

ANAPLASMA MARGINALE

(Gen. and spec. nov.)

THE MARGINAL POINTS IN THE BLOOD OF CATTLE SUFFERING FROM A SPECIFIC DISEASE

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PART I.

FURTHER INOCULATION EXPERIMENTS WITH SOUTH AFRICAN REDWATER.

IN the Annual Report for 1906-07 some experiments were enumerated which were undertaken for the purpose of showing whether cattle which went through a slight attack of English or South African redwater—inoculated in England—would be immune against South African redwater when exposed on Transvaal veld; this exposure commenced in January, 1907. Leaving the experiments with the English redwater out of the question, the result was that all cattle immune to South African redwater again showed a reaction during which some of them showed *Piroplasma bigeminum*. All showed the typical lesions of a grave anaemia indicated by the lesions of poikilocytosis, polychromasia, and basophilia. One of the animals died, and the cause of death, although the presence of *Piroplasma bigeminum* was not noted, was diagnosed as the sequel of ordinary redwater. In the blood of some of the animals the presence of the so-called marginal points were noted which hitherto were constantly considered to be a sequel of ordinary redwater. This was particularly the case in the animal which died. It may also be mentioned that spirochaetes (*Spirillum theileri*) were noted in two other instances. The conclusion from this experiment was that the animals had obtained, by a previous inoculation in London, a certain amount of immunity against *Piroplasma bigeminum*; that this immunity did not protect against redwater when exposed to the Transvaal veld and the reactions were considered to be the result of a re-infection due to the natural agency, the bite of ticks, as at this particular time of the year ticks are very numerous. The practical inference from the experiments was that cattle inoculated in England with South African redwater should be exposed in a different season of the year when the ticks would not be so abundant, namely, in the winter. For this purpose a new experiment was planned, and this time with ten animals. On their arrival in the Transvaal, and before exposing them to natural infection, it was thought advisable to increase their immunity by injecting them with a second dose of blood of an animal which had recovered from ordinary redwater. The inoculation experiments in London were carried out by Mr. Stewart Stockman, Principal Veterinary Surgeon of Great Britain, formerly Principal Veterinary Surgeon of the Transvaal, and the history is compiled from his notes.

EXPERIMENT NO. 1.

The inoculation of the ten heifers in England.

(The first inoculation.)

Pure infection of Piroplasma bigeminum, South African strain.

Ten Sussex heifers aged from eighteen months to two years were injected in London on the 18th November, 1908, subcutaneously with 10 c.c. of newly drawn defibrinated blood from heifer 108 (Stockman's heifer).

Heifer 108.—The history of heifer 108 is as follows:—

On 5th September, 1908, she had received 60 c.c. defibrinated blood from heifer 5 (Stockman's heifer), an animal which had recovered some considerable time before from a very bad attack of South African redwater. (The origin of this redwater was by ticks sent from the Transvaal.) Heifer 108 had a reaction in the usual time, which lasted eleven days, during which the temperature reached 107° F. on two occasions. She passed red urine on the 10th day and eventually recovered, and her blood was used on the 29th September, 1909, to inject fifteen heifers which were going to the East African Protectorate. In October every one of these heifers developed a more or less severe attack of piroplasmosis.

Our Sussex heifers were injected as stated above, that is, about thirteen months from the time that the blood of heifer 108 had last proved infective. The result was that with the exception of two heifers, 782 and 783, none of them showed any reactions whatever and no piroplasms could be found in their blood.

The history of the two reacting heifers in detail is as follows:—

- (1) *Heifer 782.*—From 18th to 29th November the temperature did not rise above 102° F. On 30th November, twelve days after inoculation, the temperature was 104° F. in the morning and 104·6° F. in the evening. Two piroplasms (*bigeminum*) were found after examination of the blood. The red blood corpuscles, however, showed marked differences in size (anisocytosis).

On 1st December the temperature was 105·2° F. in the morning and 108·8° F. in the evening. The morning blood showed a small number of piroplasms. None could be found in the blood drawn in the evening.

On 2nd December the temperature was 104° F. No piroplasms could be found in the blood.

From 3rd December to 29th December the temperature did not rise above 102° F.

Result.—Slight reaction with *Piroplasma bigeminum* present.

- (2) *Heifer 783.*—From 18th to 25th November the temperature was normal. On the following day—that is, eight days after the inoculation—the temperature was 102·8° F. in the morning and 103° F. in the evening. No piroplasms could be found.

The temperature fell to normal on the 27th November and remained so until the 29th. The smears during this period were negative.

On 30th November the temperature rose to 104·2° F. in the morning and 105·2° F. in the evening. The morning blood showed no piroplasms, but the corpuscles varied considerably in size (anisocytosis). Two *Piroplasma bigeminum* were found in the smears drawn in the evening.

On 1st December the temperature was 105·4° F. in the morning and 105° F. in the evening. A few piroplasms were found in the smears taken during the morning; none could be found in the blood drawn in the evening.

On the 2nd December the temperature was 102° F. in the morning and 102·2° F. in the evening, and the smears were negative.

From the 3rd to the 29th December the temperature did not rise above 102° F., and no piroplasms could be found.

Result.—Slight reaction; *Piroplasma bigeminum* present.

EPICRISIS.

The blood of heifer 108 gave clear evidence of being infective, since it had caused a reaction in heifers 782 and 783, and naturally *Stockman* sought an explanation for the negative results he obtained with the fellow heifers. He made careful enquiries on the farms in England where those heifers were bred and reared, but he found that the farmers of the district had never heard of redwater (English redwater). It seemed impossible then to him to explain the failures to react on the supposition that the animals had previously acquired immunity from a natural attack of English redwater. A subsequent experiment showed that they were not immune.

EXPERIMENT NO. 2.

The second inoculation of the ten heifers in England.

Blood was drawn from heifer 783 (of this lot) on the 2nd December, 1908, that is to say when she was in a state of reaction.

On 2nd December heifers 775, 776, 777, 778, 779, 780, 781, and 784, received subcutaneously 10 c.c. of newly-drawn and defibrinated blood from heifer 783. At the same time another fresh heifer was inoculated as a control (control heifer). As we are not particularly interested in the control, it will be sufficient to say that the animal contracted a severe attack of redwater after a short incubation period of six days and died as the result.

The activity of *Piroplasma bigeminum* of heifer 108 (*Stockman's* heifer) had been greatly activated by passage through heifer 783, although it produced a slight reaction only in the latter animal.

NOTE.—It is very important to note that the blood of heifer 108 was not infective for all heifers, and the explanation of this observation may be that the infective agency (*Piroplasma bigeminum*) was present in the blood, but so rarely that it was not contained in every dose of 10 c.c.

Details of the history of the second inoculation.

The following are the details in connection with the heifers which did not react in the first instance to the blood of heifer 108, and subsequently received on the 2nd December, 1908, a dose of 10 c.c., injected subcutaneously of the blood of heifer 783 (our lot).

- (1) *Heifer 775.*—The temperature rose on the morning of December 3rd to 104° F. Smears of this date were negative. The fever reaction remained between 101·4° F. and 102° F. until 6th December. No piroplasms could be found. On 7th December it rose to 103·6° F. in the morning, and 104·6° F. in the evening. Between this date and 10th December it fluctuated between 102° F. and 106·2° F. From the 7th to the 10th December piroplasms could be found in the blood smears, but they were never very numerous. From the 11th to the 29th December, the temperature did not rise above 102° F.

Result.—Slight reaction to second inoculation, *Piroplasma bigeminum* was present.

- (2) *Heifer 776*.—The temperature of this heifer did not rise until 7th December—that is, five days after the injection—when it was 106° F., morning and evening. It continued between 104° F. and 105·6° F. until 10th December. From the 7th to the 9th December piroplasms could be found in the blood smears, but they were never very numerous. From the 11th to the 29th December the temperature did not rise above 102° F.

Result.—Slight reaction to second inoculation; *piroplasma bigeminum* was present.

- (3) *Heifer 777*.—On 9th December the temperature rose to 103·4° F. Blood smears showed a few piroplasms (*bigeminum*). The temperature fluctuated between 104° F. and 105° F. until the 11th, when it fell to 102° F. Piroplasms were found on the 9th and 10th December, but they were never very numerous. From the 11th to the 29th December, the temperature did not rise above 102° F.

Result.—Slight reaction to second inoculation; *piroplasma bigeminum* present.

- (4) *Heifer 778*.—The temperature rose to 103° F. morning and 105° F. evening on 10th December. A few piroplasms (*bigeminum*) could be found in the morning and evening blood. On the 11th December, the temperature was 105° F. morning, and 105·8° F. evening, and a few piroplasms were still present. From the 12th to the 29th December, the temperature did not rise above 102° F.

Result.—Slight reaction to second inoculation; *Piroplasma bigeminum* present.

- (5) *Heifer 779*.—This heifer had given a slight temperature reaction on the 8th and 9th days to the blood of heifer 108 (first injection). The temperature, however, did not rise above 103·8° F. and fell to normal on the 10th day. No piroplasms could be found in smears made on the 8th, 9th, 11th, and 12th days, so it was considered that she had not reacted to the first inoculation. This conclusion was erroneous, for after receiving 10 c.c. of the blood of heifer 783 (which, as the control heifer proves, was very virulent), the temperature never rose above the normal between the 2nd and 16th December. No piroplasms could be found in her blood.

On 17th December she received again subcutaneously 10 c.c. blood of heifer 781, which was badly infected (see this experiment).

From the 17th to 29th December the temperature remained normal, and no piroplasms could be found in the blood.

Result.—Slight reaction to the blood of heifer 108; no piroplasms present. Immunity to two subsequent inoculations.

- (6) *Heifer 780*.—From the 2nd to the 10th December the temperature remained normal. On the evening of the 7th it rose to 105° F., and between the 7th and 10th it fluctuated between 105° F. and 107° F. A small number of piroplasms (*bigeminum*) were found in the blood between the 7th and 10th December. On 12th December the temperature fell to normal, and the heifer started passing red urine. The urine became clear on the 14th December. From that date to the 20th December the temperature remained normal, but on the 21st it rose to 103° F. in the morning and 103·2° F. in the evening. From the 22nd to the 29th December, the temperature remained normal.

Result.—A severe attack of piroplasmosis accompanied by haemoglobinuria; *Piroplasma bigeminum* present.

- (7) *Heifer 781*.—On the 6th December the temperature rose to 103° F. in the morning and 105·2° F. in the evening. Smears were negative. From the 7th to the 11th December the temperature varied between 103·4° F.

and 107° F. Piroplasms (*bigeminum*) in fair numbers were present in the blood from the 7th till the 10th December. On the 11th December she started to pass red urine and continued to do so until the 13th. From the following day until the 21st December the temperature remained normal. On the 22nd December it rose to 103° F., in the morning and 104.2° F. in the evening and fell again to normal on 24th December. It remained normal from the 24th to the 29th December;

Result.—A severe attack of piroplasmosis accompanied by haemoglobinuria; *Piroplasma bigeminum* present.

- (8) *Heifer 784.*—From the 2nd to the morning of the 10th December, the temperature did not rise above 102° F. On the evening of the 10th it rose to 104° F. Smears showed a few piroplasms (*bigeminum*). It fluctuated between 102.2° F. and 103.6° F. until 15th December. Piroplasms were found on the 10th and 11th December, but were never numerous. On the 12th December this heifer passed red urine. From the 16th to the 29th December, the temperature did not rise above 102° F.

Result.—A decided attack of piroplasmosis with haemoglobinuria; *Piroplasma bigeminum* present.

EXPERIMENT NO. 3.

The re-inoculation of the ten heifers in the Transvaal.

The ten heifers arrived in Pretoria on the 22nd February, 1909, and were immediately placed in the stables, bedded on sawdust, and put on a diet of bran and oathay with a change of green lucerne when they showed signs of illness. All this was done for the purpose of keeping the animals free from ticks until such period as they would be exposed to natural infection. They were divided into two lots, namely, lot No. 1, consisting of heifers 775, 776, 777, 778, and 779, and which were to receive an injection of blood of one animal, heifer 651; and lot No. 2, consisting of heifers 780, 781, 782, 783, and 784, which were to receive an injection from a second animal ox 665. Two Cape heifers, 669 and 673, acted as controls for lot No. 1 and lot No. 2 respectively.

(a) *The inoculation of lot No. 1 with blood of heifer 651.*

Heifer 651—History.—This animal was obtained from Aliwal North. On the 10th November, 1908, it was infested with blue larvae whose mothers had been collected off cattle in Nelspruit. After this infestation a temperature reaction ensued, and *Piroplasma bigeminum* was only noticed in one instance on the 29th day after infestation.

- (1) *Heifer 775.*—Injected on the 3rd March subcutaneously with 1 c.c. blood of heifer 651.

Temperature reaction and blood examination.

The temperature curve up to the 16th day had a normal course. From that date onwards a steady increase occurred, reaching 103.8° F. in the evening of the 18th day, and 104° F. two days later; 104.4° F. the 22nd day, and on the 25th day 105° F. The exacerbations remained near or above 105° F. after the 27th day up to the 34th day, the morning remission had an average of 104° F. From the date of the first slight rise (16th day) the blood examination was repeated every second day and revealed the presence of an anisocytosis. On the 27th day (two days after the first high rise) the marginal points were noted to be present, they were absent the next day, and on the 29th day they amounted to 5 per cent. of the red corpuscles. On the 30th day they numbered 5.4 per cent., on the 31st day 9.6 per cent., and increased until the time of death which

occurred during the night of the 35th day. The lesions of anisocytosis and poikilocytosis increased during the course of the fever reaction. On the 33rd day polychromatic cells and corpuscles with basophile granulations put in an appearance.

Clinical symptoms.

The first clinical symptoms were noted on the 29th day when the animal refused to feed; it still refused when lucerne was substituted for the ordinary food. On the 33rd day the loss of condition became apparent, the mucous membranes of the eyes, nose, and mouth had become very pale. On the 34th day spasms of the head were noted, and foam collected around the mouth.

NOTE.—The course of this disease was of a sub-acute character, the lesions of anaemia being distinctly pronounced at time of death.

Post-mortem examination.

The condition was good; *Rigor mortis* was present. The flesh and fat were pale, and the faeces were yellow.

The blood was thin and watery.

The pericardium contained 2 c.c. of clear liquid.

The lungs were in inspirium; there was a slight oedema present. Foam was found in the trachea.

The mediastinal and bronchial lymphatic glands were pale, and their sinuses were slightly discoloured and oedematous.

The ventricles of the heart were empty and the endocardia normal.

The myocardium was pale, also the fat along the sulcus coronarius.

The liver measured 50 cm. × 30 cm., and weighed 7.9 kilo.

The bile was thick like chewed grass. On section the parenchyma was saffron yellowish, and the cut surface had a granulated appearance; the granula were about the size of a pin's head; there were light and dark coloured areas in the parenchyma of an irregular shape.

The spleen measured 60 cm. × 20 cm., and weighed 2.5 kilo. The margin was irregularly swollen. The follicles of the pulp were distinct.

The fourth stomach had liquid contents; its mucosa was pale; the folds were slightly oedematous; the pylorus was swollen and the mucosa folded and pale. The omasus had dry and the reticulum soft contents. The caecum and colon showed bile stained contents, and the mucosa was contracted and pale. The mucosa of the ileum and jejunum was also folded, swollen, and pale.

The cortex of the kidneys was reddish brown. The capsula was easily detached.

The bladder was distended with clear urine.

The internal lymphatic glands were soft to the touch.

The lymphatic glands along the aorta posterior were swollen and soft.

The brain contained some slightly blood-stained liquid in both ventricles.

The marrow of the femur was soft; there was a slight hyperaemia of the epiphysis of the humerus.

The marrow of the ribs was slightly oedematous.

Pathological-anatomical diagnosis.

Oligocythaemia; *Icterus*; *Oedema pulmonum*. *Tumor hyperaemicus splenis*; *Hepatitis parenchymatosa*. *Gastritis catarrhalis*; *Nephritis parenchymatosa*.

- (2) *Heifer 776*.—Injected on the 3rd March, 1909, subcutaneously with 1 c.c. blood of heifer 651.

Temperature reaction and blood examination.

From the 8th day onwards a slight irregular temperature reaction was registered, occasionally with an exacerbation above 103° F., but as a rule averaging between 102° F. and 103° F. From the 23rd day onwards a high temperature set in with evening exacerbations, averaging 105° F., even reaching 106° F. on several days. This curve continued until the 35th day, the morning remissions being almost constant at 104° F. After this date the evening exacerbations reached only 105° F. and the morning remissions were below 104° F. From the 42nd day onwards a gradual descent in the curve took place, and on about the 50th day the temperature regained normal. Thus a high fever reaction was present for twenty-two days.

The microscopical examinations during the first twenty-four days revealed nothing particular. From the 25th day onwards, however, lesions of anisocytosis became distinct, and on the 27th day marginal points were seen for the first time, numbering 2·5 per cent. of the red corpuscles. They now increased daily. On the 28th day they numbered 5·2 per cent., on the 29th 5·4 per cent., on the 30th day 6·3 per cent., on the 31st day 4·7 per cent., and on the 32nd day 22 per cent. This was the maximum; they decreased in numbers during the next days, viz., 33rd day, 20·4 per cent.; 34th day, 13·6 per cent.; 35th day, 8·3 per cent.; 36th day, 6·5 per cent.; 37th day, 3·2 per cent.; 38th day 2·3 per cent. When the blood temperature had regained normal limits they numbered about 0·1 per cent. The anaemia increased constantly and became very pronounced when the marginal points had reached their maximum, the corpuscles were found to be pale and colourless, many of them showing polychromasia, cells with basophile granulations also put in an appearance on the 35th day and were succeeded by macrocytes, microcytes, and normoblasts. This picture of anaemia remained for some time. After the temperature had decreased to normal the lesions of anisocytosis alone were present, that is from the 50th day onwards.

2nd Injection.—On the 58th day this animal was injected with 5 c.c. blood subcutaneously of heifer 785 (an animal showing marginal points and *Piroplasma mutans* in its blood at that time). It must be stated here that from the very date of injection a rise of temperature commenced, reaching its maximum on the 5th day after injection of 106° F., and remaining high for about eight days. During this time there was a repetition of the anaemia described before, preceded by the appearance of marginal points, reaching the number of 5·2 per cent. on the 5th day and 6·4 per cent. on the 6th day.

After recovery from this second attack, yet a third temperature reaction appeared, which was indicated by the appearance of *Piroplasma mutans*; it is probably due to this factor that anaemic lesions were also noted during this period.

NOTE.—Heifer 776 had two attacks of fever reactions, both accompanied by marginal points. It is somewhat difficult to say whether the second attack was due to the second inoculation or whether it was a relapse. I am inclined to this latter view, since the rise of temperature had taken place from the date of injection.

- 3) *Heifer 777*.—Injected on the 3rd March, 1909, subcutaneously with 1 c.c. blood of heifer 651.

Temperature reaction and blood examination.

The temperature began to rise and showed irregular exacerbations from the 16th day onwards, surpassing 104° F. on the 16th, 18th, and 20th days, touching 105° F. on the 23rd day, dropping on the 26th below 101° F. in the morning. From this date until the 44th day the temperature was somewhat irregular, averaging about 102° F. A sharp rise occurred on the 44th day to 105·4° F., and a higher reaction ensued, during which period exacerbations above 104° F. again occurred on two occasions on two succeeding days; the temperature now dropped below 103° F. for four days, being succeeded by a rise above 103° F. for two more days and remaining below 102° F. after this period.

During the time of the first rise of the temperature the lesions of a slight anisocytosis were noted. The examination was then continued almost daily, and on the 30th day marginal points were met with, that is to say, at a time when there was no high reaction. They were, however, very rare, not infecting 1 per cent. of the corpuscles; they began to increase two days before the temperature rose above 105° F., and amounted to 1·2 per cent. on this date, after which time they increased and became more numerous at the time when the temperature was at its maximum, viz., 5·4 per cent. on the 44th day, 8·6 per cent. on the 46th day, between 9 and 10 per cent. on the 48th and 50th days.

On one occasion *Piroplasma bigeminum* was seen, namely, on the 49th day. At the time when marginal points were rare, the lesions of anisocytosis only were noted, but after they had become numerous the symptoms of acute anaemia were seen, the corpuscles showing polychromasia and basophile granulations. This picture of anaemia remained for some time after the marginal points had disappeared.

2nd Injection.—On the 58th day the animal was injected subcutaneously with 5 c.c. blood of heifer 785 (at a time when it had marginal points and *Piroplasma mutans* in its blood). Four days after this inoculation, the temperature started to rise; there was a distinct reaction with evening temperatures above 104° F. and morning remissions averaging about 103° F. for the next fourteen days, and during this time the marginal points were again present, viz., infecting 6·5 per cent. of the corpuscles on the 11th day. All the symptoms of anaemia reappeared and were equally marked as the first time. This animal also showed later a reaction, probably due to *Piroplasma mutans*, which appeared on the 32nd day after the second injection. The animal finally recovered.

NOTE.—This animal also had a reaction immediately following the inoculation of blood of heifer 785.

- (4) *Heifer 778.*—Injected on the 3rd March subcutaneously with 5 c.c. blood of heifer 651.

Temperature reaction and blood examination.

Two days later a rise to 104° F. occurred in the evening, returning to normal on the 4th day; it was succeeded by a gradual rise, which reached its maximum with 105° F. on the 8th day; a slow descent followed and normal limits were reached on the 15th day. At the time of the highest temperature on the 9th and 10th days, the blood examination gave negative results. On the 12th day a slight anisocytosis and a single *Piroplasma bigeminum* was noted. Between the 16th and 22nd day the temperature curve averaged 102° F., that is about normal. After this some evening exacerbations to 103° F. occurred. On the 27th day a rise to 103·8° F. was noted, succeeded by 106° F. on the 30th and 33rd days.* On the

following day a drop to 100° F. occurred, and the temperature now remained at 102° F., and below this for five days. The animal died in the night of the 39th to 40th day.

The examination of the blood up to the 28th day revealed the lesions of a slight anisocytosis. On the 28th day, however, marginal points appeared, infecting 5·8 per cent. of the corpuscles on the 30th day; they numbered 30·8 per cent. the next day, and coinciding with the rise to 105° F., the marginal points were recorded to infect 47·6 per cent of the corpuscles. On the 33rd day, that is on the date previous to the drop of temperature, the marginal points dropped to 17 per cent., and the symptoms of acute anaemia became well pronounced in the form of anisocytosis and poikilocytosis and corpuscles with basophile granulations. On the 34th day 9·8 per cent. of the corpuscles were infected with marginal points. On the 38th day, namely, the date of the lowest temperature, the corpuscles were but thinly distributed in the film. On the 36th day the anaemia became still more pronounced; macrocytes and microcytes were noted, showing polychromasia and metachromasia; normo and megaloblasts appeared and the few corpuscles which were present were very pale. Death was preceded by the symptoms of leucocytosis.

Clinical Symptoms.

The clinical symptoms were first noted on the 32nd day, coinciding with the second sharp exacerbation of the temperature and became apparent by the loss of appetite; lucerne, which had been given as a change of food, was refused by the animal. On the 33rd day the mucous membranes of the eyes and nose were noticed to be pale. On the 35th day the respirations had become hurried and laboured; there was a watery discharge from the nostrils. On the 38th day the respirations had increased and the animal was distinctly blowing. The heifer was constantly lying down and had a sleepy appearance. A considerable wasting was noticed to have occurred within the last two or three days.

NOTE.—The marginal points became rarer after the drop of the temperature, and disappeared completely during the last three days before death. This and the low temperature reaction indicated a recovery, but the animal died as the sequel of the infection, the blood lesions being too severe.

Post-mortem examination.

The condition was good.

The subcutaneous tissue of the thorax was slightly oedematous.

The muscles were slightly yellowish stained; the fat was also yellow.

The pleural cavities contained some liquid.

The pericardium contained 150 c.c. brown yellowish fluid.

The lungs were in inspirium; there was an oedematous infiltration of the interstitial tissue; the parenchyma had a mottled appearance. The mediastinal glands were enlarged and showed anthracosis. The bronchial glands were slightly hyperaemic.

The trachea contained bile-stained foam; there was also foam in the bronchi.

On the epicardium were some fibrous filaments, and a few ecchymoses were present on the radix of the aorta.

The blood was coagulated; the left endocardium showed imbibition. The right endocardium was yellowish discoloured. The myocardium was rather soft and discoloured.

The liver measured 55 cm. × 28 cm.; its weight was 9·5 kilo. On section it showed an ochre and saffron yellow colour; the cut surface was granulated and anaemic.

The bile was thick, of a brownish-green colour, and contained some blood. The capsula glissoni was smooth.

The spleen measured 64 cm. × 24 cm. and weighed 3·7 kilo.; it was irregularly swollen, and the margin was rounded. The follicles of the pulp were pronounced and protruding on section.

The first stomach contained liquid, the mucosa was slightly swollen; the folds were injected; the folds of the pylorus showed a black discolouration. The lining of the omasus and reticulum was pale; the mucosa of the duodenum was swollen. The caecum was contracted; the mucosa was bile-stained, slightly swollen, and showed an injection of the blood vessels. The mucosa of the ileum was swollen and covered with mucous. The mucosa of the jejunum was slightly swollen, and there were haemorrhagic streaks and ecchymoses.

The kidneys weighed 0·96 kilo.; they showed a strong oedema and were anaemic; there was a slight oedema between the capsula and parenchyma.

The internal lymphatic glands were slightly oedematous.

The pharynx showed a strong hyperaemia and the mucosa was black discoloured; the larynx was slightly hyperaemic.

There was some pus in the tonsillae; the opening of the tonsillae was of a black colour.

The brain was strongly oedematous and anaemic.

The bone marrow in femur and humerus was yellow and soft; it was oedematous, and red patches were noted in the epiphysis.

Pathological anatomical diagnosis.

Oligocythaemia; *icterus*, *oedema pulmonum*; *hepatitis parenchymatosa*; *tumour hyperaemicus splenis*; *gastro-enteritis catarrhalis*; *nephritis parenchymatosa*.

- (5) Heifer 779.—Inoculated on the 3rd March subcutaneously with 1 c.c. blood of heifer 651.

Temperature reaction and blood examination.

On the 11th day a rise to 140° F. preceded by 103° F. on the previous day was noted, and on the 12th day *Piroplasma bigeminum* appeared. On the 16th, 19th, and 21st days there were evening rises, above 104° F., reaching 105° F. on the last-mentioned date. On the 23rd day a sharp rise to 106·2° F. took place, and from that date to the 29th day the temperature dropped gradually to normal. At the time of this exacerbation the blood was examined, but showed no parasites; only the lesions of a slight anisocytosis were registered. On the 30th day the temperature was at 101·2° F. in the morning; it rose to 106·2° F. in the evening of the same day; a remission to 104° F. succeeded for two days. On the 34th day another exacerbation to 106·6° F. took place; the curve now descended gradually from this climax to about 103° F. in the evening of the 45th day. After this the temperature kept between 102° F. and 103° F. in the evening up to the 49th day. It increased again to 104° F. on the 54th day, descended again below 102° F., and reached normal on the 58th day, on which date clean ticks of a heifer were placed on in order to become infected with marginal points.

On the 29th day (viz., the day preceding the onset of the sharp rise in temperature), the marginal points were noted, averaging 5·6 per cent. On the following day they averaged 6·8 per cent.; they increased rapidly and were very prevalent during the period of the highest temperature, viz., they were registered as follows:—

On the 32nd day	5·8 per cent.
„ 33rd day	10·6 „
„ 34th day	15 „
„ 36th day	17 „

They dropped to 9·6 per cent. on the 37th day. The lesions of anisocytosis and poikilocytosis were also observed; on the 33rd day the corpuscles were noted to be pale; on the 35th day polychromatic cells put in an appearance; on the 38th day cells with basophile granulations were registered, normoblasts were also present. After this date, coinciding with the drop of temperature, a decided decrease of marginal points ensued. On the 40th day basophile and polychromatic cells were still present. After this date the picture of anaemia was still pronounced. Marginal points were noted on the 42nd, 44th, 54th, and 57th days.

On the 5th day after the tick infestation, there was another rise of temperature and a distinct reaction lasting about a week, the highest exacerbation being to 103·8° F. During this time marginal points were again present, but not so frequent, the maximum number only reaching 0·4 per cent.; the picture of anaemia became again pronounced by the lesions of anisocytosis; macrocytes were frequent, polychromatic cells and cells with basophile granulations were also present. After this the temperature remained more or less normal, but was somewhat irregular, the lesions of anisocytosis remaining present for a long time.

The animal finally recovered.

NOTE.—This case may be considered to be of a sub-acute character. In this instance also, it is difficult to decide whether the second fever reaction with marginal points was due to the tick infestation, or whether it was a coincidental relapse of the primary infection. The latter view is probably correct.

- (6) *Heifer 669 (control animal for Lot No. 1).*—Imported from Aliwal North and injected on the 3rd March, 1909, subcutaneously with 1 c.c. blood of heifer 651.

On the 8th day there was a slight rise of temperature to 102·8° F.; on examination only slight increase of eosinophile cells was noted and slight anisocytosis. After this the temperature was normal up to the 28th day, when another slight rise took place, and appearing as a somewhat irregular disturbance of the temperature curve; no higher record than 103° F. was registered. On the 30th day marginal points were found in rare numbers and again on the 32nd and 35th day, when the lesions of anisocytosis were also present.

NOTE.—This animal did not react with the appearance of *Piroplasma bigeminum*, as the first control did, and marginal points were present but rarely during a very indistinct fever reaction. It is probable that this animal was immune against both infections.

COMPARISON OF RESULTS OF INOCULATION IN ENGLAND AND OF INOCULATION IN THE TRANSVAAL OF
SIX ENGLISH HEIFERS (LOT NO. 1).

Lot No. 1 injected (1) with blood of Heifer 108, and (2) with blood of Heifer 783, both inoculations carried out in England, and (3) injected with blood of Heifer 651 in the Transvaal.

No. of Animal.	Result of Second Inoculation in England.	Result of Inoculation in the Transvaal.
<i>Heifer 775</i> ..	Temperature reaction from 5th to 8th day, between 102° F. and 106·2° F. <i>Piroplasma bigeminum</i> from the 5th to 8th day.	No reaction during the first fifteen days; no piroplasms during this time. Slight reaction from the 16th day onwards, distinct from the 25th day until death on the 35th day. Marginal points appeared on the 28th day; the animal died on the 35th day.
<i>Heifer 776</i> ..	Temperature reaction commenced on the 5th day, lasting until the 8th. <i>Piroplasma bigeminum</i> noted from the 5th to 7th days.	Slight reaction from the 9th to 22nd days; no piroplasms during this period. Distinct reaction from the 23rd to 50th day, commencing to fall from the 35th day. Marginal points appeared on the 27th day.
<i>Heifer 777</i> ..	Temperature reaction from the 7th to 9th days. <i>Piroplasma bigeminum</i> noted on the 7th and 8th days.	Rise of temperature from the 16th day, with a maximum on the 23rd day. No piroplasms seen during this period. Curve continued irregularly with second maximum record on 44th day. Marginal points were present on the 30th day.
<i>Heifer 778</i> ..	Temperature reaction on the 8th and 9th days. <i>Piroplasma bigeminum</i> on the 8th and 9th days.	First rise two days after inoculation, with maximum record on 8th day. One <i>Piroplasma bigeminum</i> seen on the 12th day. Second reaction from the 22nd to 39th day. Marginal points present on 28th day. Animal died on the 39th day.
<i>Heifer 779</i> ..	Temperature reaction indistinct. No piroplasms noted, but animal proved to be immune when tested later.	First rise on the 11th day with <i>Piroplasma bigeminum</i> on the 12th day. Distinct second rise from 22nd day onwards; a third rise on the 31st day, dropping slowly to normal on the 58th day. Marginal points present on the 30th day.
<i>Heifer 669, Control</i>	—	Slight reaction from 8th to 16th days, but no piroplasms seen. Second slight reaction from 28th to 35th days. Marginal points on 30th, 32nd, and 35th days.

Résumé—Lot No. 1.

1. With the exception of one heifer, 779, all animals inoculated in England showed distinct temperature reactions with the maximum rise between 5th and 8th day, during which time *Piroplasma bigeminum* was present in the blood.

Heifer 779 had an indistinct reaction, but did not show any piroplasms in the blood; it proved to be immune to later tests made in England with the same strain of redwater virus.

2. In England the animals were kept under observation for twenty-seven days; in no instance was a second reaction noted, nor were marginal points found.

3. The inoculation in the Transvaal produced in one case an immediate rise of temperature with a maximum on the 8th day (heifer 778). There was only one piroplasm noted. In one animal (heifer 776) a reaction was noted after the 9th day without piroplasms, and one (heifer 779) showed a reaction after eleven days with piroplasms; in two instances (heifers 775 and 777) no reaction and no piroplasms were noted during the time which would correspond to a redwater reaction.

4. In all cases after the lapse of a longer period, a distinct reaction with a very high fever ensued which, in every case, was accompanied with the appearance of marginal points.

5. The period after which the marginal points appeared varied from twenty-seven to thirty days. The appearance of marginal points was in some instances preceded by slight fever reactions.

6. Of the five heifers two died of a typical disease, equivalent in its pathological anatomy to redwater, but without haemoglobinuria.

7. The inoculation of the control heifer gave negative results as regards piroplasms and positive results concerning marginal points.

(b) *The inoculation of Lot No. 2 with blood of ox 665.*

Ox 665.—Was infested on the 10th November, 1908, with blue larvae collected off cattle running in Nelspruit. In due course these ticks caused a typical but slight attack of redwater in which *Piroplasma bigeminum* were seen but rarely, and the lesions of anaemia but slightly pronounced. The observations continued from the 17th November to the 7th January, 1909—fifty-two days. No other parasites having been found during this time, it was considered to be a pure infection of *Piroplasma bigeminum*.

(7) Heifer 780.—Inoculated subcutaneously on the 3rd March, 1909, with 1 c.c. blood of ox 665.

Temperature reaction and blood examination.

There was a sharp rise of temperature on the 8th day, reaching 104·8° F. in the evening and 105·6° F. the following evening, succeeded by a drop to 100° F. in the morning of the 16th day. From that date onwards the temperature kept normal, averaging about 102° F., with the exception of a slight morning and evening exacerbation on the 16th and 17th days, when it touched 103° F. in the evening. During the two days of the highest temperature (8th and 9th days) slight lesions of anisocytosis were noted, and on the 11th and 12th days, that is at a time when the temperature was again normal, a few piroplasms were seen. On the 10th day—that is the day when the temperature again dropped to normal—diarrhoea was noted. The temperature remained normal up

to the 32nd day, when it reached 104·4° F. in the evening. There was a slight remission on the succeeding day (102° F.), followed by a sharp rise without remission from the morning of the 34th day to the evening of the 36th day, reaching 106·8° F. on this date. The next morning the temperature had dropped to 103° F. The same evening collapse temperature was noted, and the animal died on the 38th day.

The temperature reaction was preceded by the lesions of anisocytosis and the presence of marginal points on the 29th day, infecting 3·6 per cent. of the red corpuscles. From this date the marginal points increased, viz. :—

5·4	per cent.	on the	30th day,
6·8	„	„	31st day,
7·6	„	„	32nd day,
16·0	„	„	34th day,
24·2	„	„	35th day,
28·4	„	„	36th day.

The days of the highest temperature reaction were the 35th and 36th days, and it may be noted that the temperature was the highest when the marginal points were most numerous. The lesions of anisocytosis were accompanied by those of poikilocytosis and basophilia.

Clinical symptoms.

The first clinical symptom noted was the loss of appetite on the 35th day. It did not improve the following day. On the 36th day—two days before death—the mucous membranes of the eyes, mouth, and of the nose had a very pale appearance. When the animal was found in collapse, the respirations were hurried, accompanied with groaning. There were muscular tremblings. The urine was clear.

Post-mortem.

The condition was good. Rigor mortis not present.

The flesh was pale. The subcutaneous tissue had a yellowish tinge. The blood was of a watery appearance.

The pericardium contained 5 c.c. of a clear fluid.

The lungs were half in inspirium; the pleura was folded; the parenchyma was dry, and there was but little blood present. There were traces of foam in the trachea. The mediastinal and bronchial lymphatic glands were pale and soft.

The left ventricle of the heart contained some watery blood; the left endocardium showed a few ecchymoses. The right ventricle also contained some watery blood, and the endocardium was normal.

The liver measured 55 cm. × 35 cm.; the weight was 9 kilo. A section of this organ was noted to be of a saffron colour; the cut surface had a granulated appearance, and the parenchym was slightly softened. The *Capsula glissoni* was light brown in colour; the bile was thick and dark green.

The spleen measured 60 cm. in length, and the weight was 3·1 kilo.; the parenchym was soft, the pulp was diffusely reddened and the Malpighi's bodies were distinct.

The fourth stomach contained food; the mucosa was slightly folded. The reticulum was normal. The mucosa of the duodenum was folded and pale. The caecum had dry contents, the mucosa was swollen and

flakes of mucous were present. The colon had yellow stained contents, and the mucosa was swollen. The mucosa of the ileum was slightly swollen and pale. The jejunum had yellow stained contents, and the mucosa was slightly swollen. The mesenteric glands were slightly swollen.

The kidneys were pale; their total weight was 1·4 kilo., the capsule was easily detached.

The bladder contained clear urine.

The brain was pale and had a watery appearance.

The marrow of bones in femur and humerus was soft and oedematous.

Pathological anatomical diagnosis.

Oligocythaemia; Petechiae endocardiales; Icterus; Tumour hyperaemicus splenis; Hepatitis parenchymatosa; Nephritis parenchymatosa.

- (8) *Heifer* 781.—Injected on the 3rd March, 1909, subcutaneously with 1 c.c. blood of ox 665.

Temperature reaction and blood examination.

From the 9th to the 17th day a slight rise in temperature was noted, averaging about 102° F., and, with the exception of the 10th day, remaining below 103° F. The examination during this time showed the presence of an increased number of eosinophile cells and slight anisocytosis. On the 21st day there was another rise to 104° F., succeeded by irregular exacerbations averaging about 103° F. up to the 35th day; there was one high record of 104·8° F. on the 24th day. On the 35th day a sharp rise was noted to 105° F. Up to the 40th day the evening temperature kept high, reaching even as high as 106·8° F. The morning remissions averaging about 103° F. After the 42nd day the temperature reaction dropped to normal, the curve was somewhat irregular, the evening exacerbations remaining below 103° F. and the morning remissions about 101° F. The blood examinations were continued daily from the 26th day and the lesions of anaemia were noted. On the 32nd day marginal points were noted in rare numbers:—

On the 33rd day they numbered	1·0	per cent.,
„ 34th day „ „	3·0	„
„ 35th day „ „	4·6	„
„ 36th day „ „	9·5	„
„ 38th day	the number reached the maximum,	

numbering 29 per cent. After the drop of the temperature from the 42nd day onwards, the marginal points were present but rarely, averaging about 1·2 per cent.; polychromatic cells and cells with basophile granulations were frequent, normo-blasts also appeared on the same date. These lesions were again noted during the following days, and remained present for some time, the corpuscles in addition became very pale. The anaemic lesions disappeared gradually, although basophile granulations were still noted on the 56th day.

The animal was used later (30th April, 1909), to infect brown nymphae with marginal points.

From the 65th day onwards a slight reaction was noted during which time marginal points reappeared for about three days, but only amounting to about 0·5 per cent., and the slight symptoms of anaemia, principally indicated by the presence of basophile granulations in a few corpuscles were registered.

This second reaction lasted about a week.

NOTE.—Heifer 781 showed two temperature reactions in which the presence of marginal points was registered; the first occurred thirty-five days after inoculation and lasted six days, which was preceded by the presence of marginal points in rare numbers, whilst during the reaction they were very numerous. The second reaction was much lighter, only lasting about eight days, when only a few marginal points appeared during the reaction.

It is more than probable that the appearance of points in the second reaction stands in some relation to the marginal points in the first reaction. The ticks which were placed on the animal about a week previously may be responsible for the fever, accordingly the marginal points would only be a recrudescence due to the second fever reaction, the cause of which is uncertain.

- (9) Heifer 782.—Inoculated on the 3rd March, 1909, subcutaneously with 1 c.c. blood of ox 665.

Temperature reaction and blood examination.

There was no temperature reaction up to the 10th day after inoculation. On the 9th day the examination of the blood was negative. On the 10th and 11th days a slight morning and evening elevation to 103° F. was noted, and on the following day one single round-shaped *Piroplasma bigeminum* was found. A slight disturbance of the temperature ensued from the 15th to the 20th day, with a maximum exacerbation of 103° F. on the 17th day, but the average temperature of the animal during this time was 102° F. The presence of anisocytosis was noted on the 19th day; the temperature reaction continued to be normal up to the 26th day, on which date the phenomenon of leucocytosis was pronounced. On the 27th day the curve had a slight tendency to rise. On the 28th day the temperature reached over 105·2° F.; the 30th day after the inoculation it reached 107° F. in the evening and the same again twenty-four hours later, dropping to 104° F. on the 32nd day. The animal died on the 33rd day.

Coinciding with the rise of temperature on the 28th day marginal points appeared. The examination of the blood revealed the presence of pronounced anisocytosis and poikilocytosis.

The number of marginal points amounted

On the 30th day to	1·0	per cent.
„ 31st day to	5·4	„
„ 32nd day to	11·2	„
„ 33rd day to	26·7	„

One single *Piroplasma bigeminum* was noted on the 30th day. The blood corpuscles counted on the 30th day numbered 2,560,000 per cm., and the volume amounted to 23 per cent. The day preceding death the corpuscles were noted to be pale.

Clinical symptoms.

The first clinical symptoms coincided with the high rise to 107° F. on the 30th day, when the animal refused to feed. On the 31st day the mucous membranes of the eye were noted to be pale, also the nose and mouth. During this time the animal refused to eat its food, and accordingly the diet was changed to lucerne, which was also refused. On the

33rd trembling of the muscles of the shoulders and the flanks was noted, the animal looked very distressed and a bad prognosis had to be made. It died the same day.

Post-mortem examination.

The condition was good; the blood was pale; the fat was slightly yellowish; in the fat deposits along the aorta posterior a slight swelling of the red lymphatic glands was noted. The retro-pharyngeal glands were slightly swollen.

The pericardium was empty; the lungs were in inspirium, and showed a slight yellowish colour and were oedematous.

The mediastinal and bronchial lymphatic glands were pale.

The mucosa of the trachea was pale, and the heart was flabby. The left endocardium showed a few ecchymoses, and the ventricle was empty.

The right ventricle contained some blood, and the endocardium was normal.

The epicardium showed a few ecchymoses along the sulcus coronarius; there were sub-epicardial suffusions on the left ventricle.

The liver was enlarged; its weight was 6.60 kilo. The section showed a distinct saffron yellow colour, and the cut surface was granulated. A few red spots were present in the parenchyma.

The peri-portal lymphatic glands were swollen, soft, and slightly pigmented.

The capsula glissoni was rather whitish; the bile was dark green.

The spleen measured 68 cm. × 21 cm., and its weight was 2.7 kilo.; the margins were rounded and the form irregular. A few sub-capsular haemorrhages were present; the pulp was swollen and rather hard.

The mucosa of the fourth stomach was pale and the contents of the omasus were normal. The apex of the caecum was contracted, the contents were dry, and the mucosa was pale. The colon was contracted, and the mucosa pale.

The valvula ileo-caecalis was bile-stained.

The mucosa of the ileum and jejunum was bile-stained; the contents were pale. The mesenteric glands were swollen and soft.

The fat was of a pale yellowish colour.

The kidneys were rather pale; in a few lobuli small wedge-shaped infarcts were noted. The capsule was easily detached. The bladder was filled with a clear urine.

The brain was pale.

The bone marrow in the femur and humerus was changed into a pale gelatinous mass. Also the marrow of the ribs was gelatinous and watery.

Pathological anatomical diagnosis.

Oligocythaemia; icterus; petechiae endocardii; tumour hyperaemicus splenis; hepatitis parenchymatosa; nephritis parenchymatosa.

(10) Heifer 783.—Injected on the 3rd March, 1909, with 1 c.c. blood of ox 665.

Temperature reaction and blood examination.

On the evening of the 7th day an exacerbation to 104° F. was noted. Before this the evening temperature remained below 102° F., and after it kept at 102° F. or above, reaching on three occasions 103° F. *Piroplasma*

bigeminum was noted on the 12th day but rarely; on this date the temperature was 103·2° F. in the evening, and at the same time a slight anisocytosis was present. On the 24th day the temperature rose abruptly and reached 105·6° F. that evening. It remained high, morning and evening of the succeeding day. On the 25th day it was 106·6° F., and kept above 106° F. on the 26th and 27th days; the following day it was 105·6° F., and now dropped suddenly to 100·8° F. in the morning of the 29th day. The animal entered into collapse and died the same night.

During the first three days of the high temperature nothing particular was noted in the blood. On the 27th day the marginal points were present, infecting 11·2 per cent. of the red corpuscles, accompanied by the symptoms of poikilocytosis. The marginal points were now found daily up to the time of death, infecting 15·4 per cent. on the 29th day, together with lesions of anisocytosis and poikilocytosis.

Clinical symptoms.

The clinical symptoms were noted one day after the appearance of marginal points; the animal did not feed, and the respirations were hurried. A distinct loss of condition had become noticeable. On the day of death trembling of the muscles of the shoulders and the flanks were noted. The mucous membranes of the eye were white, and the nostrils and mouth pale.

NOTE.—The presence of *Piroplasma bigeminum* hardly had any influence on the temperature reaction.

The course of the disease was very acute, and there was not sufficient time for the appearance of lesions in the blood such as are met with in cases of longer illness.

Post-mortem examination.

The condition was good. The subcutaneous tissue over the shoulder and neck showed a yellowish discolouration.

The fat was yellowish.

In the pleural cavities there was about 1 litre of yellowish liquid.

The pericardium contained 30 c.c. of red stained liquid.

The retro-pharyngeal glands were slightly swollen.

The lungs were in inspirium, and there was a slight emphysema in the anterior lobes.

There was a slight oedema in the interstitial tissue.

The bronchial lymphatic glands were slightly enlarged, the sinus of which showed a slight haemorrhagic infiltration.

The trachea contained a little foam and the mucosa was slightly injected. There was foam in the bronchi.

The left endocardium was rather whitish. The blood of the heart was well coagulated. The right endocardium showed slight imbibition and yellowish discolouration. There were petechiae and suffusions on the epicardium. The myocardium was soft.

The liver was swollen, and its weight was 7·9 kilo.

On section the parenchyma appeared brown yellowish. There was an interstitial oedema. The peri-portal lymphatic glands were pale, and there was a slight injection of their sinuses.

The spleen was swollen and irregularly shaped; it measured 63 cm. × 20 cm., and its weight was 3·6 kilo.

The pulp resembled black currant jam.

In the fourth stomach the folds were slightly swollen and hyperaemic. The blood vessels were injected. The cardiac portion was slightly oedematous. The pylorus was slightly swollen. The contents of the omasus were dry. The reticulum was normal. The intestines appeared pale; the caecum was empty, and its mucosa was slightly swollen, and was the seat of a patchy hyperaemia. The mucosa of the colon was swollen and slate coloured. The ileum was swollen; the mucosa was hyperaemic in longitudinal streaks. The jejunum was discoloured and slightly swollen. The mesenteric glands were slightly swollen.

The left kidney was pale, the parenchyma was yellowish discoloured and soft. There was a small infarct in the left kidney. The right kidney showed injection of the sub-capsular blood vessels.

The bladder was empty; there were petechiae on the walls. The thymus gland showed a gelatinous infiltration and yellowish discolouration.

The brain was pale, and had a watery appearance; the capsula was moist; the grey substance was nearly white, and there was the appearance of an oedema.

The marrow of the diaphysis in the femur was yellowish.

In the ulna the marrow of the *diaphysis* was watery and a few haemorrhagic spots were noted. The marrow of the ribs was also yellowish discoloured, oedematous and infiltrated.

Pathological anatomical diagnosis.

Oligocythaemia; icterus, oedema pulmonum; tumour hyperaemicus splenis; petechiae epicardii and endocardii; hepatitis parenchymatosa; gastritis, enteritis catarrhalis; nephritis parenchymatosa.

(11) Heifer 784.—Inoculated on the 3rd March, 1909, with 1 c.c. blood of ox 665.

Temperature reaction, and blood examination.

There was a slight temperature reaction, beginning on the 9th day and lasting until the 19th day, averaging about 102° F., only one exacerbation occurred and that was on the 16th day to 103° F. During this time the examinations of the blood proved negative. A similar curve continued between the 22nd and 58th days, an exacerbation to 103° F. was only noted on one occasion, namely, the 55th day. During this period occasionally the lesions of anisocytosis were noticed; they were but slightly pronounced; the presence of eosinophile cells was also noted, and on one occasion, that is the 36th day after inoculation, a few corpuscles with basophile cells were seen.

On the 58th day, that is the 30th April, 1909, the animal was injected subcutaneously with 5 c.c. blood of heifer 785 (*vide Part III*).

This blood was freshly drawn, and on this date contained marginal points and *Piroplasma mutans* in rather large numbers.

A disturbance in the temperature ensued eight days later, rising to 103·6° F. in the evening, but remaining below this during the succeeding days until the 15th, when another rise to 105° F. was registered. A remission to 102° F. intervened for one day and was succeeded by another sudden rise on the 18th day up to 105° F., reaching 106·4° F. in the evening of the 19th day, the evening exacerbations averaging 105° F. to the 22nd day. After this date the temperature descended gradually, regaining normal latitudes about the 26th day. Marginal points were noted for the first time on the 13th day, infecting 5·7 per cent. of the red

corpuscles, they were frequent on the 14th day, amounting to 16.6 per cent., and at the same time the lesions of anisocytosis were noted. On the 17th day the marginal points numbered 17.8 per cent. On the 18th day, the date of the highest rise, poikilocytosis was also noted, and corpuscles with basophile granulations put in an appearance subsequently. The marginal points decreased in numbers after this date, reaching 5 per cent. on the 21st day, and the cells with basophile granulations increased. On the 21st day the lesions of an acute anaemia were indicated by anisocytosis, poikilocytosis, polychromatic and basophile cells, macrocytes and a few normo blasts.

There was still another temperature reaction noticed from about the 35th day to the 50th day, after the second inoculation, which in its exacerbations and remissions was rather irregular, the maximum evening temperature on one occasion reached 105° F. During this time also marginal points were noted and the lesions of anaemia as described above. *Piroplasma mutans* was registered on the 45th day, the previous examination was seven days earlier on which date it was not yet present.

The heifer was still alive at the end of the year.

NOTE.—This heifer did not contract the marginal points from the first inoculation, but did so after the second one, and when blood was used in larger quantities and with marginal points present, which may account for the shorter incubation time. The failure of the first injection is either due to some inborn resistance, for which we have no explanation, although the result of the second injection does not support this view, or, perhaps, and what is more likely to offer a reasonable explanation, that the marginal points were not contained in the 1 c.c. of blood with which it was injected in the first instance. The last reaction was due to the *Piroplasma mutans* infection.

- (12) Heifer 673 (control animal for blood of ox 665).—This heifer was imported from Aliwal North, a country from which, hitherto, we always obtained cattle which had proved to be susceptible to the Pretoria strain of ordinary redwater.

The heifer was injected on the 3rd March, 1909, with 1 c.c. blood of ox 665.

Temperature reaction and blood examination.

There was a rise of temperature on the 6th day, and on the next day the temperature reached 105° F.; it remained so during the following day, and on the 9th day suddenly dropped to 102° F. A series of slight evening exacerbations (104.6° F.) now followed until the 14th day, when the normal limit was again reached.

Coinciding with the first rise of temperature *Piroplasma bigeminum* appeared. It was noted on the succeeding day; it was absent for the following two days, after which it reappeared. After this the temperature remained normal up to the 27th day. The curve now showed slight irregularities indicating some disturbances. The highest evening exacerbation was on the 39th day, reaching 103.8° F. During this disturbance the blood was first examined daily and latterly every second day, and on two occasions marginal points were noticed to be very rare, namely, on the 32nd and 36th days, when the lesions of anisocytosis were also present.

NOTE.—The injection of 1 c.c. blood caused in this animal a decided attack of redwater, although not clinically severe, yet microscopically it was distinct.

COMPARISON OF RESULTS OF INOCULATION IN ENGLAND AND OF INOCULATION IN THE TRANSVAAL OF SIX ENGLISH HEIFERS (LOT NO. 2).

Lot No. 2 injected (1) with blood of Heifer 108, and (2) with blood of Heifer 783, both inoculations carried out in England, and (3) injected with blood of Ox 665 in the Transvaal.

No. of Animal.	Result of Second Inoculation in England.	Result of Inoculation in the Transvaal.
<i>Heifer 780</i> ..	Reaction from 5th to 8th days. <i>Piroplasma bigeminum</i> from 5th to 8th days.	First reaction on 8th, 9th, and 10th days, with <i>Piroplasma bigeminum</i> on the 11th and 12th days. Second reaction from the 32nd to 38th days. Marginal points appeared on 29th day, and animal died on the 38th day.
<i>Heifer 781</i> ..	Reaction from 4th to 9th days. <i>Piroplasma bigeminum</i> on 4th to 8th days; haemoglobinuria.	First slight reaction from 9th to 17th days, no piroplasms being noted during this period. Second rise from the 35th to 41st days. Marginal points on 32nd day. A fourth rise after tick infestation on 62nd day with re-appearance of marginal points.
<i>Heifer 782</i> ..	<i>Result of first inoculation.</i> Reaction from 12th to 14th days. <i>Piroplasma bigeminum</i> on 12th day.	First slight reaction from 10th to 20th days, during which time one single <i>Piroplasma bigeminum</i> was seen on the 12th day. Second and high reaction from the 27th to 33rd days. Marginal points appeared on the 28th day. The animal died on the 33rd day.
<i>Heifer 783</i> ..	<i>Result of first inoculation.</i> Reaction from the 8th to 14th day. <i>Piroplasma bigeminum</i> on 12th day.	Very slight reaction from the 10th to 22nd day, <i>Piroplasma bigeminum</i> on 12th day. Distinct and high reaction from 24th to 29th day. Marginal points noted on 27th day, and the animal died on 29th day.
<i>Heifer 784</i> ..	<i>Result of second inoculation.</i> Reaction from 8th to 13th day. <i>Piroplasma bigeminum</i> noted on 8th and 9th days. Haemoglobinuria noted.	Very slight reaction from the 9th to 19th days; second reaction from 22nd to 48th days; no piroplasms and no marginal points noted during this period. Distinct reaction after a second injection of blood from the 9th to 25th days, and still another reaction from 35th to 50th day after second inoculation.
<i>Heifer 673, Control</i>	—	<i>Rise from 6th to 14th day, with Piroplasma bigeminum present on 7th and 8th days. Marginal points noted on 32nd day, but only a slight reaction noted at the time.</i>

Résumé—Lot No. 2.

1. All animals inoculated in England showed a temperature reaction with a minimum incubation time of four days, and a reaction averaging from three to four days with the presence of *Piroplasma bigeminum* in every instance and haemoglobinuria in three instances (heifers 780, 781, and 784).

2. In no instance was a second reaction observed in England due to the blood inoculation, nor were any marginal points registered.

3. The inoculation in the Transvaal caused a fever reaction with piroplasms in one instance (heifer 780), a slight reaction with piroplasms in a second case (heifer 783), a slight reaction with a single piroplasm in a third animal (heifer 782), and indications of reactions without piroplasms in heifers 781 and 784.

4. A distinct second reaction with a long incubation time was noted in four instances (one case, heifer 784, only showed a reaction after the second inoculation with sick blood). In these four animals the reaction started on the 32nd, 35th, 27th, and 24th days respectively (heifers 780, 781, 782, and 783).

5. Marginal points appeared on the 29th, 32nd, 28th, and 27th days respectively in these four animals, and in heifer 784 on the 13th day after second injection.

6. Of these five heifers three died of a typical disease, characteristic in pathological anatomy of redwater but without haemoglobinuria.

7. The control animal had a distinct fever reaction, typical of redwater and *Piroplasma bigeminum* was found.

CONCLUSIONS.

1. The inoculation of the ten Sussex heifers in England with a South African strain of redwater produced in all a more or less severe attack of this disease, followed by immunity, which protected them against a second attack of redwater in the Transvaal after an injection of immune blood. This immunity was pronounced in all animals. In no instance was there any severe reaction. In some animals fever reactions were noted, but the animals were never noticed to be ill.

2. Five of the animals, or 50 per cent., died of a disease of which the pathological blood lesions resembled those of redwater, but it differed from this disease through the absence of haemoglobinuria, and through the presence of peculiar chromatic bodies, the so-called "marginal points".

3. The appearance of these marginal points corresponds to an incubation time, varying from twenty-seven to thirty-two days, with the exception of one animal injected with sick blood containing visible marginal points, in which instance it was shorter.

4. The appearance of the marginal points coincided with the onset of a severe fever reaction. The numbers of these points increased with the progress of the fever reaction, and the temperature fell when the number of the chromatic bodies decreased or disappeared.

5. The severe pathological lesions of the blood in the form of oligocythaemia, anisocytosis, poikilocytosis, polychromasia, basophilia, micro and macrocytosis, normo and megaloblasts were preceded by the appearance of marginal points.

6. These observations point to the fact that the so-called marginal points play the role of a pathogenic organism acting in a similar way but not so acutely on the red corpuscles as *Piroplasma bigeminum*.

7. The question arises therefore whether *Piroplasma bigeminum* and these marginal points represent two different phases in the life cycle of one parasite, viz., of *Piroplasma bigeminum*, or whether they are two distinct species.

8. The facts speak for the dual nature of these parasites for the following reasons :—

- (a) Immunity against *Piroplasma bigeminum* does not protect against the marginal points. We do not know in protozoology of any analogy where a protozoon would produce a primary reaction in one form and a secondary more severe reaction in another form; the rule is that a primary reaction gives some immunity against the parasite which caused it.
- (b) If these marginal points represent a form in the life cycle of *Piroplasma bigeminum* we would have expected to see them after the inoculation in England where the animals had been injected with the South African strain of redwater. Here the infection was a pure *Piroplasma bigeminum* infection. *Stockman* who used the same strain in quite a number of experiments never met with such late reactions, which, on account of their severity and peculiarity, could not have escaped notice.
- (c) The fact that out of ten animals five died due to this second inoculation can only be interpreted by accepting that death was due to a different cause than *Piroplasma bigeminum*.

All these various reasons induce me to consider the marginal points as parasites of protozoic character. They differ from any known blood parasite by the absence of a protoplasmatic body and consist only of chromatin substance, thus resembling, to a certain extent, the bacteria. They represent, in my opinion, a new genus of protozoa which I propose to call *Anaplasma*, and the species under consideration *Anaplasma marginale*.