

Both Geissler and Wehr noticed that in some cases tumours which had developed after subcutaneous inoculation, ultimately disappeared, although portions of these tumours, when removed, showed the same structure as the original growth. This agrees with our own experience above quoted.

Geissler and Wehr appear to have been successful in obtaining secondary deposits in the viscera. We have, up to the present, no conclusive evidence of the occurrence of secondary deposits in the organs; but the infiltration of the deeper tissues in two of the cases, and the affection of the glands in one case, show that the tumours are capable of assuming a malignant course.

Conclusions.—1. The tumours we have described affect the genitals of dogs, and are probably identical with the papillomata, condylomata, and warts of veterinary surgeons, and with the infective tumours described by Geissler, Wehr, and by Duplay and Cazin.

2. The contagion is conveyed during the act of coitus, and the tumours are not dependent upon the irritation of any discharge.

3. The tumours can be transplanted artificially, not only to the mucous membrane of the genital organs, but also to the subcutaneous tissue.

4. The muscular wall of the vagina may be infiltrated, and secondary deposits may occur in the lymphatic glands.

5. The clinical identity of the infiltrating tumours and the simple outgrowths is shown by the case in which a bitch with an infiltrating tumour infected a dog with multiple simple outgrowths.

6. The structure of the tumours is identical with that of a round-celled sarcoma.

7. The tumours which have developed in the subcutaneous tissue after inoculation may disappear in the course of a few months.

ANTHRAX.

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Definition.—Since the discovery of the anthrax bacillus and the demonstration of its pathogenic properties, it has been possible to give an exact definition of the disease which is now generally known in this country under the name of anthrax. That word must now be reserved for the malady which is caused by the anthrax bacillus. Every case of disease determined by that organism—no matter what may be the symptoms manifested or the lesions induced—must be called anthrax, and no case of disease that has any other cause must be so named, no matter how closely it may resemble a case of anthrax in respect of symptoms and lesions.

Furthermore, it is very undesirable that the word anthrax, even when qualified by an adjective, should be applied to morbid conditions that are etiologically distinct from the disease caused by the anthrax bacillus. On this account the use of the words anthracoid and symptomatic anthrax ought to be avoided. The first of these is as meaningless as “glanderoid” or “tuberculoid,” and the latter tends to promote confusion by suggesting a relationship between two diseases that are etiologically quite distinct.

Discovery of the Bacillus.—The first published observation regarding the occurrence of rod-shaped bodies in the blood of animals dead of anthrax emanated from Rayer and Davaine in 1850, but Pollender is sometimes credited with the discovery because, in an article which appeared in 1855, he claimed to have observed the rods in 1849. Neither Davaine nor Pollender in recording the discovery claimed that these rods were etiologically connected with the disease in the course of which they were found, but in 1863 the previously published researches of Pasteur on butyric fermentation led Davaine to definitely declare the opinion that the rod-like bodies which he had first seen in 1855 were the actual cause of anthrax. The final and decisive proof of the correctness of this view was not furnished till 1876, when Koch discovered the phenomenon of sporulation on the part of the anthrax rods, and demonstrated the infectivity of artificial cultures.

Morphology of the Bacillus Anthracis.—In the blood of an animal recently dead anthrax bacilli occur in the form of cylindrical, straight or slightly curved, motionless rods, about 1.5μ in breadth, and varying in length from 4μ to 20μ . When unstained these rods appear structureless, and they are generally termed "bacilli," as if they represented the elementary units or individuals of the species. That, however, is a mistake. All but the very shortest rods found in the blood when appropriately stained are found to be composed of from two to ten or more segments, which are the true "bacterial cells" or individual elements. The segments of a rod are held together by a common envelope, and united end to end by a thin disc which appears to be identical in composition with the envelope.

The appearance of the rods varies according to the method of staining. When a cover-glass preparation of anthrax blood is stained with an aqueous solution of methylene blue, washed in water, dried, and mounted in Canada balsam, the rods generally appear considerably thinner than when they are stained in the same way with an aqueous solution of gentian violet. That is because the latter stain attaches itself to the envelope as well as to the central protoplasmic part of the bacterial cells, while the methylene blue leaves the envelope uncoloured and invisible. With either of these methods of staining the ends of the bacilli and the ends of their segments appear square cut, or even slightly concave. In the latter case the unstained material between the ends of the segments takes the form of a minute biconvex lens. With deep gentian-violet staining, however, the thinner septa as well as the envelope are stained, and many of the segments thus appear longer than they really are.

The best method of demonstrating the envelope of anthrax bacilli is that recommended by John. It consists in staining with 2 per cent. aqueous solution of gentian violet, washing in water, then dipping for a few seconds in water acidulated with acetic acid ($\frac{1}{2}$ to 1 per cent.), and washing again in water. The cover-glass thus stained is dried on its upper face, and then mounted for examination, not with Canada balsam, but with a drop of water. By this method of staining the axial protoplasmic substance of each segment is stained a deep violet, while the envelope and the septa between the segments retain merely the faintest tinge of violet colour, or even without that are visible, owing to their having a refractive index different from that of the water in which they are examined (Plate I., Fig. 3). Every septum

being here unstained, the segments may appear shorter than when the accidulated solution is omitted, and the ends of the rods and segments no longer appear concave, but are either flat or distinctly convex.

The presence of a thick easily demonstrable envelope to the bacilli is, as Johnes has insisted, of great value in distinguishing between the rods of anthrax and putrefactive bacteria with which they may be associated in blood that is not quite fresh. A further valuable distinction in such cases is afforded by the fact that with simple aqueous solutions of the basic aniline dyes, and particularly with methylene blue, anthrax bacilli stain less intensely than the bacilli of malignant œdema and most other putrefactive bacteria that approach the former in size and shape. This difference of tint is quite distinct even when the comparison is made between anthrax bacilli in fresh blood and the malignant œdema bacilli of putrid blood, and it is much more evident when the comparison is made between the different bacilli in the same preparation, for in blood invaded by putrefactive bacteria anthrax bacilli have, in consequence of degeneration, in considerable measure lost their staining affinities.

The bacillary or rod-like form is the common one in the blood and spleen pulp, probably because the blood current tends to break segments off the longer rods, and thus interferes with their natural tendency to grow out into the filamentous or leptothrix form. Occasionally, however, filaments of considerable length are found in the body, rarely in the blood, but usually in extra-vascular positions, such as the gelatinous œdema of a local lesion, or in the tissue of a lymphatic gland.

A point of great importance in connection with anthrax bacilli as they occur in the blood or tissues of the living animal, or in these situations in the intact carcase, is that they never proceed to the development of spores.

When blood bacilli are transferred to suitable artificial media, kept at a proper temperature and exposed to the air, they give rise to cultures in which the filamentous form of the organism predominates. These filaments are produced simply by the undisturbed growth of the shorter rods, and they may attain a great length. When examined unstained such young filaments appear glassy and structureless, like the unstained rods found in the blood, but when appropriately stained they are found to have a structure identical with that of these rods, save that the enveloping sheath is often thin or scarcely demonstrable. The existence of the envelope in the filaments of artificial cultures has even been denied, but erroneously, as Fig. 5 in Plate I. will show. As in the case of the blood bacilli, the length of the segments or bacterial cells varies in different cultures.

The segments of the rods and filaments represent the vegetative cells of the anthrax organism, and, strictly speaking, it is only by their growth and division that the germ multiplies. A single segment grows until it has attained a size approximately double that with which it started, and then by the formation of a septum across its centre it is divided into two new segments. These may remain connected, until, by repetition of the process of fission, a rod or a filament is built up, or they may separate and give rise to independent rods.

This division by fission is the only method of reproduction that takes place in the blood and tissues of the living animal, and in certain circumstances it is the only method of growth in artificial cultures

also. But when either anthrax blood or artificial cultures are freely exposed to the air and kept at a suitable temperature the phenomenon of sporulation sets in, and culminates in the formation of spores, which bear to the vegetative cells of the bacilli a somewhat similar relationship to that which seeds bear to the higher plants.

Sporulation.—The process of sporulation may without trouble be followed under the microscope in a hanging-drop preparation kept at a temperature approaching that of the body. The segments of a filament that are preparing to sporulate lose their hyaline aspect, their substance becoming granular and more opaque. Towards the centre of the segment a bright refractile speck now appears, and continues to grow at the expense of the original axial or protoplasmic substance of the bacterial cell. This is the spore, and when it has attained its full size it is oval in shape, with its long axis coinciding with that of the segment. When the spore has reached its full size it is set free by the dissolution of the last remnants of the bacterial cell from which it was formed.

The mode of formation of spores may also be studied by examining stained cover-glass preparations of sporulating cultures. With simple aqueous solutions of the basic aniline dyes, used at ordinary temperatures, the spores remain unstained, and in sporulating filaments appear as oval clear specks in the stained substance of the segments (Plate I., Fig. 5). Not more than one spore is formed in a single segment.

The possession of this power of sporulation is of immense advantage to the anthrax germ, as it enables it to maintain its existence in the stress of circumstances that would otherwise be promptly fatal to it. This arises from the fact that the spores offer much greater resistance than the vegetative elements or bacilli to such agents as heat, sunlight, and chemical disinfectants.

The circumstances necessary for sporulation are (1) *free* exposure of the anthrax rods or filaments to the air or oxygen, and (2) a temperature between 18° and 42° C. (64° and 107° F.). The necessity for a free exposure to oxygen explains why sporulation never takes place in the blood or tissues during life, or in these after death as long as the carcass remains intact. With a suitable temperature, one has only to shed the blood, or incise the skin, and the bacilli that are thus exposed to the air, or some of them, will at once begin to sporulate. The influence of oxygen on sporulation is also seen in artificial cultures. Provided the temperature is suitable, these always sporulate abundantly when the growth is a surface one, as on potato or agar, or when it is effected in a thin stratum of liquid. On the contrary, sporulation is scanty or entirely absent in the filaments grown at the bottom of a deep stratum of liquid, even although the temperature be quite suitable.

The temperature at which sporulation is most rapidly effected is from 90° to 100° F. In artificial cultures grown at these temperatures, with free exposure to the air, many filaments have resolved themselves into spores in twenty-four hours. At 65° to 70° F. sporulation requires about a week, and the rapidity of the process between 70° and 90° is proportional to the temperature. As will presently be shown, these facts are of great practical importance.

In the dried state and protected from sunlight, anthrax spores retain their vitality and power of germination for an indefinite period,

but as soon as they find themselves in suitable conditions of moisture and temperature they germinate and produce vegetative elements or bacilli.

In the process of germination the protoplasmic centre of the spore gives rise at one of its poles to a minute bud, which soon assumes the form and dimensions of one of the before-described bacterial cells, and this, in suitable conditions, multiplies by fission and leads to the development of a fresh cycle of bacilli. The protrusion of the protoplasmic bud is apparently made possible by the gradual absorption of the external envelope of the spore at the spot where the bud is to issue.

Staining of the Bacilli.—The anthrax bacillus is an easily stained organism. In cover-glass preparations of blood, tissue juices, or artificial cultures, it instantly takes the colour of any of the basic aniline dyes in aqueous solution. It is also stainable by the method of Gram, but requires a little more care to avoid decoloration than in the case of some other organisms, such as the pyogenic cocci.

As already mentioned, the spores of anthrax remained unstained when treated with simple aqueous solutions of the basic aniline dyes at ordinary temperatures. This difficulty of staining the spores appears to be due to their resistant external envelope, and it may be overcome by using carbol-fuchsin solution at a temperature of 120° C. (in the autoclave). The substance of the filaments in which the scarcely mature spores are set may afterwards be contrast-stained with aqueous solution of methylene blue. In successful preparations obtained in this way the bright red oval spores occur at intervals along the course of the blue-stained filaments.

Cultivation.—Within the limits of temperature required for its growth, the anthrax bacillus may be cultivated in a great variety of animal and vegetable infusions, provided these are neutral or slightly alkaline in reaction and are exposed to the air. The temperature limits are 16° and 44° C. (60° and 111° F.) At the lower limit growth is very slow, and at temperatures above 43° C., it is also meagre and soon ceases, the bacilli perishing without having formed spores. Growth is most rapid and luxuriant between 32° and 38° C. (90°-100° F.).

The anthrax bacillus is a strict *aërobie*. Like animal cells it requires for its growth oxygen that is either free or only loosely combined (as in the case of the red blood corpuscles). In the complete absence of oxygen no growth takes place, and the withdrawal of oxygen from growing cultures within a comparatively short period determines the degeneration and death of the bacterial cells, though it leaves the vitality of the spores unimpaired. In consequence of its *aërobic* habit the growth and multiplication of anthrax bacilli in the blood and tissues of the body ceases as soon as death takes place.

The following are the principal characters of the anthrax cultures in the nutritive media in common use in the laboratory.

In broth contained in test-tubes the growth takes the form of a fine "fluffy" material, which, if the liquid is not disturbed, entirely settles to the bottom of the tube, and leaves the supernatant liquid quite transparent.

In gelatine plate cultures the colonies develop as white opaque round grains, which when magnified are found to be composed of

intricately interwoven leptothrix filaments. The course of these filaments can only be distinctly made out at the "frayed out" surface of the colonies (Plate I., Fig. 6).

In gelatine stab cultures a white line of growth develops along the needle track, and from it processes like thistledown shoot out laterally into the gelatine. Liquefaction of the gelatine then begins at the surface, and slowly proceeds until the whole mass down to the bottom of the needle track has been liquefied.

On the surface of agar the growth is white and shining, resembling "frosted" silver or hoar frost when held between the eye and the light.

On potato the growth is luxuriant, dull white in colour, and opaque.

In all these artificial cultures the organism grows in the form of leptothrix filaments, which in the before specified conditions proceed to sporulation.

Resistance of the Virus.—In considering the resistance offered by the anthrax germ to destructive agents, it is necessary to distinguish between the spore and the spore-free bacillus.

Fresh anthrax blood, which when it is enclosed within the body or has only recently been shed contains only bacilli, is rendered innocuous by prolonged desiccation (over sixty days at ordinary temperatures), but desiccation is without effect on anthrax spores. Sunlight is fatal to the bacilli in blood exposed to the air in less than twenty-four hours, but, in the dry state, spores may resist exposure to sunlight for a month or more.

The bacilli in fresh anthrax blood are killed by a few minutes' exposure to a temperature of 60° C. A moist heat of 100° C. is fatal within five minutes to spores, but they may resist a temperature of 95° C. for ten minutes. To instantly destroy spores in the dry state, a temperature of about 160° C. is required.

Bacilli in blood or culture are quickly destroyed by exposure to 2½ per cent. solutions of carbolic acid or chloride of lime in water, or by 2 per cent. aqueous solution of mercuric chloride. On the other hand, spores are not with certainty destroyed by a week's exposure to a 5 per cent. solution of carbolic acid in water, but ½ per cent. solution of mercuric chloride is fatal to them in a few minutes when contact can be assured, as in the case of artificial cultures.

Attenuation of Virulence.—Speaking generally, it may be said that the virulence of anthrax bacilli, as they are found in the blood of animals or in artificial cultures, may be diminished by exposing them for somewhat less than the fatal period to an agent that is destructive to them. Thus when fresh defibrinated anthrax blood is heated to a temperature of 55° C. for ten minutes, some at least of the bacilli are not destroyed, but are so modified that the defibrinated blood in quantities of 3 cc. may be used as a protective vaccine for sheep (Toussaint). In this case, however, the modification impressed upon the organism is transient, and not transmitted to succeeding generations if these are grown under ordinary conditions. On the other hand, more or less permanent diminution of virulence, transmissible to succeeding generations, may be impressed upon bacilli by cultivating them under conditions that are somewhat unfavourable to growth and prevent sporulation.

An attenuated virus has thus been produced by the action of compressed air or oxygen on growing cultures (Chauveau), by cultivating

the organism in artificial media containing minute quantities of carbolic acid or potassium bichromate (Chamberland and Roux), or by maintaining artificial cultures at a temperature approaching the highest point at which growth takes place. The last of these methods was devised by Pasteur, and employed by him to prepare cultures so attenuated that they might be used as vaccins to protect animals against natural attacks of the disease.

In the preparation of these vaccins the anthrax bacillus is cultivated in bouillon at a temperature between 42° and 43° C. By maintaining the temperature slightly over 42° sporulation is prevented and that is absolutely essential in order to effect attenuation. Incubation for about a month in these conditions entails the death of the culture, but short of the period necessary to destroy the vitality of the organism such incubation renders it less and less virulent. After from fifteen to twenty days' incubation the culture is still fatal to mice, but harmless to guinea-pigs and rabbits, and it is then suitable to use as a "first vaccin." The "second vaccin" is less attenuated, since it will kill guinea-pigs as well as mice, and make rabbits ill. This degree of attenuation is obtained by incubating in the conditions mentioned for ten to twelve days. When the bacilli of these attenuated cultures are used to start fresh cultures incubated at 37° or 38° they yield spores, and these again may be employed to start further cultures which retain the attenuation although grown at ordinary temperatures.

Distribution of the Bacilli within the Body.—As a rule, which has few if any exceptions in the case of the domesticated mammals, when an animal succumbs to anthrax the blood throughout the whole body contains the bacilli. These are most numerous present in the capillaries, but they are also found in great numbers in the blood of the veins, both large and small. The spleen pulp also contains immense numbers of them. In some situations, such as the renal glomeruli, the lungs, and the intestinal villi, the capillaries are generally crammed with the bacilli to such a degree that in stained preparations these vessels appear as if they had been filled with a coloured injection.

Anthrax is thus generally a veritable septicæmia, that is to say, a disease in which the blood is the main seat of propagation of the causal organism. For the most part the tissues outside the vessels are free from bacilli, but there are always exceptions to that rule. In the first place, the rods may be found in extra-vascular positions as the result of minute hæmorrhages. They thus find their way into the uriniferous tubules and urine, a fact which is of great importance from its bearing on soil contamination. When the primary seat of the disease is the intestine, bacilli are also found in the lumen of the bowel, from which they may be passed out with the fæces. In the second place, bacilli are always found outside the blood-vessels in the neighbourhood of the point at which they first found a foothold in the body. They are thus always found in the inflammatory exudate of the local swelling at the seat of inoculation, in the tissues of the throat and its lymphatic glands when the primary point of infection is the mucous membrane of the fauces or pharynx, and in the intestinal wall and the mesenteric or other lymphatic glands of the bowel when the intestinal mucous membrane is the part first invaded.

Even in the most susceptible species—those in which the disease most rapidly becomes septicæmic—anthrax is thus to begin with a local disease, in which the bacilli are propagating in the lymphatic spaces and vessels of the part or organ first invaded. In the case of susceptible animals the bacilli, however, soon gain the nearest lymphatic glands, pass these, and reach the blood. On the other hand, in animals that offer a notable degree of resistance to the disease the invading organisms may for a considerable period be confined to the neighbourhood of their point of entrance into the tissues, or entirely destroyed there. In the one case the fatal ending is materially delayed; in the other the animal recovers.

It follows from what has been said that if an animal already showing symptoms of anthrax, such as swollen throat and elevated temperature, be killed, few or no bacilli may be found in its blood. It is also conceivable that an animal in which the throat is the part first invaded may die from asphyxia before the general invasion of the blood stream has had time to take place, but experience justifies the statement that this is a very rare occurrence in the lower animals, and the writer has never seen an indubitable instance of it.

The foregoing considerations also show why it is, that, except during a short period immediately preceding the fatal ending, the bacilli cannot by microscopic examination be found in blood taken from the living animal.

Habit of Life of the Bacillus.—There can be no doubt that in perfectly natural circumstances the anthrax bacillus frequently finds all the conditions essential for its multiplication external to the animal body, and it must therefore be classed with the facultative parasites. At the same time it is probable that at least in temperate climates, such as ours, it depends for its continued existence mainly on its power of propagating in the body. In other words, it is probable that if the germs of anthrax which are at present in existence were permanently cut off from every opportunity of invading the animal body the species would be unable to maintain its struggle for existence in the outer world. As has already been seen, the conditions necessary for its growth are (1) a suitable nutritive medium, (2) a temperature between 60° and 111° F., and (3) the presence of free oxygen. But when it is growth external to the body that is being considered a fourth condition must be recognised as essential, viz., the absence of more rapidly growing purely saprophytic bacteria, such as those of putrefaction. When anthrax bacilli are voided in urine or excrement in the summer months they find themselves in the presence of the conditions necessary for growth and multiplication, in respect alike of nutriment, temperature, and exposure to oxygen. But if the temperature is only a few degrees above 60 they are unable to sporulate, and their rate of multiplication is slow. They are therefore almost certain to be crowded out of existence in the struggle with other organisms that grow much more rapidly at the same temperature. Another factor unfavourable to the bacilli is desiccation, which first arrests the growth of the bacilli, and finally destroys their vitality. There are therefore good grounds for believing that during the greater part of the year in this country surface contamination of the soil with blood, urine, or excrement of an anthrax animal is only temporary. If, however, the temperature is over 70° F., the result

may be very different, for the bacilli may then resolve themselves into spores, and these are capable of resisting the most adverse conditions that they are likely to encounter in a natural way in soil or water.

These considerations show that the anthrax organism leads a very precarious saprophytic existence, and make it probable that most outbreaks of the disease in this country are ascribable to the ingestion of spores or bacilli that are separated by only a few generations from the bacilli voided from the body of an animal suffering from anthrax, or that have themselves been liberated from the dead body of such an animal.

The part which is played in soil contamination by the careless burial of anthrax carcasses has been much discussed. Pasteur thought that it was the main method by which the disease is spread, and he ascribed to earth-worms an important rôle in this connection, maintaining that they brought to the surface of the ground spores which had been formed in the bodies of buried anthrax carcasses. But while it is impossible to deny that earth-worms thus may be instrumental in bringing anthrax spores to the surface of the ground when these do exist in anthrax graves, it is permissible to maintain that the view which Pasteur held regarding the dangers of careless burial and the rôle of earth-worms was an exaggerated one. In the first place, it ought to be remembered that in such portions of an anthrax carcase as are not in direct contact with the air the bacilli soon perish without forming spores, even although the temperature is suitable for that process. In the second place, the prompt burial of an anthrax carcase at a depth of a few feet withdraws from the bacilli two of the conditions essential for sporulation, viz., exposure to oxygen and a temperature above 64° F.

To sum up what has been said under this head, it appears highly probable that those parts of the earth's surface at which anthrax germs at present exist owe their contamination to the excrement, urine, or other discharges of animals suffering from anthrax, or to the blood shed on the surface of the soil during *post-mortem* operations.

Methods of Infection.—Anthrax may be experimentally set up in susceptible animals by inhalation, inoculation, or ingestion. It is doubtful whether the first of those is ever the cause of natural cases of anthrax in the lower animals, but the so-called "wool-sorter's disease" is an example of that method of infection in the human subject. Inoculation is also a rare method of infection in the lower animals. On the other hand, the great majority of cases of anthrax in man are contracted in this way, either during operations with fresh anthrax carcasses, or with the hair, wool, or hides of animals dead of the disease. Either spores or bacilli suffice to infect in this way when implanted as deep as the dermis, and the slightest scratch or abrasion of the skin may furnish all the wound that is necessary to bring about infection if the germs of the disease are brought into contact with the part.

Undoubtedly the common method of infection in the lower animals is ingestion. The infective material may be in the form either of bacilli or spores. In the case of the herbivora it is probable that nearly all cases are caused by the ingestion of anthrax spores adhering to the food which they eat or suspended in the water which they drink. Since gastric digestion is capable of destroying the bacilli

but not the spores, infection is much more certainly induced by feeding experimentally with the latter than with the former, but the tissues of the alimentary canal anterior to the stomach are equally susceptible to infection with bacilli or spores. It is impossible to say with certainty whether some abrasion or defect of the mucous membrane is necessary for infection, and the point is of no practical importance, since it is probable that no animal at any time has the lining membrane of its alimentary canal absolutely devoid of epithelial defects. At the same time it is not open to doubt that actual wounds and macroscopic abrasions of the buccal or pharyngeal mucous membrane must carry with them extra risk of infection when the animal ingests contaminated food, and Pasteur found that a larger proportion of sheep contracted the disease when the infective material was sprinkled on such prickly materials as barley awns than when it was given on soft green food.

Probably the only natural method of infection in the pig and dog is the ingestion of raw flesh from some animal dead of anthrax. In animals of these species the mouth, fauces, or pharynx is frequently the primary point of invasion.

The human subject is also occasionally infected by ingestion, but such cases are apparently very rare in this country. That is not entirely, probably not even mainly, due to the fact that the flesh of anthrax animals is rarely consumed with us, but because anthrax flesh generally contains only bacilli (not spores), and these are destroyed during the process of cooking.

Susceptibility.—All the common domestic mammals are susceptible to anthrax. In this country the disease is most frequently diagnosed in animals of the ox species, and only a small number of cases in sheep figure in the official returns. It is not improbable that these returns are less trustworthy for sheep than for cattle as an indication of the prevalence of the disease, owing to the fact that farmers are more careless regarding the cause of death in the former species than in the latter, and also because there is a greater probability of the nature of the disease being overlooked in sheep if the blood is not examined microscopically. At the same time, it is not possible to believe that anthrax is a common ovine disease in Great Britain. In the human subject cases of malignant pustule contracted while dressing the carcasses of sheep appear to be unknown in this country, and that would certainly not be the case if anthrax were a common disease among sheep. The greater frequency of the disease among cattle is not very easy to explain, except on the supposition that they are more susceptible than sheep to alimentary infection. It is well known that sheep are much more susceptible to experimental inoculation than cattle, the disease when thus set up with a strong virus being almost invariably fatal in the former species, while a considerable proportion of cattle recover from inoculation anthrax.

In point of susceptibility the horse appears to stand near the ox.

The pig is readily infected with anthrax by ingestion, although it appears to be more resistant to inoculation than cattle or sheep. The dog is generally credited with a marked insusceptibility to the disease, and it has even been by some authors declared immune. In this country anthrax has frequently been observed in the dog as the result

of feeding with raw anthrax flesh, and old as well as young animals have been attacked.

The domestic fowl, it is generally stated, is quite immune against anthrax in ordinary conditions, but it may be rendered susceptible by circumstances that lower the vitality, such as immersion in cold water until the temperature is reduced several degrees.

The mouse, rabbit, and guinea-pig are very susceptible to anthrax by inoculation, but they cannot with anything like the same certainty be infected by feeding.

Neither age nor general condition appears to affect susceptibility, and no difference in the degree of resistance to infection has been noted in different breeds of the same species in this country, though Chauveau found that Algerian sheep possess a high degree of natural immunity against anthrax.

Symptoms.—In a very large proportion of cases of anthrax in the ox and sheep the period during which manifest symptoms of illness are exhibited does not exceed a few hours, and it therefore frequently happens that animals thought to be perfectly healthy when last seen are found dead. Systematic employment of the thermometer in outbreaks of the disease shows that the earliest indication of infection is a rise of temperature. This will often be found to be from 2° to 4° above the normal in animals that are still eating and ruminating. Moreover, although it is impossible to doubt that this febrile state is really a mark of infection, many animals that have for a day or two a temperature decidedly above the normal recover without developing any urgent symptom.

Clinical observation thus shows that anthrax in the ox is by no means so invariably fatal as many suppose it to be, and this view is also borne out by experiment, since a considerable proportion of cattle inoculated with virulent anthrax blood or culture recover.

The elevation of internal temperature may be associated with slight dulness, inequality of surface temperature, impairment of appetite, occasional rigors, and acceleration of the heart's action, and these also may pass off in a day or two.

In the fatal cases the urgent symptoms rapidly follow on one another, and the animal generally dies within an hour or two of their onset. These are, great prostration, profound dulness of expression, rapid action of the heart, and feeble pulsation in the arteries. Occasionally more or less blood is passed with the fæces. Death usually takes place quietly, without convulsions.

In the sheep the symptoms are very similar, but the course of the disease is even more rapid, the period during which urgent symptoms are exhibited rarely exceeding an hour or two. During this period there are signs of intense prostration, and both urine and fæces may be tinged with blood. As in the case of the ox, death usually occurs without convulsions.

In the horse a somewhat longer period, from four to twelve hours, generally elapses between the onset of pronounced symptoms and death. Indications of abdominal pain are in many cases exhibited, the animal being more or less restless, lying down and rising up again frequently, and perspiring. The temperature rises 3° or 4° , the respirations are hurried, and the heart's action is rapid. In a considerable number of cases, the signs of colic are slight or absent, but

a new symptom is added in the shape of a diffuse swelling in the region of the throat. This is often the earliest abnormality noticed. The swelling is hot, tense, and ill-defined at its margins, rapidly spreads down the neck, and may almost reach the entrance to the chest before death takes place. With this localisation of the disease, more or less urgent dyspnoea is one of the most striking symptoms.

In very rare instances the disease is ushered in by the sudden development of a brawny swelling in some other part of the horse's body, and occasionally in an animal that has displayed the earlier symptoms, such as rise of temperature, general depression, loss of appetite, etc., a number of such swellings may form in succession in different places, and ultimately disperse, the animal recovering after an illness extending over some days.

In the pig primary localisation of the disease in the throat is the rule, and it is also frequently observed in the dog. The more or less urgent dyspnoea caused by the swelling of the throat and neck is accompanied by the general symptoms seen in other animals—elevation of temperature, rapid action of the heart, rigors, complete loss of appetite, and great prostration. The duration of the illness lasts from a few hours to a day.

Lesions of Anthrax.—In an animal dead of anthrax putrefactive changes set in immediately, and proceed with great rapidity. Within a very short time after death the stomach and intestines become enormously distended with gas, in consequence of which the anus becomes everted, and the abdominal parietes occasionally ruptured. A blood-stained fluid escapes from the anus, and a similarly tinged froth is expelled from the nostrils. In sheep the skin soon shows a bluish-black discoloration, and the wool is easily pulled out. This proneness to rapid putrefaction is in no way characteristic of anthrax. It is simply due to the deoxygenated state of the blood at the moment of death, this being eminently favourable to the multiplication of the malignant oedema bacillus and other putrefactive anaerobes.

The subcutaneous veins are distended with dark imperfectly coagulated blood. If this blood be examined before putrefaction has made progress, it will be found that the white corpuscles are abnormally numerous (hyperleucocytosis). Extravasations of blood may be present under the skin, but except at the seat of local swellings, such as that of the throat, these are generally absent. Within the abdomen the most constant lesions involve the spleen, the stomach (fourth stomach in ruminants), the intestines, and the lymphatic glands.

Engorgement of the spleen is almost invariably present in anthrax in cattle (hence the term splenic apoplexy). In the great majority of cases that organ is from five to ten times the normal volume and weight, and sometimes the congestion is so great as to cause rupture of the splenic capsule. The spleen pulp is softened, often diffuent and dark in colour, though it may brighten on exposure to the air. In rare cases of bovine anthrax, however, the spleen may be normal in size, and almost normal in appearance, and exceptions to the rule are commoner in the other domestic animals. In the sheep particularly, the spleen, although more or less softened, is often normal in size, or but slightly enlarged. The stomach or intestines, or both, are

frequently inflamed, the inflammation being of a hæmorrhagic type. The intestinal contents may be mixed with more or less liquid blood. The enteritis sometimes affects the small intestines mainly, at other times the colon and cæcum are mainly involved. In the one case the mesenteric glands, and in the other the lymphatic glands of the large intestine, are swollen, deeply congested, and hæmorrhagic. The liver and kidneys as a rule are normal save that they are congested. The organs of the thoracic cavity are generally normal, but the pleura, the serous membrane of the pericardium, or the endocardium may show hæmorrhagic spots.

In those cases in which swelling of the throat is a symptom during life the subcutaneous and intermuscular tissue of the region is found to be saturated with a straw-coloured, clear, trembling, gelatinous exudate, and this may be associated with larger or smaller blood extravasations. The lymphatic glands of the throat are then swollen, congested, and hæmorrhagic. In those rarer natural cases in which circumscribed swellings are present in other parts of the body, and at the seat of inoculation in experimental cases, the swelling is found to be due to the presence of the same straw-coloured gelatinous exudate. The transparent character of this exudate is due to its poverty in leucocytes, and that again is referable to the negative chemotaxis which anthrax bacilli and their products exert on the white blood corpuscles.

Diagnosis during Life.—The diagnosis of anthrax during life is in certain circumstances comparatively easy and in others attended with great difficulty. It is easy when, as is the rule in the pig, both the history and the external manifestations point to anthrax. The writer has had brought under his notice but one outbreak of anthrax in the pig in which there was not a history of the animals having recently been fed either with slaughter-house offal, or with the raw flesh of some farm animal found dead or which had died after a very brief illness. When with such a history some of the affected pigs show swelling of the throat and neck anthrax may confidently be diagnosed. In cattle swelling of the throat or other external manifestations of anthrax are rarely present, but when there is a history of recent sudden death of one or more animals, and some of other members of the herd are found to have an elevated temperature, a probable diagnosis may be made. In the horse, unless there is swelling of the throat, the first case is not likely to be correctly diagnosed owing to the comparative rarity of the disease among horses in this country, and the difficulty in distinguishing between the symptoms displayed and those of enteritis, peritonitis, or twist of the bowel. It is unnecessary to discuss the *intra-vitam* diagnosis of anthrax in the sheep, as owing to the extremely rapid course of the disease in members of that species the veterinary surgeon rarely has the opportunity to see the patient while alive.

Diagnosis after Death.—In the great majority of cases, whatever be the species attacked, the veterinary surgeon has a dead animal at his disposal when he is first called in to an outbreak of anthrax, and in that case a perfectly certain diagnosis may quickly be made by anyone who is acquainted with the characters of the anthrax bacillus as it is found in the blood. But, in this matter there are several pitfalls into which the tyro is very likely to drop. Probably the most

dangerous of these is the resemblance between anthrax bacilli and certain putrefactive organisms, particularly the bacillus of malignant œdema, when insufficient powers of the microscope are used. This source of error is absent in the perfectly fresh carcase, and except during the warm months of the year it is also absent for twenty-four hours or more after death if the blood for examination be taken from one of the veins of an ear or a foot, as the invasion of the blood by putrefactive organisms has not then reached the extremities. The practitioner who aspires to basing his diagnosis of anthrax on microscopic examination of the blood, must before he attempts to put his knowledge into practice have satisfied himself that he can certainly distinguish between a preparation of fresh anthrax blood and one of putrid blood containing large numbers of malignant œdema bacilli. It would be a great mistake to suppose that this is easy, and it is not improbable that fully as many errors are at the present time made by those who base their diagnosis on the result of a microscopic examination as by those who rely solely on the history and the appearance of the lesions.

For simple diagnostic purposes aqueous solution of methylene blue is probably the best stain in the hands of those who are not expert bacteriologists. A convenient solution may be made by dissolving 1 part of methylene blue in 100 parts of $\frac{1}{2}$ per cent. carbolic water, filtering, and preserving in a glass-stoppered bottle. Such a solution will keep for an indefinite period. One advantage of this solution is that it stains the bacilli of malignant œdema much more intensely than the anthrax bacilli. In blood that is partially putrid the anthrax bacilli, provided they have not entirely disappeared, are by this method stained of a light blue, which is in strong contrast with the blue-black colour of the associated putrefactive organisms. Indeed, when intensely staining bacilli are found in blood that is not quite fresh it may safely be concluded that they are not anthrax bacilli.

The trouble necessary to enable one to certainly distinguish between anthrax bacilli and putrefactive bacteria is well spent, for microscopic examination of the blood is the only method that entirely obviates the necessity of opening the body in order to arrive at a diagnosis.

With a sufficient magnification anthrax bacilli may readily be detected in unstained preparations of blood, and some authors have even recommended this method of examination as preferable to the use of stained preparations. It has the obvious merit of being simpler, but in inexperienced hands it is certainly less reliable.

In all circumstances the blood for microscopic examination should be taken from one of the peripheral veins, the ear being the most convenient part. This course is commendable for a double reason. In the first place, it reduces to a minimum the amount of blood placed in contact with the air, and this, as already explained, is in the highest degree desirable. In the second place, in partially putrid carcasses anthrax bacilli may be found in the blood of the peripheral veins a considerable time after they have entirely disappeared from the veins of the great body cavities.

The experimental inoculation of small animals is not of great value in the diagnosis of anthrax, and not infrequently it conducts those who adopt it into absolute error. When the suspected carcase is still

fresh inoculation of a guinea-pig or rabbit with a trace of blood justifies the diagnosis "not anthrax" if the result of the experiment is negative, but death of the experimental animal by no means justifies an opposite conclusion. Before arriving at that opinion it would be necessary to find anthrax bacilli in the blood of the guinea-pig or rabbit, and whoever is capable of doing that might have found the bacilli in the same way in the blood of the suspected carcase.

When the suspected carcase is partially putrid, inoculation experiments uncontrolled by microscopic examination of the blood of the experimental animal are in the highest degree fallacious, because many putrefactive bacteria, including the frequently present malignant oedema bacillus, are highly pathogenic for the guinea-pig and the rabbit. A further source of error is introduced by the fact that putrefaction destroys anthrax bacilli, and inoculation with such materials as putrid spleen pulp may fail to communicate anthrax, although the case was really of that nature.

Inoculation experiments may usefully be resorted to when, as in partially putrid blood, anthrax bacilli cannot with certainty be identified by microscopic examination, but when such an experiment entails the death of the experimental animal the blood of the latter must be microscopically examined before deciding that the case is one of anthrax. Moreover, a diagnosis is justified only when the experiment has a positive result, for partially putrid anthrax blood may fail to infect owing to all its contained bacilli having perished.

When experimental inoculation is resorted to the guinea-pig ought to be preferred to the rabbit, unless the blood is perfectly fresh, as the former is almost immune against certain putrefactive bacteria that are intensely virulent for the rabbit. When partially putrid blood or spleen pulp is used it is also better to rub the suspected material into the scarified skin than to inject it hypodermically, as the malignant oedema bacillus, although readily fatal by subcutaneous injection, generally fails to infect by endermic inoculation, while the latter method is quite reliable as a means of infecting with anthrax.

The practitioner who cannot rely on his own microscopic examination as a guide to diagnosis in suspected cases has several courses open to him. (1) He may cut off an ear or a foot, send it to some competent bacteriologist, and in the meantime take all the precautions necessary in a case of anthrax. (2) He may base his diagnosis on the clinical history and the external appearance of the carcase, and abstain from a proper *post-mortem* examination. (3) He may proceed to open the body in order to base his opinion on the condition of the internal organs. The first of these three courses is undoubtedly the best, and the last is probably the worst, since it is dangerous to the person operating and to those who assist him, and involves serious contamination of the ground, with the greatly to be dreaded danger of subsequent spore formation. The opening of the carcase of an animal supposed to have died of anthrax ought to be forbidden by law until a microscopic examination by some competent person has proved that the disease was of another nature.

Assuming, however, that the practitioner has decided to open the carcase, it becomes necessary to discuss the value of the indications which he may thus obtain. In the case of cattle, if the carcase is moderately fresh, a tolerably well-founded, but not absolutely certain,

opinion may be based on the condition of the spleen. If the animal has been unexpectedly found dead, or has died after a few hours illness, and the spleen is found to be decidedly enlarged and softened, there is little chance of error in diagnosing the case as one of anthrax. The diagnosis cannot be absolutely certain because (1) in a very small proportion of cases in cattle the spleen is almost normal in appearance, and (2) because extreme splenic congestion is occasionally found in other conditions than anthrax.

What has just been said of the ox applies, for the most part, to the horse also, though in the latter species exceptions to the apoplectic condition of the spleen are probably more numerous. But, on the other hand, swelling of the throat is much more frequent in the horse, and where the peculiar straw-coloured gelatinous exudate is found in that region, and there is enteritis, with great enlargement and congestion of the intestinal lymphatic glands, anthrax may be diagnosed with little chance of error. In the pig and dog also these lesions may confidently be set down to anthrax, especially when, as is nearly always the case, there is a history of the animal having recently been fed with raw flesh.

Diagnosis based solely on *post-mortem* appearances is least satisfactory in the case of the sheep, because in that species swelling of the spleen is far from constant, and because the lesions in other parts of the body are generally seriously masked by the putrefactive changes that have taken place before the carcass is submitted for examination.

Treatment.—Notwithstanding a contrary belief entertained by a good many veterinary surgeons, it may confidently be asserted that the course of an attack of anthrax remains entirely unaffected by any medicinal treatment at our command. The belief that such agents as carbolic acid or hyposulphite of soda, administered by the mouth, exercise a curative affect on anthrax is an erroneous one, arrived at apparently because many have not recognised that the forces of nature are sufficient to bring a considerable proportion of the larger animals successfully through an attack of the disease.

Prevention.—The measures that may be taken with the object of prevention comprise (1) protective inoculation, or vaccination, and (2) those precautions against infection which are suggested by a knowledge of the methods by which the disease is generally contracted and spread.

The Pasteurian method of vaccination has had a most extensive trial in France and several other countries in which anthrax is specially prevalent, and it is claimed for it that it has effected a marked reduction in the death-rate from the disease in districts in which it has been systematically practised. The classical experiment which was carried out at Pouilly-le-Fort in 1881 proved in the most convincing manner that the Pasteurian method of vaccination, when properly performed, confers a high degree of immunity against anthrax—an immunity which enables vaccinated sheep to resist inoculation with quantities of virus certainly fatal to ordinary unvaccinated animals of the same species. Nevertheless the method has not found universal favour, and in this country it has had a very restricted trial. Probably one of the reasons why it has not been largely practised with us is that anthrax in this country is seldom such a formidable plague as it is in many other countries. Evidence of this is afforded by the fact that

the total number of animals returned in any one year as having suffered from anthrax has only once exceeded 1000 (1300 in 1893), while the average number of fatal cases in an outbreak is generally under two. Another obstacle to the general adoption of vaccination by British stock-owners, has doubtless been the large proportion of accidents that have attended the operation in some of the few instances in which it has been resorted to in this country. A notable example of such untoward consequences of vaccination was recorded by the writer in Vol. VII. of this Journal (p. 325). In that instance out of a lot of 225 ewes sixteen died as the result of the second vaccination. A still greater shock to one's faith in the usefulness of vaccination was imparted by the fact that when, less than four months afterwards, a number of the vaccinated ewes of this flock had their immunity tested by feeding or inoculation with strong anthrax virus, they all succumbed to the disease. This case, and others of the same kind that have occurred abroad, show that the preparation of an anthrax vaccin that is at once safe and efficacious is not a matter that can be regulated with mathematical accuracy, and suggest that it would be very unwise for any British veterinary surgeon to press this method of dealing with the disease on a reluctant stock-owner.

Other methods of protective inoculation, by processes of chemical vaccination, may be dismissed by saying that while some experiments on a laboratory scale encourage the hope that we may yet be provided with a germ-free vaccin that will confer a valuable degree of protection, we do not yet know any method of that sort which we can recommend to the farmer. In this connection it is interesting to recall that Pasteur himself, before he had discovered the method of vaccinating by attenuated cultures, expressed his firm conviction that anthrax could easily be exterminated by exercising proper care in disposing of animals dead of the disease.

In considering what prophylactic measures other than vaccination may be taken when an outbreak of anthrax occurs, it is necessary to remember that every animal affected with the disease is a source of soil contamination while it is still alive, by reason of the bacilli voided in its urine or excrement, and that it may be made a still more serious source of contamination after death, if its carcase is improperly dealt with. To limit the dangers arising in the first of these ways every animal suspected of anthrax (and, as previously mentioned, when an outbreak occurs many cases may be diagnosed early by the use of the thermometer) ought if possible to be confined in a place that admits of being thoroughly disinfected. The dung, bedding, etc., in the place should afterwards be destroyed by burning, and the floor and walls ought to be treated with some reliable germicide. If the disinfection is practised immediately, and the temperature does not exceed 70° F., it may safely be considered that any disinfectant capable of destroying spore-free bacilli will suffice, such as a 2 or 3 per cent. solution of carbolic acid, creolin, or chloride of lime. On the other hand, if the temperature has been 80° and a day or more has elapsed since the contamination occurred, rendering it probable that spores may have been formed, stronger agents, such as $\frac{1}{2}$ per cent. mercuric chloride, ought to be employed. It is also worth remembering that the disinfectant power of any of these solutions is intensified by using them hot.

Every case of sudden unexpected death in cattle, sheep, or horses ought to be suspected of anthrax until the contrary is ascertained. If this precaution were invariably adopted the lives of human beings as well as of the lower animals would occasionally be saved. When the case is ascertained to be one of anthrax the method of disposing of its carcase will depend upon the place where the death has occurred. If the carcase is found in a field, and the soil admits, a grave of sufficient depth (not less than six feet according to the regulations of the Board of Agriculture) should be made alongside the carcase, and in this the latter and any visibly contaminated earth in its neighbourhood should be buried. If the burial is promptly effected, even in summer time, putrefaction and the deprivation of oxygen may be relied upon to destroy the bacilli before they have had opportunity to resolve themselves into spores. As a further precaution, some reliable disinfectant, such as chloride of lime, may be applied to the surface of the soil where the carcase lay, or a thick layer of litter may be spread on the place and then set fire to.

If the death has occurred in a building, or in a field or other place not adapted for the making of a grave, the carcase must be transported to some place where it can be destroyed or buried in the manner above described. During this transport adequate precautions to prevent dissemination of infective material from the natural orifices of the body ought to be taken, and the vehicle used to convey the carcase should afterwards be thoroughly cleansed and disinfected.

As a precaution intended to prevent the transmission of the disease to the pig and dog, farmers ought to be warned against the danger of feeding either of these animals with the raw flesh of any farm animal that has suddenly died from some unknown cause.

Finally, when an outbreak of anthrax occurs among animals at grass, it is advisable to keep animals off the field in which the death occurred as long as possible, in the hope that such germs as must almost inevitably have been deposited on the surface of the soil or pasture may either be washed away by rain, or be destroyed by desiccation, sunlight, or the competition with more rapidly growing soil organisms.

DESCRIPTION OF PLATE I.

FIG. 1. Anthrax bacilli from spleen of rabbit, stained with methylene blue, mounted in water ($\times 535$).

FIG. 2. Anthrax bacilli from spleen of mouse, stained with gentian violet, washed in alcoholic solution of picric acid, mounted in Canada balsam ($\times 1000$). The envelope of the bacilli is unstained and invisible.

FIGS. 3. and 4. Anthrax bacilli from same source as in Fig. 2, stained with gentian violet, washed with acetic acid, mounted in water ($\times 1000$). In Fig. 3 the envelope of the bacilli is distinctly visible.

FIG. 5. Sporulating anthrax filaments from an agar culture, stained with carbol-fuchsin ($\times 535$). The envelope is well shown.

FIG. 6. An anthrax colony from a gelatine plate cultivation ($\times 30$).

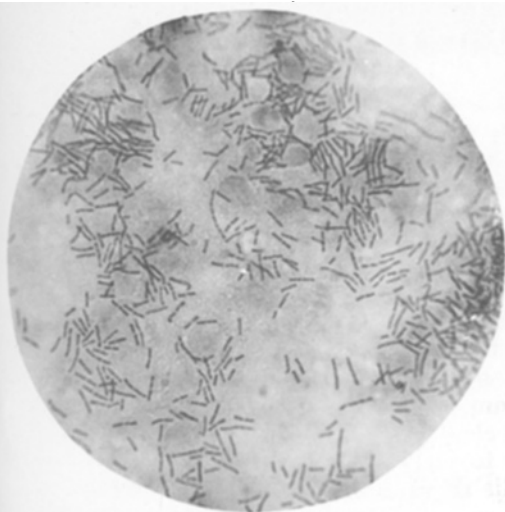


Fig. 1



Fig. 2



Fig. 3

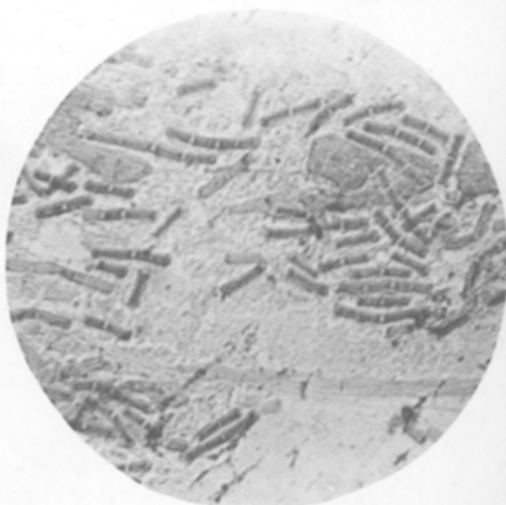


Fig. 4



Fig. 5

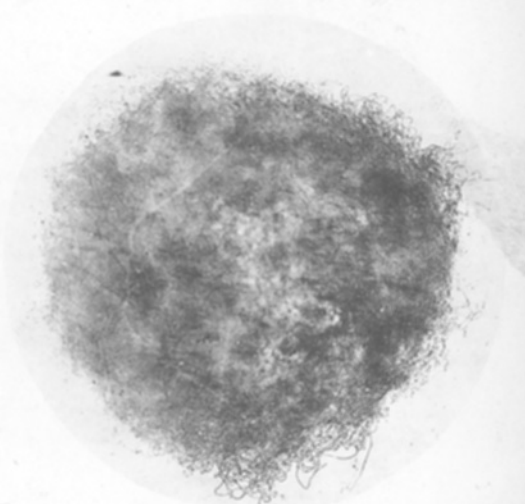


Fig. 6