of tissues to anthrax infection, but is also highly sensitive to an immunising stimulus. If immunity depends on the production of a new immune substance or the augmentation of a pre-existing immune substance, it might /

might be supposed that this principle is most readily elaborated by the skin, and that cutaneous vaccination supplies the optimum immunising stimulus.

Two agencies must undoubtedly contribute to anti-anthrax immunity - phagocytosis and some adjuvant principle which acts by inhibiting or neutralising the toxic bacterial products responsible for injury to the phagocytic cells or for the negative chemotaxis which has been postulated as a factor in successful infection. It seems likely that the phagocytic cells of the reticulo-endothelial system constitute the essential cellular protection against anthrax bacilli, and that in the immune animal this protection is specially active in the skin. As suggested, immunity (natural or acquired) must depend also on some principle which promotes phagocytosis. Such principles have been long designated opsonins, and both natural and immune opsonins have been demonstrated in relation to phagocytosis by polymorph leucocytes. The relationship of such principles to other phagocytes is more obscure, but it is obvious that toxic bacteria must exert a considerable resistance against their destruction by phagocytic body cells, whereas detoxicated organisms are easily phagocytosed like inert foreign particles. In short, as has been suggested, the opsonins may in reality be antitoxic /

antitoxic or detoxicating principles.

In the case of an organism which produces a diffusible toxin in culture media, such as the diphtheria bacillus, antitoxin can be readily demonstrated by experimental methods. The existence of an antitoxic principle for the anthrax bacillus is not, however, so readily susceptible of experimental proof for the reasons discussed above, but must remain at present hypothetical. It is only reasonable to suppose, however, that anti-anthrax immunity is dependent on such agency, and by postulating such substance as a free principle in the blood serum the therapeutic action of Sclavo's serum might be explained. Natural resistance of animals to whatever degree it is manifested may be a function of this principle, occurring as a natural immune substance, and in this connection it is of great interest how a partially resistant animal may be more readily immunised than one of the highest grade of susceptibility. It is well known how horses possessing natural antitoxin respond more readily to immunisation with diphtheria toxin than animals without natural antitoxin, (Glenny). If we presuppose antitoxin as a factor in anti-anthrax immunity, i.e. a "cell receptor" (Ehrlich) - it may be mainly "sessile" in the tissues or free in the blood plasma. If entirely sessile a local immunity (as of the skin) might be explained: on the /

the other hand passive immunisation by antiserum could only be due to free receptors. Before such questions can be fully answered further analyses are required of the protective action of antiserum particularly in animals of moderate natural resistance. The writer's own experiments have failed to demonstrate such effect in animals of high susceptibility. It is possible, of course, that in such animals susceptibility depends on an intrinsic deficiency of the phagocytic system which cannot be influenced by any opsonic or antitoxic agents, and in such case even a powerful opsonic or antitoxic serum would be ineffective. It is also noteworthy in regard to this question that it has been impossible to immunise actively highly susceptible animals by cutaneous vaccination.

Until the exact manner in which the anthrax bacillus produces its pathogenic effect in the body is more clearly understood, the mechanism of the production of immunity to the disease must remain doubtful. Besredka's conception of the action of the organism on the skin is of particular interest in relation to this problem and his method of immunisation has undoubtedly proved of great practical value in the control of anthrax. Moreover, his work on the general question of local immunity has opened up a new field of immunological study the exploration of which may lead to new principles and conceptions of resistance to disease.

SUMMARY AND CONCLUSIONS

(1) (a) A "solid" immunity to anthrax can be produced by cutaneous vaccination of animals of moderate susceptibility (rabbits). For the purpose cultures attenuated by Pasteur's method have been employed.

(b) Cutaneous immunisation is attended by less risk of fatal results following vaccination than other methods.

(c) The sera of these animals does not confer protection against the most minute doses of infection in highly susceptible animals (mice).

(2) It has not been possible to immunise animals of high susceptibility (guinea-pigs) by this method: these animals have invariably succumbed to either the "first" or "second vaccine" (after survival from the "first").

(3) It seems doubtful whether Besredka's claim that the skin is the only tissue susceptible to B. anthracis holds good.

(4) Anthrax spores may be introduced into the blood stream through the alimentary mucosa, possibly by the agency of leucocytes, and remain latent in the blood for a certain length of time only germinating and producing an active infection as a result of some additional factor.

(5) The question of local immunity and the question of anti-anthrax immunity are discussed with particular reference to cutaneous vaccination.

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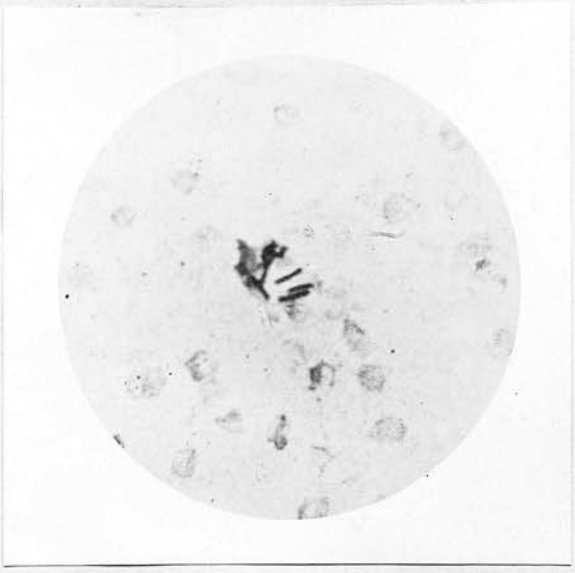
ILLUSTRATIONS /

FIGURE 1.

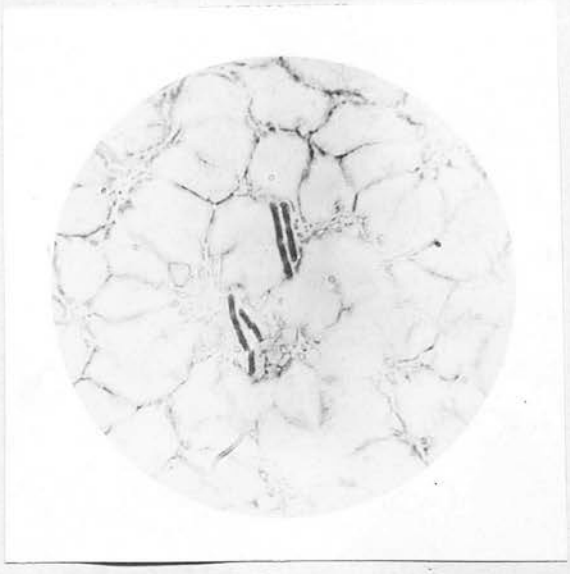


× 1000.

B. anthracoides in heart-blood of mouse, stained with polychrome methylene blue.

The bacilli are seen in small clumps scatter-:ed irregularly throughout the film. There is no evidence of capsule formation and the organism does not tend to occur in chains as in the case of B. anthracis (Figure 2).

FIGURE 2.

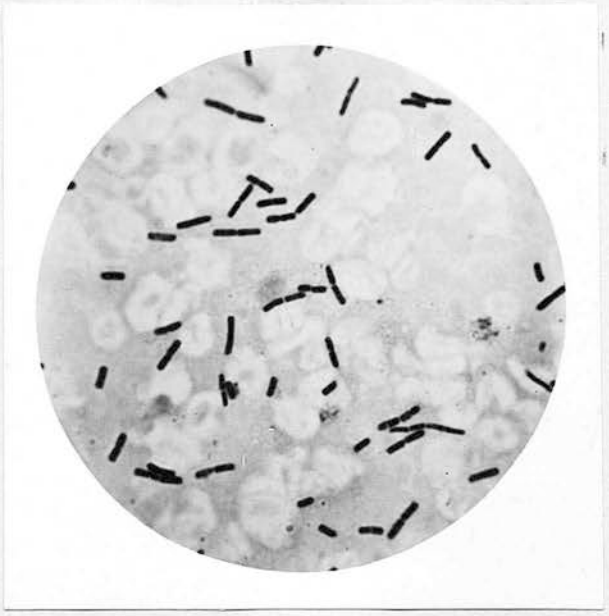


× 1000.

B. anthracis in heart-blood of guinea-pig, stained with polychrome methylene blue.

This organism occurs in chains and is cap-:sulated as contrasted with B. anthracoides in Figure 1.

FIGURE 3.



× 1000.

B. anthracoides in heart-blood of mouse after passage, stained with polychrome methylene blue.

The bacilli are more numerous than in Figure 1 and tend to occur in chains. No capsule formation visible.

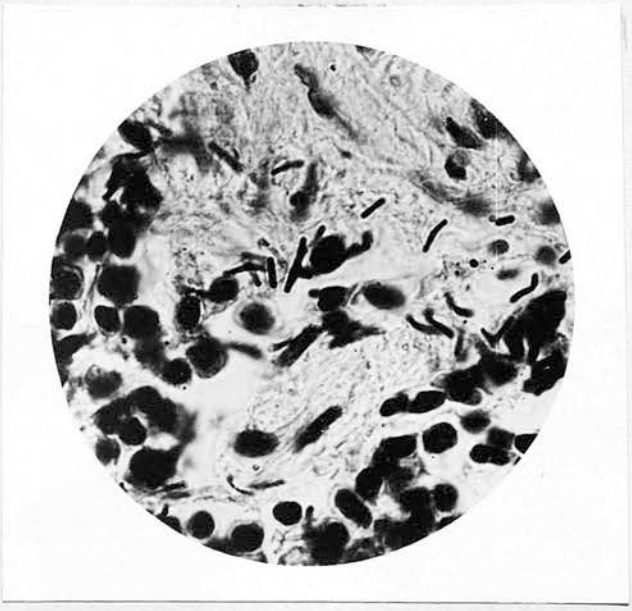
FIGURE 4.

× 1000.

B. anthracoides in peritoneal exudate of guinea-pig, stained with polychrome methylene blue.

The organisms are very numerous but do not occur in chains and do not show any capsules.

FIGURE 5.



× 1000.

B. anthracoides. Section of spleen of guinea-pig stained by Gram's method.

The bacilli are present throughout the spleen but are not so numerous as in an anthrax infection.

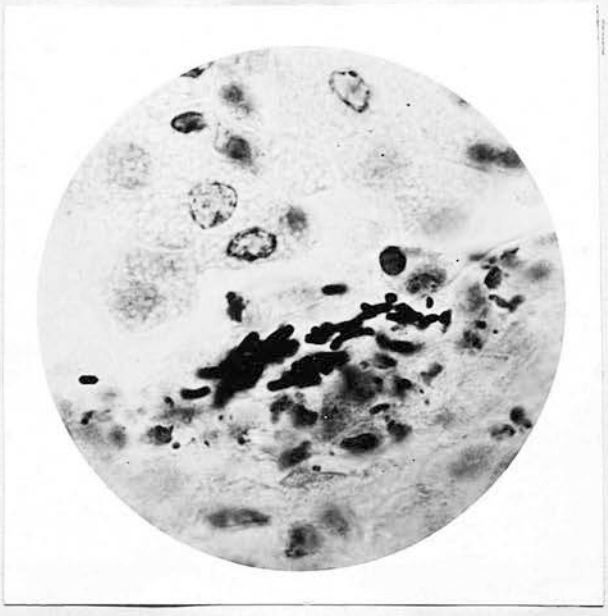
FIGURE 6.

× 1000.

B. anthracis. Section of spleen of guinea-pig stained by Gram's method.

The number of bacilli present in the spleen is much greater than occurs with B. anthracoides (Figure 5).

FIGURE 7.

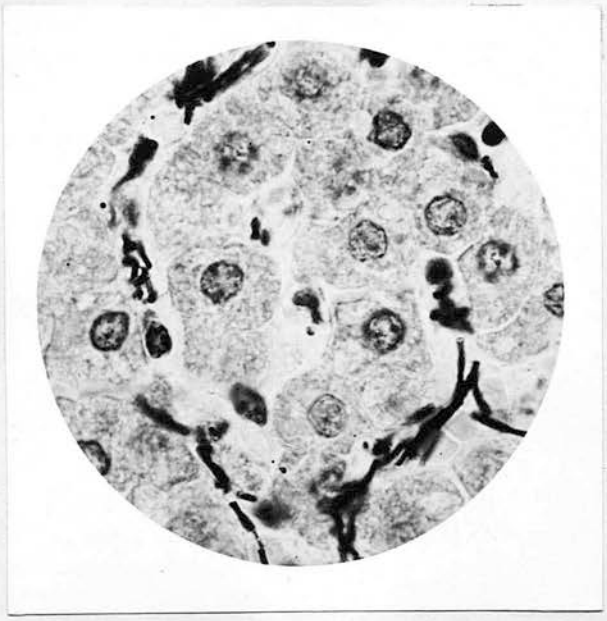


× 1000.

B. anthracoides. Section of liver of guinea-pig stained by Gram's method.

The organisms can be seen in clumps in the liver capillaries.

FIGURE 8.

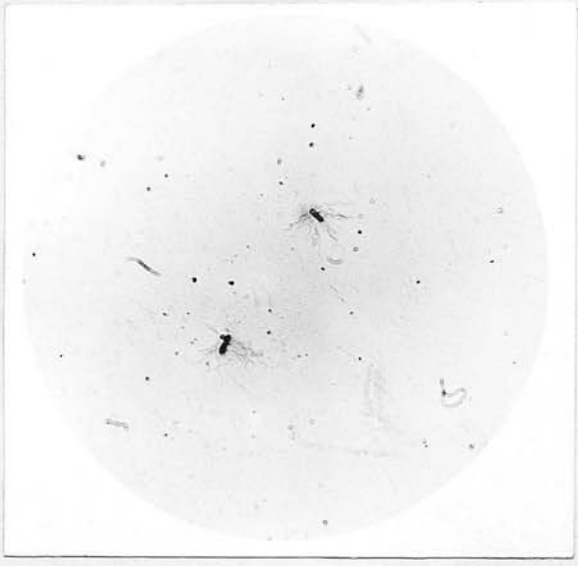


× 1000.

B. anthracis. Section of liver of guinea-pig stained by Gram's method.

The bacilli are more numerous in the liver capillaries in contrast to B. anthracoides (Figure 7).

FIGURE 9.



× 750.

B. anthracoides. Film from agar culture stained by Kirkpatrick's method to show numer-:ous peritrichous flagella.

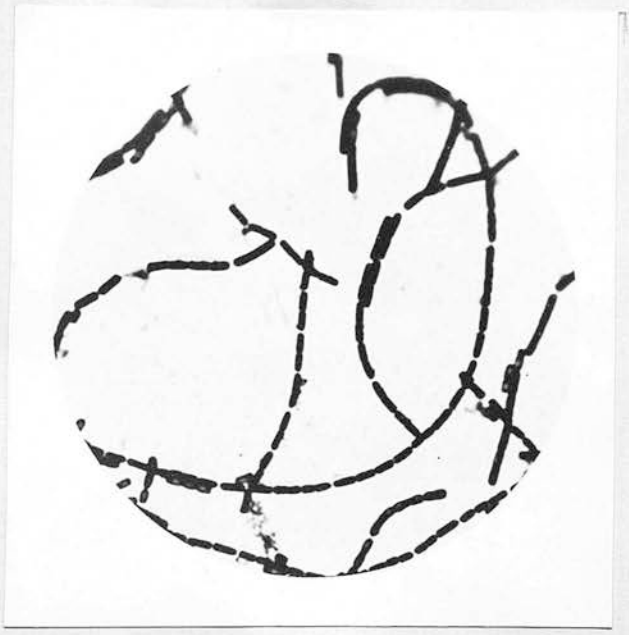
FIGURE 10.

× 1000.

B. anthracoides. Film from agar culture stained by Gram's method.

The organism shows central and also slightly eccentric spores. Chains of bacilli do not occur so frequently and are shorter than in the case of B. anthracis.

FIGURE 11.



× 1000.

B. anthracis. Film from agar culture stained by Gram's method.

This organism tends to form long chains as contrasted with B. anthracoides (Figure 10).

FIGURE 12.

× 1000.

B. subtilis. Film from agar culture stained by Gram's method.

The tendency of this organism to form chains is not so marked as in the case of B. anthracis, and the ends of the bacilli appear more rounded.

FIGURE 13.

× 1000.

B. megatherium. Film from agar culture stained by Gram's method.

The organism is rather smaller than B. anthracoides (Figure 10) but the spores are relatively larger and are subterminal.

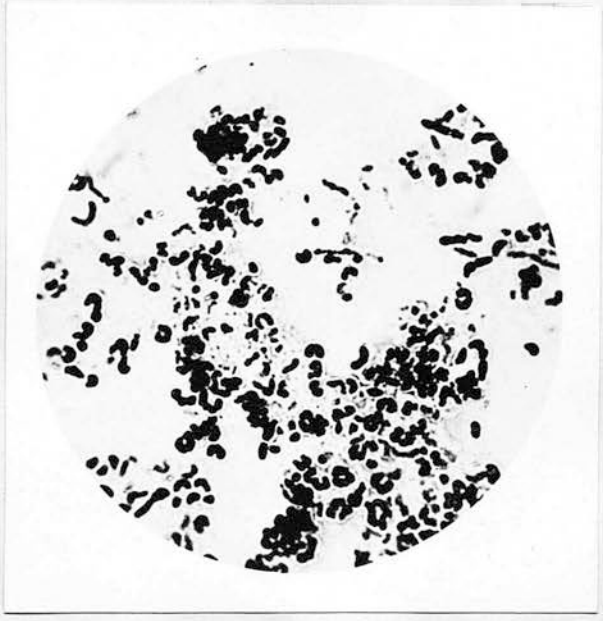
FIGURE 14.

× 1000.

B. mycoides. Film from agar culture stained by Gram's method.

This organism shows a close similarity in morphology to B. anthracoides (Figure 10).

FIGURE 15.



× 1000.

B. anthracis. Film from culture on salt agar - stained by Gram's method.

Many bizarre involution forms are seen when the organism is grown on the above medium.