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STUDIES IN BLACK DISEASE.

A BRAXY-LIKE DISEASE OF SHEEP.

By SYDNEY DODD, D.V.Sc., F.R.C.V.S., Veterinary Research
Laboratory, The University of Sydney, New South Wales.

PART I.

FOR about fifty years a disease which affects sheep and causes considerable mortality has been known on the Southern Highlands of New South Wales, but it is not until 1895 that any records appear of attempts to investigate its etiology. The early records are very meagre, and throw little or no light upon the subject except as regards its distribution and lesions.

In 1895 Mr S. Pottie, M.R.C.V.S., made an investigation and suggested that the disease be called braxy, apparently on account of its clinical resemblance to the European disease of that name. In his report, however, no description of the symptoms or lesions was given.

In 1901 Professor J. D. Stewart, then Government Veterinary Surgeon, visited the locality of an outbreak. He, however, submitted no report, and said that he was unable to make any definite statement as to the nature of the disease.

The first official record of the name "black disease" appears in 1901, when the stock inspector at Cooma submitted specimens from dead sheep to the State Bureau of Microbiology for examination and opinion. The result was negative.

The first official record of experimental inoculations in the endeavour to transmit the disease artificially occurs in 1902, but no results are given, and it is not stated what the nature of the inoculations were. One must conclude that the results were negative.

Attention somewhat later on was drawn to an article in the Sydney *Sunday Times* by Dr Brown of Melbourne, in which he described a disease of sheep in Victoria, apparently similar to black disease, and claimed that he had found the causal organism of it. He named the condition pneumo-enteritis. The claim was not substantiated.

In May 1908 black disease was reported in the district of Goulburn, twenty-five sheep having died (two or three every few days). No account of autopsies, however, appears in the report. Smears examined by the Bureau of Microbiology were negative as regards ascertaining the cause.

In May 1909 thirty wethers out of 2400 died near Braidwood. They had been depastured at Yass, on the Southern Highlands, from February until April. The stock inspector suspected black disease. At the end of the month the sheep were still dying at the rate of two daily. In this year the State Bureau of Microbiology began a systematic examination of specimens taken from sheep dead of this disease, but the efforts to ascertain the cause of the condition failed.

During the same year Mr M. Henry, B.V.Sc., M.R.C.V.S., investigated an outbreak in the Goulburn district, and reported that 500 out of 3000 sheep had died. The mortality on this special sheep station occurred every year from May to September. In a few cases the affected animals showed symptoms of illness a day or so before death. He found a ewe apparently just dead, and the following is his account of the *post-mortem* examination: "Skin and subcutaneous tissues ecchymotic. Considerable peritoneal fluid. Some bile-staining of the intestines. Stomachs normal. Intestines distended with gas. Spleen slightly enlarged and softened. Liver rather soft and friable. Gall bladder distended. Lungs slightly congested. Some pleural effusion. Pericardium distended with a pale, clear, straw-coloured fluid."

Mr Henry stated that the above were the usual lesions found in this disease. *Post-mortem* decomposition was very rapid, and sheep died without a struggle. The report is headed "Report on Braxy-like Disease, known as Black Disease in Sheep."

From 1909 to 1913 the Bureau of Microbiology, Department of Public Health, made various experiments with guinea-pigs and rabbits, and also a sheep, but were unable to produce black disease. Some feeding experiments were also made upon sheep with minced up intestines from sheep dead of the disease, but without result. At the Bureau various organisms were also isolated from material sent for examination, but none of them could be associated with the cause of the disease. Apparently they were *post-mortem* invaders. It was also stated that "Gilruth's bacillus" could not be detected.

In 1913 officers of the Bureau of Microbiology made a large number of autopsies in the field upon sheep dead from black disease. The work was of value in so far as a knowledge of the lesions are concerned, but no light was thrown upon the etiology of the disease.

The foregoing abstracts are taken from officially reported cases of black disease. They represent a very small fraction of the cases actually occurring.

Two explanations of the origin of the term black disease are

given, viz., (1) on account of the dark appearance of the liver of animals dead of the disease; (2) because of the dark colour assumed by the under surface of the skin soon after removal from the body. The latter explanation is the one given me by stockowners; and certainly, on account of the congestion of the subcutaneous vessels, the skin does assume an unusually dark appearance after removal, especially when contrasted with the skins of sheep dead from other causes. Indeed some owners diagnose the condition by the colour of the skin when removed.

Distribution of the Disease.—In New South Wales it appears to be confined to the Southern Highlands. It has been reported in localities outside this area, but in the authenticated cases the sheep had recently come from areas in which black disease is enzootic. There appears to be some reason for believing that the disease is slowly spreading, but this may be more apparent than real, and due to the fact that more attention is being drawn to the condition. Furthermore, when sheep die unexpectedly in certain areas many of the stockowners have a tendency to attribute the fatality to black disease whatever the real cause may be.

A very similar condition occurs in Victoria on similar country. Dr Gilruth, who investigated the Victorian disease, considered that the two conditions were probably identical, although he had not, to my knowledge, seen cases or specimens from sheep dead of black disease.

In its clinical features it also resembles a braxy-like disease of sheep in Tasmania.

Finally, its clinical features and lesions resemble the disease in sheep in Europe known as braxy or bradsot.

Seasonal Occurrence.—The period of the year during which mortality occurs from black disease varies somewhat, apparently in relation to climatic conditions. In the Monaro district of the Southern Highlands, where the winters are for Australia fairly severe, the elevation being roughly between 2000 and 4000 feet, the disease usually makes its appearance about February (late summer) and lasts until about May (early winter). As a rule the mortality is stated to cease to a great extent with the onset of frosty weather, although a few sheep may die at irregular intervals throughout the winter. In 1914 I saw numbers of sheep dead from the disease in paddocks which were heavily covered with frost.

Apparently in a wet, warm winter the mortality is almost as heavy as in the autumn. It is quite possible also that occasional sheep may die at any time of the year, but on account of the large size of the flocks and the large areas over which the sheep graze no special attention is drawn to the death of an odd sheep.

In other localities more inland, in what might still be termed the black disease area, where the winters although fairly cold are not so severe as in the Monaro district, the disease apparently begins to make its presence noticeable about May and continues till about September (the winter months).

It is enzootic in character, but, although it may be seen more or less on certain sheep stations every year, its incidence in any particular paddock is very erratic. A number of cases may occur in a paddock one year, whilst in the following year in the same

enclosure there may be none or only an occasional case. Some affected areas are also sharply demarcated from areas in which the disease is seldom or never seen. Not uncommonly the boundaries of such areas are artificial, such as fences or walls, whilst the geological conditions are identical and the water supply may be the same, *e.g.*, a creek flowing through, or a lagoon fronting both paddocks.

As an instance of the foregoing, in 1916, on a station running 26,000 sheep, 250 died in a paddock containing 1600. Of the remaining 24,400 sheep none were said to have died from black disease, although some of the paddocks were adjoining the former.

Age Incidence, Etc.—There does not appear to be any special age at which the disease occurs. In some districts it is said to affect principally sheep between one and two years. In the Monaro district, where most of my own investigations up to the present have been conducted, the mortality is principally among adult sheep. In this instance it may be because the young animals were not so exposed to infection, *i.e.*, were not grazing in infected paddocks.

The disease is seldom seen in animals under one year old, although in the Goulburn district, in the season 1916, young lambs from six to twelve weeks old were dying in a notoriously infected paddock. I saw two just prior to death, and the clinical symptoms and *post-mortem* appearances were identical with those of black disease.

The sheep affected are invariably in good condition.

Sex does not appear to play any part. In 1914 on one of the stations on which my researches were being conducted the mortality was chiefly among wethers, whereas in 1915 ewes were run in the same paddocks as the wethers of 1914 and suffered heavily. Rams have no immunity.

The disease appears to be peculiar to the sheep. No cases have been observed in other animals grazing in paddocks known to be badly infected.

Mortality.—This varies considerably every year, even on the same station, and the various factors influencing the incidence of the disease are not yet clearly understood. The statements of various sheep owners are often completely at variance with each other. In some flocks the mortality is from 15 to 30 per cent. per annum, while in others only a few may die during the season. Most stock-owners are of opinion that once a sheep is affected death is certain to result. This is probably because they seldom know that an animal is affected until it is not far from a fatal termination. My own experience leads me to the opinion that recoveries do occasionally take place.

In 1915 on three stations the loss from black disease was 3000, 1200, and 500 respectively, the percentage mortality being heaviest in the last. Considerable losses were experienced elsewhere, but that year was an unfavourable one for stock generally, on account of drought. It is impossible to obtain any true estimate of the actual death-rate from black disease, as owners in the majority of cases conceal their losses for various reasons, and some even deny its existence at all, although one is aware that sheep are dying in their paddocks. It is quite certain, however, that the direct annual

loss from this disease amounts to thousands of pounds. One investigator has estimated that the direct and indirect annual loss amounts to at least one hundred thousand pounds, and that estimate was made when sheep were very much cheaper than at present.

Symptoms.—As a rule, and unless the sheep are watched closely all day, no symptoms are observed. The animal is simply found dead in the morning on the spot where the flock had camped during the night. On large sheep stations, where the paddocks are of considerable size, sheep have predilection spots where they sleep, although during the day they may graze over the whole of the paddock. Consequently, these are the places where one usually finds the majority of the dead animals. Deaths, however, do take place during the day in various parts of the paddocks, although far fewer than during the night; but, on account of the great area to be covered and the nature of the ground, the bodies are frequently overlooked, and the actual losses not ascertained until the monthly count of the flock.

Nevertheless, if the flock be carefully watched, the sick animals do show some signs of illness prior to death, although such signs are not marked, and may very readily be overlooked. Furthermore, even with the greatest vigilance, the period elapsing between the first discovery of symptoms and death is generally not more than a few hours, frequently less than an hour; at times it is a matter of minutes only.

A point to be kept in mind is that there is a considerable difference in the behaviour of sheep which are kept in small areas and accustomed to the presence of human beings, as in Europe, and those living in large paddocks, often miles in area, as in Australia. The latter animals are semi-wild, and it is practically impossible to get near them on foot; and even when the observer is mounted the sick animals, although seriously ill, will make endeavours to avoid close observation and try to keep up with their fellows as they move about the paddocks. Consequently, the careful, minute study of initial symptoms in the field is exceedingly difficult, and it is not practicable to keep a flock of several thousand sheep penned up in a yard for any length of time.

During my investigations in the field in 1915-16 it was my practice to watch the grazing flocks closely from daylight to dark, following them about the paddocks as they grazed, without disturbing them in any way. Even with such measures, cases are very difficult to detect in the early stages. As a rule, they are not discovered until the animal is not very far from the point of death. In some cases the immediate cause of death appears to be cardiac embarrassment resulting from pressure of pericardial exudate, combined with the exertion required by the endeavours of the animal to keep up with its fellows as the latter are grazing during the day or moving off to their camping ground in the evening. On several occasions a sheep, apparently near the point of death, when placed in a shed where it has been compelled to keep quiet has been found the next morning to outward appearances quite recovered, and consequently been released.

If the flock be watched carefully an affected animal will be observed to lag behind the others in their migrations about the

paddock, although until the last it will always strive to keep up with the remainder. At first this appears to be accomplished with little or no undue effort, but gradually the periods of rest become more and more prolonged, and the efforts to keep up with the flock more pronounced. As a rule, when mixing with the rest of the sheep the sick animal makes no attempt to feed, yet even this is not constant. Some sheep have been seen grazing and a few hours later found dead. Instead of standing with the usual alert attitude when human beings are near, the affected sheep usually appears rather indifferent to its surroundings. When moving on uneven ground the animal will at times be observed to stagger a little. Finally it lies down and ceases to attempt to follow its fellows. At this stage on being approached the animal may or may not make an attempt to escape; it depends upon how far the disease has progressed. Speaking generally, it will be seen that until the final stages there is nothing to attract the attention of the casual observer except that the animal permits its fellows to get further and further away—a thing which a healthy sheep living under these conditions will seldom do.

As an illustration of the foregoing, several owners have told me that they have never seen a sheep sick from black disease, but have always found the animals dead, usually in the morning.

When a sheep lies down finally it does so very quietly, and there are no attempts to struggle or regain its feet.

The temperature when the animal is first discovered is usually between 104° and 107° F. The latter temperature, however, is more frequently seen at the moment just preceding death. There is no marked injection of the visible mucous membranes. The respirations are shallow. The condition of the pulse affords little or no information in these half wild and exceedingly timid animals.

If the sick animal be kept in an enclosure it may evince a little restlessness, but this may be merely from a desire to regain its fellows. Later on the respirations frequently assume the Cheyne-Stokes type. The animal lies down if it has been standing, and dies with hardly any movement. Consciousness, however, appears to be preserved until the last.

Usually the time elapsing between the appearance of marked symptoms and death is about half an hour or an hour.

Towards the end of May 1914 the writer was invited by Mr S. D. Symons, M.R.C.V.S., Chief Inspector of Stock, New South Wales, to undertake investigations into the etiology of black disease.

In 1914 the black disease season was approaching its end when the work was begun; and, although the mortality over a large area had been heavy, no sick animals could be found in spite of the watching from daylight to dark of some thousands of sheep. Five animals were, however, found dead in the early morning on various days, although on inspection of the flocks overnight no signs of illness had been detected. The nights were cold, heavy hoar frost covering the paddocks till well after sunrise. The animals could have been dead only a few hours at the most when discovered, as their bodies were quite warm.

*Post-mortem Examination of Sheep found dead of Black Disease.**Case 1.*

Aged ewe. Found dead noon, but autopsy not made until four hours later. Fat. Decomposition well advanced. Congestion of subcutaneous vessels not very marked. A few cc. of dark blood-coloured exudate in peritoneal cavity. Putrefactive changes marked in the liver, kidneys, and spleen. The last showed a few hæmorrhages under the capsule. Thoracic and abdominal lymphatic glands congested and rather œdematous. Rumen, reticulum, and omasum normal. Mucous membrane of abomasum moderately congested, but masked by *post-mortem* changes. Intestines normal in appearance. Pericardium contained about 60 cc. of blood-stained liquid.

Case 2.

Aged ewe. Found dead on arrival at paddock in morning. Body warm. Condition good. Rigor mortis setting in. No discharge from natural orifices. On skinning, marked congestion of the subcutaneous veins evident. No discoloration or other changes visible in the muscles, but there was a moderate amount of a clear, straw-coloured, gelatinous exudate infiltrating the intramuscular connective tissue of the abdominal muscles. No subcutaneous emphysema. No odour of putrefaction on opening the carcase. A few cc. of clear, gelatinous exudate in the pericardial sac. Heart blood well clotted. A number of subendocardial hæmorrhages in the left ventricle. Lungs apparently normal. About 100 cc. of clear, straw-coloured exudate in the pleural cavity. The peritoneal sac contained rather more than 200 cc. of a blood-stained exudate. Spleen slightly enlarged, pulp softer than normal. Kidneys moderately congested and softened. Liver showed a moderate degree of fluke cirrhosis. On both anterior and posterior surfaces of the organ were several necrotic areas varying in size up to an inch in diameter. Also a few hæmorrhagic foci about half an inch in diameter. Rumen, reticulum, and omasum normal. Abomasum: moderate reddening of the mucous membrane (probably *post-mortem*). No necrosis, œdema, or ulceration. Duodenum slightly, and jejunum intensely, congested in parts; elsewhere the congestion varied from slight to moderate, with a few scattered petechiæ. The ileum presented very little congestion, but numerous small hæmorrhages. The large intestine was normal save for one or two small patches of moderate congestion of the mucosa.

Case 3.

Aged ewe. Found dead 7 A.M. Condition good. Rigor mortis disappearing. Slight abdominal tympany. *Post-mortem* decomposition advanced. Subcutaneous tissues along neck and back discoloured. A few cc. of blood-stained exudate in the thoracic cavity. Lungs normal. Pericardium distended with a partly gelatinous, partly liquid, blood-tinged exudate. Blood clot soft. No subendocardial hæmorrhages. Thoracic and abdominal lymphatic glands slightly congested. Abomasum showed some patchy congestion at the pyloric extremity. Duodenal mucous membrane slightly

congested. The remainder of the small intestines showed patchy congestion. Large intestines normal. Spleen not enlarged, but pulp slightly softened. Kidneys softened and a little congested. Liver showed fluke cirrhosis. No necrotic or hæmorrhagic areas. *Post-mortem* decomposition of the organ marked. About 15 cc. of sanguinolent, turbid fluid in the peritoneal cavity.

Case 4.

Aged ewe. Found dead daylight. *Post-mortem* decomposition advanced. No congestion of subcutaneous vessels seen after removal of the skin. About 50 cc. of turbid, blood-stained liquid in the thoracic cavity, 300 cc. of a similar fluid in the peritoneal sac, and about 15 cc. in the pericardial sac. The liver presented advanced fluke cirrhosis, but no hæmorrhagic or necrotic areas. A few small areas of moderate congestion were present around the pyloric orifice of the abomasum. The bronchial and mediastinal lymphatic glands were congested, whilst the remainder were normal. No other lesions were observed.

Case 5.

Ewe aged about three years. Found dead at daylight. Had been observed ill when yarded the previous evening. Condition good. *Post-mortem* decomposition advanced. Pericardium distended with a clear, sanguinolent exudate. Heart normal. Lungs congested. About 100 cc. of a turbid, bloody exudate in the pleural cavity, and about 200 cc. of a similar exudate in the peritoneal sac.

Liver showed marked *post-mortem* decomposition. No hæmorrhagic or necrotic areas. A few ecchymoses under the capsule of the spleen. Kidneys very pulpy. The abomasum showed several hæmorrhagic areas about an inch in diameter near the pylorus. A few scattered patches of congestion of the mucous membrane were present in the intestines, which were otherwise normal.

There was no very marked congestion of the subcutaneous vessels.

Pipettes were filled with blood and exudates from the various animals for subsequent experimental work, and portions of organs, etc., were taken for microscopical examination. Six sheep were also inoculated on the station where the deaths had occurred with various materials from the dead animals. All of them succumbed as the result. The *post-mortem* findings were very much the same, consequently it is proposed to give details of only four of them here.

Sheep No. 1.

Aged ewe. Inoculated 19th May 1914 subcutaneously inside thigh with 5 cc. heart blood from Case 3.

20th May, 4 P.M. (twenty-four hours later). Animal distinctly lame; carrying inoculated leg if hustled.

Thirty-six hours later. Sheep comatose. Very little signs of struggling. Temperature 100° F. Breathing stertorous. Around the point of inoculation an area a few inches in extent was swollen, oedematous, and slightly livid. On its surface were a few droplets of reddish exudate.

Thirty-seven hours later. Sheep dead. *Post-mortem* examination made at once.

Autopsy.—Subcutaneous vessels injected. The inoculated leg showed a little subcutaneous gas formation around the site of inoculation. On incision of the skin over this area a small amount of blood-tinged liquid drained away. When the underlying superficial muscles were incised a distinct, peculiar, but not putrefactive, odour was observed. Some of the affected muscles had a dark, hæmorrhagic appearance not unlike that of black-leg. Other affected muscles had a clay-coloured appearance, and on incision had a distinctly putrefactive odour.

A small unmeasured quantity of clear, odourless, colourless fluid in the thoracic cavity. Lungs presented a few ecchymoses. The pericardium contained about 15 cc. of clear, straw-coloured exudate. A few subendocardial hæmorrhages in the left ventricle. A slight excess of blood-tinged exudate in the peritoneal sac. A few petechiæ in the omentum. Abomasum congested in patches. First portion of duodenum intensely congested, the remainder of the small intestines moderately to deeply congested.

In the large intestines congestion varied from slight to intense between the cæcum and the rectum. Lymphatic glands generally, congested. Kidneys congested. Spleen normal. Liver rather friable; no necrotic or hæmorrhagic areas, but a few shreds of fibrinous lymph on its posterior surface.

Sheep No. 3.

Aged wether, fat.

9.30 A.M., 21st May 1914. Inoculated subcutaneously into thigh with 5 cc. heart blood from Case 1.

Noon, 23rd May 1914. Temperature 105° F. Animal very ill and in pain. Lying down. Inoculated thigh swollen, œdematous, and rather livid. A blood-tinged exudate oozing through the skin over the hock.

2 P.M. Animal *in extremis*. Killed.

Autopsy.—Subcutaneous vessels congested. In the inoculated leg the inner side of the thigh was swollen, œdematous, and slightly livid. The swelling extended along the floor of the abdomen as far as the umbilicus, and down the leg to the coronet. No subcutaneous gas formation. On incision of the skin a fluid drained away, which was blood tinged around the site of inoculation but colourless elsewhere. The superficial muscles of the inner side of the thigh appeared normal save for an œdematous character, but in deep muscles of this area there were a few hæmorrhagic zones, dark and varying in size from that of a lentil to that of a cherry. There was no odour of putrefaction. No excess of fluid in the thoracic cavity. Lungs congested. A few cc. of clear, straw-coloured fluid in the pericardium. No subendocardial hæmorrhages. Mucous membrane of abomasum œdematous but not congested. Duodenum markedly, and jejunum very slightly, congested. Ileum showed distinct patchy congestion. Cæcum normal. The first half of the colon showed a moderate, diffuse congestion, whilst in the latter half the congestion was patchy, with here and there actual hæmorrhages in the mucosa. The fæces in the rectum showed shreds of

blood-stained mucus on the surface. Liver and spleen normal. Kidneys congested. Lymphatic glands slightly congested, with the exception of the precrural gland on the inoculated side, which showed intense congestion.

Sheep No. 5.

Aged ewe.

22nd May 1914. Inoculated subcutaneously into thigh with 5 cc. heart blood from Case 5.

6.30 A.M., 24th May 1914. Animal found dead.

Autopsy.—Marked rigor mortis. Subcutaneous vessels congested. No excess of fluid in thoracic cavity. Lungs congested. A small quantity of blood-tinged fluid in the pericardium. A few sub-endocardial hæmorrhages in the left ventricle. A few cc. of turbid fluid in the peritoneal sac. Abomasum showed a moderate general reddening of the mucous membrane. Duodenum deeply congested. Jejunum normal. Ileum congested in patches. Large intestines presented a moderate general congestion. Spleen and kidneys softened. The liver contained a few circumscribed necrotic foci, each about the size of a lentil. Lymphatic glands in general deeply congested.

The whole of the upper part of the inoculated leg from the groin down to the hock was swollen and oedematous. A little subcutaneous gas formation in this area. The skin for a few inches around the site of inoculation livid. On incision of the skin over the swollen area a quantity of sanguinolent fluid drained away. On section the muscles near the point of inoculation were dark and hæmorrhagic, and had a rather putrefactive odour. A few inches from this zone the muscles, although dark in colour, had a peculiar rancid odour, not at all putrefactive, and in places were emphysematous.

Sheep No. 25.

Wether aged four years.

4th May 1914. Inoculated subcutaneously with 10 cc. of citrated heart blood from Case 4. Result: slight thermal reaction only.

11th May 1914. Inoculated subcutaneously with 20 cc. of a seven days old anærobic serum broth culture of a bacillus isolated from the heart blood of Case 2. No reaction.

25th August 1914. Inoculated subcutaneously into thigh with 4 cc. of a twenty hours culture in serum formate broth (anærobic), sixth from the peritoneal fluid from Case 3.

9 A.M., 26th August 1914. Sheep very ill. Lying down; temperature 104° F. Respirations hurried. Visible mucous membranes injected. Inoculated leg swollen and oedematous. Slight lividity around the site of inoculation.

9 A.M., 27th August 1914. Swelling increased in size and extending to the coronet. Temperature 105·8° F. At one or two places on the inside of the hock a few droplets of blood-tinged exudate had oozed out. For several inches around the site of inoculation the skin was distinctly livid, the livid area being sharply circumscribed and the line of demarcation very dark. Much œdema but no emphysema.

Noon, 27th August 1914. Animal killed by chloroform. It was not *in extremis*, but probably would not have survived the night.

Autopsy.—Condition fat. Inoculated leg swollen and œdematous down to the hoof. A livid area several inches in diameter surrounded the point of inoculation. This area had a central zone of commencing necrosis, about an inch in diameter. The subcutaneous tissues of the inoculated leg saturated with an odourless exudate. This exudate down to the hock was blood-tinged, and below that clear and straw-coloured. This latter type of exudate was also present in the precrucial region of the same side. The subcutaneous vessels of the inoculated leg were intensely congested, whilst elsewhere the congestion of these vessels was moderate. The intermuscular tissue of the adductors of the thigh were infiltrated with a clear, straw-coloured exudate. The muscles of the inoculated leg appeared otherwise normal. No excess fluid in the thoracic or peritoneal cavities. The pericardium contained a few cc. of clear, straw-coloured liquid. Heart, lungs, and spleen normal. Liver and kidneys congested. Abomasum: a few small areas, varying in extent, of moderate congestion; no necrosis and no hæmorrhages. Duodenum and ileum deeply, jejunum moderately, congested. Cæcum moderately congested. Left internal iliac lymphatic glands swollen, œdematous, and congested. Mesenteric glands œdematous. Other glands apparently normal.

Bacteriological and Cultural Examination.—(This animal was inoculated at the laboratory, and the examination was therefore more complete.) The exudate around the site of inoculation showed apparently two species of bacteria, one resembling the braxy bacillus and the other being of the malignant œdema type. Heart-blood smears showed a few braxy-type bacilli. These were subsequently isolated in pure culture. The clear exudate below the hock yielded what appeared to be a pure culture of bacilli more of the malignant œdema type than of the braxy type. Smears and cultures from the liver and kidneys yielded mixed organisms.

With various materials, *e.g.*, blood, exudates, liver, kidney, from the six sheep found dead of black disease and from the animals inoculated in the field from these natural cases, and also with muscle in the experimentally infected cases, 104 inoculation and feeding experiments were performed, *viz.*: forty-three inoculation and feeding experiments upon sheep, forty-four inoculation experiments upon guinea-pigs, and seventeen upon rabbits. (This includes experiments with various organisms isolated from the different materials.)

A summary of the results of the foregoing is as follows:—

Sheep.—Five feeding experiments with mashed-up viscera of animals dead of the naturally acquired disease. Result negative.

Two feeding experiments with minced muscle from the local lesions of sheep dead as the result of experimental inoculation. The contents of the stomachs of the two sheep were neutralised before the muscle was administered. Result negative.

Thirty-three inoculation experiments with blood, exudates, emulsions of organs, etc., or cultures of organisms obtained from cases of black disease, either direct or after animal passage. Of

these inoculations eight terminated fatally, and twenty-five were entirely negative or resulted in a local lesion with slight systemic disturbance.

Three sheep were fed daily for over a week with young cultures of various organisms isolated from natural or experimental cases, the contents of the stomach being neutralised just beforehand on each occasion. Result negative.

Guinea-pigs.—All of the forty-four experiments were subcutaneous inoculations into various parts of the body with materials and cultures derived from sources similar to those in the case of the sheep. Twenty-five of these inoculations were fatal, and nineteen were either quite negative or resulted in a local lesion only.

Rabbits.—In these cases also, the experiments were all in the form of subcutaneous inoculations with similar materials as above. Six inoculations resulted in death, and eleven were negative.

In some of the foregoing inoculation experiments with bacteria isolated from various cases lactic acid was added to the inoculation fluid.

Intraperitoneal injection of emulsions or cultures was not performed, as it was recognised that such would only result in a heavy mortality of the experimental animals without any corresponding benefit.

Summary of Results obtained in 1914.

The subcutaneous inoculation of heart blood from sheep found dead of black disease resulted in death, as also did the inoculation of peritoneal fluid obtained in a similar manner. The principal *post-mortem* lesions in these experimental cases appeared to have more resemblance to those of malignant œdema than to those of black disease.

Feeding experiments with minced-up abomasum, intestines, liver, kidneys, etc., were negative.

Two main types of bacilli, both anærobes, were isolated from the sheep found dead, viz.: (1) braxy type, (2) malignant œdema type.

Inoculation experiments into sheep of the bacillus of the braxy type which was isolated from the sheep found dead of black disease had various results—sometimes death, but more frequently only a local lesion with slight systemic disturbance.

Feeding experiments on sheep with various bacilli isolated were negative.

With guinea-pigs subcutaneous inoculation of the braxy type of bacillus in young cultures in doses of .1 to .5 cc. was invariably fatal within twenty hours, often within sixteen hours. With smaller doses (.05 cc.) death might be delayed a few hours.

In a number of the fatal inoculations of guinea-pigs no cultures were made, as death had occurred an unknown although short period before discovery in the morning, and obvious *post-mortem* changes had already occurred.

The total number of experiments performed include those conducted with all the various species of organisms which were on experiment found to be non-pathogenic, or whose effects were such as to indicate that they were merely cadaver bacilli.

Remarks.

The principal result of the work performed during 1914 was the isolation of an organism that was morphologically and culturally of the braxy type, and which was fatal when inoculated subcutaneously in small doses into guinea-pigs, but not to rabbits.

Administered in the same way to sheep, it was at times fatal, at others not. It was noted that in these experiments the fatal cases presented a picture rather more approaching that of malignant oedema than that of naturally occurring black disease.

Administration of the organism per os in large and daily doses to sheep produced no evident result.

A bacillus of the malignant oedema type was also isolated and experimented with, but accumulated evidence indicated that in all probability it was not the cause of black disease.

A number of other species of organisms were isolated, both ærobes and anærobes, but these were evidently all *post-mortem* invaders.

Reasoning superficially, one might have arrived at the conclusion that one had succeeded in demonstrating the cause of black disease, and that the organism in question was identical with the braxy bacillus, or, at least, belonged to the same group; but the weak spot, and a serious one, in the train of evidence was the fact that the organism experimented with had been isolated from animals *found dead*. It had not been proved that any one of the various bacteria, including the braxy type of bacillus, was present either in the blood, exudates, or tissues of black disease sheep before the death of the animals. Consequently, it was recognised that, no matter how far the work progressed on the lines then being followed, there would always be the objection that there was no conclusive proof that the organism isolated was the cause of the disease, since it might be merely one of the so-called "cadaver bacteria."

PART II.

As the result of the experience gained in 1914, it was recognised that work on the etiology of black disease, carried out with such materials as were then available, would not be productive of conclusive results, since when such materials were obtained from sheep found dead, no matter how recently the death appeared to have taken place, the objection could always be raised that organisms so obtained, especially anærobes, were possibly *post-mortem* invaders. To meet this objection as far as possible it was decided that any materials, such as blood, exudates, and portions of organs, would have to be removed from animals affected with the disease immediately they were seen to die naturally or were killed, with, of course, every precaution to avoid contamination. The research work during the seasons 1915, 1916, and 1917 was carried out on those lines.

As the symptoms of the disease are so indefinite, and death takes place so rapidly and unexpectedly, it was realised that the only way to see sheep alive was to watch the various flocks during the whole day, from daylight to dark; and, on account of the distance of the

paddocks from the homesteads, this at times necessitated camping out with the sheep for several days at a time. Such a procedure, although attended by some inconvenience on account of frost and rain, was amply rewarded, inasmuch as a total of twenty affected sheep were detected by this means alive and in various stages of the disease, and autopsies were held immediately after they had died naturally or had been killed. Numerous *post-mortem* examinations were also made upon sheep found dead of black disease; but no materials for experimental work were taken from these latter animals except for control.

The mortality from black disease during the 1915 season was very heavy. This may to some extent have been influenced by drought conditions then prevailing. In the 1916 season the mortality on the other hand was lighter than usual, the season that year, so far as food was concerned, being very favourable for stock in general. In 1917 the losses were so light that little field work could be done.

Although, as stated, twenty cases of black disease were detected during life and autopsies held immediately after death, the *post-mortem* appearances were upon the whole so constant that it is necessary to give details of only a few in order to present a picture of the morbid anatomy of this disease. Consequently six consecutive cases are here given.

Case 8.

Wether, aged five years. Fat condition. Found ill in paddock early in the morning. The animal was able to walk, and even to run a short distance when approached. When brought to the hut where the autopsies were made the illness had visibly increased. Sensibility was retained, but the sheep made no attempt to move, lying on its side. The respirations were shallow, and the pulse hardly perceptible. About an hour later the breathing grew stertorous and deep, and the animal became comatose. This condition having lasted about fifteen minutes, the sheep was killed and a *post-mortem* examination made immediately.

Autopsy.—About 300 cc. of quite clear, straw-coloured fluid in the thoracic cavity. Lungs apparently normal. Pericardium distended with a fluid resembling that in the pleural sacs. Heart presented extensive endocardial hæmorrhages. Peritoneal cavity contained several hundred cc. of slightly turbid liquid. Connective tissue of the various compartments of the stomach markedly œdematous. Gastric, hepatic, and mesenteric glands swollen, congested, and œdematous. Kidneys congested. Spleen normal. Liver: the posterior surface was covered with flakes of fibrinous lymph. The organ was very dark and congested. Moderate fluke invasion. Two hæmorrhagic areas, each about the size of a shilling, were present; also a dirty yellow, necrotic area about the same size. Abomasum: mucous membrane a little œdematous. No hæmorrhages or congestion save immediately surrounding the pylorus, where there was a deeply congested submucous area about an inch wide. Mucosa of duodenum fairly deeply congested, with a few petechiæ. Jejunum intensely congested, mucosa showing many small hæmorrhages, with actual blood in the lumen. Ileum: con-

gestion by no means so pronounced. Large intestines normal. No muscular lesions.

Case 9.

Aged ewe, fat, pregnant. Found ill in the paddock during the day. *In extremis* when brought in. Killed, and *post-mortem* examination held at once.

Autopsy.—No excess fluid in pleural sac. Lungs normal. Pericardium distended with a clear, straw-coloured liquid. Heart showed some diffuse endocardial congestion, but no distinct hæmorrhages. Peritoneal cavity contained about 300 cc. of slightly turbid fluid. Liver congested. One hæmorrhagic area about an inch and a half in diameter. Slight fluke invasion. Spleen slightly swollen, pulp rather softer than normal. Kidneys congested. Abomasum showed many punctiform hæmorrhages on the mucous membrane and its folds, which were also slightly œdematous. Duodenum fairly deeply congested. Jejunum slightly, and ileum deeply, congested. The whole of the mucosa of the latter studded over with petechiæ. Mediastinal lymphatic glands congested and œdematous, the remainder of the lymphatic glands being swollen and œdematous, but not congested. Subcutaneous vessels deeply congested. No muscular lesions.

Case 10.

On Sunday, whilst watching a flock of about a thousand sheep being moved from one part of a paddock to another, this animal was observed in the distance to be lying down. After a short interval it rose and moved off briskly to join its fellows. Having gone several yards it turned off at right angles, and then stood looking in a direction opposite to that of the flock. Upon a man riding up to it, the animal ran several yards in the attempt to evade capture. Finally it lay down and permitted the man to seize it and place it upon the horse. When it reached the hut, about 500 yards distant, it was placed in an enclosure. It at once moved into a corner and lay down with its eyes closed. Breathing was very shallow. About fifteen minutes later dyspnœa set in, and a few minutes after this the animal died without a struggle. *Post-mortem* examination was made immediately.

Autopsy.—Ewe, aged five years. Condition good. Subcutaneous vessels greatly congested.

A few cc. of clear, straw-coloured liquid in the thoracic cavity. Lungs normal. Pericardium distended with a clear, straw-coloured fluid. There was a diffuse congestion of the endocardium, but no hæmorrhages. The peritoneal cavity contained several hundred cc. of a rather turbid liquid. Vessels of omentum very congested. Liver congested, slight fluke invasion. Surface covered with flakes of fibrinous lymph and spotted over with petechiæ. Abomasum normal save for an area of congestion, about an inch square, near the pylorus. Duodenum moderately congested and a moderate number of small hæmorrhages. Jejunum intensely congested; contents blood-stained. Ileum moderately congested. A few small, scattered areas of congestion in the large intestine. Kidneys and spleen normal. Mediastinal lymphatic glands greatly congested

and swollen. Other lymphatic glands slightly œdematous. No muscle lesions.

Case 11.

Ewe, aged six years. Found ill in paddock. Killed, and *post-mortem* examination made at once.

Autopsy.—Condition fair. Subcutaneous vessels engorged. About 100 cc. of clear, straw-coloured fluid in the thoracic cavity. Lungs normal. Pericardium distended with clear, straw-coloured fluid. A few small areas of congestion on the muscular rim of the diaphragm (no bacteria found in this subsequently). Heart normal. Some intermuscular œdema of the abdominal muscles. Peritoneal sac contained about 200 cc. of slightly turbid fluid. Mesentery deeply injected. Liver congested. Some flakes of fibrinous lymph on its surface. Slight fluke invasion. Kidneys moderately congested. Spleen normal. Abomasum showed moderate general congestion of the mucosa, more pronounced around the pylorus. No hæmorrhages or ulceration. Mesenteric, hepatic, and gastric lymphatic glands congested. No pronounced changes in the remainder. Duodenum slightly, jejunum and ileum deeply, congested. Large intestines normal. No muscle lesions save in the diaphragm.

Case 12.

On this occasion the sheep had been yarded the night before, and allowed out in the paddock during the day. They had been closely watched all day, and were again yarded at evening. Whilst examining the flock of about 1300 in the enclosure, a sheep, Case 12, was found lying on the ground in a comatose condition. Temperature 105·8° F. Respirations slow and shallow. No pulse could be felt. The animal was killed, and a *post-mortem* examination made immediately.

Autopsy.—Ewe, aged four years. Condition fat. Subcutaneous vessels congested. No muscular lesions. No excess of fluid in pleural or peritoneal cavities. A few cc. of clear, straw-coloured liquid in the pericardium. Heart normal. Thoracic and abdominal lymphatic glands swollen and congested. Lungs pale. Liver congested. On the posterior surface there was a hæmorrhagic area about half an inch in diameter; also on the same surface a dirty white, necrotic-looking focus about an inch and a half in diameter. Kidneys congested. Spleen normal. Mucous membrane of the abomasum showed moderate general congestion. Two areas around the pylorus, each about 3 by 2 inches, were deeply congested. Mucosa of duodenum deeply congested. Jejunum moderately, and ileum intensely, congested. Large intestines normal.

Case 13.

Ewe, aged. Condition good. Pregnant. Observed ill in paddock. Taken to shed in a buggy, but died during journey. *Post-mortem* examination made about fifteen minutes later.

Autopsy.—Subcutaneous vessels injected. No muscular lesions. A few cc. of clear straw-coloured liquid in the pleural sacs. Lungs normal. Pericardium distended with a similar liquid to that in the

pleural cavity. Numerous endocardial hæmorrhages in the left ventricle. About 50 cc. of slightly turbid fluid in the peritoneal cavity. Spleen normal. Kidneys slightly congested. Liver congested. A dirty white, necrotic area, about 2 inches in diameter, near the hepatic lymph gland. Abomasum normal. Duodenum showed a few small patches of congestion of the mucosa. Jejunum similar. Mucosa of ileum intensely congested. Lymphatic glands moderately congested and slightly œdematous.

From various of the twenty fresh cases blood, exudates, portions of organs, etc., were removed with the utmost care to avoid contamination, and placed immediately in sterile receptacles, or aspirated direct into pipettes, which were sealed at once. The materials so obtained were taken to the laboratory, and such as remained uncontaminated were used for subsequent experimental work—inoculations, cultures, etc. In some cases it was found, as was anticipated, that in spite of the care taken some of the specimens had become contaminated; these were discarded. A number of the sealed pipettes containing blood or exudate were incubated before being used.

In addition to the above, various specimens were placed in preservative, and smears made for histological and bacteriological examination. In some instances, as soon as the animal was dead, the abomasum and intestinal tract, portions of other organs, etc., were minced up, and a relatively large amount of the mixture administered per os to sheep.

In other cases sheep were inoculated on the spot with varying amounts of heart blood or exudate within a few minutes of death of the animal from which it was taken.

The following is a synopsis of the experiments performed during 1915, 1916, and 1917:—

<i>Sheep No.</i>	<i>Experiment.</i>	<i>Result.</i>
1	Inoc. 10 cc. subcut. heart blood, Case 9	Negative
2	Inoc. 7 cc. subcut. heart blood, Case 10	"
3	Inoc. 20 cc. subcut. citrated heart blood, Case 10	"
4	Inoc. 2 cc. emulsion kidney, Case 12	"
5	Inoc. 7 cc. emulsion mesenteric glands, Case 13	"
6	Inoc. 15 cc. citrated heart blood, Case 12	"
7	Inoc. 2 cc. emulsion liver, Case 13	"
8	Inoc. 10 cc. emulsion mesenteric glands, Case 14	"
9	Inoc. 15 cc. emulsion liver, Case 14	"
10	Inoc. 10 cc. emulsion hepatic and mesenteric glands, Case 15	"
11	Inoc. 15 cc. emulsion liver, Case 15	"
12	Inoc. 2 cc. citrated and incubated heart blood, Case 17	"
13	Inoc. intraperitoneally 160 cc. citrated heart blood, Case 17	"
14	Inoc. intraperitoneally 36 cc. pleural exudate, Case 17	"
15	Inoc. 30 cc. emulsion liver, Case 17	"
16	Inoc. 4 cc. of a 24 hours' anærobic culture of a bacillus isolated from kidney of Case 12	"
17	Inoc. 15 cc. 24 hours' anærobic culture, third from liver, Case 14	"
18	Inoc. 10 cc. 24 hours' anærobic culture, fourth from hepatic gland, Case 15	"

<i>Sheep No.</i>	<i>Experiment.</i>	<i>Result.</i>
19	Inoc. 10 cc. 24 hours' anærobic culture, second from hepatic lymphatic gland, Case 12	Negative
35	Inoc. 3 cc. 5 days' anærobic culture, second from lung, Case 17	Local reaction
37	Inoc. 1 cc. 24 hours' anærobic culture, fifth from lung, Case 17	Killed
22	Inoc. 2 cc. 24 hours' anærobic culture, first from exudate flank of Guinea-pig 74, <i>ex</i> hepatic gland, Case 15	Negative
23	Inoc. 3 cc. 20 hours' anærobic culture, second from pericardial exudate Guinea-pig 74, <i>ex</i> hepatic lymphatic gland Case 15	"
34	Inoc. 5 cc. exudate from left axilla, Guinea-pig 79, <i>ex</i> lung, Case 17	Died
35	Inoc. 3 cc. 30 hours' anærobic culture, third from left groin of Guinea-pig 76, <i>ex</i> lung, Case 17	Killed
38	Inoc. subcut. with 5 cc. emulsion mediastinal lymphatic gland, Case 19	Negative
38	Inoc. subcut. with 1 cc. 20 hours' anærobic culture, second from mediastinal gland, Case 19	Local and systemic reaction
39	Inoc. subcut. with 2 cc. 24 hours' anærobic culture, second from mediastinal gland, Case 19	Local and systemic reaction
38	Inoc. subcut. with 2 cc. 20 hours' anærobic culture, fourth from mediastinal gland, Case 19	Died
40	Inoc. subcut. with 1.5 cc. 22 hours' anærobic culture, fourth from mediastinal gland, Case 19	Severe local and general reaction
40	Inoc. subcut. with 60 cc. citrated blood from Case 19	Negative

<i>Guinea-pig No.</i>	<i>Experiment.</i>	<i>Result.</i>
73	Inoc. subcut. with 5 cc. emulsion kidney, Case 13	Negative
74	Inoc. subcut. with 5 cc. emulsion liver, Case 13	"
75	Inoc. subcut. with 2 cc. emulsion hepatic gland, Case 12	"
75	Inoc. subcut. with 5 cc. emulsion mesenteric gland, Case 14	"
74	Inoc. subcut. with 1 cc. emulsion liver, Case 17	"
74	Inoc. subcut. with 5 cc. 24 hours' anærobic culture, second from hepatic lymphatic gland, Case 15	Died
76	Inoc. subcut. with 1 cc. 24 hours' anærobic glucose culture, first from liver, Case 17	Negative
77	Inoc. subcut. with 8 cc. 24 hours' anærobic culture, second from mesenteric gland, Case 14	Died
78	Inoc. subcut. with 5 cc. 20 hours' anærobic culture, second from liver, Case 17	"
79	Inoc. subcut. with 1 cc. 24 hours' anærobic culture, second from lung, Case 17	"
80	Inoc. subcut. with 1 cc. 24 hours' anærobic culture, second from adrenal gland, Case 17	"
81	Inoc. subcut. with 2 cc. 4 days' anærobic culture, first from exudate axilla, Guinea-pig 79	"
82	Inoc. subcut. with 8 cc. 5 days' anærobic culture, first from exudate leg, Sheep 35	Local reaction
82	Inoc. subcut. with 2 cc. 30 hours' anærobic culture, third from exudate groin, Guinea-pig 78	Died

<i>Guinea-pig No.</i>	<i>Experiment.</i>	<i>Result.</i>
83	Inoc. subcut. with '15 cc. 24 hours' anærobic culture, fourth from lung, Case 17	Negative
83	Inoc. subcut. with '1 cc. 48 hours' anærobic culture, third from hepatic lymphatic gland, Case 12	"
76	Inoc. subcut. with '2 cc. 24 hours' anærobic culture, fifth from lung, Case 17	Killed
86	Inoc. subcut. with '3 cc. emulsion mediastinal gland, Case 19	Negative
87	Fed with emulsion of liver, Case 19, mixed with bran	"
88	Inoc. subcut. with '2 cc. 20 hours' anærobic culture, first from mediastinal gland, Case 19	Died
89	Inoc. subcut. with '1 cc. 72 hours' anærobic culture, second from mediastinal gland, Case 19	"
90	Inoc. subcut. with '1 cc. 48 hours' culture, second from mediastinal gland, Case 19	Killed
93	Inoc. subcut. with '05 cc. 30 hours' anærobic culture, second from mediastinal gland, Case 19	Died
94	Inoc. subcut. with '05 cc. 24 hours' anærobic culture, fourth from mediastinal gland, Case 19	"
95	Inoc. subcut. with '025 cc. 24 hours' anærobic culture, fourth from mediastinal gland, Case 19	"
96	Inoc. subcut. with '025 cc. 3 days' anærobic culture, first from heart blood, Guinea-pig 95	"

<i>Rabbit No.</i>	<i>Experiment.</i>	<i>Result.</i>
1	Inoc. subcut. with 1 cc. emulsion liver, Case 13	Negative
2	Inoc. subcut. with 1 cc. emulsion kidney, Case 13	"
3	Inoc. subcut. with 1 cc. emulsion liver, Case 17	"
4	Inoc. subcut. with 1 cc. 24 hours' anærobic culture, second from mesenteric gland, Case 14	"
5	Inoc. subcut. with 1 cc. 24 hours' anærobic culture, second from hepatic lymphatic gland, Case 15	"
6	Inoc. subcut. with 1 cc. 24 hours' anærobic culture, from liver, Case 17	"
7	Inoc. subcut. with '5 cc. 24 hours' anærobic culture, fifth from lung, Case 17	"
8	Inoc. subcut. with '75 cc. 30 hours' anærobic culture, third from left groin, Guinea-pig 78	"
9	Inoc. subcut. with 1 cc. emulsion mediastinal gland, Case 19	"
10	Inoc. subcut. with 1 cc. 20 hours' anærobic culture, first from mediastinal gland, Case 19	"
11	Inoc. subcut. with 1 cc. 3 days' anærobic culture, first from heart blood of Guinea-pig 95	"

In addition to the foregoing, six sheep at various periods were given per os emulsions of mixed-up organs and portions of alimentary tract from animals just dead of black disease, as already stated. No reaction was observed.

RESULT OF EXAMINATION, MICROSCOPICAL AND CULTURAL, OF VARIOUS MATERIALS REMOVED FROM SHEEP IMMEDIATELY AFTER DEATH.

Smears were made from the pericardial, pleural, and peritoneal exudates, heart blood, liver, kidneys, spleen, lungs, lymphatic glands,

and such other places as were thought desirable, but no bacteria could be detected in them microscopically except from the liver of Case 14. Here an occasional bacillus of the colon type was seen.

Cultures, both aerobic and anaerobic, on a variety of media from the above materials (in some cases the pipettes of fluid were incubated before opening) proved quite negative with the exception of the following. Instances of obvious contamination are not included.

(a) Liver and mesenteric glands, Case 14. A bacillus of the colon type. Experimental inoculation showed that it was not the cause of black disease.

(b) From a necrotic focus in the same liver. A long, thick anaerobic bacillus, which was not pathogenic either to guinea-pigs or rabbits on subcutaneous inoculation.

(c) Hepatic lymphatic gland, Case 15. A short bacillus, anaerobic. Fatal to a guinea-pig in dose of 5 cc., but not to sheep in doses of 2 cc., 3 cc., and 15 cc.

(d) A short anaerobic bacillus from the liver of Case 16. Produced no reaction in guinea-pigs, rabbits, or sheep.

(e) From the adrenal gland and lung of Case 17; also the lung. An anaerobic bacillus which was experimented with more extensively. Details are given later.

(f) From the mediastinal lymphatic gland, Case 19. An anaerobic bacillus. A number of experiments were also made with this organism. Details given later.

INOCULATION EXPERIMENTS WITH FRESH MATERIALS TAKEN FROM SHEEP AFFECTED WITH BLACK DISEASE IMMEDIATELY AFTER DEATH.

Sheep, guinea-pigs, and rabbits were inoculated subcutaneously with varying amounts of heart blood, pericardial, pleural, and peritoneal exudates, and emulsions in saline solution of kidneys, liver, spleen, and lymphatic glands from sheep dead of black disease. The various materials were removed directly after death and placed in sterile receptacles with every precaution. In some instances intraperitoneal injections were made into sheep, but the result was invariably negative.

MORBID HISTOLOGY.

Sections were prepared from portions of the abomasum, intestines, liver, kidneys, lymphatic glands, and other parts showing lesions, with the following results.

In the small intestines and the congested areas of the abomasum the blood vessels are greatly engorged, and there is a round-celled infiltration of the surrounding tissues. Occasionally extravasations of blood on to the surface of the mucosa are seen. In those instances where the tissues have been removed immediately after death and placed at once in fixative solution no bacteria have been demonstrated in these congested areas although some may be present in the exudate on the surface, but when the removal of the tissue has been delayed varying numbers of bacteria can almost invariably be found in such localities. The blood vessels of the kidneys are also engorged, and the parenchyma shows cloudy swelling. No

bacteria were seen when the organs had been removed directly after death.

The same condition may be observed in the liver, except in the hæmorrhagic and necrotic areas. In the former the blood vessels are often so swollen, or extravasation has occurred to such an extent, that the amount of liver tissue in the section may be relatively small. No bacteria have been detected in such areas when the material has been removed immediately after death. The necrotic foci have a rather well-defined margin which shows only a moderate leucocytic invasion. Around the circumference also, and extending over the necrotic area, are often fairly large numbers of bacilli—non-sporing, straight, rather long, but never approaching the filamentous. The rods usually appear single. These bacteria are apparently secondary invaders, as the hæmorrhagic areas which seem to be the precursors of the necrotic foci are quite free from bacteria, provided *post-mortem* invasion has not been allowed to occur. With the liver, as with other organs, various bacteria may be demonstrated if the removal of the tissue is delayed. Sections of spleen have been bacteria free. The same applies to lymphatic glands if the proviso mentioned has been observed.

From the description of the morbid anatomy and histology of sheep dead of black disease it will be seen that the indications are those of a severe toxæmia, although nothing has been demonstrated either clinically or experimentally to account for this condition.

Some of the changes which have been often described in this and similar conditions affecting sheep, and considered to be part of the morbid anatomical features of the diseases in question, viz., the production of gas, the colouring of the various exudates, rendering them either sanguinolent or very turbid, the blood-staining of the tissues, dissolution of the surface of the mucosa of the abomasum, the softening of the kidneys, and the clay-coloured appearance of the liver, are apparently purely *post-mortem* changes.

Furthermore, a fact of importance in allocating any special rôle to bacteria found in sections from sheep dead of black disease is that when such sections were prepared from material taken from sheep found dead, although such death was very recent, bacteria could generally be demonstrated in them, especially in the greatly engorged blood vessels. When, however, similar sections were taken from sheep directly death had occurred, and placed in fixative immediately, bacteria were conspicuous by their absence, except in the necrotic foci mentioned. The indications, therefore, are that such bacteria are probably *post-mortem*, or at least agonal invaders. In the latter case, a few bacteria although undemonstrable when the material was removed directly after death, would in a very short time in the carcase of the sheep have multiplied to such an extent as to become readily demonstrable.

With regard to the ecchymoses in the heart wall and other places, it is probable that such are of agonal origin. Cardiac ecchymoses appear to be rather common in sheep dying from a variety of causes.

The following are the details of experiments with sheep recorded in Part II. of this article which produced some evident results, not

necessarily positive so far as black disease is concerned, during the seasons 1915, 1916, 1917, with materials obtained from sheep immediately after death.

It has been thought unnecessary to go into the details of experiments with guinea-pigs and rabbits inoculated from the same sources, although certain cultures were very fatal to the former, even with minute doses.

Sheep No. 34.

4 P.M., 20th September 1916. Inoculated subcutaneously behind shoulder with .5 cc. exudate from axilla of guinea-pig No. 79, *ex* second culture from lung, Case 17.

21st September. Temperature 107° F. Lamé. Inoculated leg painful, swollen, and œdematous around site of inoculation. No gas formation. No discoloration. Animal very depressed. The swelling had increased a little by the evening.

7.30 A.M., 22nd September. Animal found dead, thirty-nine hours after inoculation. *Post-mortem* examination made at 10 A.M.

Autopsy.—Wether, aged two and a half years. Good condition. General discoloration of the skin. No subcutaneous gas formation. Subcutaneous vessels markedly engorged. Blood dark and firmly coagulated. A few subcutaneous hæmorrhages on abdomen. On the left (inoculated) side the subcutaneous tissues were infiltrated with a pinkish, odourless exudate. There was no exudate on the right side of the thorax. The body had no unusual odour.

There was no excess of fluid in the pleural or peritoneal cavities. The pericardium contained about 20 cc. of a pinkish, odourless exudate. The lungs presented a few congested areas. The epicardium around the coronary circle was studded with ecchymoses. There were numerous subendocardial hæmorrhages in the left ventricle, but none in the right. Spleen normal. Kidneys congested. Liver congested. The visceral surface showing two hæmorrhagic areas, each about half an inch in diameter.

Abomasum: The whole of the mucous membrane was of a deep red colour (probably *post-mortem*). A few hæmorrhagic spots were scattered over the surface. The vessels of the small intestines were congested. The mucosa was œdematous. Large intestines showed no naked-eye changes. Lymphatic glands in general slightly œdematous. Peritoneal covering of urinary bladder spotted with ecchymoses. Some of the muscles of the left shoulder and around the site of inoculation were very dark and had a butyric odour.

From the above it is very evident that *post-mortem* changes had occurred. This was confirmed by bacteriological examination of the various fluids and organs, as besides the bacteria of the type injected there were numerous filamentous bacilli of the malignant œdema type, and other species.

Sheep No. 36.

Ewe, pregnant, aged. Good condition.

10th October 1916. Inoculated subcutaneously into thigh with 10 cc. of a twenty-eight hours' anærobic serum-formate culture, second from œdema of leg, sheep No. 35. A few drops of lactic acid were also added to the fluid. A large dose was employed as the bacilli were not very plentiful. No reaction was observed.

4 P.M., 8th November 1916. Inoculated subcutaneously into thigh with 3 cc. of a thirty hours' anærobic culture (a few drops of lactic acid being added), third from left groin of guinea-pig No. 76, *ex* lung, Case 17.

9 A.M., 9th November. Temperature 104·6° F. Animal very lame and markedly ill. Whole of inside of inoculated thigh livid and œdematous.

2 P.M., 9th November. Temperature 105·2° F. Animal killed by chloroform. It had been gradually growing worse, and was in great pain. The swelling had extended to the coronet of the affected leg. Minute drops of a pinkish exudate were oozing from the skin at the coronet. Seeing that death usually took place at night or early morning, and that by the time the autopsy was held *post-mortem* changes might have occurred, it was thought advisable to kill the animal and examine it at once.

Autopsy.—Skin of inoculated thigh around site of injection very livid. Subcutaneous tissues from the groin to the hoof on the inoculated side saturated with an odourless exudate, which was deep pink in colour in the thigh, but only a faint pink elsewhere. Subcutaneous vessels congested. No evident gas formation, but the muscles around the inoculated area were in parts slightly spongy in appearance. In places these muscles were pale, and in others there were small dark hæmorrhagic patches with a moderately rancid odour.

None of the great serous cavities contained an undue amount of fluid. All the organs were apparently normal. The folds of the abomasum were, however, congested. Of the intestines, only the jejunum presented an appearance of congestion, and that was slight.

Bacteriology.—Smears from heart blood, liver, kidney, spleen, and lymphatic glands negative.

Exudates in pubic and metatarsal regions show a fair number of short, straight rods with rounded ends. None sporing. Single or double. No chains.

Exudate around site of inoculation. Many short, single, straight bacilli, and a fair number of longer, thicker elements, at times approaching the filamentous. Some sporing.

Muscle smears. Similar to inoculation site, save that fewer sporing bacilli were present. The long elements were more decidedly filamentous.

Cultures.—Twenty-four hours. Anærobic, serum-formate broth. Heart blood and exudates from various points all showed numerous bacilli varying somewhat in length, but none of the longer ones could be called filamentous. No chains. Many sporing.

Heated liquid serum coagulated. A good deal of gas formation. No odour of putrefaction.

Muscle cultures. As above, but a number of thicker, longer rods, some approaching the filamentous.

Sheep No. 37.

Aged ewe, fat, pregnant.

3 P.M., 31st October 1916. Inoculated intramuscularly into thigh with 1 cc. of a twenty-four hours' anærobic serum-formate culture, fifth from the lung of Case 17.

1st November. Temperature 105·6° F. Very lame. No distinct swelling of inoculated area, although the skin around site of puncture was slightly œdematous. No lividity.

2nd November. Animal still very lame and quiet. Feeding a little. No further changes in inoculated area.

The animal appeared likely to recover but, as it was desired to note the character of the lesions when the probability of secondary contamination was very remote, it was killed with chloroform on the evening of the 2nd.

Autopsy.—Inoculated leg. No swelling, discoloration, or gas. On incision of the skin the subcutaneous vessels around site of inoculation were found engorged, and there was a little clear, straw-coloured exudate infiltrating the subcutaneous tissues of the thigh. The muscles in this area were quite normal, save for a little clear intermuscular exudate.

There was no abnormal amount of fluid present in the pleural or peritoneal cavities, but the pericardium contained about 25 cc. of a clear, straw-coloured odourless liquid.

The lungs, heart, and all other organs were quite normal in appearance. The mucosa of the small intestines was slightly congested. Lymphatic glands were also normal.

Bacteriology.—Smears from heart blood, pericardial exudate, and various organs were negative.

Smears from the intermuscular and subcutaneous exudate showed a few short, straight, single bacilli; but the majority of them were disintegrating.

Cultures.—Heart, pericardial fluid, and organs negative.

Subcutaneous exudate. Numerous bacilli, anærobic, short, straight, many sporing, single or in pairs. No chains or filaments.

Sheep No. 38.

Wether, aged two years, fat.

11 A.M., 20th March 1917. Inoculated intramuscularly into thigh with 2 cc. anærobic culture, fourth from mediastinal gland, Case 19.

9 A.M., 21st March. Animal found dead.

Autopsy.—Body almost cold. The skin in general had a livid appearance. Considerable gas infiltration of the subcutaneous tissues of the groin. Subcutaneous tissues of inoculated thigh saturated with a sanguinolent, watery, odourless liquid. Inoculated muscles hæmorrhagic, almost black. Odour rancid. No putridity. These muscles appeared rather drier than normal. Not much intramuscular gas formation. Subcutaneous vessels in general very congested. No excess fluid in pleural or peritoneal sacs. The pericardium contained a few cc. of sanguinolent liquid. Lungs congested. Heart chambers distended with gas. Numerous epicardial ecchymoses. Liver, spleen, and kidneys showed pronounced *post-mortem* changes, otherwise normal. Lymphatic glands in general congested. Mucous membrane of abomasum congested in patches, especially around the pylorus. Small intestines congested throughout. The colon was moderately congested, but the remainder of the large intestine was normal.

Bacteriology.—Smears from the heart blood showed many short, single, straight bacilli and a few filamentous organisms. None

sporing. Smears from the pericardial exudate and organs showed a variety of species (cadaver bacteria).

Affected muscle from inoculated leg: many short, single bacilli, some sporing. A few in pairs and an occasional chain of three elements. No definite filamentous organisms, although a few of the elements were four or five times as long as the average.

In the case of three sheep, Nos. 35, 39, and 40, although the inoculations were not followed by death, the local and systemic reactions were severe.

Sheep No. 35.

Inoculated subcutaneously into the thigh with '3 cc. of a five days' anærobic culture, fifth from the lung of Case 17.

On the morning following the injection the inoculated leg was swollen and œdematous, and there was pronounced systemic disturbance. At no time, however, was there any lividity or necrosis of the skin around the point of inoculation. Several cc. of clear, straw-coloured fluid were aspirated from the swollen leg and cultures made. The organism obtained in pure culture was subsequently inoculated into sheep, guinea-pigs, and rabbits; but no reaction, local or general, followed in these animals.

The swelling gradually subsided, and the animal recovered.

Sheep No. 39.

Inoculated into thigh with 1 cc. of a twenty hours' anærobic culture, second from mediastinal gland, Case 19.

A week later this animal was reinoculated with 2 cc. of a twenty-four hours' culture from the same material.

Two inoculations were made, because it was seen that the first would not prove fatal and the reaction was not very pronounced.

The local reaction consisted of swelling of the inoculated leg, commencing around the site of injection, and gradually spreading to the hoof. The swelling was œdematous and painful, the animal at times carrying the limb. There was no lividity of the inoculation area. A few days after injection the skin around the site ruptured in several places, and a few droplets of a pinkish fluid exuded. Later on necrosis of the skin for some inches around the point of the needle puncture set in, followed by suppuration, the latter no doubt being due to secondary infection through the broken skin. The necrotic part eventually sloughed, and the lesion gradually healed up. The swelling had, however, gradually disappeared some time prior to this. The general symptoms were hyperthermia, loss of appetite, dulness, increased respirations, etc.

Sheep No. 40.

Inoculated subcutaneously with 1.5 cc. of a twenty-two hours' anærobic culture, fourth from mediastinal gland, Case 19.

In this case the leg became greatly swollen and œdematous the morning following the inoculation, and the usual symptoms of general disturbance were evinced. In addition, however, a livid area developed around the point of inoculation and gradually spread until it had a diameter of about 4 inches. The periphery was

sharply demarcated from the normal skin. The animal was very ill for some days, after which the swelling and the lividity gradually disappeared. There was no necrosis or suppuration in this case. The animal completely recovered.

PRÉCIS OF EXPERIMENTAL WORK, BOTH FIELD AND LABORATORY,
PERFORMED DURING THE SEASONS 1915, 1916, AND 1917.

Twenty sheep were discovered ill with black disease on various stations at different periods. They were autopsied immediately after they had died naturally or had been killed. Morbid anatomical pictures were thus obtained before *post-mortem* changes could have supervened. Various materials were removed with the greatest precautions to prevent contamination, and taken to the laboratory for examination and experiment. Smears were prepared at the same time from blood, exudates, organs, etc.

In addition to the above twenty cases, numerous *post-mortems* were made upon sheep found dead in the morning although apparently well overnight. In view of the results already obtained in dealing with such cases, and of the liability to draw erroneous conclusions in experimenting with materials taken from them, the decision to abstain from using materials for cultivation and experimental inoculation unless they were obtained immediately after death and from animals seen to die, was rigidly adhered to, except that occasionally control cultures were made from sheep found dead.

Microscopic examination of smears taken from the twenty cases in question failed to reveal the presence of any bacteria except with regard to the liver of Case 14, where a bacillus of the colon type was seen. Experiments showed that this was not the cause of black disease.

Microscopic examination of smears taken from animals found dead showed various species of bacteria in greater or fewer numbers. At times none could be demonstrated microscopically even in these. It depended chiefly upon the length of time the animal had been dead.

Histological examination of sections from various parts removed from the cases immediately after death showed profound changes, but even in the morbid areas no bacteria could be demonstrated save in the necrotic foci, where the organism was apparently a secondary invader, and also in the liver of Case 14, where a bacillus of the colon type was demonstrated.

In sections of affected parts taken from animals found dead, although death may have appeared only to have just taken place, *e.g.*, the congested areas in the intestines, stomach, or liver, etc., bacteria of varying species, but in which those having the morphology of the braxy type predominated, could almost always be demonstrated. At times this type appeared to be the sole invader of the tissue under examination.

A great number of tubes of culture media were inoculated from various exudates, organs, blood, etc. Frequently the blood and exudates were incubated in the originally sealed pipettes before planting out in tubes. Tubes showing growths which were obviously the result of accidental contamination are omitted from mention.

A variety of media was employed, both aerobically and anaerobically. As the indications were that if the cause were bacterial at all it would probably be an anaerobe, most attention was paid to anaerobic cultivation, and also because the majority of the bacteria isolated were anaerobes. The medium finally decided upon as being the most satisfactory for growth, sporulation, and maintenance of virulence of such anaerobes as were isolated, was a serum-formate-bouillon, the serum being non-coagulable by heat. Although a medium containing glucose and serum gave an abundant and very rapid growth, its use was abandoned for sub-cultivation or purposes of inoculation, as it was found that the cultures quickly lost their virulence, and that very few spores were formed.

Excluding instances where the growths were manifestly the result of accidental contamination, tubes inoculated from various materials from the twenty cases mentioned remained sterile except in the following instances:—

Liver and mesenteric lymph gland, Case 14. A bacillus of the colon type was isolated. From a necrotic focus in the liver of the same animal an anaerobic bacillus which was non-pathogenic to the experimental animals was also isolated.

Hepatic lymphatic gland, Case 15. A short anaerobe, pathogenic to guinea-pigs in relatively large doses, but not to sheep in large doses.

Liver, Case 16. A short anaerobe, non-pathogenic to sheep, guinea-pigs, or rabbits.

Lung and adrenal gland, Case 17. An anaerobe which upon subcutaneous inoculation into sheep produced varying results, was fatal to guinea-pigs in rather large doses, but non-pathogenic to rabbits.

From mediastinal lymph gland, Case 19. An anaerobe, morphologically of the braxy type, which upon subcutaneous inoculation produced varying results in sheep, was very fatal to guinea-pigs even in very small doses; but non-pathogenic to rabbits.

The feeding of sheep with blood, exudates, and minced-up viscera produced no evident effects.

Subcutaneous injection of varying amounts of blood, exudates, and emulsions of organs, etc., taken from animals dead of the naturally acquired disease directly after death, into sheep, guinea-pigs, and rabbits was quite negative. Intraperitoneal injection of certain material into sheep was also without positive result.

No bacilli of the hæmorrhagic septicæmia type were detected at any time, either microscopically or culturally, from natural or experimental cases.

Serological tests with various bacteria isolated and the serum of sheep dead of black disease were carried out; but the results were not such as to encourage one to persevere.

REMARKS UPON THE FIELD AND LABORATORY WORK CONDUCTED DURING THE SEASONS 1915, 1916, AND 1917.

The point which immediately attracts attention is the striking difference between the results obtained in 1914, and related in Part I. of this communication, and those obtained during the

subsequent seasons. In the former case the animals from which the materials for experimental work had been obtained were not seen alive, but had been dead an unknown although not very long period—at the most only a matter of a few hours and during a cold season of the year. In the latter case the animals were seen alive, and the materials were taken from them immediately after they had died naturally or had been killed.

The results show that a number of the changes observed at the autopsy of animals found dead were merely *post-mortem* in origin, and not part of the morbid anatomy of black disease.

Materials taken from sheep found dead and inoculated subcutaneously into healthy sheep invariably resulted in death, and bacteria could be isolated and cultivated from such sheep, and also from those dead as the result of experimental inoculation with these bacteria, sometimes in purity and sometimes mixed. Certain of these anærobic organisms were either fatal on injection into sheep or resulted in pronounced local lesions. No effect, however, could be produced by oral administration of minced-up organs or of portions of the alimentary tract of naturally dead animals, or of large doses of cultures of organisms which were so fatal on parenteral injection. Such bacteria, too, were very fatal upon inoculation to guinea-pigs, but not to rabbits.

On the other hand, the subcutaneous or intraperitoneal inoculation of blood, exudates, or emulsions of organs, etc., removed from animals directly they had died of black disease, with every care to avoid contamination, failed to produce any distinct reaction, even although large doses were often injected.

The feeding of sheep with minced-up viscera from such cases was without evident effect, as in the case of similar material from the animals dead an unknown period.

In the case of sheep found dead of black disease certain bacteria could in most instances be demonstrated microscopically in the blood, exudates, and organs, the number depending upon the length of time the animal had been dead. In the congested areas in the stomach, intestines, and liver, sections also showed fewer or larger numbers of bacteria, often apparently of only one species. The commonest organisms were (1) a bacillus morphologically of the braxy type, and (2) a bacillus of the malignant œdema type. The former organism when injected subcutaneously could invariably be recovered by cultivation from the heart blood of the experimental sheep or guinea-pigs after death, and also from the subcutaneous exudates. If smears of the heart blood, and in some instances pericardial, pleural, or even peritoneal exudate, were taken directly the experimental animal had died, it was often impossible or difficult to detect any bacteria by microscopic examination, although, as just stated, they could always be obtained by cultivation of such apparently sterile materials. Large doses of such bacteria, also administered per os to sheep, failed to produce black disease.

If one compares these results with those obtained with materials taken from sheep immediately after death, it will be seen that in the majority of the latter cases no bacteria at all could be demonstrated, either in smears or sections, from the congested areas in the

stomach and intestines, or in smears, sections, cultures, or by animal experiment in the liver, kidneys, lymphatic glands, spleen, heart blood, etc. Also that, whereas in the former cases bacteria could be demonstrated in the submucosa and the engorged blood vessels, in the latter, where the material had been removed and placed in fixative directly after death, no bacteria could be seen at all in such situations. Of course, bacteria could often be found on the surface of the alimentary mucous membranes, particularly in the exudate. Cultivation of various materials from these sheep were usually negative, and the exceptions were either apparently instances of secondary invasion, or, where the organism proved to be pathogenic, the number of cases from which it was isolated were too few to permit one to decide definitely what rôle, if any, it played in the cause of black disease.

In some instances, such as with the liver and mesenteric glands, the growths obtained were of a mixed character. This does not necessarily indicate that contamination had occurred after the material had been removed from the body. In fact, in the light of present-day knowledge, one would not be surprised to be able to obtain growths of certain bacteria from these situations even from a healthy carcase. At first, however, these organisms were tested by animal inoculation, in order to make sure that such was not the cause of black disease.

Another feature worthy of note is the result of inoculations with, and attempts to cultivate bacteria from, the heart blood of various animals. Bacteria primarily isolated from sheep found dead could, upon the death of an experimental sheep or guinea-pig, always be cultivated from the heart blood, even if such material were aspirated directly the heart had stopped. Only occasionally could such results be obtained from the heart blood of sheep taken directly after death from black disease, and even then such bacteria did not produce any serious results on inoculation into sheep. Bacteriological workers will need no reminding that the mere finding of an anærobic organism in the heart blood of a dead animal by cultural method, especially in such an one as the sheep, is no proof whatever that it was the cause of death, even although it proves to be highly pathogenic to certain animals upon parenteral injection.

If from the first one had been content to proceed on the assumption that the organism of the braxy type isolated during the work of 1914, which was so markedly pathogenic to sheep and guinea-pigs parenterally, was the cause of the disease under investigation, one might no doubt have built up a very nice structure based upon experimental work performed with the organism in question, but unfortunately it would have been erected upon a very insecure foundation, as no proof had been furnished that the organism was present in the tissues or fluids of the body before death. The experiments conducted during 1915, 1916, and 1917 show that the critical attitude of mind in refusing to accept the evidence obtained from sheep found dead of black disease as conclusive was amply justified. They also confirm the opinion formed as the result of experimental work in 1914, viz., that no conclusion of any value is to be drawn in connection with black disease from the cultivation

and experimentation with anærobes isolated from animals found dead.

The conclusion to be drawn from the research work narrated is that the bacteria isolated from the blood, exudates, organs, etc., of sheep found dead of black disease, including that of the braxy type, are agonal or *post-mortem* invaders. They may also invade the blood, etc., just prior to the death agony in such diseases as the one in question. It may be held that the latter type of bacillus was actually the causal factor, but that during life it was localised at some spot, and was confined thereto until the death of the animal, when it rapidly spread throughout the body, somewhat like the bacillus of blackleg, death being brought about by the production of a very powerful toxin. This might be considered a reasonable explanation if one could demonstrate that the particular bacterium is constantly present in lesions. This could be done with regularity in the case of tissues removed from animals found dead, *e.g.*, in the submucosa of the congested areas of the abomasum or small intestines; but, unfortunately, the bacteria could not be found at all in similar tissues taken directly after death. Furthermore, contrary to what is usually the case in blackleg, although there are exceptions in this latter disease, no subcutaneous muscular lesion was detected.

There remains the instances of Cases 17 and 19, where certain bacteria were isolated from fresh carcasses and proved to be pathogenic. The one obtained from the lung and adrenal of Case 17 was apparently a malignant œdema type of bacillus. Experimentally it did not produce black disease, and the conclusion is that it was a pre-agonal or agonal invader. The bacillus isolated from Case 19 might at first sight be thought to substantiate the view that the braxy type of bacillus was the cause of the disease. It, however, might just as well be considered to have the same origin as the malignant œdema type of bacillus isolated from Case 17. Even if it does play the premier rôle in the causation of black disease, it will have to be found more constantly in animals immediately they have died from that disease before its etiological significance can be considered to be established.

In considering the importance to be attached to the results of inoculation experiments with the braxy type of organism, it must be remembered that most of the intestinal anærobes will produce very serious results when injected into certain animals parenterally. Furthermore, examination of smears from the intestinal walls and contents of affected animals has not shown the bacillus in question to be unduly numerous.

Finally, it has been shown that attempts to convey the disease by feeding sheep with minced-up viscera or cultures have hitherto failed, although undue importance should not be attached to this when discussing the possible bacterial origin of black disease. It does not prove that infection *cannot* occur *viâ* the alimentary tract.

I am aware it is considered by many bacteriologists that the malignant œdema bacillus, the braxy bacillus, and the bacillus of blackleg belong to one group. All that is intended to be conveyed here at present, when speaking of a braxy type and a malignant

œdema type of organism, is that culturally and morphologically the two species of bacteria had characteristics which served to differentiate them into these two classes.

HYPOTHESES AS TO THE CAUSE OF BLACK DISEASE.

Various explanations have been advanced as to the cause of the disease in sheep known as black disease. They may be grouped under four chief headings, viz.: (1) bacteria, (2) plant poisoning, (3) a deficiency disease or absence of a vitamine, (4) a filterable virus.

The fourth hypotheses, that the condition is due to an ultra-visible or filterable virus, may be dismissed very briefly. The negative results following injection of large amounts of blood, exudates, or emulsions of organs from affected sheep apparently rule this view out from present consideration.

The other three views need fuller discussion.

(1) Bacteria. The morbid anatomy of sheep dead of black disease indicates a condition of toxæmia; but up to the present researches have failed to demonstrate that, with any degree of constancy, bacteria are present, at or before the time of death, in the blood, tissues, or exudates of diseased animals. The instances in which bacteria have been demonstrated to have been present before death are too few, when compared with those in which none were found, to permit of any generalisation with regard to their etiological importance. In spite of this, however, it is quite possible that the cause is bacterial, but that the organisms are confined to the lesions, or to the intestinal tract, whence a very powerful toxin is given off. Unfortunately, however, up to the present examination of sections from the lesions has failed to reveal any bacteria which can be said to play a causative rôle in the disease under consideration.

Furthermore, the injection of emulsions of affected portions of liver were without positive result. It need hardly be stated that the injection of an emulsion of congested portions of abomasum or intestine would have had no useful result. The bacteria present on the surfaces would in all probability have killed the experimental animal or produced a serious local lesion; but so would probably the injection of similar material from a normal sheep.

(2) Although, as has been remarked, the indications are that the cause of black disease is probably bacterial, this has not caused one to shut one's eyes to other possible causes. The next possibility, and the one held by the majority of stockowners concerned, was plant poisons. During the whole period of field investigations I have endeavoured to find plants which were common to the various localities in which the disease had occurred, and also what plants had been eaten in these areas.

A number of plants were fed to sheep and other animals, not because they were suspected to be harmful in every case, but because they had the common factors mentioned above. They were all proved to be innocent so far as black disease is concerned.

In one district many stockowners firmly hold the view that the common water-cress, which grows abundantly in the small water courses, is the cause of the trouble. In order to settle the question definitely six sheep were fed for a fortnight upon fresh water-cress.

The result was negative. One is quite prepared to admit, however, that the water-cress hypothesis may be an instance of correct observation, but wrong deduction, *i.e.*, the cause of the disease may be present more particularly in the wet situations where water-cress grows so abundantly.

The field observations and the various experiments indicate that black disease is not due to plant poisoning.

(3) The third theory, *viz.*, that the condition is a deprivation or deficiency disease, due to the absence of a vitamine in the food, and that the lesions resemble those of "wet" beri-beri in the human being, appears to have been arrived at, not from any direct evidence, but mainly because a tangible cause has not yet been demonstrated. It is not wise nowadays to dismiss any hypothesis because it is strange or does not fit in with one's own views; but there are factors in connection with black disease which, although ignored in the "deprivation" theory, need to be explained away if the latter is to receive more than academic attention. Some of these factors are the following:—

(a) Various species of animals graze continually over the same country, and in some instances in paddocks where sheep are dying, but only sheep are affected.

(b) Similar country, both in its physical geography and its geology, exists in other parts of the state; but black disease has not been reported in these places.

(c) What appears to be the seasonal character of the condition. A few isolated cases may occur throughout the year, but the heavy mortality occurs at one particular period, and apparently it stops abruptly soon after the onset of frost. Often there is plenty of feed, and that of good quality.

(d) The erratic nature of the incidence of the disease. Adjoining paddocks may be only separated by artificial divisions, and in one the losses may be heavy, whilst in the other the mortality may be nil or very light.

(e) The considerable annual variations in the incidence of the disease. During the season 1915 losses were very heavy. This, as I have suggested elsewhere, may have been partly due to drought conditions; but in 1916 (a good season for feed) they were again heavy, although not so great as in the preceding season. On the other hand, during the past season, 1917, when losses were heavy in sheep from parasitic troubles, the disease has been practically non-existent. Even on sheep stations where the annual loss often runs into four figures the owners have informed me that this year their losses from black disease have been negligible, only occasional sheep dying from this condition. During January 1917 I spent my vacation visiting various sheep stations in a black disease district where the annual losses are usually considerable, in order that I might arrange for immediate investigation as soon as the disease commenced that season. In the cases where the owners have corresponded, they have informed me that this season the losses were so sporadic that they could not be certain I would be able to find cases on my arrival at the place—the district being several hundred miles from Sydney.

(f) There is no loss of condition in sheep dead of black disease.

In every undoubted case coming under my own observation the condition of the animal has been good, or even fat. Such also is the experience of sheep owners.

It is true that one or several of these objections to the acceptance of the deprivation theory might be explained away; but it appears to be difficult to meet the majority of them satisfactorily.

IS BLACK DISEASE IDENTICAL WITH THE BRAXY-LIKE DISEASE OF SHEEP IN TASMANIA AND VICTORIA, AND WITH BRAXY OR BRADSOT IN EUROPE?

At first sight the answer to the above question appears simple. In black disease, up to the present, no bacterium has been shown to be present with sufficient constancy in animals immediately after death has taken place to permit of one assigning to it any rôle in the causation of the disease. As a matter of fact, it has been the rule under such conditions to be unable to demonstrate any organism at all, whereas with the other diseases mentioned a bacillus has been readily isolated and considered to be the causal organism. If, however, one considers the matter more carefully, it will be recognised that the question is not so easy of disposal, and it is proposed to indicate briefly where the difficulty lies.

In the first place, it may be noted that a comparison of the clinical characters and morbid anatomy of black disease with the braxy-like disease in Victoria, and with braxy or bradsot in Europe, indicates that these variously named diseases are, if not identical, at least varieties of the same disease. It must be remembered, however, that some workers on bradsot have expressed the view that several distinct diseases of the sheep, having common clinical features and similar *post-mortem* appearances, are at present grouped together under the name of bradsot.

In comparing the diseases mentioned, the first to be considered is naturally that occurring in a neighbouring state, viz., Victoria. Gilruth has gone very fully into the etiology of this disease, and, as the result of his researches, isolated a braxy-like bacillus which he considers to be the cause of the condition investigated by him. Later on, he concluded that the sheep disease in Victoria is but a variety of braxy or bradsot of Europe, and also probably identical with that known as black disease in New South Wales.

Taking all the circumstances into consideration, viz., nature of country, seasonal occurrence, ill-defined symptoms, rapidity of death, morbid anatomy, etc., there is every probability that the two diseases are identical, in spite of the fact that in the Victorian condition a bacillus has been definitely assigned as the cause, and that a good deal of experimental work has been performed to substantiate the rôle assigned to that organism. It will be conceded that the same argument can be applied here as in my own case with black disease.

In the latter condition it was shown that in the early part of the investigations sheep were not seen alive, but certainly very soon after they had died. Various experiments *in vivo* and *in vitro* were performed with material taken from them, and if one had been satisfied that the bacillus isolated from such cases was the cause of black disease it would have been additional confirmation of Gilruth's

conclusions ; but, as has been seen, subsequent work failed to substantiate the early findings.

In perusing the account of Gilruth's researches, it has become apparent that the rôle of the bacillus he regards as the cause of the disease in Victoria has not been completely established, since the series of experiments conducted was built up with an anærobic organism obtained from an animal found dead. It has not been proved that this organism was constantly present in the body (speaking in the stricter sense) before death.

Under the circumstances it is not unreasonable to suggest that further research is needed upon the Victorian disease, before it can be considered as conclusively proved that the braxy-type of bacillus in question is the cause of that disease.

In the case of European braxy or bradsot it does not appear to be universally accepted that the so-called braxy or bradsot bacillus is the cause of that condition, and I have been given to understand that further research work is contemplated in Scotland to elucidate the matter as regards the disease in that country. The conclusions of workers in Scotland and Ireland regarding the bacteriology of the disease appear to be based upon specially unsatisfactory evidence. The bacteriological results of the British Departmental Committee on braxy appear now to be largely discounted, as it appears possible that Hamilton was working with one or more of the so-called cadaver bacilli.

From what I can gather, the work in Ireland upon braxy is or was based upon the findings of the above committee, and the same remark may be applied to its conclusions. Indeed, Mellon's description of the morbid anatomy of the disease in Ireland contains a good deal of what is *post-mortem* change.

Concerning the disease on the continent of Europe, controversy is still going on as to the rôle played by the so-called bradsot bacillus, some maintaining that it is not the cause of bradsot, but merely a *post-mortem* or agonal invader, while others hold that all the evidence necessary to prove that this bacillus is the cause of the disease has been furnished.

In going through such literature upon this subject as is available to me here, it would appear that the advocates of the bradsot bacillus have not yet shown convincingly that this organism is invariably present in the tissues or body fluids of an animal affected with bradsot, before, or at the time of death. The fact that an organism may be found occasionally within the body before *post-mortem* invasion can have occurred is not altogether conclusive ; nor is the fact that one may at times be able to demonstrate microscopically in sections of certain organs removed directly after death a bacillus which is there in numbers, and apparently the sole organism present in the lesion, the only piece of evidence necessary to prove that this organism is the cause of the disease under investigation. An instance of this is seen in the examination of some necrotic foci in the liver of animals dead of black disease.

Finally, it may be stated that it is quite probable that the disease of sheep in New South Wales known as black disease is identical with the braxy-like disease in Victoria, and with bradsot or braxy in Europe ; but, until the rôle of the bradsot or braxy bacillus is estab-

lished upon a more unassailable footing, no definite conclusion one way or the other can be arrived at.

With regard to M'Gowan's claim that the primary cause of braxy in Scotland is the *Bacillus bipolaris septicus ovium*, I have not seen the original article, and no comment is made here except to say that no bacilli having the morphology of the hæmorrhagic septicæmia group have been detected in cases of black disease.

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