

Splenectomy in Domesticated Animals and its Sequelae, with special reference to Anaplasmosis in Sheep.

By G. DE KOCK, M.R.C.V.S., Dr.Med.Vet., Research Officer, Onderstepoort, and JOHN QUINLAN, M.R.C.V.S., Dr.Med.Vet., Research Officer, Onderstepoort.

IN a paper on "Equine Infectious Anaemia," De Kock (1) discussed the rôle played by the liver with reference to the elimination of degenerated erythrocytes from the circulation. No satisfactory explanation could be given why, in a "gradual progressive oligocythaemia" produced by a virus, the liver should assist the function of the spleen in the elimination of erythrocytes, whereas in a disease like equine nuttalliosis, where there is a "great destruction of erythrocytes" in the circulation, no such process occurred in the liver. In nuttalliosis, where the oligocythaemia is sudden and very acute, no lesions of erythrophagocytosis and desquamation of stern cells were seen, i.e. the reticulo-endothelial apparatus of the liver was not brought into action. A further series of experiments was undertaken to continue the study of the relation of the liver to the spleen, and to ascertain the rôle of the reticulo-endothelial apparatus, especially in animal diseases where an abnormal breaking down of erythrocytes occurred.

The first series of experiments was based on the work of Kiyono (2), to ascertain to what extent the reticulo-endothelial apparatus could be affected in normal horses and in animals suffering from equine infectious anaemia, by the injection intravenously of lithion-carmine solutions. The preliminary experiments were unsatisfactory. It was found that normal horses withstood large quantities intravenously, and "hoarded" the pigment in the cells of the reticulo-endothelial apparatus to a fair extent (especially in the liver), but in case of infected animals, e.g. horse 15635, 15488, 15675, and 15631, even though much smaller doses were injected, marked symptoms of shock were observed, followed by the death of the animal.

Probably this disturbance might be of the nature of a "previous blocking" of the reticulo-endothelial apparatus by degenerated erythrocytes. According to Lepehne (3), it has been possible to produce "blocking" of the stern cells by means of collargol, preventing thereby phagocytosis of erythrocytes. This experiment was not further investigated, because the subject of splenectomy, also introduced to study the nature of the reticulo-endothelial apparatus, was followed by important results, which form

the theme of this paper. It is hoped to continue the experiments on the "hoarding of pigment," especially in connexion with splenectomy and anaplasmosis of sheep.

The splenectomy experiments first carried out in connexion with horses, then in sheep, cattle, and goats, gave results of far-reaching importance. It was found that one had to discriminate between (a) "susceptible animals," i.e. those which were not the carriers of any form of piroplasms or anaplasms, and (b) "infected animals," i.e. the carriers of piroplasms or anaplasms or both, as the case may be. In susceptible animals, no important sequelae followed splenectomy, whereas in cases of "carriers" relapses followed, viz., in cases of horses, nuttalliosis; in cattle, anaplasmosis, piroplasmosis, gonderriosis; in sheep, anaplasmosis. It may be stated that this is the first time that anaplasmosis has been recorded in sheep in South Africa.

With reference to the work carried out, Dr. De Kock was responsible for the series of experiments undertaken and the haematological and pathological observations, whereas Dr. Quinlan did all the operations of splenectomy and clinical observations. On that account the subject-matter has more or less been arranged as follows:—

I.

A Description of the Operation of Splenectomy of Susceptible "Ungulates," dealing with—

1. The Literature;
2. Preparation of the Animal;
3. Anaesthesia;
4. Technique of the Operation:
 - (a) In Equines,
 - (b) In Bovines,
 - (c) In Ovines;
5. Enlargement of the Spleen;
6. After-treatment.

II.

A Consideration of the *Sequelae* following the Splenectomy of *Ungulates*, "Carriers" of Piroplasmosis or Anaplasmosis, with special reference to Anaplasmosis in sheep; dealt with as follows:—

- (i) The Literature;
- (ii) Splenectomy of Equines, "Carriers" of Nuttallia;
- (iii) Splenectomy of Bovines, "Carriers" of Piroplasma or Anaplasma;
- (iv) Splenectomy of Ovines, "Carriers" of Anaplasma.

Anaplasmosis of Sheep.

1. Anaplasmosis in sheep, either produced by splenectomy of "carriers" of anaplasma, or by the infection of susceptible sheep, or by the infection of splenectomized susceptible sheep, with infected blood.
2. Transmission of ovine anaplasmosis to ovines (including "passage" in a series of sheep), to bovines, to goats, or do such animals only become "carriers"?
3. Transmission of bovine anaplasmosis to ovines, or do such animals only become "carriers"?
4. A study of the nature of the anaplasma of sheep:—
 - (i) As regards filterability;
 - (ii) As regards its resistance "in vitro";
 - (iii) A comparison of sheep "anaplasma" with "jolly-bodies" produced by drugs;
 - (v) General conclusions.

III.

References.

IV.

Appendices, including—

1. Summary of the experiments;
2. Clinical observations, temperature charts, and photographs;
3. Tables of blood counts.
4. Plates, temperature charts, graphs, figures (follow page 480).

I.

A DESCRIPTION OF THE OPERATION OF SPLENECTOMY.

1.—THE LITERATURE.

Splenectomy has been successfully carried out in the case of dogs on innumerable occasions. No reference, however, could be traced whether this operation had actually been undertaken in equines and bovines. In cases of sheep and goats only one reference, viz., that of Warthin (4) was found. Splenectomy was carried out on sheep and goats by Warthin, but the results were not satisfactory, 50 per cent. of the animals operated upon dying of shock. This author states:—"The animals were allowed to fast for several days preceding the operation, as experience showed in the case of one animal not so treated that the distended stomach greatly increased the difficulty of the operation. In the fasting animal the operation was performed with ease and without important loss of blood. Chloroform was used as an anaesthetic, the animals not taking ether well. The abdomen was shaved before operation and asepsis carried out as far as possible. The animals were fastened to the operating table on the right side, this being found more favourable for the operation than the dorsal position. The incision was made in the "left hypochondrium" in the splenic region, about a hands breadth below and parallel to the edge of the ribs. . . . All sheep operated upon suffered severely from shock during the first twenty-four hours following the operation, 50 per cent. of the cases dying from this within that time. The survivors reacted quickly, and by the third day were apparently as well as before the operation. Because of the great mortality it was decided to make use of goats instead, the two operated upon for the series of experiments showing no symptoms of shock and recovering quickly. . . . With the exception of local abdominal hernia developing at the seat of operation and the rapid development of goitre in one of the goats, no bad effects were observed to result from the operation. All the animals kept over a month after the operation showed a great increase of fat."

The Operation of Splenectomy.

The horses and cattle selected for splenectomy experiments were of small size, as it was anticipated the operation would be extremely difficult if even medium-size adult animals were used. This anticipation of difficulty was fully justified after the first splenectomy operation on the horse had been completed. An adult mare of small size with a fairly wide open "coupling" was operated upon. The operation was successful, but it was extremely tiresome and tedious, and, as will afterwards be pointed out in the paragraph on technique, the danger of peritonitis was very real, because of the protracted manipulations which were necessary in the abdomen. After this first experience, immature animals were used, that is, horses and cattle under two years. The best results can be obtained with very small animals, as the operation can be expeditiously carried out, and short intra-abdominal manipulation only is necessary.

The condition of the horses used in two cases was fairly good, while two others were poor. The cattle were in fair "store" condition.

The sheep and goats used for the experiments were selected at random from the flocks maintained at the station for general experimental purposes. Very fat sheep are unsatisfactory, as the fat somewhat impedes a rapid laparotomy. This, however, is the only objection.

2.—PREPARATION.

The following precautions were taken before bringing the animals to the operating theatre. The preparation included dieting, purging and starving, with the object of reducing as far as possible the volume of the stomach and intestines. The animals were put on to a concentrated, non-bulky diet eight days prior to operation. Horses were fed on oats and bran with a few pounds of green lucerne daily. The cattle ration consisted of maize-meal and bran with a small amount of lucerne daily. Sheep and goats received crushed maize and a small quantity of lucerne hay. Food was withheld and a mild purgative given thirty-six hours prior the operation, while water was withheld for twenty-four hours. The day previous to operation the horses and cattle were clipped, and when considered necessary the sheep were similarly treated. A large area over the left flank was shaved and painted with tincture of iodine. From this period sterile bedding only was used in the box.

3.—ANAESTHESIA.

General anaesthesia only was adopted. In the case of horses and cattle, chloral hydrate intravenously and chloroform inhalation was used. The dose given varied from $2\frac{1}{2}$ to 3 grams per 100 pounds body weight for horses, and 3 to 4 grams for cattle. The chloral hydrate was dissolved in normal saline to make a 10 per cent. solution. When necessary, the depth of the anaesthesia required was reached by using inhalations of Merck's chloroform given openly through a mask. For sheep a similar procedure was adopted in a few cases. The dose used was $1\frac{3}{4}$ to 2 grams of chloral hydrate per 50 pounds. The greater number of the sheep and goats were anaesthetized by using chloral hydrate per os. The dose used was about 4 grams per 50 lb. body weight. Only in a few cases of those operated upon was it necessary to use chloroform to any extent to cause a sufficiently deep anaesthesia.

This method of anaesthesia gave complete satisfaction. In no case was there the slightest cause for anxiety as to the welfare of the patient. The animals recovered rapidly from the effects of the anaesthetic, and did not show symptoms of shock.

4.—TECHNIQUE OF THE OPERATION.

(a) *In Equines.*—An intravenous injection of chloral hydrate was administered and the patient immediately placed on the operating table on the off-side. The fore limbs and head were fixed in the most convenient position, while the hind limbs with all the joints well extended were drawn slightly backwards so as to make the region of the flank as approachable as possible. Anaesthesia was then completed with chloroform inhalation.

The skin over the shaven area in the near flank was then swabbed with ether. The area surrounding the operation field was covered with sterile linen held in position by means of cloth forceps. The operator's hands, after cleaning with soap and running water, were thoroughly washed with alcohol corrosive sublimate solution (1-1000), and finally rinsed in sterile physiological saline.

The instruments, after sterilization in the autoclave, were transferred to sterile physiological saline, or used direct from the sterilizer.

Laparotomy.—A vertical incision was made through the skin and muscles down to the peritoneum in the region of the left flank, one and a half to two inches behind the last rib. The incision was begun as close to the transverse processes of lumbar vertebrae as possible. The length varied (from 15 to 25 cm.) according to whether the operation was being carried out on a large or a small animal. In large animals the longer incision is necessary, as both hands must be used inside the abdomen at the same time.

All haemorrhage was now arrested. The peritoneum was caught up in a forceps and cut in the direction of the cutaneous incision for a distance of about half an inch. A large piece of sterile gauze was then placed over the field of operation and an incision made through it over the length of the wound. The gauze was then sutured by occasional interrupted sutures all round the lips of the incision, the peritoneum being gradually opened as the sutures extended towards the extremities. This would seem to be an excellent precaution, as it prevents any danger of infection from the skin. Further, the sutures prevent stripping of the peritoneum during abdominal manipulation and their continuations can be used as loops for dilating the incision when necessary.

It was intended when removing the spleen to disturb the relations of the abdominal organs as little as possible. To maintain this normal relationship it was necessary to leave intact the cavity of the omentum by laying a line of ligatures which would unite the gastro-splenic with the greater-omentum,

The laparotomy having been completed, the operation differed somewhat in the medium-size and small animals. In medium-size horses only a very small portion of the apex of the spleen can be withdrawn through the incision, so that almost all the ligatures have to be laid on within the abdomen. In consequence, both hands must be used for intra-abdominal manipulation. Using fine silk for ligaturing, operations were begun at the apex of the spleen. The gastro-splenic omentum and the greater-omentum were caught between the thumb and fingers of the left hand, while the right hand carrying a half-circle needle and ligature silk was passed over the parietal surface of the spleen. The needle was passed from before backwards through the omentum, and then, having isolated a portion, it was again passed forward and withdrawn through the laparotomy incision. The ligature was then completed by knotting the silk. For the purpose of applying tension on the silk and also to facilitate knotting, a short piece of nickel-plated steel, one and a half inch long, was attached to the free end of the ligature silk. By this means the fingers of the left hand working inside the abdomen could easily knot the ligature silk, and apply sufficient tension to prevent haemorrhage. Proceeding in this way, a double row of sutures was laid on uniting the gastro-splenic and greater-omentum along the entire length from the apex to the hilus of the spleen until the splenic vessels were reached. (In this manner about 6 or 7 double ligatures were laid on the omentum.) After isolation and double ligation of each portion of the omentum, it was divided between the ligatures by means of a uterine scissors. This facilitates further and more rapid progress. The suspensory ligament of the spleen was

treated in a similar manner until the spleen was attached only by the splenic artery, vein and nerves. A last ligature was passed around the vessels and nerves at the hilus. The spleen was then detached with the uterine scissors and withdrawn through the incision in the abdominal wall.

The laparotomy wound was closed by suturing the peritoneum and muscular coats separately with (No. 4, 12 days) chromatinized cat-gut. The skin was united by interrupted silk sutures, and a small gauze drain inserted; at first two tape button tension sutures were used in addition, but it was found that these were of little use and were very likely to suppurate. Better results were obtained by the use of narrow strips of gauze soaked with mastisol passed across the wound. The skin wound was sealed by a dressing of collodium and iodoform.

The operation was completed by the application of a sterile protective bandage.

In small animals the operation is not so difficult, as a large portion of the spleen can be withdrawn through the incision in the abdominal wall. An incision 15 cm. in length is sufficient, since it is necessary to introduce one hand only into the abdominal cavity.

In two foals, after the laparotomy was completed, the apex of the spleen was withdrawn and most of the omental ligatures as well as those on the suspensory ligament laid on outside the abdominal cavity. After having laid on as many ligatures as possible, the left hand was introduced and the remaining structures still attached to the spleen—small portions of the gastrosplenic and great-omentum and suspensory ligament with the splenic vessels—were pressed together and a last ligature was laid on these compressed tissues and the spleen removed with the uterine scissors.

Outside this small deviation the operation in both large and small horses was identical.

(b) *Operation in Cattle*.—The preparation, anaesthesia, and the laparotomy are similar to what has been described in horses. A slight modification was, however, necessary with the position of the incision, which was made parallel with the last rib and about $1\frac{1}{2}$ inch posterior to it. It was begun as high up as possible and extended downwards and backwards for a distance of about 15 cm.

The laparotomy having been completed, the left hand was introduced into the abdominal cavity between the rumen and the visceral surface of the spleen. Beginning at the antero-inferior aspect of the area of attachment, the peritoneum was perforated along the line of its reflection from the rumen to the spleen and the connective tissue gradually broken through. Where difficulty was experienced in breaking through the tissue with the fingers it was cut through with the uterine scissors. Proceeding by means of blunt dissection, the detachment was completed over the left face of the rumen and the adjacent area of the reticulum, as well as the left crus of the diaphragm, until it remained attached only by the splenic vessels and nerves at the hilus. The spleen was then withdrawn and the splenic vessels ligatured with silk. It was afterwards removed with the scissors. The completion of the operation was similar to the description which has been given for the horse. In the bovines operated upon no drain was used when closing the laparotomy wound.

Manipulations should be gentle during the breaking down of the connective tissue attachments of the spleen, as rupture of this organ causes troublesome haemorrhage.

(c) *The Operation in the Sheep and Goat.*—The technique of the operation in the sheep and goat differs little from the operation in bovines. The preparation, anaesthesia, and laparotomy are similar to what has been described for bovines. The laparotomy having been performed, the left hand is inserted over the parietal surface of the spleen. The attachment between this surface and the diaphragm is broken down with the fingers and thumb. Then, beginning at the most inferior portion of its attachment to the dorsal curvature of the rumen, the spleen is loosened up to the hilus. Its remaining attachment to the left crus of the diaphragm is detached in a similar manner, proceeding from behind forward. When only the splenic vessels remain the spleen is drawn out through the laparotomy wound, the vessels caught in a forceps, and slight tension applied so as to bring the splenic vessels nearer the opening. A silk ligature is then applied around the vessels, and the organ removed with the scissors.

The operation is completed in the same manner which has been described in equines and bovines.

It was observed that the spleen is very easily ruptured in sheep and goats. In consequence, the manipulations during detachment of its attachments must be gentle and patient, as it bleeds profusely. When it is removed without rupture of its capsule, the operation can be performed with a loss of not more than 15 to 20 cm. of blood.

5.—ENLARGEMENT OF THE SPLEEN.

It may be mentioned that in every case in horses, cattle, goats and sheep, where splenectomy was performed, there was a considerable enlargement of the spleen. This enlargement was due to engorgement with blood. The enlargement was not caused by any pressure on the splenic vein during the operation, as it was noted immediately the hand was introduced into the abdomen. The weight of many of the removed spleens is given in the appendix. It will be seen that a spleen of 3.4 kg. was removed from one of our horses, one of 3.2 kg. from a bovine, and one of 315 grams from a sheep. It may be stated that in almost every case the weight of the removed spleen was more than twice what one expects to find in a clinically healthy animal of relatively equal weight. As to the cause of this engorgement, the authors suggest that it may be due to anaesthesia.*

6.—AFTER-TREATMENT.

After the operation the animals were placed in boxes in which sterile bedding was used. This precaution was not taken entirely with a view to getting healing of the wound *per primam intentionem*, but also to insure against the possibility of tick infections, which would have rendered the experiments with tick-borne diseases, which were being conducted, unsatisfactory. Two or three hours after the operation small quantities of laxative food, such as bran and green lucerne, were given and water was allowed *ad lib.*

* With reference to such an enlargement of the spleen, the following reference in Kitt (35) is interesting:—

“Congestive hyperaemia is due to paralysis of the splenic nerves, or to stimulation of the splenic vaso-dilator branches, and therefore is the direct result of dilatation of the splenic vessels. It is produced by toxic substances such as curare, narcotic drugs, or the organisms of infectious disease, and consequently the condition is a neuroparalytic hyperaemia.”

The wounds were inspected daily and when considered necessary the external dressing renewed. The wounds in the case of cattle, sheep and goats gave no trouble and always healed *per primam intentionem*. The skin sutures were removed in every case on the seventh or eighth day after operation.

The wounds in the four horses did not heal by first intention. Probably the difficulty was not due to conditions in the wound itself, but to the nature of the intercurrent disease where changes in the blood prevented healing. Even in those cases the peritoneum and internal oblique muscle had united, but there was no adhesion between the severed edges of the external oblique muscle or the skin. These structures were healing under granulation when the animals died from the intercurrent disease. In the horse, which was free from *Nuttallia equi* infection, and which recovered, the upper two-thirds of the wound healed beautifully *per primam intentionem*, but the skin over the lower third suppurated in consequence of two stitches rupturing when the animal was struggling to rise after recovering from anaesthesia. However, this portion of the wound healed nicely under granulation. The resulting cicatrix was scarcely observable when the animal died 4 months later as a result of an intravenous injection of haemolytic serum.

II.

A CONSIDERATION OF THE " SEQUELAE " FOLLOWING SPLENECTOMY OF ANIMALS, " CARRIERS " OF PIROPLASMS OR ANAPLASMS,

WITH SPECIAL REFERENCE TO ANAPLASMOSIS OF SHEEP DEALT WITH AS FOLLOWS :—

- (i) The Literature;
- (ii) Splenectomy of Equines, " Carriers " of *Nuttallia*;
- (iii) Splenectomy of Bovines, " Carriers " of Piroplasms or Anaplasms;
- (iv) Splenectomy of Sheep, " Carriers " of *Anaplasma*.

(i) THE LITERATURE.

With reference to the literature relating to the splenectomy of animals, " carriers " of piroplasms, only one publication could be traced, viz., that of Ciuca 1921 (5), regarding the effect of total splenectomy upon the evolution of canine piroplasmosis. It would appear that infection subsequent to the removal of the spleen, provided the dog has completely recovered from the operation, does not affect the course, whereas splenectomy during the evolution of the disease aggravates the symptoms, especially in young animals. Moreover, when animals are on the way to recovery, splenectomy in the great majority of cases does not lead to any reappearances of the parasites in the blood.

An earlier publication, n.l. of Gonder and Rodenwalt (6), somewhat related to the above, deals with the splenectomy of apes, affected with *Plasmodium kochi*, and dogs with *Babesia canis*. These authors maintain that plasmodium usually disappears quickly from the blood, whereas after splenectomy they occur in large numbers in blood for months.

In case of human malaria, Ziemann (7) asserts that, with the disappearance of malaria, tumour splenis recedes. In old cases, however, tumour splenis remains and may set up acute complications

such as torsion, etc. Lately in such cases, where therapy has proved to be useless, removal of the spleen has been recommended. He concludes that splenectomy is indicated in those cases, where the spleen becomes the locality of toxic substances and where therapy gives no result. However, it appears that many patients without a spleen become affected with new malaria relapses, in some cases ending in death.

W. J. Mayo (8) discusses certain blood conditions depending on pathological processes in the spleen, and concludes, in the light of clinical experience, a *chronically enlarged spleen must be regarded as a menace* to the well-being of the human-carrier. Mayo refers to 255 cases of splenectomy performed in the Mayo clinic for splenic anaemia, haemolytic jaundice, pernicious anaemia, polycythaemia and purpura. He regards the spleen as a kind of "old fool" that acts as an agent of some, as yet little understood, pathological processes elsewhere in the body, and when set in motion does not know when to stop. When the spleen is once stimulated to action it develops a pathological condition of its own, and splenectomy under these circumstances removes a rebellious agent, but does not necessarily cure the original disease.

Gay (9) and others refer to the undoubted importance of the spleen (probably its reticulo-endothelial apparatus) in the disposal of foreign cells and in antibody formation. Holler (10) and other investigators believe that the breaking up of bloodcells is associated with monocytes, in the first place in the pulpa sinus of the spleen. Such monocytes, which have phagocyted, are eventually themselves held back and filtered off.

Summary of the Literature.

(a) It appears that the course of canine piroplasmiasis in splenectomized susceptible dogs is not more acute than in non-splenectomized susceptibles.

(b) According to some, splenectomy of a "carrier" of canine piroplasmiasis results in a "relapse," whereas others maintain that it does not necessarily lead to the reappearance of parasites in the circulation.

(c) Splenectomy of apes, carriers of *Plasmodium kochi*, leads to the reappearance of parasites in the circulation for months.

(d) In case of the human being, splenectomy is recommended in chronically enlarged spleen, either as result of malaria or some other disease elsewhere in the body. Patients without a spleen become affected with new malaria relapses, and in some cases ending in death. In other diseases it is stated that splenectomy does not necessarily cure the original disease.

(e) It appears that certain functions can be attributed to the spleen, probably mainly involving the reticulo-endothelial apparatus:—

- (i) Participation in the destruction of degenerated blood-cells.
- (ii) Elaboration of protective substances.
- (iii) Formation of endotheliocytes (monocytes) and lymphocytes.

(ii) SPLENECTOMY OF EQUINES, "CARRIERS" OF NUTTALLIA.

From Appendix I it will be seen that altogether four horses were splenectomized. Of these, three, viz., Hs. 15186, 15420, and 16032 were found to be "carriers" of Nuttallia and showed relapses of a very severe nature. The remaining horse, 16072, a "non-carrier," showed little impairment as the result of the operation. In the "carriers," parasites appeared in the blood three to four days after splenectomy.

Changes in the Blood.

(i) *Normal Horse.*—In Horse 16072 splenectomy was followed by a leucocytosis from about 8/3/24-7/6/24, at times reaching 55,000 per cubic mm. The leucocytosis in the first instance affected the neutrophiles, and later on the monocytes, with slight erythrocytosis.

There was a slight reduction in the number of erythrocytes, from 11 millions to 8 millions, but this might have been due to the fact that the animal was brought from the veld to the stable conditions [*vide* Nesor (14)]. Jolly-bodies appeared more frequent than normal.

(ii) *Infected Horses.*—In case of infected horses, splenectomy was followed by a similar neutrophilia, and later on by a monocytosis. The majority of monocytes showed either erythrophagocytosis or the presence of "vacuoles" in the cytoplasm. Marked reduction of erythrocytes occurred, from about 5½ millions per c.m.m. to about 2 millions and 1½ million in the blood collected from vena cava caudalis, under chloroform. The micro-photographs (*vide* Appendix 4) of Horse 16072 show the presence of large monocytes of the mononuclear type or "endothelials," with light iron-grey cytoplasm, not well circumscribed, namely, with a frayed-out appearance, and a leptochromatic nucleus.

Symptoms.—The symptoms associated with these relapses developed rapidly. There was a sharp rise in temperature up to 105° F., accompanied by an increased pulse-rate and increased respirations. The mucous membranes were at first somewhat congested, but towards the sixth day after the operation anaemia and a very slight icterus appeared. This late and slight appearance of icterus in the splenectomized horses, which showed relapse, was very characteristic when compared with the intense lemon-yellow icterus observed in natural cases. A similar observation has been recorded by Pearce, Austin, and Krumbhaar (15). According to these authors, the injection of haemolytic serum caused in control dog and splenectomized dog well-marked haemoglobinuria, accompanied in the normal dog by a persisting excretion of large amounts of bile pigments in the urine, but without evidence of jaundice in the splenectomized dog. They gave no satisfactory explanation why this should be so. The matter is still under investigation.

Haemoglobinuria with incontinence of urine occurred in horses on the sixth day. They stopped feeding and lost condition very rapidly. They were in extremis on the seventh or eighth day after the operation and either died or were destroyed under chloroform for pathological study.

Pathological Anatomy.—The pathology of these cases will be considered in greater detail in a subsequent paper. The following is a short résumé of the chief changes: anaemia, slight icterus,

enlargement, stasis, and pigmentation (bile) of the liver, degeneration of the myocardium, blood intravasations of epicardium and endocardium, degeneration and pigmentation (haemoglobin) of kidneys, haemoglobinaemia, haemoglobinuria, small haemorrhages in the lungs, swelling and hyperaemia of the majority of lymphatic glands. No appreciable changes were noticed in connexion with the haemal lymph glands. One case, viz., H.s. 15420, was complicated with a diffuse fibrino-purulent peritonitis.

Summary.

(a) Splenectomy of "carriers" of nutallia showed fatal "relapses," whereas in case of a "non-carrier" very little impairment was noticed besides slight alterations in leucocytes.

(b) In "carriers," splenectomy was followed by a recurrence of the parasites in the blood in three to four days and a neutrophilia. The latter was followed by a monocytosis associated with erythrophagocytosis and "vacuole" formation.

(c) Symptoms and post-mortem changes were those of a very acute attack of nuttalliosis, except in case of splenectomized animals, the late appearance of the characteristic lemon-yellow icterus was noticed.

(iii) SPLENECTOMY OF BOVINES, "CARRIERS" OF PIROPLASMS AND ANAPLASMS.

Altogether five bovines came under our observation, viz.: C. 711, C. 758, C. 893, C. 1027, and C. 1034 (*vide* appendices). It would appear that C. 711 was a carrier of *Anaplasma centrale* at the time when it was splenectomized. It showed the appearance of anaplasma about fifty days after the operation, with slight symptoms of oligocythaemia, the number of erythrocytes decreased from about 8 millions on the 27th March, 1924, to about $4\frac{1}{2}$ millions on the 2nd of June, 1924. Anaplasma was still present on the 16th February, 1925. That this animal was not a carrier of piroplasma at the time of the operation was shown to be the case by subsequent inoculations of infected blood on the 26.1.1925. *Gonderia mutans* occurred in the blood on the 3.3.1925, and *Piroplasma bigeminum* was frequent in the blood after a second injection of infected blood on the 6.3.1925. During the redwater reaction gonderia was very frequent and acute symptoms of oligocythaemia (due to *P. bigeminum*) were observed, viz.: number of erythrocytes decreased to 1 million per c.m.m., anisocytosis, polychromasia, erythroblasts, normoblasts frequent. There was a leucocytosis, mainly affecting the monocytes. Erythrophagocytosis in connexion with the latter was observed. C. 711 died of shock as the result of trypan blue injected to counteract the acute reaction of redwater.

C. 893 injected with infected blood on 9.10.24 showed an acute reaction of *Anaplasma marginale*, followed later on by a fair number of gonderia. Oligocythaemia was marked; the number of erythrocytes decreased from about 11 millions to $2\frac{1}{2}$ millions per cubic mm. The animal did not show the presence of *P. bigeminum* in its blood. It recovered on 15.12.24, and was splenectomized. This was followed by a reaction of redwater, and *Gonderia mutans* were again frequent in the blood. Anaplasms appeared in the blood on the 5.1.25. On the 26.3.23 *P. bigeminum*, *Anaplasma marginale*, and gonderia were still present with slight symptoms of oligocythaemia.

In case of C. 1027 and C. 1034, fairly acute reactions followed splenectomy, in which that of redwater was observed three days after the operation and *Anaplasma marginale* four days afterwards. *Gonderia mutans* were very numerous soon after splenectomy.

C. 1027, splenectomized on the 24.12.24, still showed the presence of all three parasites on the 9.3.25, gonderia being the more numerous. Oligocythaemia was most marked from about the 11.1.25 to 16.1.25, when the number of erythrocytes had decreased to less than 2 millions; on the 17.3.25 it was still at about $2\frac{1}{2}$ millions. (See Graph 4, Appendix 4.)

C. 1034 showed symptoms of acute oligocythaemia from 11.2.25 to 23.3.25, when the number of erythrocytes remained below 2 millions; on the 16.2.25 it reached 1.3 million c.mm.

In case of cattle 758, splenectomized on the 6.6.24, the presence of *Anaplasma centrale* in the blood on the 18.6.24, and a slight infection of *P. bigeminum* on 19.6.24 was noted. The animal died on the 26.6.24 of a paratyphoid infection, characterized by a miliary necrosis in liver, spleen, and lymph glands.

Symptoms, etc.—No animals died directly from the effects of piroplasmosis or anaplasmosis as result of splenectomy. Two animals succumbed; one as the result of shock, the other as the result of an intercurrent disease, viz., paratyphoid. In case of a pure anaplasmosis reaction following splenectomy, symptoms only involving paleness of the mucous membranes were observed. There was little, if any, constitutional disturbance beyond a slight loss in condition. *Gonderia mutans*, although very frequent, causes little disturbance to an animal beyond slight anaemic changes, but in case of a relapse of redwater produced by splenectomy, acute symptoms of anaemia were observed, viz.: palor of mucous membranes, rapid loss of condition, etc. The reactions, however, were controlled with an intravenous injection of trypan blue.

Summary.

(a) It appears that all bovines are not necessarily the carriers of the three parasites, viz.: *P. bigeminum*, *P. mutans*, and *Anaplasma*.

(b) Some animals after splenectomy only showed mild reactions of anaplasmosis, whereas when subsequently infected with blood containing *P. bigeminum*, they showed acute reactions of redwater, in which oligocythaemia was very marked.

(c) Carriers of the three parasites, when splenectomized, showed the presence of gonderia in great numbers without any apparent symptoms. *P. bigeminum* was mainly responsible for the acute symptoms of oligocythaemia.

(d) In all cases in animals without a spleen the course of the disease became a chronic one, especially as regards the blood changes.

(iv) SPLENECTOMY OF SHEEP, "CARRIERS" OF ANAPLASMS.

Ovine anaplasmosis has not yet been described in South Africa, and for that reason it is necessary to consider this disease, the various experiments, and the literature, etc., in greater detail.

The Literature.—*Anaplasma*, mainly as the result of experiments of Theiler (1910) in South Africa, Lignieres (1914) in South America, and other workers in other parts of the world (as recently

as 1924 by Helm in Germany), have been incriminated as the actual cause of a serious disease, especially in bovines. Very little, at least definite, information has so far been published about the occurrence of anaplasma in sheep. The first reference to the appearance of such parasites in sheep seems to be that of Schellhase (16), who in 1909 describes a disease of sheep in German East Africa which was characterized by symptoms of catarrh of the conjunctival and nasal mucous membranes, loss in condition, followed by diarrhoea and irregular temperatures. The animals died of poverty. *Haemonchus contortus* were frequent. The blood, besides anaemia, in case of one sheep showed numerous *coccus-like bodies of various sizes*; the majority were in the corpuscles. It would seem that Schellhase was apparently not dealing with anaplasmosis, but probably with some intercurrent disease (catarrhal symptoms) complicated with anaemia, probably as the result of a verminosis. With reference to the coccus-like bodies, it might here be mentioned that on innumerable occasions (see below) cocci, diplococci, free or adherent to erythrocytes, were seen in smears of healthy and infected sheep. In another publication, 1912, Schellhase (17) refers to a similar disease; the chief symptoms were diarrhoea, rhinitis, conjunctivitis; and, in case of eighteen sheep, he could only show the presence of "marginal points" in six. No other details as regards blood changes, etc., are given.

In 1912 Bevan (18) mentions the occurrence of anaplasmas in the red corpuscles of sheep, associated with all the changes of the blood met with in anaplasmosis of cattle. Unfortunately, as none but locally bred sheep were available for sub-inoculation, an experimental strain could not be established, the most noticeable symptom in every case being a dropsical condition of the throat. The latter symptom described seems to indicate that these animals were probably suffering from *Haemonchus contortus* infection, resulting in anaemia with the appearance of jolly-bodies and anasarca. In 1913 Trautmann (19) confirmed the observations made by Schellhase. He again mentions the peculiar catarrhal changes of the eyes, nose, buccal mucous membranes, emaciation, and alopecia. In a lamb, stomatitis with ulceration and bluish-red coloration of the m.m. are mentioned. It would seem that the symptoms, especially the latter, resemble those of blue-tongue of sheep of this country. Anasarca was also evident. Blood showed "marginal points," coccus-like bodies of various sizes, mostly peripheral, inside and outside the cells, very often in the form of diplococci. An increase in the number of blood platelets with tail-forms were observed. At post-mortem, the chief changes noticed by Trautmann were the presence of *Haemonchus contortus*, gelatinous changes in the fat and cachexia. It would appear that neither Schellhase nor Trautmann were dealing with anaplasmosis of sheep, but most probably with some other specific disease characterized by catarrhal symptoms, complicated with anaemia, cachexia, and anasarca, the result of a verminosis. In our cases of anaplasmosis, with a progressive fairly acute anaemia, there was very little evidence of cachexia and no catarrhal symptoms, whereas icterus and oligocythaemia were always present. Schellhase and Trautmann do not refer to an icterus. The first reliable description of anaplasma in goats (he does not mention sheep) is that of Domizio (21). In 1913 he saw the presence of "marginal points" in a goat in Eritrea, with symptoms

of progressive anaemia, extreme palor of the mucous membranes, slight fever, and a weak pulse. Microscopic examination revealed severe anaemic changes and a particular striking picture of "marginal points," polychromasia, poikilocytosis, punctate degeneration, anisocytosis, normoblasts, and leucocytosis. About one-half or more of the red cells contained two or three bodies, although the greater majority only contained one. These were seen as minute, more or less rounded or oval bodies from 0.4μ to 1μ in diameter, with *indistinct, sometimes irregular margins*, situated for the most part towards the periphery of the corpuscle. Others were more or less adjacent to the margin of the corpuscle, whilst some appeared to be completely extra-corpuseular. A very small minority were situated towards the centre of the corpuscle. The bodies stained of a purplish-red, and some forms on closer examination showed a very minute sprouting process. Domizio compares this anaplasma with jolly-bodies, produced by the methods of Aragao and Dias (20). These jolly-bodies were small, spherical-looking masses, staining a deep dark violet, like the chromatin of the nuclei of normoblasts. Domizio concludes that there exists among goats in the colony of Eritrea an intracorpuseular blood parasite, which in all probability represents a species belonging to the genus anaplasma (*Anaplasma ovis*).

According to Finzi and Campus (22), the observations made by Tibaldi in Sardinia, and their own observations, did not allow them to confirm the existence of anaplasms in Sardinian sheep. The intracorpuseular "marginal" and "central" bodies are frequently signs of changes of the blood, usually encountered in acute secondary anaemia following grave distomatosis.

Lignieres (23) reports that the injection of *Piroplasma bigeminum*, *Piroplasma argentinum*, and *Anaplasma* causes in sheep and goats no visible symptoms. If the blood of these animals is injected into healthy cattle, after two months these cattle contract anaplasmosis, but not piroplasmosis. These experiments of Lignieres were confirmed by Kraus, Dios, and Oyarzabal (24) in Buenos Ayres, and they were of the opinion that there may be an "invisible stage" in the sheep and that anaplasma may be a product of haemolytic poisons. Later on Lignieres (25) was able to isolate anaplasms from piroplasms through goats and sheep. He demonstrated the possibility of immunizing cattle by means of blood from infected sheep, in one case with an anaplasma strain maintained for eleven generations in sheep. Sergent and his co-workers (26) also confirm Lignieres work *re* preservation of anaplasma in sheep, *but not constantly*. They have been unable to transmit the infection in sheep in series. Theiler (27) was not able to infect sheep and horses with bovine anaplasmosis. Just recently Lestoquard (28) reports that Algerian sheep may be infected with five different viruses, viz.: *Piroplasma ovis*, *Babesiella ovis*, *Gonderia ovis*, *Theileria ovis*, and *Anaplasma ovis*. The similarity between the piroplasma of bovines and of ovines and goats is thus complete in Algeria.

The above is a brief consideration of the publications of some authors who admit the *parasitic nature of anaplasma*. There are, however, many workers of repute who have refused to admit the parasitic natures of these so-called "marginal points" and regard them either (a) as *disintegrated nuclear remains* (jolly-bodies), often

encountered in oligocythaemia; or (b) as the degenerated "protoplasm-free remains" of blood parasites such as *Piroplasma bigeminum*; or (c) as "reaction products" of ultraviolet agencies. The point raised in (b) does not concern us, because anaplasma occurred in sheep in our experiments, unassociated with any form of piroplasma, type bigeminum, or other blood parasites.

In the case of (a), Jowett (29) and others mention the appearance of coccus-like bodies in several erythrocytes of cats experimentally infected with trypanosomes and also in the blood of healthy cats. In the case of anaemic subjects, these bodies were, as a rule, more numerous, and polychromasia and anisocytosis were also present. These bodies give the chromatin staining reaction. Nesor mentions the presence of jolly-bodies in clinically healthy horses, and De Kock (1) maintains that jolly-bodies are more numerous in equines showing reactions of infectious anaemia. Normoblasts and jolly-bodies are also more frequent in acute forms of nuttalliosis. Dodd (30) thinks that it is debatable whether the intracorpuscular bodies giving reactions of chromatin and found in certain other animals, can be considered to belong to anaplasmas. The evidence at present appears that the chromatin bodies observed in the erythrocytes of marsupials, lemurs, mousedeer, monkey, etc., are more probably identical with jolly-bodies.

In case of (c), viz., that these "marginal points" are the products of "ultraviolet agencies" or poisons, Dias and Aragao, by means of the injection of phenylhydrazin, pyrogallie acid, nitrobenzol, and trypan-blue into rabbits, guinea-pigs, dogs, and bovines, were able to produce anaplasma-like bodies associated with anaemia. Theiler (31), in case of bovine anaplasmosis, failed to obtain a "filter-passer virus" through a Berkefeld filter.

Summary of the Important Points raised in the Literature.

(1) On account of: (i) the close resemblance of "jolly-bodies" and "anaplasma," (ii) the cachexia, anasarca, anaemia associated with *Haemonchus contortus*, (iii) the occurrence of catarrhal symptoms, (iv) the absence of icterus, (v) the absence of positive transmission experiments, it is doubtful whether anaplasma was actually seen in sheep in German East by Schellhase and Trautmann, and in Rhodesia by Bevan.

(2) Probably some of the coccus-like bodies described associated with the erythrocyte, or free in the blood, or as diplococci, are of the nature of bacteria, whereas others may have been jolly-bodies associated with an anaemia.

(3) Domizio's description of anaplasma in goats seems to be the first reliable information of the presence of these parasites in animals other than bovines.

(4) Lignieres in the Argentine (confirmed by Sergent and others) was able to make the sheep a "carrier" of bovine anaplasma, but not piroplasma, without the appearance of parasites in the circulation. In this way he was able to separate bovine anaplasma from piroplasma.

(5) Many investigators deny the "parasitic nature" of anaplasma and regard them either—

- (a) as disintegrated nuclear remains; or
- (b) stages in the life-cycle of piroplasms; or
- (c) "reaction products" of ultraviolet viruses, although no reference in the literature was actually found to prove this.

The experiments undertaken in the study of anaplasmosis in sheep can be conveniently considered under the following:—

(1) Anaplasmosis in sheep, either produced by splenectomy of "carriers" of anaplasma, or by the infection of susceptible sheep, or by the infection of splenectomized susceptible sheep with infected blood.

(2) Transmission of ovine anaplasmosis to *ovines* (including passage in a series of sheep), to *bovines*, to *goats*, or do such animals only become "carriers"?

(3) Transmission of bovine anaplasmosis to *ovines*, or do such animals only become "carriers"?

(4) A study of the nature of anaplasma of sheep—

- (i) as regards filtrability;
- (ii) as regards its resistance *in vitro*;
- (iii) a comparison of sheep "anaplasma" with "jolly-bodies" produced by drugs.

(1) The preliminary experiments indicated that splenectomy of *local* sheep was followed by a "relapse" of anaplasmosis, whereas in case of sheep introduced from some other localities, e.g. the Free State Province, no such relapse occurred. Such susceptible sheep could easily be infected by the injection of blood of sheep which are the carriers of anaplasma.

The following classes of sheep were utilized for the study of the reactions, incubation period, blood, etc.:—

- (a) Susceptible sheep (from the Free State Province);
- (b) splenectomized susceptible sheep (from the Free State Province);
- (c) splenectomy of "carriers" which have passed through a reaction;
- (d) "carriers" of anaplasma which have passed through a reaction.

Tables have been compiled from the various experiments (*vide* appendices) to compare the reactions, etc., in these various classes.

By origin of virus is meant whether the blood used for inoculation was taken from a susceptible sheep, infected by blood-inoculation, or from a "carrier" splenectomized. This is included in column 3, Table I, to ascertain whether splenectomy may increase the virulence or otherwise of the anaplasma. (S.P.=Blood derived from a splenectomized carrier. S.U.=Blood from a susceptible sheep infected by blood-inoculation.)

From Table I (and appendices) it will be seen—

- (i) that in case of susceptible sheep infected with blood, the incubation period varied from the fourth day to the thirty-first day, the majority showed the presence of parasites from the ninth to eleventh day:

- (ii) that the duration of the disease in susceptible sheep was about two months;
- (iii) that symptoms varied from a slight anaemia to a more acute anaemia, in which case the mucous membranes were white with a slight tinge of icterus;
- (iv) that the blood-changes in some cases were slight, whereas in others acute; besides anisocytosis, polychromasia, punctate degeneration, erythroblasts, normoblasts, and jolly-bodies, the number of erythrocytes decreased to about 3 millions per cubic m.m.;
- (v) that the source of the blood did not seem to have any bearing on the reaction, i.e. whether the blood was taken from a splenectomized "carrier" or an infected susceptible;
- (vi) that the reaction was not influenced, whether the blood was taken early in the reaction or late;
- (vii) no mortality from anaplasmosis occurred amongst the thirty-two susceptible sheep infected.

TABLE I.
(a) *Susceptible Sheep.*

No. of Sheep.	Date of Inoculation	Origin of Virus.	First appearance of Parasites.	Whether Frequent or Otherwise.	Lowest Number of Erythrocytes per cubic m.m. Registered.
8427	19/3/24	S.P.	11 days	Infrequent...	4 millions.
8428	"	S.P.	13 "	Infrequent..	4½ " N.
8429	"	S.P.	14 "	Frequent....	3.9 " N.
8434	"	S.P.	11 "	Infrequent...	2.7 " E.N.
9099	2/8/24	S.P.	9 "	Frequent....	
9100	"	S.P.	9 "	Rare.....	
8432	5/4/24	S.P.	17 "	Frequent....	3.7 " N.
8433	"	S.P.	4 "	Frequent....	3.3 " N.
8455	24/4/24	S.P.	11 "	Infrequent...	3.3 " N.
8458	"	S.P.	18 "	Infrequent...	3.3 " N.
9122	7/10/24	S.U.	15 "	Not infrequent	
8454	"	S.U.	18 "	Rare.....	
10929	10/2/25	S.U.	8 "	Frequent....	
10921	"	S.U.	10 "	Not infrequent	
8461	"	S.U.	10 "	Rare.....	
8473	"	S.U.	10 "	Frequent....	
9111	"	S.U.	10 "	Not frequent.	
8453	"	S.U.	10 "	Not frequent.	
7109	30/4/24	S.P.	16 "	Frequent....	
8447	30/9/24	S.P.	11 "	Not infrequent	
6350	8/10/24	S.P.	20 "	Not infrequent	
6543	"	S.P.	15 "	Frequent....	
8459	14/6/24	S.U.	11 "	Frequent....	
9128	26/6/24	S.U.	11 "	Infrequent...	
9102	24/7/24	S.U.	12 "	Rare.....	
9131	26/8/24	S.U.	15 "	Not rare.....	
9130	7/10/24	S.U.	15 "	Not infrequent	
9120	8/7/24	S.U.	31 "	Not rare.....	
9116	"	S.U.	9 "	Infrequent...	
9121	10/11/24	S.U.	21 "	Rare.....	
10229	26/3/25	S.U.	8 "	Rare.....	
10250	"	S.U.	8 "	Not infrequent	

N = Normoblasts.

E = Erythroblasts.

TABLE II.
(b) *Splenectomized Susceptible Sheep.*

No. of Sheep.	Date of Inoculation.	Course of Disease.	When Splenectomized.	Origin of Virus.	First appearance of Parasites.	Whether Frequent.	Lowest No. of Erythrocytes.
8430	15/9/24	Killed for Pathology	8/4/24	S.P.	7	Frequent	—
8457	30/6/24		15/5/24	S.U.	10	Frequent	1·9
8456	26/6/24		15/5/24	S.U.	4	Frequent	1·5

From the Table II (and the appendices) it appears—

- (i) that the incubation period varied from four to ten days, it was difficult to say whether this would have remained so short if a large number of sheep were utilized;
 - (ii) that the symptoms and blood-changes were slightly more acute than those observed in Table I, the number of erythrocytes decreased to below 2 millions per cubic m.m.;
 - (iii) that erythrophagocytosis was very evident in the circulating blood (to be referred to below);
 - (iv) that the source of the virus did not influence the reaction;
 - (v) that the course of the reaction was decidedly extended.
- All these animals were, however, killed during the reactions for pathological study.

TABLE III.
(c) *Splenectomy of "Carriers."*

No. of Sheep.	Date of Splenectomy.	Course of Disease.	First appearance of Parasites.	Whether Frequent.	Lowest No. of Erythrocytes per cubic m.m.
7369	28/2/24	Died.....	11	Frequent.....	—
7443	"	Killed <i>in extremis</i>	14	Frequent.....	—
8427	3/7/24	Still reacting Dec., 1925	18	Frequent.....	3
8428	"	" "	8	Frequent.....	1·6
8429	22/9/24	" "	7	Frequent.....	4
8434	3/7/24	Killed for Pathology	8	Frequent.....	1·8
8451	28/1/25	Still reacting Dec., 1925	8	Frequent.....	4·6
9119	"	" "	31	Not infrequent	3
10511	13/3/25	" "	17	Frequent.....	1·9
10743	—	" "	21	Not infrequent	2·8

More or less the same remarks apply for (c) as for (b), except here, actually, mortality from anaplasmosis occurred, and in some of those cases, which have survived, the disease has actually been in progress for eighteen months and still showing the presence of parasites, e.g. sheep 8427 and 8428.

(d) No reaction could be produced in sheep which have passed through anaplasmosis by blood-inoculation.

In the study of anaplasmosis in sheep it was found that the reaction could more or less be divided *into a number of stages*, however, without sharp lines of division, viz. :—

- (i) occurrence of anaplasms in the circulation, the number gradually increasing;
- (ii) appearance of changes in the blood, commencing with slight anisocytosis, passing to a more marked anisocytosis, polychromasia, punctate degeneration; the number of erythrocytes reached its lowest level with the presence of a few erythroblasts;
- (iii) anisocytosis, polychromasia, etc., with the regeneration forms frequent, i.e. erythroblasts, normoblasts, and the appearance of jolly-bodies, especially larger ones, but various stages present. Increase in the number of erythrocytes; anaplasma distinctly rare;
- (iv) anisocytosis and polychromasia less marked, fewer normoblasts, jolly-bodies more frequent and of more uniform size, especially small forms; anaplasma may be absent. This stage may last for some time, and in the non-splenectomized animal eventually lead to recovery. In the splenectomized sheep the course is protracted, with slight "relapses" and recurrence of parasites in the blood.

The above remarks might be amplified in the following cases:—

- Sheep* 7109 (non-splenectomized) third stage:
 on 26.6.24. Few anaplasma. S.A. (slight anisocytosis), S.P. (slight polychromasia) S.P.D. (slight punctate degeneration). Jolly-bodies very frequent. No difficulty in distinguishing J.B. from anaplasma.
- Sheep* 8428 (splenectomized) first stage:
 on 17.7.24. Anaplasma frequent, the majority appear to be attached to the corpuscle, or part of them seem to protrude beyond the margin of the erythrocyte. They appear as dark violet-blue compact masses (stained with Pappenheim's modification of Giemsa), not absolutely spherical, appear to have minute protrusions from the periphery. In some erythrocytes more than one parasite present, not all the same size; where more than one in a corpuscle, some are situated nearer the centre.
 21.7.24. Second—third stage. Anaplasma less frequent. A; P; erythroblasts, normoblasts, jolly-bodies various stages from normoblast.
- Sheep* 8428 (splenectomized)
 on 23.7.24. Fourth stage.
 Jolly-bodies frequent.
 26.7.24. Jolly-bodies frequent, some erythrocytes more than one, some situated near the periphery, few nuclear remains of normoblasts, which lie free in the circulation, some protruding from the normoblast.
 27.7.24. Jolly-bodies smaller, many situated near the periphery.
 1.8.24. Jolly-bodies small, not frequent. At this stage it would be difficult to distinguish jolly-bodies from anaplasma *if the previous reaction was not known*.
 11.8.24. *Relapse*: Anaplasma more frequent, anisocytosis, jolly-bodies rare.

- Sheep 8457 (splenectomized susceptible infected)
 on 16.7.24. First stage.
 Anaplasma frequent.
 21.7.24. Second-third stage.
 Anaplasma rare, A; P; P.D., erythroblasts, normoblasts. J.B.
 23.7.24. Third stage.
 A; P; erythroblasts, normoblasts frequent, jolly-bodies.
 26.7.24. Fourth stage.
 A; P; normoblasts, jolly-bodies very frequent, protruding and
 free nuclei of normoblasts.
 29.7.24. S.A; S.P; jolly-bodies not infrequent, small forms.

In considering the reaction of anaplasmosis in sheep in this way, it was found that morphologically anaplasma could easily be distinguished from jolly-bodies, and in no way confused for them. Anaplasma make their first appearance without changes in the blood, whereas a crop of jolly-bodies was always preceded by the appearance of normoblasts in the circulation. In the drug experiment, as will be shown below, stages 2, 3, 4, etc., are set up *without* any evidence of anaplasma.

Besides this method of differentiation between anaplasma and jolly-body, other criteria might be considered:—

(i) Anaplasma are fairly characteristic, and with Pappenheim's stain are of an intense violet-blue colour. They are not completely spherical, the contour here and there show slight protrusions, as if "sprouting." (See figures 19, Appendix 4.)

(ii) When only a few are present, their position is more or less peripheral. When more erythrocytes are affected, the number of anaplasma in the corpuscles increase, and their position, besides peripheral, may be more towards the centre. As many as four anaplasma were seen in one erythrocyte.

(iii) Their sizes may vary, especially when they are very frequent, but usually this discrepancy is slight when compared with the variations in size observed in case of jolly-bodies during some of the stages.

(iv) With the dark field illumination, many of the anaplasma appeared to be structures hanging on or attached to the corpuscles.

(v) Various stains were employed to ascertain whether a method could be evolved which would be specific, either for anaplasms or for jolly-bodies. Smears were made during the first stage and during the third and fourth stages for comparison. It was found that when such smears are stained with Giemsa for about six minutes, anaplasma always stain an intense violet-blue colour, whereas the chromatin of the nuclei of normoblasts and jolly-bodies stained not at all, or only of a slight red chromatin colour. The jolly-bodies were completely spherical and their contour were sharp and well defined.

(vi) It was found that with the appearance of pathological regenerating forms in the circulation, i.e. erythroblasts and normoblasts, and with the decrease in the number of anaplasma, jolly-bodies gradually increased in number. Various stages in their development could be traced, i.e. from normoblasts to jolly-bodies of various sizes, and finally to jolly-bodies of the size of anaplasma. (See figures 6, 12, 13, 14, 20-25, Appendix 4.)

(vii) As the number of jolly-bodies decreased and the anæmia improves, they become smaller in size and their position within the corpuscle more variable, often forms are found which are situated near the periphery of the erythrocyte. At this stage, if one is not

acquainted with the finer detail of these structures, and without a previous history of the reaction, great difficulty might be encountered in deciding whether one is dealing with anaplasma or jolly-body.

Besides jolly-bodies, the appearance of *cocci* in the smears may be mentioned in connexion with the differential diagnosis of anaplasma. Cocci were seen in the blood-smears of infected as well as healthy sheep on innumerable occasions, e.g.—

Sheep 8464 (splenectomized):

17.5.24. Lying free, as well as attached to the erythrocytes, perfectly circumscribed and spherical bodies, larger than anaplasma. They do not occur uniformly in the smear, but in patches.

Sheep 8462 (susceptible and infected with blood):

29.5.24. Coccus-like bodies free or in close contact with the margin of the erythrocyte. In places double, much larger than anaplasma, etc.

A differentiation between such *cocci* and anaplasma could always be made on account of—

- (i) their spherical appearance and regular contour;
- (ii) their association with the erythrocytes and the appearance of single and double forms free in the circulation;
- (iii) their uneven and irregular distribution over the smear, i.e. a patchy distribution;
- (iv) their irregular occurrence, i.e. they make their appearance for one or more days, and then no further trace of them.

The exact nature of these cocci is not yet understood, i.e. whether they occur in the blood of healthy sheep without setting up pathological changes, or whether they are accidental inclusions at the time when the smears are made, or whether they are present in the fluids used for staining purposes.

CHANGES AFFECTING THE ERYTHROCYTES.

It would appear that the number of erythrocytes in cases of clinically healthy sheep kept under stabling conditions varies between 10 to 12 millions per cubic mm. Schantz (32) gives an average of 9 million per cubic mm. On reference to Appendices 3 and 4 it will be seen that a polycythaemia occurred for a few days after splenectomy. The decrease in the number of erythrocytes, either when injected with infected blood or when carriers and splenectomized, is more or less the same, i.e. reaching the lowest level in about 15 to 25 days. In some cases less than $1\frac{1}{2}$ million were recorded. The non-splenectomized infected sheep reached the normal level in about 100 days and over, whereas recovery in the splenectomized animals, e.g. sheep 8427 and 8428, was very protracted with slight relapses at intervals. After eighteen months these sheep had not passed 8 millions per cubic m.m. In some sheep, in spite of such low counts as $1\frac{1}{2}$ million, etc., relatively slight clinical manifestations were observed; anisocytosis, polychromasia, and punctata degeneration appeared before the erythrocytes had reached their lowest level; changes in shape, i.e. poikilocytosis, and in haemoglobin-content were practically absent. It may be recorded that in case of two sheep (9129 and 9122) not associated with anaplasma reaction, there was a distinct oligochromæmia, and in case of sheep 9122 also a marked poikilocytosis, with the absence of pathological regenerating forms, e.g. erythroblasts and normoblasts. At post-mortem it was found that both sheep were heavily infected with *Haemonchus contortus*.

Another observation to be recorded in connexion with the erythrocytes of some sheep is the appearance of a peculiar irregular reticular-like network. These varied in shape from long irregular threads to network-like masses, sometimes almost resembling large piroplasms. No chromatin, nor typical cytoplasm, could be identified in them. In case of sheep 8454, on the 30th May, 1924, such pyramidal-shaped bodies were seen unassociated with anaemia. They were about the size of large piroplasms, some irregularly quadrilateral in shape, some lying free. They were dissociated from thrombocytes, nor was it due to faulty staining, because none of the other smears of that date showed it. In cases of sheep 10743, infected with anaplasma, showing changes of anaemia, these appearances in connexion with the erythrocytes were observed in February, 1925, and were still present in April, 1925; and on some days the greater number of erythrocytes were affected.

Besides the above, small chromatin points were from time to time observed in the erythrocytes of infected sheep as well as of clinically healthy sheep. The exact nature of these appearances in connexion with the erythrocytes is at present not understood.

CHANGES AFFECTING THE LEUCOCYTES AS REGARDS :

- A. *Number.*
- B. *Morphological Peculiarities.*
- C. *Internal Circulation.*

A. *Number of Leucocytes.*

The number of leucocytes per cubic mm. in the case of clinically healthy sheep kept under stabling condition, varied from about 7,000 to about 10,000. In one or two instances more than 14,000 per cubic mm. were recorded. Schantz refers to this variability, and gives 5,900 as the lowest and 19,800 as the highest figure recorded by him, with 8,000 to 9,000 as a fair average.

On account of these variations, changes affecting the numbers of leucocytes were studied in each case separately: first in the clinically healthy state and afterwards during the disease.

The differential counts in case of clinically healthy sheep are also somewhat variable. Schantz enumerates them as follows:—

<i>lymphocytes</i>	= 50-60	per cent.	
<i>monocytes</i>	= 3-5	"	(large mononuclears).
	8-14	"	(transitionals).
<i>neutrophiles</i>	= 20-40	"	
<i>eosinophiles</i>	= ±1	"	
<i>basophiles</i>	= ±1	"	

A fair average for clinically healthy sheep at Onderstepoort would be the following:—

<i>lymphocytes</i>	= 60	per cent.
<i>monocytes</i>	= 4	"
<i>neutrophiles</i>	= 34	"
<i>eosinophiles</i>	= 2	"
<i>basophiles</i>	= ±1	"

With reference to changes in the circulation after splenectomy, very variable and incomplete data are given by many authors. This is probably due to the fact that splenectomy was carried out in pathological cases.

Naegeli (11) gives the following changes, which occur some months after splenectomy, e.g. (a) lymphocytosis, (b) eosinophilia, (c) increase of monocytes (but this most probably associated with pathological processes), (d) polyglobuly.

Musser (12) states that in the dog splenectomy is followed by a gradual progressive decrease in red blood-cells, reaching the lowest level about the 26th day. Accompanying this change in the red cells is a leucocytosis, most marked after the operation and diminishing gradually. There was absence of the eosinophiles from third to eleventh day, but followed later on by an increase varying from 6 to 20 per cent. Krumbhaar and Musser (13) again observed the blood at various periods after splenectomy in the dog, and found that all animals developed a leucocytosis (26,000 to 38,000) with a rapid fall in a few days, reaching the normal level at the end of four months. The initial leucocytosis was mainly due to an increase in neutrophiles, then in eosinophiles (10 to 32 per cent.). According to Mayr and Moncorps (36), there exists a relation between the function of the spleen and the appearance of eosinophiles in the circulation. Suspension of spleen function, e.g. after splenectomy, leads to the accumulation of those substances which work productively on the origin of these A-cells. On the other hand, an increased function of the spleen leads to an accumulation of "eosinophilic anti-substances," followed by a fall in the number of eosinophiles in the circulation.

Analysis of the leucocytic counts and charts (see Appendices 3 and 4) in the following cases:—

- (i) Susceptible sheep infected with anaplasma blood, e.g. sheep 8428, 8429, 8455.
- (ii) Splenectomized susceptible sheep infected with anaplasma blood, e.g. sheep 8457.
- (iii) Splenectomized susceptible sheep, e.g. sheep 8457.
- (iv) Splenectomized carriers of anaplasma, e.g. sheep 8428.

The absolute values of the various leucocytes, as well as the total number of erythrocytes and leucocytes, per cubic mm. were charted.

(i) Susceptible Sheep infected with Anaplasma Blood.

(a) In case of all three sheep, variations occurred in the number of leucocytes after inoculation, first a tendency to a decrease, followed later on by an increase more or less to the normal level.

(b) The decrease seemed to affect the neutrophiles more particularly, whereas the increase in the number of leucocytes subsequently seem to affect the lymphocytes, which runs more or less parallel with the return to normal of the erythrocytes.

(c) In all three sheep the decrease in the number of erythrocytes was also associated with a monocytosis, and this was more or less accompanied by an increase in the number of eosinophiles. (See Graphs 1 and 2, Appendix 4.)

(ii) Splenectomized Susceptible Sheep infected with Anaplasma Blood.

(a) The injection was associated with a decrease in the number of leucocytes (chiefly neutrophiles), but with the onset of oligocythaemia there was a leucocytosis, chiefly affecting the monocytes, most marked at the time when the number of red cells had reached their lowest level.

(b) The monocytosis was associated with an erythrophagocytosis and accompanied by an increase in eosinophiles.

(c) The increase in number of erythrocytes was much protracted and was accompanied by a more or less parallel lymphocytosis.

(d) Several relapses occurred in which the above changes were repeated. (See Graph 3, Appendix 4.)

(iii) *Splenectomized Susceptible Sheep.*

In case of sheep 8457, a polyglobuly followed splenectomy accompanied by a neutrophilia. There was a return to normal after a time.

(iv) *Splenectomized Carriers.*

(a) A marked leucocytosis (in one case 58,000 per cubic millimetre), chiefly associated with an almost precipitous neutrophilia.

(b) This was followed by an equally sudden monocytosis with erythrophagocytosis, the monocytosis was most marked at the time when the number of erythrocytes had reached its lowest level. There was also an eosinophilia at this period.

(c) The return of the erythrocytes to the original level was protracted and accompanied by a lymphocytosis, monocytosis, and eosinophilia.

(d) These protracted cases of anaemia seem to be more or less associated with a permanent leucocytosis affecting the monocytes, the eosinophiles, and the lymphocytes.

B. *Morphological Peculiarities.*

Monocytes.

Great difficulty was experienced, especially in those cases with a definite monocytosis, in deciding whether certain forms of leucocytes belonged to the monocytes or to the lymphocytes. Schantz, in his consideration of sheep's blood, divides the lymphocytes into two groups: (i) *small forms*, slightly larger than erythrocytes; and (ii) *large lymphocytes* with an oval eccentric nucleus, which possessed a lighter basophilic blue-violet colour and a badly recognizable irregular net-like structure, whereas the cytoplasm was very bulky and of a diffusely bluish-grey colour, often containing 3 to 8 azure granules. *Schantz maintains that the large mononuclears (monocytes) resemble this second group of lymphocytes closely.* In a number of instances one could not even rely on the characteristic appearance of the chromatin in these cells to make a differentiation.

For the blood-study, Pappenheim's modification of Giemsa was used. Infected non-splenectomized animals showed practically no morphological changes as regards the monocytes. In case of the splenectomized "carriers" and infected splenectomized susceptibles, the monocytosis was accompanied by erythrophagocytosis. The remains of erythrocytes, as well as of normoblasts, were seen in the cytoplasm of these cells in all stages of degeneration (see figures 1, 2, 3, 4, 36-44, Appendix 4). This occurred especially at a time when the number of erythrocytes had reached its lowest level. As the oligocythaemia improved, so these forms disappeared from the circulation.

The stages of degeneration of the phagocytosed cells, or might one call it the digestion of the cell-inclusions, varied in size from that of erythrocyte to the appearance of much smaller structures. In the majority more than one cell-inclusion was present. A number of monocytes were characterized by the presence of large granules,

e.g. in case of sheep 8428 on the 12th July, 1924, the presence of numerous granules in the cytoplasm of some monocytes were seen, varying in colour from a light chromatin-red to an intense dark purple, and varying in size from a pin-point to granules several μ in diameter.

In several instances monocytes were encountered with chromatin-inclusions several μ in diameter and more or less pyramidal in shape (see figures in Appendix 4). It would appear (i) that these inclusions are probably the remains of degenerated erythrocytes, normoblasts, and probably also leucocytes; (ii) that in the monocytes these cellular-inclusions undergo digestion which would account for the variability in size, colour, shape, etc.; (iii) that in splenectomized animals the filter of such phagocytic monocytes has been removed, that is why they are so prevalent in the general circulation [see Holler (10) referred to above].

Erythrophagocytosis by monocytes has been described by De Kock (1). Domagk (33) mentions it in connexion with endothelials, in which the most variable inclusions, e.g. erythrocytes, leucocytes, etc., are recorded. Hirschfeld and Sumi (34) states that three different types of cells are associated with erythrophagocytosis, viz.: *neutrophiles*, *monocytes*, and cells identified as *endothelials*. With reference to the latter, it may be pointed out that from time to time large cells were encountered in the blood of splenectomized "carriers" which resembled "endothelials." These cells showed a relative large amount of a light-stained cytoplasm with a somewhat irregular-stained nucleus. In some instances "vacuoles" were seen in the cytoplasm of monocytes of splenectomized infected sheep. These "vacuoles" probably represent a stage in the process of erythrophagocytosis. [See figure 44 (1).]

Neutrophiles.

In cases of "neutrophilia" after splenectomy a number of neutrophiles (young forms) were seen, characterized by a rather compact, hoof shaped, non-lobulated nucleus. In many instances the neutrophile granules were abnormally distinct. Erythrophagocytosis as such was not observed in case of neutrophiles, but in some of the splenectomized sheep and also in some of the splenectomized horses (see figure 48, Appendix 4), "vacuoles" were seen in the cytoplasm. Whether these vacuoles represent a stage in erythrophagocytosis, or whether they represent degeneration of the neutrophiles, is not clear.

Cells of a pathological nature (figures 45-47, Appendix 4) of a neutrophilic character were also encountered.

Lymphocytes.

Besides the difficulty encountered in differentiating between certain forms of lymphocytes and monocytes already referred to above, other peculiar cells, probably of the nature of "lymphocytic types" were from time to time seen in the blood of sheep.

Such cells closely resemble Cell No. 4, described by Piney (37) as a *Türk cell* in a case of plasma cell leukaemia in man.

The second type of cell, which was seen in the blood of sheep (splenectomized, infected), resembles cells Nos. 4 and 7, described by Piney as *plasma cells*, and characterized by a fine "vacuolation" appearance of the cytoplasm. Whether these latter cells seen in the blood of sheep are of the nature of "plasma cells" (see also under internal circulation below) has not yet been decided.

Regularly lymphocytes were encountered in the blood of healthy as well as infected sheep, whose cytoplasm showed the presence of peculiar granules, e.g.—

Sheep 7754 (clinically healthy), 21st July, 1924, lymphocytes with peculiarly intensely dark-stained chromatin-like granules, about $\frac{1}{2}\mu$ to 1μ in diameter, more or less of the same size.

Sheep 8464 (splenectomized), 28th July, 1924, lymphocytes with oval, darkly stained chromatin-like granules.

Sheep 8457 (splenectomized and infected), 1st August, 1924, granules in lymphocytes frequent, various shapes; about 1μ in diameter, many of them appear to be surrounded with what looks like a "halo."

The exact nature of these granules is not understood. They are not of the nature of typical acidophile granules, on account of their number and more intense character of staining. The granules described by Du Toit (38) in the lymphocytes of normal bovine blood (*vide* Nos. 10 and 12) resemble the above to a certain extent, especially as regards the number and distribution. It is quite possible, therefore, that these granules observed in the lymphocytes of sheep are entities of these cells, and characteristic for this species of animal.

C. Internal Circulation.

A study was made in various instances of the distribution of the erythrocytes and leucocytes in different parts of the body. Sheep in various stages of anaplasmosis were chloroformed and samples of blood and smears collected from the jugular, vena cava caudalis, aorta, and vena portae. It would appear that the variations of cells, etc., in these parts were insignificant, e.g. in case of sheep 8456 the following were found:—

	R.C.	W.C.	L.	M.	N.	E.	B.
Jugular.....	1.5	3,4400	30	24	45	1	—
Vena cava.....	1.2	3,4200	36	20	42	2	—
Vena portae.....	1.1	3,3200	35	19	45	1	—
Aorta.....	1.1	3,5800	41	17	41	—	—

Some of the cells appearing in the smears from some of these situations were interesting:—

Sheep 7369 (splenectomized carrier), vena carva caudalis: large cells in the smears exceeding the size of monocytes, characterized by intense basophile cytoplasm, whereas the nucleus had a more granular, net-like arrangement of the chromatin. Some of these cells showed the presence of inclusions, either in the form of engulfed erythrocytes or large irregular chromatin stained masses. These cells appear to be of the same nature as the cells encountered in the intralobular capillaries and central veins of the liver, where marked erythrophagocytosis and desquamation of stern cells occurred. These cells will be more fully discussed in connexion with the pathology.

Thrombocytes.

In the splenectomized sheep, not only was there an apparent increase in the number of thrombocytes, but also variations from the normal size and shape. Naegeli (11) contends that not sufficient notice is taken of an increased number of thrombocytes in cases of splenectomy. Very often a marked increase was present in the blood of some sheep in the form of a white flocculent layer above the leucocytes in the tubes utilized for the red precipitate. Smears made from this showed a pure accumulation of thrombocytes. In the smears made from the blood it was found that the thrombocytes were more "diffusedly" dispersed in great numbers, instead of the usual "clusters." Their shape was more variable. Instead of the spherical discs they become kidney-shaped, with their poles drawn out into fine threads. Naegeli refers to similar anomalies in his book (11), page 184. Great richness in thrombocytes was especially evident in cases of anaemia in the reconvalescent stage (e.g. sheep 9119 and 8451). This is regarded by Naegeli as a hyperactivity of the megakaryocytes. This question of the relation of the thrombocytes to the spleen has not yet been settled. According to Sutherland and Williamson (39), it is still uncertain whether the platelets are actually destroyed by the spleen or are merely withdrawn from the circulation and stored up in the spleen. In some cases of splenectomy for "essential thrombocytopenic purpura haemorrhagica," the blood in the spleen has been found to contain many more platelets than red cells.

Symptoms.

In case of splenectomized "carriers," recovery from the operation was uneventful. Two types of anaplasmosis were observed:—

- (i) *A very acute and rapidly fatal form*, as exemplified in the splenectomized sheep. The condition is the same whether the sheep harboured the parasite, having recovered from the mild form and splenectomized, or whether non-infected splenectomized sheep were inoculated with blood containing anaplasma.
- (ii) *A mild form of the disease* from which sheep invariably recovered.

(i) *The acute disease* is ushered in with listlessness and a rapidly rising temperature. The parasites can be seen in the blood for a few days previously, when there is a very rapid increase from day to day until 75 per cent. of the corpuscles show parasites. About twenty-four hours after the appearance of clinical symptoms the animal refuses food and lies most of the day with head stretched out and the chin resting on the ground. The mucous membranes become anaemic, quite white, and later on show slight icterus. The skin also becomes anaemic, so much so that at a casual examination it is possible to pick out affected sheep standing with healthy sheep in the box, on account of the extreme paleness of the face and lips.

The pulse rate is increased up to 148 to 168 p.m. The heart-beat is slightly throbbing and irregular. Respirations vary from 40 to 60 p.m. and are shallow. The superficial lymphatic glands are not changed. The faeces appear normal in some cases, in others they are soft. Towards the later stages the animal is unable to rise or stand when placed upon its feet. It lies extended on the side and shows slight nervous twitching of the eyelids and ears. It dies of *profound anaemia* after a course of about four days.

In some cases the disease is not quite so acute, even in splenectomized sheep. The symptoms are not so well marked and affected sheep gradually recover after showing protracted symptoms of anaemia for a period of 12 to 20 days.

(ii) *The mild form of the disease* is seen in susceptible sheep which are inoculated with the blood of infected sheep, and probably this is the form of the disease which occurs naturally *under veld conditions*. The only well-marked symptoms observed clinically is an anaemia of the mucous membranes accompanied by listlessness. Feeding is not much disturbed. The bowels are unaffected. The pulse rate is somewhat increased, sometimes up to 158 p.m. The respirations may also be slightly increased. Sheep gradually recover after a course of about 12 to 18 days.

Post-mortem Changes.

The pathology of these cases will be considered in greater detail in a subsequent paper.

Three animals, viz., 7369, 8434, and 8430, succumbed to anaplasmosis after splenectomy. Sheep 8430, however, was bled extensively the day before death for experimental purposes. One sheep, viz., 7443, was killed in extremis as result of anaplasmosis. Several animals were killed at various intervals for collections of blood and material for pathology.

The chief changes observed at post-mortem were the following:—

The condition of the animals was good, if one takes into account the amount and character of adipose tissue. Even in those cases where oligocythaemia was acute and protracted, adipose tissue was present in fair quantities and was of normal colour and consistence. One of the most constant lesions was the presence of anaemia, and signs of icterus evidenced in connexion with mucous membranes, etc. The blood was not properly coagulated and hydraemic. Enlargement and hyperaemia of the majority of lymphatic glands. Haemal lymph glands gave the impression that they were more prominent, but could not be definitely associated with enlargement. Swelling, degeneration, and pigmentation of the liver, the pigmentation was mainly of the nature of icterus. Degenerative changes and pigmentation of the kidneys. Oedema and hyperaemia of lungs, and in some cases accompanied by a pigmentation in the form of a slight iron-grey sheen. In some cases hydropericardium; in the majority of cases the presence of blood extravasations associated with epicardium, right and left endocardium; degenerative changes of myocardium; absence of spleen; localized fibrous adhesive peritonitis.

Summary of Observations in connexion with Ovine Anaplasmosis.

1. *In case of susceptible sheep infected with blood—*
 - (a) the average incubation period was about 9 to 13 days; the shortest period recorded was 4 days, and the longest 31 days;
 - (b) the course of the disease was about 2 months and sometimes slightly longer;
 - (c) the blood-changes varied from a slight anaemia to a more acute oligocythaemia, viz., anisocytosis, polychromasia, punctate degeneration, and the presence of regenerating forms, e.g. erythroblasts, normoblasts, and jolly-bodies;

- (d) no mortality occurred;
 - (e) the source of the blood, whether derived from a susceptible infected animal or from a splenectomized "carrier," did not affect the course of the disease;
 - (f) the disease was transmitted in series of sheep for eleven generations, passage of anaplasma did not diminish or accentuate its virulency.
2. *In case of splenectomized susceptibles infected with blood*—
- (a) the symptoms and blood-changes were decidedly more acute; there was a reduction in the number of erythrocytes below 2 millions per cubic millimetre;
 - (b) the course of the disease was much protracted;
 - (c) the majority of these sheep were killed for pathology.
3. *In case of splenectomized "carriers"*—
- (a) the symptoms and changes affecting the erythrocytes were more or less the same as in (2);
 - (b) there was a fairly heavy mortality.
4. "*Carriers*" of anaplasma, either natural or previously infected, did not show a reaction when reinfected with blood.
5. *The reaction of anaplasmosis in sheep could more or less be divided into a number of stages*:—
- (a) *First stage*: anaplasma in the blood, from rare to frequent, appearance of slight oligocythaemia;
 - (b) *second stage*: anaplasma less frequent, oligocythaemia appearance of regenerating forms;
 - (c) *third stage*: anaplasma rare, oligocythaemia less acute, regenerating forms more frequent, especially normoblasts; jolly-bodies in various stages;
 - (d) *fourth stage*: anaplasma may be absent, further improvement in oligocythaemia, regenerating forms rare; jolly-bodies frequent, especially small forms;
 - (e) *fifth stage and so on*: anaplasma rare, slight anisocytosis and punctate degeneration, jolly-bodies rare.

In case of splenectomized sheep, relapses occurred from time to time, with anaplasma frequent and signs of oligocythaemia.

6. Anaplasma could easily be differentiated from jolly-bodies when considered in light of stages as regards position in the erythrocyte, size, shape, contour, structure, and staining characteristics.

7. Anaplasma could be distinguished from cocci, which occurred in the blood-smears of healthy and infected sheep, as regards the free and double forms of cocci, their irregular distribution in the smears, their irregular occurrence in the blood.

8. Splenectomy of susceptible sheep was followed by a polyglobuli and neutrophilia, and a return to normal levels occurred after a while.

9. In *infected susceptible sheep* a decrease in the number of erythrocytes was accompanied by a decrease in the number of neutrophils. This was followed by an increase of lymphocytes, which was more or less parallel with the return to normal of the erythrocytes. The decrease in the number of the erythrocytes was associated with a slight monocytosis.

10. In case of *splenectomized sheep* subsequently infected, similar leucocytic changes as in 9 were seen, except that, with the onset of oligocythaemia, it was accompanied by marked leucocytosis,

especially characterized by a monocytosis with erythrophagocytosis and especially marked when the number of erythrocytes had reached their lowest level. This monocytosis was also associated with an eosinophilia which ran more or less parallel with the monocytosis.

11. In case of *splenectomized carriers*, the picture was more or less the same, as in case of 10, except that splenectomy was followed by a marked leucocytosis, which, in the first instance, was of the nature of a neutrophilia, followed later on by a monocytosis, etc.

12. In all splenectomized sheep the oligocythaemia became chronic and a more or less permanent leucocytosis followed, chiefly affecting the lymphocytes and monocytes.

13. Difficulty was often experienced in distinguishing between monocytes and certain forms of lymphocytes.

14. In case of erythrophagocytosis by monocytes, various cell-inclusions were seen, varying in size from erythrocytes, normoblasts, to minute granules, probably the remains of "digested" cells.

15. In some monocytes and neutrophiles "vacuoles" were identified in the cytoplasm.

16. In splenectomy cases there was an increase in the number of thrombocytes; their distribution was more diffusely scattered, instead of in clusters; atypical shapes were seen in great numbers.

Transmission of Ovine Anaplasmosis to Ovines, Goats, and Bovines.

1. It was found that ovine anaplasma (*vide* 2, Experiment S. 1613, Appendix 1) could successfully be transmitted to susceptible sheep in series for ten generations. No decrease or increase in the virulence of the parasites could be detected. In case of the eleventh generation, sheep 10743 showed oligocythaemia, no parasites were detected in the blood. This sheep was subsequently splenectomized, was followed by a relapse of anaplasmosis, which, however, was more protracted and not acute.

2. An experiment was carried out to ascertain to what extent sheep kept at Onderstepoort for a number of years become carriers of anaplasma. Of six sheep inoculated, five reacted, whereas the sixth (e.g. sheep 7754) failed to react and proved to be a carrier of anaplasma. Its blood set up anaplasmosis in two susceptible sheep. If we include sheep 7443 and 7369 as carriers, it would appear that only a certain percentage of sheep kept at Onderstepoort for a number of years become infected with anaplasma.

3. In case of bovines (*vide* Experiment S. 1613, Appendix 1), it was found that blood infected with ovine anaplasma failed to infect cattle. Three of the animals, e.g. 844, 893, and 894, subsequently tested as regards their immunity to bovine anaplasmosis, reacted positively. C. 711 proved to be a carrier of bovine anaplasma *centrale* after splenectomy, and failed to react to ovine anaplasma *type marginale*, but subsequently reacted to bovine anaplasma *marginale*.

4. These cattle (*vide* 8, Experiment S. 1739, Appendix 1), injected with ovine anaplasma, did not even act as "carriers," because susceptible sheep injected with their blood failed to react to anaplasmosis.

5. No reaction of ovine anaplasmosis were observed in local goats (*vide* 2, Experiment S. 1613, C, Appendix 1). This may have been due to the fact that local goats were carriers, but splenectomy of such

goats (e.g. 8304, 8280) failed to reveal a "relapse" of anaplasmosis. These splenectomized goats subsequently injected with ovine anaplasma showed the presence of anaplasma rare in the blood after a very long incubation period. There were practically no blood changes.

6. Experiments carried out to transmit these anaplasma from goats to sheep (e.g. 10936, 10943) were positive, fairly good reactions were set up, with anaplasma frequent, e.g. in sheep 10936. Local goats failed to react.

Summary of the Transmission of Ovine Anaplasma to Ovines, Goats, and Bovines.

1. By means of blood-inoculation it was possible to infect with ovine anaplasmosis a series of susceptible sheep for eleven generations.

2. Only a certain percentage of local sheep become "carriers" of anaplasma.

3. Non-splenectomized and splenectomized susceptible bovines failed to react to ovine anaplasma, but subsequently readily reacted to bovine anaplasma.

4. Such bovines did not become "carriers" of ovine anaplasma.

5. Non-splenectomized goats could not be infected, whereas in splenectomized goats parasites appeared in the blood practically without signs of oligocythaemia.

6. Susceptible sheep could easily be infected from goat carriers.

Transmission of Bovine Anaplasma to Sheep.

1. An attempt was made (*vide* 4, Experiment S. 1665, Appendix 1), but without success, to infect susceptible sheep with bovine anaplasma. The attempt also failed in case of splenectomized susceptible sheep (e.g. sheep 8430, 8464). Sheep 10944 was first injected with bovine anaplasma and six months afterwards it was splenectomized. No reaction of anaplasmosis occurred either before or after splenectomy. These sheep subsequently reacted to ovine anaplasmosis, and in every instance with a definite reaction and the presence of parasites.

2. An experiment carried out to ascertain whether sheep injected with bovine anaplasmosis become "carriers" also proved to be negative.

Summary of the Transmission of Bovine Anaplasma to Sheep.

1. Susceptible and splenectomized susceptible sheep did not become infected with bovine anaplasma.

2. Sheep injected with bovine anaplasma did not become "carriers" of that parasite.

3. Sheep which failed to react to bovine anaplasma were found to be susceptible to ovine anaplasma.

A STUDY OF THE NATURE OF ANAPLASMA OF SHEEP—

- (i) as regards filtrability;
- (ii) as regards its resistance in vitro;
- (iii) a comparison of sheep "anaplasma" with "jolly-bodies" produced by drugs.

(i) *A Study of the Nature of Anaplasma as regards Filtrability.*

Blood was taken from a sheep which was showing a heavy infection of anaplasma. The blood was defibrinated and washed

erythrocytes obtained. Some of the washed corpuscles were haemolysed with distilled water, and as soon as haemolysis was complete, sodium chloride was added to bring the solution up to 0.75 per cent. saline. Some of this solution was passed through: (a) Berkefeldt filter; and (b) E. K. Schichten size 14 filter. The following solutions were then injected intravenously into susceptible sheep (*vide* 9, Experiment S. 1775, Appendix 1) to ascertain whether "anaplasma" observed in the circulation are not perhaps of the nature of "reaction products" of an ultraviolet, filtrable virus:—

- (1) Defibrinated blood (control).
- (2) Serum.
- (3) Washed corpuscles.
- (4) Haemolysed washed corpuscles used as a control to ascertain whether distilled water does not destroy the "anaplasma" or the "virus."
- (5) Filtrates of haemolysed blood corpuscles through a Berkefeldt and through a E. K. Schichten filter.

It was found that susceptible sheep injected with defibrinated blood, serum, and washed corpuscles of infected sheep, reacted with the appearance of parasites in the blood. In case of serum, defibrinated blood, and washed corpuscles, the reactions were typical of anaplasmosis. In case of those sheep injected with haemolysed washed corpuscles, symptoms of oligocythaemia preceded the appearance of anaplasma in the blood, which was fairly late.

The filtrate (through a Berkefeldt) injected into a number of susceptible sheep produced no reactions, whereas these sheep subsequently reacted when injected with infected blood. The filtrates through the E.K. filter in some instances seem to allow anaplasma to pass through, probably due to an error in the technique. When this experiment was subsequently repeated and greater care taken in the preparation of the filtrate, such filtrates failed to set up reactions of anaplasmosis.

Summary.

1. It would appear that anaplasma are not of the nature of "reaction products" set up by ultraviolet, filterable viruses.

2. As long as anaplasma are present in a solution, whether it be in serum, washed blood corpuscles, haemolysed washed blood corpuscles, reactions of anaplasmosis with such solutions can be produced in susceptible sheep.

(ii) *A Study of the Nature of Anaplasma of Sheep as regards its resistance in Vitro.*

An experiment was undertaken (*vide* 12, Experiment S. 1844, Appendix 1) to ascertain how long anaplasma could retain its "vitality" when stored in a 7½ per cent. citrate solution.

Splenectomized sheep 8430, heavily infected with anaplasma, was bled, and blood collected into a number of sterilized bottles containing a quantity of 7½ per cent. citrate solution.

Susceptible sheep were injected with this blood at intervals of two weeks.

It was found possible to infect susceptible sheep with citrated blood stored for one week, three weeks, and five weeks. It seems that the incubation period becomes more and more extended the longer the blood was kept, whereas the severity of the reaction remained

more or less the same. Citrated blood kept for seven weeks failed to produce a reaction of anaplasmosis, whereas at nine weeks, in case of sheep 10511, anaplasma appeared in the blood after a long incubation period with practically no symptoms of oligocythaemia, and in case of the other sheep, 10497, nothing happened. Sheep 10511, subsequently splenectomized, showed an acute relapse of anaplasmosis, indicating that the animal was infected with the citrated blood, whereas sheep 10497, tested with infected blood, showed a reaction of anaplasmosis, indicating that this sheep was susceptible and did not become infected with citrated blood.

This method of preventing the vitality of anaplasma in citrate solutions might perhaps be thought of in the immunization of cattle with the more virulent forms of anaplasma.

Smears made from citrated blood collected in 30th September, 1924, were examined from time to time, e.g.—

7th November, 1924, i.e. after nearly two months, the erythrocytes stained well, anaplasma not infrequent, in the form of compact masses of a deep violet colour; irregular contour, due to minute protuberances, as if in the process of "budding"; position with reference to the erythrocytes mainly peripheral;

2nd January, 1925, i.e. after about three months, erythrocytes well stained, anaplasma present and well stained, but appear less frequent;

11th February, 1925, i.e. after more than four months, erythrocytes with anaplasma, some of the later smaller than usual;

18th April, 1925, i.e. after five and a half months, the majority of the erythrocytes still stained fairly well, they are smaller in size, a number of them can be made out as "ghosts"; no anaplasma associated with erythrocytes could be identified, thrombocytes still prominent.

Summary.

1. It was possible to infect susceptible sheep with blood containing anaplasma and collected in citrate solution after it was stored for one week, three weeks, five weeks, and in one instance for nine weeks.

2. Anaplasma associated with erythrocytes could still be identified in smears made from citrated blood stored for more than four months.

(iii) *A Comparison between "Anaplasma" and "Jolly-bodies" produced by drugs.*

It was shown above that anaplasma could easily be differentiated from jolly-bodies if the reactions of anaplasmosis are considered in stages.

A number of experiments (*vide* 13, Experiment S. 1841, Appendix 1) were carried out in case of susceptible sheep to ascertain whether such "jolly-bodies" could be produced by drugs, and whether these could be differentiated from "anaplasma."

Three drugs, viz.: phenylhydrazin, pyrogallie acid, and nitrobenzine, were used for this purpose. The following doses intravenously were repeated on different days:—

Phenylhydrazin, 0.2 c.c. per kilo body weight of a 5 per cent. solution.

Pyrogallic acid, 0.2 c.c. per kilo body weight of a 50 per cent. solution.

Nitrobenzine, 2 c.c. per kilo body weight of a 5 per cent. solution.

It was found that nitrobenzine solution produced symptoms of shock, followed by slight symptoms of oligocythaemia. In case of phenylhydrazin, the symptoms of oligocythaemia were more pronounced, but the best results of all were obtained with pyrogallic acid. This brought about a pronounced oligocythaemia, with more or less a repetition of the various stages described in connexion with anaplasmosis, except with the complete absence of anaplasma, e.g.—

sheep 8469 on 11th October, 1924, showed distinct anisocytosis, polychromasia, punctate degeneration, erythroblasts, normoblasts; jolly-bodies in various stages, in some corpuscles double, of different sizes (see figures 15-18, Appendix 4).

Nothing in the nature of anaplasma were observed.

An experiment (14, S. 1814) was carried out to ascertain whether sheep which were "carriers" (e.g. sheep 8433, 8458, 8455) would show a relapse of anaplasmosis during the stage of pronounced oligocythaemia produced by drugs. No such "relapse" occurred, but the following observations were made in connexion with the leucocytes of one of the sheep (sheep 8455, see chart, Appendix 3). The picture differed somewhat from that observed in case of anaplasmosis. The reduction in the number of erythrocytes was rather precipitous, and its return to normal was almost as sudden. The initial stage of the return to normal was associated with a leucopenia. With the decrease in the number of erythrocytes, no monocytosis was observed as was seen in the case of anaplasmosis. (See Graph 5, Appendix 4.)

Summary.

1. Jolly-bodies appeared in the blood during a stage of an oligocythaemia produced by a drug like pyrogallic acid.
2. The jolly-bodies were typical of those encountered in forms of oligocythaemia.
3. Such jolly-bodies bore no resemblances to and had no connexion with anaplasma.

GENERAL CONCLUSIONS.

1. A certain percentage of South African sheep kept locally become "carriers" of anaplasma. The course of the disease is evidently of such a mild nature that it has never been identified naturally.

2. When sheep introduced from some other centres in Union are injected with the blood of such carriers, reactions of anaplasmosis are set up. Such a disease, as regards its course, symptoms, and the nature of anaplasma, resembles a mild form of bovine anaplasmosis described by Theiler. The disease was propagated in sheep for eleven generations by means of blood-inoculations.

3. When carriers of ovine anaplasma are splenectomized, grave symptoms of anaplasmosis are set, which in some instances prove fatal.

4. In the same way splenectomy of equines, carriers of nuttallia, was followed by a relapse of nuttalliosis with fatal results. In case of bovines, relapses of anaplasmosis alone, or combined with piroplasmosis and gonderiosis, followed. In the latter instance it was shown that bovines reared locally were not necessarily carriers of anaplasma and piroplasma simultaneously.

5. The reactions of anaplasmosis in splenectomized sheep were protracted over long periods, with remissions from time to time.

6. The blood-changes in the splenectomized infected sheep were associated with a marked oligocythaemia, the appearance of erythroblasts, normoblasts, and jolly-bodies. With reference to the leucocytes, first a neutrophilia was noted, then a monocytosis with erythrophagocytosis and a lymphocytosis developed, and in some instances an eosinophilia.

7. It would appear that the *neutrophilia* is associated with the operation of splenectomy, the *monocytosis* with the removal of "degenerated" and "damaged" erythrocytes, the *lymphocytes* seem to have some association with the return to normal of the erythrocytes, and the *eosinophilia* seem to stand in some relation to the removal of the spleen.

8. The operation of splenectomy in susceptible equines, bovines, goats, and ovines was carried out with practically no impairment. A transitory polyglobuly and neutrophilia were seen in some of the cases.

9. Susceptible and splenectomized susceptible bovines failed to react to "ovine" anaplasma, nor did such "bovines" become carriers of "ovine" anaplasma.

10. Non-splenectomized local goats could not be infected with "ovine" anaplasma, whereas splenectomized local goats only showed the presence of parasites in the blood after a prolonged incubation period.

11. Susceptible and splenectomized ovines could not become infected with "bovine" anaplasma, nor did such ovines become carriers of "bovine" anaplasma.

12. Anaplasmosis in sheep, except for a few instances where *Gonderia ovis* was seen, could in no way be connected with a stage in the life-cycle of a piroplasmosis.

13. All attempts made to show that anaplasmosis of sheep was associated with a filterable virus failed.

14. It was shown that anaplasma retained its vitality to infect susceptible sheep when stored in citrate for one week, three weeks, five weeks, and in one case for nine weeks.

15. No difficulty was experienced in differentiating between "anaplasma" and "jolly-bodies."

16. The "*chromatin-bodies*" produced by drugs, like pyrogallie acid, were "*jolly-bodies*" and associated with an oligocythaemia and had nothing whatsoever to do with "anaplasma."

17. In view of the results arrived at in this paper, and on account of fatal "relapses of malaria" reported to have occurred in human beings after the removal of the spleen, *splenectomy in man* ought to be carefully considered when undertaken in malarial regions.

III.

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APPENDIX I.

SUMMARY OF THE EXPERIMENTS.

Abbreviations.

Sh.....	Sheep.
Intraj., intrav. inj.....	Intrajugularly, intravenously, injected.
Anapl.....	Anaplasma.
P. bigem.....	Piroplasma bigeminum.
Gond.....	Gonderia.
A.....	Anisocytosis.
S.A.....	Slight anisocytosis.
P.....	Polychromasis.
S.P.....	Slight polychromasis.
P.D.....	Punctate degeneration.
E.....	Erythroblasts.
N.....	Normoblasts.
J.B.....	Jolly-bodies.
Freq.....	Frequent.
Infreq.....	Infrequent.
R.C.....	Red count.
W.C.....	White count.
Hs.....	Horse.
C.....	Bovine.

I.—EXPERIMENT S. 1605.—TO ASCERTAIN THE EFFECT OF SPLENECTOMY OF DOMESTICATED ANIMALS.

A. *Equines.*

- (1) Infected=Hs. 15186, 15420, 16032.
- (2) Non-infected=Hs. 16072.

B. *Bovines.*

- (1) Infected=C. 711, 758, 893, 1027, 1034.
In case of C. 758, an intermittent disease occurred, viz.—Multiple necrosis of liver. Sub-inoculations from this animal into susceptible sheep (*vide* Exp. S. 1707) proved to be negative.
- (2) Non-infected=Animals not available.

C. *Sheep.*

- (1) Infected—
(a) Natural, Sh. 7369, 7443.
(b) Artificial, Sh. 8429, 8434, 8427, 8428, 8451, 9119, 10511, 10743.
- (2) Non-infected=Sh. 8430, 8431, 8456, 8457, 8464, 10944.

D. *Goats.*

- (1) Infected=Not available.
- (2) Susceptible=G. 8280, 8304=Negative.

For observations, etc., of the above cases, see Appendix 3.

Conclusions.

1. Non-infected equines recovered completely after splenectomy, whereas infected animals showed relapses of nuttalliosis with very severe symptoms of oligocythaemia, which would have proved fatal. Animals were killed *in extremis*.

2. Bovines infected with anaplasmosis after splenectomy showed relapse with slight symptoms of oligocythaemia, whereas animals infected with *Piroplasmosis bigeminum* and *Gonderia mutans* as well showed relapses (in case of gonderia more than 90 per cent. of erythrocytes were infected) with severe symptoms of oligocythaemia.

3. Non-infected sheep recovered completely after splenectomy, whereas carriers of anaplasma showed relapses of anaplasmosis with severe symptoms of oligocythaemia, which proved fatal in some cases.

4. Goats recovered completely after splenectomy. The experiment was not carried out on known carriers of anaplasma.

II.—EXPERIMENT S. 1613.—TO ASCERTAIN THE NATURE OF THE ANAPLASMA-LIKE BODIES OBSERVED IN THE BLOOD OF SHEEP ON:

A. *Ovines.*

(1) *Non-splenectomized Susceptible Sheep.*

1st generation,	[Sheep No. 8427, 8428, 8429, 8434.
2nd	” ” ” 8432, 8433.
3rd	” ” ” 8455, 8458.
4th	” ” ” 8462, 8463.

(For observations on the above cases, see Appendix 3.)

5th generation,	Sheep No. 8459.
6th	” ” ” 9128.
7th	” ” ” 9102.
8th	” ” ” 9131.
9th	” ” ” 9130.
10th	” ” ” 9116.
11th	” ” ” 10743.

Fifth Generation.

Sheep 8459.	14.6.24.	Inj. intraj, 20 c.c. bld. Sheep 8463.
	25.6.24.	Anapl. rare.
	26.6.24.	Anapl. not rare.
	28.6.24.	Anapl. freq.; A.; P.; J.B.
	2.7.24.	Anapl. rare; P.; S.A.; J.B. freq.
	16.7.24.	S.A.
	23.7.24.	S.A.

Sixth Generation.

Sheep 9128.	26.6.24.	Inj. intraj. 20 c.c. bld. Sheep 8459.
	7.7.24.	Anapl. rare.
	9.7.24.	Anapl. not infreq.; S.A.; S.P.
	16.7.24.	” ” A.; S.P.; S.P.D.
	21.7.24.	” few; Gond. few, S.A.
	29.7.24.	” ” S.A.
	31.7.24.	” ” S.A.; S.P.A.; S.P.
11.8.24.	” ”	

Seventh Generation.

Sheep 9102.	24.7.24.	Inj. intraj. 10 c.c. bld. Sheep 9128.
	5.8.24.	Anapl. rare.
	12.8.24.	” ”
	14.8.24.	” ” A.; P.
	19.8.24.	” ” S.A.; P.; J.B. few.
	25.8.24.	” ” S.A.; S.P.
	8.9.24.	” few; S.A.; S.P.

Eighth Generation.

Sheep 9131.	26. 8.24.	Inj. intraj. 20 c.c. bld. Sheep 9102.
	10. 9.24.	Few anapl.; S.A.
	15. 9.24.	Anapl. rare; S.A.
	19. 9.24.	" not rare; A.; P.; P.D.
	24. 9.24.	" few; S.A.; S.P.
	29. 9.24.	S.A.; few J.B.
	7.10.24.	Anapl. few; S.A.; J.B.

Ninth Generation.

Sheep 9130.	7.10.24.	Inj. intraj. 20 c.c. bld. Sheep 9131.
	22.10.24.	Few anapl.; S.A.
	29.10.24.	" " S.A.; P.
	31.10.24.	Anapl. not infreq.; A.; P.; P.D.
	4.11.24.	S.A.; S.P.
	11.11.24.	Anapl. not infreq.

Tenth Generation.

Sheep 9166.	10.11.24.	Inj. intraj. 20 c.c. bld. Sheep 9130.
	19.11.24.	Few anapl.
	24.11.24.	Anapl. rare; S.A.; S.P.
	26.11.24.	" " S.A.; P.
	28.11.24.	" " S.A.; S.P.
	4.12.24.	" " S.P.
	6.12.24.	Few anapl.; S.A.
	9.12.24.	S.A.; P.
	11.12.24.	Anapl. infreq.
	24.12.24.	Anapl. rare; S.A.

Eleventh Generation.

Sheep 10743.	2.1.25.	Inj. intraj. 20 c.c. bld. Sheep 9116.
	16.1.25.	S.A.; P.; S.P.D.
	19.1.25.	A.; S.P.; few J.B.
	21.1.25.	S.A.; S.P.; N.
	26.1.25.	S.A.; S.P.; irregular threadlike deposit in erythrocytes.
	28.1.25.	S.A. "
	3.2.25.	S.A.; R.P.=25; R.C.=4.9; irregular thread-like deposit in erythrocytes.
	6.2.25.	A.
	12.2.25.	S.A.; irregular thread-like deposit in erythrocytes.
	16.2.25.	" " " "
	20.2.25.	" " " "
	23.2.25.	S.A.
	27.2.25.	S.A.
	13.3.25.	No anaplasma yet observed, and, therefore, Sheep 10743 was splenectomized to ascertain whether it is a carrier. For further observations, see Appendix 3. (Animal showed a relapse of anaplasmosis.)

(2) Non-splenectomized Local Sheep.

Nos. 7109, 7754, 7338, 7298, 6543, 6350.

Sheep 7109.	30. 4.24.	Inj. intraj. 20 c.c. bld. Sheep 8432.
	8. 5.24.	S.A.; P.D.
	15. 5.24.	Anapl. few.
	17. 5.24.	" " S.A.; S.P.; P.D.
	23. 5.24.	" freq.
	26. 5.24.	" rare; S.A.; P.; P.D.; N.; J.B.
	2. 6.24.	" very rare; S.P.; S.P.D.
	10. 6.24.	" "
	27. 6.24.	Few anapl.
	21. 7.24.	Discontinued.

Sheep 7754.	30. 4.24.	Inj. intraj. bld. Sheep 8432.
	21. 7.24.	Negative; discontinued.

Sheep 7338.	8. 7.24.	Inj. intraj. bld. Sheep 8459.
	1. 9.24.	Negative; discontinued.
Sheep 7298.	8. 7.24.	Inj. intraj. bld. Sheep 8459.
	1. 9.24.	Negative; discontinued.
Sheep 6350.	8.10.24.	Inj. intraj. bld. Sheep 8428.
	28.10.24.	Anapl. few; S.A.; S.P.D.
	30.10.24.	„ not infreq.; A.; P.
	7.11.24.	A.; P.
	18.11.24.	Anapl. few; A.
Sheep 6543.	8.10.24.	Inj. intraj. bld. Sheep 8428.
	23.10.24.	Anapl. freq.; S.A.
	30.10.24.	„ not infreq.; A.; P.
	3.11.24.	A.; P.; J.B. (all stages).
	7.11.24.	S.P.; S.P.D.; J.B.

Addendum.—*Sheep 7754 (vide Expt. 1613) failed to react. Is it a carrier of anaplasma?* Two susceptible sheep inoculated from it.

Sheep 9120.	8.7.24.	Inj. intraj. bld. Sheep 7754.
	9.8.24.	Anapl. very rare; J.B.; P.; P.A.
	19.8.24.	„ rare; A.; P.
	28.8.24.	„ not rare; S.A.; J.B.
	1.9.24.	„ rare; P.; P.D.
Sheep 9121.	8.7.24.	Inj. intraj. bld. Sheep 7754.
	29.7.24.	Anapl. few; S.A.; P.
	31.7.24.	„ very rare; P.; P.D.; J.B.
	19.8.24.	„ rare; A.; P.
	1.9.24.	Few anapl.

(3) *Splenectomized Susceptible Sheep.*

Sheep 8456, 8457, 8430.

For observations see Appendix 3.

(4) *Sheep which are the Carriers of Ovine Anaplasma.*

Nos. 8454, 9131, 9122, 9116, 9123, 8471, 9125, 8465, 8459, 9106: Intraj. bld Sheep 8451 on 11.2.25.

Sheep 8454.	10.2.25.	Anapl. few.
	24.2.25.	„
	16.3.25.	Negative.
Sheep 9131.	10.2.25.	v. S.A.
	24.2.25.	„
	16.3.25.	„
Sheep 9122.	10.2.25.	v. S.A.
	21.2.25.	S.A.; S.P.
	24.2.25.	„ „ points in erythrocytes.
	26.2.25.	Intra corp. points, poikilocytosis, A.P.; erythrocytes lighter in colour.
	2.3.25.	A.; poikilocytosis, erythrocytes vacuolated; absence of regenerated forms; R.C. 2·17; precipitate=5; W.C. 4,100. Died of acute haemonchosis.
Sheep 9116.	10.2.23.	v. S.A.
	24.2.25.	S.A.
	16.3.25.	Negative.
Sheep 9123.	10.2.25.	v. S.A.
	24.2.25.	„
	28.2.25.	S.A.; few anapl.
	6.3.25.	S.A.
	16.3.25.	v. S.A.
Sheep 8471.	10.2.25.	Negative.
	23.2.25.	„
	16.3.25.	„