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# **On the Nature of Anaplasma.**

**By P. J. DU TOIT, B.A., Dr.Phil., Dr. Med. Vet., Deputy-Director  
of Veterinary Education and Research, Onderstepoort.**

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## INTRODUCTION.

SINCE Theiler's description of *Anaplasma marginale* in 1910, quite a literature has grown up around the subject of Anaplasmosis. The problem which occupied most authors was the nature of the anaplasmata. Some (especially those who had never seen a case of anaplasmosis) held the view that these bodies were nothing else but artificial products or degenerative changes, if not normal constituents, of the red blood cells; others (especially the older authors who wrote before Theiler's work appeared) regarded the "marginal points" as a definite stage in the development of *Piroplasma bigeminum*; whereas the third group of authors supported the view, first advanced by Theiler, that the anaplasmosis of cattle was a disease *sui generis* and that *Anaplasma marginale* was a protozoan blood parasite responsible for this disease.

It is unnecessary for the purpose of this paper to review the entire anaplasma literature. This has been done by various writers; a full discussion of the literature up to 1918 can be found in Knuth's and Du Toit's "Handbuch der Tropenkrankheiten der Haustiere" (1921); the later publications are discussed by Helm (1924) and De Kock and Quinlan (1926).

The present paper concerns itself exclusively with the nature of the anaplasmata. As stated above, Theiler regarded them as true endoglobular blood parasites belonging to a distinct genus *Anaplasma*. It is now customary to place this genus in the family *Anaplasmatidae*, which, together with the *Babesidae*, the *Theileridae*, the *Plasmodiidae*, and the *Haemosporidae*, constitute the order *Haemosporidia*. Most protozoologists group the *Haemosporidia* with the *Coccidia* in the sub-class *Coccidiomorpha* of the class *Sporozoa*.

Soon after the discoveries of Theiler, some authors began to express their doubt whether these bodies were really protozoa. The arguments most commonly used were that the anaplasmata differed from all other protozoa in so much as they consisted of nuclear substance only and were entirely devoid of cytoplasm; and, further, that bodies similar to *Anaplasma marginale* could be found in perfectly healthy animals belonging to species ranging from the marsupials to the anthropoid apes. Prominent amongst these authors was Schilling-Torgau (1912), who made a minute study of the internal constitution of the mammalian erythrocyte and was able to demonstrate certain structures in it which, when suitably stained, bore a resemblance to the anaplasmata. By injecting guinea-pigs and cats with a poison like phenylhydrazin, Schilling-Torgau produced bodies in the red blood-corpuscles which he compared with the anaplasmata.

The work of Schilling-Torgau was followed up by Dias and Aragao (1914). These authors injected rats, guinea-pigs, rabbits, dogs, and cattle with a variety of poisons. The best results were obtained by injecting nitrobenzene, pyrogallie acid, or phenylhydrazin into rabbits or guinea-pigs; in the case of dogs pyrogallie acid proved most satisfactory, and in cattle trypan blue. The authors emphasize the importance of accurate dosage; if the quantity given is too small, no changes are observed in the blood; if too large, the animal dies before showing the typical picture. However, in cases where the doses were just big enough, Dias and Aragao observed anaemic changes in the blood with the appearance of small chromatin bodies in the red blood-corpuscles, which they regard as identical with the anaplasmata described by Theiler. In particular, do they refer to the results obtained in cattle by the injection of trypan blue. Since this drug is extensively used in the treatment of piroplasmosis of cattle in South Africa and elsewhere, they suggest that many cases of so-called anaplasmosis may be due to the injection of trypan blue. In this connexion it should be pointed out that Theiler's original observations on *Anaplasma marginale* were made before the introduction of trypan blue as a cure for piroplasmosis in cattle.

In the appendix to their article, Dias and Aragao give the experimental record of an eighteen months' old calf which was treated with trypan blue. In view of the importance of this experiment and in order to facilitate a comparison with the experiments described below, it seems justified to give a full summary of the case:—

- 1st day: Intravenous injection of 1.5 gm. trypan blue in 150 c.c. distilled water.
- 2nd to 9th day: Same dose repeated daily.
- 12th day: First appearance of anaplasmata in the blood.
- 13th day: 1.5 gm. trypan blue. Anaplasmata more frequent.
- 14th and 15th day: 2 gm. trypan blue in 150 c.c. water.
- 19th to 21st day: 2.5 gm. trypan blue in 150 c.c. water.
- 26th day: 3 gm. trypan blue. Anisocytosis, few anaplasmata.
- 27th day: 3.5 gm. trypan blue.
- 28th day: Basophilia, polychromatophilia, normoblasts. Anaplasmata very rare.
- 29th day: 4 gm. trypan blue.
- 30th day: Disappearance of basophilia and normoblasts. Anaplasmata rare.
- 39th day: Anaplasmata rare. Animal is emaciated and weak.
- 48th day: Advanced cachexia. Anaplasmata more frequent.
- 49th day: Anaplasmata increasing in number.
- 50th day: Animal dies. Advanced cachexia. Mucous membranes blue. Anaplasmata in heart-blood.

It will be seen that this animal received altogether 39 gm. of trypan blue.

The experiments of Dias and Aragao with other animals (guinea-pigs, rabbits, dogs) need not be quoted in detail, but will be referred to in the discussion of the results of similar experiments carried out at Onderstepoort.

The main conclusions of Dias and Aragao are—

- (1) that “anaplasma” is not a protozoan, but a degenerative product of the red blood-corpuscles, which can be produced by various haemolytic poisons; and
- (2) that “anaplasmosis” is not a separate disease, but merely a clinical form of piroplasmosis.

This second conclusion need hardly be discussed here. Volumes of evidence have been brought forward to show that anaplasmosis is a disease *sui generis* and does not stand in any direct relation to piroplasmosis of cattle. Amongst the authors who, in recent years, have brought additional evidence in support of this view may be mentioned Lignières (1914 and later) in South America, Brumpt (1920) in France, Sergent and his co-workers (1924) in North Africa, Helm (1924) in Germany, De Kock and Quinlan (1926) in South Africa.

The first conclusion of Dias and Aragao, however, still merits serious discussion. Their view has been accepted by a number of authors, and the experimental work upon which it was based has been regarded as a refutation of Theiler's conception of anaplasmosis. It is because of the important rôle which this article of Dias and Aragao has played in the anaplasma controversy that it has been quoted at such length. The later work on similar lines by Laveran and Franchini (1914), Finzi and Campus (1916), Di Domizio (1919), and Helm (1924) need not be quoted here.

Most of the experiments described in the present paper were undertaken with a view to test the accuracy of Dias' and Aragao's conclusions. The work has been conducted throughout in close co-operation and consultation with my esteemed chief, Sir Arnold Theiler, to whom my sincerest thanks are due.

#### FIRST SERIES OF EXPERIMENTS.

*Object of the experiments.*—To observe the changes produced in the blood of cattle by the injection of trypan blue and other chemicals, and to compare the changes, if any, so produced with the blood-picture in true anaplasmosis.

##### *Experiment 1.*

Two calves were selected, a two-and-a-half years old true hermaphrodite calf 316 and a young five-and-a-half months old female calf 426. These two animals received increasing doses of trypan blue (the size and frequency of the doses are given in the appendix, page 174). It will be noted that the initial doses were comparatively small, corresponding to the no mal dose which would be administered in the case of a *Piroplasma bigeminum* infection to calves of the respective ages mentioned. Calf 316 first received 150 c.c. of a 1 per cent. solution and calf 426, 50 c.c. The dose was gradually increased to 400 c.c. in the case of calf 316, whereas in the case of the smaller animal the maximal dose was 75 c.c.

Altogether calf 316 received ten injections, amounting to 3,250 c.c. 1 per cent. solution or 32.5 gm. of trypan blue, and calf 426 thirteen injections, amounting to 8.5 gm. of the drug.

*Result.*—During the course of the experiment, calf 316 showed a comparatively high temperature (105–105.8° F.) on three occasions, the 9th

14th, and 20th day. On each occasion the temperature returned to normal within two days. Blood smears examined on these and other days failed to reveal any parasites. Anaplasmata or bodies resembling anaplasmata were never seen. It is also remarkable that, in spite of the large quantities of trypan blue, the blood remained normal throughout the experiment; degenerative or regenerative changes (basophilia, poikilocytosis, normoblasts, etc.) were never observed. Figure 1 shows a microphotogram of the blood of calf 316, which was taken on the 34th day of the experiment, i.e. two days after the last injection of 400 c.c. trypan blue solution. It will be noticed that there is not even an anisocytosis present.

The result of the injections of trypan blue in calf 426 stands in marked contrast with that obtained in 316. On the 12th day of the experiment, after calf 426 had received four injections of  $\frac{1}{2}$ -gram trypan blue each, parasites were observed in the blood. Both *Anaplasma marginale* and *Gonderia mutans* appeared on this day. These parasites persisted in the blood for a considerable time, without producing any serious symptoms. This is in accordance with the usual observation that the *Anaplasma marginale* and *Gonderia mutans* infections run a relatively mild course in young calves. The temperature of calf 426 was scarcely ever above the normal. There was a slight anisocytosis, but no other blood changes.

*Discussion.*—There are two outstanding features in this experiment: (1) The difference between the results obtained in the two calves, and (2) The fact that *Anaplasma marginale* actually appeared in calf 426 after the injection of trypan blue.

It will be convenient to start with the second point. At first sight it would appear that our experiment lends support to the view of Dias and Aragao, that *Anaplasma marginale* can be produced by injection of trypan blue. However, in calf 426 it so happened that another parasite, *Gonderia mutans*, appeared together with *Anaplasma marginale*, and it is not likely that anybody would seriously contend that the former parasite was also produced by the trypan-blue injection. How then can we account for the appearance of these parasites? The explanation seems obvious: Calf 426 must have had a previous infection of the two parasites named, and the intoxication produced by the trypan blue brought about a recrudescence of the infection.

If this explanation is correct, we would have to assume, in order to account for the different behaviour between the two calves (point one, above), that calf 316 had never had an infection of *Anaplasma marginale* or *Gonderia mutans*. To test the correctness of this assumption, it is necessary to explain the earlier history of these calves.

Calf 426 had been injected with blood of a heifer recovered from anaplasmosis on the 9th November, 1922, when it was only four days old. After the lapse of ten days it showed a febrile disturbance suggestive of a piroplasmosis, but the parasites were not demonstrated. On the 38th day after the injection the temperature again showed a disturbance, lasting about ten days with a maximum of 105·6° F. on one occasion. With the rise of the fever, anaplasmata were noted in the blood. They increased in number and towards the end of the fever disappeared, and only the lesions of anisocytosis were registered. On the 58th day, however, *Gonderia mutans* made its appearance and was present in fairly large numbers.

Calf 316 was sent to Onderstepoort by the Uitval Hereford Farm at Vereeniging, a district which is practically free from anaplasmosis, as an interesting specimen showing externally both male and female characters. The calf was 15 months old at the time of its arrival and was kept during its entire stay at Onderstepoort in a stable reserved for redwater and gall-sickness vaccine cattle. All bedding and foodstuffs used in this stable are sterilized in a steam autoclave so as to prevent the introduction of ticks and the accidental infection of the vaccine cattle with tick-borne diseases. Calf 316, therefore, had every opportunity of escaping such infection, and the result of Experiment 1 above would seem to prove that it actually did escape.

*Conclusion.*—From the above data it would seem justified to conclude that the injection of trypan blue may bring about a relapse of anaplasmosis (and *Gonderia mutans* infection). In animals which have never had these diseases the injection, even of very large doses of trypan blue, produces no changes in the blood.

### *Experiment 2.*

*Object.*—To test the accuracy of the conclusion arrived at in Experiment 1, namely that trypan blue will produce no change in the blood of calves known to be free from anaplasmosis and other blood infections.

*Method.*—Two young calves, 607 and 709, which, since birth, had been kept in the stable referred to above, and thus kept free from all tick-borne diseases, were given increasing doses of trypan blue solution. In the appendix, details of the injections can be found. Both calves were treated in the same way. They received twenty-six injections each, beginning with  $\frac{1}{2}$  gm.; the dose was increased to  $\frac{3}{4}$ , then 1, then  $1\frac{1}{2}$ ,  $2\frac{1}{2}$ , 3, 4, and finally 5 gm. This latter must be considered an enormous dose for so small a calf. Each of the two calves received  $36\frac{1}{2}$  gm. altogether.

*Result.*—On the 9th day after the first injection, both calves showed a high temperature (above  $106^{\circ}$  F.) which, however, subsided after two or three days. This is apparently the same phenomenon which was observed in calf 316 (Experiment 1) and would seem to be due to the toxic action of the trypan blue. As soon as the organism accustoms itself to the drug the temperature returns to normal.

During the fever period and throughout the course of the experiment, the blood of the two calves was examined regularly. Parasites were never observed, neither did the blood show any noticeable morphological change.

*Conclusion.*—Trypan blue as such, even when injected in very large quantities, does not produce any change in the blood of healthy calves.

In view of the great importance of the results obtained in Experiments 1 and 2, it was decided to repeat the test once more.

### *Experiment 3.*

*Object.*—To observe again the blood changes, if any, produced by the injection of trypan blue in calves which had passed through an anaplasmosis (and possibly other) infections.

*Method.*—As carriers of anaplasmosis, two calves were selected (424 and 755) which were supposed to have had this disease. Calf 755 had been injected on the 8th September, 1924 (i.e. about two months before the beginning of the present experiment), with blood of another calf (924) which, before death, had had *Piroplasma bigeminum* and *Gonderia mutans* in its blood, but had shown symptoms which seemed to indicate heartwater as the cause of death. The blood-inoculation into calf 755 was really made for the purpose of confirming or excluding the diagnosis heartwater. Calf 755 showed *P. bigeminum* about a week and *G. mutans* about a fortnight after the injection. Its temperature reaction was again suspicious of heartwater and it was at this stage (23rd September, 1924) that its blood was injected into calf 424. About another fortnight after this subinoculation, calf 755 showed *Anaplasma marginale* in its blood (see Figure 2) with slight fever and marked anaemic changes (Figure 3). However, it made a rapid recovery as was to be expected in so young a calf. The blood of calf 424 was not examined microscopically since the inoculation had been made for the sole purpose of confirming or excluding the diagnosis heartwater. It is therefore impossible to say with certainty whether any of the above-named parasites appeared in its blood. It is, however, important to note for the purpose of our present experiment that only in the blood of calf 755 was *A. marginale* actually observed, and that its blood was injected into calf 424 about a fortnight before this parasite appeared.

The history of these two animals has been given in some detail because it is essential for the interpretation of the results of the present experiment.

The two calves (424 and 755) received increasing doses of trypan-blue solution.

Calf 424 received an initial dose of 1 grm., which was increased to 1½, 2, and 2½ grm. In all fourteen injections were given amounting to 23 grm. trypan blue.

Calf 755 received the same initial dose which was ultimately increased to 3 grm. Fifteen injections, totalling 30½ grm. trypan blue, were given.

*Result.*—On the 10th day of the experiment *Anaplasma marginale* and *Gonderia mutans* appeared in the blood of calf 755, the temperature still being normal. From the 16th to the 19th day the animal showed a distinct temperature reaction with both parasites still present in the blood. Apart from anisocytosis no morphological changes were observed in the blood (see Figure 4). The two parasites persisted for a considerable time, the animal being otherwise normal.

Calf 424 first showed *Gonderia mutans* on the 11th day of the experiment. The parasite was still present on the 83rd day, but produced practically no changes in the blood. A slight elevation of temperature now and then must probably be ascribed to the trypan blue rather than to the *G. mutans* infection.

*Discussion.*—The results obtained in the case of calves 424 and 755 harmonize very well with the earlier history of these two animals. 755 was known to have had an infection of *P. bigeminum*, *G. mutans*, and *A. marginale*. *P. bigeminum* could not reappear, because trypan blue has a specific effect on this parasite and would, in the enormous quantities

administered, easily prevent a recrudescence of this disease. On the other hand, *Gonderia mutans* and *Anaplasma marginale* infections can, as observed in Experiment 1, be provoked by the injection of large quantities of trypan blue. Here, then, we have again a relapse of anaplasmosis under the influence of trypan blue.

Calf 424 showed only *G. mutans*. In the analysis of its previous history above, it was shown that this calf was previously injected with blood in which only *P. bigeminum* and *G. mutans* had been found up to the date of inoculation. (*Anaplasma marginale* appeared in the blood of the donor about a fortnight later.) It was thus to be expected that only *G. mutans* would reappear under the influence of trypan blue, and this actually happened.

*Conclusion.*—This experiment again proved that the injection of large quantities of trypan blue can produce a relapse of anaplasmosis and other blood infections (*Gonderia mutans*). In animals which have not had an *Anaplasma marginale* infection, this parasite cannot be produced by the injection of trypan blue.

#### *Experiment 4.*

*Object.*—To compare the results obtained by the injection of pyrogallie acid into calves with the results obtained with trypan blue.

The experiment was so arranged that the result obtained with trypan blue (namely that the drug would produce a relapse in calves which had previously had an infection of *anaplasmosis* or *gonderiosis*, but no change in calves free from such infection) would also be tested with pyrogallie acid.

Two calves were therefore selected, one of which (No. 928) was known to have had an *Anaplasma marginale* infection in October, 1924 (i.e. one month before the present experiment), whereas the other (No. 1026) belonged to a group of calves brought from the Karroo, a country free from such infection.

*Method.*—Since pyrogallie acid is far more toxic for cattle than trypan blue, it was found necessary to calculate the size of the dose accurately. In dogs and rabbits (see below) 0·1 gramme pyrogallie acid per kilogram bodyweight was found to be a fairly safe dose, and the same was accordingly applied to cattle.

Both calves received intravenous injections of pyrogallie acid corresponding to 0·1 grm. acid or 0·2 c.c. of the 50 per cent. solution per kilogram bodyweight. The injections were repeated at intervals of one week or less. Calf 928 received altogether six injections amounting to 120 grm. pyrogallie acid, and calf 1026 nine injections amounting to 153 grm. acid.

*Result.*—In calf 928 anaemic changes of the blood (anisocytosis) were observed on the 6th day of the experiment. On the 10th day, after the second injection of pyrogallie acid, these changes were very marked, the blood showing polychromasia, basophilia, jolly bodies, normoblasts, etc. After two further injections oligocytosis and poikilocytosis were observed in addition to the changes already noted. *Anaplasma marginale* appeared on the 10th day and *Gonderia mutans* on the 13th day of the experiment. The animal made a complete recovery.



Calf 1026 showed the same blood changes as 928. It is interesting to note that in spite of the continued injection of pyrogallic acid the anaemic changes gradually disappeared, so that after the eighth and ninth injections the blood was practically normal.

*Conclusion.*—Pyrogallic acid proved to be a far more active blood poison for cattle than trypan blue. Marked anaemic changes appeared in both calves after the injection of relatively small quantities. The body seems to “accustom” itself to the toxin fairly rapidly.

In a calf with a previous record of anaplasmosis, pyrogallic acid produced a relapse, whereas in the control calf no parasites appeared.

### RÉSUMÉ.

In order to facilitate reference, the results of the above series of experiments have been summarized in the following table:—

Exp. No.	Calf No.	Age.	Previous History.	Treatment.			Result.	Remarks.
				Drug.	Number of Injections.	Total Quantity.		
1	316	2½ years	Kept in tick-free stable; presumably free from blood parasites	Trypan blue	10	32½ grm.	Negative	
	426	5½ months	Previously infected with <i>A. marginale</i> and <i>G. mutans</i>	Trypan blue	13	8½ grm.	Infection of <i>A. marginale</i> and <i>G. mutans</i> with anaemia.	Obviously a breakdown in immunity.
2	607	4½ months	Kept in tick-free stable since birth	Trypan blue	26	36½ grm.	Negative	
	709	2½ months	Kept in tick-free stable since birth	Trypan blue	26	36½ grm.	Negative	
3	755	1½ years	Previous infection of <i>A. marginale</i> , <i>P. bigeminum</i> , and <i>G. mutans</i>	Trypan blue	15	30½ grm.	Infection of <i>A. marginale</i> and <i>G. mutans</i>	Obviously a relapse.
	424	2 years	Previous infection of <i>P. bigeminum</i> and <i>G. mutans</i>	Trypan blue	14	23 grm.	Infection of <i>G. mutans</i>	Relapse.
4	928	2 years	Previous infection of <i>A. marginale</i>	Pyrogallic Acid	6	120 grm.	<i>A. marginale</i> and <i>G. mutans</i> infection with anaemic blood changes	Relapse.
	1026	1 year	Brought from a district supposed to be comparatively free from tick-borne diseases	Pyrogallic Acid	9	153 grm.	Anaemic blood changes, otherwise negative	

### SECOND SERIES OF EXPERIMENTS.

*Object.*—To determine whether in the smaller mammals (dogs, rabbits, guinea-pigs) changes can be produced by the injection of blood poisons which can be compared with the blood-picture observed in anaplasmosis of cattle.

*Experiment 5.*

*Object.*—To observe the blood-picture produced in dogs by the injection of pyrogallic acid.

*Method.*—As observed by Dias and Aragao (see above), it was found to be very difficult to adjust the dose of the more active blood poisons accurately. Too small a dose yields no result, too large a dose kills the animal before any results are obtained.

Dog 158 first received 0.1 gm. pyrogallic acid per kilogram body-weight, then two days later 0.15 gm., and another four days later 0.2 gm. per kilogram. It died on the 10th day of the experiment.

Dog 139 received 0.1 gm., then two days later the same dose, and another two days later 0.2 gm. per kilogram bodyweight. It died two days after the third injection.

In the case of dog 157, the injections were given at longer intervals, as can be seen from the Appendix. In this way the dog seems to have accustomed itself better to the poison and withstood an injection of 0.4 gm. per kilogram on the 18th day without showing marked changes. When the dose was again doubled on the 28th day the dog died.

*Results.*—In all three dogs the pyrogallic acid produced very noticeable changes in the blood. One of the first changes to be observed was a marked leucocytosis. The red blood corpuscles showed anisocytosis, poikilocytosis, oligochromasia, polychromasia, basophilia, Jolly bodies, normoblasts, etc. Figure 5 shows the blood of dog 158 just before death.

In the blood of these dogs Jolly bodies were to be found which occasionally bore a resemblance with *Anaplasma marginale* of cattle. In Figure 5 one such body can be seen near the edge of the picture. They were, however, never so frequent as in the cases observed by Dias and Aragao. De Kock and Quinlan (1926) have shown how Jolly bodies can be distinguished from true anaplasmata. In isolated blood-smears there may be some doubt about the nature of such bodies, but if the blood of an animal is examined daily there can never be the slightest doubt whether it is a case of anaplasmosis or not.

*Conclusion.*—Pyrogallic acid produces a severe anaemia in dogs with all the characteristic blood changes. Jolly bodies which in individual specimens could be mistaken for anaplasmata appear in the blood.

*Experiment 6.*

*Object.*—To observe the blood-picture produced in rabbits by the injection of nitrobenzene.

*Method.*—Rabbit 1 was injected with 0.1 gm. nitrobenzene per kilogram bodyweight; two days later it received double the amount (0.2 gm. per kg.), which dose was repeated four days later without fatal result.

Rabbit 3 also received 0.1 gm. per kg., the following day the same dose, and the next day double this dose. This latter dose was again doubled

(0.4 gm. per kg.) two days later, and the injection repeated after intervals of four and seven days. A few hours after the last injection the rabbit died. Altogether it had received about  $4\frac{1}{2}$  gm. nitrobenzene.

Rabbit 4 received 0.1 gm. per kg. on the 1st and 2nd day, 0.2 gm. on the 3rd, 0.4 gm. on the 5th, and 0.5 gm. on the 12th day. A few hours after the last injection it died, having received 4.1 gm. nitrobenzene altogether.

*Results.*—In rabbit 1 the amount of nitrobenzene was scarcely enough to produce marked changes. Polychromasia, basophilia, and a few normoblasts were observed for a time, and the blood-picture then returned to no mal. This rabbit had only received a little over 1 gm. nitrobenzene. The other two rabbits which had received more than four times this amount showed very severe changes in the blood: leucocytosis, oligocythaemia, anisocytosis, basophilia, Jolly bodies, normoblasts.

*Conclusion.*—Nitrobenzene, if injected in sufficient quantity, produces a severe and typical anaemia in rabbits. Blood-pictures resembling anaplasmosis in cattle were not observed.

#### *Experiment 7.*

*Object.*—To observe the blood-picture produced in rabbits by the injection of phenylhydrazin.

*Method.*—Rabbit 2 was injected with 0.1 gm. phenylhydrazin per kg. bodyweight, and two days later with double this dose. Soon after the second injection the rabbit died.

The dose was thereupon reduced considerably, and rabbit 5 received 0.01 gm. per kg. bodyweight. The next day the same dose was given and the following day the dose was doubled. Two days later the latter quantity was again doubled (0.04 gm. per kg.), but the day after that the rabbit died.

In the case of rabbit 6 the dose was increased from 0.02 gm. per kg. bodyweight to 0.08 gm., the rabbit dying soon after the last injection.

In rabbit 7 the injections were given at longer intervals, so that the animal could accustom itself better to the poison. This rabbit lived for more than a month after the first injection, during which time it received more than 1 gm. phenylhydrazin.

*Result.*—As in the case of the rabbits injected with nitrobenzene (Experiment 6), the typical blood-picture of a severe anaemia was observed in the rabbits injected with phenylhydrazin. Figure 6 shows a photogram of the blood of rabbit 7 taken on the 32nd day of the experiment.

Here, again, occasional Jolly bodies were seen, which could be mistaken for anaplasmata. One such body appears in Figure 6 near the edge. Pictures such as those published by Dias and Aragao were, however, never seen.

*Conclusion.*—Phenylhydrazin when injected into rabbits produces typical anaemic changes of the blood.

*Experiment 8.*

*Object.*—To observe the blood-picture produced in guinea-pigs by the injection of nitrobenzene or phenylhydrazin.

*Method.*—Only two guinea-pigs were used, one being injected with nitrobenzene and the other with phenylhydrazin. Guinea-pig 1 received an initial dose of 0.1 grm. nitrobenzene per kg. bodyweight. Two days later it received 0.2 grm. and four days later again 0.2 grm. per kg., without fatal results.

Guinea-pig 2 was injected with 0.1 grm. phenylhydrazin per kg. bodyweight and two days later with a double dose. Soon after the second injection the animal died.

*Result.*—In guinea-pig 1 a slight anaemia with polychromasia and a few Jolly bodies was observed. Guinea-pig 2 died before any blood changes had been seen.

*Conclusion.*—Nitrobenzene produces the usual anaemic changes in the blood of guinea-pigs.

## THIRD SERIES OF EXPERIMENTS.

*Object.*—To investigate the nature of anaplasma by means of experiments which will show whether the "virus" of anaplasmosis is contained in the red blood-corpuscles, whether it can be destroyed by haemolysis, and whether in haemolysed blood it will pass through a bacterial filter.

*Experiment 9.*

*Object.*—To determine whether the "virus" of anaplasmosis can be destroyed by means of distilled water, and whether a Berkefeld filtrate of such haemolysed blood is still virulent.

*Method.*—Ox 1077, which at the time was suffering from a severe attack of anaplasmosis and showed numerous *Anaplasma marginale* "parasites" in its blood was bled; the blood was defibrinated and used in the present series of experiments as the "virus" of anaplasmosis.

All the calves used in these experiments were very young and had been kept since birth in tick-free stables; they were therefore, without exception, susceptible to anaplasmosis and other tick-borne diseases.

In Experiment 9 a quantity of defibrinated blood of ox 1077 was mixed with an equal quantity of distilled water. After complete haemolysis, 20 c.c. of the mixture was injected into calf 1052.

Another portion of the haemolysed mixture was passed through a Berkefeld candle and 20 c.c. of the filtrate injected into each of two calves 1099 and 1101.

The control calf 1066 received 10 c.c. of the fresh defibrinated blood of ox 1077.

*Result.*—Calf 1052, which received the haemolysed unfiltered blood, developed a typical infection of *Piroplasma bigeminum* and *Anaplasma marginale*.

Calves 1099 and 1104, which received the filtrate, remained healthy; parasites were never found in their blood.

The control calf 1066 also developed the double infection of *P. bigeminum* and *A. marginale*.

*Conclusion.*—Haemolysis with distilled water does not destroy the virus of anaplasmosis, nor does it kill *Piroplasma bigeminum*.

A Berkefeld filtrate of such haemolysed blood does not contain the virus of either disease.

#### *Experiment 10.*

*Object.*—To determine whether the virus of anaplasmosis can be destroyed by haemolysis with a haemolytic serum, and whether a Berkefeld filtrate of such haemolysed blood contains the virus.

*Method.*—Haemolytic serum was prepared in a horse (16234) by means of repeated injections of large quantities of washed cattle red blood-corpuscles (see appendix). The serum of the horse was tested from time to time and was found to produce complete haemolysis of cattle red blood-corpuscles in a dilution of 1 in 8.

A quantity of defibrinated blood of ox 1077 was mixed with one-and-a-half times the quantity of serum of horse 16234. After complete haemolysis, 20 c.c. of the mixture was injected into calf 1101.

The remainder of the mixture was passed through a Berkefeld candle and 20 c.c. of the filtrate injected into calf 1073.

*Result.*—Twenty days after the injection calf 1101, which received the unfiltered haemolysed blood, showed *Anaplasma marginale* in its blood. It went through a typical mild reaction, such as one would expect in so young a calf.

The blood of calf 1073, which received the filtered liquid, remained negative and its temperature normal.

*Conclusion.*—Haemolysis with haemolytic serum does not destroy the virus of anaplasmosis.

A Berkefeld filtrate of the haemolysed blood does not produce anaplasmosis.

#### *Experiment 11.*

*Object.*—To determine whether the virus of anaplasmosis can be removed from the blood by washing the red blood-corpuscles repeatedly.

*Method.*—A quantity of defibrinated blood of ox 1077 was allowed to stand and the red blood-corpuscles collected. These were mixed with normal saline solution in the usual way and centrifuged. The process was repeated six times so as to remove all traces of serum.

After six washings 5 c.c. of the blood-corpuscles was injected into each of the two calves 1062 and 1067.

*Result.*—Calf 1062 showed both *Piroplasma bigeminum* and *Anaplasma marginale* in its blood on the 9th day after the injection. In the case of calf 1067, *P. bigeminum* appeared on the 13th day and *A. marginale* on the 18th day. The infections in both cases took a normal, mild course.

*Conclusion.*—The virus of anaplasmosis cannot be removed from the blood by washing the blood-corpuscles; it would therefore seem to be contained in the blood-corpuscles.

It may again be convenient to have the results of the above series of experiments in tabular form.

Experiment No.	Calf No.	Age.	Injected with.	Filtered or Unfiltered.	Result.	Remarks.
9	1052	2½ months	20 c.c. haemolysed virulent blood (Haemolysis by means of distilled water)	Unfiltered	<i>A. marginale</i> and <i>P. bigeminum</i> infection	
	1099	1 month	20 c.c. haemolysed virulent blood (Haemolysis by means of distilled water)	Filtered	Negative	
	1104	3 weeks	20 c.c. haemolysed virulent blood (Haemolysis by means of distilled water)	Filtered	Negative	
	1066	1 month	10 c.c. virulent blood	Unfiltered	<i>A. marginale</i> and <i>P. bigeminum</i> infection	Control calf.
10	1101	1 month	20 c.c. haemolysed virulent blood (Haemolysis by means of specific haemolytic horse serum)	Unfiltered	<i>A. marginale</i> infection	
	1073	1½ month	20 c.c. haemolysed virulent blood (Haemolysis by means of specific haemolytic horse serum)	Filtered	Negative	
11	1062	2 months	5 c.c. virulent red blood corpuscles freed from serum by repeated washings	Unfiltered	<i>A. marginale</i> and <i>P. bigeminum</i> infections	
	1067	2 months	5 c.c. virulent red blood corpuscles freed from serum by repeated washings	Unfiltered	<i>A. marginale</i> and <i>P. bigeminum</i> infections.	

#### GENERAL DISCUSSION OF RESULTS.

It has been stated in the introduction that the main purpose of the above experiments was to test the accuracy of Dias' and Aragao's conclusion, namely, that "anaplasma" is not a protozoan, but a degenerative product of the red blood-corpuscles, which can be produced by various haemolytic poisons, notably by means of trypan blue in cattle.

In the *first series of experiments* a number of calves were accordingly subjected to this treatment, and the very first experiment yielded a result which seemed to throw entirely new light on the experiment which Dias and Aragao carried out with a calf, and which has since played such an important rôle in the anaplasma literature, and has therefore been quoted in full in the introduction (page 158). It will be recalled that these authors injected large quantities of trypan blue into a calf and then observed numerous anaplasmata in the blood. The same procedure was followed in our experiment: two calves were injected with increasingly large doses of trypan blue; one of them developed a typical attack of anaplasmosis (like Dias' and Aragao's case), the other not, its blood remaining normal

throughout the course of the experiment. The first calf had previously had an infection of *Anaplasma marginale* and had to be regarded as a "virus reservoir." The obvious interpretation of the result seemed to be therefore that the trypan-blue injections had simply brought on a relapse of anaplasmosis in the first calf. Further support was lent to this view by the observation that during this relapse the calf showed not only *Anaplasma marginale* in its blood, but also *Gonderia mutans*, an unquestioned protozoan parasite which could not very well be regarded as a degenerative product of the blood.

It would appear, therefore, that the experiment of Dias and Aragao has to be interpreted on the same basis. If we assume that their calf (2) had previously had anaplasmosis, then the appearance of anaplasmata in its blood after the trypan-blue injections must be regarded as a simple breakdown in immunity due to the toxic action of the drug.

Our further experiments adduced more evidence in favour of this view. Calves which were known to be free of anaplasmosis were injected with large quantities of trypan blue without producing any changes in the blood.

On the other hand, calves which were known to have had anaplasmosis or other blood infections (gonderiosis), when injected with trypan blue, showed relapses of the particular parasites which they previously had. In this connexion it must be remembered that a previous infection of *Piroplasma bigeminum* cannot be expected to reappear under the influence of trypan blue, since this drug has a specific destructive action on *P. bigeminum*.

The result obtained by Dias and Aragao can theoretically be explained in two ways (in addition to their own explanation): (1) that the calf had had a previous infection of anaplasmosis, which infection showed a relapse under the provoking influence of trypan blue; or (2) that the calf was accidentally infected with anaplasmosis in the course of the experiment.

The first explanation is the more likely. In countries like South Africa and South America, where anaplasmosis is a very widespread disease, few calves escape infection in their early youth. Usually the infection takes a mild course, and if such calves are examined later (e.g. at the beginning of an experiment such as those discussed here) their blood appears perfectly normal.

Similar results to those obtained with trypan blue were also obtained with pyrogallie acid. A calf that had previously had an infection of anaplasmosis and gonderiosis showed a relapse after the injections, whereas the blood of a calf which had not had these infections remained free from parasites. Pyrogallie proved to be a far more active blood-poison than trypan blue, severe anaemic changes appearing after injections of the former, whereas the blood remained practically normal even after the injection of enormous quantities of trypan blue into healthy calves.

The severe blood changes which Dias and Aragao observed in their calf (see p. 158) must undoubtedly, in the light of our experiments, be ascribed to the relapse of anaplasmosis and not to the action of the trypan blue.

In the *second series of experiments* the work of Dias and Aragao on small mammals (dogs, rabbits, and guinea-pigs) was repeated. The best results were obtained with pyrogallic acid in dogs, and nitrobenzene and phenylhydrazin in rabbits. Very severe anaemia with all the characteristic changes (oligocythaemia, anisocytosis, poikilocytosis, polychromasia, basophilia, normoblasts, Jolly bodies, etc.) appeared in the blood. However, in no instance was a blood-picture observed which could be mistaken for anaplasmosis. The Jolly bodies which appeared certainly resembled anaplasmata in some cases, but no person with a knowledge of anaplasmosis in cattle would ever be in doubt about the diagnosis. Never were Jolly bodies observed in such numbers as are shown in the illustrations to the article of Dias and Aragao.

For a fuller discussion of the essential differences between Jolly bodies and anaplasmata, the article of De Kock and Quinlan (1926) should be consulted.\*

The *third series of experiments* was intended to cast further light on the nature of the anaplasmata. The first series had proved to our satisfaction that anaplasmata could not be produced in the blood of cattle by means of the drugs employed. On the contrary those experiments brought additional evidence of the specificity of the anaplasmata, and proved afresh that *Anaplasma marginale* appeared in the blood only in conjunction with the disease "anaplasmosis."

A further question required investigation, namely, whether these "marginal points" were the actual parasites responsible for the disease or merely the *product* of the disease. This latter view would receive support if it could be shown that in the etiology of anaplasmosis a filterable virus played a rôle. In that case it would be reasonable to explain the anaplasmata as a *symptom* of the disease produced by the action of the "anaplasmosis virus" on the red blood-corpuscles. All the other known facts about the disease could be brought into harmony with this view. It may be mentioned here that in their textbook on the tropical diseases of domestic animals Knuth and Du Toit (1921) advocated this view.

However, the experiments outlined above proved conclusively that the "virus" of anaplasmosis does not pass through a Berkefeld candle. In earlier experiments Theiler had also carried out filtration experiments with negative results, but the objection has since been raised that the "virus" might be included in the blood-corpuscles in such a way that it is only liberated when the blood-corpuscles are destroyed. Accordingly, in our experiments virulent blood containing anaplasmata was haemolysed both with distilled water and with haemolytic horse serum, and the haemolysed product filtered through a Berkefeld candle. Calves which received this filtrate remained healthy, whereas the control calves which received the unfiltered product contracted the disease in the typical way.

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\* In this connexion it may be mentioned that Aragao and Dias submitted smears of their cases to Theiler for an expression of opinion. In his absence at the time in Europe (1912-13), Mitchell submitted the smears to an examination and replied to the two authors that the smears did not show anaplasmata at all, but only nuclear debris (Jolly bodies). On the return of Theiler, the smears were examined by the various officers of the Division, and nobody had any doubt about the nature of these artefacts.



In another experiment red blood-corpuscles containing anaplasmata were washed repeatedly so as to free them from all traces of serum or other substances which might adhere to them. These blood-corpuscles were then injected into susceptible calves, which contracted anaplasmosis in the normal way.

This latter experiment indicated definitely that the "etiological factor" of anaplasmosis is *contained in the red blood-corpuscles*; and the preceding experiments showed that if such blood-corpuscles are broken up by haemolysis, the liberated "etiological factor" will not pass through an ordinary bacterial filter, thus proving that it must be of appreciable size.

The simplest and most reasonable explanation for these facts seems to be that the anaplasmata themselves which are contained in the virulent red blood-corpuscles represent the etiological factor and as such must be regarded as true blood parasites. All other explanations which have been advanced seem far-fetched and forced.

It is true the view advanced by Knuth and Du Toit has not been entirely refuted, and it is difficult to see how it could ever be entirely refuted. But the above experiments have certainly brought an additional argument against this view, namely, that if anaplasmosis is caused by an ultra-visible virus it cannot be a filter-passing virus. The only remaining possibility is, therefore, that it must be an ultra-visible non-filterable virus which causes anaplasmata to appear in the blood.

One consideration seems to us to speak against this "virus view." If the anaplasmata had to be regarded merely as a *symptom* of the disease, it would be reasonable to assume that occasionally a case must arise where this particular symptom is absent. However, such a case has never been described. At the Onderstepoort Laboratory, in addition to the hundreds of cases of anaplasmosis observed in South African cattle, about 150 imported pure-bred cattle have, in the course of sixteen years, been infected with this disease and kept under careful control. In no single instance has the disease appeared without the simultaneous appearance of the anaplasmata in the blood. There seems to be no more and no less reason to regard *Piroplasma bigeminum* as the true cause of "redwater" in cattle than there is to regard *Anaplasma marginale* as the true cause of "anaplasmosis."

We conclude that the most natural and logical explanation for all the observed facts is that *Anaplasma marginale* is a blood parasite which actually causes the disease anaplasmosis in cattle. The further question remains to be answered whether these parasites are *protozoa* or not. A direct proof in favour of or against their protozoan nature cannot be expected, but indirectly, especially by analogy with the piroplasms, we are led to regard the anaplasmata as true endoglobular protozoa. Almost in every respect the anaplasmata behave like the piroplasmata; the symptoms and pathological changes they produce are in many respects similar; their mode of transmission through ticks is the same; the epizootology of the two diseases is practically identical; and immunologically they bear a great resemblance to each other. The recent work of De Kock and Quinlan brings out this similarity still further. In splenectomised animals the parallelism between anaplasmosis and piroplasmosis (*in sensu lato*) is indeed most striking.

In conclusion, it may be stated that, taking all available evidence into consideration, the view originally advanced by Theiler, namely, that the anaplasmata are true parasitic protozoa and the actual cause of anaplasmosis has in no way been refuted, but, on the contrary, still offers the simplest and most natural explanation for all facts in connexion with these interesting bodies.

#### SUMMARY OF MAIN CONCLUSIONS.

1. The result of injecting large quantities of *trypan blue* into calves varies according to the previous history of these calves:—

(a) In calves which have never had a blood infection, practically *no changes* are produced.

(b) In calves which have previously had an infection of *Anaplasma marginale* or *Gonderia mutans*, the trypan blue will bring about a *relapse* with reappearance of these parasites in the blood.

2. Trypan blue as such cannot be regarded as a specific blood-poison for cattle; even in large quantities it produces practically no blood changes; prolonged injections may, however, lead to a gradual intoxication with cachexia and death.

3. These results throw new light on the experiment of Dias and Aragao (1914) in which a calf injected with trypan blue showed anaplasmata in its blood, which the authors regarded as the product of the trypan-blue injections.

In view of the above results, this case must be regarded as a simple relapse of anaplasmosis provoked by the trypan blue.

The anaemia observed in the calf was undoubtedly due to the anaplasmosis and not to the trypan blue.

4. *Pyrogallic acid* when injected into calves which have previously had an *Anaplasma marginale* or a *Gonderia mutans* infection will also provoke a relapse of these diseases. In calves free from such infections severe anaemic changes are produced in the blood.

5. In *dogs*, *rabbits*, and *guinea-pigs*, severe anaemia (and death) may be produced by the injection of *pyrogallic acid*, *nitrobenzene*, or *phenylhydrazin*. Jolly bodies appear in the blood, but a blood-picture which could be compared with that of anaplasmosis in cattle was never observed.

6. *Anaplasma marginale* of cattle cannot be regarded as a degenerative product of the red blood-corpuscles. It cannot be produced by the injection of poisonous substances.

7. If virulent anaplasmosis blood is *haemolysed* and *filtered* through a Berkefeld candle, the filtrate is non-virulent, whereas the unfiltered haemolysed blood produces anaplasmosis.

8. The "virus" of anaplasmosis cannot be separated from the red blood-corpuscles by means of *washing* and centrifuging. The washed red blood-corpuscles produce the disease in the typical way.

9. The "virus," therefore, is contained in the red blood-corpuscles and, when liberated, does not pass through a bacterial filter.

10. The simplest and most natural explanation for these facts is that "*Anaplasma marginale*" is an *endoglobular parasite* and the actual cause of anaplasmosis.

11. By analogy with "piroplasmosis" and in the light of recent work, it is reasonable to regard "*Anaplasma marginale*" as belonging to the protozoa.

## APPENDIX.

Being the experimental records of the animals referred to in the foregoing pages.

## EXPERIMENT I.

*Hermaphrodite Bovine No. 316.*

2½ years old.

1st day (6th April, 1923). Intravenous injection of 150 c.c. 1 per cent. trypan blue solution. Blood before injection normal.

5th	200 c.c. trypan blue solution.
7th	Blood normal.
8th	250 c.c. trypan blue solution.
9th	Blood normal. Temperature 105·8° F.
11th	„ „
13th	300 c.c. trypan blue.
14th	Blood normal. Temperature 105° F.
15th	350 c.c. trypan blue.
18th	400 c.c. trypan blue. Blood normal.
19th	Blood normal.
20th	„ „ Temperature 105·4° F.
21st	„ „ „ 104·8° F.
22nd	„ „ „ normal.
25th	400 c.c. trypan blue.
27th	400 c.c. trypan blue.
28th	Blood normal.
29th	400 c.c. trypan blue.
32nd	400 c.c. trypan blue.
36th	Blood normal.
40th	„ „
47th	„ „
58th	„ „
60th	„ „

*Calf No. 426.*

Female, 5½ months old.

1st day. (30th April, 1923). Intravenous injection of 50 c.c. of a 1 per cent. solution trypan blue. Blood normal.

3rd day.	50 c.c. trypan blue. Temperature 102·4° F.
5th	50 c.c. „ „ 102·8° F.
8th	50 c.c. „ „ 103·6° F.
12th	<i>Anaplasma marginale</i> and <i>Gonderia mutans</i> rare. Temperature 102·4° F.
15th	50 c.c. trypan blue. Temperature 103·2° F.
16th	<i>Anisocytosis</i> . <i>A. marginale</i> and <i>G. mutans</i> rare. Temperature 102° F.
17th	75 c.c. trypan blue. Temperature 103° F.
19th	75 c.c. „ „ 102·4° F.
20th	75 c.c. „ „ 103·4° F.
23rd	75 c.c. „ „ 103° F.
24th	75 c.c. „ „ 101·6° F.
25th	75 c.c. „ „ 104·6° F.
26th	75 c.c. „ „ 102·6° F.
27th	75 c.c. „ „ 102·2° F.
29th	<i>A. marginale</i> rare, <i>G. mutans</i> fairly frequent. Blood otherwise normal. Temperature 102·6° F.
34th	<i>G. mutans</i> fairly frequent. Blood normal. Temperature 104° F.
36th	<i>A. marginale</i> and <i>G. mutans</i> not rare. Temperature 103° F.
43rd	<i>G. mutans</i> not rare. <i>A. marginale</i> rare. Temperature 102·4° F.
47th	Slight <i>anisocytosis</i> . <i>G. mutans</i> fairly frequent. <i>A. marginale</i> rare. Temperature 103° F.
54th	No parasites seen. Temperature 102·6° F.
62nd	<i>G. mutans</i> rare. Blood normal. Temperature 103° F.

This calf was born on the 5th November, 1922. On the 9th November, 1922, it was injected subcutaneously with 5 c.c. fresh blood of heifer No. 183; from the 18th to the 24th it had a distinct temperature curve indicative of *Piroplasmosis*, but *Piroplasma bigeminum* was not found during the daily examination extending for ten days. On the 17th December, another rise of temperature occurred which developed into a typical curve lasting until the 26th December. During this time *A. marginale* was observed in fairly large numbers. The lesions that accompanied it were those of *Anisocytosis*, towards the end of the reaction. On the 6th January, *Gonderia mutans* appeared and was present in fair numbers.

## EXPERIMENT 2.

*Calf No. 607.*

Male, 4½ months old.

1st day	(28th June, 1923).	Intravenous injection of 50 c.c. 1 per cent. trypan blue solution.	Blood normal.
3rd	„	50 c.c. trypan blue.	
4th	„	Blood normal, no parasites seen.	
5th	„	50 c.c. trypan blue.	
6th	„	50 c.c. „	Blood normal.
7th	„	50 c.c. „	
8th	„	50 c.c. „	
9th	„	50 c.c. „	Temperature 106·4° F.
10th	„	50 c.c. „	„ 106° F. Blood normal.
14th	„	No parasites present.	Blood normal.
15th	„	75 c.c. trypan blue.	
17th	„	75 c.c. „	Blood normal.
19th	„	100 c.c. „	„ „
21st	„	100 c.c. „	„ „
23rd	„	100 c.c. „	„ „
26th	„	150 c.c. „	„ „
28th	„	150 c.c. „	„ „
30th	„	150 c.c. „	„ „
33rd to 38th day.		150 c.c. trypan blue daily.	
41st day.		No parasites present.	Blood normal.
43rd	„	250 c.c. trypan blue.	Blood normal.
44th	„	300 c.c. „	
45th	„	400 c.c. „	„ „
47th	„	Blood normal.	
50th	„	500 c.c. trypan blue.	Blood normal.
61st	„	No parasites present.	Blood normal.
69th	„	„ „	„ „
72nd	„	„ „	„ „

*Calf No. 709.*

Female, 2½ months old.

1st day	(28th June, 1923).	Intravenous injection of 50 c.c. 1 per cent. trypan blue solution.	Blood normal.
3rd	„	50 c.c. trypan blue.	No parasites present.
5th	„	50 c.c. „	
6th	„	50 c.c. „	Blood normal.
7th	„	50 c.c. „	
8th	„	50 c.c. „	
9th	„	50 c.c. „	Temperature 106·6° F.
10th	„	50 c.c. „	„ 105·4° F. Blood normal.
14th	„	No parasites seen.	Blood normal.
15th	„	75 c.c. trypan blue.	
17th	„	75 c.c. „	Blood normal.
19th	„	100 c.c. „	„ „
21st	„	100 c.c. „	„ „
23rd	„	100 c.c. „	„ „
26th	„	150 c.c. „	„ „
28th	„	150 c.c. „	„ „
30th	„	150 c.c. „	„ „
33rd to 38th day.		150 c.c. trypan blue daily.	
41st day.		No parasites seen.	Blood normal.
43rd	„	250 c.c. trypan blue.	Blood normal.
44th	„	300 c.c. „	
45th	„	400 c.c. „	Temperature 105·4° F.
47th	„	Temperature 106·6° F.	Blood normal.
50th	„	500 c.c. trypan blue.	Temperature 105·4° F. Blood normal.
61st	„	No parasites present.	Blood normal.
69th	„	„ „	„ „
72nd	„	„ „	„ „

## EXPERIMENT 3.

Bull No. 424.

2 years old.

1st day	(12th November, 1924).	Intravenous injection of 100 c.c. 1 per cent. trypan blue solution.	Blood normal.
6th	„	150 c.c. trypan blue.	Blood normal.
10th	„	150 c.c.	Animal showed symptoms of shock after injection.
11th	„	Slight <i>anisocytosis</i> .	<i>Gonderia mutans</i> very rare. Temperature 101.2° F.
13th	„	150 c.c. trypan blue.	
15th	„	Slight <i>anisocytosis</i> .	<i>G. mutans</i> rare. Temperature 102.4° F.
22nd	„	150 c.c. trypan blue (shock).	
23rd	„	<i>G. mutans</i> rare.	Temperature 101.8° F.
25th	„	150 c.c. trypan blue.	<i>G. mutans</i> rare. Temperature 103° F.
28th	„	Slight <i>anisocytosis</i> .	No parasites seen.
29th	„	„	<i>G. mutans</i> rare. Temperature 102° F.
31st	„	150 c.c. trypan blue.	
32nd	„	<i>G. mutans</i> very rare.	Temperature 102.2° F.
36th	„	No parasites seen.	
37th	„	150 c.c. trypan blue (slight shock).	
47th	„	<i>G. mutans</i> rare.	Temperature 101.6° F.
48th	„	150 c.c. trypan blue (slight shock).	
50th	„	250 c.c.	(shock). <i>G. mutans</i> fairly frequent. Temperature 104° F.
52nd	„	<i>G. mutans</i> rare.	Temperature 103° F.
55th	„	150 c.c. trypan blue (shock).	<i>G. mutans</i> fairly frequent. Temperature 102° F.
64th	„	200 c.c.	„ „ <i>G. mutans</i> rare. Temperature 101.4° F.
66th	„	200 c.c.	(slight shock).
67th	„	200 c.c.	„ „ <i>G. mutans</i> rare. Temperature 101° F.
70th	„	Very slight <i>anisocytosis</i> .	<i>G. mutans</i> rare. Temperature 102.2° F.
79th	„	<i>G. mutans</i> fairly frequent.	Temperature 102° F.
83rd	„	„	rare. Temperature 101.2° F.
85th	„	No parasites seen.	Blood normal.

Heifer No. 755.

1½ years old.

1st day	(12th November, 1924).	Intravenous injection of 100 c.c. 1 per cent. trypan blue solution.	Blood normal.
6th	„	150 c.c. trypan blue.	No parasites seen. Temperature 102.6° F.
10th	„	150 c.c.	(shock). <i>Anaplasma marginale</i> and <i>Gonderia mutans</i> not very rare. Temperature 102.6° F.
11th	„	<i>G. mutans</i> fairly frequent.	<i>A. marginale</i> not rare.
13th	„	150 c.c. trypan blue.	<i>A. marginale</i> fairly frequent. <i>G. mutans</i> rare. Temperature 103° F.
15th	„	Slight <i>anisocytosis</i> .	<i>A. marginale</i> fairly frequent. Temperature 102.8° F.
18th	„	Marked <i>anisocytosis</i> .	<i>A. marginale</i> not rare. Temperature 105.4° F.
22nd	„	150 c.c. trypan blue (shock).	<i>A. marginale</i> and <i>G. mutans</i> rare. Temperature 102° F.
25th	„	150 c.c. trypan blue.	<i>A. marginale</i> rare. Temperature 102.2° F.
29th	„	<i>A. marginale</i> not rare.	<i>G. mutans</i> rare. Temperature 101.4° F.
31st	„	150 c.c. trypan blue.	
32nd	„	Slight <i>anisocytosis</i> .	<i>A. marginale</i> very rare. Temperature 102.4° F.
36th	„	<i>G. mutans</i> and <i>A. marginale</i> rare.	Temperature 101° F.
37th	„	150 c.c. trypan blue (slight shock).	
48th	„	150 c.c.	„ „ <i>G. mutans</i> rare.
50th	„	250 c.c.	„ „ No parasites seen.
52nd	„	300 c.c.	(slight shock). <i>A. marginale</i> rare.
55th	„	300 c.c.	„ „ „
60th	„	<i>G. mutans</i> rare.	
64th	„	300 c.c. trypan blue (shock).	<i>G. mutans</i> rare.
66th	„	300 c.c.	„ „ <i>G. mutans</i> rare.
67th	„	300 c.c.	(slight shock). <i>G. mutans</i> rare.
70th	„	No parasites seen.	
79th	„	„	„
85th	„	<i>Anaplasma marginale</i> very rare.	

## EXPERIMENT 4.

Heifer No. 928.

2 years old: weight about 200 kilograms.

1st day	(12th November, 1924).	Intravenous injection of 40 c.c. of a 50 per cent. solution of pyrogallie acid, i.e., 0.2 c.c. solution per kilogram bodyweight.
6th "	40 c.c. 50 per cent. pyrogallie acid.	<i>Anisocytosis</i> . Temperature 102.6° F.
10th "	40 c.c. "	" " <i>Anisocytosis, polychromasia, basophilia, normoblasts, Jolly bodies.</i> <i>A. marginale</i> rare. Temperature 102.6° F.
13th "	40 c.c. 50 per cent. pyrogallie acid.	Marked anaemic changes. Normoblasts and Jolly bodies more frequent. <i>G. mutans</i> and <i>A. marginale</i> rare. Temperature 103° F.
15th "	40 c.c. 50 per cent. pyrogallie acid.	
16th "		<i>Oligocytosis, Anisocytosis, Basophilia, Jolly bodies.</i> Temperature 105° F.
17th "		<i>Poikilocytosis, Jolly bodies</i> rarer. Temperature 102.8° F.
18th "		Marked <i>anisocytosis, Basophilia, punctate cells.</i> Temperature 103.4° F.
20th "		" " <i>Jolly bodies</i> rare. Temperature 103° F.
22nd "	40 c.c. 50 per cent. pyrogallie acid.	<i>G. mutans</i> rare. Temperature 103° F.
23rd "		<i>Anisocytosis. G. mutans</i> rare. Temperature 103.6° F.
24th "		<i>Polychromasia.</i> Temperature 104.4° F.
25th "		<i>G. mutans</i> and <i>A. marginale</i> very rare. Temperature 103.4° F.
27th "		<i>Anisocytosis.</i> Anaemic changes disappearing. Temperature 102° F.
32nd "		" " <i>G. mutans</i> rare. Temperature 103° F.
36th "		" " Temperature 102° F.

Tollie No. 1026.

1 year old; weight about 170 kilograms.

1st day	(12th November, 1924).	Intravenous injection of 34 c.c. of a 50 per cent. solution of pyrogallie acid, i.e., 0.2 c.c. solution per kilogram bodyweight.
6th "	34 c.c. 50 per cent. pyrogallie acid.	Blood normal. Temperature 103.6° F.
10th "	34 c.c. "	" " " " " " 103° F.
11th "		Slight <i>anisocytosis.</i> Temperature 103.6° F.
13th "	34 c.c. 50 per cent. pyrogallie acid.	<i>Anisocytosis, Polychromasia, Basophilia.</i> Temperature 103.6° F.
15th "	34 c.c. 50 per cent. pyrogallie acid.	Marked <i>anisocytosis, basophilia, Jolly bodies</i> rare. Temperature 103° F.
17th "		No change. Temperature 103° F.
20th "		Anaemic changes less marked. Temperature 103° F.
22nd "	34 c.c. 50 per cent. pyrogallie acid.	Slight <i>anisocytosis.</i> Temperature 102.2° F.
24th "		<i>Anisocytosis, macrocytes. Jolly bodies</i> of various sizes not numerous. Temperature 103.8° F.
25th "	34 c.c. 50 per cent. pyrogallie acid.	
29th "		Slight <i>anisocytosis, polychromasia, normoblasts</i> rare. Temperature 102.2° F.
31st "	34 c.c. 50 per cent. pyrogallie acid.	
32nd "		<i>Anisocytosis.</i> A few <i>punctate cells</i> and a few <i>Jolly bodies.</i> Temperature 103.8° F.
34th "		Blood normal. Temperature 102.4° F.
35th "	34 c.c. 50 per cent. pyrogallie acid.	Slight <i>anisocytosis.</i>
46th "		Blood normal. Temperature 102° F.

## EXPERIMENT 5.

Dog No. 158.

Weight 30 kilograms.

1st day	(6th September, 1923).	Subcutaneous injection of 6 c.c. of a 50 per cent. solution of pyrogallie acid, i.e., 0.1 gram pyrogallie acid per kilogram bodyweight. Blood normal.
3rd "		Subcutaneous injection of 9 c.c. 50 per cent. pyrogallie acid, i.e., 0.15 gram per kilogram.
7th "		12 c.c. 50 per cent. pyrogallie acid (0.2 gram per kilogram.)
8th "		No parasites seen. <i>Jolly bodies</i> rare.
10th "		Very marked <i>Leucocytosis. Anisocytosis, Poikilocytosis, Polychromasia, Oligochromasia,</i> numerous <i>Normoblasts. Jolly bodies</i> very rare. Dog dies.

*Dog No. 139.*

Weight 16 kilograms.

- 1st day (30th October, 1923). Subcutaneous injection of 3.2 c.c. of a 50 per cent. solution of pyrogallic acid, i.e., 0.1 gram per kilogram bodyweight. Blood normal.
- 3rd ,, 3.2 c.c. 50 per cent. pyrogallic acid.
- 4th ,, Blood normal.
- 5th ,, Subcutaneous injection of 6.4 c.c. 50 per cent. pyrogallic acid (0.2 gram per kilogram). Blood normal.
- 6th ,, Slight *leucocytosis*.
- 7th ,, Marked *Leucocytosis* and *anisocytosis*. Dog dies.

*Dog No. 157.*

Weight 12 kilograms.

- 1st day (6th November, 1923). Subcutaneous injection of 2.4 c.c. of a 50 per cent. solution of pyrogallic acid, i.e. 0.1 c.c. per kilogram bodyweight. Blood normal.
- 2nd ,, Blood normal.
- 3rd ,, " "
- 4th ,, " "
- 5th ,, 2.4 c.c. 50 per cent. pyrogallic acid. Blood normal.
- 6th ,, Blood normal.
- 7th ,, " "
- 8th ,, " "
- 9th ,, 4.8 c.c. 50 per cent. pyrogallic acid. Blood normal.
- 11th ,, Slight *anisocytosis*.
- 12th ,, " " *basophilia*.
- 13th ,, " " "
- 14th ,, 4.8 c.c. 50 per cent. pyrogallic acid. *Anisocytosis, basophilia*.
- 15th ,, *Basophilia, anisocytosis*.
- 16th ,, " "
- 17th ,, " "
- 18th ,, 9.6 c.c. 50 per cent. pyrogallic acid (i.e. 0.4 gram per kilogram bodyweight). *Basophilia, anisocytosis, a few Jolly bodies*.
- 19th ,, No change.
- 20th ,, Anaemic changes less marked.
- 21st ,, *Leucocytosis, basophilia, anisocytosis, normoblasts, Jolly bodies*.
- 22nd ,, *Basophilia, normoblasts*.
- 23rd ,, " " *Jolly bodies rare*.
- 24th ,, Anaemic changes less marked.
- 25th ,, " " "
- 27th ,, Blood almost normal.
- 28th ,, Subcutaneous injection of 19.2 c.c. 50 per cent. pyrogallic acid, i.e., 0.8 gram per kilogram.
- 29th ,, Dog dies.

## EXPERIMENT 6.

*Rabbit No. 1.*

Weight 2,500 grams.

- 1st day (6th September, 1923). Subcutaneous injection of 5 c.c. of a 5 per cent. solution of nitrobenzene, i.e., 0.1 gram nitrobenzene per kilogram bodyweight. Blood normal.
- 3rd ,, Subcutaneous injection of 10 c.c. 5 per cent. Nitrobenzene (0.2 gram per kilogram).
- 7th ,, 10 c.c. 5 per cent. nitrobenzene (0.2 gram per kilogram).
- 8th ,, *Polychromasia, basophilia*.
- 10th ,, " "
- 12th ,, " " few normoblasts.
- 14th ,, " "
- 15th ,, Blood almost normal.
- 27th ,, Blood normal.

## Rabbit No. 3.

Weight 2,800 grams.

- 1st day (30th October, 1923). Subcutaneous injection of 5.6 c.c. of a 5 per cent. of nitrobenzene, i.e., 0.1 gram per kilogram bodyweight. Blood normal.
- 2nd ,, 5.6 c.c. 5 per cent. nitrobenzene. Few *basophilic* cells.
- 3rd ,, 11.2 c.c. 5 per cent. nitrobenzene (i.e., 0.2 gram per kilogram).
- 4th ,, Few *basophilic* cells and normoblasts.
- 5th ,, 22.4 c.c. 5 per cent. nitrobenzene (0.4 gram per kilogram). *Basophilia, anisocytosis.*
- 7th ,, *Basophilia, anisocytosis.*
- 8th ,, " " normoblasts.
- 9th ,, 22.4 c.c. 5 per cent. nitrobenzene. Marked *anisocytosis* and *basophilia.*
- 10th ,, Marked *anisocytosis* and *basophilia.*
- 11th ,, " " " "
- 12th ,, *Basophilia, anisocytosis,* normoblasts.
- 14th ,, Slight *basophilia* and *anisocytosis.*
- 15th ,, " " " "
- 16th ,, Blood almost normal. 22.4 c.c. 5 per cent. nitrobenzene. Rabbit dies.

## Rabbit No. 4.

Weight 3,200 grams.

- 1st day (30th October, 1923). Subcutaneous injection of 6.4 c.c. of a 5 per cent. solution of nitrobenzene, i.e., 0.1 gram per kilogram bodyweight. Blood normal.
- 2nd ,, 6.4 c.c. 5 per cent. nitrobenzene.
- 3rd ,, 12.8 c.c. 5 per cent. nitrobenzene, i.e., 0.2 gram per kilogram. Few *basophilic* cells.
- 4th ,, *Anisocytosis, basophilia.*
- 5th ,, 25.6 c.c. 5 per cent. nitrobenzene (0.4 gram per kilogram). Marked *basophilia* and *anisocytosis.*
- 6th ,, Marked *basophilia* and *anisocytosis, oligocythaemia.*
- 8th ,, *Basophilia anisocytosis, normoblasts.*
- 9th ,, Marked *anisocytosis* and *basophilia.*
- 10th ,, *Leucocytosis* and *basophilia.*
- 11th ,, Marked *basophilia, anisocytosis, normoblasts.*
- 12th ,, 32 c.c. 5 per cent. nitrobenzene (0.5 gram per kilogram). Rabbit dies.

## EXPERIMENT 7.

## Rabbit No. 2.

Weight 1,770 grams.

- 1st day (6th September, 1923). Subcutaneous injection of 3.5 c.c. of a 5 per cent. solution of phenylhydrazin, i.e., 0.1 gram phenylhydrazin per kilogram bodyweight. Blood normal.
- 2nd ,, *Oligochromasia, polychromatophilia, normoblasts.*
- 3rd ,, Subcutaneous injection of 7 c.c. 5 per cent. phenylhydrazin (0.2 gram per kilogram). Rabbit dies.

## Rabbit No. 5.

Weight 2,450 grams.

- 1st day (30th October, 1923). Subcutaneous injection of 0.49 c.c. of a 5 per cent. solution of phenylhydrazin, i.e., 0.01 gram per kilogram bodyweight. Blood normal.
- 2nd ,, 0.49 c.c. 5 per cent. phenylhydrazin. Blood normal.
- 3rd ,, 0.98 c.c. 5 per cent. phenylhydrazin (0.02 gram per kilogram). *Leucocytosis.*
- 4th ,, *Leucocytosis, slight basophilia.*
- 5th ,, 1.96 c.c. 5 per cent. phenylhydrazin (0.04 gram per kilogram). *Leucocytosis, basophilia, anisocytosis.*
- 6th ,, Marked *leucocytosis, basophilia, anisocytosis.* Rabbit dies.

## Rabbit No. 6.

Weight 2,000 grams.

- 1st day (30th October, 1923). Subcutaneous injection of 0.8 c.c. of a 5 per cent. solution of phenylhydrazin, i.e., 0.02 gram per kilogram bodyweight. Blood normal.
- 2nd ,, 5 per cent. phenylhydrazin. Blood normal.
- 3rd ,, 1.6 c.c. 5 per cent. phenylhydrazin (0.04 gram per kilogram). Slight *leucocytosis, anisocytosis, basophilia.*
- 4th ,, *Leucocytosis, anisocytosis, normoblasts.*
- 5th ,, 3.2 c.c. 5 per cent. phenylhydrazin (0.08 gram per kilogram). Rabbit dies.





29th day	Temperature 105·8° F.
32nd "	<i>A. marginale</i> fairly frequent. Marked <i>anisocytosis</i> . <i>Jolly bodies</i> rare. Temperature 104·8° F.
34th "	No change. Temperature 104·8° F.
39th "	<i>P. bigeminum</i> rare. Slight <i>anisocytosis</i> . Temperature 103·2° F.
49th "	Blood normal. Temperature 103° F.

## Calf No. 1,099.

Female, 1 month old.

1st day (30th January, 1925).	Defibrinated blood of Ox No. 1077 (showing <i>anaplasmosis</i> ) was mixed with an equal quantity of distilled water and the mixture passed through a Berkefeld filter. 20 c.c. of the filtrate was injected intravenously into Calf No. 1,099.
3rd "	Blood negative.
5th "	" "
11th "	" "
13th "	" "
15th "	" "
18th "	" "
20th "	" "
25th "	" "
26th "	" "
29th "	" "
32nd "	" "
34th "	" "
46th "	" "

## Calf No. 1,104.

Male, 3 weeks old.

1st day (30th January, 1925).	Intravenous injection of 20 c.c. of the same filtrate which Calf No. 1,099 received.
3rd "	Blood negative.
7th "	" "
11th "	" "
13th "	" "
15th "	" "
18th "	" "
20th "	" "
25th "	" "
26th "	" "
29th "	" "
32nd "	" "
34th "	" "
46th "	" "

## Calf No. 1,066.

Male, 1 month old.

1st day (30th January, 1925).	Intravenous injection of 10 c.c. defibrinated blood of Ox No. 1,077 (showing <i>anaplasmosis</i> ).
4th "	Blood negative. Temperature 102° F.
7th "	" " " 103·8° F.
9th "	<i>Piroplasma bigeminum</i> and <i>Anaplasma marginale</i> rare. Temperature 103·4° F.
11th "	<i>P. bigeminum</i> rare. <i>A. marginale</i> more frequent. Temperature 104° F.
12th "	" " and <i>A. marginale</i> fairly numerous. <i>Anisocytosis</i> . Temperature 105° F.
13th "	<i>A. marginale</i> numerous. <i>P. bigeminum</i> fairly numerous. Temperature 106° F.
17th "	" " fairly numerous. <i>Anisocytosis</i> . Temperature 102·6° F.
21st "	Slight <i>anisocytosis</i> . <i>A. marginale</i> numerous. Temperature 102·4° F.
26th "	<i>A. marginale</i> fairly numerous. Temperature 105·6° F.
28th "	" " " " 104·2° F.
33rd "	Slight <i>anisocytosis</i> . <i>P. bigeminum</i> rare. <i>A. marginale</i> frequent. Temperature 104° F.
39th "	Slight <i>anisocytosis</i> . <i>A. marginale</i> rare. Temperature 104° F.
49th "	No parasites seen, blood normal. Temperature 104° F.

## EXPERIMENT 10.

Calf No. 1,101.

Male, 1 month old.

1st day (30th January, 1925). Defibrinated blood of Ox No. 1,077 (showing *Anaplasmosis*) was mixed with  $1\frac{1}{2}$  times the quantity of haemolytic serum of Horse No. 16,234 (see below), and, after complete haemolysis, 20 c.c. of the mixture was injected intravenously into Calf No. 1,101.

4th	„	Blood negative.	Temperature	102.6° F.
7th	„	„	„	102.8° F.
9th	„	„	„	103.8° F.
11th	„	„	„	102.4° F.
13th	„	„	„	104° F.
15th	„	„	Slight <i>anisocytosis</i> .	Temperature 102° F.
18th	„	„	„	104.6° F.
20th	„	Slight <i>anisocytosis</i> , <i>Anaplasma marginale</i>	rare.	Temperature 103.2° F.
23rd	„	„	„	fairly numerous. Temperature
		103.4° F.		
24th	„	Temperature	104.4° F.	
25th	„	Slight <i>anisocytosis</i> . <i>A. marginale</i>	rare.	Temperature 105.2° F.
26th	„	„	„	104.6° F.
29th	„	„	„	105° F.
24th	„	<i>Anisocytosis</i> . <i>A. marginale</i>	rare.	Temperature 103.2° F.
39th	„	Slight <i>anisocytosis</i> . <i>A. marginale</i>	rare.	Temperature 102° F.
48th	„	Blood negative.	Temperature	103.4° F.

Calf No. 1,073.

Female,  $1\frac{1}{2}$  months old.

1st day (31st January, 1925). A portion of the haemolysed blood mentioned above (Calf No. 1,101) was passed through a Berkefeld filter and 20 c.c. of the filtrate injected intravenously into Calf No. 1,073.

3rd	„	Blood negative.
6th	„	„
10th	„	„
12th	„	„
14th	„	„
17th	„	„
19th	„	„
23rd	„	„
25th	„	„
28th	„	„
32nd	„	„
33rd	„	„
38th	„	„
45th	„	„
48th	„	„

Horse No. 16234.

Gelding, 15 years old.

1st day	(19th June, 1924).	Subcutaneous injection in five different places of 100 c.c. of a 25 per cent. suspension of red blood corpuscles of a healthy heifer (No. 840) in normal saline.
8th	„	Subcutaneous injection of 50 c.c. red blood corpuscles of Heifer No. 840 in 150 c.c. saline.
10th	„	Painful swelling on neck and chest.
15th	„	Subcutaneous injection of 40 c.c. red blood corpuscles of Heifer No. 840 in 120 c.c. saline.
19th	„	Swelling disappeared.
27th	„	Subcutaneous injection of 100 c.c. red blood corpuscles of Heifer No. 840 in 300 c.c. saline.
28th	„	Swelling on neck and sternum.
33rd	„	Swelling disappeared.
36th	„	Haemolytic test carried out with serum of Horse No. 16234 and cattle blood corpuscles. Complete haemolysis in dilutions, $\frac{1}{2}$ , $\frac{1}{4}$ , and $\frac{1}{8}$ th, partial haemolysis in dilution $\frac{1}{16}$ .

- 48th day Subcutaneous injection of 100 c.c. red blood corpuscles of Heifer No. 840 in 300 c.c. saline.  
 51st ,, Slight swelling at seat of inoculation.  
 118th ,, Subcutaneous injection of 120 c.c. red blood corpuscles of Heifer No. 840 in 360 c.c. saline.  
 120th ,, Swelling at seat of inoculation.  
 134th ,, ,, still present.  
 146th ,, ,, disappeared.

## EXPERIMENT 11.

Calf No. 1,062.

Male, 2 months old.

- 1st day (30th January, 1925). A quantity of blood corpuscles of Ox No. 1,077 (showing *anaplasmosis*) was mixed with normal saline and centrifuged; the process being repeated six times, so as to free the corpuscles from all traces of serum. 5 c.c. of the washed corpuscles were diluted and injected intravenously into Calf No. 1062.
- 4th ,, Blood negative. Temperature 103.8° F.  
 7th ,, ,, ,, 104.2° F.  
 9th ,, *Piroplasma bigeminum* and *Anaplasma marginale* rare. Temperature 104.6° F.  
 11th ,, *P. bigeminum* fairly numerous. *A. marginale* rare. Temperature 104.4° F.  
 13th ,, *Anisocytosis*. *P. bigeminum* and *A. marginale* rare. Temperature 104° F.  
 19th ,, ,, *A. marginale* rare. Temperature 104.6° F. Mucous membranes very pale.  
 21st ,, Slight *anisocytosis*. *A. marginale* rare. Temperature 103.4° F.  
 26th ,, *A. marginale* fairly frequent. Temperature 103.0° F.  
 28th ,, ,, ,, ,, *P. bigeminum* rare. Temperature 104.8° F.  
 34th ,, ,, ,, ,, Temperature 104.4° F.  
 39th ,, Slight *anisocytosis*. *P. bigeminum* rare. Temperature 104.4° F.  
 47th ,, Blood negative. Temperature 103° F.

Calf No. 1,067.

Male, 2 months old.

- 1st day (30th January, 1925). Intravenous injection of 5 c.c. blood corpuscles of Ox No. 1,077 (*anaplasmosis*) washed and diluted in the same way as in the case of Calf No. 1,062.
- 4th ,, Blood negative. Temperature 104° F.  
 7th ,, ,, ,, ,, 102.8° F.  
 9th ,, ,, ,, ,, 103.6° F.  
 11th ,, ,, ,, ,, 103° F.  
 13th ,, *Piroplasma bigeminum* rare. Temperature 103.2° F.  
 18th ,, *Anaplasma marginale* rare. Temperature 104° F.  
 21st ,, *A. marginale* rare. *Anisocytosis*. Temperature 105° F.  
 22nd ,, ,, ,, ,, 105.4° F.  
 26th ,, ,, ,, ,, fairly frequent. *P. bigeminum* not rare. Temperature 104° F.  
 28th ,, *Anisocytosis*. *P. bigeminum* numerous. Temperature 104° F.  
 33rd ,, Slight *anisocytosis*. *P. bigeminum* rare. Temperature 103.6° F.  
 34th ,, *A. marginale* rare. Temperature 103.8° F.  
 39th ,, *P. bigeminum* rare. Temperature 103.2° F.  
 48th ,, Blood negative. Temperature 103.2° F.

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[Full lists of references to *Anaplasma* literature can be found in the publications of Knuth and Du Toit (1921) and Helm (1924)—see below.]

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## EXPLANATION OF FIGURES.

FIG. 1.—Microphotogram of the blood of Calf 316 (Experiment 1) taken on the 34th day of the experiment after the animal had received 32½ grams of trypan blue. The blood is perfectly normal and free from parasites. Magnification of all figures 660x.

FIG. 2.—Blood of Calf 755 taken during the first attack of *anaplasmosis*, about a month before Experiment 3 was started. The blood shows a fairly heavy infection with *Anaplasma marginale*.

FIG. 3.—Microphotogram of blood of Calf 755 taken a few days after Fig. 2, and showing anaemic changes (*anisocytosis*, *basophilia*, *normoblasts*, *Jolly bodies*).

FIG. 4.—Blood of Calf 755 (Experiment 3) showing a relapse of *anaplasmosis* after four injections of trypan blue (5½ grams). Apart from *Anaplasma marginale*, the blood shows only a slight *anisocytosis*.

FIG. 5.—Blood of Dog 158 (Experiment 5) after three injections of pyrogallie acid (13½ grams) and just before the death of the animal. The picture shows extreme anaemic changes of the blood. A *Jolly body* which could be mistaken for an *Anaplasma* can be seen near the edge of the microphotogram.

FIG. 6.—Blood of Rabbit 7 (Experiment 7) on the 32nd day of the experiment, after nine injections of phenylhydrazin. Anaemic changes marked.