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ON A SEPTICÆMIC BACILLUS FOUND IN THE OX.

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THIS bacillus was isolated from portions of the carcase of an ox which had been exposed for sale as human food at Smithfield Market, and subsequently seized. The meat was received from Dr Bond, Medical Officer of Health for Holborn, and the following note by his sanitary inspector describes the appearances of disease which led to the seizure of the carcase. "Four quarters of a young ox, weighing 50 stones, from Wavenden, Bucks, were found to be extremely soft, sodden, and wet, magenta coloured (varying from light to dark), and smelling strongly of drugs; there was much serous effusion and inflammation in the intermuscular and other tissues in many parts (muscles, glands, and kidney); the glands were enlarged and badly congested; the left kidney was inflamed, full of blood, and a large cyst was embedded in it; the right kidney had been removed. The meat was ill-bled, and the animal was evidently killed when moribund." The carcase, it should be added, was perfectly fresh.

Immediately the meat was seized Dr Bond sent the following portions to the British Institute of Preventive Medicine for bacteriological examination. (1) A kidney which showed a number of small hæmorrhages into its substance. There was a simple retention cyst in the cortical portion of the gland, which had obviously nothing to do with the fatal illness. (2) A lymphatic gland with some surrounding fat; the former was distinctly congested, and there were one or two small hæmorrhages into its substance. (3) A small portion of the diaphragm, which also showed a few minute hæmorrhages into the muscular substance. (4) A portion of a skeletal muscle sodden and dark-coloured.

In a stained cover-glass preparation of the juice expressed from the kidney a few bacilli were to be seen. Gelatin plates were inoculated with muscle and kidney juice, and from the resulting cultures a coccus and a motile bacillus were isolated. At the same time two guinea-pigs were inoculated under the skin with an emulsion made by shaking up small portions from the interior of the muscle with sterilised normal saline solution. Both these guinea-pigs died on the fifth day with double pneumonia, and a bacillus identical with that isolated from the plate cultures was obtained in pure culture from their heart-blood. The bacillus isolated from the plate cultures was similarly tested on guinea-pigs, and gave exactly corresponding results. In stained cover-glass preparations made from the pleural juice of each of these animals a bacillus could be seen which morphologically and in its staining properties resembled the bacillus seen in the cover-glass preparation made from the original kidney. The coccus isolated from the gelatin plates proved to be non-pathogenic for guinea-pigs, and its presence was assumed to be due to an accidental *post-mortem* contamination of the meat.

MORPHOLOGY.

The micro-organism when seen in the blood or tissues of an inoculated animal appears as a shortish bacillus with rounded ends. Under these circumstances it is of fairly constant length, and individuals may exhibit a tendency to bi-polar staining. When grown on artificial media, on the other hand, a marked degree of pleomorphism prevails, as a reference to Plate III., Fig. 1 will show. The predominant type is a bacillus measuring from $1.25\ \mu$ to $1.5\ \mu$ in length with rounded ends. Shorter rod shapes, almost oval bacilli, and occasional coccal forms also occur. Two bacilli may rarely be seen joined end to end, but chains of greater length are never seen. Spore formation was not observed; the bacillus is actively motile.

STAINING PROPERTIES.

The bacillus stains well with the ordinary basic anilin dyes. Methyl-violet and watery carbol-fuchsin particularly gave very good results. The bacillus is rapidly decolourised by alcohol after staining by Gram's method. Although culture experiments show that it is always present in large numbers in the heart-blood of inoculated animals, it is extremely difficult to demonstrate its presence there by staining methods. With well-prepared blood films it is only now and again that one may see a single or perhaps two bacilli in the microscope field. In the pleural, peritoneal, and tissue juices of inoculated animals, which have been killed before becoming actually moribund, the bacillus can be readily demonstrated.

CULTURAL CHARACTERISTICS.

The following are the principal points noted in connection with the culture of the bacillus on various media :—

Gelatin plate cultures, at 22°C. ; the colonies are visible to the naked eye at the end of the second day, and attain their maximum size by the end of the third or fourth day. They appear as small raised

white spots with sharp-cut edge, and are quite round, measuring on the third day about 2.5 mm. in diameter. Viewed under a low power and by transmitted light, they are seen to be finely granular in substance and distinctly brownish in colour. Colonies in the depth of the gelatin are of a uniform deep brown colour and rather opaque; surface colonies show a deeper brown central portion and a lighter brown peripheral zone. The edge of the colony is quite even and well defined (Fig. 2).

Gelatin streak, at 22° C.; very faint indication of growth at the end of twenty-four hours; at the end of forty-eight hours the growth appears as a narrow raised streak with well-defined edges which show no tendency to spread. The growth attains its maximum between forty-eight and seventy-two hours. Viewed by direct illumination the streak has an opaque white appearance, viewed by transmitted light it appears of a brownish colour. There is no liquefaction of the medium.

Gelatin stab at 22° C.; at the end of forty-eight hours there is a fine white growth along the entire length of the needle track with a small "pin-head" expansion at the surface end.

Gelatin shake, at 22° C.; on the second day a faint wavy cloudiness is seen in the medium. By the fourth day small round colonies may be seen in the depth of the gelatin, whilst the same are sprinkled over the surface.

Agar streak, at 37° C.; free, moist-looking, opaque, greyish-white looking growth with spreading edges at the end of sixteen hours. The growth increases in extent up to the end of about forty-eight hours, by which time the condensation water at the foot of the agar slope is opaque with growth. On agar at 22° C. growth is much slower than at 37° C., but considerably more active than on gelatin at the same temperature.

Glycerine-agar streak; growth is perhaps a little more active than on plain agar at corresponding temperatures.

Alkaline litmus-agar streak, at 37° C.; distinct colour change at end of twenty-four hours, deep red colouration at end of forty-eight. No subsequent discharge of colour. At 22° C. no colour change is obvious until the end of at least forty hours.

Alkaline litmus-sugar agar streak, at 37° C.; reddening much more pronounced at the end of twenty-four hours than in the case of plain litmus-agar. After seventy-two hours a gradual discharge of colour occurs, commencing at the bottom of the tube. This reducing action extends about half-way up the agar, and above that level the red colouration is reduced in intensity, but not discharged.

Sugar-agar stab, at 37° C.; active gas development by twentieth to twenty-fourth hour. At 22° C. gas development occurs some hours later.

Potato, at 37° C.; towards the end of the second day an almost invisible moist-looking patch may be distinguished on the surface; the area of the patch does not seem to increase in extent, and after several days assumes a faint yellowish-brown tinge. Growth on potato is feeble and uncertain, being not always obtainable.

Alkaline litmus-milk, at 37° C.; distinct colour change at end of twenty-four hours. The red colouration then gradually decreases and all colour is discharged by the fifth or sixth day. At 22° C. the

colour change does not occur in the first instance until several hours later than at 37° C.

Peptone beef broth, at 37° C.; at the end of sixteen hours there is a general cloudiness of the broth. By the end of the second day there is a small stringy deposit at the bottom of the tube. By the sixth or seventh day a considerable number of bacilli have lost their motility, and fallen to the bottom in small clumps. By the tenth or eleventh day there is a thick sediment of clumped bacilli, and the upper part of the fluid is appreciably clearer. By the twentieth day the upper part of the fluid is to the naked eye quite clear. On examining a hanging drop preparation of the clear fluid a few bacilli may still be seen in it. One or two of these may show a little sluggish movement, but the majority are absolutely motionless. A few days later the clear upper part of the fluid will be quite free from bacteria. The growth does not at any time form a pellicle on the surface, nor does it show any tendency to adhere to the sides of the tube. The reaction of the broth, originally alkaline, becomes strongly acid within twenty-four hours of inoculation. The acidity, as roughly tested by the reaction with blue litmus paper, begins to decrease on the third or fourth day, and by the fifth or sixth day the broth has again become feebly alkaline. The cultures were tested for indol up to the twenty-first day with negative results.

Figures 3 and 4 in Plate III. show subcultures from infected animals. Figure 3 shows the free growth obtained on agar at 37° C., and Figure 4 the much scantier growth on gelatine at 22° C. The tubes were inoculated by passing a platinum wire lightly over the surface of the medium, and were formalised at the end of twenty-four and seventy-two hours respectively.

EXPERIMENTAL INFECTION OF ANIMALS.

The pathogenic action of this bacillus was tested on tame mice, white rats, guinea-pigs, rabbits, and young bull calves. For the purpose of inoculation either an actively virulent subculture (between two and six days old) from the heart-blood of a previously inoculated animal, or a bacillary emulsion, made by shaking up a forty-eight hours' old culture on agar with 5 cc. of sterilised normal saline solution, was used.

Tame mice died between fourteen and eighteen hours after the subcutaneous injection of .1 cc. of the culture. Death was the result of a septicæmia, and the bacilli were recovered from the heart-blood and pleural juice. The only change noted *post-mortem* was some excess of fluid in the peritoneal cavity.

White rats were less susceptible to the action of the bacillus than any of the other animals used. They were obviously very ill twenty-four hours after the subcutaneous injection of .3 cc. of a culture, but death did not result from this dose and the animals were usually quite well again in three or four days.

In the case of medium-sized guinea-pigs also a dose of .3 cc. was used, and was invariably followed by a fatal result. When the injection was made into the muscles of the thigh death occurred within forty-eight hours, and usually towards the end of that period. The only changes to be seen *post-mortem* were a bloody œdema at the site of inoculation, and injection of the peritoneal membrane, with some

excess of fluid in its cavity. When a similar dose was injected into the subcutaneous tissue death occurred on the fourth or fifth day with double pneumonia. The animals became comatose towards the end, and often died in convulsions. Subcultures from various organs and from the blood showed that the bacillus was universally distributed. A doe guinea-pig which was inoculated proved to be pregnant, and in that case the bacillus was found in the amniotic fluid. Microscopically the condition of the lungs varied from one in which a number of small hæmorrhages were scattered throughout their substance to that in which large patches of pneumonia involved one or two lobes. Other changes usually present were capillary injection of the peritoneal and pleural membranes, with excess of fluid in the cavity of the former. Less commonly small hæmorrhages into the kidney substance were seen. Microscopic examination of sections of the lungs showed that the pneumonia was primarily a hæmorrhagic one.

With large rabbits a dose of .5 cc. was invariably followed by death within twenty-four hours, the greater number of those inoculated dying within eighteen. The animals died comatose, and death was preceded by a marked fall in body temperature. The bacillus was found universally distributed in the blood, and the only tissue change found after death was an extensive bloody oedema at the site of inoculation. In the case of rabbits it seemed to make no difference in the fatal result whether the injection was an intra-muscular or a purely subcutaneous one.

Three bull calves were inoculated with the bacillus. One survived the operation and ultimately recovered after a long period of ill health, one died a few hours after inoculation with a general infection of the blood, the third died on the eighth day with a well-marked septic pneumonia. The following notes give the more important details with regard to these several calves.

Experimental Calf No. I., about six weeks old. This calf was inoculated with 2 cc. of a broth culture (twenty-four hours old) from the heart-blood of a rabbit. The injection was made on 26th January 1897 into the subcutaneous tissue on the left side of the neck. On 27th January the animal seemed poorly, there was some swelling about the site of inoculation, temperature in rectum 39.8° C. On 28th January there was no change in the general condition, but there was now at the site of inoculation a definite flattened swelling with a circular outline measuring about 8 cm. in diameter; temperature 39.8° C. On 29th January the swelling was slightly more prominent; the animal had diarrhoea and was voiding a little blood-stained mucus; temperature 40.5° C. On 30th January there was still free scouring; the swelling was very hard and presented no sensation of fluctuation; temperature 40.2° C. During these days the animal had been taking its food fairly well, but had lost a great deal of flesh. Nothing special occurred until 15th February when an attack of synovitis developed in the left fore knee. In the middle of April the calf was still in very poor condition; the knee joint was still distended with fluid, and the character of the swelling on the neck remained unaltered. Shortly after this the animal's condition improved, and the synovitis passed away leaving an apparently good joint. On 14th May, the calf being in very good condition, I decided to explore the swelling on the neck, which presented itself as a hard, flattened, circumscribed tumour with

circular outline and freely movable between the skin above and the neck muscles beneath. On cutting into it I found a hard, white, oblong mass of necrotic tissue measuring about 4 cm. by 2.5 cm., and about .75 cm. in thickness. This was lying quite loosely in a smooth-walled cavity which was free from pus or fluid of any kind. The operation wound healed up very quickly, and all trace of swelling disappeared. The calf was kept under observation until the end of July, and appeared to have made a perfect recovery.

The necrotic mass when removed was quite free from any smell, and contained no living bacteria. Microscopically it consisted chiefly of altered striated muscle fibre derived from the panniculus carnosus and a certain amount of connective tissue. Here and there faintly stained degenerated forms of bacilli could be seen. The specimen was interesting from a pathological point of view as being a perfect example of necrosis without suppuration, a condition which in the human subject, at anyrate, is quite uncommon, but is met with occasionally in the "quiet necrosis" of bone.

Experimental Calf No. II., aged about three weeks. In the case of this animal 2 cc. of a broth culture (forty-eight hours old) from the heart-blood of a rabbit were injected into the external jugular vein at 11.15 A.M. on 15th February. At 3.30 P.M. the calf was found to be moribund; it was bringing up a little blood-stained fluid, apparently from the lungs. It died at 4.30 P.M., just five and a quarter hours after the injection. The body was opened at six o'clock the same evening. There was no sign of thrombosis in the vein into which the injection had been made. Both lungs were extremely congested and œdematous. The right lung showed a small irregularly shaped hæmorrhage at its internal border; this had not the appearance of being a hæmorrhagic infarct, and it may be added, in view of the rapidity with which death occurred, that there was no sign of pulmonary embolism. There was a little blood-stained fluid in the right pleural cavity. There was also a recent hæmorrhage into the ligament representing the remains of the umbilical vein. A subculture on agar taken from the blood an hour and a half after death gave a very free growth of the bacillus (Fig. 3).

Experimental Calf No. III. This was a particularly fine looking pedigree animal between four and six weeks old. It was inoculated on 12th April with .75 cc. of an emulsion made by shaking up a two-days-old culture on agar from the heart-blood of a rabbit with 5 cc. of sterilised normal saline solution, the injection being made into the external jugular vein. The animal seemed fairly well up to 17th April. During the six days it took its food well, its respiration was rather hurried, but there was no abnormal chest sounds. There was no swelling or induration at the site of inoculation. The temperature was elevated, but unfortunately the record for these days was mislaid. On 18th April the animal was obviously very ill, lying down and refusing to stir. Its respiration was very hurried, its temperature was 40.5° C., and it was still taking its food. On the morning of 19th April it seemed a little better, but its temperature was 41.2° C. At mid-day it was moribund, and it died in the evening after its temperature had risen to 41.8° C. The body was opened next day. A double pneumonia was found, the greater part of both lungs being in a condition of red hepatisation. Here and there were distinct small hæmorrhages

into the lung substance. The pneumonic process was especially marked at all the borders, and throughout the upper lobes. There were several small yellowish-white necrotic patches along the borders. A central part in each lower lobe was not pneumonic, but merely congested and œdematous. The bronchial glands were swollen, and appeared to be in a condition of almost purulent infiltration. There was a slight excess of peritoneal fluid. The kidneys were rather paler than is normal; scattered throughout their substance and especially numerous in the pyramidal portion were a number of greyish nodules rather larger than a pin's head. Cultures of the bacillus were obtained from the heart-blood, the pleural juice, and the kidney. The microscopic examination of sections of the lungs showed that the general condition was one of fibrinous pneumonia. Sections through a necrotic area showed patches of altered tissue which stained a faint yellowish-green colour with methylene blue. In the necrotic patches isolated bacilli which had taken the stain deeply were seen lying about. Sections through the grey nodules in the kidney showed the same appearance of necrotic tissue and isolated bacilli. Had the animal survived a little longer these nodules would doubtless have assumed the form of multiple miliary abscesses. There was no thickening about, or thrombosis in, the vein into which the injection was made.

The results of these inoculation experiments and the conclusions to be drawn from them may be summarised as follows:—

(1) White rats were not very susceptible; they suffered a temporary illness and recovered.

(2) Rabbits and tame mice with a subcutaneous, and guinea-pigs with an intra-muscular, injection, died within forty-eight hours. The cause of death was an acute simple septicæmia unaccompanied by any gross tissue change; in other words the decomposition of their own proteid constituents and the consequent evolution of toxic substances was so rapid that the animals were poisoned before reaction effects in the tissues had time to show themselves.

(3) Guinea-pigs, after an injection into the subcutaneous tissue, died on the fourth or fifth day with a double septic pneumonia added to the general septicæmia. In these cases the bacilli entered the general circulation more slowly, the guinea-pigs were rather less "susceptible" than the rabbits, the septic process was more deliberate, and sufficient time elapsed before death occurred for the local effects of the poisonous decomposition products to become manifest.

(4) With regard to the calves—Calf No. I., survived a subcutaneous inoculation. I think in this case it is clear that the bulk of the bacilli injected into the subcutaneous tissue never travelled beyond the site of inoculation, but were checked by the intensity of the local reaction—to speak in general terms. The actual dose of bacilli which entered the general circulation was so small that the natural powers of resistance were in the result equal to the stress thrown upon them, and the animal recovered after a prolonged illness, including an inflammation of a synovial membrane and necrosis of the area of tissue into which the bacilli were injected. Calf No. II., had a very large number of bacilli thrown directly into the circulation; the bacilli, as the culture experiments show, are capable of extremely rapid increase at the body temperature, and the result was a septicæmia even more acute than

in the cases of the rabbits and mice. Calf No. III., which appeared to be a stronger animal than either of the others, received a medium dose directly into the circulation. But sufficient time elapsed before death occurred, not merely for a local inflammatory reaction in the lungs to manifest itself, as in the case of the guinea-pigs which survived inoculation for five days, but for a further stage to be reached—necrosis of the poisoned tissues.

In carrying out the inoculation experiments it was found that there was a considerable diminution in the virulence of broth cultures after the seventh day; a diminution which, it may at once be said, was probably due to the rapid dying out of the bacilli rather than to the lowering of the pathogenic activity of individual bacilli. Thus a beef-broth culture 5 cc. of which on the sixth day killed a large rabbit in sixteen hours appeared to be quite inert on the tenth day in a dose of 2 cc. This result was tested several times, and no variation occurred. Such a culture of ten days' growth, however, proved fatal to a rabbit after two injections of 2 cc., each with an interval of sixteen hours. The animal died within twenty-four hours of the second injection, and a subculture from its blood gave a characteristic growth which possessed the normal virulence. After passing the original culture through a considerable number of guinea-pigs and rabbits, it was found at the end of eleven months to possess just the same degree of virulence for these animals and for mice as it did at the commencement.

All attempts to infect mice, rats, and guinea-pigs by the mouth failed.

SOME BIO-CHEMICAL REACTIONS.

Some experiments were carried out to determine the decomposition products resulting from the action of this bacillus upon carbo-hydrates and proteids.

For the former the medium was prepared as follows :—

Glucose	40	grammes
Peptone	10	„
Sodium chloride	7.5	„
Calcium carbonate	20	„
Distilled water	1000	„

The flask containing this was steamed on two successive days for forty-five minutes. After inoculation the flask was incubated for twenty-one days at 37° C. The following results were arrived at. A large proportion of the glucose had undergone decomposition, only about 4.5 grammes remaining unaltered in solution. From the contents of the flask succinic and lactic acids were recovered, but were not estimated quantitatively. About 1 cc. of alcohol was recovered by distillation; this gave the iodoform test and was probably ethyl alcohol. The volatile fatty acids recovered were small in amount, about .1 gramme expressed in terms of acetic acid; they were not separated qualitatively. There was no evolution of sulphuretted hydrogen.

In order to study the proteid decomposition the following medium was prepared. Five hundred grammes of lean beef were finely minced and added to 1000 cc. of a .75 per cent. sodium chloride solution in a flask. The flask was steamed as before. Its sterility having been tested, the flask was inoculated and kept at 37° C. for twenty-eight days, during which time active growth of the bacilli took place. The results

(For description of Figs. 1 to 5, see opposite page.)

DISEASED NAVICULAR BONES.
VET. COLLEGE, DUBLIN, IRELAND.

ARTIFICIAL TEETH FOR DOG.
PROFESSOR HOBDAV'S CASE.

of the examination of the contents of the flask were mainly negative. There was no production of indol, phenol, or skatol, nor were mercaptans or sulphuretted hydrogen present. There appeared, however, to have been some production of ammonia during the growth of the culture. The exact nature of the acid produced in proteid media, as noticed in reference to beef-broth cultures, was not ascertained owing to the dying out of my cultures during a temporary absence from the laboratory, and before my researches in this subject were completed. This production of acidity from proteid matter is of some interest when considered in relation with the rapid pathogenic action of this bacillus in the rabbit, an animal which is known to be extremely sensitive to any alteration of the reaction of its blood in that direction. That the bacillus has no peptonising action is indicated by the fact that no liquefaction of gelatine occurs.

In conclusion I have only to add that this bacillus does not belong to the so-called "hæmorrhagic septicæmia" group of bacteria, to which it may possibly bear some superficial resemblance. Nor can I ascertain that it corresponds with any other pathogenic bacillus hitherto described. It has, then, been thought worth while to record its characteristics rather minutely in the hope that it may be identified again.

My very best thanks are due to Professor M'Fadyean of the Royal Veterinary College for the facilities afforded me at that Institution for carrying out the calf inoculations which have been referred to herein.

DESCRIPTION OF PLATE.

FIG. 1. Cover-glass preparation from culture on agar $\times 1000$.

FIG. 2. Colonies from gelatin plate on fourth day $\times 20$.

FIG. 3. Smear culture on agar from blood of calf, incubated for twenty-four hours at 37° C. (Natural size.)

FIG. 4. Smear culture on gelatin from pleural juice, incubated for seventy-two hours at 22° C. (Natural size.)

FIG. 5. Section through necrotic patch in lung of calf dying on eighth day after inoculation, stained with mythylene blue, $\times 1000$.

Photographs by Mr Bernard from Mr Foulerton's preparations.

(For description of the other two figures see pages 370 and 362.)

THE PROPHYLAXIS OF GLANDERS OF THE HORSE.¹

By Professor NOCARD, Alfort Veterinary College.

THEME.

1. Every horse which presents any symptom whatever indicating the existence of glanders or farcy ought to be submitted to the mallein test. If the mallein provokes a complete reaction (both organic and thermal) the horse ought to be declared glandered, and slaughtered without delay; if, on the contrary, the mallein does not provoke any reaction, the horse ought to be declared not glandered, whatever may

¹ A communication made to the Thirteenth International Congress of Medicine, held at Moscow, from the 19th to the 26th September 1897.