Further Investigations into Anaplasmosis of South African Cattle.

By Dr. A. THEILER, C.M.G., Acting Director of Veterinary Research.

IN the Annual Report for 1908–09 * and elsewhere † I have demonstrated that the so-called peripheral coccus-like body of *Kilborne* and *Smith* ‡ or the marginal points of my previous publications § had to be considered as a parasite *sui generis*, and from the fact that its biology and some of its staining properties considerably resembled that of a protozoon I concluded that it belonged to this group of parasites, being a protozoon consisting in the main of a nucleus and devoid of protoplasma. Hence the name "anaplasma", which should indicate this condition.

I really considered this anaplasm to be an organism which, by reason of its parasitism, no longer required a protoplasmatic substance, having adapted itself to an intra-cellular life.

In a series of staining reactions, *Dr. Sieber* || was able to separate the substance of the anaplasm into a central body with a surrounding envelope, suggesting that the parasite is closely related to the group of chlamydozoa, to which no definite place has as yet been allotted in the system. Whatever may be the final finding, there is no doubt in my mind that the anaplasm represents a parasite which biologically is most closely related to the piroplasms (Babesiae).

For the last six years I have shown that cattle born and bred in certain parts of South Africa, which I had used for my experiments, contained these parasites (or as I then called them "marginal points") in their blood in a latent form; although they were not always visible in the blood, they were transmitted by the injection of such blood into susceptible imported animals or calves born in a stable and kept free from ticks. In nearly all these cases, however, *Babesia bigemina* appeared as a result of such injections, and accordingly from the fact that the *Babesia bigemina* (with its shorter incubation time) appeared first and was followed by the appearance of the anaplasm, it is evident that such a regular succession of the two naturally led to the supposition that the Babesia and the anaplasm were somehow connected, the latter or the "coccus-like body" being considered to belong to the life cycle of one and the same blood parasite.

It was due to a lucky coincidence that I was enabled to demonstrate the dual nature of these organisms in my report for 1908–09. Cattle which had been inoculated in England with our South African redwater (*Babesia bigemina*) contracted a grave disease when injected in Pretoria with blood taken from Transvaal animals: this disease presented many differences to redwater, chiefly by reason of its long incubation time, its prolonged duration, and the appearance of the characteristic coccus-like bodies. It had thereby been proved that the two diseases could be separated; that the cattle which had been inoculated in England with *Babesia bigemina* and were immune against redwater did not possess any immunity against anaplasmosis.

* Report of the Government Veterinary Bacteriologist, 1908–09.

† Journal of Comp. Pathology and Therapeutics, 1910; Royal Society of South Africa, Capetown, 1910.

‡ Eighth Annual Report, Bureau of Animal Industry, U.S.A.

§ Report of the G. V. B., Transvaal, 1905–06.

|| Annual Report G. V. B., Transvaal, 1909–10.

In my last report I was able to give one experiment in which a pure infection of anaplasmosis was obtained. I therefore considered it advisable to repeat these experiments in order to obtain a pure infection with *Anaplasma marginale*, and then to prove that such animals are still susceptible to redwater, and if this was correct it would complete the evidence of the dual nature of the two infections in the reverse order.

My previous experiments had shown that when cattle obtained from the Aliwal North district of the Cape Province were injected with redwater they usually contracted this disease, and in all my previous experiments I used such heifers known to be susceptible to redwater. Accordingly, if blood of such animals was injected into freshly imported English cattle, it could reasonably be expected that redwater would not be produced, and it remained to be seen whether such injections would give rise to the appearance of anaplasmosis. Should this deduction prove to be correct, then the proof would have been given that anaplasmosis is not of necessity and under all circumstances associated with redwater.

For this purpose an imported Ayrshire heifer, No. 790*, had been inoculated with blood from an Aliwal North animal and developed a pure infection of anaplasmosis. Unfortunately no more imported heifers were available at that time for the purpose of carrying on a pure infection, and as this imported heifer died from anaplasmosis as a result of the infection, that particular strain was lost. Subsequently a new batch of imported heifers, this time of the Sussex breed and varying in age between twelve to eighteen months, was imported, and, needless to repeat, on their arrival at the Laboratories they were immediately placed in the stables, kept free from ticks, and fed on oat forage and bran food, which one could reasonably expect to be free from ticks.

The new experiments, of which I give the details in this article, were naturally on the same lines as those undertaken with the imported heifer No. 790: the Sussex heifers were to be injected with blood taken from Aliwal North animals, to note the effect of a pure infection of anaplasmosis, and to continue the strain by inoculation as soon as a pure infection was obtained, and to show by a series of inoculation experiments that the anaplasma infection remained unassociated with *Babesia bigemina* or any other blood parasites; also to show that such animals could easily be infected with *Babesia bigemina* at any later period. However, before running any risks with expensive English heifers, preliminary experiments were undertaken with Africander calves, born and kept in tick-free stables.

PART I.

THE SEPARATION OF ANAPLASMOSIS FROM BABESIOSIS.

EXPERIMENT NO. 1.—*To note whether the blood of heifers obtained from Aliwal North would produce a pure infection of anaplasmosis when injected into susceptible calves.*

(a) *Blood of Heifer (Aliwal North)* 901.

NOTE.—Heifer 901, about eighteen months old, born and bred in the Aliwal North district, arrived at the Laboratory on the 7th October, 1909; she was immediately placed in a tick-free stable and kept under such conditions as would prevent all possibilities of tick infection.

* Annual Report G. V. B., Transvaal, 1909–10, page 48.

On the 7th December—sixty-one days after arrival—she was tapped and 20 c.c. defibrinated blood were injected into calf 893.

(*A*) *Calf* 893.—Born on the 9th September in a stable of the Laboratory and kept tick-free.

Treatment.—Injected as above at a time when it was nearly three months old.

Remarks.—A very slight reaction set in on the 8th day after injection, hardly noticeable except for one sharp rise on the 10th day to 104·2° F. On the 8th, 11th, and 12th day *Babesia bigemina* appeared in rare numbers.

On the 21st day a second reaction commenced of an irregular nature and without high evening rises. Anaplasms appeared for the first time on the same day and were noted almost daily until the 55th day, but never in large numbers. With the exceptions of the lesions of anisocytosis and the reappearance of *Babesia bigemina*, no other blood changes were noted.

(b) *Blood of Heifer* (*Aliwal North*) 902.

NOTE.—Heifer 902, about eighteen months old, born and bred in the Aliwal North district, arrived at the Laboratory on the 7th October, 1909; she was immediately placed in a tick-free stable and kept under such conditions as would prevent all possibilities of tick infection.

On the 7th December—sixty-one days after arrival—she was tapped and 20 c.c. defibrinated blood were injected into calf 892.

(*B*) *Calf* 892.—Born on the 20th August, 1909, in a stable at the Laboratory and kept tick-free since birth.

Treatment.—Injected as above at a time when the calf was just over three months old.

Remarks.—A slight reaction commenced on the 6th day, with a temperature reaching 103·4° F. on the evening of the 7th, 8th, and 10th day; *Babesia bigemina* was noted on the 8th, 10th, 11th, and 12th day.

From the 21st day another reaction started, but somewhat of an irregular nature, continuing for over three weeks. The temperature averaged between 103–104° F. in the evening and seldom exceeded 104° F. Anaplasms were detected from the 21st day, increasing daily, and were most prevalent about the 26th–33rd days; they were still present on the 55th day.

At the time when the anaplasms were becoming very frequent blood changes appeared, and on the 30th day microscopical examination of the blood revealed the picture of an acute anaemia, characterized by the presence of anisocytosis, polychromasia, poikilocytosis, and basophilia. Nucleated cells were noted and the red corpuscles stained very badly.

The animal eventually recovered.

(c) *Blood of Heifer* (*Aliwal North*) 906.

NOTE.—Heifer 906, about eighteen months old, arrived in Pretoria on the 28th October, 1909, and was immediately placed in a clean stable and kept free from ticks. The temperature was taken twice daily and a detailed record kept; no deviations from normal were observed.

On the 7th December, 1909—thirty-eight days after arrival—she was tapped and 20 c.c. defibrinated blood were injected into calf 881. [For subsequent history of heifer 906, see (*D*).]

(*C*) *Calf* 881.—Born on the 20th August, 1909, in a stable at the Laboratory and kept tick-free.

Treatment.—Injected as above at a time when it was just over three and a half months old.

Remarks.—The temperature of this calf at the time of injection and twenty-four hours previously was 103·2° F. On the morning of the 3rd day there was a remission to 101·2°, with a rise to 103° F. the same evening, rising 0·4° F. the following evening and reaching 103·8° F. on the morning of the 5th day.

The temperature now descended and remained at about 100·2° F. until the 14th day; some evening exacerbations to 103° F. were noticed during the next few days.

On the 21st day a reaction commenced, in which the temperature averaged between 101°–102° F. in the morning and reaching 103·8° F. and 104° F. on two occasions in the evening.

The blood was examined daily from the day previous to inoculation until the 45th day, excepting the 28th and 35th days; from the 46th day the blood examination was made every third day.

Anaplasms in rare numbers were noticed for the first time on the 22nd day, remaining in about the same number for the next few days, and were noted rather more frequently from the 30th day, but at no time were they ever registered as numerous. Blood changes were hardly noticeable; a slight anisocytosis was noted.

Test for Immunity against Redwater.—Calf 881 was again injected on the 30th March, 1910, 112 days after the first injection, with 20 c.c. blood of heifer 926. This is a heifer which was inoculated in England with the South African strain of redwater and developed a pure infection of *Babesia bigemina.*

Remarks.—A slight reaction followed from the 5th–10th days, with both morning and evening maxima of 103° F. on the 5th days.

Babesia bigemina were seen on the 6th day, increasing in numbers during the succeeding twenty-four hours, and reaching their maximum frequency on the 8th and 9th days; the parasites then disappeared for a few days, reappearing on the 13th and 14th days.

Results.—Of the three calves, Nos. 892, 893, and 881, born in the Laboratory stables and kept tick-free, which were injected with blood from heifers imported from Aliwal North (a district considered to be free from redwater), two developed redwater and anaplasmosis and the third (881) developed a pure infection of anaplasmosis. It must be mentioned here that heifers 901 and 902 did not come from the same farm in the Aliwal North district as heifer 906. The two former belonged to a batch of animals which arrived at the Laboratory on the 7th October, 1909, and the latter to a batch which arrived on the 28th October, 1909.

The fact that calf 881, subsequent to its recovery from anaplasmosis, could successfully be infected with redwater, showing the Babesiae in the blood in fair numbers, should indicate that this Aliwal North heifer had no immunity against redwater, the interpretation of which fact would be that it did not contain the parasite of *Babesia bigemina* in the blood previous to the second inoculation.

Test of Heifer 906.—In order to prove that heifer 906, from which the anaplasma infection was obtained, represented a pure infection of anaplasmosis, it was decided to show its susceptibility to a redwater infection by the following injection :—

(d) *Injection of Blood of English Heifer* 926.

NOTE.—Heifer 926 (redwater infection) was an English beast, which had been injected with South African redwater in London and arrived in Pretoria on the 13th December, 1909.

(*D*) *Heifer* 906.—(See previous animal.)

Treatment.—On the 27th April, 1910—six months after arrival and four and a half months after it had been tapped for the first time—it was injected with 10 c.c. blood of heifer 926.

Remarks.—On the 6th day a slight rise of temperature occurred, reaching 104·8° F. the following evening and developing into a definite curve, lasting from the 7th to 14th days, with a maximum evening temperature of 103° F.

For the three days previous and six days subsequent to the injection of blood all microscopical examination proved negative; *Babesia bigemina* were found daily from the 6th to 20th days; the maximum frequency was noted on the 11th day; anisocytosis and basophilia appeared after the 10th day, but were never strongly pronounced.

Results.—The fact that heifer 906 could be successfully inoculated with blood of English heifer 926 (a pure strain of South African redwater) and developed a typical reaction lasting for some days, with the presence of *Babesia bigemina* and the typical characteristics of anaemia, supports the conclusion that heifer 906 was susceptible to a redwater infection, and accordingly the conclusion is warranted that on the date of tapping (7th December, 1909) she was not infected with *Babesia bigemina.*

EXPERIMENT NO. 2.—*To note whether the blood of heifer* 906 *and succeeding generations would produce a pure infection of anaplasmosis when injected into freshly imported English heifers.*

FIRST GENERATION.

(a) *Blood of Heifer* 906—*Anaplasmosis Infection* (vide *Previous Experiments*).

(*A*) *Sussex Heifer* 928.—Imported from England and arrived at the Laboratory on the 13th December, 1909. Her temperature was taken twice daily from the date of arrival; the highest record given was 102·4° F., which was reached on one occasion only.

Treatment.—On the 20th January, 1910, heifer 928 was injected subcutaneously with 1 c.c. defibrinated blood of heifer 906 (anaplasmosis infection).

Remarks.—For the next forty days the temperature averaged about 101° F. in the morning and between 101°–102° F. in the evening; the only abnormal records were 102·2° F. and 102·4° F. in the evening of the 12th and 30th days respectively.

From the 40th day onward the curve became somewhat irregular, with an evening exacerbation on the 50th day to 103·2° F. From the 55th to 60th days nothing above 102° F. was noted. Taken as a whole the reaction can be said to indicate a slight fever only.

The blood examinations were made daily during the first six days, twice daily during the next four days, and after that at intervals of two days until

the 42nd day. On the 42nd day anaplasms appeared and the daily percentage of infected corpuscles was as follows :—

Day.	Per cent.	Day.	Per cent.
42nd	2·3	51st	3·0
43rd	4·2	52nd	3·0
44th	4·8	53rd	2·3
45th	5·4	54th	1·9
46th	5·6	55th	1·6
47th	11·4	56th	1·4
48th	9·9	57th	1·0
49th	9·25	58th	1·0
50th	3·4		

On the 45th day two anaplasms were frequently found in one corpuscle ; three days later polychromasia was noted, and the following day basophilia was registered and anisocytosis was well pronounced ; macrocytes were recorded on the 50th day, and an increase of basophile cells was noted during the following days.

These symptoms remained until the 56th day, when the basophilia began to disappear ; on the 59th day basic cells were but rarely found ; the following day anisocytosis with numerous macrocytes were seen.

The blood picture now showed an improvement, and a normal condition was noted on the 80th day (10th April, 1911).

Test.—On the 27th April, 1910—ninety-six days after the first injection—heifer 928 was injected with 10 c.c. blood of heifer 926 (an English heifer immunized against *Babesia bigemina* in England).

Remarks.—On the 8th day there was a sharp rise to 104° F. in the evening On the 9th day the morning temperature was 103° F. and twelve hours later it had reached 106·6° F. On account of this high fever she was injected with a 1 per cent. solution of trypan blue on this date. A remission had occurred by the next morning to 102·4° F.

The blood was examined previous to the injection of redwater blood and found normal ; *Babesia bigemina* made their appearance on the 7th day after injection ; the following day the parasites had increased in numbers and were still noted on the 10th day ; they disappeared after the animal was treated with trypan blue.

Results.—Heifer 928 developed a pure attack of anaplasmosis, from which she recovered. Although the attack was a slight one, all the typical lesions were present in the blood. When tested with blood of an animal immune to South African redwater she promptly contracted this disease and developed a severe attack, but recovered as the result of the treatment with trypan blue.

(*B*) *Sussex Heifer* 940.—An English heifer, arrived in Pretoria on the 13th December, 1909 ; she was kept in a tick-free stable ; the temperature was taken twice daily, and periodical rises to about 103° F. were noted weekly during the first month, but which could not be accounted for, otherwise the temperature remained normal.

Treatment.—On the 28th February, 1910, heifer 940 was injected subcutaneously with 20 c.c. fresh blood of heifer 906 (anaplasmosis infection) as above.

Remarks.—For the next twenty-four days the temperature remained normal and did not deviate in any way from a healthy record ; on the following day a slight fever reaction commenced, reaching 103·2° F. in the evening ; the morning records for the next fortnight averaged 101° F., and were one degree higher in the evenings.

Microscopical examination of the blood was commenced on the 3rd day, and excepting on the 12th and 13th days, was continued until the 32nd day. On the 20th day the anaplasms were first noted to be present, but in rare numbers. They were traced in every subsequent examination, but were never found to be very frequent.

Test for Immunity to Redwater.—On the 2nd September, 1910, heifer 940 was infested with blue larval ticks collected from heifers 922 and 925 (*vide* Experiment No. 4). Both heifers had shown *Babesia bigemina* and anaplasms in their blood, heifer 922 also having been infected with *Spirochaeta theileri.*

Remarks.—There was a slight reaction between the 7th and 11th days, and from the next day a second reaction began, reaching the maximum temperature of 106° F. on the 19th day.

A remission due to the injection of trypan blue followed, and the temperature continued somewhat irregularly for the next fourteen days.

Examination of the blood at intervals up to the 17th day gave negative results. On the 18th day *Babesia bigemina* were found in rare numbers; the following day the parasites were more frequent, and as this was the day of the highest fever and the symptoms rather alarming, the animal was treated with trypan blue, resulting in a decrease of parasites and a remission of the temperature. *Babesia bigemina* was noted for the last time two days after injection of trypan blue.

Results.—The inoculation of 10 c.c. fresh blood of heifer 906 (anaplasmosis infection) into heifer 940 caused the appearance of a slight fever reaction during which the anaplasms were found regularly, but in rare numbers. The infestation of infected larval ticks caused the appearance of redwater in the time typical for a tick infestation, and which would probably have been responsible for the death of the animal had the trypan blue not been injected.

Accordingly, in this instance it must be concluded that the preceding anaplasmosis reaction did not in any way give immunity to the subsequent infection of babesiosis.

SECOND GENERATION.

(b) *Blood of English Heifer* 928 (*Anaplasmosis Infection*).

NOTE.—Heifer 928, an English heifer, showed a pure infection of anaplasmosis as the result of injection of blood of heifer 906 [*vide* Experiment No. 2 (*A*)].

(*C*) *Sussex Heifer* 933.—An imported English heifer, which arrived in Pretoria on the 13th December, 1909, was immediately placed in a tick-free stable and temperatured twice daily; its temperature remained perfectly normal during the observation period.

Treatment.—On the 5th February, 1910—fifty-four days after arrival—heifer 933 was injected subcutaneously with 50 c.c. fresh blood of heifer 928.

Remarks.—A slight curve ensued from the 13th to the 27th day, characterized by slightly elevated morning temperatures and slight evening rises to 102° or 103° F.; on one occasion 104° F. was registered.

With the exception of the 6th day, the blood was examined daily up to the 27th day, but with negative results for the first ten days. On the 11th day rare chromatic points were noticed to appear which had to be considered as anaplasms.

These parasites were now noticed daily, increasing slightly, but were never present in great numbers; forms undergoing division were occasionally seen. On the 21st day the lesions of a slight anisocytosis were recorded; rare basophile granulations were noted the following days, remaining for a few days, accompanied with polychromasia on the 23rd day.

The maximum frequency of anaplasms was reached on the 14th day, when 4·5 per cent. of the corpuscles were infected.

Test for Immunity to Redwater.—On the 27th May, 1910, heifer 933 was infested with blue larval ticks collected off heifer 871, an animal which was immune to *Babesia bigemina*, *Babesia mutans*, and anaplasmosis.

Remarks.—On the 6th and 19th days, evening rises to above 103° F. were recorded, and a reaction commenced on the 21st day, lasting for ten days, of a well-pronounced but not severe character, with the reappearance of anaplasms on the 29th, 30th, and 31st days. *Babesia bigemina* appeared on the 29th, 30th, and 32nd days.

The animal eventually recovered without any treatment.

Result.—The injection of 50 c.c. fresh blood of heifer 928 (anaplasmosis infection) only produced a slight attack of anaplasmosis in heifer 933. It is true that the incubation time was a short one (even shorter than in any other animal we have noticed so far), and is probably due to the fact that a large quantity of blood—and with it a larger number of dormant parasites—was injected.

Heifer 933 was then infested with infected blue larval ticks and a typical attack of redwater followed, from which the animal recovered, indicating again that recovery from an attack of anaplasmosis does not give immunity against redwater transmitted in a natural way by means of ticks.

(c) *Blood of English Heifer* 940 (*Anaplasmosis Infection*).

NOTE.—Heifer 940, imported from England, contracted anaplasmosis from the injection of heifer 906 [*vide* Experiment No. 2 (*B*)].

(*D*) *Sussex Heifer* 938.—Imported from England and arrived at the Laboratory on the 13th December, 1909; she was immediately placed in a tick-free stable and temperatured twice daily. No abnormal records were noted before injection.

Treatment.—Injected subcutaneously on the 9th April, 1910 (eighty-six days after arrival), with 10 c.c. fresh blood of heifer 940 (anaplasmosis infection).

Remarks.—The temperature remained normal until the 25th day, when a distinct reaction set in, lasting for ten days, with morning temperatures averaging 102° F. and evening rises to above 103° F.; on one occasion 104·6° F. was registered.

Anaplasms were noted in but rare numbers during this reaction.

Test for Immunity against Redwater.—On the 10th November, 1910, heifer 938 was infested with blue larval ticks collected from redwater-immune cattle on the station (Ref. 357). A sharp reaction followed on the 17th, 18th, and 19th days, reaching 105° F. A slight fever reaction continued up to the 30th day.

Babesia bigemina was present on the 27th, 28th, and 29th days, being especially frequent on the 28th day, but disappearing somewhat during the next twenty-four hours.

The animal eventually recovered without treatment. This observation further demonstrates the susceptibility of anaplasmosis-immune cattle to redwater.

(*E*) *Sussex Heifer* 932.—Imported from England and arrived at the Laboratory on the 13th December, 1909; she was immediately placed in a tick-free stable and temperatured twice daily. No abnormal records were noted.

Treatment.—On the 27th May, 1910—165 days after arrival—this animal was injected subcutaneously with 10 c.c. fresh blood of heifer 940 (anaplasmosis infection).

Remarks.—Nothing unusual occurred during the following four weeks, but from the 29th to 50th days an irregular curve was noted, with evening records of over 103° F. between the 40th and 50th days.

Generally speaking, the reaction was a slight one.

Anaplasms were noted for the first time on the 31st day, increasing in numbers in the course of the following days, but never being found in excessive numbers; divisional forms were noted.

The symptoms of anisocytosis were also noticed during the reaction, together with basophilia, the latter being registered daily between the 42nd and 50th days.

Tests for Immunity to Redwater.—On the 24th May, 1911, heifer 932 was tested by the subcutaneous injection of 5 c.c. blood of heifer 1216 [an English heifer which arrived at the Laboratory in January, 1911, and was injected on the 23rd January, 1911, with 5 c.c. blood of heifer 926, redwater infection, *vide* Experiment No. 1 (*D*)], and developed a pure attack of redwater.

Remarks.—A slight temperature reaction began on the 6th day, with a sharp rise to 105·4° F. two days later, on which date the animal was injected with trypan blue; the temperature regained normal within forty-eight hours.

Babesia bigemina were noted on the 7th day, increasing considerably in numbers the following day; only once *Babesia bigemina* was recorded, the day subsequent to the injection of trypan blue. On the 14th day polychromasia and basophilia were registered; seven days later the parasites were noted in fair numbers, but without any temperature disturbance. Here also the fact is demonstrated that an animal which passed through an attack of anaplasmosis remained susceptible to redwater.

THIRD GENERATION.

(d) *Blood of Heifer* 933 (*Anaplasmosis Infection*).

NOTE.—English heifer 933 contracted a pure infection of anaplasmosis from the injection of blood of heifer 928 [*vide* Experiment No. 2 (*C*)].

(*F*) *Sussex Heifer* 935.—Imported from England and arrived at the Laboratory on the 13th December, 1909; she was immediately placed in a tick-free stable and temperatured twice daily. Occasional exacerbations were noted during the first four weeks, but no cause could be assigned. At the date of injection the temperature was normal.

Treatment.—Injected subcutaneously on the 30th March, 1910, with 50 c.c. fresh blood of heifer 933 (anaplasmosis infection).

Remarks.—The temperature remained normal until the 18th day, when a slight curve started, with evening rises and morning remissions, reaching 104° F. on the evening of the 27th day, thence descending to normal.

Considered as a whole the reaction was of a slight character.

The examination of the blood was undertaken daily from the 5th to the 30th day; no changes were noted during the first sixteen days. On the following days chromatic points were recorded, which in the course of time proved to be anaplasms, since divisional forms were noted as their numbers increased.

A slight anisocytosis was noticed on the 20th day; basophilia was noted five days later.

Tick Infestation.—On the 29th day (28th April, 1910) heifer 935 was heavily infested with clean blue larval ticks from heifer 929, with a view of infecting them with anaplasmosis.

Unfortunately this heavy infestation resulted in the death of the animal twenty-one days later from anaemia at a time when the females had commenced to engorge and were present in extraordinarily large numbers, the whole carcass presenting a whitish appearance, due to the quantity of blood withdrawn by the ticks.

NOTE.—In the second generation of this pure strain of anaplasmosis produced by the injection of 50 c.c. of blood, the disease again had a short incubation time, corresponding to that observed in the case of heifer 933 [*vide* Experiment No. 2 (*C*)].

(e) *Blood of Heifer* 938 (*Anaplasmosis Infection*).

NOTE.—English heifer 938 contracted a pure infection of anaplasmosis from the injection of blood of English heifer 940 [*vide* Experiment No. 2 (*D*)].

(*G*) *Sussex Heifer* 937.—Arrived at the Laboratory on the 13th December, 1909; she was immediately placed in a tick-free stable and temperatured twice daily. In February and April, 1910, this heifer was used in tick experiments with brown adults and nymphae.

Treatment.—On the 27th May, 1910—165 days after arrival—she was injected subcutaneously with 10 c.c. fresh blood of heifer 938. The temperature remained normal until the 27th day, when a slight but distinct curve commenced, reaching its maximum on the 33rd day of 104·6° F. and returning to normal about the 40th day.

Anaplasms were noticed for the first time on the 20th day, and from this date increased gradually until they became fairly frequent on the 32nd day, remaining present for the next three or four days; at the time the temperature returned to normal they were only present in very rare numbers.

The symptoms of anisocytosis appeared with the anaplasms, and on the 30th day a few basophile cells were noticed, remaining for the next nine days.

Test for Immunity to Redwater.—Injected on the 28th February, 1911, with 5 c.c. blood of heifer 926 (redwater infection). A doubtful reaction was noticed, but the examination of the blood gave negative results. *Retested* on the 14th June, 1911, with 5 c.c. blood of heifer 1216 (redwater infection). No reaction followed. It is possible that the ticks used in February and April, 1910, transmitted *Babesia bigemina* in such rare numbers that the parasites escaped notice.

(f) *Blood of Heifer* 932.

NOTE.—Heifer 932 contracted a pure attack of anaplasmosis from the injection of blood of heifer 940 [*vide* Experiment No. 2 (*E*)].

(*H*) *Sussex Heifer* 1212.—An imported English heifer, which arrived from England on the 9th January, 1911; she was immediately placed in a tick-free stable and temperatured twice daily; no abnormal records were noted during this observation period.

Treatment.—On the 23rd January, 1911—fourteen days after arrival—heifer 1212 was injected subcutaneously with 100 c.c. fresh blood of heifer 932.

Remarks.—The temperature remained within normal limits until the 21st day, when a slight rise of a definite character was noted, lasting until the 35th day; the morning records averaged 101° F. and 103° F. was the maximum evening limit.

Anaplasms appeared on the 23rd day, followed by the lesions of polychromasia and basophilia two days later. The anaplasms were noted but in rare numbers until 36th day.

Test for Immunity against Redwater.—On the 24th May, 1911, heifer 1212 was tested by the subcutaneous injection of 5 c.c. fresh blood of heifer 1216 (an animal belonging to the same batch as heifer 1212, and which had developed a pure attack of redwater as the result of the injection of blood of heifer 926).

Remarks.—There was a sharp rise of temperature on the 5th day, reaching 105·6° F.; the following day the heifer was noted to be off her feed; a slight anisocytosis and a few *Babesia bigemina* were noted in the blood. The temperature remained high until the 8th day, when it was considered advisable to inject trypan blue, the parasites having been noted in the blood the previous day in large numbers.

Two days after the injection of trypan blue the temperature regained normal, but *Babesia bigemina* and basophile cells remained present until the 10th day; polychromatic and basophile cells were again noted on the 14th day, and four days later two parasites were detected.

(*I*) *Sussex Heifer* 1220.—An imported English heifer, which arrived from England on the 9th January, 1911; she was immediately placed in a tick-free stable and temperatured twice daily. No abnormal records were noticed during this observation period.

Treatment.—Injected on the 23rd March, 1911—seventy-three days after arrival—with 10 c.c. fresh blood of heifer 932 (anaplasmosis infection—see above).

Remarks.—Nothing abnormal was noticed until the 27th day, when a temperature reaction commenced of a definite character, lasting until the 45th day, with evening exacerbations on several occasions to 105° F. Anaplasms were noted on the 26th day, and from this date onwards they rapidly increased in numbers, reaching the maximum on the 38th day. Anisocytosis was recorded on the 28th day. On the 32nd day polychromasia and basophile cells were noted. About the 40th day oligocythaemia was pronounced.

The animal was not feeding well on the 36th day, but soon rallied and did not lose in condition.

Not tested for immunity to redwater.

(*J*) *Sussex Heifer* 1221.—An imported English heifer, which arrived from England on the 9th January, 1911; she was immediately placed in a tick-free stable and temperatured twice daily. No abnormal records were noticed during this observation period.

Treatment.—Inoculated on the 23rd March, 1911—seventy-three days after arrival—subcutaneously with 50 c.c. fresh blood of heifer 932 (anaplasmosis infection—see above).

Remarks.—A definite temperature reaction commenced on the 32nd day, lasting for thirteen days. The appearance of anaplasms preceded the reaction, and with the onset of fever they increased in numbers. Polychromatic cells and anisocytosis were registered on the 33rd day. Basophilia was noted for the first time on the 36th day, when the anaplasms began to disappear and the anaemic lesions diminished. No signs of distress were noticed in the animal.

Not tested for immunity to redwater.

FOURTH GENERATION.

(g) *Blood of Heifer* 1212 (*Anaplasmosis Infection*).

NOTE.—Heifer 1212 [*vide* Experiment No. 2 (*H*)] contracted anaplasmosis as the result of the subcutaneous injection of 100 c.c. fresh blood of heifer 932.

(*K*) *Sussex Heifer* 1222.—An imported English heifer, which arrived at the Laboratory on the 9th January, 1911; she was immediately placed in a tick-free stable and temperatured twice daily; no temperature disturbances were noted during this observation period.

Treatment.—Injected on the 23rd March, 1911—seventy-three days after arrival—with 10 c.c. fresh blood of heifer 1212 (anaplasmosis infection).

Remarks.—The temperature remained normal until the 28th day, when a definite reaction ensued, lasting until the 50th day, with evening records over 104° and 105° F. on several occasions.

Anaplasms were noted on the 26th day; they rapidly increased in numbers, and during the first few days of the temperature reaction were present in very large numbers. Anisocytosis was recorded on the 28th day.

On the 34th day polychromatic and basophile cells were noted, increasing in numbers during the following days. On the 28th day oligocythaemia was fairly well pronounced and large numbers of macrocytes appeared. The animal presented clinical symptoms of illness on the 35th day: the muzzle was pale, the animal refused to feed and lost condition.

The heifer remained in somewhat poor condition for some time after recovery.

Not tested for immunity to redwater.

(*L*) *Sussex Heifer* 1223.—An imported English heifer, which arrived at the Laboratory on the 9th January, 1911; she was immediately placed in a tick-free stable and temperatured twice daily; no temperature disturbances were noted during this observation period (see note below).

Treatment.—Injected on the 23rd March, 1911—seventy-three days after arrival—with 50 c.c. fresh blood of heifer 1212 (anaplasmosis infection).

Remarks.—There was an immediate rise of temperature from the 2nd day, developing into a typical curve and returning to normal on the 19th day From the 3rd day anaplasms were noted in great numbers, accompanied with all the lesions of oligocythaemia, anisocytosis, polychromatic, and basophile cells.

From the 20th to the 50th day another reaction ensued, when anaplasms, together with anisocytosis and macrocytes, were present.

This animal was noted to lose rapidly in condition.

NOTE.—The immediate appearance of anaplasms a few days after the injection is an extraordinary occurrence and of a paradoxical nature. Noticing that after the injection of a large quantity of blood in some previous experiments the incubation time was shortened, it is possible that the same explanation holds good in this instance; further, the increased virulency of the parasites after passing through three highly susceptible animals, may have had some influence.

The heifer was not tested for immunity to redwater.

CONCLUSIONS (EXPERIMENTS NOS. 1 AND 2).

The inoculation of the blood of heifer 906 into Africander calf 881, born in the stable and kept tick-free, and subsequently into heifers 928 and 940, imported from England, was succeeded in all three instances by a pure infection of anaplasmosis.

Through a series of four generations the anaplasms were present as a pure infection, as was proved by the injection of large quantities of blood (50 c.c. to 100 c.c.) of animals which had recovered from anaplasmosis, and if any other parasites had been present in the blood it could rightly have been expected that out of thirteen animals which were all susceptible to red-water and other blood diseases, some at least would have developed one or the other disease. It has accordingly been proved that a pure strain of anaplasmosis can be obtained and can also be continued through a number of animals.

Special attention must be drawn here to the striking fact that of twelve English heifers which were susceptible to both babesiosis and anaplasmosis none died, whereas in the experiments enumerated in my first report, out of ten animals, all had severe reactions and five of them died.

The fever reactions in these twelve new animals were not so severe, and the temperature only occasionally reached a high record. An increase in virulency was noted by passing the infection through subsequent generations and using larger quantities of blood for the injection.

In each of the animals referred to in this report blood lesions were registered, succeeding (as in previous cases) the anaplasms; basophilia was also noted. Only in one or two exceptional cases did the changes of the blood indicate a more severe anaemia due to a heavy destruction of corpuscles, as described previously, not sufficient, however, to cause death. Generally, recovery and restitution were observed to occur much quicker than in the experimental animals referred to in previous reports.

I also noticed a difference in the appearance of the anaplasms, both in size and position in the corpuscles. In recording the presence of anaplasms in the foregoing experiments, I have purposely omitted to specify *Anaplasma marginale*, the parasite described in previous reports as being a body of the dimensions of a coccus, usually situated *on the border of the red corpuscles*, varying slightly in size.

In all the foregoing experiments the anaplasms which I noticed were, as a rule, not so regularly and constantly situated on the margin, but were frequently found within the corpuscle somewhat at a distance from the margin.

Although it is difficult to accurately measure the diameters of such small bodies, I am decidedly under the impression that the anaplasms which I found in heifer 906 were, taken as a whole, somewhat smaller to those described in my previous reports. The remaining characteristics are the same, namely, the parasites easily take the protozoic stain, and divisional forms could be recognized.

In order to distinguish between these two anaplasms in the further course of this report, I propose to designate the one *Anaplasma marginale* (as described previously) and the other, which is more centrally situated in the corpuscle and causes only a slight attack of the disease, *Anaplasma marginale* (variety *centrale*), indicating that, in my opinion, this latter is only a variety of *Anaplasma marginale*.

The characters shown—namely, somewhat smaller size, different position, and less virulency—support this view. It may, *a priori*, be expected that *Anaplasma marginale* (variety *centrale*) confers immunity against an infection of *Anaplasma marginale* proper, but by analogy with other protozoan diseases this need not be the case. Should the immunity not be complete, then this fact may be interpreted to indicate a slight difference between the two parasites, and this would support the view of different varieties.

A further point of importance is clearly brought out by this experiment—and this was the one at issue in the first instance—that recovery from anaplasmosis did not prevent a reinfection with *Babesia bigemina*. With one exception (an English heifer), the animals promptly reacted to the *Babesia*

bigemina infection, introduced either by subcutaneous injection of blood or in the natural way by ticks. In some instances the reactions were so severe that if medicinal treatment with trypan blue had not been adopted the animals would probably have died.

The proof is accordingly given in the reverse order that redwater and anaplasmosis are two different diseases.

PART II.

TO TEST THE IMMUNITY CONFERRED BY *ANAPLASMA MARGINALE* (VAR. *CENTRALE*) AGAINST *ANAPLASMA MARGINALE* PROPER.

EXPERIMENT No. 3.—*To note whether animals which had an attack of anaplasmosis, due to* Anaplasma marginale (*variety* centrale) *will react to an injection of blood taken from an animal infected with* Anaplasma marginale *proper.*

(a) *Origin of Blood: Calf* 917.

NOTE.—Calf 917 was born on the 8th November, 1909, had been kept in a stable, and was bedded on veld hay. On the 9th February, 1910, it showed *Babesia mutans* in its blood. It was thought at that time that it might represent a pure infection of *Babesia mutans*; accordingly, it was decided to verify this by injecting a susceptible English heifer. It was proposed to use a very small quantity of blood in order to minimize the risk of a possible *Babesia bigemina* infection, which naturally had also to be anticipated.

(*A*) *Sussex Heifer* 934.—Imported from England and arrived at the Laboratory on the 13th December, 1909. She was immediately placed in a tick-free stable and temperatured twice daily. The animal remained in good health and no temperature disturbance occurred.

Treatment.—On the 10th February, 1910—fifty-nine days after arrival—heifer 934 was injected subcutaneously with 10 c.c. of a mixture comprising 2 parts blood of heifer 917 and 10,000 parts normal saline solution.

Remarks.—A slight curve was noticed after injection, the highest record of which was 102·4° F. on the 12th day. The morning temperatures averaged 101° F.

From the 17th day onwards, the morning records averaged between 100°–101° F. On the 35th day a distinct reaction began, with evening exacerbations as high as 105·2° F., and morning records of about 104° F. on the next two days, the reaction lasting until the 45th day.

From the 55th–64th day another reaction occurred, very much resembling the previous curve, and with a maximum record of 105° F. in the evening of the 59th day.

During the first four weeks the blood was examined every second or third day, but nothing abnormal could be found. On the 33rd day *Anaplasma marginale* made its appearance and infested about 5·7 per cent. of the red corpuscles. The next day their number had increased to 9·1 per cent., on the 35th day 15·2 per cent., the following day 20·4 per cent., and twenty-four hours later 21·4 per cent.

Anisocytosis was noted on the 37th day, and the next day basophile macrocytes put in an appearance. The corpuscles now became very pale;

polychromasia and basophilia increased, and a typical picture of an oligocythaemia, with all the accompanying lesions of the blood corpuscles, developed fully.

Babesia mutans appeared on the 40th day; it increased in numbers and remained present during the second reaction.

Anaplasms disappeared with the descent of the temperature, but *Babesia mutans* remained, and was still noted on the 100th day, although in rare numbers.

During the first reactions the animal was decidedly ill, refused to feed, and lost condition considerably.

Results.—It is proved by this experiment that at the time the blood was taken from calf 917 it must have been infected with *Anaplasma marginale*, and the incubation time being slightly shorter than that of *Babesia mutans*, it naturally appeared first of all in heifer 934, although it was not noticed at the time of the blood examinations of calf 917; even the precaution taken of diluting the blood did not have any effect, except perhaps that of excluding *Babesia bigemina*.

Babesia bigemina was not observed; accordingly it had to be expected that the injection of blood containing this parasite would produce an attack of redwater in heifer 934. She was therefore injected on the 28th June, 1910, with 10 c.c. blood of heifer 926, an animal immune to redwater.

A reaction followed from the 9th to 13th day, with evening records of 103·6° F., and during which time *Babesia bigemina* was noted.

The attack was a slight one and the animal easily recovered.

(b) *Blood of English Heifer* 934 (Anaplasma marginale *Infection*).

(*B*) *Calf* 881.—About eight months old, and immune against redwater and anaplasmosis [*vide* Experiment No. 1 (*C*)—*Anaplasma marginale* (variety *centrale*) infection].

Test for Immunity to Anaplasmosis.—On the 21st April, 1910, calf 881 was injected subcutaneously with 10 c.c. fresh blood of heifer 934.

Remarks.—A temperature reaction, somewhat irregular in its character and with evening exacerbations, followed from the 19th to 40th day; *Anaplasma marginale* was noted on the 20th day for the first time, and infesting 3 per cent. of the corpuscles; the following day the same percentage was infested, the 22nd day 2·9 per cent., the 23rd day 2·0 per cent., and the following day 1·3 per cent., after which time the anaplasms disappeared.

Babesia mutans appeared on the 31st day, on which date *Anaplasma marginale* was again noted; *Babesia mutans* remained present, but in rare numbers, until the temperature regained normal limits.

(*C*) *Sussex Heifer* 940.—Imported from England and immune against *Anaplasma marginale* (variety *centrale*) and *Babesia bigemina* [*vide* Experiment No. 2 (*B*)].

Test for Immunity to Anaplasmosis.—Injected on the 29th October, 1910, subcutaneously with 10 c.c. fresh blood of heifer 934 (*Anaplasma marginale* proper).

Remarks.—From the 25th to 35th day a distinct reaction was noted, with evening rises to 104·4° F. and morning records of 103° F. On the 26th day *Anaplasma marginale* appeared and was registered as very frequent the following day.

The symptoms of basophilia were also noted on the 27th day; a few days later *Babesia mutans* put in an appearance, and remained until the temperature reached normal limits.

(*D*) *Sussex Heifer* 933.—Imported from England and immune against *Anaplasma marginale* (variety *centrale*) and *Babesia bigemina* [*vide* Experiment No. 2 (*C*)].

Test for Immunity to Anaplasmosis.—On the 8th August, 1910, heifer 933 was injected subcutaneously with 5 c.c. fresh blood of heifer 934 (*Anaplasma marginale* proper).

Remarks.—A temperature reaction started about the 33rd day, lasting for about fourteen days, never reaching high figures, but characterized by marked differences between the morning and evening records.

Anaplasma marginale were noticed on the 36th day, remaining present for some days. On the 42nd day a slight basophilia was noticed. Two days later the presence of *Babesia mutans* was registered, remaining for a considerable length of time, and probably being responsible for the irregular temperatures which were noted later. This animal was exposed on the Potchefstroom Experimental Farm on the 5th November, 1910, and was still alive on the 31st August, 1911.

(*E*) *Sussex Heifer* 924.—The following account of the history of this animal in England was supplied by *S. Stockman*:—

Heifer 924.—On the 16th October, 1909, this heifer received subcutaneously 10 c.c. of blood from calf 211. The temperature varied only within physiological limits until the morning of the 21st October, when it rose to 103·6° in the morning and 104·4° in the evening. No piroplasms, however, were found in the smears.

On the evening of the 21st October the heifer received subcutaneously 1 gramme of trypan blue dissolved in 150 c.c. of water.

Date.	Temperature.		Remarks.
1909.			
October.	Morning.	Evening.	
22	102	102·6	Smears negative.
23	103	102·6	„ „
24	101·4	102	„ „
25	101·2	104	„ „
26	103	102·2	„ „
27	102·2	102·4	„ „
28	102·2	102·6	„ „
29	102·2	102·2	„ „
30	102	102·2	„ „
31	101·2	101·8	„ „
November.			
1	101·2	101·6	A few piroplasms (bigeminum) found in smears, i.e. 16th day.
2	101·2	101·8	A few piroplasms (bigeminum) present in smears.
3	101	102	Principally round forms found in the morning, bigeminum in the evening.
4	101·6	102	No piroplasms found in smears.
5	102	102	A few piroplasms, round and bigeminum, in the smears.

Heifer 924 arrived at the Laboratory on the 13th December, 1909; she was immediately placed in a tick-free stable and temperatured twice daily. A slight irregular record was noticed during the first week, but from then no unusual temperatures were registered during the observation period.

(c) *Blood of Heifer* 933.

NOTE.—Heifer 933 contracted *Anaplasma marginale* (variety *centrale*) from the injection of blood of heifer 928 [*vide* Experiment No. 2 (*C*)].

Treatment.—On the 30th March, 1910—107 days after arrival—heifer 924 was injected subcutaneously with 10 c.c. blood of heifer 933.

Remarks.—The temperature remained normal up to the 35th day, with the exception of one evening record of 103·4° F. on the 29th day. From the 35th day onwards a slight reaction set in, with morning temperatures above normal, but the evening records were chiefly within the usual limits.

The first anaplasms were noted on the 30th day. The following figures show the percentage of red blood corpuscles infected during the ensuing days :—

Day.	Per cent.	Day.	Per cent.
38th	4·7	47th	15·2
39th	3·2	48th	15·5
40th	3·9	49th	10·5
41st	5·0	50th	8·4
42nd	9·6	51st	9·6
43rd	15·0	52nd	9·0
44th	14·0	53rd	7·2
45th	13·8	54th	5·0
46th	15·2	55th	1·5

Anisocytosis appeared at the beginning of the reaction; the maximum percentage of anaplasms of 15·5 on the 48th day coincided with the highest temperature record of 103·4° F. Basophilia appeared on the 41st day, but did not acquire any importance.

The animal quickly recovered after the 50th day.

Test of Immunity for Anaplasmosis.—Injected on the 28th June, 1910, subcutaneously with 10 c.c. fresh blood of heifer 934 (*Anaplasma marginale* infection).

Remarks.—A distinct reaction ensued from the 25th to 38th days, with evening records of 103° F. on several occasions.

On the 30th day *Anaplasma marginale* put in an appearance, remaining for some days. On the 32nd day a slight polychromasia was recorded. Piroplasms were not noticed during this reaction, and as the temperature regained normal limits on the 38th day, the blood examinations were discontinued.

(*F*) *Sussex Heifer* 927.—The history of this animal in England, as furnished by *S. Stockman*, is as follows :—

Heifer 927.—On 16th October, 1909, this heifer received subcutaneously 10 c.c. of blood from calf 211. The temperature remained within physiological limits until the 26th October, when it rose to 103·2° in the morning and 105° in the evening. Smears made in the morning showed a few round piroplasms and a good number of bigeminum. The blood had been examined morning

and evening from the 22nd October, i.e. from the 6th day, but no piroplasms were found until the 26th October.

Date.	Temperature.		Remarks.
1909.			
October.	Morning.	Evening.	
27	105·4	107·2	Piroplasms were present in the morning and evening smears, both round and bigeminum.
	On this day	the heifer	
	began to pa	ss red urine.	
28	104·2	104	Morning smears showed piroplasms, both round and bigeminum.
29	102·8	102·2	Smears showed a few piroplasms, round and bigeminum, in the morning; none in the evening.
30	102·2	102·2	No piroplasms could be found in the smears.
31	102	102·2	No piroplasms could be found.
November.			
1	102	102·8	No piroplasms could be found in the smears, but basophile cells were present.
	By this time	the urine	
	had become	quite clear.	
2	101·8	103·2	Basophile cells present in the smears, also punctate cells, but no piroplasms.
3	101·2	102	Smears same as on the 2nd November.
4	102	102·2	Smears were the same as on the 2nd November.
5	102·0	—	Basophile and punctate cells and a few piroplasms, round and bigeminum, were present in the smears.

Heifer 927 arrived at the Laboratory on the 13th December, 1909; it was immediately placed in a tick-free stable and temperatured twice daily; *no abnormal* records were noted during this time.

Treatment.—The heifer was injected on the 30th March, 1910—107 days after arrival—with 10 c.c. fresh blood of heifer 933 [*Anaplasma marginale* (variety *centrale*)].

Remarks.—With the exception of a slight rise on the 8th day to 103° F., the temperature remained normal until the 31st day, on which date, however, a decided curve commenced, with a sharp rise to 105° F. on the 42nd, 43rd, and 44th days, and gradually returning to normal.

Anaplasms were noted to be present in rare numbers on the 34th day. The following is the record of their frequency during the next few days:—

Day.	Per cent.	Day.	Per cent.
35th	Very rare	45th	6·3
36th	,,	46th	9·7
37th	0·8	47th	8·9
38th	0·6	48th	8·6
39th	2·3	49th	7·4
40th	3·4	50th	10·0
41st	5·3	51st	6·4
42nd	6·0	52nd	7·3
43rd	8·3	53rd	5·3
44th	6·8		

The symptoms of anisocytosis were present throughout the reaction. Basophilia were noted on the 41st day, remaining until the 48th day.

Test for Immunity to Anaplasmosis.—Injected on the 28th June, 1910, subcutaneously with 10 c.c. fresh blood of heifer 934 (*Anaplasma marginale*).

Remarks.—On the 33rd day a sharp reaction commenced, reaching 105° F. the following day and returning to normal about the 40th day.

Anaplasma marginale were noticed with the first rise of the temperature, increasing rapidly in numbers, but decreasing quickly from the 38th day until they completely disappeared five days later; anisocytosis and a slight basophilia accompanied the anaplasms.

Before *Babesia mutans* had time to appear the examination of the blood was discontinued as the temperature had regained normal.

(G) *Heifer* 906.—Imported from Aliwal North and immune against *Anaplasma marginale* (variety *centrale*) and *Babesia bigemina* [*vide* Experiment No. 1 (*D*)].

Treatment.—Injected on the 4th July, 1910, subcutaneously with 10 c.c. fresh blood of heifer 934 (*Anaplasma marginale*).

Remarks.—No temperature reaction followed, and accordingly no blood examination was made.

Results.

Of six animals which were all immune against *Babesia bigemina* and *Anaplasma marginale* (variety *centrale*), five English heifers reacted when injected with *Anaplasma marginale* proper; the reactions were of a mild type, accompanied with the appearance of anaplasms, which from their situation in the corpuscles had to be considered as *Anaplasma marginale* proper.

The temperature reactions cannot be considered to be due to the simultaneous *Babesia mutans* infection, inasmuch as in every instance when *Anaplasma marginale* made its appearance it preceded *Babesia mutans*, and in two instances the *Babesia mutans* infection was not noted, as owing to the fact that the temperature had returned to normal, the examination of the blood was discontinued before the incubation time of *Babesia mutans* had elapsed.

This is not an unusual observation, especially in South African born cattle, which may show an infection of *Babesia mutans* without any rise of temperature at all.

It may be concluded that the previous inoculation of *Anaplasma marginale* (variety *centrale*) gave sufficient immunity to protect the animals from a severe attack of anaplasmosis.

In five instances a reinfection was not prevented by the previous immunity; hence the conclusion finds a further support that *Anaplasma marginale* (variety *centrale*) and *Anaplasma marginale* proper represent two varieties of the same species. The immunity given by variety *centrale* protects against a severe illness or death (a frequent result of the variety *marginale* injection), but does not prevent the infection completely in every instance.

These observations suggest the possibility of using an infection with variety *centrale* to protect animals against an infection contracted in the field.

It is further brought out by this experiment what has already been shown before, that an anaplasma infection does not protect against a subsequent *Babesia mutans* infection, a fact which speaks for the duality of the two parasites.

PART III.

THE TRANSMISSION OF ANAPLASMOSIS BY TICKS.

EXPERIMENT No. 4.—*To convey anaplasmosis by means of ticks collected from a heifer immune to redwater and anaplasmosis.*

(*A*) *Heifer* 922.—The following is the history of this animal in England, recorded by *S. Stockman*:—

Heifer 922.—On 16th October, 1909, this heifer received subcutaneously 10 c.c. of blood from calf 211. The temperature in the morning and evening varied only within physiological limits until the morning of 21st October, when it rose to 105° F. Blood smears taken at this time showed no piroplasms.

The animal immediately received subcutaneously 1 gramme of trypan blue dissolved in 150 c.c. of water. On the evening of the 21st October the temperature was still 105° F. and the blood smears were negative. On the morning of the 22nd October the temperature had fallen to 102·6° F. In the evening of the same day it was 102° F., and from this time onwards until the 5th November it varied only between 101·2° F. and 102·2° F. Blood smears were examined daily, but no piroplasms could be found until the 28th October, that is to say, twelve days after inoculation and seven days after the rise of temperature. On the morning of the 28th October a very few piroplasms (bigeminum) were present in the blood smears: temperature 102° F.

No piroplasms were present in the blood smears taken on the 2nd November. A few bigeminum were found in blood withdrawn on the morning of the 3rd November. They could not be found in the smears of blood withdrawn on the evening of the same day. From that time up to the 5th November the examination of this heifer's blood gave negative results.

NOTE.—Heifer 922 was subsequently exported to South Africa, and arrived at the Laboratory on the 13th December, 1909. She was immediately placed in a tick-free stable and temperatured twice daily.

No abnormal records were noted during this observation period.

Treatment.—On the 1st March, 1910—seventy-seven days after arrival—heifer 922 was infested with a considerable number of blue tick larvae, the mothers of which had been collected off cattle in Natal; the eggs from which the larvae emerged were laid at the Laboratory.

Remarks.—After an incubation time of ten days a slight reaction occurred, lasting for four days, with a maximum evening record of 104° F. *Babesia bigemina* were noted on one occasion only, and then only in exceedingly rare numbers. Some irregular records were noted subsequently, but all blood examinations proved negative.

On the 55th day a reaction started, lasting for eighteen days, with high temperatures in the early part of the reaction (105° F. and over) and averaging between 103°–104° F. during the rest of the time. On the 55th day microscopical examination of the blood showed a very few chromatic points; they

were still rare the following day. Two days later their number had reached 8·9 per cent. and they were identified as anaplasms. The following day a remission was noted to 2·4 per cent.; they were very rare on the 60th day.

On the 61st day they had increased to 18·8 per cent. and they decreased to 16·3 per cent. the following day. Their number now dwindled down again rapidly, and a corresponding remission in the temperature occurred. Their numbers for the next few days were :—

Day.	Per cent.	Day.	Per cent.
65th.....	0·6	71st.....	0·02
66th.....	0·5	72nd.....	0·01
67th.....	0·3	73rd.....	0·005
68th.....	0·1	74th.....	0·003
69th.....	0·06	75th.....	0·002
70th.....	0·01		

The anaplasms had the character of the variety *centrale* and the anaemic lesions in the blood were in no way marked; the anaemia was characterized by a slight basophilia, which remained for seven days; it was succeeded by anisocytosis and complete recovery.

On the 71st day *Spirochaeta theileri* was noted, and another slight reaction was registered from the 76th to 85th days, when *Anaplasma marginale* (variety *centrale*) reappeared, infesting 5·4 per cent. of the red corpuscles.

Tests.—Heifer 922 was tested subsequently on two occasions with blood of animals which had recovered from anaplasmosis, namely, on the 20th May, 1910, when it was injected with 10 c.c. blood of heifer 928 [an animal immune against redwater and anaplasmosis, variety *centrale*—*vide* Experiment No. 2 (*A*)], without any results; and again on the 28th June, 1910, no reaction followed the injection of 10 c.c. blood of heifer 934 [an animal immune to anaplasmosis, variety *marginale*, *Babesia mutans*, and redwater—*vide* Experiment 3 (*A*)].

NOTE.—The infestation of ticks produced a slight temperature reaction, during which *Babesia bigemina* were seen but in rare numbers. Seeing that in subsequent examinations *Spirochaeta theileri* was found, it is doubtful whether the first fever reaction, during which the examination of the blood was negative, was caused by the *Babesia bigemina*. It is probable that it was caused by spirochaetes, against which the animal was not immune, spirochaetes being sometimes so rare that they are overlooked. The presence of *Babesia bigemina* must be considered to be more of an accidental nature (breakdown in immunity). This animal showed immunity to a subsequent *Anaplasma marginale* infection.

(*B*) *Sussex Heifer* 925.—The following is the history of this animal in England, as given by *S. Stockman* :—

On 16th October, 1909, heifer 925 received subcutaneously 10 c.c. of blood from calf 211. The temperature varied only within physiological limits until the evening of the 21st October, when it rose to 103·8° F. Smears, however, were negative.

Date.	Temperature.		Remarks.
1909.			
October.	Morning.	Evening.	
22	101·4° F.	103° F.	A few piroplasms both morning and evening, round forms.
23	103·2° F.	104° F.	Smears showed a few piroplasms, round and bigeminum, morning and evening.
24	101·8° F.	101·2° F.	In the morning a few piroplasms were found, in the evening there were very few.
25	101·8° F.	101·4° F.	Smears the same as on the 24th.
26	102° F.	101·2° F.	Smears showed a few piroplasms, mainly bigeminum.
27	102° F.	102° F.	Smears showed a few piroplasms, mainly bigeminum.
28	101·8° F.	102° F.	Smears stained as on 27th.
29	102° F.	102° F.	Smears showed a few piroplasms, mostly round forms, in the morning; none in the evening.
30	101·4° F.	102° F.	Smears showed a few piroplasms.
31	101·8° F.	102° F.	A few piroplasms were present in the morning, mainly bigeminum.
November.			
1	101·8° F.	102° F.	A few piroplasms (bigeminum) found in the smears.
2	101·6° F.	102° F.	No piroplasms found in the smears.
3	102° F.	102° F.	Smears showed a few piroplasms, round and bigeminum, both morning and evening.
4	101° F.	101·5° F.	No piroplasms found in smears.
5	101·2° F.	—	No piroplasms found in smears.

NOTE.—Heifer 925 arrived at the Laboratory on the 13th December, 1909; she was immediately placed in a tick-free stable and temperatured twice daily.

Treatment.—On the 1st March, 1910—seventy-seven days after arrival—this heifer was infected with blue larval ticks, belonging to the same batch as used on heifer 922 (*vide* previous animal); a fairly heavy infestation was made in this instance.

Remarks.—There was a temperature reaction from the 9th to 16th day, immediately succeeded by a second reaction, but of an irregular character, lasting up to about the 30th day. A few *Babesia bigemina* were noted on the 12th day only. At later intervals a slight anisocytosis and very rare numbers of basophile cells were detected.

A high reaction set in from the 45th day, with evening temperatures reaching 105·8° F., and followed by irregular records. The blood was frequently examined during this period, but with the exception of a slight anisocytosis, it was found quite normal.

It was not until the 114th day that another reaction occurred, when a few anaplasms of the type *centrale* were found.

Test.—Heifer 925 was tested on the 8th August with blood of heifer 934, an animal immune to redwater, *Babesia mutans*, and anaplasmosis [*vide* Experiment No. 3 (*A*)].

A reaction followed, lasting from the 20th until the 40th day with exacerbations over 105° F., and during which time anaplasms, variety *marginale*, were found in large numbers for a few days; they then slowly disappeared, corresponding with the fall of the temperature. Anisocytosis appeared at the onset of the fever reaction and was soon succeeded by basophilia; the symptoms of anaemia were, however, never strongly pronounced, the animal recovering quickly.

Towards the end of this reaction a few *Babesia mutans* put in an appearance; they increased in numbers daily and reached their maximum between the 45th and 55th days after injection.

The animal was sent to Potchefstroom on the 5th November, 1911, and was still alive on the 31st August, 1911.

NOTE.—In this instance the infestation of ticks produced a reaction, during which *Babesia bigemina* was noted on one occasion. The irregular reaction was not typical of a redwater infection, and it has again to be surmised that a spirochaetosis infection was responsible which escaped observation.

It is, however, also possible that the heavy infestation of ticks caused a reinfection with *Babesia bigemina*, from which the animal easily recovered.

In this particular instance the first anaplasmosis infection was very slight, and would have passed unnoticed if the examination of the blood had not been continued over a long period. Anaplasms were found in rare numbers and with a long incubation time of over 100 days. The subsequent injection of blood of heifer 934 (which contained *Anaplasma marginale*) produced a fever reaction, accompanied with a larger number of parasites.

EXPERIMENT No. 5.—*To convey anaplasmosis by means of the black pitted tick.*

(*A*) *Sussex Heifer* 930.—This heifer belonged to the same batch as 922 and 925, but had not been treated in England. She arrived at the Laboratory on the 13th December, 1909, and was immediately placed in a tick-free stable. The temperature was taken twice daily and no abnormal records were noted.

Treatment.—Infested on the 30th March, 1910—107 days after arrival—with black pitted larvae, the mothers of which were collected in Natal.

NOTE.—Black pitted larvae do not readily become attached on cattle, but nevertheless a small number were found hanging to the ears the following day.

Remarks.—Nothing unusual occurred in the temperature until about the 75th day, when a gradual rise was noted, developing into a typical curve and lasting until the 100th day; the fever was at its height between the 83rd and 92nd days, with evening records reaching 105° F. Anaplasms appeared with the rise of temperature, increasing and decreasing in numbers corresponding with the course of the fever; counting was not undertaken in this instance.

The symptoms of anaemia began with the appearance of anisocytosis, and on the 86th day polychromasia was noted. These symptoms developed in a marked manner, and were later accompanied with poikilocytosis and basophilia.

Nothing further happened with this animal, and in order to prove that it was a pure infection of anaplasmosis, it was tapped on the 23rd January, 1911, and 50 c.c. fresh blood was injected into heifer 1213. (For continuation of history of 930, see later.)

Test of Blood of Heifer 930.

(*B*) *Sussex Heifer* 1213.—An English heifer, arrived at the Laboratory on the 9th January, 1911, and was kept in a tick-free stable.

Treatment.—Injected as above (fourteen days after arrival).

Remarks.—A severe temperature reaction commenced on the 16th day, with evening rises to over 105° F., and lasting until the 30th day.

During this period anaplasms appeared in great numbers, and all the symptoms of anaemia developed to such an extent that, in addition to polychromasia and basophilia, nucleated cells appeared. The heifer showed visible symptoms of illness, with pale mucous membranes, refused to feed, frequently lay down, showed hurried respirations, and lost condition.

She was treated and eventually recovered.
(See Temperature Curve and Plates Nos. 1, 2, and 3.)

Heifer 930 (*continued*).—On the same day as this heifer was tapped for the injection of heifer 1213 (23rd January, 1911), 930 was infested with blue tick larvae, whose mothers were collected off cattle running at Onderstepoort.

Nothing unusual occurred until the 20th day, when a slight fever reaction occurred, during which *Spirochaeta theileri* were noticed.

On the 28th day another reaction set in, reaching 105° F. in the evening of the 34th and 36th day and returning to normal on the 40th day; *Babesia bigemina* appeared in rare numbers on the 29th day, becoming fairly frequent two days later; polychromatic and basophile cells were noted on the 35th day, and a slight anisocytosis with rare macrocytes appeared two days later.

NOTE.—The black pitted ticks transmitted a pure infection of anaplasmosis to heifer 930, and the blood of heifer 930, when subsequently injected into heifer 1213, also produced a pure infection. When heifer 930 was submitted to a subsequent tick infestation, *Babesia bigemina* appeared.

EXPERIMENT NO. 6.—*To convey anaplasmosis by means of blue ticks to susceptible heifers.*

ORIGIN OF BLUE TICKS.

NOTE.—Heifer 934. This animal had been used previously [*vide* Experiment No. 3 (*A*)], when, as a result of the injection of blood 917, it developed the disease due to *Anaplasma marginale*; *Babesia mutans* had also appeared. Later, as the result of a second injection with blood of heifer 926, heifer 934 developed an attack of babesiosis, it having been clearly shown that anaplasmosis was not complicated by babesiosis.

Accordingly I expected that by infesting this animal, previous to the appearance of *Babesia bigemina*, with blue ticks which were free of any infection, they would only become infected with anaplasmosis and would then transmit this disease alone, since in all my previous experiments I failed to convey a *Babesia mutans* infection by means of this tick.

On the 27th May, 1911, heifer 934 was infested with blue tick larvae collected from heifer 931 (heifer 931 was an English beast which had been infested on the 23rd February, 1910, with blue tick larvae collected from horses; no disease developed in 931 as a result of this infestation, proving that these blue larval ticks were clean).

The infestation of heifer 934 was a fairly heavy one; the engorged females dropped between the 21st and 29th days and were collected in great numbers.

(*A*) *Africander Calf* 1168.—Born in the Laboratory stables on the 7th November, 1910, and kept tick-free.

Treatment.—Infested on the 12th November, 1910, with the progeny of the blue ticks which had developed on heifer 934, and whose mothers had dropped from the 17th to 24th June, 1910. (Reference No. 322.)

Remarks.—Nothing unusual occurred after this infestation. The engorged ticks commenced to drop on the 24th day; the blood was occasionally examined during this time, but with negative results.

On the 52nd day a temperature reaction started, continuing for eighteen days. Although this reaction was well marked, with evening records of occasionally 104° F., it could not be called severe.

Anaplasma marginale appeared with the rise of temperature, being present in the greatest numbers at the onset of the fever, i.e. infesting 3·3 per cent. on the first day, 7·8 per cent. the 2nd day, 4·7 per cent. 4th day, 3·1 per cent. 5th day, 2·2 per cent. 6th day, 1·9 per cent. 7th day, 1·2 per cent. 8th day, and remaining at about 1 per cent. throughout the remainder of the reaction (*vide* Temperature Curve and Plates Nos. 4 and 5).

In addition to anisocytosis, the lesions of polychromasia and basophilia were noted on the 58th day; the infection was not heavy, and disappeared rapidly whilst the animal was recovering.

NOTE.—As was expected in this case, the *Anaplasma marginale* alone was transmitted by means of the ticks, proving that this parasite can be separated from *Babesia mutans* by blue ticks.

In order to prove that calf 1168 was a pure infection of anaplasmosis, unaccompanied by anything else, the calf was tapped and 5 c.c. fresh blood was injected into English heifer No. 1217.

Test of Blood of Calf 1168.

Heifer 1217 arrived at the Laboratory from England on the 9th January, 1911, and was immediately placed in a tick-free stable. The temperature remained within physiological limits during the time of observation.

Treatment.—Injected on the 25th January, 1911 (sixteen days after arrival) as above.

Remarks.—A temperature reaction set in from the 22nd day, with evening exacerbations to 105° F. on the 25th and 26th days, and returning to normal on the 31st day; *Anaplasma marginale* were noted on the 22nd day, increasing in numbers during the succeeding days.

On the 26th and 27th days the lesions of anisocytosis were noticed, and on the 28th day polychromatic and rare basophile cells were noted. A few nucleated cells were detected the following day, and from this date until the 44th day anaplasms were found in rare numbers, accompanied with rare polychromatic and basophile cells.

Test.—Injected on the 24th April, 1911, with 5 c.c. blood of heifer 1216, an imported heifer belonging to the same batch as 1217, and which developed a pure attack of redwater as the result of the injection of blood of heifer 926 on the 23rd January, 1911.

Remarks.—There was a sharp rise on the 6th day, reaching 104° F. and rising to 106° F. on the 8th day; *Babesia bigemina* were noted in fair numbers on the 7th day. The animal was injected with a 1 per cent. solution of trypan blue on the 8th day, and the temperature dropped to normal the following morning.

On the 14th day anisocytosis, macrocytes, slight basophile, and polychromatic cells were recorded.

NOTE.—The subsequent inoculation proves that the ticks transmitted a pure infection of *Anaplasma marginale.*

(*B*) *Sussex Heifer* 1218.—An English heifer, which arrived at the Laboratory on the 9th January, 1911; she was immediately placed in a tick-free stable and temperatured twice daily. No deviations from a normal record were noted.

Treatment.—Infested on the 25th January, 1911—sixteen days after arrival—with blue larval ticks from heifer 934 (Reference No. 322).

Remarks.—The adult females were collected from the 21st–28th day in great numbers; there was one sharp rise of temperature to 103·8° F. on the 22nd day, but blood examinations proved negative. Seventy days after infestation a slight but definite reaction, lasting about fifteen days, ensued. *Anaplasma marginale* were noted in the blood for the first time on the 70th day and remaining present throughout the reaction; anisocytosis, polychromatic, and basophile cells were noted at different intervals.

On the 24th April, 1911—eighty-nine days after the tick infestation—heifer 1218 was injected with 5 c.c. blood of heifer 1216 (immune to redwater, *vide* previous test).

Remarks.—A temperature reaction set in from the 8th day, and on the following three days microscopical examination of the blood showed the presence of *Babesia bigemina*. The animal was injected with trypan blue on the latter date, when the temperature returned to normal and the blood regained its healthy appearance.

Test of Blood of Heifer 1218.

NOTE.—In order to prove that heifer 1218 was a pure infection of anaplasmosis, it was tapped on the 24th April, 1911, and 10 c.c. fresh blood were injected into English heifer 1224.

Heifer 1224 arrived at the Laboratory from England on the 9th January, 1911, and was immediately placed in a tick-free stable. No abnormal temperatures were noted during this observation time.

Treatment.—Injected on the 24th April—105 days after arrival—subcutaneously with 10 c.c. blood of heifer 1218 (as above).

Remarks.—On the 18th and following days *Anaplasma marginale* were detected in the blood, coinciding with the fever reaction, which lasted from the 16th to 35th days.

The symptoms of anisocytosis, accompanied with polychromatic and basophile cells, were also noted during the reaction.

NOTE.—This case also proves that the infection was a pure one.

CONCLUSIONS.

Five English heifers which were susceptible to *Anaplasma marginale*, of which three, in addition, were susceptible to South African redwater (the other two having been inoculated against this latter disease in England), were infested with ticks, four with blue ticks and one with black pitted ticks. In every instance this infestation was followed by the appearance of anaplasmosis. For the two animals which were immune to South African redwater, I purposely used ticks known to be infected with *Babesia bigemina*. In one animal (922) the infection was typical, commencing on the 55th day, and in the other (925) the presence of the anaplasms was only noted at a later period. In the fourth and fifth animals infested with blue ticks (1168 and 1216), ticks exclusively infected with anaplasms as a result of feeding on an English heifer were used. In the case of the infestation with black pitted ticks, no other blood parasites besides anaplasms were transmitted.

In the previous experiments I have shown that the incubation time of anaplasmosis transmitted by ticks is rather a long one, varying from less than two months to over three months.

This experiment proves that by luck and a careful selection of both ticks and animals it is possible to obtain a pure infection of anaplasmosis.