EQUINE PIROPLASMOSIS, OR "BILIARY FEVER."

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THE disease known as equine piroplasmosis is one possessing great importance to the farming community of certain portions of Cape Colony, especially to importers, for the reason that recently imported horses sometimes develop a very acute and often fatal form of this malady. A similar disease attacks mules. The recently reported outbreak of piroplasmosis amongst donkeys in the Transvaal, if not identical, is probably very closely allied to the malady affecting horses and mules. A great mortality amongst donkeys at Lydenburg was due to piroplasmosis. Equine piroplasmosis requires further investigation, it being unknown how the disease is disseminated, although most authorities consider that the parasite is transmitted by the agency of a tick.

Nomenclature, History, Geographical Distribution, etc.

Equine piroplasmosis, commonly known throughout South Africa as biliary fever, was first observed in Natal, in 1883, by Wiltshire, who named the malady anthrax fever. Subsequently Hutcheon described it in the Cape Colony as biliary fever of the horse—the name by which it is most commonly known at this date. Hutcheon also states, "individual cases occur all over the Colony, but it is most prevalent in the Cape Peninsula, and along the East Coast to about 110 miles inland. It may occur at any season of the year, but most frequently during the summer and autumn months. It is fully as common amongst stable-fed horses as amongst those which are never inside a stable." Guglielmi (1899) described the disease in Italy, as horse

malaria, and was the first to discover endoglobular parasites in the blood of the infected animals. Rickmann (1902) described endoglobular parasites in the blood of horses that succumbed to horse sickness, and concluded that horse sickness was similar or closely allied to pernicious malaria in man. Bowhill (1904) observed *Piroplasma equi* in a horse that died of naturally acquired horse sickness at Grahamstown (see Fig. 6).

Koch (1904), in the report on the recent horse sickness experiments in Rhodesia, states that one of his young animals developed a fatal attack of biliary fever after inoculation with blood from a salted horse, and that amongst the other experimental animals he had six cases of biliary fever.

Theiler (1902), described the disease as equine malaria, owing to the parasites in specimens he sent to Laveran being classified by that authority as *Piroplasma equi.*

Edington (1901), on the other hand, states, "I am strongly of the opinion that a disease in horses in South Africa commonly described as biliary fever is in the majority of instances naturally acquired malarial horse sickness. I believe however that a true biliary fever also exists," a very inclusive statement. According to Hutcheon, "biliary fever has also been described by veterinary surgeons in Natal as the biliary form of horse sickness—but although it bears a strong resemblance to horse sickness in some points, I am convinced that it is distinct in its origin from that disease and amenable to different treatment." Hutcheon's conclusions are as far as I am able to ascertain from the literature in my possession, accepted by all recent investigators, both here and abroad, with the exception of Edington (1901), who persists in dividing horse sickness into two forms, (1) virulent form with continued fever, "in which no parasites are visible," and (2) a non-virulent form with a malarial type of fever, "and in which parasites are found." Bruce (1902), considers Edington's so-called visible parasites of accidental occurrence as far as their relation to true horse sickness is concerned. He states, "(1) the hypothetical germ of horse sickness undoubtedly does pass through a porcelain filter—the experiment has been made by Dr John McFadyean, Principal of the Veterinary College, here in London, and by H. Watkins Pitchford and myself in Natal amongst others. (2) The phase of the germ which does not, according to Edington, pass through the filter is in my opinion not the germ of horse sickness at all, but a totally different well-known parasite, the *Pyrosoma equi*, which Edington evidently

accidentally met with when working at horse sickness, and thought a phase in the life history of the parasite of horse sickness." Bruce's contention is supported by the afore-mentioned association of biliary fever parasites with true horse sickness by Koch, Rickmann, and Bowhill. It is also important to note that Theiler (1902), who studied equine malaria in a horse sickness district, mentions no co-relation between equine malaria and horse sickness, but describes the former as a distinct type of disease, thus qualifying Hutcheon's statement, "I am convinced that it is distinct in its origin." It is a tribute to Hutcheon's powers of observation, that a view promulgated a long time ago from purely clinical observation is now corroborated by scientific research, and accepted by all investigators capable of determining an issue of this nature.

Finally, Ziemann (1902) has described equine piroplasmosis as occurring in Germany, and states that a similar disease prevails in Venezuela.

Cause of the disease:
The Parasite and probable agent of transmission.

The parasite causing piroplasmosis of the horse, mule, and donkey, belongs to the haemocytozoa or endoglobular haematozoa.

Of these haemocytozoa the genus Piroplasma is the most important to veterinary surgeons as parasites of this genus cause devastating diseases amongst domesticated animals in many parts of the world. The various known species and their hosts are enumerated by Nuttall (1904).

The parasite found in the donkey in the Transvaal was discovered by Theiler (p. 316), who states "it is very closely related to if not identical with the Piroplasma found in malarial or biliary fever of the horse and mule."

By what means the parasites are transmitted to horses, mules, and donkeys, is at present unknown, but judging by analogy it seems probable that the parasites are conveyed by species of ticks, as has been amply demonstrated experimentally in the cases of bovine and canine piroplasmoses. I have usually found a great number of the common blue ticks (*Rhipicephalus decoloratus*) present on the infected animals that have come under my notice. This species of tick is known to transmit the *Piroplasma bovis* in South Africa. Theiler also considers this tick as probably responsible for the propagation of equine piroplasmosis.

The Parasite.

In prepared blood films, stained with a modified Romanowsky stain, Laveran's stain, or Azur II and Eosin, the parasites can be demonstrated without much difficulty when the examination is made in the earlier stages of the disease. In some cases the parasites are difficult to detect in the peripheral circulation. With any of the above-mentioned staining reagents the cytoplasma is stained blue, and the karyosome a bright red. The following is a description of the various forms of the parasite I have observed in Albany and adjoining coast districts.

1. *Large and small spherical forms* (sometimes of varying sizes in the same corpuscle), the latter about half the diameter of the former, the karyosome being situated at the edges of the parasite in both forms. See Figs. 1, 2, and 8.

2. *Large and small pyriform parasites*, single and in pairs.

3. *Large and small rod-like bodies*, some of them extending across the whole diameter of the corpuscle, sometimes in pairs; in one corpuscle three were observed lying parallel to each other. See Fig. 3.

4. The *Rosette* form, consisting of four bodies connected in the centre by very fine threads, each body usually containing a karyosome at the distal extremity. Some of the parasites resemble a Maltese cross, others a St Andrew's cross, and where the rosette is formed of three leaves it can be compared to the Manx coat-of-arms. In some specimens only one body has been observed to contain a red stained karyosome. The rosette form is a phase in the reproduction of the parasite which eventually divides into four, although division into two sometimes also occurs. See Figs. 4 and 5.

5. *Flagellate forms.* These bodies have been observed free (extra-corpuscular). They consist of a distinct pear-shaped head, possessing a clearly defined red stained karyosome, and a long flagellum ending in a bulbous protuberance. Flagellate forms have also been observed where the body and flagellum were situated within a blood corpuscle. In one instance the flagellum could be traced passing out of the corpuscle and terminating in a bulbous protuberance a considerable distance from the infected corpuscle. In the latter case the corpuscle measured 3μ, the length of the body of the parasite 1μ, and that of the flagellum $3{\cdot}5\mu$. See Fig. 10. In other films longer flagella were observed, but their structure was so delicate and difficult to focus, that while a faint print could be obtained from the negative, repro-

duction would not yield sufficient detail to enable the figure to be of any special interest or instructive value. See Figs. 9 and 10.

6. A great number of parasites being present in one case, some blood was drawn from the same animal under aseptic precautions into a flask containing some sterile citrate of potash solution and kept at room temperature.

Two days later, when films were prepared from this blood, the parasites, which were previously nearly all intracorpuscular and of varied shape, were now observed to be nearly all spherical or ovoid in form, extracorpuscular, and arranged in irregular masses, the karyosome being stained a bright red and the cytoplasm a light blue. See Fig. 7. The karyosome stained red up to the 4th day. Theiler (1902) observed that the parasites in incubated blood disappear as soon as the corpuscles lose their haemoglobin, and that they keep their colourability longer at room temperature. Even after being kept 12 days in an ice box he found that stained specimens showed the piroplasmata as distinctly as fresh ones, both karyosome and plasma being distinctly stained by Laveran's method; the colourability of the nucleus being an indication of its being alive. He also observed that nearly all the parasites were spherical. Similar results were not obtained with blood kept for 12 days in an incubator. The proliferation of *Piroplasma bovis* observed by Lignières (1870) in South America, was not seen by Theiler in transplantations made into fresh serum. In some experiments I made, fresh serum was added at different times to infected blood, both at room temperature and in the incubator at 29° C. up to 40° C., but no definite proliferation was observed, although a slight amoeboid movement of the parasites was noted on one or two occasions. The changes of form described by Lignières in *Piroplasma bovis*, were not observed by me in *Piroplasma equi*.

Inoculation Experiments.

Theiler (1902) failed to transmit the disease by direct inoculation, not even by the transfusion of about 1 litre of blood from the jugular vein of a sick horse into the vein of a healthy one. Koch (1904) was also unsuccessful; he states, "while fortifying our immune animals I injected intentionally on several occasions blood containing the organism of biliary fever and never succeeded in inducing an attack of the disease, but one of our young animals inoculated with 20 c.c. of blood taken from an old salted horse developed a severe and fatal attack of

biliary fever after an incubative period of nine days." Edington (1904) found that the blood obtained from donkeys previously inoculated with virulent horse sickness blood (without being severely affected) when drawn about the 10th or 11th day after inoculation and injected into clean horses was capable of setting up a fever accompanied by definitely marked remissions and intermissions. In two of these animals the blood corpuscles were found to be infected with a parasite resembling the organism present in Texas fever. In a later publication Edington states he managed to regenerate a virulent form of horse sickness from the malarial type. The disappearance of the parasites in the virulent disease, and their reappearance in the non-virulent or malarial type, as stated by Edington, seems to me highly doubtful. Spreull inoculated a sheep and a goat intravenously with negative results.

Theiler injected blood from horses suffering from equine malaria into cattle with negative results. Such blood had no effect on rabbits, dogs and guinea-pigs. Injected into the peritoneal cavity of guinea-pigs it has no effect. As I have also found, according to Nicolle and Adil-Bey, blood of cattle suffering from piroplasmosis is toxic to guinea-pigs, 1 c.c. killing these animals rapidly when injected intra-peritoneally. Guinea-pigs resist an injection of normal cattle blood in quantities up to 5 c.c.

Symptoms.

The period of incubation is unknown. Koch stated that it was nine days in one of his experimental animals. The malady occurs in an acute and a chronic form, the infected animals exhibiting the following symptoms:—

Temperature. The onset is ushered in by intense fever reaching to 104·2°—107° Fahr., in one instance I recorded 108° Fahr. In chronic cases the temperature is very fluctuating. The temperature becomes subnormal shortly before death takes place.

Prostration. In acute cases the animal hangs its head, there is increased lachrymation, disinclination to move, a stumbling gait. In the later stages there is a partial loss of power in the hind limbs, the animal becomes comatose, and death follows in a few hours.

Appetite. Sometimes the animal only picks at its food, in other cases anorexia is complete or the animal may be voracious.

Icterus and Anaemia. The mucosa of the eye, sclerotic, and other visible mucosa, also portions of the skin devoid of hair, are coloured yellow. In acute cases, the conjunctiva and nictitating membrane are

studded with numerous reddish-brown spots. This lesion was well marked amongst the transport mules during the late war on the return march from Barberton. In donkey piroplasmosis the mucosa are stated to be clean and pale, and not yellow or dirty as in the equine disease. I have also observed a blanched condition of the mucosa of the eye in this district, the diagnosis of piroplasmosis being confirmed microscopically. Anaemia is well marked in most cases, it is progressive especially in the chronic form.

Pulse. This varies according to the state of the heart, sometimes it is weak and irregular, at other times its action is tumultuous and palpitations are easily discerned.

Respiration. Accelerated at first, sometimes abdominal.

Faeces. Diarrhoea is sometimes apparent at first, in other cases the bowels are constipated, the faeces being offensive and black coloured, and covered with slime.

Urine (*haemoglobinuria*). The urine is usually high coloured, and in some of the mild cases an intense polyuria is sometimes observed. I have seen haemoglobinuria in three cases, all of the animals recovering.

The duration of the disease is variable; death sometimes occurs in 2—5 days. Sometimes the fever lasts for nine days when it ceases, the animal becoming convalescent a few days later. The longer the disease lasts the more uncertain is the prognosis.

Mortality. In my districts the death-rate has not been excessive, out of 150 cases that have passed through my hands during the last two years I know of only three deaths, and these were due to complications or terminal infections.

Treatment. A great many cases recover without any special treatment, some appear benefited by small doses of bicarbonate of soda in the food or drinking water. Hutcheon recommends the use of belladonna and ammonium chloride. A dose of calomel and raw linseed oil, followed next day by bicarbonate of soda and ammoniated quinine (where great depression is present), will usually be found sufficient in cases running an ordinary course. Where complications arise treatment requires to be modified according to the extent and character of the complications.

Pathology.

External appearances. The carcase is very much emaciated, the mucosa and subcutaneous tissue stained yellow, the muscles a brownish-

red with a yellowish tinge in certain parts. All the tissues are anaemic, but this condition is somewhat obscured by the icterus.

The blood is thin and watery; it coagulates quickly after death, the clot being small and soft. The serum exuding from blood kept in a test-tube is brownish-yellow in colour and contains a great many red blood corpuscles, which are deposited by degrees at the bottom of the tube. *Spleen:* enormously enlarged (may weigh up to 5 kilogrammes), capsule distended, pits slightly on pressure, pulp softened and of a tarry colour. *Liver:* Yellow coloured, congested, bile capillaries dilated and full of fluid. *Kidneys:* Frequently enlarged, anaemic, and cortex infiltrated. *Bladder:* contains usually normal urine except in cases associated with haemoglobinuria, when it is dark brown coloured and small petechiae are present on the mucosa of the bladder. *Lymphatic glands:* Those of the spleen, liver and kidneys are tumefied and haemorrhagic, and in the mesentery and sub-lumbar region there is a serous infiltration. *Stomach:* Usually empty, mucosa sometimes congested. *Intestines:* Mucosa pale coloured, swollen, and in some cases the seat of haemorrhagic patches. *Serous cavities:* The pleurae are stained yellow and there is sometimes a slight effusion into the peritoneum. In donkeys this lesion is well marked, the effusion being stated to appear like an acute dropsy (Dale). *Heart:* This organ is sometimes enlarged and flabby, small ecchymoses are sometimes observed on the parietal layer of the pericardium, small endocardial punctiform haemorrhages are usually present associated with a gelatinous infiltration, which also involves the valves and origin of the aorta. The cavities are usually filled with blood. *Lungs:* Usually normal in appearance, the anterior lobes are sometimes emphysematous, occasionally, when complications have arisen, broncho-pneumonic and allied septic changes are in evidence.

Immunity.

There is some evidence that South African veldt horses are more or less immune to this disease. My experience has led me to believe that this immunity depends upon the animal being reared in an infected area. I have seen the disease in Cape bred horses in the Albany district; imported horses are however the greatest sufferers. I am not certain that one attack produces immunity, but I am inclined to believe it does.

Secondary or terminal infections.

A catarrhal condition of the bronchi, hypostatic pneumonia, etc. arising in the course of the disease render the animals prone to secondary infections. Germs or parasites bearing no direct causal relation to the primary disease find conditions favourable for their development, and give rise to lung complications, which have no connection with the original disease *per se*. Secondary infections may also take place through the digestive tract. Secondary infections may be local or general, and consequently may give rise to conditions of great complexity, this especially when the piroplasmosis attacks an animal primarily suffering from another perhaps chronic disease which may show renewed activity.

Theiler, speaking of the sequelae of equine piroplasmosis, states, "it is exceedingly rare to find that only the Piroplasma is present in a horse suffering from or dying of biliary fever. In nearly every case I found a bacterium which was present sometimes in the blood and always in the spleen." The organism is described as a cocco-bacillus, showing bi-polar staining. It is not unlikely from the description given that the complication was a Pasteurella infection. Dale, in piroplasmosis of the donkey, observed different kinds of pneumonia, principally of a septic nature, also intestinal lesions which in some cases hastened death.

As already mentioned, I have observed a case of typical acute horse sickness (naturally acquired) in which *Piroplasma equi* was present. See Fig. 6. Again, in a case of equine piroplasmosis, I observed Filariae together with the *Piroplasma equi* in the blood. The Nematode may be the *Filaria sanguinis equi* of Sonsino or larval forms of the Filaria which causes the blood-sweat disease, "Haemathydosis" (see Fig. 8). I have similarly met with a Spirochaete in the liver of an ox that died from ordinary coast red-water. (See Fig. 15.) Theiler has observed Spirochaetes in cattle, also in the blood of a sheep and horse, in the Transvaal.

The photomicrographs which accompany this paper were made by me from specimens I have prepared and stained, and represent a selection from an extensive series. A flagellate body seen in a case of Rhodesian fever of cattle was figured in an earlier paper in this *Journal* (Vol. IV. p. 218), where flagellate bodies of similar character in the case of *Piroplasma canis* are also described and figured.

EXPLANATION OF PLATES I TO III.

Piroplasma equi.

Plate I. Figs. 1, 2 and 3. Blood film preparations. Different forms of intracorpuscular parasites. × 1200.

Figs. 4, 5. The same as above. Forms of parasites resembling St Andrew's and Maltese crosses respectively. × 1500.

Plate II. Fig. 6. Bigeminate form of parasite seen in blood of a horse suffering from horse sickness and piroplasmosis infections combined. × 1000.

Fig. 7. Free parasites seen in citrated blood kept two days at room temperature. × 1000.

Fig. 8. Blood of a horse suffering from mixed infection: Piroplasmosis and Filariasis, three Filariae being visible in the field. × 1000.

Figs. 9, 10. Flagellate forms. × 1500.

Piroplasma canis, Piroplasma bovis, Spirochaete.

Plate III. Fig. 11. Piroplasma canis in section of dog's kidney. The parasites intracorpuscular. × 1200.

Fig. 12. P. canis. Rosette-like grouping of intracorpuscular parasites. × 1800.

Fig. 13. Piroplasma bovis in blood of cattle. × 1500.

Fig. 14. P. bovis in spleen smear. Flagellate body. × 1200.

Fig. 15. Spirochaete in liver of cow dead of red-water. × 1200.

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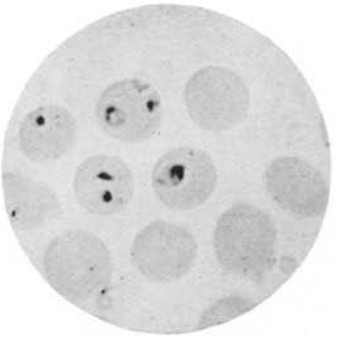
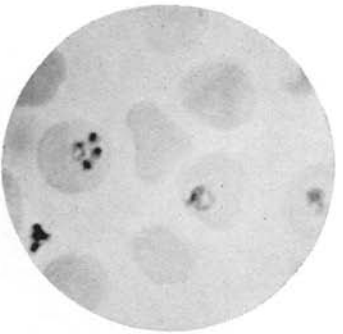


Fig. 1.



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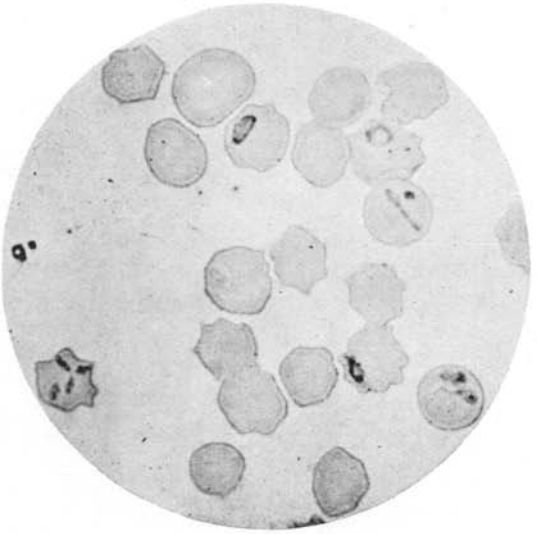

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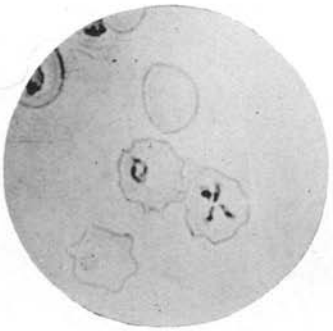

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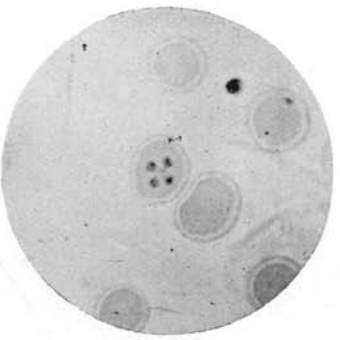

Fig. 5.

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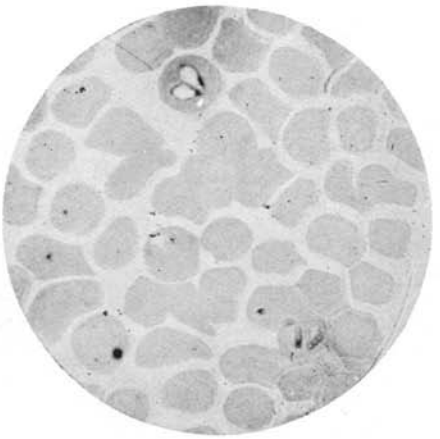

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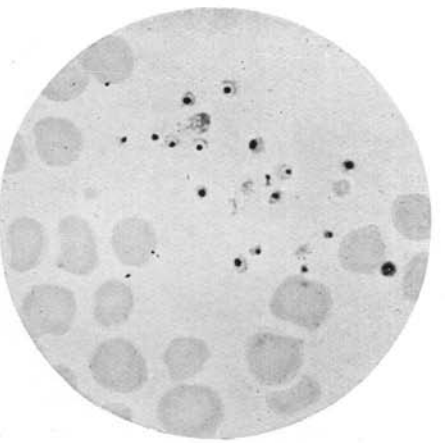

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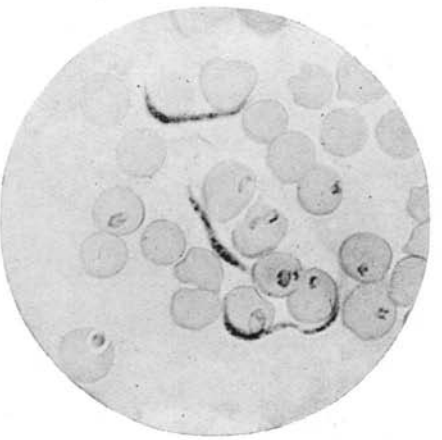

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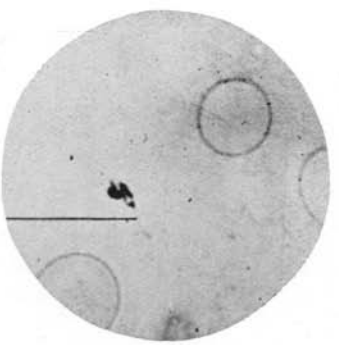

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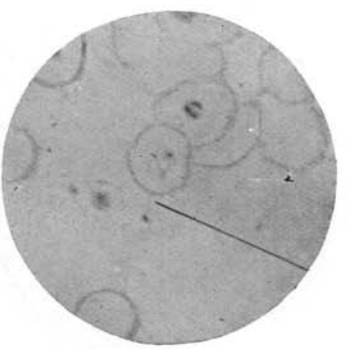

Fig. 10.

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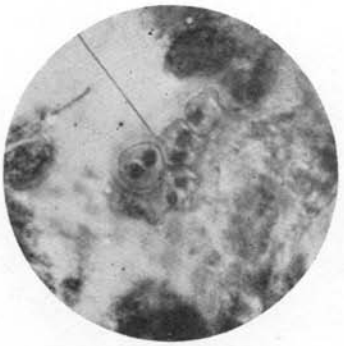

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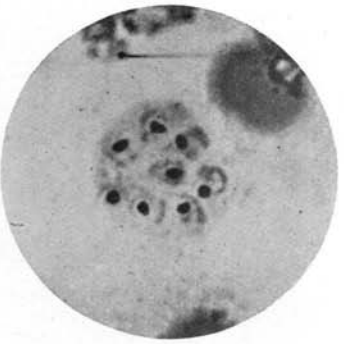

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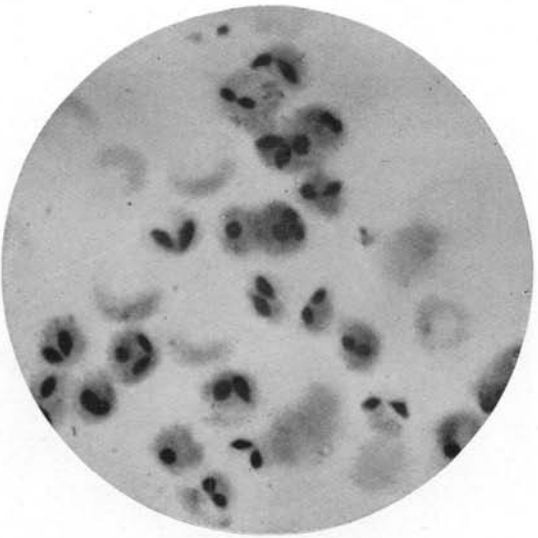

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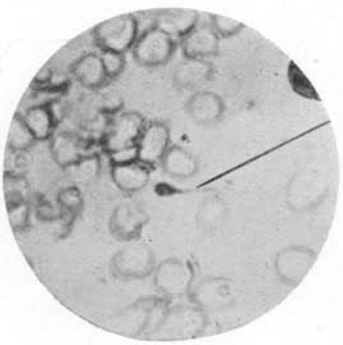

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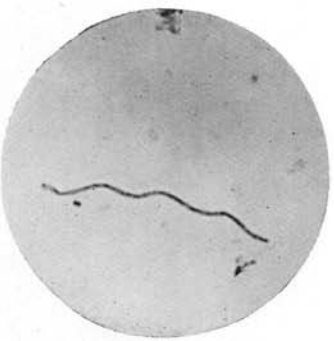

Fig. 15.

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