

T H E

Journal of Medical Research.

(NEW SERIES, VOLUME XVIII.)

VOL. XXIII., No. 3. NOVEMBER, 1910. Whole No. 123.

TRYPANOPLASMA RANÆ N. SP. AND ITS LIFE-CYCLE IN CULTURES.*

ERNEST LINWOOD WALKER.

(From the Department of Comparative Pathology, Harvard University
Medical School.)

(Plate X.)

The hemoflagellates include at least two well defined genera: *Trypanosoma* Gruby, 1843, and *Trypanoplasma* Laveran and Mesnil, 1901. The Trypanoplasmata differ morphologically from the Trypanosomata chiefly in possessing, in addition to the flagellum that is attached to the undulating membrane, a flagellum that extends free from the kintonucleus end of the cell. The Trypanoplasmata are parasitic for the most part in the blood of the lower vertebrates, the fishes. Léger (1904) found one species (*Trypanoplasma intestinalis*), however, in the intestinal tract of a fish; and Friedrich (1909) has recently described under the name *Trypanoplasma helicis* a flagellate from the receptaculum seminis of a snail, which appears from the investigations of Crawley (1909) to be the type species of this genus, first described by Leidy in 1846 under the name *Cryptobia helicis*. If Crawley be correct the valid name of this genus would be, according to the rules of zoölogical nomenclature, not *Trypanoplasma* but *Cryptobia*.

Trypanoplasma ranæ was obtained from a frog (*Rana palustris* Leconte) in a culture made post-mortem from the intestinal contents on Musgrave and Clegg's medium. In cultures from a considerable number of frogs it has been

* Received for publication July 28, 1910.

found only once. This flagellate has been isolated in "pure mixed culture" with bacteria and studied in cultures on Petri plates and on cover-glasses (Walker 1908a and 1909b). Preparations for staining were made by streaking clean cover-glasses, that had been flamed, with a platinum needle charged with a culture of the organism, and floating the preparations before they were dry upon the surface of Zenker's fluid. After five minutes' fixation the preparations were floated upon the surface of distilled water until the fixing fluid was thoroughly washed out of them. They were then stained with Giemsa's stain.

Trypanoplasma ranæ possesses the characters of the genus *Trypanoplasma* as given by Laveran and Mesnil (1901, amended 1904): Flagellates with elongated body, presenting laterally (dorsally) an undulating membrane, the thickened edge of which is prolonged posteriorly as a flagellum and turns back anteriorly to reach a mass which is as large as, and which has up to a certain point the same structure as, the nucleus. An anterior free flagellum has its origin in the same mass. Multiplication is probably by binary, equal, longitudinal division.

Specifically *Trypanoplasma ranæ* (Fig. 1) is an elongated, more or less flattened cell, twelve to eighteen micra long and two to two and five-tenths micra wide. It tapers to a blunt point anteriorly and to an acute point posteriorly. Whatever be the orientation in the Trypanosomata, both movements and development of *Trypanoplasma ranæ* indicate that the end in which is located the kinetonucleus and which bears the free flagellum is the anterior end. The free flagellum arises near the dorsal edge of the anterior end of the cell and extends forward. The attached flagellum arises dorsal to but near the free flagellum, bends backward, is attached to the undulating membrane and extends free beyond the posterior end of the cell. The undulating membrane varies considerably at different stages of development of the trypanoplasm, but it is never very wide or much frilled. The trophonucleus is round or oval in outline, about one and five-tenths micra in diameter, and is located near

the middle of the cell. The kinetonucleus is an elongated mass located at the dorsal border of the anterior end of the cell. It does not have so definite an outline nor does it stain so intensely as the trophonucleus. With Giemsa's stain it is colored pink while the trophonucleus is colored deep purple. In properly fixed and stained individuals it can be made out that the two flagella arise from two granules situated side by side anterior to the kinetonucleus. The free flagellum extends straight to the more ventrally placed granule, while the attached flagellum enters the anterior end of the cell and bends backward to reach the dorsal granule. The position and structure of the kinetonucleus and the relation of it to the flagella correspond in general with that of *Trypanoplasma borreli* as described by Keysselitz (1906) and with *Trypanoplasma helicis* as described by Friedrich (1909). A small noncontractile vacuole is located just posterior and ventral to the kinetonucleus. At the blunt anterior end of the trypanoplasma a minute notch is usually discernible ventral to the free flagellum which may represent a cytostome; for the flagellate is phagocytic in its feeding habits, ingesting living bacteria which are digested within its cytoplasm. This protozoon presents, therefore, an interesting transition, having the habitat and the feeding habits of the intestinal flagellates and the morphology of the hemoflagellates. The movements of this trypanoplasma are active but of an aimless wriggling character.

Trypanoplasma ranæ, apart from its significance in the phylogeny of the hemoflagellates, is of interest particularly for the light that it throws on the life-cycle of the hemoflagellates. It has been found to be a particularly suitable species for the study of these processes. Not only do the cultures of this trypanoplasma show most of the forms that have been described in both the intermediary and the definitive hosts of other trypanoplasms and trypanosomes; but, since its life history is not complicated by an alternative host and since it is readily cultivable on a relatively simple medium, it has been possible to follow the evolution and fate of these forms with a considerable degree of certainty.

In 1904 Schaudinn published an account of an elaborate life-cycle of *Trypanosoma noctuæ*, which lives in the blood of an owl (*Athene noctua*), consisting of an alternation of generations and of hosts somewhat similar to that of the malarial parasites. From the indifferent or asexual trypanosome in the blood of the owl are developed macrogametes and microgametocytes which penetrate the red blood corpuscles of the host and constitute the *Halteridium* stage of the parasite. When the blood containing these sexual forms of the trypanosome are taken into the stomach of the mosquito (*Culex pipiens*) the gametes are set free from the remains of the red corpuscles that enclose them, the macrogamete undergoes changes preparatory to fertilization, the microgametocyte gives rise by multiple division to eight microgametes and these copulate with the macrogametes to form the oökinet. From the oökinet are developed through division, asexual, male and female trypanosomes. Periods of activity and multiplication of the trypanosomes when the gut of the mosquito is filled with blood, alternate with periods of rest during which the trypanosomes are attached to the intestinal epithelium when the gut of the mosquito is empty. If the mosquito be starved for a time the asexual and male trypanosomes may die off, while the female trypanosomes persist and reproduce parthenogenetically when the gut of the mosquito is again filled with blood. Only after the mosquito has fed on blood three times do the trypanosomes reach that part of the intestine, the curvature of Basili in the colon, where the nature of the epithelium is such that they are able to penetrate the wall and enter the circulation, by which some of them are carried to the lacunoma around the pumping organ. Here the trypanosomes collect and by injuring the tunica elastico-muscularis, which is very delicate here, pass into the pharynx. They are then injected into the blood of the fresh vertebrate host during the evacuation of the pumping reservoirs.

The female form of *Trypanosoma noctuæ* is distinguished from the indifferent trypanosome, according to Schaudinn, by its plumper figure, by a deeper staining of its cytoplasm,

which is loaded with reserve food, and by a feebler development of its flagellar apparatus, the kintonucleus being smaller and the flagellum shorter than in the asexual form. The movements of this form are sluggish and the trypanosome ordinarily comes to rest attached to the intestinal epithelium of the mosquito. The male trypanosome, on the other hand, is distinguished by its smaller size, slenderer figure, a hyaline cytoplasm, and by a strong development of its flagellar apparatus, the kintonucleus being relatively larger and the flagellum longer than in the asexual form. The movements of this form are especially active. The oökinet, resulting from the copulation of the male with the female trypanosome, is an elongated, flagella-less, uninucleate cell, capable of changing its form and of executing gregarine-like movements.

Schaudinn's life-cycle of *Trypanosoma noctuæ*, although discredited by Novy and MacNeal (1904) and other investigators, has been confirmed by the Sergents (1905) and has had a profound influence on the subsequent investigations of the life-cycles of the hemoflagellates.

Prowazek (1905) and Baldry (1909) have described a sexual process in *Trypanosoma lewisi* that takes place in the mid-gut of the louse (*Hematopinus spinulosus*) of the rat, which differs from that of *Trypanosoma noctuæ* only in that the gametes have no intracorpuseular stage in the blood of the vertebrate host and that the products of the sexual generation do not leave the lumen of the intestine, but wander forward into the proboscis of the blood-sucking invertebrate host. According to Prowazek the gametes of *Trypanosoma lewisi* are differentiated only after the parasites have been taken with the blood into the mid-gut of the louse, and the differentiation is accompanied by reduction divisions of the trophonucleus and the kintonucleus of both male and female gamete. The ripe gametes do not show so pronounced dimorphism as is described by Schaudinn in *Trypanosoma noctuæ*. The male is, however, smaller and slenderer, its trophonucleus assumes the form of a long band which at a certain stage is spirally twisted, and its cytoplasm

stains a peculiar sky-blue with Giemsa's stain. In the copulation of the male with the female the kintonuclei fuse first, followed by a union of the cell bodies and the trophonuclei, while the flagella degenerate. The product of the copulation is an elongated, flagella-less cell, having only one nucleus, and is designated the oökinet. The further development of the oökinet was not followed. Baldry describes the female form of *Trypanosoma lewisi* as ordinarily large, having a round trophonucleus containing a distinct karyosome, a similar but smaller kintonucleus, a cytoplasm containing inclusions and staining deeply and a flagellum stretched close to the body, somewhat indistinct and short, the male form as equal in length to the female but much more slender, having the long trophonucleus described by Prowazek, a thick kintonucleus and a very distinct flagellum of which the free end is very long. According to this author only the nucleus of the male fuses with that of the female gamete, the rest of the male gamete degenerating. From the oökinet are developed *Crithidia*-like forms which by repeated division become smaller and smaller. Infection of other rats is supposed to take place with these small forms.

Similar sexual processes have been described in part in *Trypanosoma barbartulæ* (Léger, 1904), *Trypanosoma brucei*, (Koch, 1905, and Stuhlmann, 1907), *Trypanosoma dimorphon* (Hindl, 1909), *Trypanosoma gambiense* (Minchin, 1908, and Klein, 1909), and in *Schizotrypanum cruzi* n. gen., n. sp. (Chagas, 1909).

In the Trypanoplasmata Keysselitz (1906) has described a sexual process of *Trypanoplasma borreli* in the intestine of the leech (*Piscicola geometra*). A differentiation of gametes from the indifferent trypanoplasms takes place in the blood of the fish. These gametes are characterized by their great richness in plasmatic substance that is concentrated in the posterior half of the body, which is consequently increased in breadth and which stains dark blue with Romanowsky stains. Male and female can be distinguished among the gametes, although the difference in size between them is less marked than in most trypanosomes. The male form

possesses a much larger kintonucleus and a relatively smaller trophonucleus. The female form, on the other hand, has kintonucleus poor in chromatin and a relatively large trophonucleus. The male and female trypanoplasms copulate in the mid-gut of the leech. From the copula are derived by division indifferent, male and female trypanoplasms as in the case of *Trypanosoma noctuæ*. There male and female forms show more distinctly than do the corresponding forms developed in the blood of the fish the characteristic differences in shape, size and nuclear structure, described by Schaudinn. They soon disappear from the intestine of the leech, and the indifferent forms that persist serve to infect other fishes.

In the cultures of *Trypanoplasma ranæ* Schaudinn's three types are well differentiated. The indifferent or asexual trypanoplasma (Plate X., Fig. 1) possesses characters more or less intermediate between the male and female forms about to be described, and is the typical trypanoplasma which has served for the description of the species. The female trypanoplasma (Fig. 2) has a broad plump body loaded with more or less granular reserve food and staining dark blue with Giemsa's stain, a large trophonucleus rich in chromatin and staining deep purple, and a feebly developed flagellar apparatus, the kintonucleus being relatively small, the flagella short and slender and undulating membrane closely applied to the obese body. The movements of this form are slow and clumsy. The male trypanoplasma (Fig. 3) on the other hand has a long slender body with a hyaline cytoplasm that stains feebly with Giemsa's stain, a small trophonucleus, and a strongly developed flagellar apparatus, the kintonucleus being relatively large, the undulating membrane distinct and the flagella, especially the anterior one, long and stout. The movements of this form are very active.

What appears to be copulation of the trypanoplasms is very abundant at certain times in the cultures. The two trypanoplasms are seen to approach one another and become attached by their anterior ends, as described by Prowazek (1905) in the copulation of *Trypanosoma lewisi*, but

continue in active motion (Fig. 4). The two gametes later become entwined and exhibit writhing and revolving movements. They finally become more or less rounded, cease all movements except agitation within their cytoplasm, and appear to be in the process of fusion (Fig. 5). The ultimate product of copulation, the oökinet, is represented in these cultures by a large oval or pyriform cell having neither undulating membrane nor flagella and only a single nucleus (Fig. 6).

These forms that have been described in the cultures of *Trypanoplasma ranæ* correspond morphologically with the forms of other trypanoplasms and trypanosomes that have been interpreted male, female, and oökinet forms and as stages of copulation by Schaudinn, Prowazek, Keysselitz, and other authors. And since such forms constitute the evidence of the sexual process that has been described in these hemoflagellates, one would be justified on this same evidence, I believe, in concluding that there exists in *Trypanoplasma ranæ* a similar sexual process which does not require an alternative host for its development.

A more careful study of the growth cycle of the cultures of this trypanoplasma leads me, however, to an entirely different conclusion. When *Trypanoplasma ranæ* is transplanted to fresh culture medium there takes place, in consequence of the abundant food supply, an active growth of the trypanoplasms which is preparatory and is at first disproportionate to multiplication by fission. As a result of this the culture at the end of twenty-four hours shows almost exclusively large obese trypanoplasms loaded with reserve food and having the other characteristics of the so-called female gametes. After forty-eight hours longitudinal fission begins to catch up with the growth of the trypanoplasms and there appear in the culture, first, long forms of medium thickness, which perhaps correspond with the long forms of *Trypanosoma brucei* described by Stuhlmann (1907) in the gut of tsetse flies and the long forms of *Trypanosoma gambiense* described by Minchin (1908) in the gut of *Glossina palpalis* on the third day after infection, and later, typical

indifferent or asexual trypanoplasms. After four to seven days, in consequence of the constantly increasing concentration of the products of protozoan and bacterial growth in the culture, the growth of the trypanoplasms becomes retarded, while multiplication by fission appears to be carried, as it were, by its momentum ahead of growth, giving rise to slender forms free from reserve food and having the other characteristics of the so-called male gametes. Finally the products of growth become so concentrated in the culture that they inhibit both growth and multiplication. The trypanoplasm then bends upon itself ventrally and the cytoplasm of the two opposed halves fuse, forming a pyriform cell, from the more pointed pole of which the two flagella appear to arise as in flagellates of the genus *Bodo*. A more careful examination shows, however, that the posterior flagellum has maintained its attachment to the undulating membrane, which forms a frill about the circumference of the rounded-up trypanoplasm, just as is the case in the rounded forms of *Trypanosoma lewisi* described by Breinl and Hindl (1909), and its free end appears to arise beside the anterior flagellum. The flagella are finally cast off, the undulating membrane becomes fused with the body of the cell, the kinetonucleus unites with the trophonucleus, and we have a flagella-less, uninucleate cell, the so-called oökinet. This stage in *Trypanoplasma ranæ* represents reductions preparatory to encystment. The cell becomes still further condensed, takes a rounded form, and secretes a resistant wall about itself, within which the trypanoplasm rests dormant until transplanted to fresh culture medium.

The growth of the cultures of *Trypanoplasma ranæ* may therefore be said to be cyclical, in that there is a definite sequence of forms developed in the cultures, a return to the initial form at each transplant of the culture, and a repetition of the forms in successive cultures. This cycle appears to be purely vegetative, and to be due to the progressive decrease in food and the concomitant increase in the products of growth in the cultures, which cause fluctuations in the balance between growth and fission of the trypanoplasms. The

so-called female and male forms appear to represent the two extremes, of which the indifferent form is the mean, in this fluctuating balance between growth and fission, while the oökinet represents the cessation of both vital processes and regressive changes preparatory to encystment.

A careful and extended study of the so-called copulation in the cultures of *Trypanoplasma ranæ* has failed to convince me that a sexual process is involved. The "copulation" of the trypanoplasms may occur at any time during the growth of the culture, but it is more prevalent in the early periods of growth. It is not limited to the union of a "male" with a "female" trypanoplasma, but may take place between "male," "female," or "indifferent" forms indiscriminately and in any combination, and it is most apt to occur between two "female" trypanoplasms. Moreover, the trypanoplasms frequently aggregate into larger groups composed of several individuals, and the process is, as we shall see, largely influenced by external conditions. Many pairs of "copulating" trypanoplasms have been kept under constant observation in cultures on cover-glasses, and it has been found that, although the union of the two individuals might appear for a time to be progressive, in no case did actual fusion take place. The process appears to be a spontaneous agglutination rather than a sexual act, a phenomenon common in the protozoa and which has undoubtedly been mistaken for copulation or conjugation. Such forms as are represented in Figure 5, in which there appears to be an actual continuity of the cytoplasm of the two trypanoplasms, and the quadri-flagellate forms sometimes found in stained preparations represent, in all probability, stages of fission. These stages of fission, taken with the stages of agglutination, together make up a picture that might readily be interpreted as copulation.

If the trypanoplasms be subjected to unusual conditions, as occurs when the culture is allowed to dry out, is transplanted into a medium of a different temperature, reaction, or tonicity, or is transferred to the microscope slide, there is an increased tendency for the development of obese and

flagella-less forms and for the appearance of agglutination among the trypanoplasms. The forms appearing under these conditions are not developmental, but represent reactions to the unfavorable conditions. The obese form results from a shortening and broadening of the trypanoplasm due to the change in osmotic tension or to a contraction of its myomeres. The agglutination of the trypanoplasms is generally progressive and results in an aggregation into groups composed of several individuals. The process appears to be connected with the encystment of the trypanoplasms which takes place in clumps. The rounded flagella-less forms, as in the normal growth cycle, represent the regressive changes preparatory to encystment.

The question naturally arises, how far may forms similar to these that have been described in *Trypanoplasma ranæ*, and which have been interpreted as gametes, stages of copulation and oökinets in other trypanoplasms and trypanosomes, be accounted for by vegetative changes or reactions to unfavorable conditions. These forms have been observed for the most part in the intestinal tract of the blood-sucking invertebrate hosts. The multiplication of the hemoflagellates, which Schaudinn, Gray and Tullock, Koch, Stuhlmann, Léger, Keysseltz, Minchin and other authors have found to take place in the gut of the invertebrate host, must be associated with the multiplication of intestinal bacteria and under conditions of a decreasing food supply and an increasing concentration of the products of bacterial and protozoan growth similar to the conditions in the cultures of *Trypanoplasma ranæ* on artificial media. It seems quite possible, therefore, that these conditions might produce a vegetative cycle of the trypanosomes in the cultures *in vivo* similar to that of *Trypanoplasma ranæ* in the cultures on artificial media. In the transfer from the blood of the vertebrate to the gut of the invertebrate host the trypanosomes must be subjected to changes in temperature, reaction and tonicity, to the action of the digestive juices and to the possible repeated feeding of the invertebrate host; conditions which, we have seen, increase the tendency for the development of

obese and flagella-less forms and for the appearance of agglutination among the trypanoplasms in the cultures, but which at the same time interrupt the normal growth cycle of the cultures, so that one would not find the sharp separation of the different types of trypanosomes corresponding to the different periods of growth of the cultures *in vivo* that one does find in the cultures of *Trypanoplasma ranæ* on artificial media.

In certain of the cultures of *Trypanoplasma ranæ* there appear from the third to the seventh day thin, "shadowy" trypanoplasms, having peculiar disjointed wriggling movements, which contain two spheroidal, slightly refractive bodies that form distinct protuberances in the body of the trypanoplasma. With Giemsa's stain the cytoplasm of these forms stain slightly or not at all, while the spheroidal bodies are stained deep purple (Fig. 7). Such forms might be interpreted as early stages of fission did not some of them show evidence of further development in another direction. In certain of them the two chromatin bodies appear to be condensed and surrounded by a layer of cytoplasm (Fig. 8). Others have rounded up and have lost their flagella. In one or two cases in unstained preparations rounded forms have been seen that contained four instead of two of the spore-like bodies. These bodies appear to be set free by a disintegration of the trypanoplasms, sometimes in the elongated or curled up (Fig. 8) as well as in the rounded condition. The free bodies are minute hyaline spherules about one micron in diameter. In stained preparations the smaller of these bodies are seen to be uninucleate; slightly larger ones (1.8 microns) are distinctly binucleate, having a larger and a smaller chromatin granule eccentrically located in the body; still larger ones (2.3 microns) are provided with two flagella (Fig. 9), one extending free and the other attached to the periphery of the spherule, similar to the attachment of the flagella in the rounded adult trypanoplasms. These flagellated spherules probably elongate and become small trypanoplasms. On account of the relative infrequency of these forms, the minute size of the bodies,

and the fact that their development is largely intracellular and cannot be followed in the living trypanoplasm but interpreted from stained preparations, I have not yet succeeded in working out the details of this reproductive process.

Of the somewhat voluminous literature on the trypanosomes two papers especially interest us in this connection. The first of these is a paper by Salvin-Moore and Breinl (1907) who describe the development of what they call resting bodies in *Trypanosoma gambiense*. In the development of these bodies there is first an interaction of the kinetonucleus with the trophonucleus in the form of a chromatin band that grows out from the kinetonucleus to the trophonucleus. The nucleus that results from this interaction between kinetonucleus and trophonucleus then becomes more compact and is surrounded by a vacuole. This nucleus and vacuole surrounded by a thin layer of cytoplasm is finally set free by a disintegration of the trypanosome. These bodies, according to Salvin-Moore and Breinl, have a definite relation to the life-cycle of the parasite and to the course of the disease in the host. They appear only after a certain number of generations by fission in the peripheral blood, are developed only in the capillaries of the internal organs such as the spleen and bone marrow, coincident with the disappearance of the trypanosomes from the peripheral circulation, and correspond with the latent period characteristic of trypanosomiasis. At the end of the latent period the resting bodies elongate and develop into trypanosomes which reappear in the peripheral blood.

The other paper of interest in relation to the formation of intracellular reproductive bodies in *Trypanoplasma ranæ* is that by Chagas (1909), who describes a new trypanosomid, *Schizotrypanum cruzi* of man in Brazil. According to Chagas longitudinal fission of this trypanosomid is entirely absent in the peripheral circulation of the host and is replaced by a schizogony in the capillaries of the lungs. The schizont loses its flagellum and undulating membrane, bends upon itself, and fuses to a round or oval cell. The kinetonucleus is in some cases shed with the flagellum, in

other cases it unites with the trophonucleus. The nucleus divides into eight and there are developed eight club-shaped merozoites within the cell. These merozoites are said to be dimorphic: those formed in the schizont that shed its kinetonucleus are uninucleate and are supposed to develop into female trypanosomes; those formed in the schizont that retained the kinetonucleus are binucleate and are supposed to develop into male trypanosomes. These merozoites penetrate red blood corpuscles and have an intracorpuseular stage of development.

How far the "resting-bodies" of *Trypanosoma gambiense* and the "merozoites" of *Schizotrypanum cruzi* are comparable with one another and with the reproductive bodies which have been described in *Trypanoplasma ranæ* it is, of course, in the present state of our knowledge, impossible to say with certainty. These reproductive processes have, however, certain fundamental characters in common. The reproductive bodies are in each case developed intracellularly, and in their development not all of the parent cell is used up but a part remains as a residual or restiform body. Such a method of reproduction is not a multiple division, such as is described by Dutton, Todd and Toby (1907) in *Trypanosoma loricatum*, nor is it strictly speaking a schizogony, but rather a sporulation. I should, therefore, interpret all three reproductive processes as sporulation, which results in the formation of one spore in *Trypanosoma gambiense*, two spores in *Trypanoplasma ranæ* and eight spores in *Schizotrypanum cruzi*.

REFERENCES.

Baldrey (1909). Versuche und Beobachtungen über die Entwicklung von *Trypanosoma lewisi* in der Rattenlaus, *Hematopinus spinulosus*. Archiv. f. Protistenkunde., xv, 326-332, 2 Textf.

Breinl, A., and Hindle, E. (1909). Observations on the life history of *Trypanosoma lewisi* in the rat louse (*Hematopinus spinulosus*). Ann. Trop. Med. and Parasitol., iii, 553-564, Pl. XIX-XX.

Chagas, C. (1909). Ueber eine neue Trypanosomiasis des Menschen. Studien über Morphologie und Entwicklungszyklus des *Schizotrypanum cruzi* n. gen., n. sp., Erreger einer neuen Krankheit des Menschen. Memoras do Instituto Oswaldo Cruz, i, 160-218, Taf. 9-13, 10 Textf.

Crawley, H. (1909). The priority of *Cryptobia Leidy*, 1846, over *Trypanoplasma* Laveran and Mesnil, 1901. U.S. Dep't Agric., Bureau An. Ind., Bull. 119.

Dutton, J. E., Todd, J. L., and Tobey, E. N. (1907). Concerning certain parasitic protozoa observed in Africa. Ann. Trop. Med. and Parasitol., i, 285-370, Pls.

Friedrich, L. (1909). Über Bau und Naturgeschichte des *Trypanoplasma helicis* Leidy. Archiv. f. Protistenkunde., xiv, 363-395, 48 Fig. in Text.

Gray, A. C. H., and Tulloch, F. M. G. (1905). The multiplication of the *Trypanosoma gambiense* in the alimentary canal of *Glossina palpalis*. Rpt. Sleeping Sickness Com., Roy. Soc., Lond., No. 6, 282-287, 4 Fig.

Hindle, E. (1909). The life history of *Trypanosoma dimorphon*. Univ. California Publ. in Zoöl., vi, 127-144, Pls. 15-18, 1 text-fig.

Keysselitz, G. (1906). Generations- und Wirtswechsel von *Trypanoplasma borreli* Laveran et Mesnil. Archiv. f. Protistenkunde., vii, 1-74, 162 Fig.

Klein (1909). Weitere wissenschaftliche Beobachtungen über die Entwicklung von Trypanosomen in Glossinen. Deutsche med. Wochenschr., xxxv, 924-925.

Koch, R. (1905). Vorläufige Mittheilungen über die Ergebnisse einer Forschungsreise nach Ostafrika. Deutsche med. Wochenschr., xxxi, 1865-1869.

Laveran, C. L. A., et Mesnil, F. (1901). Sur les flagellés à membrane ondulante des poissons (genres *Trypanosoma* Gruby et *Trypanoplasma* n. g.). Compt. rend. Acad. d. Sci., Paris, cxxxiii, 670-675, Figs. 1-4.

Léger, L. (1905). Sur la présence d'un *Trypanoplasma* intestinal chez les poissons. Compt. rend. Soc. d. Biol., Paris, lviii, 511-513.

Leidy, J. (1846). Description of a new genus and species of entozoa. Proc. Acad. Sci., Phila., iii (v), Sept.-Oct., 100, 101, Figs.

Minchin, E. A. (1908). Investigations on the development of trypanosomes in tsetse flies and other diptera. Quart. Jour. Micro. Sci., lii, 159-260, 6 pls., 2 Figs.

Novy, F. G., and MacNeal, W. J. (1904). Trypanosomes and bird malaria. Am. Med., viii, 932-934. Also, Jour. Inf. Diseases, 1905.

v. Prowazek, S. (1905). Studien über Säugetiertrypanosomen. Arb. a. d. kais. Gesundheitsamte, Berl., xxii, 351-395, 6 Taf.

Salvin-Moore, J. E., and Breinl, A. (1907). The cytology of the trypanosomes. Jour. Trop. Med. and Parasitology, i, 441-472, Pls.

Salvin-Moore, J. E., Breinl, A., and Hindle, E. (1908). The life history of *Trypanosoma equiperdum*. Proc. Roy. Soc., Lond., Series B, lxxx, 290-298, Pl. VIII.-IX.

Sergent, Ed. et Et. (1905). Evolution des Hématozoaires de l'Athéna noctua, d'après F. Schaudinn. Recherches expérimentales. Compt. rend., vi Congr. internat. de Zoologie, Berne, 384-388.

Schaudinn, F. (1904). Generations- und Wirtswechsel bei *Trypanosoma* und *Spirochaeta*. Arb. a. d. kais. Gesundheitsamte, Berl., xx, 3.

Stuhlmann, F. (1907). Beiträge zur Kenntniss der Tsetsefliege. Arb. a. d. kais. Gesundheitsamte, Berl., xxvi, 1-83, Taf. 1-4, 28 text-figs.

Walker, E. L. (1908a). The parasitic amebæ of the intestinal tract of man and other animals. *Jour. Med. Research*, xvii, 379-460, 4 pl.

Walker, E. L. (1908b). The cultivation of the parasitic flagellata and ciliata of the intestinal tract. *Jour. Med. Research*, xviii, 487-495.

EXPLANATION OF PLATE X.

(All of the figures in this plate are reproductions of photomicrographs made by Mr. Fairfield of the Department of Pathology, Harvard Medical School, from preparations fixed wet and stained with Giemsa's stain. The magnification is uniformly one thousand five hundred diameters.)

FIG. 1. — "Indifferent" or "asexual" trypanoplasm.

FIG. 2. — "Female" trypanoplasm.

FIG. 3. — "Male" trypanoplasm.

FIG. 4. — Early stage in the "copulation" of the trypanoplasms.

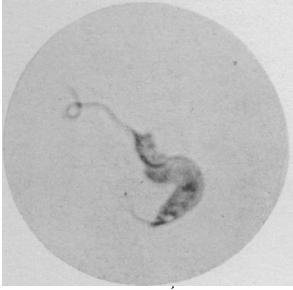
FIG. 5. — Late stage in the "copulation" of the trypanoplasms.

FIG. 6. — The "oökinet."

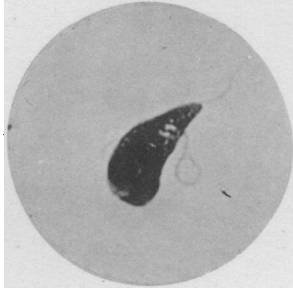
FIG. 7. — Early stage in the sporulation of a trypanoplasm.

FIG. 8. — Late stage in the sporulation of a trypanoplasm.

FIG. 9. — A flagellated spore.



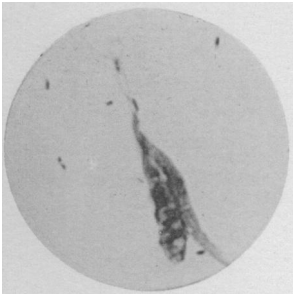
1



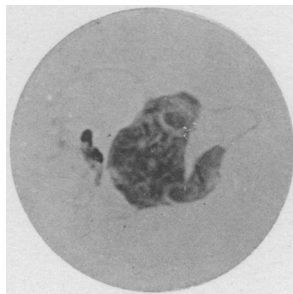
2



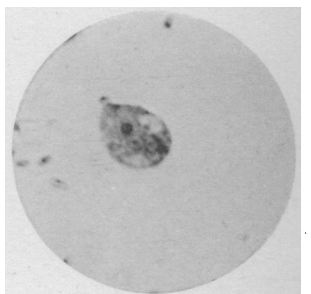
3



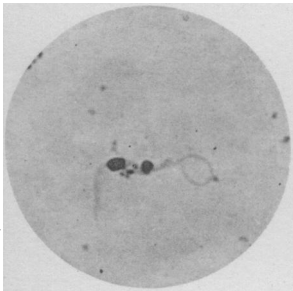
4



5



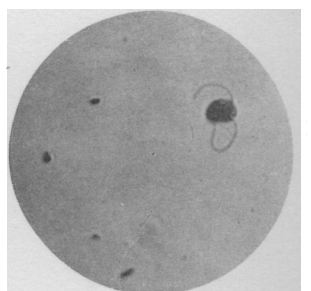
6



7



8



9