number of bands concerned is about 96 per atmosphere of air, or about 62 over the range actually used. It is wise always to include a match with pressures about midway between the extremes. If the results harmonise, an error of a single band is excluded, and it is hardly possible to make a mistake of two bands.

As regards accuracy, independent final results usually agree to one-thousandth part.

"On Nagana, or Tsetse Fly Disease. (Report, made to the Tsetse Fly Committee of the Royal Society, of Observations and Experiments carried out from November, 1896, to August, 1898.)" By A. A. KANTHACK, H. E. DURHAM, and W. F. H. BLANDFORD. Received October 27, 1898.

At the request of the Colonial Office, the Royal Society of London appointed a Committee to co-operate with Surgeon-Major Bruce in his research upon Nagana or the Tsetse Fly disease. This Committee entrusted us with the actual experimental work. The object was to study Nagana systematically in ordinary laboratory animals, to investigate the life-history of the hæmatozoon discovered by Bruce, and, if possible, to discover methods of prevention, cure, or immunisation.

The material for our observations was obtained in the first instance from the blood of a dog infected by the disease on the voyage from Africa, and brought to England in November, 1896, by Dr. Waghorn.

The investigation was begun at once at the pathological laboratory of St. Bartholomew's Hospital, but in February, 1897, was transferred to the pathological laboratory of the University of Cambridge.

The Hæmatozoon of nagana has already been described by Bruce, and is closely allied to the *Trypanosoma* of Surra. We have had no opportunity of studying the latter disease, the relation of which to nagana is referred to later. The parasite discovered and described by Rouget* in a horse in Algeria is also similar. In English sewer rats (*Mus decumanus*) a *Trypanosoma* (*T. sanguinis*) is occasionally found, but this is quite distinct from the hæmatozoon of nagana, both in its morphological appearance and in its pathogenic effects (*vide infra*).

I. *Susceptibility.*

Cats, *dogs*, *mice*, *rabbits*, *rats*, both sewer rats (*Mus decumanus*) and white and piebald rats (*Mus rattus*), are highly susceptible, and in these animals the disease has proved fatal in every case of infection which has been allowed to run to a close.

* 'Annales de l'Institut Pasteur,' 1896, p. 716.

A single *hedgehog* inoculated was readily infected and died in seventeen days, so that this animal probably possesses a high susceptibility.

A single *donkey* was inoculated and was killed twelve weeks later, being then in a weak condition and near dying.

Two *horses* have been inoculated, one a strong and well-fed cart horse ("Russian"), which survived seven weeks, the other a rather old animal (see under zebra hybrids) which survived only eight days.

A *bosch-bok* has also been inoculated; it died seven months afterwards without showing any lesions. All the inoculations made from it proved negative.

Two hybrids of zebra and horse (♂ zebra and ♀ horse, and ♂ horse and ♀ zebra) and one hybrid of zebra and ass (ass ♂ and ♀ zebra) have also been inoculated. These were kindly put at the disposal of the Royal Society by Professor Cossar Ewart, of Edinburgh, in order to see whether such hybrids are refractory to nagana.

The two former were infected by plunging a needle wetted with nagana blood beneath the skin; the latter received a dose of 1 cubic centimetre of the same blood. All of them died in about eight weeks. During the course of the disease they showed irregular rises of temperature, sometimes up to 41·6° C. Variations in the number of hæmatozoa were ascertained in the case of the horse hybrids; on some occasions they were abundant (66,000 per cubic millimetre). Whenever the donkey hybrid was examined at the earlier stage of the illness the hæmatozoa were found to be either scanty or absent. A horse which was inoculated as a control died in eight days, with very abundant hæmatozoa in its blood; this animal must have been peculiarly susceptible to nagana, as no other cause for death could be found at *post-mortem* examination. There is no reason for supposing that the hybrids exhibited any more refractoriness than other horses or asses.

Koch* reports on attempts which he made to infect two Masai donkeys, and two crosses from Muscat and Masai donkeys. None of these showed any symptoms of the disease up to three and a half months, nor were hæmatozoa discovered in their blood at any time, although repeated examinations were made. Consequently there is no proof that these animals were really infected. In our experience scratch inoculations sometimes, though rarely, fail; on the other hand, inoculations by puncture with a needle or by actual injection do not fail; Koch's animals were inoculated by the scratch method. It should be added that all his control infections were successful. He did not find that ordinary mules showed any immunity.

With regard to *guinea-pigs*, at first we thought that they were refractory under normal conditions, and that it was possible to infect them only after their resistance had been reduced by bleeding or other interferences.

* 'Reiseberichte,' pp. 69 and 88.

We found, however, that guinea-pigs are susceptible to nagana under ordinary conditions, but that, as a rule, the disease in them is more protracted than in rabbits, rats, mice, cats or dogs, and even horses; so that they are distinctly more resistant than these animals. In no instance, however, has recovery ensued after hæmatozoa have once appeared in the blood.

According to unpublished observations by Bruce upon the Tsetse Fly Disease in South Africa, it appears that native *goats* and *sheep* are to some extent refractory, the disease, as a rule, running a chronic course (five months).

A *monkey* (*Macacus rhesus*) was also tried. It died in about two weeks in an advanced condition of pulmonary tuberculosis, but the presence of abundant hæmatozoa had been determined in the blood during life up to the time of death.

A *weasel* was injected. It showed hæmatozoa in its blood, and died a few days later, but death almost certainly was hastened by the effects of captivity.

Pigeons are the only *birds* which have yet been tried. The pigeons after inoculation did not show signs of the disease, nor was their blood infective. It may be mentioned that Bruce tried South African *hens* without success. Further experiments with birds are in hand.

Young animals, if susceptible (kittens and puppies), as a rule have died earlier than adults, and while suckling they are still more highly predisposed; young guinea-pigs, however, are comparable to older ones in their resistance.

The *fœtus in utero* of infected rabbits, guinea-pigs or rats, is not infected, although the mother's blood may contain a large number of hæmatozoa. The latter are to be found in the placenta, but not in the fœtal blood. Similar observations have also been made by Lewis,* Lingard,† and Rouget‡ in their investigations on allied hæmatozoa.

II. *Duration of Disease in the different Animals.*

As will be seen from the figures given below, the lethal period varies somewhat in each species of susceptible animal. The duration of the disease appears to depend principally upon the individual susceptibility rather than on the mode of inoculation or the quantity of infective material introduced. Thus, of four rabbits inoculated in the same manner with the same material, three died on the 12th, 21st, and 24th days respectively, whilst the fourth was killed on the 41st day; many similar instances could be cited. Nor does a larger quantity necessarily determine a more rapid death; thus a rabbit which has

* 'Physiol. and Pathol. Researches,' p. 630.

† 'Summary of Further Report on Surra,' 1895.

‡ 'Annales de l'Institut Pasteur,' 1896, p. 716.

received the whole blood of another rabbit containing numerous hæmatozoa may survive longer than the minimal lethal period for rabbits. We have not been able to define the conditions which determine these variations in susceptibility. Rouget has noted similar variations in the lethal period.

The ratio of the minimal to the maximal lethal periods is about 1 to 5 or 1 to 6 in rabbits and rats, and 1 to 9 in guinea-pigs. The number of other animals inoculated has not been sufficiently great to determine a satisfactory ratio.

In our experiments dogs survived an infection 14—26 days, cats 22—26 days, rats 6—26 days, mice 8—25 days, rabbits 13—58 days, and guinea-pigs 20—183 days; the average duration being for dogs 18 days, for cats 24 days, for rats 12 days, for mice 13 days, for rabbits 30 days, and for guinea-pigs 50 days.

Since the commencement of these experiments, a large number of animals have been dealt with, and thus an extensive series of cross-inoculations has been carried out; but we have found that the duration of the disease is not dependent on the kind of animal from which the hæmatozoa are derived. No constant modification is, therefore, effected by passages, either in the direction of attenuation or of increased virulence. This statement is completely borne out by Bruce's observations on wild animals, as well as African sheep and goats, for he found that the hæmatozoa of these animals were as infective as those obtained from highly susceptible animals, such as dogs.

III. *Mode of Inoculation.*

Inoculations have been made with the blood of an infected animal, subcutaneously, intravenously, or intraperitoneally, or by applying a minute and often minimal quantity of infected blood to a superficial scratch. Rabbits have also been inoculated in the anterior chamber of the eye, and rats directly into a lymphatic gland.

Blood taken from diseased animals, although showing no hæmatozoa when examined microscopically, has frequently been proved to be fully infective, so that it appears that a single hæmatozoon, or at any rate a very small number of them, successfully introduced, are capable of producing the disease. At present no method of graduating the dose appears possible, since a minute quantity is as effective as much larger quantities, though the lethal period may be somewhat prolonged. It is also possible that unrecognised forms are present in these cases, though it should be added that in some instances where no hæmatozoa are found in simple films, we were able to detect them by means of centrifugalising the blood.

Successful inoculations have also been made with lymphatic gland, spleen, bone-marrow, aqueous humour, serous fluid, œdema transudation, and testicular juice.

The incubation period and the duration of the disease, as already pointed out, are not entirely dependent upon the number of hæmatozoa in the material injected, or the source of the infective material. Thus the hæmatozoa of lymphatic glands, &c., are as infective as those of the blood. The duration of the disease is not materially affected by the mode of inoculation adopted, and is about the same, whether the infection was brought about by subcutaneous, intravenous, or intra-peritoneal injection, or by a superficial scratch.

Material taken from the bodies of animals twenty-four hours, or sometimes less, after death, is hardly ever infective, even when several cubic centimetres are injected, so that we have no evidence of a resisting or sporing form which survives in the tissues or blood of the dead animal, and is inoculable into other mammals. It must be added that putrefactive changes often set in with great rapidity in the bodies of animals dead of nagana.

Blood drawn from the living infected animal and kept *in vitro* in an aseptic condition, retains its infective power *at most* for three or four days, but this period is generally less. Complete drying also renders blood non-infective.

Blood heated to 50° for thirty minutes invariably becomes non-infective, even in large doses (such as 4 c.c.), while when heated to 46° C. for half an hour it proved infective in one out of two cases, although apparently the hæmatozoa had become non-motile—at least no motile forms were detected under the microscope. But even in this case the lethal period was not prolonged.

Infection by feeding has been attempted by means of a number of experiments. Sometimes it was successful, in most cases unsuccessful, so that it has seemed to us that the possibility of infection by the mouth depends on accidental lesions about the mouth, nose, ears (in rats), or alimentary tract.

Of a number of rats fed on organs of nagana animals, only a few acquired the disease, and these invariably showed superficial lesions of the snout and ears, due to lice. When fed upon infective material, they bury their snouts in it as well as scratch their ears with their blood-stained forepaws. Furthermore, in the rats which acquired the disease through feeding, the cervical glands were always enlarged most, which proves that the hæmatozoal infection must have taken place in the head, for, as we shall show, the primary infection travels by the lymphatics.

A cat fed repeatedly on soft tissues of the bodies of infected dogs and cats, and subsequently on the bodies of dead rats, died at a time corresponding by lethal period to an infection at the first meal on rats. We regard it as probable that some splinter of bone caused a superficial lesion through which the hæmatozoa were enabled to enter.

One rabbit, fed carefully by means of a pipette with large quantities

of infected blood, never showed the slightest sign of the disease. Rouget (*op. cit.*) also failed to infect animals by the mouth.

Two rabbits, into whose conjunctival sacs several drops of blood containing very abundant hæmatozoa, and a third rabbit whose eye was brought into contact with one of these, did not become infected. We presume that Rouget's positive results by this method were due to some accidental lesion.

A dog suffering from the disease did not infect her puppies during the last fourteen days of her life, nor did these puppies infect their foster mother (she-cat) after they had been inoculated.

Nor have we observed transmission of the disease through the mother's milk in guinea-pigs. Rouget alludes to a doubtful instance of infection by coitus in rabbits by means of the spermatic fluid. We have not detected hæmatozoa in spermatic fluid obtained from the vesiculæ seminales, and believe that in Rouget's single positive case there may have been direct infection from the penis, which suffers considerably in rabbits and may become excoriated, so that it easily bleeds.

We therefore do not believe that it is possible to infect an animal by feeding in the absence of superficial lesions, and in this respect we differ from Bruce, who seems to imply that the hæmatozoa can pass through the unbroken surface of the alimentary tract.

IV. *Symptoms and Course of the Disease.*

These vary somewhat according to the nature of the animal, but there are certain striking symptoms which commonly occur in different groups of animals. These may therefore be regarded as the most characteristic.

1. *Muscular wasting* and *loss of power* are evident in all but the small animals. In rats, mice, and guinea-pigs they are but little marked or absent altogether. In the horse, dog, cat, and rabbit the wasting is very conspicuous. In the cat, dog, rabbit, and hedgehog there is marked loss of weight, amounting to 20—30 per cent.

2. *Fever.*—In most animals which have been examined, there is a smart rise of temperature about the time of appearance of hæmatozoa in the blood. (Horse, 41·5° C.; dog, 40° C.; rabbit, 41° C.; guinea-pig, not constant.)

Paroxysms of fever are common in the *horse*, as has already been shown by Bruce. The temperature may rise to a considerable height (41·6° C.); the same is true of the zebra-horse hybrids. In a horse upon which daily observations were made, quick and sudden rises of temperature immediately followed an increase of the hæmatozoa in the blood. At the time of death there was marked pyrexia.

In the single *donkey* which we examined the temperature was generally raised throughout the course of the disease.

In *dogs* there is also fever, the temperature becoming subnormal on approach of death.

In *rabbits* pyrexia is common, and generally the temperature is elevated throughout the disease, but it may fall suddenly to normal. The temperature curve is always irregular, and no relation between the temperature curve and the hæmatozoal curve could be established.

In *cats* also the fever is well marked, the temperature falling quickly towards the end.

It is difficult to speak with certainty of the temperature in such small animals as *rats* and *mice*.

In *guinea-pigs*, representing less susceptible animals, fever as a rule is not a special feature. The temperature is as irregular as it is in the normal animal, but the animal shows paroxysmal rises from time to time, sometimes above 41°; these may be accompanied by an accession of hæmatozoa into the circulation, but this is not a constant feature.

3. *Œdema* is common in certain animals, such as the horse, rabbit, cat, and dog, and is most marked about the head, legs, belly, or genitals. In smaller animals, such as rats and mice, it is not usual, and in guinea-pigs it has not been observed. In dense tissues, as the rabbit's ear, there may be a local œdema at the site of inoculation.

Rabbits exhibit a special tendency to œdema of the external genital organs. There is often great and progressive swelling of the prepuce or labia, as the case may be. The swollen parts often excoriate and become sore and covered by crusts, so that the animal is in a sorry condition.

4. *Changes in the Eyes and Nose.*—In cats, dogs, rats and rabbits turbidity of the aqueous humour, fibrinous plaques in the anterior chamber, and corneal opacities are occasionally observed. In rabbits a muco-purulent conjunctivitis is common, and this may be followed by an opacity of the cornea and a turbidity of the aqueous humour, which under such conditions shows hæmatozoa microscopically as well as leucocytes. Hæmatozoa have also been discovered in the conjunctival discharge in the earlier stages of the disease. Vascular corneal ulcers sometimes occur in dogs, and the conjunctivitis of cats, dogs, rats and rabbits is frequently associated with œdema of the eyelids and face. In rabbits the eyelids and nose frequently become almost entirely closed up by the drying of the secretion; in the latter case they breathe with great difficulty, keeping their mouths open. This condition has been described by Rouget (*op. cit.*).

5. *Anæmia.*—Some degree of anæmia is always present, but it does not seem to be so extreme as to be the sole attributable cause of death, and points rather to a disturbance in the hæmatopoietic or the hæmatolytic mechanisms.

The number of red blood corpuscles steadily diminishes and nu-

cleated red corpuscles (normoblasts) often appear, especially in rats. According to observations on rabbits the diminution of hæmoglobin is roughly proportional to that of the blood corpuscles.

Leucocytosis is not a constant feature and when present is apparently due to the febrile temperature. An excessive leucocytosis, such as occurs in leukæmia, was never observed; 15,000—34,000 leucocytes being the highest numbers recorded per cubic millimetre.

Blood drawn from an animal seriously ill, when clotted, generally exhibits a marked buffy coat; the serum is often turbid and may undergo secondary clotting.

Instead of forming rouleaux, the red corpuscles tend to clump into masses and to lose their outlines, especially when the anæmia is pronounced (rabbit, ass, and horse).

The serum of such blood, when mixed with normal blood of the same species of animal, causes the red corpuscles to clump together also.

The urine of infected dogs spectroscopically examined often shows an intense *urobilin* band.

6. Wounds do not heal well, and tend to break down and become septic, even though the operation has been performed with strict aseptic and antiseptic precautions. The hæmatozoa may be abundant in the discharge from the wounds. Many animals, especially dogs, are apt to become infected with pyococci and other bacteria in the later stages of the disease, even when the inoculated material has been proved to be free from bacteria. We conclude that a spontaneous terminal bacterial infection may occur when the marasmus has reached a certain degree. This may accelerate death and is probably fairly often the case in the naturally acquired disease. But we have often proved by cultures that bacteria are absent in uncomplicated cases of experimental inoculation.

7. A voracious appetite has not been observed in the infected laboratory animals; some animals refuse their food and the stomach is not seldom empty after death.

8. Rats and guinea-pigs often exhibit convulsive or eclamptic seizures shortly before death, but otherwise guinea-pigs, rats, and mice show no symptoms of disease, except dulness in the later stages.

9. Transmission from one animal to another, without direct inoculation, has never been observed. Nor have we come across instances of infection by coitus or through suckling, although we have dealt with large numbers of animals.

V. *Morbid Anatomy.*

In *rats* and *mice* exactly the same conditions may be observed. The most striking changes are:—

(1) Enlargement of the lymphatic glands, the glands corresponding

to the seat of inoculation being always largest. This observation is important, because from the relative size of the glands it is possible to determine the seat of infection. To this allusion has already been made, when the effects of feeding were discussed. The glands are generally red, congested, juicy, and œdematous; in a few instances hæmorrhagic extravasation has been observed. In some cases all lymphatic glands in the body are enlarged, in others a particular series only. If a rat be inoculated in the right thigh, the glands in the left axilla and left groin suffer last.

(2) The spleen is much enlarged, with but few exceptions, and it is generally firm, friable, and dark coloured.

(3) The liver generally shows some enlargement and may be fatty.

(4) Wasting of the muscles and atrophy of the fat is, as a rule, not well marked.

(5) Sub-pleural ecchymoses are sometimes present in the lungs, accompanied by a small amount of pleural fluid.

In *rabbits* the general enlargement of lymphatic glands is less noticeable. The spleen is generally enlarged. Petechial ecchymoses are rare. Fatty degeneration of the liver is always present, and muscular wasting is often extreme. Enlargement of testes has been observed.

In *dogs* muscular wasting is well marked, the animal being often reduced to a skeleton, but the fatty tissues are generally not much affected, except at the base of the heart, where the fat may undergo œdematous degeneration. The general enlargement of the lymphatic gland is well marked, and, as in the rat, the glands are œdematous and congested, yellowish, or even show hæmorrhagic extravasations.

The spleen is also greatly enlarged, granular, firm and friable.

Pericardial effusion is common, pleural effusion may be present.

Sub-pericardial petechiæ and hæmorrhages occur frequently, sub-peritoneal occasionally, and sometimes also sub-mucous in the intestines and stomach.

In *cats* wasting is pronounced, the glands are greatly enlarged, the spleen is also enlarged, the liver is slightly enlarged. Hæmorrhages beneath the pleura and pericardium have been noticed.

In *guinea-pigs*, which clinically often show no changes or symptoms at all, the morbid changes after death are not very well marked. The spleen is generally moderately enlarged, and occasionally even considerably; it is often very soft and rather pale. The lymphatic glands are distinctly, but as a rule only slightly, enlarged, those corresponding to the seat of inoculation being always the most affected.

Hæmorrhages have been observed in the lungs and in the stomach; serous effusions and œdema have not been noted.

In all these animals the bone-marrow is sometimes dark red in colour, at other times natural, or paler than it should be. In the shafts of the long bones the fat disappears and becomes replaced by "red" marrow.

In many cases an iron reaction has been obtained with the liver, spleen, and kidney (ammonium sulphide; and $K_4FeCy_6 + HCl$).

VI. *Distribution of Hæmatozoa.*

A. *Blood.*

After a latent period of some days, hæmatozoa are invariably found in the blood at some time or other during the course of the illness.

1. *Rats.*—When the animal is inoculated with small quantities of infective blood, the latent period averages 3—4 days. When, however, a large number of hæmatozoa is inoculated into the peritoneal cavity, the parasites may be found in the blood even after a few hours.

When the hæmatozoa have once appeared in the blood, they are generally found therein to the end, gradually increasing in number till the blood literally teems with them. During the early stages of the disease, however, variations are frequently noted, inasmuch as an increase on one day may be followed by a marked decrease on the next. In a few cases they have even temporarily disappeared from the circulation for a day or two, but this is distinctly rare in rats and mice, although common in other animals.

At the later stages the hæmatozoa may amount to 2,000,000—3,000,000 per cubic millimetre.

2. *Mice.*—What has been said of rats applies also to mice.

3. *Rabbits.*—In these animals, after inoculations with minute quantities of blood, the parasites first appear in the blood in about eight days, about the same time as the pyrexial attack. They remain in the general circulation for a day or two in small numbers; this is followed by a disappearance and reappearance for a variable number of days at irregular intervals. In the animals which have been systematically examined the hæmatozoa do not appear abundantly until towards the close of the disease; the largest number which has been estimated near the time of death has been 60,000 per cubic millimetre (compare rats and mice), but even at that time they may be scanty and difficult to find. They are also to be found in the fluid of the local œdema and discharge from wounds, conjunctiva, or genitals. Although hæmatozoa may be so scanty that they cannot be discovered by the microscope (sometimes even after centrifugalising), the animals show marked clinical symptoms. Their blood has often been proved to be infective.

4. *Dogs.*—Early in the disease, from 4—6 days, the hæmatozoa may be absent from the blood, but observations on their presence during life in the lymphatic glands have not been made. Towards the end they become very numerous (100,000—300,000 per cubic millimetre). Variations in the number of hæmatozoa are common, but as a rule hæmatozoa are numerous throughout the disease.

5. *Cats.*—The latent period is about five days; then the hæmatozoa

appear in the blood, and, with daily variations, quickly increase in number. The variations are sometimes remarkable; thus on one day the hæmatozoa may be extremely numerous, while on the next day they will have become scanty.

6. *Horse.*—Systematic observations on this animal, as well as on the donkey, have been made by Bruce. In our first horse the latent period was seven days. The first appearance of hæmatozoa in the blood was followed by a sharp rise of the temperature. After the hæmatozoa once showed themselves they were generally scanty and often absent (the centrifuge was not used), but an appearance of the hæmatozoa in the blood was generally followed immediately by a paroxysm of fever. A few days before death, however, the number increased greatly, falling again to zero two days before death and being low at the time of death.

7. *Guinea-pigs.*—After a subcutaneous inoculation, a few hæmatozoa will generally be found in the blood about the fifth to seventh day. They may then again disappear and reappear from time to time, to disappear again after a few days. This alternation may go on for weeks. Then suddenly the hæmatozoa become numerous and gradually increase, sometimes with irregular variations, till the blood is almost crowded, 200,000—500,000 per cubic millimetre being present. The guinea-pigs die, generally without showing any symptoms, except perhaps convulsive attacks a day or two before death.

In some cases no hæmatozoa have been found in the blood for over six weeks, although it has been examined daily. They then appeared in small numbers, and after remaining scarce for a week or so, suddenly and rapidly increased as the disease approached its fatal termination. It is, however, more common to find a few hæmatozoa about a week after inoculation, this being followed by a more or less prolonged period of absence.

In cases where the disease runs a less protracted course, the hæmatozoa become numerous about four weeks after the inoculation, when they are often present in large numbers; but, as in the case of other animals, the number of hæmatozoa may be very variable, being almost enormous one day and very considerably less, or even very small, the next. In a case where the guinea-pig had been bled before inoculation the disease ran a rather short course; hæmatozoa appeared nine days after the infection, rapidly rising in number to over 128,000 per cubic millimetre, the animal dying after twenty-two days. In a few cases where the lymphatic gland corresponding to the seat of inoculation was examined, hæmatozoa were found in the gland whilst they were absent in the blood.

B. *Lymphatic Glands.*

In the rat the superficial lymphatic glands may be readily examined by piercing them with fine capillaries or sharp needles; they may also be excised and examined more thoroughly. Although a considerable number of observations have been made by these means, and also after killing the animals at various periods after inoculation, we wish to speak somewhat guardedly, since the appearances are not quite constant. Moreover, we are at present unable to be certain that unrecognised developmental forms have not been overlooked.

By the study of the right inguinal glands after subcutaneous inoculation in the right thigh, we find that the hæmatozoa are present from one to three days before they are discoverable in the blood (taken from the ear or right leg). Again, they may be very abundant in the gland when they are still scanty in the blood. Moreover, the number in the blood may increase, whilst that in the gland decreases. In these earlier stages the hæmatozoa may be extremely numerous, forming tangles and clusters in the lymph gland, whilst only a few scattered ones are to be found in the blood. The first appearance of hæmatozoa in the gland of the other side is apparently associated with their appearance in the blood.

The observations, fewer in number, which have been made upon guinea-pigs, also point to a multiplication in or about the nearest chain of lymphatic glands in the first instance.

We have not yet determined whether these hæmatozoa pass directly into the blood through the local blood vessels, or whether they are distributed by means of lymphatic paths into the main circulation.

The animals may appear comparatively well whilst large numbers of parasites are present in their blood and glands, this is especially the case with rats and guinea-pigs. On the other hand they may be seriously ill whilst the hæmatozoa are scanty in their blood; this obtains usually in rabbits, in which animals, as already stated, the glands do not become so much enlarged, and it is possible that the main effect of the parasites is borne by other organs. For instance, at times the bone-marrow has shown the presence of hæmatozoa, although search in other organs and in the blood proved negative.

After death in the various animals, hæmatozoa are to be found in most cases in the bone-marrow and spleen. The adult hæmatozoa may be common in these situations when but few are present in the blood; but this is not constant, for the reverse may be the case. Multiplication of the parasites certainly takes place in the lymphatic glands (rat) as well as in the infected area of connective tissue; it may also occur in the above-mentioned organs as well, and perhaps too in the blood, but of this we have no certain evidence.

The hæmatozoa are also found in the fluids of the serous cavities, at any rate when they are present also in the blood.

They have not been found in the intestinal contents, nor have they been seen in the urine, except in one case in which hæmaturia and submucous petechiæ of the bladder were present (rat).

It is evident from these observations that, in order to investigate the development of the hæmatozoa in the rat, &c., special attention must be paid to the seat of inoculation and the nearest lymphatic glands.

Dead and non-motile forms may frequently be found in the circulation and in the lymphatic glands, when the disease is advanced. These are less defined and are ghost-like, being somewhat swollen in appearance; they are also generally in an extended condition.

VII. *Toxic Power of the Blood.*

The fact that animals may appear to be well for days while hæmatozoa are abundant in their blood, suggests that the hæmatozoa do not secrete much, if any, specific toxin, and indeed so far no direct evidence has been obtained of a potent poison manufactured by the hæmatozoa, either by secretion or by chemical changes induced in the blood.

Fresh serum after filtration through Berkefeld filters, and blood or serum which had been kept for days in a sterile condition till the hæmatozoa had died, have had no specific toxic effects, even when large quantities have been injected into dogs, rats, or rabbits. Blood in which the hæmatozoa have been killed by exposure to 50° C. has had no more effect. The extracts of organs obtained from diseased animals have also shown no poisonous properties.

The whole available blood of highly diseased rabbits has been injected immediately after removal into healthy rabbits, without producing immediate symptoms of acute intoxication.

The bile of diseased animals does not appear to be more toxic than that of healthy animals.

A cat, into the peritoneal cavity of which a collodion sac full of fresh infected blood had been inserted, showed no signs of illness. It was fully susceptible on subsequent inoculation.

Dogs, when injected with large quantities of filtered serum from an infected dog, showed no symptoms of a profound toxæmia.

Our experiments do not point to the presence of any intense specific toxin or poison in the blood.

VIII. *Immunisation and Cure.*

The endeavours to produce immunity, or to cure the disease after its establishment, are shortly summed up as follows:—

1. Animals which have been repeatedly injected with blood or serum of nagana animals, such blood or serum having been previously freed from living hæmatozoa, either by filtration, heat or by allowing it to stand for a week or longer, have not shown the slightest degree of an acquired immunity. Rats, rabbits, and dogs have been tested in this manner, but none of these animals have shown any diminution in susceptibility.

2. Animals repeatedly injected with extracts of the organs of diseased animals have acquired no resistance.

3. The blood of almost full-term fœtuses, prematurely born of highly diseased rabbits, has been tried, but without the slightest success in prevention or cure.

4. The guinea-pig being a comparatively resistant animal, its serum has been used, but it also has no immunising action.

5. Repeated inoculations of bile of diseased animals have been without preventive or curative effects, although *in vitro* bile, which is always free from hæmatozoa, rapidly destroys the hæmatozoa. Infective blood mixed with sufficient bile becomes non-infective, but confers no immunity.

6. Previous inoculations with the hæmatozoon of the ordinary rat (*T. sanguinis*) have also been valueless.

7. Sewer rats and white rats which have been repeatedly, but unsuccessfully, inoculated with the ordinary rat-hæmatozoon (*T. sanguinis*), and have been proved to be refractory to further inoculations with this hæmatozoon, have all contracted nagana when subsequently inoculated, and have died in the same time as control animals treated with an equal dose of infective blood.

8. The young born of infected mothers (dog, guinea-pigs), are no more resistant than those born of normal animals.

9. As already stated, by constant transmission through different species, the nagana hæmatozoon has shown no definite loss or gain in the intensity of virulence.

10. Of immunising sera the diphtheria antitoxin and antistreptococcus serum have been used, but, as we expected, without the slightest effect: they neither protect nor cure.

11. *Dieting.*—Rats have been fed, on the one hand, exclusively with meat, and on the other, with green vegetables; in neither case has any increased resistance or prolongation of life resulted from this alteration in diet.

12. Excision of the lymphatic glands immediately after inoculation or after they have begun to show enlargement has been of no avail.

13. Feeding with hæmatozoa also conveys no immunity.

IX. *Allied Hæmatozoa.*

1. *Trypanosoma Sanguinis.*

As already mentioned, in sewer rats (*Mus decumanus*) a trypanosoma may be found in a certain percentage of individuals. This hæmatozoon is distinct from that of nagana morphologically, and also as regards its pathogenic effects. Thus (1) the *T. sanguinis* has not been communicated to the dog, cat, rabbit, or mouse, even when large quantities of blood were used for inoculation. (2) In some guinea-pigs it has been found in very small numbers in the blood for two or three consecutive days, usually from about the fifth day after injection; but there has been no persistence. (3) In white rats many unsuccessful inoculations have been made with the *T. sanguinis*, even with considerable doses, and it appears that the minimal infective dose is larger than with the nagana *Trypanosoma*. (4) White rats may lose the hæmatozoon after they have been proved to have been successfully inoculated with the *T. sanguinis*. (5) Some of those which had had, and then lost, the parasite proved refractory on re-inoculation. Black and white (piebald) rats have never been successfully inoculated in our experience. (6) No rat has been successfully inoculated with the *T. sanguinis*, except at the first attempt. (7) We have not been able to recognise any illness after successful inoculations with *T. sanguinis*. Any pathogenic effect it may have must be slight. Infected rats remained alive for months; we have not observed any instance where death was to be ascribed to the hæmatozoon.

R. Koch* examined rats in Dar-es-salam, and also recognised differences between the hæmatozoon of the local rats and the *Trypanosoma* of the Tsetse disease. Whether the *Trypanosoma* occurring in the African rats examined by him is identical with that occurring in our English rats we cannot decide, although from the brief description given by Koch they certainly closely resemble each other. Nor did Koch succeed in infecting animals other than rats with the African rat Trypanosoma. He therefore showed that the parasites which occur in the blood of rats (in Dar-es-salam) do not stand in any relation whatever to the Tsetse disease of horses and cattle. In this connexion the observations of Bruce are of great value, because they prove that in big game the Tsetse parasite certainly does occur without apparently causing acute, fatal, or even obvious disease; in the same manner, this parasite may sojourn in the body of the guinea-pig for weeks and months without interfering with the health and development of the animal for a long time. Furthermore, Bruce (in an unpublished report) has made similar observations on the South African goat and sheep; he shows that in these animals the disease runs an extremely protracted course, and lasts for months.

* 'Reiseberichte,' 1898, pp. 70 and 71.

2. *Trypanosoma of Surra* (Trypanosoma Evansi).

Koch announces that the disease known as Surra in India and the Tsetse disease of Africa are produced by the same parasite. His reasons for this assumption are apparently not based on extensive personal comparative observation.* Lingard, working in areas naturally infected with surra, has not clearly distinguished between the Trypanosoma of surra and that occurring in rats in India. His account of surra as it occurs in rabbits, and guinea-pigs, and rats is suggestive of this disease being closely similar to nagana, but it is impossible for us to pretend to give any final decision in the matter. To illustrate the confusion in which Lingard has placed the matter, it may be pointed out that he writes that cows, horses, monkeys, and field rats are susceptible to inoculation with the ordinary Indian rat hæmatozoon, but that rabbits, guinea-pigs, dogs, cats, and donkeys are insusceptible, but he adds that the latter animals are susceptible after this rat hæmatozoon has been passed through the horse. He also asserts that surra can be produced in horses by feeding on the excrement of rats. These observations are calculated to create a certain amount of suspicion, particularly when it is remembered that Vandyke Carter failed to infect horses with the Indian rat Trypanosoma.† For the present therefore the question must be left open till an opportunity arises of studying the various parasites at the same time side by side, both in their morphology and pathogeny.

3. *Trypanosoma of Rouget.*‡

Rouget describes Trypanosoma disease in Algeria, which apparently is identical with the disease described by Bruce. Judging from the drawings and descriptions, his parasite agrees with that of nagana. He will not commit himself as to the identity of his parasite with that of surra. White rats and mice, rabbits, and dogs exhibited a considerable susceptibility, while guinea-pigs, he says, were refractory (possibly because he did not recognise the chronicity of the disease). Sewer rats were not always susceptible, while some showed a relative immunity. It seems, however, that he did not re-inoculate them so as to test their immunity again. His description of the symptoms and anatomical appearances in mice, rats, rabbits, and dogs agrees almost exactly with our own observations. He claims to have succeeded in immunising a number of mice by injecting them with the serum of an infected rabbit previous to inoculating the Trypanosoma; six mice survived altogether,§

* 'Reiseberichte,' p. 66.

† 'Scientific Memoirs of Med. Officers of the Army of India,' 1887, Part III, p. 56.

‡ 'Annales de l'Institut Pasteur,' 1896, vol. 10, p. 716.

§ It does not appear whether they were again tested.

while others lived for 17—23 days. As a curative agent this serum was useless; guinea-pigs serum also had no preventive action.

X. *Biology of the Nagana Hæmatozoon.*

So far our knowledge of the nagana parasite, as that of other Trypanosomas, is very incomplete. Rouget* has failed to find forms corresponding to those described by Danilevsky in birds and by Shalashnikov in rats, and Lewis and others have been equally unsuccessful, while it is difficult to follow Lingard in his description of young forms. We have not succeeded in tracing a life history, and we are still in search of developmental forms, a task which at present occupies our special attention.

1. Most commonly in blood, &c., drawn from infected animals the forms described by Bruce are found. They are generally in active movement, and can sometimes be observed in locomotion with their flagellated end forwards, as Lewis described in the case of other hæmatozoa; in many cases they do not change their position by free swimming, but tend to fix themselves by one or other end to the coverslip or to corpuscles or cells in the specimen; they then exhibit more or less rapid oscillations, and may change their position by apparently drawing or pushing themselves in one direction or the other. Meanwhile the vibratile membrane waves rapidly and the protoplasmic body alters in shape, becoming thicker and shorter or thinner and longer; in the case of the English rat hæmatozoon free swimming is the rule; changes in the shape of the body like those of the nagana organism are not observed.

2. The nagana parasites vary considerably both in size and form; they may be long and pointed or blunt-ended and somewhat stouter; some individuals are short and thick with a short flagellum, their protoplasm being crowded with rounded granules. Still larger forms possessing more than one vibratile membrane are sometimes, though rarely, met with.

3. Especially in specimens taken from lymphatic glands, but also in specimens obtained from the blood, &c., there is a clear vacuole at the thick end; this does not become stained with staining reagents; it varies much in size in the different individuals, but we have watched in vain for evidence that it is of a contractile nature.

4. By means of hæmalum or hæmatoxylin a nuclear body can be demonstrated in the middle of the parasite; it is usually oval, but may be more saddle-shaped. The protoplasm also contains a number of granules which stain with basophil reaction (methylene-blue, thionin, &c.); these are somewhat variable in number, being fewer in specimens in which the protoplasm is more refractive, *e.g.*, from lymphatic gland.

* *Loc. cit.*, pp. 722 and 723.

These granules are distributed irregularly throughout the body of the parasite; they do not occur in the membrane or the flagellum. The chromatin spot situated close to the non-flagellated end in the *T. sanguinis* is not defined in the Nagana *Trypanosoma*. The *T. sanguinis* also does not stain at all readily even by basic aniline dyes (dahlia, fuchsin, &c.), whilst that of Tsetse disease is more readily coloured by these reagents.

5. Examples joined by the poles opposite the flagellum are common at times, but although they suggest perhaps conjugation, we have no evidence that this process does really occur. After prolonged observation no further changes have been noticed in these joined individuals.

In freshly drawn blood, or in the lymph of lymphatic glands, or in pleural and peritoneal fluid, when the hæmatozoa are common, tangles made up of numerous hæmatozoa have been observed; this has been already described by Lewis. The hæmatozoa often converge with their non-flagellated ends towards one common point. In lymphatic glands, before the hæmatozoa are found in the blood, such tangles may be present in great numbers.

6. Forms consisting apparently of two individuals joined side by side by their bodies, the flagella being free, have been observed on rare occasions; from prolonged observation in the living state we have no reason to suppose that these are undergoing longitudinal fission. Especially in kept blood, &c., many of the hæmatozoa may present a rhomboid outline whilst still motile.

In drawn blood or serous fluids the hæmatozoa eventually become motionless; this may occur rapidly, for instance in twenty minutes, but generally some motile specimens can be found after two to three days—sometimes, indeed, after as long as five or six. When they are abundant, the tangles above noted are formed. Then the bodies of the organisms become rounded, the nuclear bodies becoming more distinct and readily stainable (hæmatoxylin or basic aniline dyes). At the same time the vibratile membrane or fin and the flagellum separate, forming a rather rigid filament. Eventually (generally after three to four days) masses of spherules alone remain; these apparently correspond to the nuclear bodies. In numerous experiments these structures have uniformly proved to be non-infective, and it must therefore be inferred that if they are not simply degeneration products, they require other conditions for their further development than those that are found in warm-blooded animals. All individuals, however, do not pass through these changes; some, or even all, may simply become non-motile, stiff, and pale, at the same time retaining their form. In numerous attempts at cultivation in normal blood, similar phenomena are observed without any evidence of multiplication. Within a corpse, the blood and organs become non-infective in about twenty-four hours, the changes in the hæmatozoa being similar to those just described.

Exposure for several hours in an atmosphere of hydrogen or CO_2 has no appreciable effect on the motility of hæmatozoa in blood-free serous fluids. The hæmatozoa are also fully active in hosts killed by ether, chloroform, or coal-gas.

7. Oval forms, smaller than the ordinary hæmatozoa, with (or without) a short flagellum, and often with a "beak" at the opposite pole, have been observed in the organs, but rarely in circulating blood.

8. Small rounded or ovoid bodies, about 1—2 μ in diameter, hyaline, sometimes with a refringent chromatin spot or bipolar spots, or irregular (? amœba-like) bodies, also sometimes with a chromatin spot and of the same size or rather larger, have been observed, especially in the lymphatic glands and bone-marrow, or spleen. It is possible that these are early stages in the development of the hæmatozoon. No forms have been seen at any time within the red blood corpuscles.

9. Neither sporocystic nor larger distinctly amœboid forms have been observed.

As our observations on the development of the parasites are still in an incomplete condition, this short statement must suffice, and a more detailed description must be left for a future occasion, when we submit a full report upon our work.