The Operation of Splenectomy in Horses, Cattle, Sheep, Goats, Pigs, Dogs, and some South African Antelopes: A Summary of the Results of 98 Splenectomies.

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WITHIN recent years the advance in our knowledge of the functions of the spleen and its rôle in certain diseases of the blood have justified the operation of splenectomy as a recognised treatment in certain forms of anaemia in human medicine; removal of the spleen is no longer confined to cases of splenic trauma.

In the lower animals the operation of splenectomy, as a curative measure, has been undertaken only in the dog in the case of traumatic injury and in splenic newgrowths. There is little doubt that the use of the operation can be successfully extended and will be in the future, with the progress of surgery in the lower animals. The operation would save the lives of dogs that suffer from spontaneous splenic rupture, which sometimes occurs as a sequel to canine piroplasmosis. However, splenectomy in canine medicine is confined to veterinary specialists and has not yet become a general practice.

The operation of splenectomy in veterinary medicine has been mostly of an experimental nature. For this purpose it has been carried out on dogs in many countries. De Kock and Quinlan (1926) operated successfully on horses, cattle, sheep, and goats. The literature appears scanty in reference to the operation except in the dog. Warthin (1903) published the results of splenectomy in sheep and goats. His results were not satisfactory, as 50 per cent, of the animals operated upon died from shock. In this connection Warthin's results are surprising, as shock following the operation has been a rare occurrence in over ninety splenectomies performed by one of us (Q) in the conduction of a series of experiments on piroplasmosis, theileriosis, eperythrozooniasis, anaplasmosis, and trypanosomiasis.

Removal of the spleen was first introduced at this Institution to study the nature of the reticulo-endothelial system, in which one of us (de K.) was interested. It was begun on horses and was followed by such important results that it was extended to cattle, sheep, goats, and antelopes with equally important sequelae (de Kock and Quinlan, 1926).

Since the publication of the abovementioned work the use of the operation has been extended at this Institution as a means of rendering animals susceptible to certain protozoal diseases and also to demonstrate whether they were carriers of infection. For this purpose it is highly successful.

The operation has been applied in the study of the life cycle of certain blood parasites. It has been used in the diagnosis and the study of susceptibility and immunity to diseases such as anaplasmosis, piroplasmosis, trypanosomiasis, and eperythrozooniasis. Further the value of splenectomy in the study of the distruction of crythrocytes, the metabolism of the pigment of the blood, and the reticulo-endothelial system has been appreciated.

The results of completed studies have already been published in the Reports of the Director of Veterinary Services and Animal Industry and in the Onderstepoort Journal. The experimental work now in progress will be published later in the Onderstepoort Journal.

Splenectomy has been successfully performed on horses, cattle, sheep, goats, pigs, dogs, and a few South African antelopes, viz.: the bleshok (Damaliscus albifrons) and the duiker (Sylvicapra grimmi grimmi L.).

Since de Kock and Quinlan (1926) published their paper some other workers, using almost similar operative technique, have splenectomized bovines (Meissner, 1931, Rees, 1933). These are the only references that can be traced in the literature to the operation in the larger domestic animals. However, it appears likely, now that the operation has been performed extensively and successfully, that it will facilitate the task of research workers in tropical and subtropical countries.

In this paper it is not intended to recapitulate in detail the results of removal of the spleen in the lower animals as already published by de Kock and Quinlan (1926, 1927), de Kock (1929), du Toit (1931), Meissner (1931), Rees (1933), Neitz and du Toit (1932). The object of the paper is to give an account of the operative technique and the methods of anaesthesia used, as well as a summary of the result obtained in animals splenectomised subsequent to the first publication of de Kock and Quinlan (1926).

THE OPERATION.

GENERAL REMARKS.

In the case of equines it is advisable to select young and small animals as the operation is very difficult even in medium-sized horses. The first operation performed was on an adult mare of small size with a wide open "coupling". The operation was successful, but it was

extremely tedious and tiresome and, as will afterwards be pointed out in the paragraph on operative technique, the danger of peritonitis is very real, because of the protracted manipulations which are necessary within the abdomen. After this first experience immature animals were used, when selection was possible, that is horses under two years and cattle under eighteen months. In one case an ox weighing seven hundred pounds was operated upon. Such an operation requires considerable physical strength as well as technical skill and should not be undertaken by surgeons with a fragile constitution. The best surgical results can be obtained with very small animals as the operation can be carried out expeditiously and short abdominal manipulation only is necessary.

The subjects, where selection is possible, should be in good hard condition. Obesity is undesirable, while very poor animals may show shock. In the case of the smaller animals such as sheep, goats, pigs, and dogs obesity is objectionable as the subcutaneous and intraabdominal fat somewhat impede laparotomy. This, however, is the only objection.

A. The Operation in Horses.

Preparation.

The following precautions are taken before bringing the animals into the operating theatre. The preparation includes dieting, purging, and starving with the object of reducing as far as possible the volume of the stomach and intestines. The horses are put on a concentrated non-bulky diet eight days prior to the operation. The diet consists of oats and bran with a few pounds of green lucerne daily. Water is given ad libidum. Food and water are withheld and a mild purgative administered 36 hours before the operation. The horses are clipped and washed the day previous to operating and a large area over the left flank shaved and painted with fincture of iodine. From this time sterilized bedding only is used in the box.

Anaesthesia.

General anaesthesia is adopted. Chloral hydrate in a solution of 10 per cent, with normal saline is used in company with chloroform inhalation. The dose given varies from $2\frac{1}{2}$ to 3 grams per 100 pounds body-weight. It is given intraveneously at the rate of 50-75 c.c. per minute. Deep anaesthesia is produced by chloroform inhalation following the injection of chloral hydrate. The chloroform is administered through an open mask.

This method of anaesthesia for equines has been used at this Institution for a number of years with complete success. In no case was there the slightest cause for anxiety as to the welfare of the patient during the operation. The horses recovered rapidly from the effects of the anaesthetic and did not show symptoms of operative shock.

Technique.

The patient is placed on its right side on the operating table. The fore limbs and head are fixed in the most convenient position, while the hind limbs, with the joints extended, are drawn slightly backwards so as to make the region of the flank as approachable as possible.

The skin over the shaven area in the left flank is then liberally swabbed with ether. The area surrounding the field of operation is covered with sterile linen which is held in position by cloth forceps. The hands and arms, after a thorough cleansing with synol soap and warm water, are bathed with alcohol-corrosive sublimate solution (1—1,000). They are finally rinsed in sterile physiological saline.

The instruments, after sterilisation in the autoclave, are used dry or transferred to sterile physiological saline.

Laparotomy.

A vertical incision is made through the skin and muscles down to the peritoneum in the region of the left flank about five centimetres behind the last rib. The incision is begun as close to the transverse processes of the lumbar vertebrae as possible, as this position facilitates the approach to the splenic vessels when the remainder of the organ has been mobilised. The length of the incision varies from 15 cm. to 25 cm. according to whether the patient is a small or large animal. In large animals the longer incision is necessary as both hands and arms must be used inside the abdomen at the same time. Haemorrhage is now arrested. The peritoneum is caught up in a forceps and incised in the direction of the cutaneous incision for a distance of about half an inch.

A large piece of sterile gauze is placed over the field of operation and an incision made through it over the length of the wound. The gauze is attached to the lips of the wound by sutures or forceps. Sutures are preferable as they do not interfere with later manipulations. The peritoneum is included in the sutures. It is gradually opened as the suturing extends towards the extremities of the incision. The suture material is not cut off short but is looped so that it may be used for dilating the wound when necessary.

When mobilising the spleen the relations of the intra-abdominal organs are disturbed as little as possible. To maintain the normal relationship it is necessary to leave the cavity of the omentum intact by laying on a line of sutures which will unite the gastro-splenic and the greater omentum.

The mobilisation of the spleen differs somewhat in small and medium-sized animals. In the latter only a small portion of the apex of the spleen can be withdrawn, so that all the ligatures have to be completed within the abdomen. In consequence both hands must be used for intra-abdominal manipulation. Using fine silk for ligaturing, operations are begun at the apex of the spleen. The gastrosplenic and greater omentum are caught between the thumb and fingers of the left hand, while the right hand carrying a half-circle needle and ligature silk is passed over the parietal surface of the The needle is passed from before backwards through the omentum, and then, having isolated a portion it is again passed from back to front and withdrawn through the laparotomy opening. The ligature is completed by knotting the silk. For the purpose of applying tension and also to facilitate knotting a short piece of plated steel, one-and-a-half inches long, is attached to the free end of the ligature silk. By this means the fingers of the left hand working inside the abdomen can easily apply the knot. Proceeding in this way a double row of sutures is laid on uniting the gastro-splenic and the greater omentum along the entire length from the apex to the hilus of the spleen until the splenic vessels are reached. The attachment is divided between the double row of ligatures by means of a long blunt-pointed scissors. The suspensory ligament is then treated in a similar manner until the spleen is attached only by the splenic vessels and nerves. A last double ligature is passed around the vessels and nerves at the hilus. The spleen is then detached with the scissors close to the distal ligature and withdrawn.

In ligating, the close connection of the stomach with the spleen must be remembered and the organ protected from injury. While ligating the splenic vessels care must be exercised that too much tension is not applied as the splenic vein may rupture and cause considerable haemorrhage. To obviate this accident the vessels are first clamped with a long forceps and then ligatured. This method facilitates the application of a reinforcing ligature for extra safety after the spleen has been removed.

The abdominal incision is then closed in three layers without drainage. Chromatised catgut is used for the peritoneum and the muscles and silk for the skin. At first two tape tension sutures as well as as small gauze drain were used, but they appear to be unnecessary and have a disadvantage in that there is more likelihood of suppuration. The wound is sealed with collodion and iodoform and covered with gauze and mastisol.

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

In small horses the operation is not so difficult as a large portion of the spleen can be withdrawn through the abdominal incision, and the application of many of the ligatures can be done under direct observation. An incision 15 cm. in length is sufficient, since it is necessary to introduce only one hand into the abdominal cavity. In foals the apex of the spleen can be withdrawn and most of the omental ligatures as well as those on the suspensory ligament can be applied outside the abdominal cavity. In this way the organ can be considerably mobilised. Then the left hand is introduced and the remaining attachments—small portion of the gastro-splenic and great omentum and the suspensory ligament surrounding the splenic vessels—are pressed together and caught in a strong forceps. A silk ligature is applied on the compressed tissues and the spleen removed with the scissors.

Outside this small technical change the operation in both small and large horses is identical.

Difficulties.

These are chiefly concerned with the application of the ligatures in large horses, as the intra-abdominal manipulations are not under direct observation. The mobilisation of the organ should be completed as far as possible before attempting withdrawal, as it very easily ruptures and causes unpleasant haemorrhage. The operation causes considerable fatigue on account of the slight elasticity of the thoracic wall and the position which necessarily has to be assumed by the operator.



Fig. 1.—Equine 16072, 2 weeks after operation.



Fig. 2.—Equine 16072, 5 weeks after operation.

B. The Operation in Cattle.

The preparation, anaesthesia, and the laparotomy are similar to what has been described for horses. A slight modification is, however, necessary with the position of the cutaneous incision, which is made parallel to the last rib and about 5 cm. posterior to it. It is begun as high up as possible in the angle between the posterior rib and the transverse processes of the lumbar vertebrae; a length of 15 to 20 cm. is sufficient.

The left hand is introduced into the abdomen between the rumen and the visceral surface of the spleen. Beginning at the anteroinferior aspect of the area of attachment the peritoneum is perforated along the line of its reflection from the rumen to the spleen and the connective tissue broken down by careful blunt dissection. When difficulty is experienced in breaking through the tissue with the fingers, as is sometimes the case in adult animals, it is cut through with a long-handled scissors. Proceeding by means of blunt dissection the detachment is completed over the face of the rumen and the adjacent face of the reticulum, as well as over the left crus of the diaphragm, until it remains attached only by the splenic vessels and nerves at the hilus. The spleen is then withdrawn and the vessels caught in a strong forceps. A double ligature is placed on the vessels about 2 to 3 cm, from the hilus and the spleen is detached by cutting between the ligatures with a scissors. The forceps is opened slowly to observe whether the ligature has completely occluded the vessels before releasing the stump.

Intra-abdominal manipulation should be gentle and careful as rupture of the capsule of the spleen will cause profuse and troublesome haemorrhage.

The abdominal wound is closed by suturing the peritoneum and muscles with catgut and the skin with silk. Post-operative protection of the wound is carried out as in equines.

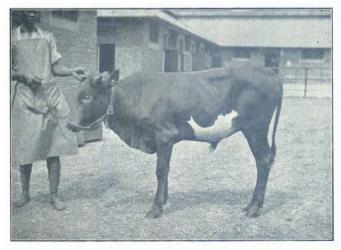


Fig. 3. Bovine 894, 3 weeks after operation.



Fig. 4.—Bovine 894, 7½ months after operation.



Fig. 5.—Bovine 894, 2 years after operation,



Fig. 6.—Bovine 893, 1 month after operation.

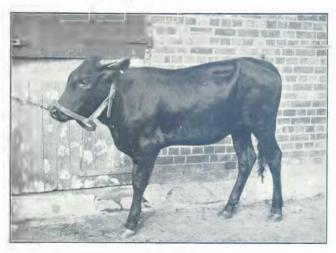


Fig. 7.—Bovine 893, 5 months after operation.

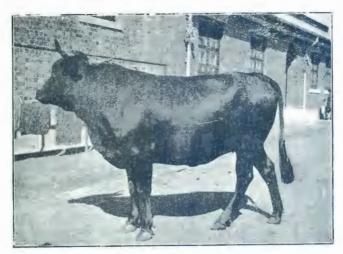


Fig. 8.—Bovine 893, 2 years and 4 months after operation.

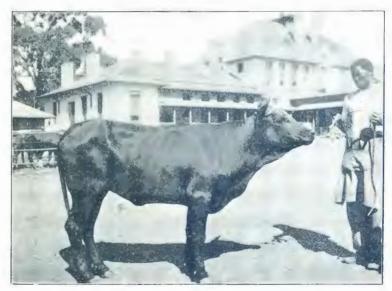


Fig. 4.—Bovine 2611, 10 months after operation.



Fig. 10.—Bovine 2611, 3½ years after operation.

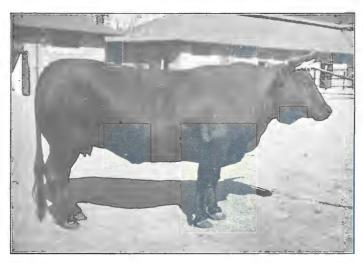


Fig. 11.—Bovine 2611, 4 years and 2 months after operation.

C. THE OPERATION IN THE SHEEP, GOAT, AND ANTELOPE.

The technique of the operation in the sheep, goat, and antelope differs little from that described in bovines. The oral administration of chloral hydrate for anaesthesia, which was used in the commencement, was later superceded by intravenous injection. The laparotomy is done in the same situation. The left hand is inserted into the abdomen and passed over the parietal surface of the spleen. The attachment between this surface and the diaphragm is first broken down by blunt dissection. Then beginning at the most inferior portion of its attachment to the dorsal curvature of the rumen the tissue is broken down up to the hilus. So as to prevent stripping of the peritoneum from the surface of the rumen a long curved scissors is used as an aid to blunt dissection with the fingers. Small portions of peritoneum and fibrous tissues are isolated and cut through. The remaining attachment to the left crus of the diaphragm is broken down in a similar manner proceeding cranially. The detachment of the spleen is carried out in a similar way to that described in the bovine. The wound in the abdominal wall is also closed in a similar manner. Post-operative protection is carried out as in equines.

The capsule of the spleen in sheep and goats is very easily ruptured. If such an accident occurs there is profuse haemorrhage, consequently dissection of the splenic attachments must be carried out with gentleness and patience.

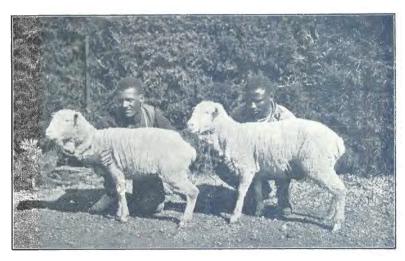


Fig. 12.—Ovines 28498 and 28578, nine months after splenectomy.



Fig. 13.—Group of splenectomised sheep, one of which, No. 8429 had been splenectomised 6 years 7 months previously.



Fig. 14.—Group of splenectomised sheep, one of which, No. 16023, had been splenectomised 6 years 5 months previously.



Fig. 15.—Caprine 8304, 3 weeks after operation.



Fig. 16.—Caprine 8280, 3 weeks after operation.



Fig. 17.—Caprines 8280 and 8304, $7\frac{1}{2}$ months after operation.



Fig. 18.—Caprines 8280 and 8304, 2 years and 10 months after operation.



Fig. 19.—Caprine 8280, 6 years 11 months after operation.



Fig. 20.—Duiker (Sylvicapra grimmi grimmi L.) 32806, one day after operation.

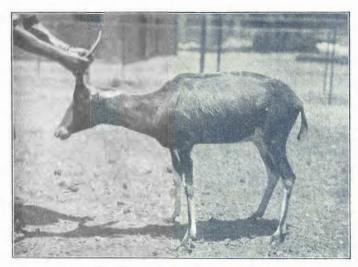


Fig. 21.—Blesbuck (Damaliscus albifrons) 33606, 8½ months after operation.

D. THE OPERATION IN THE PIG.

The pig is also carefully prepared for the operation. Pernocton, by the intravenous route, using one of the auricular veins, is used as an anaesthetic. This method of anaesthesia for the pig is excellent. The dose used is 0.03 grams per kilogram body-weight.

The route of approach to the spleen is immediately behind the last rib on the left side, beginning as close to the lumbar transverse processes as possible. The incision runs parallel to the rib. A short incision, about 8 cm., is sufficient as the spleen can be easily and completely withdrawn through such a small opening. One or two fingers are introduced into the abdomen and the spleen drawn through the opening. A double series of fine silk ligatures are then placed along the splenic attachment at the hilus and the organ detached by cutting between the ligatures with the scissors. The abdominal wound is closed by three series of sutures; those for the peritoneum and the muscles being of catgut and those for the skin silk. Post-operative protection of the sutured wound other than the usual seal is unnecessary.

E. THE OPERATION IN THE DOG.

There is no difference between the technique of the operation in the dog and that described for the pig except that the spleen can be withdrawn either through a mid-ventral or a low flank incision. Of the two routes of approach the flank incision is preferred and will be used in future operations.

Anaesthesia is accomplished in canines by the use of subcutaneous eukodal followed one hour later by intravenous pernocton; 1 c.c. of eukodal is used for each 5 kilograms body-weight and 0.03 grams of pernocton per kilogram body-weight. The previous injection of eukodal frequently reduces the amount of pernocton necessary for deep and prolonged anaesthesia.

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Fig. 22.—Porcines 903, 904, 905 and 906, 16 days after operation.



Fig. 23.—Porcine 904, 10 months after operation.

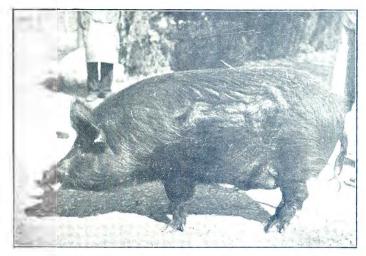


Fig. 24.—Porcine 905, 10 months after operation.



Fig. 25.—Canine 1073, 31 months after operation.

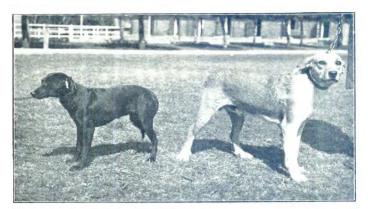


Fig. 26.—Canines 1044, 1143, 81 months after operation.

AFTER-TREATMENT.

After the operation the animals are placed in boxes in which sterilised bedding is used. It appears to be a useful precaution, especially for equines in which the tendency to wound infection appears to be far greater than in the other species of animals operated upon. The precaution was not taken entirely with a view to obtaining healing of the wound per primam intentionem, but also to ensure against the possibility of tick infection, which would have rendered the experiments which were being conducted with tick-borne diseases unsatisfactory.

The food following operation consisted of a light laxative ration, composed chiefly of small quantities of crushed oats with bran and green lucerne for horses, and maize meal with bran and green lucerne for cattle, sheep, goats, and antelopes. Water was allowed ad libidum.

Dogs and pigs were fed on milk and maize meal porridge, with the addition of beef-extract in the case of dogs.

The wounds were inspected daily and the dressings renewed. In the case of cattle, sheep, goats, pigs, and dogs there was no trouble with the wounds. They invariably healed per primam intentionem. The cutaneous sutures were removed on the seventh or eighth day following the operation.

In the case of equines the wounds did not heal by first intention. Probably the difficulty did not lie in the wound itself but rather in the nature of the disease which occurred as a sequel to the removal of the spleen, where changes in the blood were sufficient to prevent healing. Even in those cases the incisions in the peritoneum and the internal oblique muscle had united, but there was no adhesion between the incised lips of the external oblique muscle or the skin. These structures were healing under granulation when the animals died of the concurrent disease. In one horse which was free from Nuttallia equi infection, and which recovered, the upper two-thirds of the wound healed by first intention but the skin over the lower

third suppurated. However, this portion of the wound healed nicely under granulation. The resulting cicatrix was scarcely visible when the animal died four months later as a result of an intravenous injection of haemolytic serum.

SUMMARY OF THE RESULTS OF SPLENECTOMY.

De Kock and Quinlan published the results of their first series of experiments on splenectomised horses, cattle, sheep, and goats in 1926. Since then observations have been continued and extended to other species of domesticated animals as well as to wild antelopes. The results of the later splenectomies have been published mostly as isolated papers in various subsequent Reports of the Director of Veterinary Services. (De Kock and Quinlan, 1927; de Kock, 1929; du Toit, 1931; Neitz and du Toit, 1932.)

Horses.

Of the four horses splenectomised three were immune "carriers" of Nuttallia equi and all three showed fatal relapses of Nuttalliosis in from 3 to 8 days after the operation. The fourth animal was a young foal, not a carrier of Nuttalliosis. It was kept under tick-free conditions for four months following the operation when it was killed by injections of haemolytic serum.

Cattle.

Five animals were observed by de Kock and Quinlan (1926 report) and their findings may be summarised briefly as follows:--

- (1) Relapses of Theileriosis (*Theileria mutans*), anaplasmosis, and piroplasmosis (*P. bigeminum*) occurred in immune "carriers" of these parasites. Piroplasmosis relapses were in these cases particularly severe
- (2) These diseases in splenectomised animals assumed a chronic course particularly with regard to the blood changes.

A total of 23 bovines were splenectomised of which 20 recovered from the operation. One animal died three days afterwards, and on post mortem no apparent cause of death could be established; one died as a result of haemorrhage and one from surgical shock.

Some of the animals were used in East Coast fever transmission experiments and two animals which had recovered from East Coast fever were splenectomised in order to try and break down their immunity (du Toit, 1931). Removal of the spleen was found to have no effect on the course of this disease or on the immunity.

Two bovines were splenectomised for further study of the blood changes in anaplasmosis. One animal, No. 4627, infected with the mild A. centrale strain, reacted so severely after splenectomy that it died as the result of the extensive blood changes.

Two calves, Nos. 4658 and 4676, susceptible to anaplasmosis and piroplasmosis, were injected with blood from sheep showing anaplasma oris and then splenectomised to see whether the anaplasma oris would appear in the calves' blood, or whether the calves could

become "carriers" of the disease. After the operation, however, both animals became accidentally infected with bovine piroplasmosis and anaplasmosis and sheep sub-inoculated from them showed no reaction.

Sheep.

Fifty-four sheep have been splenectomised of which seven died as the direct result of the operation. In one of the latter, namely No. 8462, the attachments of the spleen were loosened and the splenic vessels ligated, but the organ itself left in situ.

The most striking result of de Kock and Quinlan's observations on sheep was the discovery of the hitherto unsuspected presence of an anaplasma, A. ovis, and a Theileria, T. recondita (called Gonderia ovis by them) present in the blood of some South African sheep.

Anaplasmosis of Sheep.—The result of their observations can be briefly summarised as follows:—

- (1) Non-splenectomised susceptible sheep can be infected by means of blood inoculation. The disease then runs a mild course with distinct anaemic changes and the appearance of parasites in the blood in large numbers, but no mortality.
- (2) In splenectomised "carriers" and splenectomised susceptible sheep the course is much more severe and protracted and there is a fairly high mortality.
- (3) The disease could not be transmitted to splenectomised or non-splenectomised bovines nor did these animals become carriers of the parasites.
- (4) Non-splenectomised goats could not be infected but in splenectomised goats parasites appeared in the blood with very slight anaemic changes, and such animals became carriers of the disease.

Sheep, splenectomised and non-splenectomised, did not react to bovine anaplasmosis nor did they become carriers of the parasites.

Theileriosis of Sheep.—De Kock and Quinlan (1927) reported on the occurrence of Theileria (T. recondita, Gonderia ovis) in the blood of some splenectomised sheep. Transmission to non-splenectomised sheep and to bovines failed. The parasites did not produce any symptoms and appeared to be quite harmless.

Histological Changes.—De Kock (1929) reported on the appearance of haemo-lymphoid-like nodules in the liver of sheep, bovines and a goat killed three years after splenectomy. The nodules varied in size from \(\frac{1}{4} \) in. to 1\(\frac{1}{2} \) in. in diameter, were dark red in colour, and circumscribed. In some instances a distinct capsule could be recognised, and in others there was no demarcation between this foreign tissue and the liver substance. Lymphoid nodules could be recognised in some of these structures. The haemo-lymphoid-like nodules were not seen in the animals which died or were killed within the first two years following splenectomy. The first indications being found in a sheep which died 33 months after the spleen was removed. From the microscopic appearances de Kock concludes that this newly formed tissue resembles most closely that of haemolymph glands.

Goats.

Before splenectomy the two animals were injected with blood from sheep showing anaplasma but no reaction followed nor could anaplasma be demonstrated. Splenectomy failed to produce a relapse as in the case of immune carriers. Subsequent to the operation, however, when reinjected the blood showed the presence of anaplasma with practically no anaemic changes.

The histological changes which were found at autopsy on the one goat which died $8\frac{1}{2}$ years later have been mentioned above.

Canines.

Seven dogs were successfully splenectomised. One animal showed the presence of *microfilaria* in the blood three days later and continued to show these parasites until it died from other causes. In no case did *piroplasma* appear in the blood as the result of removal of the spleen. However, it was only assumed that the animals were carriers of *Piroplasma canis* as they had been exposed to tick infestation, but there can be no certainty with regard to this. Subsequently three of these dogs were injected with blood from a known carrier of the disease. One of them died from piroplasmosis and two recovered after treatment, but continued to show parasites for several weeks afterwards.

Owing to the uncertainty with regard to the previous immunity it is not possible to draw definite conclusions on the effect of the removal of the spleen on canine piroplasmosis. The disease, however, appeared to affect the splenectomised dogs more severely than is experienced in clinical cases of the disease in normal dogs. One of the animals showed a severe haemoglobinuria. Miessner (1931) reports on a few cases of relapses of piroplasmosis following splenectomy in dogs. The experiment will be repeated with known carriers of *Piroplusma canis*.

Four dogs were injected with the virus of African horsesickness after splenectomy but did not show any reaction to the disease.

Porcines.

Four pigs were splenectomised and all made uneventful recoveries. No parasites appeared in the blood following the operation. Attempts were made to infect the splenectomised pigs with African horsesickness (two cases) and with ovine blue tongue (two cases) without success.

Antelopes.

Neitz and du Toit (1932) reported on a method of obtaining pure strains of Anaplasma marginale and centrale by transmission through antelopes. The antelopes were injected with blood from bovine carriers of Piroplasma bigeminum, Theileria mutans, and either Anaplasma marginale or A. centrale. In each case only the anaplasma appeared in the blood. These antelopes were then splenectomised in order to exclude the possibility of the other parasites from the donor's blood being present in a latent form. In the cases of the two that survived the operation only the anaplasma reappeared in the blood after splenectomy. Three blesbuck (Damaliscus albifrons) and one grey duiker (Sylvicapra grimmi grimmi I.) were operated on.

One blesbuck died soon after the operation and the other two recovered. The duiker seemed to recover completely but interfered with the wound on the eighth day and died as a result.

One of the blesbuck, No. 33606, was later injected with the virus of ovine blue tongue and although it showed no reaction it was found to be a carrier on subinoculation of susceptible sheep.

Spirochaeta theileri could also be transmitted to the blesbuck.

Heartwater.—Finally the animal was injected with heartwater from a sheep and died 23 days later of this disease. Rickettsia could be demonstrated and susceptible sheep were infected from it.

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Table I shows details of all the animals splenectomised and their subsequent history.

TABLE I.

		Woioht	Date of		•	Anaestnesia.			Weight of	f Rosult	Experi-	Subsequent History.
Species.	Number	Number 115.	0	Chloral	Chloral Hydrake.	Chloroform.	rm.	Duration.	splecn.	The same of the sa	ment.	
Equine	15186	650	25/10/23	15 gm. inti	15 gm. intravenously	15 c.c. by inhalation	halation	13 hours	s 2.70 Kg.	Recovered	S.1602	Killed in extremis, 31/10/23. Recurrence of putalliosis.
:	15420	680	21/11/23	18 gm.	,	45 c.c.	33	14 hours	3.40 Kg.	"	33	Died 24/11/23. Recurrence of
:	16032	400	23/10/24	10 gm.	,,	45 c.c.	33	2½ hours	s 2·10 Kg.			Killed in extremis, 31/10/24. Recurrence of untailiosis.
:	16072	320	7/8/24	6 gm.	33	60 c.c.	3.3	4 hours	s 2.00 Kg.		33	Died 15/6/24 from effect of injection of paemolytic serum.
Bovine	711	380	27/3/24	10 gm.	33	120 c.c.	33	3 hours	s 1.65 Kg.		2 1613	Recurrence of anaplasma centrale in blood. Animal recovered.
											S. 1983	Injected with blood from bovine showing nironhasmosis, 14/3/25.
: : :	758 828 893	325 410 350	$\frac{6/6/24}{20/11/24}$ $\frac{15/12/24}{1}$	8 gm. 12 gm. 15 gm.	2 2 2	Few drops 60 c.c. 30 c.c.	2 2 2	1½ hours 2 hours 1½ hours	3 1.70 Kg. 8 2.10 Kg. 3 3.10 Kg.	Died	S. 1602	
											S. 2117 etc.	and recovered. Infected with east coast fever, recovered and was used in various cast coast fever experiments.
:	1029	390	22/12/24	15 gm.	4	30 c.e.	,	1½ hours	s.	Died	S. 1602	Died trypanosomiasis, 7/10/27. Died the same day as the result of
:	1027	425	24/12/24	15 gm.	33	60 c.c.	33	14 hours	s 1.85 Kg.	Recovered	33	Showed relapse of piroplasmosis and theileriosis (mutans), was treated
										_	8.2117	and recovered. Infected with East Coast fever 24/4/25,
:	1034	397	20/1/25	15 gm.	6	30 c.c.		2 hours	в 1-75 Кg.		S. 1602	Showed relapse of pirophasnosis and their frequency without treatment but continued without treatment but continued to the continued to the continued to the continued to the pirophasnosis in the blood for the continued to the pirophasnosis in the blood for the pirophasnosis and the p
											S. 3460	9 months. Used in East Coast fever experiment, 9/5/28, and recovered. Died of piroplasmosis on being exposed to
:	894	375	17/3/25	12 gm.	***	50 c.c.		24 hours	ç.	- 22	s. 2061 etc. s. 3460	natural infection, 5/7/28, East Coast fever transmission and innumity experiments. Exposed on tick infested veld, 2/4/28,
:	1032	150	21/4/25	20 gm.	23	15 c.c.	33	2 hours	S.		S. 1602	Them and the control of picture of price of pric
:	870	780	30/4/25	16 gm.	P.	15 c.c. ther	2.	2 hours	\$.	4	S. 2061	East Coast fever recovered animal and was splenectomised to try and break down the framinity
:	3000	320	22/7/29	10 gm.	**	15 c.c. ether 30 c.c.	6 6	1 hour	٥.	6	8, 3758	Infected with piroplasmosis and anaplasmosis. Died 12/8/29 of anaplasmosis.

Table I (continued).

Number	Wumber Weight.			Anaesthesia.		Weight of	Result.	Experi-	Subsequent History.
200	ė.	operation.	Chloral Hydrate.	Chloroform.	Duration.	spieen.		ment.	
2637	700	9/4/30	85 gm. intravenously 7 gm.	gm. intravenously 150 c.c. inhalation gm.	4 hours 1½ hours	۵.۵.	Recovered ",	S. 3802 S. 4488	Died of anaplasmosis, 25/4/30. Died of anaplasmosis, 25/4/30. mosis 6.1./33
4658	22 X	9/6/32	8 gm. "	80 c.c. ,,	21 hours	٠.	**	S. 4664	Injected with blood from sheep 26331 showing anaplasmosis but did not react. Then splenectomised and showed bovine thelleria and anaplasma, possibly result of acci-
1676	186 270	9/6/32	8 gm. ",	30 e.c. ,,	3 hours 2 hours	٥٠٥٠	Died	S. 4666 S. 4665	Died 29/6/32 apparently of surgical
5425 5432 5435	00†	6/9/34 11/9/34 20,9/34	18·5 gm. 20 gm. 20 gm.	75 c.c. ,, 75 c.c. ,, 90 c.c. ,,	3 hours 2 hours	1,700 gm. 1,487 gm. 1,097 gm.	Recovered Died Recovered	S. 5170 S. 5170	show, a carriers. After Augulusma centrale carriers operation these parasites reappeared and also eper, throzon tennomi. Used in further observations.
60509	350 450	14/11/34	9 gm. ". 11 gm. ".	30 c.c. ether ,, 75 c.c. ,, ,,	2 hours 3 hours	969 gm. 1,185 gm.	Recovered	8.5517	parasite. tick-free clean operation eper, appeared.
6289	180	15,11 34	7 gm. "	Chloroform.	1 hour	575 gm.	:	S. 5517	intected with A. centrate and P. bigeniaum. Blood remained negative until 30/1/35. Then used in other eveneriments.
6295	130	15,11 34	6 gm. "	7 0.0. ,,	1½ hours	333 gm.	ź	8,5517	Blood remained negative until 20/2/35, Injected with blood from sheep 11830 corrier of energhyozoon and
7369	02	59 /2 54	12 gm. per os		3 hours	188 gm.	٠,	8, 1602	the pursafter appeared in the call's blood seven days later. Developed anaplusma ovis ou 13th day, Killed for post mortem
2++3	7.	29 2 24	12 gm	2 c.c. ,,	24 hours	315 gm.	í	S, 1602	19/2/34. Developed anaplasma ovis on 13th dev Died of anaemia 22/3/24.
0878 8430	65	t7/18	8 gm	10 e.e. , ,,	1 hour	2.	4.3	S. 1602 S. 1655	Remained normal for seven weeks. Injected with blood from bovine carrying anaplasmosis with no
								S. 1825	result. Injected with blood from sheep showing theileria and anal.lasma ovės. Theileria appeared in 5 and anaphasma in 7 days. Died after bloeding 30/9/24.
8431 8456	65 56	8 4 24 15 5/24	s gm. " sonly	15 c.c. ,,	1 hour 1} hours	? 210 кт.	Died Rcovered	8, 1705	Died from pneumonia, 12/4/24. Injected with blocd from sheep showing anaplasma and died 15
8457	63	15/5/24	x gm. per os	Few drops "	1½ hours	194 gm,	\$	S. 1705	days later from anaemia. Injected with blood from sheep showing anaplasma ovis; showed marked reaction and recovered. Killed for pathological examination, 16(4/9).

Table I (continued).

Virginia Transfer	Subsequent History.	Injected with blood from bovine affected with anaplasmosis; no	Used in T. mutans and anaplasma	Subsequently used in black quarter and anthrax vaccine tests. Died	19/z/20. Cause inknown. Used in various anaplasma experiments but failed to react. Killed 1/4/25 for pathological examina-	tion. Before splenectomy was injected with blood of sheep showing anaplasma ovis. Showed anaplasma and	recovered. Beveloped pneumonis, showed relapse of theilers and anaplasma only and recovered. Killed for pathological examination.	Treated in the same way as above. Showed anaplasma infection before spierectomy and anaplasma and	the leave 8 days after operation. Treated in same way as above and died of anaplasmosis 16 days after	Disputationary anaesthesia. Treated in same way as No. 8434, but showed only slight reaction both before and after splenteromy. Observed for 7 years afterwards.	Spleen not removed but attachments loosened and vessels ligated. Died	25/9/24, three days later, Died 6 hours later without regaining	Aprimal collapsed suddenly 5 days	Kept under observation for 10 months.	Kept under observation for 16 months. Injected with bacterial icterus 17/5/26. Died of icterus	ZU/5/Z6. Kept under observation. Injected with cultures producing	pacterial referus; no resur. Injected with blood from case enzootic leferus with no result. Used in jaagsiekte and other experi- ments. Died 24/1 31 of toxacenda.
Experi-	mênt.	S. 1655	S. 1870	S. 1999	S. 1842 S. 1999	S. 1613	S. 1602	S. 1613 S. 1602	S. 1613 S. 1602	S. 1613 S. 1602	S. 1602		1	S. 1602 S. 9214	S. 1602 S. 2523	S. 1602 S. 2523	S. 2729
470	Result.	Recovered	**		ŝ	2		\$	6	Died Recovered	Died	23	;	Recovered	6	**	
Weight of	spieen.	205 gm.	٥.		\$~;	125 gnu.		184 gm.	195 gm.	176 gm.		270 gm.	220 gm.	280 gm.	70 gm.	240 gm.	
	Duration.	2 hours	\$ hour		hours	2 hours		4 hours	14 hours	2 hours	14 hours	1	2 hours	1 hour	3 hours	1 hour	
Anaesthesia.	Chloroform.	6.5 c.c. by inhalation	Drops ,,		Drops ",	7.5 e.e.		7.5 c.c. "	7.5 c.c. "	None	Few drops	5 c.c. "	Drops "	Few drops	Few drops "	Few c.c. "	
	Chloral Hydrate.	s gm. per os	8 gm. "		8 gm. ,,	8 gm. "		s gm. "	8 gm. "	8 gm,	6 gm. "	6 gm. "	s gm	8 gm. "	6 gm. ,,	6 gm. "	
Date of	operation.	15 5,24	19/5/54		19/5/24	3.7/24		3/7/24	3/7/24	3/7 24 22/9/24	FZ/ 6/ ZZ	16,1,25	16/1 25	28/1 25	28.1.25	12/3/25	
Weight.	IP.	1 9	79		200	70 X		09	56	20 cc 20 cc	26	258	13	80	10	89	
Weight.	Number	F9F8	8545		8454	8427		8428	8434	8429	8462	8455	x 10.40	8451	9119	10743	
200	Species.	Ovine			•				•			:			:		

Table I (continued).

		Weight	Date of		Anaesthesia.		Weight of		Experi-	Galmonnant History
Species.	Number Ib.	TP.	operation.	Chloral Hydrate,	Chloroform.	Duration		wesure.	ment.	· črosem granbasimo
Ovine	10511	75	12/3/25	6 gin. per os	. Few e.e. by inhalation	on 1 hour	300 gm.	Recovered	S. 2039	Anaplasma ovis experiment. Kept under observation for 14 months. Injected with culture producing
	10944	89	11/5/25	6 gm. intravenously	y 7½ c.c	1 hour	210 gm.	66	8, 2069	bacterial icterus with no result. Died 7/11/27 of uracmia result of urethal calculus. Injected with bovine blood harbouring anaplasma. Then splenectomised 6 weeks later. No relanse occurred.
:	91601	20	11/5/25	6 gm. "	7½ 0.0.	2 hours	s 300 gm.		S. 2069	Died 21/2/28. Used as No. 10944. Died 3rd day
	9414	06	6/11/25	5 gm. ,, only	1	1½ hours	ç.	÷	S. 2315	Five days later, 11/11/25, anaesthe- tised with chloroform and blood samples taken from various organs.
	13952	85	21'2/26	4 gm. ,,	Few c.c. ,,	1 hour	6-	66	8.3109	Killed with chloroform. Injected with material from a case of enzootic icterns and died 29/6/27
:	13853	70	21/5/26	3½ gm. ,,	Few c.c.	1 hour	ō-1	,,	8, 2523	of toxacma. Killed for collection of material,
(6	15987	50	12/4/27	3 gm. ,,	4 c.c.	1 hour	63 gm.	Died	1	Died of Shock 2 days after operation,
	16023	50	12/4/27	2½ gm. "	Few drops ,,	1 hour	81 gm.	Recovered	S. 3051	Injected with blood from sheep, carrier of anaplasma, and blood, studied chemically and histologi-
									S. 3339 S. 3599	cally. Used for transmission of jangsiekte. Kept under observation. Still alive and reserved for further path-
	24355	80	13 7/29	ō gm. ,,,	Ether 10 c.c. by in-	1- 13 hours	e- -	**	S. 3599	ological study after death. Kept under observation. Died 23/9.31 from uraemia, due to
:::::::::::::::::::::::::::::::::::::::	24358	683	13/7/29	Pernocton.	33	1 hour	6-1	33	S. 3599	urethral calculus. Kept under observation. Still living.
:	24356	80	16 7/29	9 0.0.	33	1 hour	٥٠	33	S. 3599	Kept under observation. Died 21/4/33 from anaemia. Metaplases
	24357	1 1 1	65 2,91	8-8 c.c. 22	9.6	1 hour	٥.,	2	S. 3599	in liver. Kept under observation. Still living
:	24613	02	- 4 '30	Chloral nyarate. 4.2 gni. intra- venously	Ether 2 c.c. by in-	n 1 hour	460 gm.	÷	S. 4167	Injected with blood from sheep, carrier of anaplasma ovis and T. recondita. Died 18/6/30 of
:	No	7.4	-/4/30	4.2 gm. "	25 c.c. ,,	1 hour	368 gm.	66	S. 4167	anaemia. Showed A. oxis. Injected with para- typhoid culture and died 6 days

Table I (continued).

SDECIES.	The state of the state of	Weight.	Date of			Anaesthesia.		Weight of	f	Experi-	
	Summed Ib.	ď	operation.	Chloral Hydrate.	ate.	Chloraform.	Duration.	spleen.	Kesult.	ment.	Subsequent History.
Ovine	24637	10	-/4/30	4 · 5 gra. intravenously	enously	20 c.c. by inhalation.	1 hour	360 gm.	Recovered.	S. 4167	Injected with blood from sheep, carrier of A. ovis and T. reconsida. Both parasites appeared after 24 and 60 days respectively. Injected
	23601	£ 5	-/4/30			30 c.c. ,,		225 gm.	ŝ	S. 4167	with paratyphoid culture and died 20 days later. Injected with paratyphoid culture and died 2 days later.
	28±98 28578 26331	55 51	14,7,30	4 gm. ,,		None None None	hours bours	215 gm. 119 gm. 315 gm.	; ;;	S. 3599 S. 3599 S. 4167	Kept under observation to study the formation of metaplases in the liver. Died 29/10/32. Stilliving. Sames above Ro. 22449. Stilliving. See above. A. ovis and Y. reconditu
:	25805	69	17 7/30	5 gm. "		None	3 hours	462 gm.		S. 4167	appeared. Discharged 9/8/31. See above, A. ovis and T. recondita
	32729	43	28/4/32	ō gm. "		None	4 hours	103 · 9 gm.	13	S. 3599 S. 4665	appeared. See above. Still living. Injected with blood from culf showing A. centrale but did not show
	32732	88	28/4/32	5 gm. "		None,	4 hours	99 gm.		S. 4666	parastres. Injected with blood from calf showing A marginale but did not show para-
	33004	31	18, 5, 32	2 gm		15 e.c. by inhalation	2 hours	45 gm.	33	S. 4664	Keps. Keps. under observation. Died
2.5	32674 33013	401 011-	18/5/32 26/5/32	4 gm 2½ gm. ,,		None 8 c.c. by inhalation	2½ hours 2½ hours	? 115 gm.	Died Recovered	S. 4664	Died under anesthesia. Daily smear examination. No para-
	33014	35	26/5/32	3 gm. "		Ether 15 c.e. by in-	2½ hours	68 gm.		8, 1665	Sites seen. Died 1/9/32 of gastritis. Daily smear examination. Dis-
2 2	33044	32 40	26.5 32 11/5/32	3 gm. ",		None	2½ hours 3 hours	80 gm. 214 gm.	; :	S. 4665 S. 4665	Sec ovine No. 32729. Discharged
	32730	4.2 2.4	11/5/32	5 gm. "			3 hours	209 gm.	*	8, 4664	1/2/33. See Ovine No. 32732. Discharged
	28335	95	24,11,31	6 gm. "			3 hour	460 gm.	ê	S. 4896 S. 3509	1/2/00:
	35000 35004 37862	522.	6/2/34 6/2/34 6/2/34	6 gm. ", 5 gm. ", 5 gm. ",			5½ hours 6 hours 3 hours	562 gm. 333 gm. 188 gm.	: : :		Experiment in progress.
:	37429	533	6/2,34	gm.			3 hours	337 gm.		I	

Table I (continued).

	Sirbsequent History.	Under observation for four months. Injected with blood from sheep har- bouring anaphasma and showed a	S	Blood showed presence of microfilaria 3 days after operation. Died	28/9/32 From Incoxpandion and to be stings. Daily smear examination. No parasites seen for 31 days. Then infected with blood from carrier of	P. comis. After seven days parisites appeared and animal died 17/11/32 of billary fever (P. comis). Same as No. 1150 except that animal was freated on the 4th day after neasiles annoared and recovered.	Transferred to experiment S. 4931. Transmission of horsestekness. 8till alive. Same as No. 1137. Transmission of horsestekness. 8till Transmission of horsestekness. 8till	alive. Died before injection with horse-sickness. 31/1/33. result of per-	forating duodenal ulcer. Transmission of horsesickness. Still	Transmission of horsesickness, Still alive,
Experi-	ment.	S. 1602 S. 1613	S. 1602 S. 1613	S. 4790	S. 4790	S. 4790	S. 4931 S. 4790 S. 4931	8, 4931	S. 4931	S. 4931
100	Kesur.	Recovered	6	Recovered	e.		*	-		*
Weight of	spleen.	190 gm.	··	259 gm.	34 gm.	250 gm.	125 բm.	٥.	٥.	∞ -
	Duration.	11 hours	12 hours	2, hours	2½ hours	21. hours	3 hours	24 hours	1½ hours	3 hours
Anaesthesia.	Chloroform.	2 c.c. by inhalation	2 c.c. "	Pernorlon, 4-8 c.c	1.2 6.6	4.2 c.c	3.8 6.6.	1.3 e.c	3.6 e.c	1.2 c.c
	Chloral Hydrate.	30/5/24 8 gm. per os	8 gm. "	Enkodal.	1 6.6	3 c.c.	3 c.c	Ether 15 c.c	Eukodal 3 c.c	19/1/33 Kther trace
Date of	operation.	30/5/24	30/5/24	13/9/32	7/10/32	7/10/32	13/9/32	19/1/33	19/1/33	19/1/33
Weight.	D	99	26	46	11.2	40	43	1.3	=	12
	Number D.	8280	8304	1127	1150	11511	501	1001	1044	1143
2	Species.	Caprine	£ .	Canine	:			:	:	

Table I (continued).

		Weight.	Date of	A	Anaesthesia.		Weight of	2	Experi-	(7-1) (2-1)
Species.	Number Ib.	É	operation.	Chloral Hydrate.	Ether.	Duration.	spleen.	Result.	ment.	Subsequent ristory.
Blesbuck, Danaliscus abifrons	32055	F9	13/8/31	4 gm. intravenously	Few c.c by inhalation	1½ hours	355 gm.	Recovered	S. 4417	Injected with blood from bovine harbouring anaplasma marginals, and the leiferia mutans. Anaplasma marginale appeared in blood 11th day, and could be demonstrated for 14 days. Other parasites did not appear. Spiectonised and showed relapse of anaphasmosis. Died as result of anaphasmosis.
Blesbuck	32054	09	4/12/31	5 gm. "	None	2 hours	٥٠	Died	S. 4417	verminosis. Verminosis. Verminosis. With blood from bovine harbouring I: Löttenkum, T. matuns, and A. entrade, and the latter appeared in the blood accompanied by slight amannie changes hut no chineal symptoms. Then splenectomised
Duiker, Sylvicapra grimmi	32806	36	7/4/32	3 gm.	None	1½ hours	80 gm.	Recovered	S. 4521	25,5
grimmi, L. Blesbuck	33606	20	12/4/32	6 gm.	45 c.c. by inbalation	2 hours	619 gm.	£	× 4 63 6	contents. contents. contents. bouring P bigenium, T. madravas, and A. endrale. No parasites and A. endrale. No parasites appeared, hijected with blood from calf harbouring A. marginale which showed in the blood for 18 weeks. Injected with blue tongue virus. Showed no reaction but susceptible animals subinoculated from it inhowed typical blue tongue. Injected with blood from calf harbouring sprochere their tong it is nowed typical blue tongue. Injected with blood irom calf harbouring sprochere their. Paraselites appeared on 6th day and were seen for 8 days. Injected with heartwater virus and died 23 days later, 16/2/33, with lesions of heartwater. Rickettsin could be demonstrated and subinoculated animals died with
		1		Pernocton.	Ether and Chloroform.	Duration.				TOSTORES OF INCREMENTAL
Porcine	† 06	† †	13/12/32	4 · 4 c.c intravenously	8 c.c. of each by inhalation	3 bours	Sl gm.	Recovered	8, 4914	Daily blood examination for 6 weeks. No parasites appeared. Injected with horsesickness virus but
	903 905	421 39	13/12/32 15/12/32	4.4 c.c ", 4.4 c.c ",	None Trace by inhalation	3 hours	128 gm. 120 gm.	: :	S. 4914 S. 4914	showed no reaction. Still alive. Same as No. 904. Injected with blue tengue virus. No reaction Still alive
	906	53	15/12/32	5.8 c.c	Trace by inhalation	4 hours	155 gm.	,,	8, 4914	Same as No. 905.