

PART III.

**THE ASSOCIATION OF PIROPLASMA BIGEMINUM,
ANAPLASMA MARGINALE, AND PIROPLASMA
MUTANS IN SOUTH AFRICAN CATTLE.**

Origin of Blood Used for Inoculation.

Heifer 641, from Aliwal North, had been injected on 15th October, 1908, with blood which contained *Piroplasma mutans*. As a result of this injection *Piroplasma mutans* was noticed. When tested later, on the 19th April, 1909, on its immunity against redwater with 5 c.c. blood of ox 573, heifer 641 developed a severe attack of redwater, *Piroplasma bigeminum* appearing on the 5th day after inoculation, remaining for eight days, and red urine was passed on the 9th day. After the disappearance of the *Piroplasma bigeminum* the lesions of acute anaemia accompanied by basophilia were noted for some time. This test was made to prove that the heifer was not immune against redwater.

The blood of heifer 641 was used for the injection of an English heifer, 785, on the 3rd of February, 1909, viz. before she had gone through the acute attack of redwater. The dose given was 5 c.c. On the 10th and 11th days after this injection there was a slight temperature reaction, reaching at its maximum, 103° F., and during this reaction the blood examinations gave negative results. The temperature rose again on the 18th day and now continued in a prolonged curve, remaining high and averaging 105° F. up to the 38th day. The examination of the blood revealed the presence of marginal points on the 27th day. These marginal points now increased. On the 29th day they numbered 15 per cent. of the red corpuscles, two days later 15·3 per cent., and dropped after this date, and the usual picture of anaemia developed with basophile cells, normo-blasts, macrocytes, etc. The animal was noticed to be ill on the 22nd day by not feeding as usual. On the 28th day a distinct loss of condition was noticed; the animal was undoubtedly ill, grinding of the teeth was a pronounced symptom, saliva ran from the mouth and there was a slight discharge from the nose. The mucous membranes of the eyes were pale, and the nostrils had a yellowish tinge. On the 30th day there was an increased respiration and more profuse salivation; on the same day the number of blood corpuscles was counted and found to be 2,160,000 per cm., the volume being 21 per cent. The animal began to improve slightly after the 26th day, from which day the temperature descended, and about the 40th day, when the temperature had reached normal, it was undoubtedly recovering. Coinciding with that date *Piroplasma mutans* appeared in great numbers. There was a second rise of temperature, lasting about fourteen days, in which evening exacerbations were not so high as during the previous reaction; the microscopical examinations of the blood again revealed the picture of anaemia, but not so pronounced as during the first reaction. Marginal points were occasionally found, and on the 51st day they numbered 2 per cent. of the red corpuscles. The blood lesions were anisocytosis, poikilocytosis, basophile granulations, and rare normo-blasts, but this time the animal did not show symptoms of a severe disease.

For the purpose of infecting blue ticks (*Boophilus annulatus*) with marginal points, larvae, whose mothers were collected from horses, were placed on the beast on the 42nd day. On the 23rd day after this tick infestation a sudden rise of temperature occurred, reaching 106° F. the next morning. The animal showed a severe illness, developing all the symptoms of a very acute redwater. *Piroplasma bigeminum* was found in almost every corpuscle; free parasites were present in the blood plasma.

The animal died in the night of the 25th to 26th day.

Post-mortem.

The condition was good. Rigor was not present.

The subcutaneous tissue was yellow. The flesh was pale. The blood was thin, not coagulated.

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

The lungs were in inspirium; the pleura had a glossy appearance, the parenchyma was oedematous.

The mediastinal and bronchial lymphatic glands were normal. There was foam in the trachea. Both ventricles of the heart contained coagula, in which only a few blood corpuscles were present. The liver was swollen. The weight was 10.2 kilo.; it had a yellowish-brown appearance. The parenchyma was friable, yellow, and had a fine granulated and glossy appearance. The bile was thick, dark green; the mucosa of the gall-bladder was green, discoloured.

The spleen was swollen. The pulpa was soft, measuring 62 cm. × 19½ cm. On section it had a granulated appearance, Malpighi's bodies were protruding and enlarged.

The mucosa of the fourth stomach was slightly swollen. There were some irregular-shaped ulcers on the mucosa of the pylorus with black margins, their size ranged from that of a split pea to that of a shilling. The contents of the omasus were dry.

The mucosa of the caecum, colon, and ileum was folded and slightly hyperaemic and covered with brown mucus.

The mucosa of the jejunum was slightly swollen, and of a brown discolouration; there were ecchymoses and ramifications of the blood vessels.

The kidneys were black. The surface had a mottled appearance like a fine meshwork, and on section red streaks were noted. The capsula was easily detached. The bladder was distended with red urine. There were haemorrhagic streaks in the mucosa.

The pharynx was hyperaemic.

The brain was anaemic. The marrow of femur was oedematous, there were haemorrhagic foci in the diaphysis, and they were also present in the humerus.

The spongiosa of the ribs had a moist appearance.

Diagnosis: Redwater (Piroplasmosis due to *Piroplasma bigeminum*).

NOTE.—From the evidence of this case, one could conclude that it represented in the first instance an infection of anaplasmosis complicated by *Piroplasma mutans*, and this view would be supported by the fact that the animal had no immunity at all against redwater, since the infestation of ticks brought on a very acute case from which the animal died. The *post-mortem* was typical of acute redwater, and when compared with that of anaplasmosis, it will be noted how few differences there are between these two diseases when red urine is left out of consideration. The subsequent experiments do not support the view that the infection of heifer 785 with *Anaplasma marginale* was unassociated with *Piroplasma bigeminum*. Unless some uncontrollable accident has occurred which of necessity must lead to a different interpretation,

the subsequent inoculation experiment proved that *Piroplasma bigeminum* had been present in 785. The experiment is, nevertheless, of interest, as it demonstrates the fallacies which do and can occur in an experiment where blood of an immune animal is used.

EXPERIMENT WITH BLOOD OF HEIFER 785 TAKEN BEFORE TICK INFESTATION.

Heifer 792. This animal was injected on the 13th April, 1909, with 1 c.c. blood of heifer 785, not showing any piroplasms at all at that time, and the blood was taken previous to the infestation of ticks. On the 9th and 10th days after inoculation a slight temperature exacerbation was noted, reaching 103.4° F. On the morning of the 13th and on the evening of the 14th days there were exacerbations to 104° F. and 105° F., and from the 15th day the temperature steadily increased, forming a regular curve and returning to normal about the 20th day. From the 20th to the 27th day the morning and evening temperatures oscillated between 104° to 108° F., but returned to normal three days later.

On the 31st day the animal was infested with red and brown larvae and nymphae taken off heifer 650, immune to redwater and anaplasmosis.

There was another rise of temperature directly succeeding this infestation, this time more irregular, and reaching 106° F. on the 34th day after inoculation, or three days after infestation. It returned to normal about the 50th day, to be followed again by a slight reaction, reaching a maximum of 104.4° F. During the onset of the first reaction, on the 19th day, *Piroplasma bigeminum* was noted; it only remained present for three days and was succeeded by anisocytosis, poikilocytosis, and basophile granulations on the 24th day; the macrocytes were noted to be increasing on the 25th day; anaplasma appeared on the 22nd day in rare numbers, averaging about 1 per cent. of the red corpuscles. They reached their maximum during the second reaction—the 35th day—when they numbered 19.5 per cent., and were succeeded by an enormous decrease of red corpuscles, by polychromatic and basophile punctated cells; three days later mormo-blasts appeared. This picture of anaemia was apparent during the whole time of the second reaction. *Piroplasma mutans* appeared on the 42nd day. During the third reaction the anaplasms were also noted and *Piroplasma mutans* was very frequently met with. The anaemia remained for a considerable length of time and during this period a daily examination of the blood proved the presence of *Piroplasma mutans* in rather frequent numbers, accompanied by anaplasms. On the following days *Piroplasma mutans* was still met with, but the general picture of the blood improved. About the 130th day after the inoculation the animal again showed normal blood.

NOTE.—This heifer was injected with blood of an animal in whose blood *Piroplasma bigeminum* was never seen. Nevertheless an infection with *Piroplasma bigeminum* succeeded, true not after the typical period. The interpretation of this observation would be that we were dealing here with an extremely mild strain of *Piroplasma bigeminum* whose virulency was almost lost. This observation will explain some of the American's experiments, when they observed none or only mild primary reaction (due to *Piroplasma bigeminum*) and more severe reaction due to peripheral cocci (*Anaplasma marginale*).

EXPERIMENTS WITH BLOOD OF HEIFER 785 TAKEN AFTER TICK INFESTATION.

Heifer 788 was injected on the 5th May, 1909, with 5 c.c. blood of heifer 785; the blood was taken from this heifer three days before the *Piroplasma bigeminum* appeared. In this experiment, therefore, it is not safe to

conclude that heifer 785 was not infected with redwater at that time; the experiment is of value, inasmuch as it again shows the uncertainty of the appearance of *Piroplasma bigeminum* in the first instance, and the certainty of the presence of *Anaplasma marginale*. At the time of the withdrawal of the blood the animal was infected with *Piroplasma mutans*; these parasites therefore also put in an appearance.

The first slight temperature reaction started on the 6th day, the evening exacerbation reaching 104° F. The next morning the temperature was 102° F. and remained so; on the 7th and 8th days a few *Piroplasma bigeminum* were noticed. They were succeeded by the symptoms of anisocytosis; on the 13th day *Piroplasma bigeminum* was seen again. Poikilocytosis was now registered and the presence of cells with basophile granulations in rare numbers. From the 15th day onwards a steady rise of temperature occurred. *Piroplasma mutans* appeared on the 18th day and anaplasma on the 19th day. Their maximum number was reached on the 26th day, infecting about 22 per cent. of corpuscles. Both parasites were now present during the following time and increased in numbers, the temperature remaining between 105° F. in the morning and above 106° F. in the evening, until the 28th day, when the collapse occurred and the animal died on the 30th day.

During this reaction, the picture of an acute anaemia was present, the corpuscles were pale, almost shades; their numbers decreased, macrocytes and basophile granulations were present. The animal showed clinical symptoms on the 20th (when the temperature was very high), by refusing to feed. On the 23rd day loss of condition became noticeable, and on the 29th day the faeces were covered with mucus of a very yellow and sticky nature. No red urine was passed.

Post-mortem examination.

The condition was good; rigor mortis was present.

The flesh was pale.

The pleural cavities contained a little liquid; also the pericardium contained a slightly abnormal amount of liquid.

The retro-pharyngeal glands were swollen and softened.

The lungs were in inspirium; the interlobular tissue was the seat of a gelatinous infiltration; both anterior lobes showed a strong oedema.

The mucosa of the trachea contained ecchymoses; there was foam present.

The left endocardium was pale, and the right one showed ecchymoses.

The myocardium was pale and soft.

The liver weighed 9·5 kilo.; its colour was yellow; the section showed a fine granular appearance; the parenchyma was soft. The lobuli were yellowish with a brown centre. The bile was brown, thick, and had a granulated appearance.

The spleen was enlarged and thickened; length, 65 cm.; breadth, 20 cm.; thickness, 5 cm. The pulpa was protruding on section and soft. The follicles were distinct.

The fourth stomach contained some food; the mucosa was slightly swollen, oedematous, and pale. There were ramifications of the capillaries. The omasum had dry contents. The mucosa of the duodenum contained some fine ecchymoses.

The caecum had liquid contents; its mucosa was pale; there were ramifications of the blood vessels. The mucosa of the colon was yellow and folded; there were ramifications of the blood vessels. The ileum was the seat of a patchy hyperaemia and the mucosa was covered with mucus. The mucosa of the jejunum was pale; the small blood vessels were filled.

The kidneys were pale, moist on the surface and on section.

The internal lymphatic glands were enlarged and soft.

The brain was oedematous. The pia was injected.

Diagnosis : Anaplasmosis.

NOTE.—Although heifer 788 showed but a slight infection with *Piroplasma bigeminum*, from which it recovered, the supervening anaplasma infection was severe and accompanied by *Piroplasma mutans*. All these three parasites were in the injected blood and succeeded each other corresponding to the length of their separate incubation times.

EXPERIMENTS TO SEPARATE THE PIROPLASMA FROM THE ANAPLASMA INFECTION.

Heifer 789 was injected with 5 c.c. blood of heifer 788 on the 13th of May, 1909, that is at the time when *Piroplasma bigeminum* was rare in heifer 788 and before *Anaplasma marginale* and *Piroplasma mutans* had appeared. The idea of the experiment was to see whether it was possible to get a pure infection of *Piroplasma bigeminum*, as it was considered that the developments of *Piroplasma bigeminum* being shorter, *Anaplasma marginale* and *Piroplasma mutans* would not be present in the blood when such blood was known to contain *Piroplasma bigeminum*.

On the 3rd day after inoculation a rise of temperature occurred to 104·4° F. In the evening of the 5th day the exacerbation reached 106° F.; on the 7th day it touched 107° F., on the 8th day it was 106·4° F.; it now dropped to sub-normal and the animal died on the 10th day.

Large numbers of *Piroplasma bigeminum* were noted on the 5th day and remained present until death. Red urine was passed on the 8th day; muscular tremors in the flanks were noted on the 9th day, and at the same time a foamy discharge from the nostrils was in evidence.

The animal died of a pure infection of *Piroplasma bigeminum*.

It was expected that the blood of heifer 788 would only contain *Piroplasma bigeminum* and that therefore the succeeding animal, when injected with such blood, would produce a pure infection of *Piroplasma bigeminum*. Accordingly, a fresh English animal (786) was injected with blood taken on the 5th day when *Piroplasma bigeminum* was present for the first time.

Heifer 786.—Inoculated on the 18th April with 1 c.c. blood of heifer 789, withdrawn on the same date.

On the 4th day there was a slight exacerbation to 104° F. The temperature rose to 107° F. on the 6th day. It dropped to 104° F. in the morning, but the evening exacerbations remained up to the 16th day when a normal record was obtained. On the 5th day *Piroplasma bigeminum* was seen for the first time; on this and the following day it was very frequent; it was still present on the 8th and 9th days; but disappeared after this date. On the 7th day the symptoms of anisocytosis were registered. On the 9th day poikilocytosis appeared and the following day macrocytes were present. On the 11th day polychromatic cells and basophile granulations were registered as being rare. On the 12th day macrocytes were noticed to be fairly frequent. On the 15th day the macrocytes were dominant, the polychromatic cells rather rare and on the 16th day macrocytes were still dominant, polychromasia and basophilia being pronounced. These lesions now gradually disappeared, and when the temperature had almost reached normal on the 20th day the basophile granulations were noticed but rarely.

Red urine was passed on the 7th day; diarrhoea was noted the following day; the animal lost condition and showed distress; hurried respirations were noted. During this time it was treated with quinine.

From the 20th day onwards a daily examination of the blood revealed only the presence of a slight anisocytosis; no parasites were present. On the 34th day, the temperature having remained normal, the animal was injected with 5 c.c. blood of heifer 792, that is the same strain of blood, and heifer 786 being now immune to this strain of redwater should show *Anaplasma marginale* and not *Piroplasma bigeminum* if the former is really independent of the latter. From the 34th day to the 53rd no change in the temperature took place. On the 54th day, however, a sharp rise up to 106° F. ensued, the morning record averaging 105° F. and in the evening about 107° F. This remained so for about ten days when the curve gradually descended culminating in a record of 101° F. on the 70th day. There was a slight recurrence of the reaction on this date—a third reaction—with an average evening temperature of 103° F. and a morning record of 102° F. This lasted until the 80th day. From the 84th day onwards another slight temperature reaction was noticed. The rise of temperature on the 54th day after the first injection coincided with the appearance of *Anaplasma marginale*, which numbered on this day 9.5 per cent. of the red blood corpuscles, the corpuscles were noted to be pale at this date, and poikilocytosis became marked, the macrocytes were frequent and the red corpuscles only appeared as shades. On the 62nd day corpuscles with basophile granulations put in appearance. On the 64th day nucleated cells were noted, and on this date *Piroplasma mutans* was seen for the first time. During the following days this picture of acute anaemia remained. *Piroplasma bigeminum* appeared again on the 72nd day, and on that date *Piroplasma mutans* was also present. *Piroplasma mutans* increased and anaplasma was still present. The blood improved slightly notwithstanding the presence of anaplasma and *Piroplasma mutans*.

The animal recovered.

NOTE.—In this animal we have first a distinct reaction due to the *Piroplasma bigeminum*, and it can be noted that to a very great extent the picture of anaemia caused by the parasite resembles that caused by *Anaplasma marginale*. Indeed, I do not think that in the absence of the parasite the cause of the anaemia could, with certainty, be traced to a definite infection of either anaplasma or piroplasma. There is no other interpretation possible than that with the first injection only *Piroplasma bigeminum* was injected. The blood of the second injection contained *Piroplasma bigeminum*, *Anaplasma marginale*, and *Piroplasma mutans*. The animal being immune to *Piroplasma bigeminum*, this parasite did not appear, but after the incubation time typical of *Anaplasma marginale* and *Piroplasma mutans* these put in appearance.

In previous experiments I have shown that it is possible to separate *Piroplasma bigeminum* and *Piroplasma mutans* infection, and these experiments should prove that it is also possible to separate the *Piroplasma bigeminum* infection from its association with *Anaplasma marginale*. It will, however, be difficult to bring about the reverse experiment on account of the much longer incubation time of the parasite under discussion.

THE ASSOCIATION OF SPIROCHAETE THEILERI WITH PIROPLASMA MUTANS AND ANAPLASMA MARGINALE.

Heifer 624 was an animal which had proved not to be infected with *Piroplasma bigeminum*. During a fever reaction it had shown *Spirochaete theileri* and subsequently *Piroplasma mutans*. The injection of its blood into other animals had constantly caused the appearance of *Piroplasma mutans*. For this reason it was considered that it contained this parasite unassociated with *Piroplasma bigeminum*. The experiment was, in the first instance, undertaken for the purpose for continuing the pure infection of *Piroplasma mutans*.

On the 5th of May, 1909, this animal was tapped and 5 c.c. blood was injected into the English heifer 794.

On the 4th, 5th, and 6th days there was a slight reaction with an evening exacerbation of 103° F., but the examination of the blood remained negative. It is likely that this reaction was due to spirochaetes, although they could not be demonstrated, a not unusual occurrence. On the 18th day an exacerbation to 105° F. was noticed. There was a remission on the 19th day to normal, succeeded the day after by an exacerbation to the same record, and from this date up to the 37th day the temperature remained high, after which time it kept at an average of about 102° F. to rise again about the 50th day to an average of about 103° F. Between the 60th and 66th days the evening exacerbations transgressed to 104° F., then a steady decrease took place, and the animal died on the 75th day.

On the 24th day after inoculation *Anaplasma marginale* appeared for the first time, infecting 6.5 per cent. of the red corpuscles; it increased on the 26th day to 8 per cent. and continuing to do so, reaching the maximum on the 33rd day, from which date a steady decrease took place, and on the 40th day it had become very rare, being below 1 per cent. With the appearance of the marginal points anisocytosis was noticed. On the 29th day a distinct reduction of the numbers of the corpuscles became apparent, the corpuscles themselves were rather pale. On the 33rd day polychromatic cells and basophile granulations were present. On the 34th day the presence of macrocytes was registered, and the number of the cells with basophile granulations had increased. These lesions were now present throughout the rest of this reaction; when the temperature had reached its normal average the lesions of an anisocytosis were still pronounced. There was an interval up to the 50th day where no parasites of any kind were found. On this date *Piroplasma mutans* appeared and was present up to the 69th day. It increased in numbers and was very frequent during the time the fever lasted. Coinciding with the appearance of *Piroplasma mutans*, anisocytosis again became more pronounced, the basophile granulations, which had previously disappeared, were noted again. Polychromatic cells were present daily. The corpuscles lost their colour; on the 63rd day normo-blasts were noticed. From this date until death the lesions indicating the destruction of red corpuscles increased.

During this latter period and coinciding with the appearance of *Piroplasma mutans*, anaplasms again appeared. On the 58th day their number reached the maximum, infecting 8.5 per cent. of the corpuscles; they were present daily up to the time of death, on the 75th day after inoculation.

The first clinical symptoms were noticed on the 32nd day, when the animal appeared dull, had hurried respirations and refused to feed. On the 33rd day the same symptoms were pronounced. On the 34th day the excreta was of a dark yellowish colour. On the 36th day the mucous membranes of the eyes and the muzzle appeared pale, and loss of condition became very apparent. The animal rallied, and during the following days showed a marked improvement in health. On the 55th day diarrhoea was present, continuing for three days; the animal became worse notwithstanding medicinal treatment, and refused to feed on the 60th day. The symptoms of illness became more noticeable every day, and just previous to death the heifer showed symptoms of cerebral irritations.

Post-mortem examination.

The condition was fair, rigor mortis was present.

The visible mucous membranes were pale, the flesh had a greyish appearance.

The pericardium contained about 50 c.c. blood stained liquid.

The lungs were in inspirium, the right lung was the seat of a strong hyperaemia. In the left anterior lobe was a slight emphysema.

The trachea and bronchi contained some foam, the mucosa was red stained (imbibition).

The mediastinal and bronchial lymphatic glands were normal.

The epicardium and both endocardia showed imbibition. There was some coagulated blood in both ventricles.

The liver was slightly swollen and showed a brown discolouration. The bile was green-yellowish.

The spleen was slightly contracted, 46 cm. long by 15 cm. broad; the capsula was slightly hyperaemic. The pulpa was pale.

The fourth stomach had liquid contents; its mucosa was black discoloured; there was strong hyperaemia and oedema of the folds. The omasum had liquid contents. The mucosa of the caecum was pale, there were some petechiae present. The mucosa of the colon and ileum was pale; there were a few petechiae present in the ileum. The jejunum showed a black discolouration.

The kidneys were soft, friable, and showed signs of advanced putrefaction, and on section hyperaemia could be noticed.

The brain showed strong pigmentation of the frontal lobes; around the hypophysis and in the surrounding tissue were blood coagula. The ganglion semilunare was enlarged and showed strong hyperaemia.

The pia showed a milky discolouration and a very strong injection of the small blood vessels.

The plexus corioideus on both sides showed a strong hyperaemia. In the grey and white substance there were star-like injections of blood vessels and some small haemorrhages in the grey substance. In the fourth ventricle was some blood tinged liquid. Hyperaemia was also present in the cerebellum and medulla oblongata.

The larynx and pharynx were hyperaemic.

The tonsillae showed a haemorrhagic infiltration.

The bone marrow in the femur and humerus was yellowish. There was a strong hyperaemia of both diaphyses. The epiphysis was spotted with hyperaemic patches.

The red bone marrow of the ribs was gelatinous.

The dura of the spinal cord showed imbibition.

Diagnosis.—Chronic anaplasmosis.

NOTE.—In heifer 624 we did not see any *Piroplasma bigeminum*, neither did we see any in heifer 794, although an almost daily examination of the blood was carried out during the whole time the animal was under observation. Heifer 794 could not have been immune to redwater, since it was an imported English beast. We therefore can conclude with a certain amount of confidence that *Piroplasma bigeminum* was not present in this animal. The appearance of *Anaplasma marginale* was unexpected. The lesions described were due, in the first instance, to the presence of *Anaplasma marginale*, and, in the second instance, to the presence of *Piroplasma mutans*, the presence of which latter parasite towards the end apparently caused a recrudescence of the *Anaplasma marginale*. Together they were responsible for the fever, anaemia, and death.

PURE INFECTION OF ANAPLASMA MARGINALE.

Before we imported the cattle from England for experimental purposes, we had obtained our animals in South Africa from the Aliwal North district of the Cape Colony, which was considered to be free from redwater. Indeed, all our animals which were obtained from this place always proved promptly susceptible to our strain of redwater, so susceptible that an acute disease was noticed in almost every instance. It stood to reason to expect that the blood of such animals when injected into imported English heifers would not produce redwater, and it remained to be seen whether such blood would give rise to the appearance of *Anaplasma marginale*, which we noticed occasionally in Aliwal North cattle in connection with our former experiments. If such were the case then it would be a proof that anaplasma does exist unassociated with *Piroplasma bigeminum*.

For this purpose the blood of Aliwal North heifer 867 was used. This animal was imported on the 26th May, 1909.

After this heifer had been tapped for the inoculation of an English heifer, in order to prove its immunity against redwater, heifer 867 was inoculated with 5 c.c. blood of heifer 786, an animal which apparently had a pure infection of *Piroplasma bigeminum*. The blood had been taken at the time when the parasites were present. A reaction set in from the 5th to the 9th day, reaching 105° F. on the 8th day, and during this reaction *Piroplasma bigeminum* was noticed. It was succeeded by the appearance on the 12th day of cells with basophile granulations, the lesions of anisocytosis and poikilocytosis became pronounced and macrocytes were frequent.

The animal recovered.

These experiments would show that heifer 786 was susceptible to our strain of redwater, and apparently had but little immunity against this disease, which fact may be interpreted that *Piroplasma bigeminum* was absent from its blood.

As stated above, the blood of heifer 867, which previous to its inoculation with blood of heifer 786 had been collected, was injected into English heifer 790 in the dose of 5 c.c. subcutaneously.

History of heifer 790.

There was a slight temperature disturbance on the 11th and 12th days, the evening exacerbation reaching 103° F., and the morning remission being 102° F. This occurrence did not give the impression that there was any serious deviation from the course of a normal temperature record. The examination of the blood was made daily. No *Piroplasma bigeminum* was noticed, nor any other parasite, not even the lesions of anisocytosis. On the 27th day the temperature began to rise and an acute fever developed which only had a short duration. The evening exacerbations on the 28th day reached 105° F., and the morning remissions 104° F. On the 29th day the evening exacerbation was 105·6° F., the remission on the following morning was 105° F. This high fever continued until the 33rd day, on which date the collapse was noticed, and the animal died on the following day.

The rise of temperature was preceded by the appearance of *Anaplasma marginale*, infecting on the first day 12·8 per cent. of the red blood corpuscles, and of a slight anisocytosis on the 26th day. The next day the anaplasma increased in numbers, reaching 19·6 per cent., and continued increasing, reaching 35 per cent. on the 30th day. Some corpuscles contained two and three parasites. The lesions of poikilocytosis became very apparent on the 32nd day, on which date also corpuscles with basophile granulations appeared. These lesions of a grave anaemia were pronounced, macrocytes were frequent, and normo-blasts were also noted on the 33rd day.

The first symptoms of illness were noticed on the 32nd day, when the animal refused to feed. It was treated with quinine, but without any effect.

Post-mortem examination.

The condition was very good. The visible mucous membranes were white. The subcutaneous tissue and the fact were slightly yellowish.

The pericardium was empty.

The lungs were in inspirium; the pleura was whitish; there was a slight oedema of the interstitial tissue. The mucosa of the trachea was pale; some mucus was present. The bronchial lymphatic glands were slightly enlarged and slightly congested. The mediastinal glands were pale and a slight anthracosis was present.

In the epicardium along the sulcus coronarius and near the apex were suffusions and petechiae.

The left endocardium was pale and showed a few ecchymoses on the musculus papillaris.

On the right endocardium were a few ecchymoses.

The weight of the liver was 8.3 kilo.; this organ was swollen. On section it was brown yellowish and showed a stasis of the bile.

The bile was thick and of a brown yellow colour.

The periportal lymphatic glands were slightly swollen.

The spleen weighed 1.5 kilo., and was enlarged, measuring 55 cm. in length and 15 cm. in breadth. The pulpa was protruding on section and had a granular appearance.

The fourth stomach contained a small quantity of food, the mucosa was slightly swollen and congested. The contents of the omasum were somewhat dry.

The caecum and colon had dark yellow contents, their mucosa was pale and bile stained. The mucosa of the ileum and jejunum was bile stained and covered with mucus.

The kidneys were pale.

The pia was slightly pigmented, the grey white substance was pale.

The mucosa of the pharynx and larynx were pale, some mucus was found in the larynx. The tonsillae were slightly swollen.

The bone marrow of the humerus was gelatinous, pale, and showed a few hyperaemic spots.

The bone marrow of the femur was watery and white.

Diagnosis: Acute anaplasmosis.

NOTE.—In the instance of heifer 790 all indications lead to only one deduction, viz., it represents a pure infection of *Anaplasma marginale*. Since, however, we have met cases in which we have not seen *Piroplasma bigeminum*, notwithstanding its presence was proved in a succeeding test experiment, a similar objection must be raised in connection with this experiment.

Considering that the temperature of the animal did not show any disturbance during the incubation period and that no lesions due to piroplasma infection were noticed during this time, I am inclined to consider it as a pure infection of *Anaplasma marginale*, the animal dying of what I may call the acute form of anaplasmosis. The fact that heifer 867, which supplied the blood, reacted so promptly to *Piroplasma bigeminum* infection supports this view. Unfortunately there were no more English heifers available to continue this pure strain of anaplasma.

The deduction is justified that a pure infection of *Anaplasma marginale* unassociated with any other parasite does exist in South Africa. This should be the *experimentum crucis* to show the independence of *Anaplasma marginale* from any other parasite.

PART IV.

TRANSMISSION OF A. MARGINALE BY TICKS.

Since the *Anaplasma marginale* is so closely associated with the appearance of *Piroplasma bigeminum*, and since nearly all Transvaal animals are infected with both parasites, and further, since the anaplasma is found in animals in all altitudes of the Transvaal, it had to be expected that the blue tick would be the carrier of the infection.

The experiments of *Smith* and *Kilborne* with Texas fever and *Boopophilus annulatus* had the same results, as we have already seen before. This fact was indeed one of the reasons why the parasites were formerly considered to be identical.

An English heifer, 787, was infested with the progeny of blue ticks collected off animals on this station which had previously been running in the veld, and which accordingly had to be considered to be immune to *Piroplasma bigeminum* and *Anaplasma marginale*. The ticks were placed on the animal on the 15th May, 1909. Care was taken to prevent any gross infestation, and only a limited number were placed in order to prevent a severe attack of redwater.

About 100 larvae in all were used.

They came up in due time. There was on the 11th and 12th days a morning exacerbation of the temperature of 104° F. and 105° F. *Piroplasma* was not seen until later, viz. on the 26th day, and again on the 48th day. *Anaplasma marginale* appeared only after the 75th day, starting with the infection of 4.5 per cent. of the corpuscles. It increased in number, and on the 86th day amounted to 15.4 per cent. A regular reaction ensued from the 85th day onwards, lasting to about the 100th day, during which time the lesions of anaemia were indicated by the presence of poikilocytosis, polychromasia, basophilia, and nucleated cells. The animal recovered.

NOTE.—In this animal we have but a slight infection of *Piroplasma bigeminum* and a distinct infection of *Anaplasma marginale* after a very long incubation time. In this respect it would correspond with what is known in *Piroplasma bigeminum*. In redwater the incubation time after tick infections lasts about 17 to 25 days; due to blood inoculation only 5 to 7 days.

If such should be the case it would help to throw still more light on the experiments of the Americans who noted marginal points late in the season.

They started their experiments in July and August. When we take into consideration that the tick infection in our case required seventy-seven days before *Anaplasma marginale* appeared, we can understand that the appearance of these peripheral points of the Americans would thus fall in the autumn. Similarly to what has been noted by the Americans occurred in this experiment, some of their animals have had only a slight infection of *Piroplasma bigeminum*, which passed unnoticed just as ours would have passed unnoticed if we had not examined the blood daily, and the subsequent anaplasma infection was only noted by them. Our animal was only noticed to be ill after *Anaplasma marginale* had started to develop.

PART V.

THE ANAPLASMOSIS OF CATTLE.

(A summarized description of the disease resulting from the foregoing and observations from practice.)

Anaplasmosis is a disease of cattle caused by a protozoon, *Anaplasma marginale*, which invades and destroys the red blood corpuscles causing primarily an acute oligocythaemia accompanied by high fever, and secondly a degeneration of all parenchymatous organs. Recovery from the disease gives resistance to subsequent infections. The immune animal acts as a reservoir for the virus and the blue tick (*Boophilus decoloratus*) acts as host or transmitter of the parasite.

Cause.—The cause of the anaplasmosis is a protozoon of a new genus, *Anaplasma*, species *marginale*, which so far has no simile in protozoology, inasmuch as it consists of only a chromatic substance, a nucleus, a plasmatic substance as far as can be ascertained yet being absent. The parasite has hitherto been described as “peripheral coccus-like bodies” or “marginal points”, and was always considered to be of protozoic nature, mainly due to the fact that it takes the typical chromatine when stained by any of the *Romanowsky* modifications. Its shape is either that of a round or oval body, certainly resembling a coccus. Occasionally round the body a paler zone is noted, the globule apparently not staining here as well as the rest. The production of this parasite seems to take place by fission. In many instances when the number of parasites increases rapidly, double forms are frequently met with, either separated and placed closely together or splitting forms in which the two bodies have not yet completely separated. As a rule these parasites are situated on the periphery of corpuscles, sometimes reaching over the margin; they are also seen more in the centre of the corpuscles, but this is more seldom the case.

Anaplasma marginale multiplies rapidly in a susceptible animal, and invades a large percentage of corpuscles, amounting to about 50 per cent. in severe cases, and then three and four are frequently met with in one corpuscle.

The blood lesions which are thus caused and hence also the severity of the disease stand in a certain relation to the number of parasites.

Geographical distribution.—Anaplasmosis has been seen in various parts of the world. It was first seen and described in North America, although it was not recognized as an independent disease. It is present in South America, as shown by *Knuth*. It is found all over Africa. I have mentioned its existence in the Transvaal ever since I undertook the examination of blood smears on a large scale, and its frequency is shown in our annual returns of examinations. I saw it in blood smears sent to me from Rhodesia. Recently *Spreull* found it in the Eastern Province of the Cape Colony. *Pitchford* informed me that he saw it frequently in Natal. I have seen it both in British East Africa and in Uganda. *Balfour* in his report mentions its existence in the Soudan. It is present in the Trans-Caucasus, as *Dshunskowsky* and *Luhs* have shown. I do not know whether it has yet been found in India, where redwater exists, and which it usually accompanies, like, for instance, *Piroplasma mutans*, which has been seen in various parts of Asia.

Susceptibility of species of animals, breed, age, etc.—So far, the anaplasmosis has only been noticed in cattle. Experiments to transmit the disease by inoculation of blood of an animal suffering from an acute attack have failed to produce any reaction in a freshly imported Argentine horse, which are highly susceptible to equine piroplasmosis, and in a merino sheep of South African origin.

The susceptibility of cattle varies greatly. We can distinguish an absolute non-susceptibility on the one side and a high susceptibility on the other. The former is represented by the old native cattle, born and bred in infected areas, and the latter by the freshly imported high bred pedigree stock. So far, the observations point to the fact that all cattle born in South Africa are susceptible to the anaplasmosis, but the calves recover easily from the disease, and it passes almost unnoticed whilst they are very young.

This statement is based on a more recent experiment; calves of Afri-cander cows were after birth immediately placed in a tick-free stable, and reared on milk. They were injected with blood containing *Anaplasma marginale*; they contracted the disease in due course, and, although the microscopical examination revealed a very grave disease, which was clinically noted by the paleness of all mucous membranes, the animals easily recovered. It must be stated here that the inoculation of blood also caused the appearance of *Piroplasma bigeminum*, which appeared first and made no impression on the animals. This observation may account for some of the diseases known as "kalverziekte", of which it is stated that the liver on *post-mortem* is found to be very yellow, because we have to accept that in practice where the sick animals are at the same time exposed to obnoxious influences of environment, not all animals recover. Further, it may also account for the stunted growth of some of the calves which have been more or less severely infected during an earlier period of their life. In this respect, the attacks of the two diseases piroplasmosis (redwater) and anaplasmosis of the red corpuscles must interfere with the normal growth of the young animal. Although, as a rule, a calf born in South Africa passes through the anaplasmosis early in its life, yet there are exceptions, judging by the observations both in the field and experimentally obtained. The former are usually made after moving cattle from higher altitudes to lower, or from one part of the country to another, and we can imagine without any difficulty that regions exist in which the infection is present but to a slight extent; accordingly not necessarily all cattle become infected whilst calves. But this is especially the case with cattle which are born and bred in stables and in paddocks which are, more or less, kept tick-free.

Anaplasmosis is observed in South African cattle of almost any age, and the explanation just given does not explain all these cases. What is known about the virulency of redwater applies similarly to this disease. There exists a difference in the virulency of certain strains of anaplasma, and although, generally speaking, the recovery from a first attack of the disease confers a high degree of resistance, it does not give complete immunity. Thus in former inoculation experiments I have seen that the inoculation of blood from an animal gave rise to the appearance of the disease, and when the animal which supplied the blood was injected in turn with blood from a different animal it also developed the infection. This fact gives another explanation of the observations already alluded to and will account for such cases where the affected animals only show a mild infection, the parasites being met with in rare numbers.

The imported English pedigree cattle show the greatest susceptibility, as experiments will show, the morbidity averaging about 100 per cent. and the mortality amounting to over 50 per cent. of the infected cattle. It is not yet certain to what extent this high mortality is influenced by youth; the animals under my observations averaged about eighteen months old.

THE VARIOUS FORMS OF ANAPLASMOSIS AND THE LESIONS.

The disease, which is produced by injection of blood or tick infection, has a typical incubation time, the length of which seems to be influenced by various factors. It seems to be shorter in cases caused by blood inoculation than by tick infection (so far only one exact observation regarding ticks is to hand). In the cases caused by blood injection, it seems to make a difference whether an animal has been injected with immune blood or blood which does not show any parasites under the microscope, or by virulent blood, namely, blood which shows the parasites present. It varied between twenty-seven and thirty-two days in the former instance, and lasted for about sixteen days in the latter one.

(a) *Anaplasmosis ending with death.*

After an incubation time of twenty-seven and thirty-two days respectively the fever reaction started. In some instances the anaplasma was noted before the onset of the fever. In all cases the parasites increased rapidly in numbers and the number of red corpuscles decreased, the remaining corpuscles becoming quite pale and colourless. In the shortest cases death occurred about the seventh day after the onset of the fever. The lesions of polychromasia and basophilia were not present in the cases of shortest duration.

In cases of death occurring at a later period (eight to twelve days) after the appearance of anaplasma, polychromasia and basophilia were constantly noticed for some days (four, six, and seven days before death). In these instances normo-blasts also made their appearance.

(b) *Anaplasmosis ending with recovery.*

In recovery, the usual period of the fever reaction lasted about fourteen days to three weeks. The temperature reaction was very high. The parasites increased in numbers. They either disappeared before or at the same time as the temperature reaction returned to normal; sometimes they remained even longer. About eight days after the appearance of anaplasma the blood lesions of polychromasia and basophilia put in appearance; they were succeeded a few days later by normo-blasts. The blood did not show normal conditions for a considerable length of time.

(c) *Acute anaplasmosis ending in recovery.*

There were some instances where the course of the disease ran very quickly, ending with recovery, both the typical and high fever reaction only lasting about seven days. The parasites did not seem to increase to such great numbers, and decreased again with the drop of the temperature. This was eventually succeeded by the appearance of polychromasia and basophilia; rare normo-blasts were seen in one instance.

(d) *Mixed infections.*

Mixed infections were noticed with *Piroplasma bigeminum* which appeared with *Piroplasma mutans*. *Piroplasma bigeminum* appeared either before the anaplasma infection or during the reaction. *Piroplasma mutans* usually appeared during or after the anaplasma reaction. The picture of anaemia, as described before, was present in all instances and well pronounced.

Clinical symptoms.

The fever reactions is the first clinical symptom noticed and high fever is present for some days before the animal is actually noticed to be ill. Capricious appetite or refusing to feed are therefore the first symptoms which will draw attention. The severity of these symptoms varies, of course, with the susceptible animals. Calves, as a rule, do not show such high fever and continue to feed, although sometimes but sparingly. In full-grown cattle generally these symptoms are more pronounced, more particularly in imported stock. A careful observer will soon notice that the sick beasts lose in condition, and the wasting is sometimes so rapid that a marked difference becomes visible within one day.

At this period the symptoms of anaemia become very pronounced.

The muzzle appears pale, the sklera of the eyes is quite white, the conjunctiva is bloodless, also the mucous membranes of the mouth lose their pink colour. All severe cases in calves and adults begin like that, in animals which die late or recover, the mentioned parts take a slight yellow colour which may become very pronounced. A yellowness of the skin can be noticed, where it is transparent, such as in the ears or where the hair is not thick, along the flanks in the region of the udder and between the hind legs. An occasional abrasion of the skin has quite a yellow surface.

The intestinal tract also becomes involved. There is first a slight constipation. Later the faeces lose their normal colour and take a brown yellow stain. Diarrhoea with evacuations of yellow faeces intermixed with mucus is occasionally observed. The urine is distinctly yellow, but never blood stained. As the disease advances the animal frequently lies down and finally permanently. Both the heart action and respiration become hurried and a peculiar groaning noise accompanies every breath. This state of affairs, which indicates the approaching end, is followed by muscular tremblings of the flanks and shoulders.

Nervous symptoms are sometimes noticed shortly before death, such as spasms of the extremities and the head, and sometimes the animal becomes very aggressive.

In cattle which recover the symptoms appear in the same order and may be as pronounced as in an animal which is going to die. Generally, however, the sick animal soon resumes feeding again. The wasting continues for some time. The paleness and the icteric condition only wear off gradually. Convalescence is a slow process.

Diagnosis.

The clinical picture thus described is by no means typical for anaplasmosis. It applies also to cases of redwater, when the acute attack is over, the haemoglobinuria is either absent or has passed unnoticed and then the symptoms of anaemia and jaundice also become pronounced. Here also the approach of death is characterized by similar symptoms.

Microscopically the picture of anaemia is identical. The lesions of anisocytosis and poikilocytosis, polychromasia and basophilia and the presence of normo- and megaloblasts is also found in the sequel of redwater. Whereas, however, in redwater as a rule *Piroplasma bigeminum* has altogether disappeared when the above symptoms are noted, the *Anaplasma marginale* is present for a longer period and at a time when all the anaemia lesions are well pronounced. But also in anaplasmosis a stage exists (after the temperature has returned to normal) when all blood parasites may be absent.

The presence of *Anaplasma marginale* may give rise to the recrudescence of *Piroplasma bigeminum* and then we find both parasites together. The

reverse may also take place, and although the *Piroplasma bigeminum* infection is generally the primary one, it will, as a rule, be difficult or even impossible to say so with certainty, which was the first infection. *Piroplasma mutans* may also be found, especially in inoculated animals which have been injected with blood taken at random from an animal born and bred in the country.

Pathological anatomy.

Animals which died of an acute attack were found in good condition, although a noticeable wasting was observed during the few days of illness. On *post-mortem* examination the presence of fat deposits was apparent, which was generally of a distinctive yellow colour. The subcutaneous tissue was also of a yellowish discolouration and occasionally on some parts a gelatinous infiltration was noticed; for instance, neck, sternum, abdomen.

The flesh had a pale bloodless colour and looked as if it originated from a carcass bled to death.

The blood was thin and watery, poor in corpuscles, and did not stain.

In some instances the pleural cavities contained an abnormal amount of liquid, in one instance about one litre.

The pericardium was either found empty or else contained some clear straw-coloured liquid, and in only one instance was the maximum quantity of 30 c.c. found to be present.

The lungs were found in half or full inspirium; there was emphysema of the anterior lobes in one instance. The colour of this organ was pale yellow; in every instance the symptoms of an oedema were indicated, either by the presence of some foam in bronchi and trachea or in an interstitial infiltration. In one instance the parenchyma was dry.

The bronchial and mediastinal glands were usually slightly swollen, soft to the touch and bloodless; in one instance there was also found a haemorrhagic infiltration of the lymph sinus.

On the epicardium were found petechiae, ecchymoses, and haemorrhagic suffusions. The ventricles were found with and without coagulated blood. The left endocardium was whitish and showed some ecchymoses. The right endocardium was found normal in most cases; in others it showed some ecchymoses.

The myocardium was pale and of soft consistence.

The liver was in every instance enlarged; its minimum weight was found to be 6.5 kilo. and the maximum 8.6 kilo.; it had a swollen appearance with rounded margins and was of a yellow colour; the sections showed either a brown yellow or a saffron yellow tinge; the cut surface was granular and had a peculiar glossy shine; the organ also contained but little blood.

The gall-bladder contained inspissated viscid dark green bile.

The periportal glands were pale.

The spleen was enlarged in every instance; its minimum weight was found to be 1.4 kilo., and the maximum 3.3 kilo., it averaged 2.2 kilo.; its length varied from 55 to 68 cm., and the width averaged 20 cm.

The capsula was distended and sometimes showed ecchymoses.

The pulp was soft, protuding on section; the colour was deep red to black; in one instance it was of the consistence of jam, in another like hard elastic. The trabeculae had disappeared, and the Malpighi's bodies were swollen.

The third stomach usually contained dry food.

In only two instances was food found in the fourth stomach, usually liquid was present. The mucosa was slightly swollen; there were flakes of mucus present in two instances, a patchy hyperaemia in another, otherwise the mucosa was found to be pale.

The mucosa of the duodenum was usually bile stained. The mucosa of the jejunum and ileum was pale, bile stained, slightly swollen, and there were also red streaks found.

The caecum was either empty or showed bile stained contents; the mucosa was found to be pale and folded; in the apex was a patchy hyperaemia.

The colon had yellow stained food; the mucosa was slightly swollen, pale or yellow.

The mesenteric glands were swollen, pale and soft.

The kidneys were easily taken out of their capsule; the parenchyma was bloodless, pale, and yellow.

The urinary bladder was found to be empty in one instance; it usually contained clear straw-coloured urine.

The brain was pale, and so bloodless that the grey substance appeared almost white.

The bone marrow of the diaphysis was soft, of a gelatinous consistence and yellowish in colour.

Transmission of the disease.

This takes place by means of the blue tick. I have so far only one experiment to that effect, but it is convincing and has no other interpretation. In this instance the incubation time was very long, and it remains to be seen whether this is the rule or the exception. In this respect it resembles *Piroplasma bigeminum*, where the incubation time of the tick-transmitted disease lasts longer than in the inoculated one.

It is probable that *Anaplasma marginale* and *Piroplasma bigeminum* can be transmitted not only by the same species of ticks but by one and the same individual. This will, however, have to be proved first of all, then, although one lot of ticks taken off infected cattle transmitted both diseases, yet it is possible that amongst these some were infected with anaplasma only and others with piroplasma only. It will further be interesting to know whether other species of South African ticks will transmit the anaplasma, as is occasionally the case with *Piroplasma bigeminum*. Since the blue tick (*Boophilus decoloratus*) is a one host tick, it follows that the infection passes through the egg as is known to occur with *Piroplasma bigeminum*.

The virus reservoir.

All the experiments—both inoculation and tick transmission—have proved that the immune animals act as the reservoirs of the virus. From these the ticks obtain the virus and reinfect the cattle, and in this respect both *Anaplasma marginale* and *Piroplasma bigeminum* behave in exactly the same way.

This fact explains the constant infection of a veld both for anaplasmosis and piroplasmosis.

The immunity.

Animals which have recovered show a great resistance to a reinfection. There is, however, no complete immunity present. An animal may be successfully inoculated more than once, although the second inoculation will only cause slight reaction. This second reaction might be due to a difference in the strain of the anaplasma, the primary reaction gives sufficient ground immunity to protect an animal against severe lesions and death from a subsequent infection. Further the disease may reappear under the influence of any other disease; although as a rule it does not take a very acute course, and in which case the blood examinations do not show large numbers of parasites. In this respect it resembles *Piroplasma bigeminum*, *Piroplasma mutans*, and *Spirillum theileri* infections.

Complications with other diseases and mixed infections.

It is evident from my observations that immunity against redwater in no way influences the susceptibility against anaplasmosis. Under the condition of an inoculation or tick infection, the agency of redwater, *Piroplasma bigeminum*, appears first and the animal goes through the distinct redwater disease. After it has recovered, the incubation time of the anaplasmosis has elapsed, and then anaplasmosis makes its appearance from which the animal may even die. Generally speaking, this second disease was considered to be only a relapse of redwater. Indeed, since the immune animal retains the *Piroplasma bigeminum* in its blood a recrudescence of this parasite may occur, and then the microscope shows the double infection of *Anaplasma marginale* and *Piroplasma bigeminum*. In some cases this recrudescence of *Piroplasma bigeminum* may lead to the reappearance of red urine, a sure sign of the disease of redwater. And what happens with *Piroplasma bigeminum* may also happen with *Piroplasma mutans*, so that an animal at a given period of its illness may show these parasites in its blood as well. This accounts for the reason why it was difficult in the past to identify the clinical and pathological lesions found in a sick animal, with any of the parasites present.

Preventive treatment.

The great majority of animals born and bred on South African veld become immune against anaplasmosis (and also piroplasmosis) as they grow up. This is due to the fact that the disease does not attack them so severely as it does the older and fully-grown animals. The experimental inoculation of susceptible calves has a similar result, and it seems the younger the animal the better the disease is borne. This, at least, is the cause with South African calves born of the Africander or cross-bred stock. It remains yet to be seen whether the same is the case with the imported thoroughbred pedigree stock of Europe. Probably it will also be so, and the practical deduction would be to import pedigree stock as young as possible, at least under one year old, if one wishes to save the greatest possible number both from redwater and gall-sickness.

Against redwater, we can inoculate cattle almost safely in England, and the animals retain their immunity when arriving here, but piroplasmosis (redwater) does not protect against anaplasmosis, and for this reason the inoculated cattle will contract and may die from the second disease when exposed to the veld. Pedigree stock for South Africa—and we can state for Africa generally—must have immunity against the two diseases before their importation is accompanied by the best results.

There is reason to hope that this object will also be achieved.

PART VI.

GALL-SICKNESS.

In a paper of mine, "A New Trypanosome and the Disease caused by it", published in September, 1903, in the *Journal of Comparative Pathology and Therapeutics*, I have referred to the existence of the term "Gall-sickness", with which the South African farmer identifies a disease or diseases, characterized by a derangement of the liver, jaundice, and thick bile. It is naturally to be expected that a number of causes may be responsible for similar pathological lesions, and for this reason the term gall-sickness does not necessarily mean an etiological entity. Indeed, we have only to refer to our experience in the past and the history of some of the plagues imported to demonstrate this. Redwater, rinderpest, East Coast fever, have all in turn been called gall-sickness, until their true nature was recognized. If we ask a farmer what is the common cattle disease of South Africa, he will undoubtedly reply "gall-sickness". We do not go wrong when we include under gall-sickness all intestinal troubles, such as acute indigestion, gastritis, and enteritis, all of which may be accompanied by symptoms of icterus. Nevertheless, the icteric conditions seem to have been, and are still, considered to be typical of gall-sickness.

I have no doubt that the disease, which I have described under the name of anaplasmosis also belongs to this group of gall-sickness. I am inclined to believe seeing that the icteric lesions are so markedly pronounced and govern the whole pathological picture that anaplasmosis is the disease which was originally called gall-sickness by the farmers. The very fact that the term existed before the appearance of redwater—for which it could easily be mistaken—indicates that anaplasmosis was in the country before the latter appeared and it is easily understood how the two diseases became confused. For a number of years I have tried to differentiate the various diseases grouped together under the term gall-sickness, and as a result of continuous microscopical examinations of blood smears, have found several blood parasites in South African cattle, viz. *Trypanosoma theileri*, *Spirochaete theileri*, *Piroplasma mutans*, all of which were not known previously, and *Piroplasma bigeminum*, whose existence had already been recorded.

Of these parasites, *Piroplasma bigeminum* alone could be identified with a recognized disease, namely redwater, the characteristic symptom of which is the red urine. Frequently when this symptom is absent the clinical diagnosis of redwater is not made, yet the microscopical examination reveals the presence of *Piroplasma bigeminum*. Sub-acute attacks of this disease may pass over without any change in the urine, clinically they show the symptoms of anaemia and icterus, and are accordingly diagnosed as gall-sickness. A differential diagnosis between the anaplasmosis and piroplasmosis is therefore, as a rule, only reliable by microscopical examination.

As already stated, in some fevers I have come across other blood parasites, and in order to study their nature and pathological effect, I have had to take recourse to inoculation experiments. Since these parasites could not yet—or at least at the time of the experiment—be cultivated artificially outside the animal's body, the blood of sick and immune animals had to be used. And it so happened, that by the inoculation of such blood I introduced in many instances the disease which resembled "gall-sickness".

Consideration was naturally given to that form of redwater, which could only be recognized as such microscopically. But not all the observations could be brought into line with this fact, and there yet remained a disease which corresponded to the description of anaplasmosis. Looking through the records of former investigations in the various blood diseases, I find that in nearly every instance the presence of the then so-called "marginal points" intervened, and I have no doubt that most of my experiments were masked by the occurrence of these parasites.

The pathology of *Trypanosoma theileri*, *Spirochaete theileri*, *Piroplasma mutans* must therefore be worked over again, and with material in which these infections are present in an absolutely pure state. Nevertheless, it cannot be denied from observations in practice that these parasites are occasionally the cause of fevers and diseases. We must remember that *Piroplasma bigeminum*, *Anaplasma marginale*, *Piroplasma mutans*, and *Spirochaete theileri* are present in the immune animals, although microscopically not traced. If any new infection occurs, caused for instance by heartwater, we see one or the other parasite reappearing and causing blood lesions which mask the clinical picture of the primary infection. This happens occasionally with *Trypanosoma theileri*, the infection of which causes the recrudescence of parasites, and the picture of gall-sickness results.

Gall-sickness can therefore also mean mixed infections with severe blood lesions and succeeding jaundice. It is nevertheless likely that the term was originally applied to a definite disease, and from what we can deduct from observations both in the laboratory and in the practice, the one under discussion, the anaplasmosis, is the true gall-sickness.

The jaundice or biliary fever described by *Hutcheon* in his Annual Report for 1897, which was first noticed by *Spreull* in Barkly East and found to occur after the inoculation of virulent rinderpest blood is, in my opinion, the same disease, which was also noticed under similar conditions on Robben Island. At that time either the blood was not examined, or else the marginal points were not recognized as parasites, as the protozoic staining was not so well developed then as it is at present.

Recently *Leipziger* published in the *Deutsche tierärztliche Wochenschrift*, a paper on "Gallenkrankheiten der Rinder" in German South-West Africa. His description of the clinical and pathological lesions correspond with our anaplasmosis and so does the record concerning the anaemia, but we miss any reference to the presence of marginal points. These were either mistaken for basophile granulations, which were frequently in his preparations, or else they were not present, viz., the disease was either the sequel of redwater or a sequel of anaplasmosis, both of which show similar blood lesions.

The name "gall-sickness" will be used as a popular term indicating the disease described herebefore as anaplasmosis.

DESCRIPTION OF PLATES.

TEMPERATURE CURVES.

Plate 1. *Heifer* No. 790.—Pure infection of *Anaplasma marginale*.
Acute case, ending in death.

Plate 2. *Heifer* 782.—Acute anaplasmosis, ending in death.

Plate 3. *Heifer* 775.—Sub-acute anaplasmosis, ending in death.

Plate 4. *Heifer* 786.—Two separate infections :—

1. Piroplasmosis.

2. Anaplasmosis.

Plate 5. *Heifer* 776.—Sub-acute anaplasmosis with relapse.

Plate 6.—*Anaplasma marginale*.

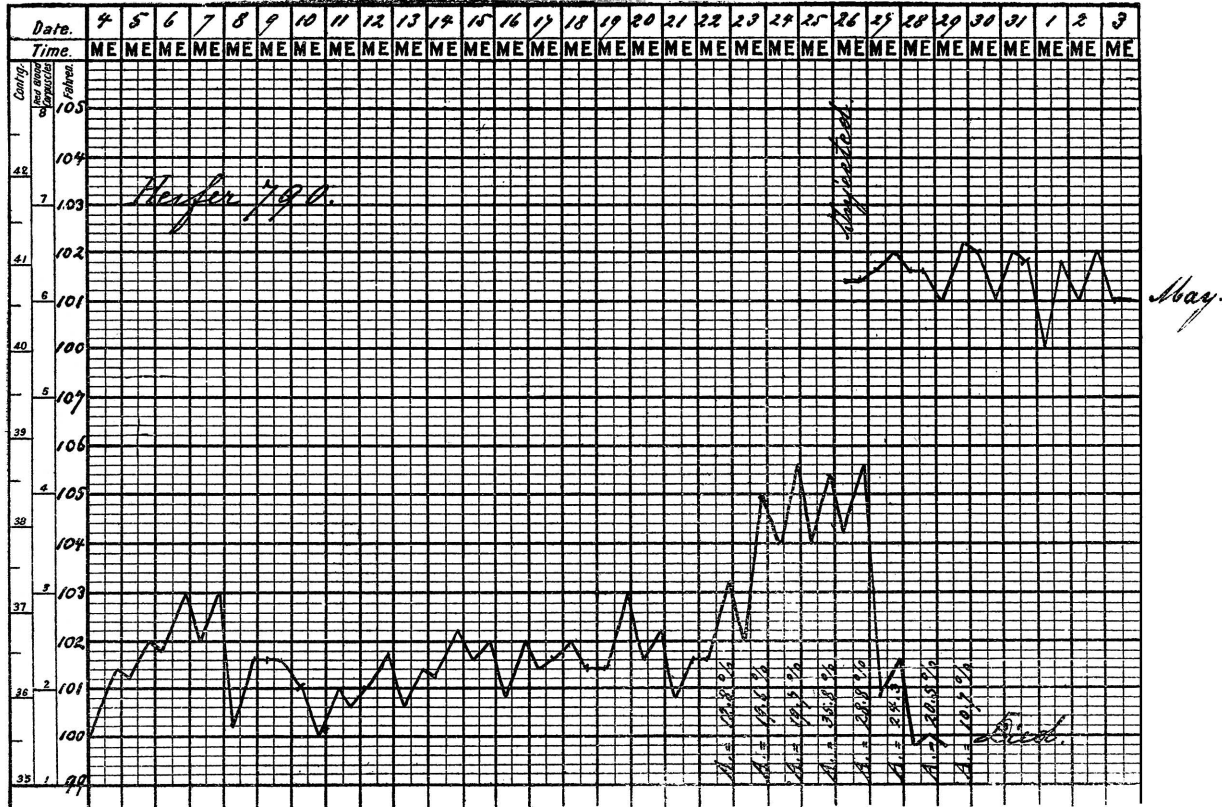


Plate I.

HEIFER No. 790.—Pure infection of *Anaplasma marginale*. Acute case ending in death.

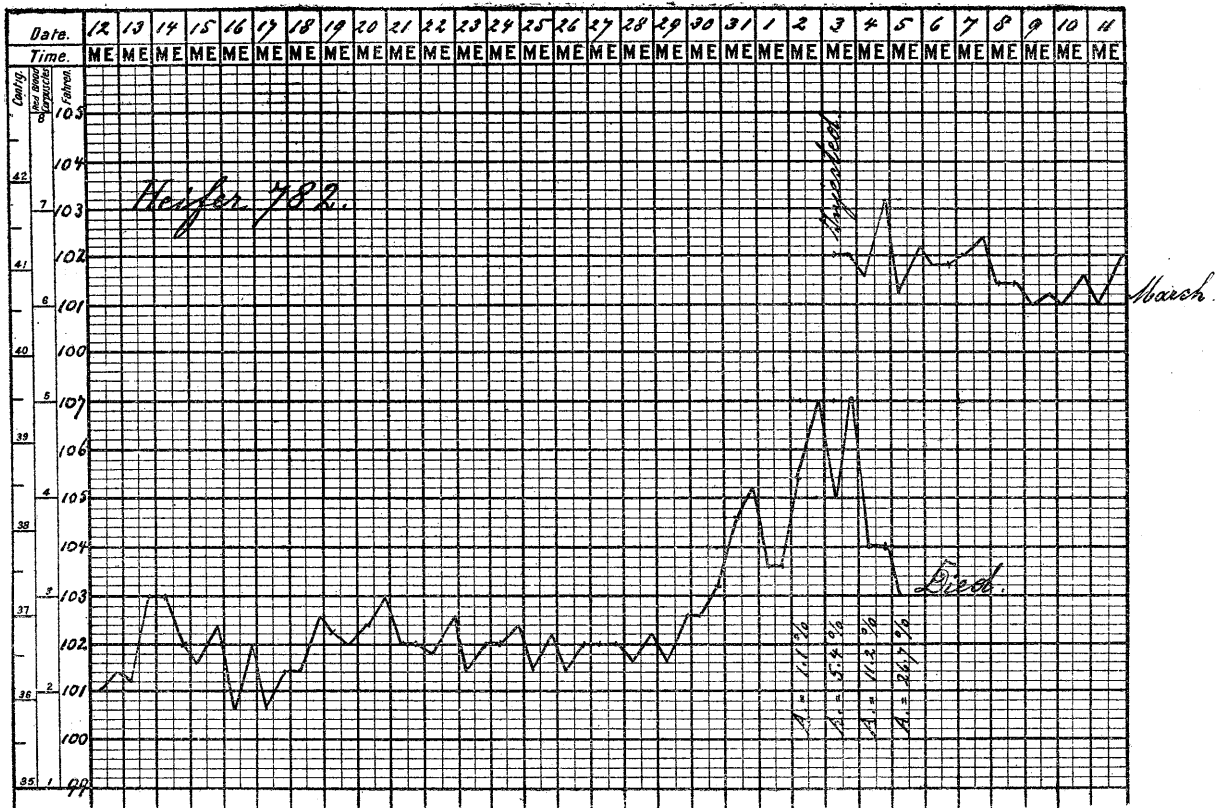


Plate II.

HEIFER No. 782.—Acute anaplasmosis ending in death.

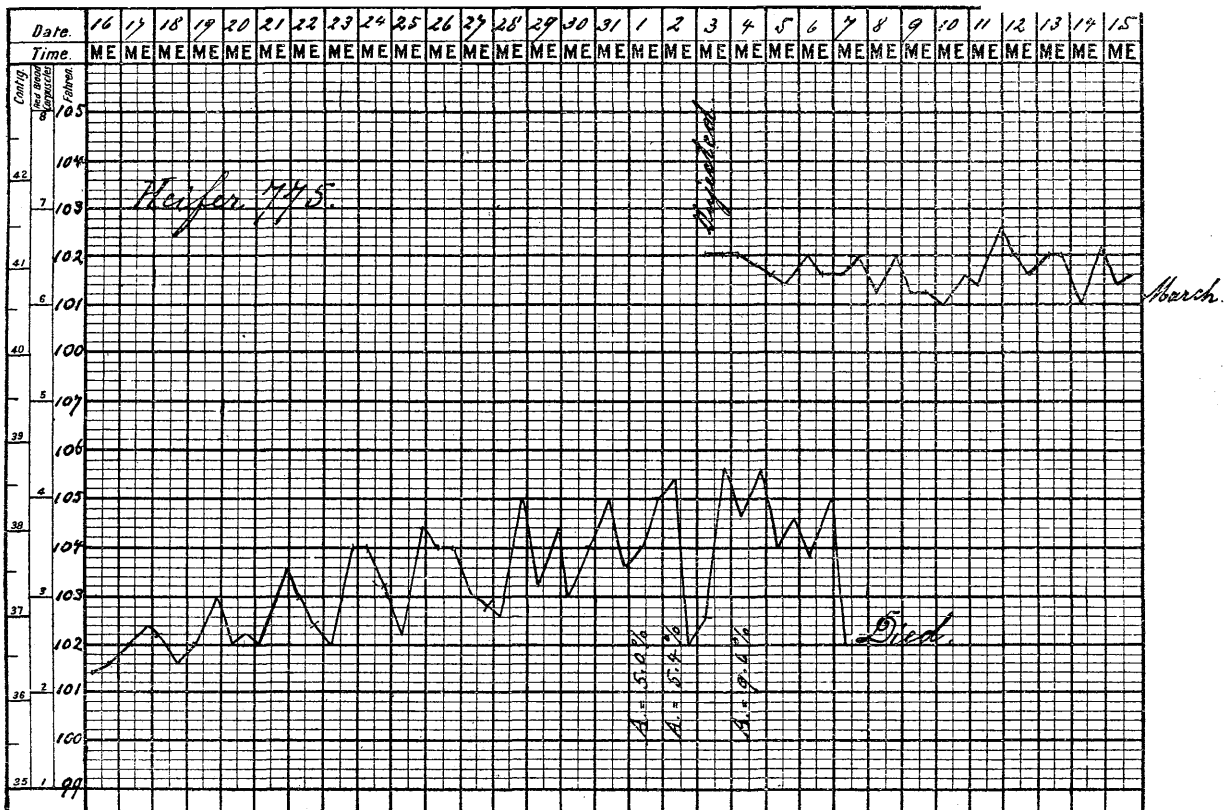


Plate III.

HEIFER No. 775.—Sub-acute anaplasmosis ending in death.

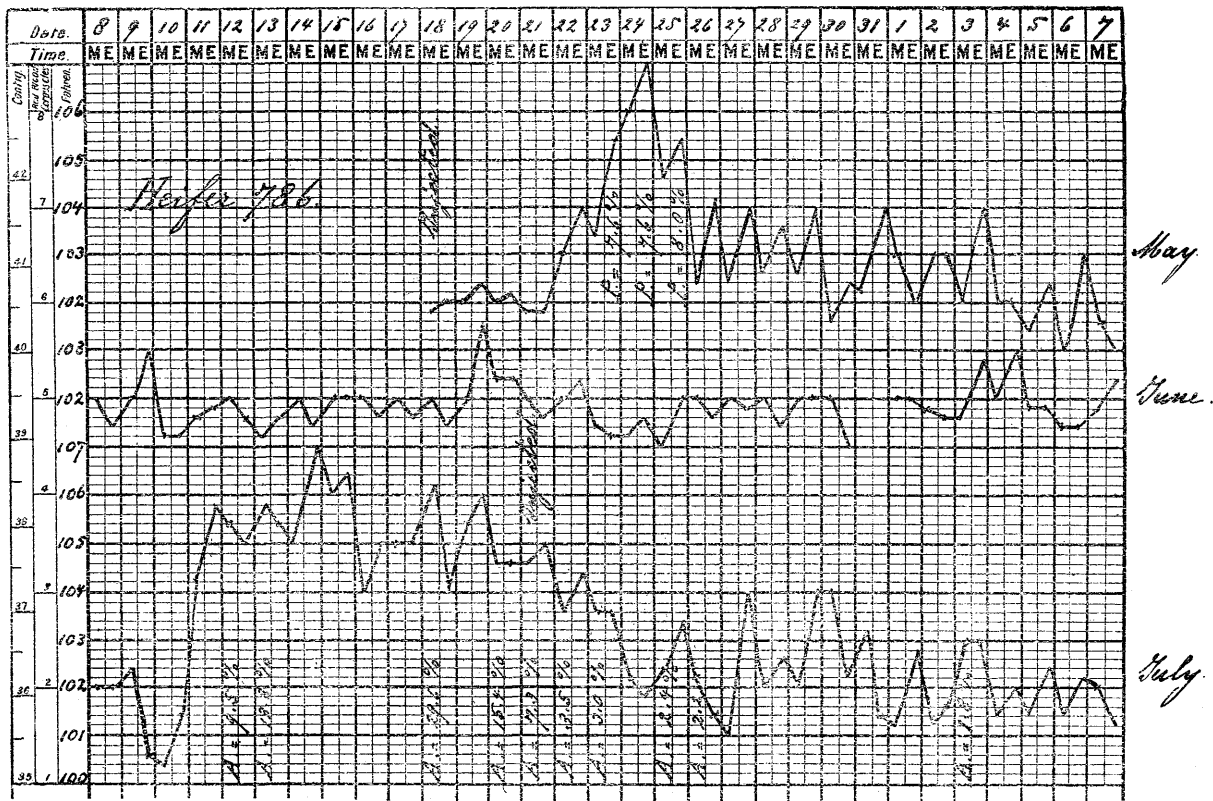


Plate IV.

HEIFER No. 786.—Two separate infections:—(1) Piroplasmosis. (2) Anaplasmosis.

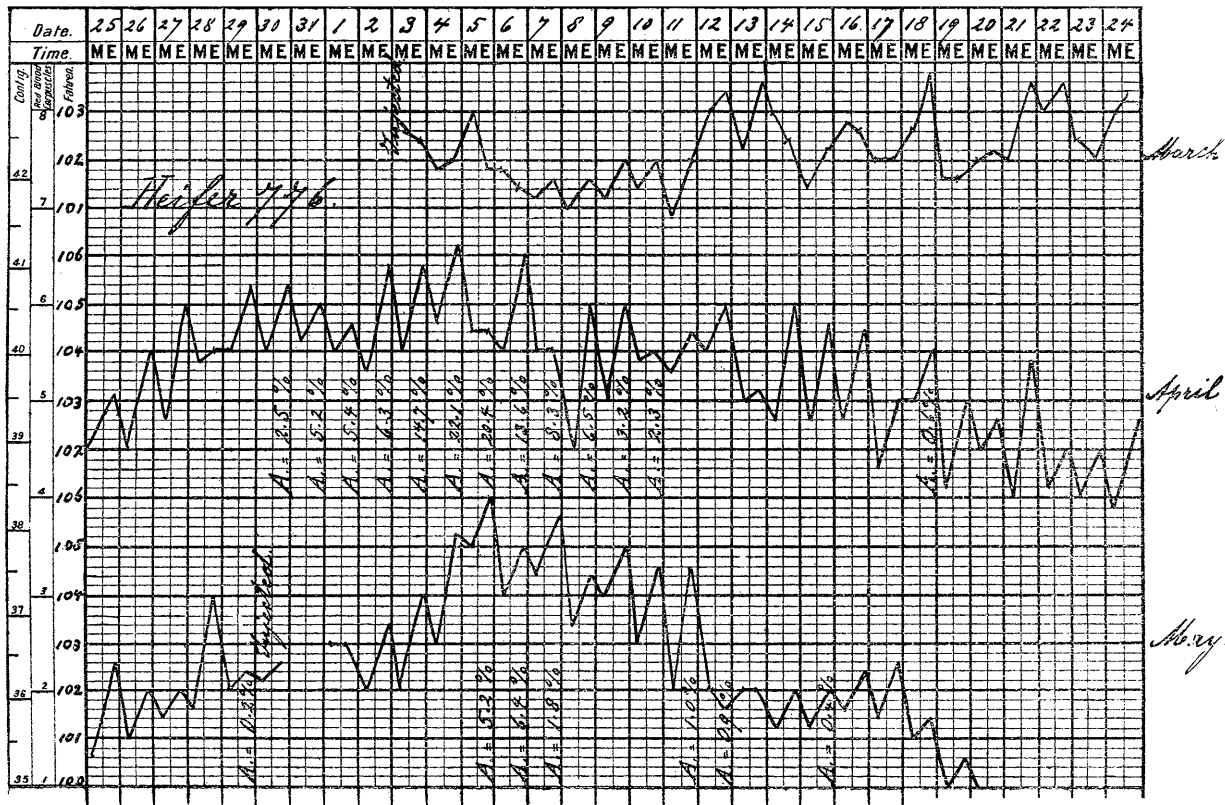


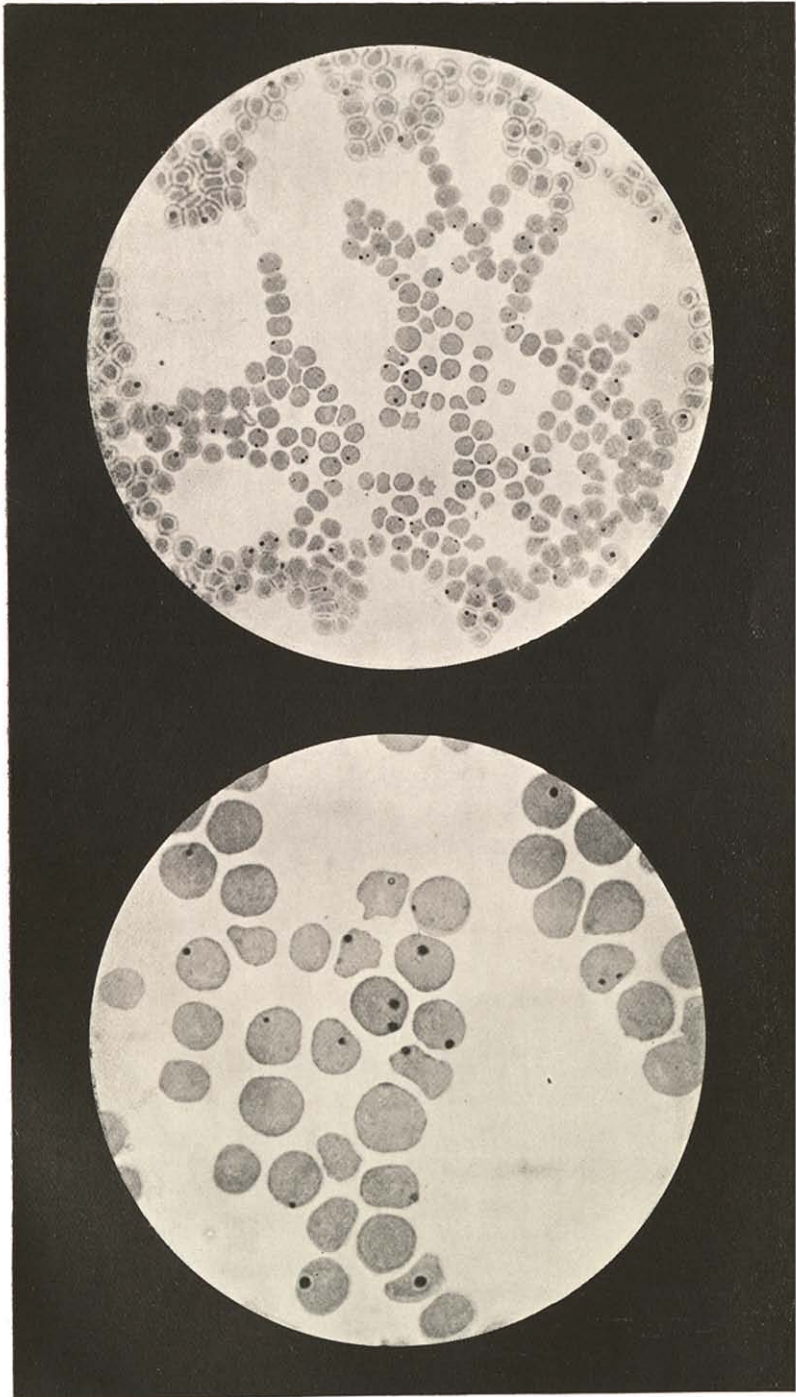
Plate V.

HEIFER No. 776.—Sub-acute anaplasmosis with relapse.

ANAPLASMA MARGINALE (*gen. et spec. nov.*)

Zeiss objective ... 2 mm.
Project. ocular ... 2 "
Extension ... 50 cm.

Short Extension.



Zeiss objective ... 2 mm.
Project. ocular ... 2 "
Extension ... 150 cm.

Long Extension.