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THE CYTOLOGY OF THE TRYPANOSOMES

THE CYTOLOGY OF THE TRYPANOSOMES

PART I*

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(A) INTRODUCTORY

The trypanosomes belong to a group of organisms of great practical importance, since they are related to numerous diseases, not only affecting many valuable animals, but, in the case of sleeping sickness, man also. Notwithstanding the facts, the nature and

* A preliminary account of the observations relating to *T. gambiense* contained in the present paper was published in the *Lancet*, p. 1219, May 4, 1907. In a subsequent paper by Plimmer and Thomson received by the Royal Society, July 20, 1907, these authors appear to have encountered either the encysted Trypanosomes, or the resistant bodies (latent bodies) which we had previously described. But from the vagueness of their reference, Pro. Roy. Soc. B. Vol. 79, p. 509, it is impossible to be certain to which order of structures already described by us they do refer.

morphology of these organisms are as yet but little understood. Information upon these matters, as well as upon the various life cycles they appear to present, is greatly to be desired. Especially is this the case with regard to their morphology. The numerous descriptions of their structure and their metamorphoses already in existence have been drawn in general from the results of methods of research not calculated to produce any correct appreciation of their true cytological features. With very few exceptions, the study of the structure of the trypanosomes has been based, either upon what can be made out of the live animals, or else upon observations made upon material after it has been heated and dried, a method which, to say the least of it, may be shown, so far as the finer details of any cell structure are concerned, to be particularly barbarous.

Trypanosomes, like other unicellular organisms, can, however, be fixed in a great variety of ways which are commonly used during cytological research. The chief manipulative difficulty they present is the unreadiness with which they can be made to take any sort of differential stain. Still this difficulty is not insurmountable; and ordinary preparations may be produced which stain as completely as can be desired.

We have used the following fixatives:—Fleming's fluid; sublimate acetic acid; osmic acid vapour; osmic acetic acid vapour; and formalin vapour.

When the animals have been fixed, it is in all cases desirable to use somewhat special precautions in relation to the stain which may have to be employed, the process adopted depending upon the principle of applying a mordant, or mordants, before the actual stains are used. On the whole, we have found that the fixation with Fleming's fluid is unquestionably the best from a morphological point of view, while the staining methods through which we have obtained the sharpest colouration have been, on the one hand, the double safranine orange methylene blue stain invented by Breinl (see Appendix I); on the other, a slight modification of the Heidenhain iron haematoxylin process (see Appendix II).

As we are dealing in this Memoir (and in future publications which will be related to it) with many different specific forms, it is necessary in the first place to consider the cytology of the trypanosomes, as far as is now possible from a general point of view.

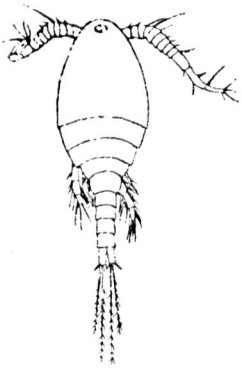

Fig. 7

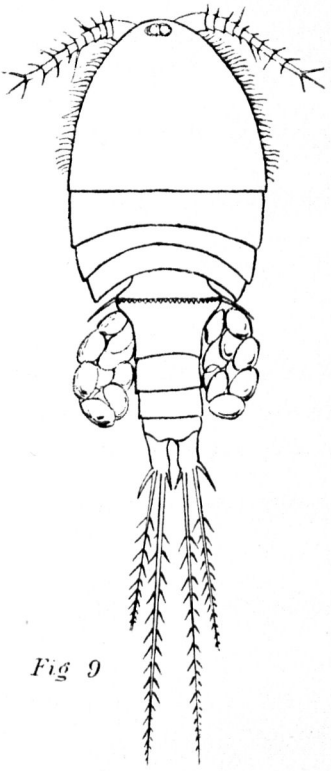

Fig. 9

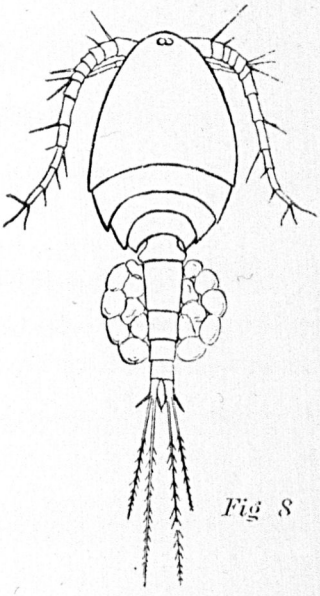

Fig. 8

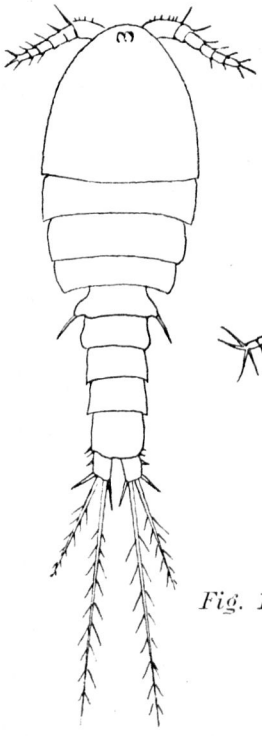

Fig. 10

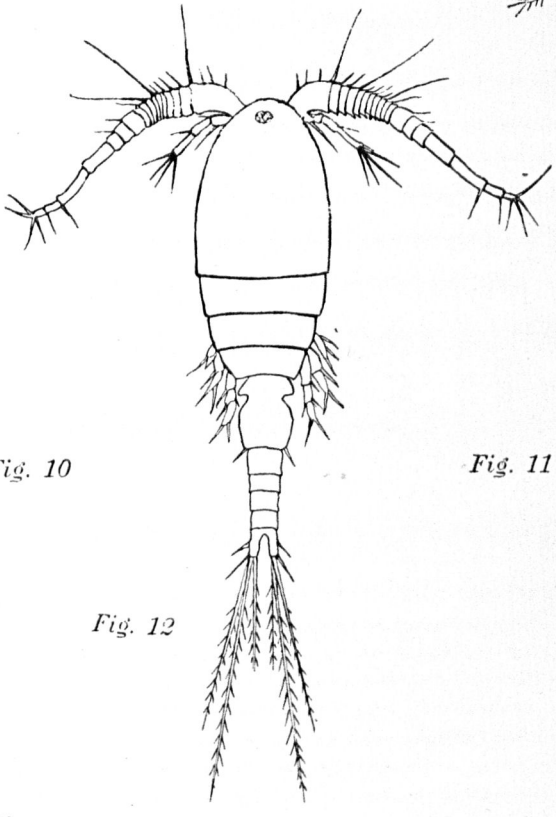

Fig. 12

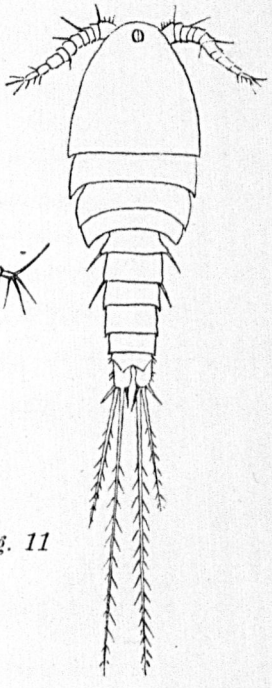

Fig. 11

W. M. Graham, ad. nat. del.

This course is unavoidable owing to the confused terminology which has grown up in the literature, and also on account of the present necessity of making clear the meaning we attach to different names. We have further to define our present conception of the nature of several structures which the trypanosomes possess.

When properly fixed, all the animals we have examined present an elongated cell form. No anterior or posterior extremity can, except in the most arbitrary sense, be defined. The exterior of the protoplasm is differentiated into a thin outer layer or ectosarc (periplast). Among the species with which this paper is concerned, the ectosarc is smooth, and does not present any definite ridges or stripes corresponding to the structure often described in the larger trypanosomes, such as those of the frog, and others.

The protoplasmic structure within the ectosarc consists of a very coarse *spongioplasm* (schaumplasma) containing fine staining granules embedded in its substance, the meshes of this spongioplasmic network being filled by a less stainable cytolymph. It is sometimes said that within the ectosarc, and distinct from the deeper portions of the spongioplasm, there exists a layer—the endoplasma. We have, however, not been able to demonstrate the existence of this subdivision.

The permanent cell structures contained within the ectosarc consist of a more or less central area, which, when subjected to Breinl's stain, assumes a purple colour (see figs. 4, 5, 11, 12, 13). We propose to call the whole of this area the *nucleus*. Within the nucleus there is always to be found a clearly-defined body which stains under the same conditions red, and we propose, for reasons which will become more apparent later, to term this body the *intra-nuclear centrosome* (Karyosome, Innenkorper).

It does not appear to be the case, when the animals are not dividing, that the nucleus can be correctly said to be bounded by any definite membrane. In most instances it appears more correct to say, that there is no definite membrane, but rather that there is a very sharp division between the spongioplasmic network and the finer network of the nucleus.

Near the broad end of the animal's body there is usually to be found a granule, or small group of granules, which stain like the intra-nuclear centrosome. These, whatever their numbers at any particular

period, we propose to call the *extra-nuclear centrosomes* (blepharoplasts). From one or more of these granules there springs a staining core, or flagellum, which lies in a thin expansion of the ectosarc, forming the so-called undulating membrane.

For present purposes we have thus the following terminology:—

Ectosarc = (Periplast).

Spongioplasm = (The substance of the network of the protoplasm).

Cytolymph = (The substance between the meshes of the spongioplasm).

Intra-nuclear centrosomes = (Karyosomes, Innenkorper).

Extra-nuclear centrosomes = (Blepharoplasts, micro-nuclei centrosomes, nucleoli).

Flagellum.

Undulating membranes.

In none of the trypanosomes which we have studied have we found the slightest indication of the existence of the so-called males, females, and indifferent forms. We have found that the often-asserted existence of these three types in the blood, a suggestion originating chiefly from Schaudinn,* is totally misleading.

So far as is at present known, trypanosomes are parasites inhabiting the blood, and body fluids, of a great variety of animals. Hitherto no non-parasitic forms have been discovered. As is the case with other parasites of this description, their life histories appear to have become modified to secure their transference from one host to another in different ways. When introduced into the blood and tissues of a suitable host, they usually multiply by fission until either the noxious influence of the infection destroys the host, as in the case of some strains of *T. gambiense* introduced into rats, or the infection runs a different type of course. In the latter type of infection the multiplication of the parasites in the blood rises to a first maximum, and then falls, so that the numbers may decrease to zero. After this fall, which we may speak of as the first negative phase, the parasites reappear, and reach a second maximum, and so on. In such cases, the infection follows an irregular course, which can be easily understood from the diagram given on page 449.

* For further information respecting this matter see Thomas and Breinl, Liverpool School of Tropical Medicine, Memoir XVI.

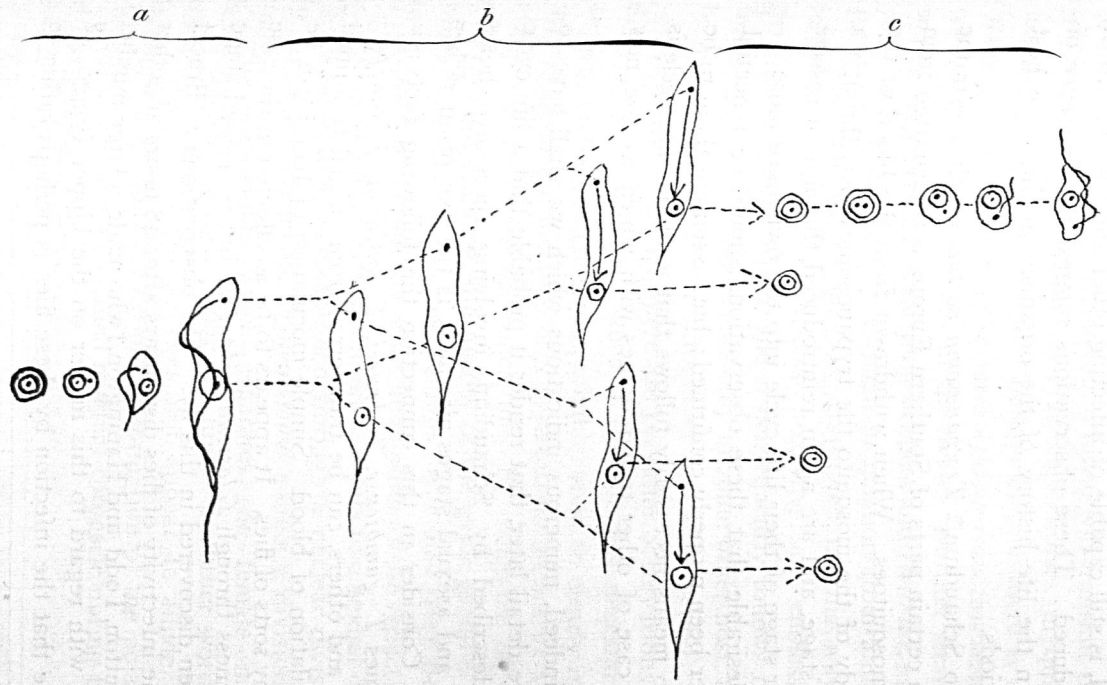

DIAGRAM.—Showing the life cycle of *Trypanosoma gambiense* in a rat:—

a. Development of the Trypanosome from the latent body.

b. The fission of the Trypanosome and the formation of the black line.

c. Reproduction of the latent bodies and the development of the Trypanosome therefrom.

It is a very remarkable fact that in some examples of the latter type of infection the blood during the negative phases, although apparently containing no trypanosomes at all, and even if it be properly filtered, is still capable of infecting other animals into which it may be introduced. These observations suggest the existence of another stage in the life history of the organism in the same host during such periods.

According to Schaudinn,* *Trypanosoma noctuæ* which infests the blood of owls in certain parts of Southern Europe is transmitted from owl to owl by mosquitoes. When withdrawn from the blood of the owl into the body of the mosquito the trypanosomes pass through a definite sexual stage, and are again reintroduced through successive bites, in another stage of their life cycle into the owls once more.

It is very desirable that these observations should be repeated. They have never been properly confirmed; but, assuming them to be correct, it by no means necessarily follows that a similar life cycle is pursued in the case of other trypanosomes with which we are now acquainted.

There are, indeed, numerous indications which we shall have to consider in more detail later, that render it probable that a life cycle such as that described by Schaudinn, involving successive hosts wherein sexual and asexual stages alternate, is the exception rather than the rule. Consider in this connection the following facts:— The trypanosomes *T. gambiense, T. brucei, T. equinum, T. equiperdum, T. lewisi* and others, can be transferred from animal to animal by simple inoculation of blood. Simple inoculation of blood can be effected by many sorts of flies. It appears to be so effected in the case of sleeping sickness through *Glossina palpalis*; but no sexual stage has hitherto been discovered in this fly. The observations of Bruce† showing that the infectivity of flies disappears after 48 hours, together with those of Dutton, Todd and Hanington,‡ who made a large number of experiments with regard to this matter on the Upper Congo, all seem to indicate that the infection by these flies is perhaps rather in

* Schaudinn. Generations und Wirtswechsel bei Trypanosoma und Spirochaete. Arb. a. d. kaiserl. Gesundheitsamte, Bd. XX, H. 3.

† Bruce, Nabarro & Greig. Reports of the Royal Society. Sleeping Sickness Commission, No. 4, 1903.

‡ Dutton, Todd and Hanington. Trypanosome transmission experiments. Ann. Trop. Med. and Parasit., Vol. I, No. 2, p. 199.

the nature of an accident than a necessary process involved in the normal life cycle of the parasites. Some such conclusion is borne out by other facts in relation to trypanosome infection. Thus in the case of *Dourine*,* simple inoculation of blood will transmit the disease, but it is habitually communicated amongst horses in quite a different manner, namely by coitus. Consequently, if there is a sexual stage in the life history of *Trypanosoma equiperdum*, this sexual stage must occur normally in the body of the horse. Further strains of trypanosomes, such as those of sleeping sickness and *Dourine*, may be kept for years in our laboratories through inoculation from animal to animal. In fact, such strains may be continued in this way for a quite indefinite period, a process involving an endless number of generations in the blood, and it consequently follows that if in such forms the sexual stage occurs only in some other host, this phase can be dispensed with for an altogether indefinite period.

As a matter of fact, there are yet other observations bearing upon Schaudinn's researches, which if they do not necessarily render his account of the sexual act improbable, seem to clearly indicate that it may exist in the instance of *Trypanosoma noctuæ* as a very unusual exception, an exception which may be incapable of throwing any general light upon the life history of the great group of organisms to which *Trypanosoma noctuæ* belongs. We may refer also particularly to the author's account of what he regards as the reduction process.

This, according to Schaudinn,† amounts to a sexual determination, or differentiation, accomplished through a nuclear division. That is to say, there occurs in *Trypanosoma noctuæ* a division (heteropolar mitosis, Schaudinn), which separates the female moiety of an hermaphrodite nucleus from the male. In other words, Schaudinn resuscitates (although he does not appear to allude to this fact) Balfour's and Minot's view of the formation of the polar bodies, and the extrusion of the so-called residual corpuscle during the formation of the spermatozoa.

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* Rabinowitsch and Kempner. Centralblat für Bakt. Bd. XXXVII, H. 5. See also Minchin, Gray and Tulloch. Pro. Roy. Soc., London. Vol. 78, 1906. See further Laveran and Mesnil. Trypanosomes et trypanosomiases. Paris, 1904.

† Schaudinn: Neuere Forschungen über die Befruchtung bei Protozoen. Verhandlungen der deutschen zoologischen Gesellschaft auf der XV. Jahresversammlung. Leipzig, 1905.

It is necessary to be quite clear about this matter. The hypothesis respecting the function of the polar bodies, and so-called residual corpuscle of the spermatozoa, as the means by which the opposite "sex-stuff" is got rid of from the ovum, and the spermatozoon, has for various reasons collapsed some years ago. In the first place, the polar bodies cannot be homologised in any way with the residual vesicle. In the second place, it has been clearly demonstrated that in the vast majority of animals and plants the sexual reproductive cells, ova, and spermatozoa are precisely equivalent so far as their nuclei are concerned.* Reduction as now understood in animals and plants is not sex differentiation, but a process which results simply in the halving of the number of chromosomes in those cells which are destined to conjugate.

A great deal of confusion has been produced by Schaudinn's inaccurate use of the term reduction, a term which in general biology has long had a limited and a definite meaning. In dealing with these matters it must, therefore, be clearly understood that by reduction Schaudinn means sex differentiation, and that the term reduction in general biology does not mean sex differentiation, and stands for something quite different.

(B) THE MORPHOLOGY AND LIFE CYCLE OF TRYPANOSOMA GAMBIENSE

Trypanosoma gambiense (Dutton) is a parasite associated with the disease appearing in Equatorial Africa and known as Sleeping Sickness. It can be transferred by simple inoculation into nearly all the animals generally used for laboratory experiments. The infection may run a very varied course. Thus the strain used in Liverpool for inoculation into rats may simply increase until, within a period of a few days, the rat's blood is teeming with parasites and the animal dies from the effect of the invasion. In other cases among the rats, as in man, the number of parasites in the blood rises and falls in a

* It is unnecessary to refer to the vast literature of this subject. The repeatedly confirmed observations upon which the above statements are based, being so well known as to render it unintelligible why Schaudinn did not himself make it clear that the process he describes, under the term reduction, can have nothing in common with the reduction process as described and studied in animals and plants for the last 20 years. It is equally unintelligible why this author did not point out the identity between the process he discussed and that erroneously supposed to exist among animals and plants by Balfour and Minot.

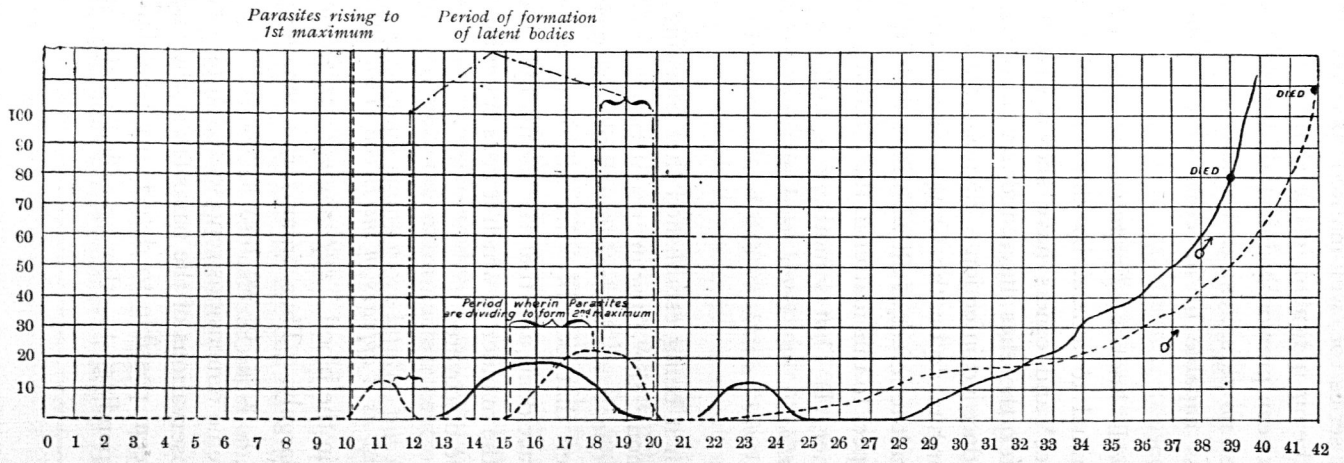

CHART OF TWO MALE RATS INOCULATED WITH *Trypanosoma gambiense*.

The horizontal figures represent the days after inoculation: the vertical figures the numbers of parasites in a microscopic field of blood; the curve representing the variation in this during the course of the infection. The two different curves represent two different infections.

somewhat irregular series of well-marked periods, the kind of oscillation produced being indicated in the chart given on page 449. When an animal has been previously infected, it has been found that even at a period when no parasites can be detected in the blood, the blood is nevertheless capable of infecting other animals by sub-inoculation.

As is already well known, *Trypanosoma gambiense* can be transmitted from animal to animal by the bites of flies; but the observations of Bruce* and others have shown that if more than 48 hours elapse after the flies have fed on an infected animal, subsequent bites produce no infection. The observations of Dutton, Todd and Hanington† made on the Congo, show further that it is often extremely difficult to infect at all with flies, and the authors sum up the position in respect to this matter in the following paragraph:—

"We believe either, (I) That something is wrong in the way in "which *Glossina palpalis* has been used in these experiments, or, "(II) That *Trypanosoma gambiense* can be conveyed by some other "means than by it."

So far, then, from it being established that Sleeping Sickness is normally spread among the African population by the bites of *Glossina palpalis* alone, it would seem that the most recent work on this subject indicates that possibly the infection through flies is in the nature of an accident, and that the means by which Sleeping Sickness spreads, in the manner in which it does spread in the African interior, has yet to be discovered.

Trypanosoma gambiense as it appears in the Blood of an Infected Animal

When examined in the blood *Trypanosoma gambiense* is found to vary in size very largely. Thus from forms smaller than those represented in figs. 1, 2, 4, 6, we may select a series increasing gradually to the extreme dimensions represented in figs. 8, 9, 10.

From our own observations of the parasites as they appear in the blood, it does not seem possible to detect any true dimorphism, or trimorphism, corresponding to the so-called male, female, and

* Bruce, loc. cit.

† Dutton, Todd and Hanington, loc. cit.

indifferent forms described by Schaudinn,* Minchin,† and many other authors. The present examination of the forms appearing in the blood leads us to believe that there is to be found among these trypanosomes a series extending from those which are relatively small, to those which are relatively immense. The three forms often described and alluded to as distinct, consequently appear to be arbitrarily chosen examples in a continuous series of dimensions.

Multiplication in the Blood

From the time when the parasites appear in the blood of an infected animal until their numbers reach any particular maximum, rapid multiplication takes place by longitudinal fission of the individual trypanosomes, the multiplication being most rapid near the successive periods of maximum numbers. When the parasites are not dividing, they present the appearance represented in figs. 1 and 4.

The nucleus is nearly in the middle of the long axis of the cell, and consists of an outer stainable mass, enclosing generally a lighter central area, within which there lies a small, sharply-definable body, which stains red in contrast to the purple colour of the outer mass when subject to Breinl's stain. This central structure forms the intra-nuclear centrosome (karyosome). At the broad end of the cell there exists another staining granule which, when the cell is not dividing, remains single, the granule in question forming the extra-nuclear centrosome (blepharoplast, micro-nucleus, centrosome). It stains, under the above conditions, like the intra-nuclear centrosome. Arising directly from the extra-nuclear centrosome, there extends a delicate thread, which stains more faintly, but in the same manner as the centrosomes. It is enclosed in a thin expansion of the ectosarc running along the entire length of the cell. The thread projects at the narrow end of the cell as a long stained whip-lash. This thread forms the so-called flagellum, and the ectosarcal expansion the undulating membrane.

The first sign of an approaching fission is generally apparent in relation to the extra-nuclear centrosome. From this there buds out a small fragment, figs. 1, 2, and 3, which may become flattened, as in

* Loc. cit.

† Pro. Roy. Soc., 1907.

figs. 1 and 7, and can be seen to be attached to the original extra-nuclear centrosome by faint unstaining strands, fig. 7. At a later period there grows out from the new extra-nuclear centrosome a young flagellum, which gradually extends parallel with the old flagellum, as in figs. 2, 3, 6.

At a period of the fission roughly corresponding to the above, it can be observed that the intra-nuclear centrosome has also divided in the manner represented in figs. 2, 3, and that the staining outer portion of the nucleus has gradually collected around, and beyond, the dividing portions of the intra-nuclear centrosome. In this form of nuclear division characteristic of *Trypanosoma gambiense*, no chromosomes are formed at all, and the intra-nuclear centrosome behaves precisely as the so-called karyosome, or centrosome, during the division of the nucleus in *Euglena*, *Eimeria schubergi* (Schaudinn), and other protozoa. At a later stage the division of the intra-nuclear centrosome becomes complete, and the outer staining portion of the nucleus collects round the two intra-nuclear centrosomes as in figs. 3, 7, 8, forming together with these two bodies two daughter nuclei, having just the same appearance as the parent nucleus. For all practical purposes the nuclear division of *Trypanosoma gambiense* is thus amitotic; but it is a form of amitosis somewhat complicated by the presence of an intra-nuclear centrosome.*

In the remainder of the cell, the process of division proceeds by the extension and growth of the new flagellum through stages such as represented in figs. 11, 12, 13, 14, so that two apparently similar flagella are produced, each enclosed in an expansion of the ectosarc. They form separate undulating membranes and stretch from one end of the animal's body to the other, fig. 12. The extremities of the flagella become eventually separate, and the animal gradually splits from the narrow end towards the broad end, figs. 13, 14. The daughter forms finally separating, pass through stages such as those represented in figs. 13, 14. In this way we have eventually two trypanosomes, each exactly resembling the parent form, but smaller.

During the growth and division of the parasites in the blood, it is frequently possible to find large numbers of cells in all stages of

* In connection with this matter it should be noted that in many of the metazoa where the centrosomes are always extra-nuclear, these bodies may be definitely related to amitosis when this form of division occurs.

division, and rest, wherein there exist, especially towards the narrow end of the cell, quantities of granules that stain under the action of Breinl's stain an intense red. The colour of these granules is quite distinct from that of either the intra-nuclear centrosome or the extra-nuclear centrosome, and they cannot be stained with any satisfaction at all by means of the iron haematoxylin method. They are quite irregular in size and number, and also in their appearance, that is to say, they may appear in all the trypanosomes in all their stages at one period, and not at another. From these circumstances, we are inclined to regard these granules as of metabolic origin, and we can find no evidence that they arise from the nucleus at any time.

These bodies should, however, not be confounded with the minute granules always seen, more or less throughout the spongioplasmic network of the entire body. These latter may, and very often do, stain in the same manner as the intra- and extra-nuclear centrosomes, but we have been unable to find any indications as to their origin, or that they any more than the large metabolic granules have any special relationship with the nucleus.

It is possible, indeed probable, that some of these granules may correspond to the vegetative and trophic chromidia observed in *Rhizopods* by Schaudinn,* and by Hertwig† in *Actinosphærium eichhorni*, but owing to the very different methods we have used, we are not in a position to make any definite statement with respect to this matter.

At a late stage of division, such as that represented in figs. 14 and 13, the appearance of the organisms at first sight very much suggests an act of conjugation, but in all such cases that we have examined we have found no indication in relation to the nuclei, or the centrosomes which would suggest a conjugational act.

Along with the regular method of division just described certain modifications are frequently observed, which, although producing the most striking appearance (see figs. 8, 9, 10) are nevertheless apparently always capable of being explained through a disparity in the stage simultaneously reached by different parts of the cell. Thus the nucleus may divide completely and then divide again so as to form

* Schaudinn. Arbeiten aus dem kaiserlichen Gesundheitsamte, Vol. XIX, 1903.
† Hertwig. Archiv. für Protistenkunde, Vol. 1.
See also Mesnil. Chromidies et questions connexes. Bull. Past., Tome III, p. 313.

four nuclei, without, however, the extra-nuclear centrosome having divided more than once. Or the extra-nuclear centrosome may divide and form three or four flagella without the nucleus having divided at all. When these unusual methods are adopted, the gigantic trypanosomes figured on Plate I are frequently produced.

Changes in the Trypanosomes relative to the Stage of Infection

The appearances described in the preceding paragraphs are those which are encountered among the parasites during multiplication after their first appearance in the blood of the infected animal. If, however, other stages of the infection be studied there are to be found different morphological appearances among the parasites, which are apparently of the greatest importance. In the case of an animal which has become infected with *Trypanosoma gambiense*, and shows a well-marked oscillation in the course of the disease, it is found that as the number of the parasites is rising in the blood—that is to say, along the ascending portions of the curve in the diagram (page 449)—the phenomena presented are the same as those found in the initial stages of the infection which we have just described. If, however, we study preparations made at or near the maxima of the curve, other changes are found to be taking place in the trypanosomes besides those of simple division.

At the time the curve approaches any maximum, there are to be found trypanosomes which present the appearance shown in figs. 15 to 20. From this figure it will be seen that such trypanosomes are distinct from those which have been previously described, in that a relatively-thick stainable band proceeds from the extra-nuclear centrosome. This thick band is found to be most readily stainable by iron haematoxylin; it is less readily, but still stainable by the various aniline colours which we have employed. It grows from the extra-nuclear centrosome not along the surface of the animal as in the case of the new flagella, but down the interior of the cell towards the nucleus (see figs. 15, 16).* This stainable band, which appears near

* It is probable that the band we here refer to is the same as the structures described by Prowazek, Studien uber Saugethier trypanosomen. Arb. a. d. kaiserl. Gesundheitsamte, Band XXII, 1905, and also Miss Robinson, Notes on certain blood-inhabiting protozoa. Proceedings of the Royal Society, Ed. Vol. XVI, No. 6.

the periods of the greatest number of parasites in the blood, is fully twice as thick as the flagellum. As it increases in length it may reach, or even pass the nucleus; or it may become coiled upon itself, as in figs. 16, 17, 18. But whatever form it takes at first, the later appearance suggests that the band eventually becomes in one way or another definitely connected up with the nucleus. This suggestion is strengthened by the subsequent behaviour of the band, for it is seen eventually to become gradually less stainable, to break up into a string of fragments, and finally to disappear. Through all these later stages of its existence it is most certainly suggested, as in figs. 19, 20, that it is directly related to the nucleus, that is to say, to have been, or to be actually connected up with it.

We appear then, at or near the maximum number of the parasites in the blood, to have direct evidence of some sort of interaction taking place between the extra-nuclear centrosome and the nucleus. This phenomenon occurs only among animals in which no evidence of nuclear division, or cell division, is apparent. In such cases, we do not find that either the extra-nuclear centrosome or the nucleus is divided, and throughout the whole development of the stainable band the nucleus remains in a condition of complete repose.

If now we examine the portions of the curve of infection where the trypanosomes are decreasing in number, still other phenomena make their appearance. The numbers of the parasites gradually decrease in the peripheral blood, and at this time, in such organs as the lungs, the spleen, and the bone marrow, we find curious changes to be taking place in vast numbers of the trypanosomes encountered in these situations. Parasites showing the present changes are rarely found in the organs named above during the period when the number of the parasites is increasing in the blood, but at the time when the numbers are beginning to decrease we see in the lungs that numbers of trypanosomes show alterations in their nuclei. The protoplasm of the animal's body becomes detached from the periphery of the nucleus which lies in a clear space, while the nucleus itself contracts somewhat, and at the same time a large clear vesicle gradually grows up in connection with it, in the manner represented in figs. 22, 23. Round the outside of this vesicle and the nucleus there may be seen a layer of protoplasm enclosing in a delicate sheath both nucleus and vesicle together. When this stage has been reached, the rest of the cell body

rapidly disintegrates, the outline of the cell becomes lost, and the flagellum together with the extra-nuclear centrosome may be frequently seen detached, and lying loose among the cells of the organs examined (see fig. 26).

These phenomena, as we have said, are encountered in the lungs, but they are also found in the bone marrow and the spleen. After the above stage has been reached the nuclear bodies and the disintegrating remains of the parent cells disappear to a large extent from the lungs, the nuclear bodies being subsequently found in very large numbers both in the spleen and the bone marrow.

At first sight, the appearance we have just described might be supposed to be simply a phase in the disintegration of the parasites, and this was, as a matter of fact, the view which we were at first inclined to adopt. There are, however, reasons for thinking that, although the greater part of the protoplasm forming the bodies of the parasites undoubtedly does disappear, the peculiar nuclear structures we have just been considering do not follow the same course. After their detachment from the rest of the disintegrating cell body, the nuclear corpuscles become impacted in great numbers in the spleen and bone marrow. Here, instead of disappearing in the course of a few hours, they may remain intact for as much as ten days or more, in fact, throughout the whole of the negative or latent period of the infection, when no parasites are present in the peripheral blood. From the time of their formation, these peculiar bodies,* which we propose to term *latent bodies*, may be found in small numbers in the blood, and although they thus remain intact for a relatively long period, it

* The formation of these bodies appears not to have been previously observed at all in *Trypanosoma gambiense*; but similar bodies have been seen in relation to other trypanosomes, and have been variously interpreted. Thus, Rodet and Vallet, Arch. des Med. expériment., Vol. 18, No. 4, describe bodies which appear to be similar to the latent bodies of *Trypanosoma gambiense* in *Trypanosoma brucei* in the blood of the organs and regard them as degenerate forms. Plimmer and Bradford (Quar. Journ. Micro. Sci., Vol. 45) seem also to have found them in the spleen, &c., during *Nagana* infections, and describe them as apparently the nuclei of a plasmodial production. Laveran and Mesnil describe apparently similar bodies as "formes d'involution." Lingard (Journ. Trop. Vet. Sci., Vol. 2, No. 1) appears to have seen them in the blood of cattle infected with *Trypanosoma indicinum*, and to regard them as forming part of the developmental cycle. Holmes (Journal of Comparative Anatomy and Therapy, Vol. 17, 1904) figures in detail the formation of precisely similar bodies in the case of *Trypanosoma evansi*, and regards these bodies as true portions of the life cycle, the illustration of the details of the formation of the latent bodies in this paper being extremely accurate. The latent bodies we have dealt with in *Trypanosoma gambiense* probably also correspond to the bodies figured by Prowazek in the rat louse.

was still possible that they might eventually simply disappear altogether, the nuclear constituents of the trypanosome body being perhaps more resistant than the rest of the protoplasmic structure of the cell.

In order to throw some light upon this matter, we examined a number of infected animals which had been treated with atoxyl, this substance having the effect of destroying the parasites in the blood in the course of a very few hours at the most. When the blood of such animals was treated by injection of atoxyl and examined during the time when the parasites were still increasing in number, it was found that a large percentage of the trypanosomes could be observed dead among the corpuscles of the blood, but during the rapid disintegration which follows nothing comparable to the formation of the latent bodies is encountered. On the contrary, the nuclei in these instances are among the first of the organs to be affected by a general disintegration, which rapidly produces masses of débris, wherein it is only just possible to recognise from their shapes the remains of trypanosome cells (see fig. 41).

The same appearance is produced through the disintegration and death of the trypanosomes in the blood of an animal which has been killed by the disease.

From all this, it would appear that the latent bodies are not produced during the ordinary course of cell disintegration, and must be considered from some other point of view.

At this point it is, however, necessary to explain that when animals are injected with atoxyl at a time when the trypanosomes are decreasing in numbers in the blood, the disintegration does not necessarily overtake all the trypanosomes present. It is found, in fact, that a certain number of trypanosomes under such conditions do not succumb to the effects of the drug, but round themselves up and become encysted (see figs. 37, 40). These cysts are, however, very much larger than the latent bodies. They appear to be true resistant forms produced directly in response to the drug, and are not in any way comparable to the latent bodies we have just described.

With regard to the latent bodies which are produced at the maxima of an oscillating infection (structures which at first sight might, and probably would, be taken for disintegration products), these are, as we have seen, eventually collected in the spleen and

the bone marrow, and do not necessarily degenerate there. They persist in such situations in very large numbers, and each consists of a flattened nucleus with an intra-nuclear centrosome. There is also a vesicle attached to the nucleus, and the whole nuclear apparatus is surmounted by a thin film of protoplasm, figs. 27, 28.

At the periods when there are no trypanosomes to be found in the blood, these peculiar latent bodies are all the evidence of the existence of the parasites in an infected animal to be detected microscopically.

At the period of the infection when a few parasites begin to reappear in the blood, it is possible to still find numbers of latent bodies in the spleen, and in the bone marrow, wherein the intra-nuclear centrosome has divided into two, fig. 28.

Again, at this period it is possible to find forms in which one-half of the dividing intra-nuclear centrosome has passed out of the nucleus, fig. 28, forming an extra-nuclear centrosome. Still further, at a later period, we find forms in which a short flagellum has grown from the extra-nuclear centrosome, and these bodies subsequently appear to gradually transform themselves into small trypanosomes in the manner represented in figs. 28 to 32.

As the latent bodies are gradually transformed into small trypanosomes, the number of these bodies in the spleen and bone marrow diminishes, but it appears to be the case that a proportion of what are apparently latent bodies never really develop into trypanosomes and disappear altogether, or, in other words, that only a proportion of these bodies are under the circumstances above described capable of surviving the negative period, and once more forming themselves into complete trypanosomes.

The changes which we have now described are all those which we have hitherto been able to detect in relation to the succeeding stages of the infection in rats.

At first it was anticipated that further changes might have been encountered during different periods of the day and night, but although we have had the parasites in various animals under observation continuously at all periods of the curve of infection throughout several days and nights, no regular nocturnal alteration was discernible. However, it was found that the rapid diminution of the number of parasites almost invariably took place between 2 and 5 a.m.

So far as the above observations upon the life cycle of the

parasite of sleeping sickness have been carried, they appear thus to indicate a complete cycle in the blood of a single host, and the stages of such a life cycle can be semidiagrammatically represented in the manner given on page 445.

(C) THE MORPHOLOGY AND THE MULTIPLICATION IN THE BLOOD OF TRYPANOSOMA BRUCEI

The appearance of *Trypanosoma brucei* in the blood is represented in figs. 42, 43, 44, 45, 46. The chief morphological distinctions which the parasite of the disease Nagana presents when compared with *Trypanosoma gambiense* are found in relation to the distribution of the nuclear substance, and the characters of the extra-nuclear centrosomes.

The division of this trypanosome in the blood is longitudinal, as in the case of *Trypanosoma gambiense.* The nucleus divides amitotically. The division being first marked by a lengthening of the extra-nuclear centrosome until this body finally separates, and to two minute beads. At the same time the nuclear substance also elongates until we observe forms such as those represented in figs. 43, 44, 45, 46.

As in *Trypanosoma gambiense*, the stages of the division of the extra-nuclear centrosome and that of the nucleus may not be the same, at any particular time, and through this circumstance we observe the same sort of multiple, and abnormal forms as in the case of *Trypanosoma gambiense.*

(D) THE MORPHOLOGY AND THE MULTIPLICATION IN THE BLOOD OF TRYPANOSOMA EQUINUM

In the blood, *Trypanosoma equinum* possesses much the same shape as that of either *Trypanosoma gambiense* or *Trypanosoma brucei*; the nucleus is, however, usually placed nearer to the broad end of the cell. The extra-nuclear centrosome is large, and the nuclear division which takes place during the fission of the cells possesses points of much interest, since the centrosomes are more conspicuous in *Trypanosoma equinum* than in many other forms we have examined. The changes which take place in the intra-nuclear centrosome during the division of the nucleus can be studied with great clearness.

When the nucleus is at rest the intra-nuclear centrosome is surrounded by a light space, which is in turn enclosed by the stainable nuclear substance (fig. 47). During division the intra-nuclear centrosome divides, as in figs. 48 to 52. The nuclear substance becoming at the same time collected up in the region of the dividing intra-nuclear beads. As this process continues, the nuclear substance eventually forms itself into two cup-shaped masses situated around, and beyond the intra-nuclear centrosomes. Owing to this, when *Trypanosoma equinum* is dried the edges of these cups becoming flattened down produce at least two irregular bands on each side of the intra-nuclear centrosomes, which under these circumstances may suggest the existence of nuclear chromosomes. This appearance is, however, misleading. The nuclear division of *Trypanosoma equinum* being, as can be seen from figs. 47 to 53, really amitotic, as in the case of *Trypanosoma gambiense* and *Trypanosoma brucei*.

(E) CONSIDERATION OF THE FOREGOING OBSERVATIONS

For the sake of convenience, it is desirable to consider the latent forms as the starting point in the life cycle of *Trypanosoma gambiense*. These bodies, which in an ordinary fluctuating infection may remain unchanged for long periods in the organs (and, to a less extent, in the blood), consist at first of a nucleus containing an intracellular centrosome. The nucleus is related to a vesicle, and the whole nuclear apparatus is surrounded by a delicate film of protoplasm. At a later stage, the intra-nuclear centrosome divides into two, and one of these bodies passes out of the nucleus into the outer layer of protoplasm, which gradually increases in extent.

The above process results in the formation of an extra-nuclear centrosome. The extra-nuclear centrosome, and nucleus together with the intra-nuclear centrosome, henceforth form two entirely distinct sets of structures, which remain distinct through a very long series of divisions, as represented in the diagram on page 445, under the letter B.

After the first separation of the extra-nuclear centrosome from nuclear apparatus, both these sets of structures multiply independently throughout the succeeding series of generations until the

period at which the black line is formed. At this period the extra-nuclear centrosome develops a bridge, as it were, and connects itself for the time with the nucleus. It may be assumed that during this period some substance from the extra-nuclear centrosome passes into the nucleus. Anyhow, after this has taken place the remains of the extra-nuclear centrosome are very shortly cast away, together with the greater part of the protoplasm forming the rest of the cell, and the old flagellum.

Thus, if we consider the nuclear apparatus in the latent body as a whole, this would seem to be divided into two parts during the development of the trypanosome. After the formation of the cell is complete, these two structures, the nucleus and the intra-nuclear centrosome, remain in the same state, and multiply independently into similarly distinct bodies contained in the cells produced by all the longitudinal fissions. In other words, there arises from a nucleus A, two new structures, B and C, both of which differ from A. B and C multiply independently as the animals divide, but at a subsequent stage a portion of each B unites again with the C in all the cells, and the condition of the organism immediately reverts to A once more.

We have thus, after the formation of the latent bodies, an unequal division of the nuclear apparatus of the latent body, so as to form two different sets of structures, the nucleus with one centrosome, and the other centrosome by itself. Each of these then multiplies indefinitely in number. In individual cells these structures subsequently unite temporarily, and later the nucleus characteristic of the latent body is produced once again. In other words, dissimilar structures are formed from a nucleus by division, both derivatives multiply by division, and after a time unite in pairs, and the first type of structure is again produced. There is in this process, when contemplated from the present standpoint, an obvious similarity to the two forms of sexual elements in the higher animals and plants; to two sorts of gametic nuclei, or to a sexual dimorphism. A dimorphism in the trypanosomes which is in like manner followed by a reunion, or conjugation between the dissimilar elements, and succeeded by a reversion to the conditions obtaining before the dimorphism was produced.

The procedure in the case of the trypanosome nuclear apparatus differs, however, from that of apparently all other known organisms

where the phenomena of sex are discernible, in that the dimorphic products into which the nuclear apparatus of the latent forms separate remain contained within the same animal during its successive fissiparous generation. With the exception of this difference, however, the phenomena observed are certainly comparable to the production of sexual gametes and their conjugation. In the forms with which we have been hitherto familiar, the retention of the nuclear apparatus related to both sexes in one cell, may thus be nothing more or less than a morphological curiosity, and in no way necessarily suggests that the process in the trypanosomes we have been considering is fundamentally different from an ordinary sexual differentiation.*

Assuming the phenomena with which we have just been concerned to be of the nature of a sexual process, still another view could be held with regard to them. It may be suggested that the metamorphoses connected with the appearance of the black line is an attempt on the part of the trypanosomes to become sexually differentiated, but this attempt is not completed, the cells reverting to their primary condition, in which case the process could be regarded as an example of a special form of parthenogenesis. In relation to the above suggestion, it should be noted that, at the time of the formation of the black line, the whole of the extra-nuclear centrosome does not reunite with the nucleus, but that only one portion of the extra-nuclear centrosome does so. One moiety of the extra-nuclear centrosome is detached from the other, and one of these portions with the flagellum disappears together with the cytoplasm forming the trypanosome body.

We thus start with the separation of an extra-nuclear centrosome from an intra-nuclear centrosome through the division of an original intra-nuclear centrosome in the latent body. But at the end of the cycle, the nucleus enters again into connection with the extra-nuclear centrosome, yet it only does so with regard to a part of this body. On the other hand, it may be that the extra-nuclear centrosome

*It should be noted that there is no nuclear reduction in this process. The intra-nuclear centrosome divides, and one half of this body passes out of the nucleus. Both nucleus and extra-nuclear centrosome then divide in the production of the succeeding fissions. Afterwards the extra-nuclear centrosome or a part of it re-unites with the nucleus, and the rest of the cell body disappears. It is known that during fertilization a centrosome is often brought in with the male element: to this extent the process of fertilization is similar to the above.

divides normally, and that it is the half so produced which enters into connection with the nucleus during the formation of the black line. In this case, it may be that the nucleus receives once more a morphologically complete extra-nuclear centrosome produced in the ordinary way by division. Our observations are not conclusive with regard to these matters.

Considering the whole question from another point of view, it should be remembered that it is only among a percentage of the trypanosomes present during the periods of the maximum numbers of the parasites in the blood which can be found at any time to exhibit the formation of the black line, and we have to assume either that all the trypanosomes which ultimately form the latent bodies have passed through this metamorphosis, or that only some of them have done so.

We have no conclusive evidence upon this question. If, however, it should eventually be shown that only a fraction of the latent bodies are produced from trypanosomes which have passed through the black line metamorphosis, then it will become clear that there are two series of latent bodies, one class produced after the black line formation, and the other without this taking place. Should this be the case, the suggestion that the black line metamorphosis represents a peculiar form of the sexual act, or conjugation, which we have merely provisionally considered, will be found probably to be inaccurate. There will be an actual dimorphism among the latent bodies, and it will in this case be strongly suggested that the actual sexual act has yet to be discovered, and has been overlooked. We think it would be unprofitable to pursue this question further in the present Memoir. It is obviously a question that can only be properly considered after the phases of the life cycles of other trypanosomes are available.

In the introductory portion of this paper it was pointed out that there exists a complete discrepancy between "reduction" as apparently understood by Schaudinn,* and reduction as understood by biologists in general. Without throwing any reflection whatever upon the correctness of Schaudinn's observations in relation to the phenomena exhibited in the life cycle of *Trypanosoma noctuæ*, it is clear that, whether the process he describes exists or not, this process

* Loc. cit.

has nothing to do with chromosome reduction as ordinarily understood. His conception is a resurrection of Balfour and Minot's idea regarding the function of the polar bodies in the egg, and supposed corresponding structures in the male cells, as machinery whereby the physical representative of the opposite sex is got rid of more or less completely before fertilization.

In our observations upon *Trypanosoma gambiense* and other forms, in which the life cycle is possibly a complete cycle within the body of one host (see diagram, page 449), we have encountered nothing at all resembling the process stated to take place in the life cycle of *Trypanosoma noctuæ*. Prowazek,* dealing with the morphology and life cycle of *Trypanosoma lewisi*, maintains that the sexual act (in the form of ordinary conjugation) may take place either in the body of the rat-louse, or in the blood of an infected animal—the latter more rarely. According to this author, conjugation is preceded by a reduction process, which he describes in the following words:—

"Wie bereits erwähnt wurde, findet auf den mittleren Stadien "der Verdauung in Mitteldarm die Reduktion der Flagellaten statt, "die aber nicht gleichzeitig die beiden Kerne, den Blepharoplast und "den centralen Kern, erfasst; bald ist der letztere schon völlig "reduziert, während der erstere erst in den Prophasen dieser "Vorgänge steht und umgekehrt.

"Im centralen Kern wird vor der Reduktion zunächst das "Karyosom bedeutend deutlicher und intensiver färbbar, das "Chromatin wird körnig doch vereinigen sich bald diese Körner zu "einzelnen Strängen (Taf. II, fig. 23, 24), die schliesslich nach Art "von vier Reifen das inzwischen geteilte Karyosom umgeben. Dieses "Stadium möchte ich mit den Stadien der Chromosomen Paarung vor "der ersten Teilung der Metazoenspermatogenese vergleichen (Taf. II., "fig. 24). Später findet man in Kernhohlraum wiederum acht mehr "zerstreut liegende Chromosomen (Taf. II, fig. 26). Ein Stadium der "Vierergruppenbildung ist nicht sehr deutlich ausgebildet obgleich "Andeutungen in diesem Sinne vorhanden waren (Taf. II, fig. 25); "doch kann man wegen der Kleinheit des Objektes nichts sicheres "diesbezüglich aussagen, obzwar in dem abgebildeten Fall doch

* Prowazek, loc. cit., page 372.

"16 Chromosomen, die durch die zwei Teilungen auf vier reduziert "werden, gezählt werden könnten. Deutlicher waren die Bilder bei "*Herpetomonas*. Durch die endlich effektiv gewordene Teilung des "Karyosoms wird der erste Reduktionskorper gebildet, der selten als "ein dunkles, körniges Gebilde gegen das spitze Ende der Zelle "abdrückt, sondern meistens dicht am Kern selbst liegen bleibt "(Taf. II, fig. 30). Bald darauf vollzieht sich noch eine Teilung, "durch die der zweite Reduktionskorper gebildet wird. In fig. 31 der "Taf. II, bemerkt man terminalwärts diesen Reduktionskern, der ein "kleines Karyosom und die vier dicht verbackenen Chromosomen "enthält. Demnach muss der reduzierte Kern nur vier Chromosomen "besitzen. An dem sich reduzierenden Blepharoplast kann man "nicht so viel Details erkennen ; zunächst teilt sich der Blepharoplast "in zwei Teile (Taf. II, fig. 29), von denen der eine Teil durch eine "heteropole Spindel noch einer Reduktionsteilung unterliegt. Der "este Reduktionskorper übernimmt manchmal die undulierende "Saumgeissel und degeneriert erst ziemlich spät. In anderen Fallen "bleibt die Geissel an dem reduzierten Blepharoplast haften (Taf. II, "fig. 28, 29)."

Prowazek describes the nuclear division taking place during the fission of the parasites in the blood in infections with *T. brucei* and *T. lewisi* as being mitotic, and the nuclei as containing eight chromosomes. Our observations upon *T. brucei* and the other trypanosomes with which we have been working, are all similar in regard to this matter. When these animals have been properly preserved there appear to be no chromosomes produced, and the type of nuclear division during the fission of the animals is invariably amitotic, the extra nuclear centrosome and the nuclear substance dividing like drops. It is possible, of course, as in the case of the Ciliata, that the divisions become characteristically mitotic immediately before reproduction, but in none of the trypanosomes which we have examined (one of which, *T. equiperdum*, certainly runs its life cycle in a single host) have we encountered anything of the kind.

We have had these forms under continuous observation for more than a year, and for long periods at all hours of the day and night In the case of *Trypanosoma equinum*, we were at first inclined tc think that the division was of the mitotic type, but this inference was soon found to be simply due to the defective manner in which the

animals had been preserved and dried, or to other forms of indifferent fixation. In other words, there are often produced during fission of the animals under such circumstances appearances in their nuclei due to coagulation effects which may readily be mistaken for nuclear chromosomes. As the fixation becomes better, in all the forms with which we have hitherto dealt, such appearances can, however, be clearly shown to be illusive, and the division of the nucleus during fission to be invariably amitotic in character. The same inference is borne out by a study of the living animals.

Among the illustrations given by Minchin [Proceedings of the Royal Society, Vol. 78, Series B, No. 20, Plate 12], figs. 4, 8, 9, and 17 have been produced from specimens that have been dried and stained, and suggest the existence of chromosomes, but we are inclined to think that these appearances are simply due to the bad fixation methods employed, and are really quite misleading.

With regard to the nature of the nuclear division accompanying fission of the above-mentioned trypanosomes, our results are in complete accord with those of Laveran. Indeed, with regard to the nuclear reduction described by Schaudinn,* and finally by Prowazek, our observations have revealed nothing suggesting anything even analogous to these descriptions. Prowazek gives a series of figures illustrating this process in *Trypanosoma lewisi* (Pl. II, figs. 23, 24, 25). Here nuclei with eight chromosomes are said to be apparent. We cannot detect in the figures themselves the slightest suggestion of this being the case, and are inclined to think that the irregular blotches and strands, undoubtedly correctly drawn, have nothing to do with chromosomes, but are due to the manner in which the specimens have been preserved. In figs. 27, 28, 29, 30 and 31, the so-called reduction of the nucleus as well as the extra-nuclear centrosome (blepharoplast) is represented; but in none of these figures do we see any indication of either true chromosomes, true mitotic division, or true reduction as ordinarily understood. In fact, as far as the illustrations are concerned, we are unable to find, or to see, any indication of a reduction process.

It will thus be observed that the results we have obtained, especially in relation to *Trypanosoma gambiense*, but also equally

* Generations und Wirtswechsel, &c.

† Loc. cit.

among other forms to be described later, differ not only in degree but also in kind from those obtained on the one hand by Schaudinn, and on the other by Prowazek. The descriptions of Schaudinn, in so far as they bear at all upon the present work, do so, however, through the investigations of *Trypanosoma noctuæ*, which since it appears to be a form of trypanosome requiring more than one host for the completion of its life cycle, may very likely differ in the features of this life cycle from the more ordinary forms with which we have been concerned. On the other hand, Prowazek's results obtained in the case of *Nagana* (that is to say, from one of the trypanosomes considered in the present paper), so far as the nuclear changes during fission are concerned, differ entirely from our own; these latter fall directly into line with the observations we have made upon other forms, and are quite incompatible with the description of this process given by Prowazek in the case of *T. lewisi* or *T. brucei*. The question which now confronts us is upon what cause this difference of results depends. We are inclined to think that the difference of result is due to the methods which have been employed. We may as well say here, that from what we have gathered with respect to the different methods that have been generally in use, it appears that all the methods involving the drying of the blood before staining, or, in fact, any method involving drying at all, is, so far as nuclei are concerned, absolutely useless from a cytological point of view. Nothing relating to the delicate mechanism of mitotic division is generally preserved in cells, whether they belong to unicellular or multicellular organisms, when dried and stained with Romanowsky, Giemsa, or in any other manner. Even the resting nucleus itself under such conditions becomes a mere caricature of the actual structure.

When treated in this way, the irregular or regular blotches and streaks of stainable matter have nothing in common with, and do not represent, even in a relative or equivalent sense, the structures actually present in the cells. Anyone who wishes to verify this fact for himself will have no trouble in doing so if, for example, he makes a smear preparation from the testis of a rat, stains after the manner of Romanowsky, and then compares this with a properly fixed and stained smear, in the production of which ordinary cytological precautions have been observed. It is a curious fact that in a rat's testes under these conditions certain cells which really contain

sixteen *gemini* or heterotype chromosomes, when subject to the action of drying and Romanowsky, very often present (within the ill-formed area representing the nucleus) six irregular masses of stainable stuff resembling the so-called chromosomes of the dried trypanosomes. Such appearances are, however, certainly due to regularity of coagulation and shrinking during the drying of the cells, and have nothing in common with the real morphological structures, (the chromosomes), which either the living cells, or successfully-preserved cells possess. In view of these circumstances, we are inclined to regard many accounts of the existence of chromosomes, spindles, and even the assumed existence of mitotic division among trypanosomes, as conclusions which appear to be most questionable, and as requiring in all cases confirmation in a variety of ways which do not involve the violence dealt to the finer details of cell structure by drying.

Finally, we see in the case of *Trypanosoma gambiense* that the life history of this parasite as it lives in rats seems to be complete in the blood of the rat, and not in any way dependent for its completion upon the transference of the parasites into the blood of any other kind of host. In rats the latent forms pass gradually into trypanosomes, these in turn divide through many generations, and their multiplication is followed by a metamorphosis which, whether we regard it as a special form of sexual process, as a form of pathogenesis, or as a sexual stage, the fuller details of which have not yet been elucidated, seems undoubtedly to stand in one of these relationships to the normal cell multiplications preceding the formation of latent bodies. The stage in question results in the production of the latent bodies once more, and the cycle is complete.

It may be objected to this conception that, notwithstanding the cyclic development of *Trypanosoma gambiense*, still there may exist a possibility, or probability, of the transference of the trypanosomes into some other host where a further metamorphosis representing the sexual stage of the organisms is passed through. This, of course, may be so, but we have in the case of the trypanosome of *Dourine* a clear instance of a trypanosome life history, which, under normal circumstances, is not transferred into any other kind of host; and, under normal circumstances, *Trypanosoma equiperdum* must pass through whatever sexual stage it may possess, its whole life history in fact, in

the body of the horse. *Dourine* can, however, like sleeping sickness, be inoculated from host to host by simple transmission of blood as well as by coitus; in other words, the faculty of being transmitted by simple inoculation of blood is shared by *Trypanosoma equiperdum*, wherein no other host is usually involved, as well as by *Trypanosoma gambiense*. In these circumstances, it is simply natural, assuming flies to be the agents by which sleeping sickness is transmitted, to admit that this form of transmission may be merely in the nature of a mechanical transference, and have no more relation to the sexual act in the life cycle than has the artificial withdrawal of blood from a horse infected with *Dourine*. In other words, it would seem that the transference by flies in the case of sleeping sickness may have no more significance with respect to the life history of the parasite than has the direct inoculation of *Dourine* from horse to horse by means of a needle.

As we have already pointed out, the observations of Bruce, Dutton, Todd and Hanington* and others seem to indicate that the transference of sleeping sickness, when it is brought about by flies, is in the nature of a simple inoculation of blood, while it would appear that Dutton, Todd and Hanington incline further to believe that flies are not necessarily the normal means by which the propagation of sleeping sickness takes place.

They sum up the situation in this respect as follows:—"It seems "that all the results are in conformity with the hypothesis that "*Glossina palpalis* transmits *Trypanosoma gambiense*, and that it "is probably not able to do so when the space between the trans- "mitting feeds exceeds 48 hours; this conclusion is, nevertheless, to "our minds a most unsatisfactory one, if we are to regard these "Glossinae as the chief or only carriers of *Trypanosoma gambiense*. " . . . It certainly seems possible that mechanical transmission "by tsetse flies cannot alone be responsible for the rapid spread of "sleeping sickness of recent years."

These questions, however, open out a wide field of enquiry, which it is at present unprofitable further to discuss.

* Loc. cit.

APPENDIX I

METHOD OF PREPARING AND STAINING WET FILMS, USED DURING THE FOREGOING INVESTIGATIONS

Place a very thin layer of albumen-glycerine on a clean slide. The best method is perhaps to put a drop the size of a large pin's head on the slide, and to spread this with a clean duster over the slide. On top of this layer spread a drop of blood in the usual way, and dip the slide, while wet, into the fixing solution (Flemming's strong solution was usually used). Leave it for about five to ten minutes, wash immediately in water, and pass the slide through alcohols in consecutive order, increasing by 10 per cent. at a time to absolute alcohol. Then back from absolute into 80 per cent. alcohol in which is contained iodine and potassium iodide. In order to prepare the solution of 80 per cent. alcohol containing iodine and potassium iodide, make up a concentrated solution of potassium iodide in water, and iodine in alcohol, mix them together, and add them to some 80 per cent. alcohol until the mixture becomes a dark brown colour. Leave the slide in this from five to ten minutes, and then bring it into 30 per cent. alcohol. Use for staining either aniline safranine (nach Babes), or the following solution:—Prepare a concentrated watery solution of safranine (Grübler) and a concentrated alcoholic solution of safranin, mix them in equal parts, and then add pure aniline oil. Shake from time to time, and leave the solution to ripen for three to six months. Stain in this solution for from half an hour to two hours. Wash off the safranin, and stain afterwards with polychrome methylene blue [one gramme methylene blue purissimum medicinale (Höchst) 100 c.cm. distilled water and ·5 gramme sodium carbonate, left in an incubator to ripen; the older the solution the better the staining properties]. Wash off the methylene blue, and differentiate with Unna's orange tannin, as long as the blue stain comes out. Bring the slide up through alcohols, as above, into absolute alcohol. The film has then a reddish colour. Now dip the slide into aniline oil until the reddish colour changes to a purple-blue tinge; the aniline oil takes out at the same time the excess of blue stain left by the orange tannin. Clear in xylol. Mount in Canada Balsam under a coverslip.

APPENDIX II

MODIFICATION OF HEIDENHAIN'S HAEMATOXYLIN METHOD, USED DURING THE FOREGOING INVESTIGATIONS

Fix and treat the film in the way described in Appendix I. Clear the slide from the alcohol containing iodine and potassium iodide, and pass it through successive alcohols (as in Appendix I) into water. Stain in a $3\frac{1}{2}$ per cent. solution of iron alum for one hour, wash this off, and stain with the following solution:—·5 gramme haematoxylin dissolved in 100 c.cm. distilled water, to which, after the haematoxylin has dissolved, a few drops of concentrated watery solution of lithium carbonate is added. Stain for half an hour, and then differentiate in the usual way with iron alum.

DESCRIPTION OF FIGURES

In all cases, unless otherwise stated, the figures have been drawn with a long tube Zeiss, 2 mm. apo. objective and 18 or 27 eyepiece.

PLATE XXXVIII

Trypanosoma gambiense

Figs. 1, 2, 3 from peripheral circulation stained with iron haematoxylin.

Figs. 4 and 5 stained with Breinl's stain.

Fig. 1.—Trypanosome in the resting condition. Nucleus single, not dividing. Intra-nuclear centrosome single.

Fig. 2.—Trypanosome showing early stages in division of nucleus, intra-nuclear centrosome divided; extra-nuclear centrosome also divided, new flagellum growing.

Fig. 3.—Trypanosome in the same stage as preceding. Intra-nuclear centrosome completely divided; the new flagellum is also seen during the course of its development.

Fig. 4.—Trypanosome is in the same condition as fig. 1, showing also the metabolic granules in different parts of the cell.

Fig. 5.—Trypanosome wherein two undulating membranes have been formed, showing also the metabolic granules.

Fig. 6.—Trypanosome in much the same condition as in fig. 1, but showing early stages in development of the new flagellum.

Fig. 7.—Later stage in the division, showing the mode of division of the intra-nuclear centrosome, amitotic fission of the rest of the nucleus, and the duplication of the extra-nuclear centrosome.

Fig. 8.—Trypanosome showing three flagella and three nuclei in different stages of division.

Fig. 9.—The same showing three flagella and four nuclei.

Fig. 10.—The same showing two flagella and two nuclei.

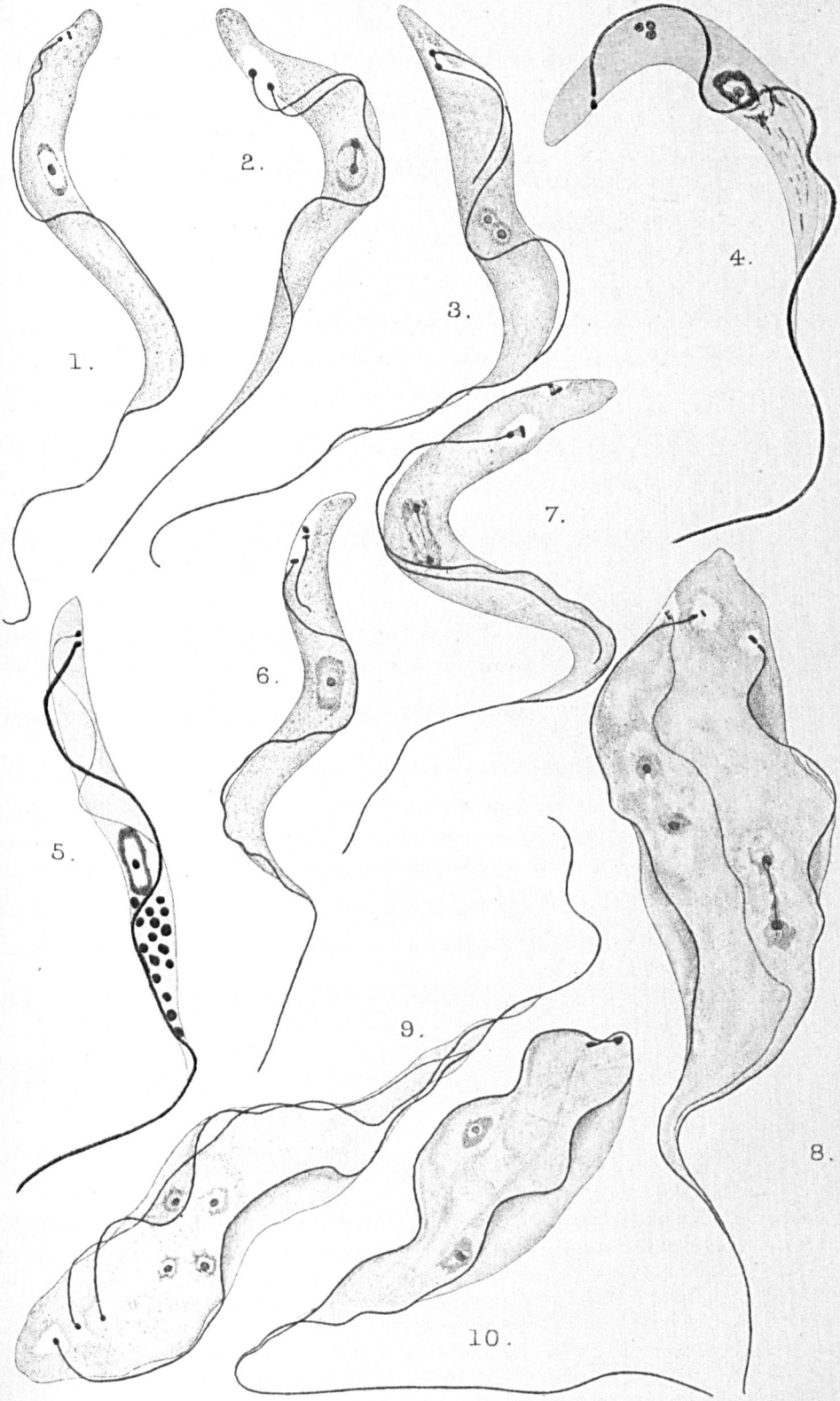

PLATE XXXIX

Trypanosoma gambiense

Figs. 11 to 14 stained with Breinl's stain.
Figs. 15 to 21 stained with the modification of Heidenhain's haematoxylin.

Fig. 11.—Trypanosome showing division of the intra-nuclear centrosome, and the nuclear substance. Also multiplication of the intra-nuclear centrosome so as to form a group.

Fig. 12.—Trypanosome showing mode of amitotic separation of the nuclei. Multiplication of the intra-nuclear centrosome, and the formation of an independent group of these bodies. In this and the preceding figures metabolic granules are also seen.

Fig. 13.—Later stage in the fission of a trypanosome. The flagella are being detached from one another at the thin end of the cell. The cell body is dividing from this end towards the other. The nuclei are already divided. Metabolic granules are scattered throughout the spongioplasm.

Fig. 14.—Later stage in the division of a trypanosome, showing the manner in which the daughter cells separate.

Fig. 15.—Trypanosome, at one of the maximum periods of the infection, showing a single flagellum and resting nucleus, and also the origin of the black line from the extra-nuclear centrosome.

Fig. 16.—A similar stage wherein the black line has reached the neighbourhood of the nucleus.

Fig. 17.—The same.

Fig. 18.—Trypanosome showing the black line coiled upon itself towards the intra-nuclear centrosome.

Fig. 19.—Trypanosome showing early stages in the degeneration of the black line, and its later direct association with the nucleus.

Fig. 20.—Similar stage, wherein the intra-nuclear centrosome has become divided.

Fig. 21.—So-called involution stage, showing resting nucleus and multiplication of the intra-nuclear centrosome.

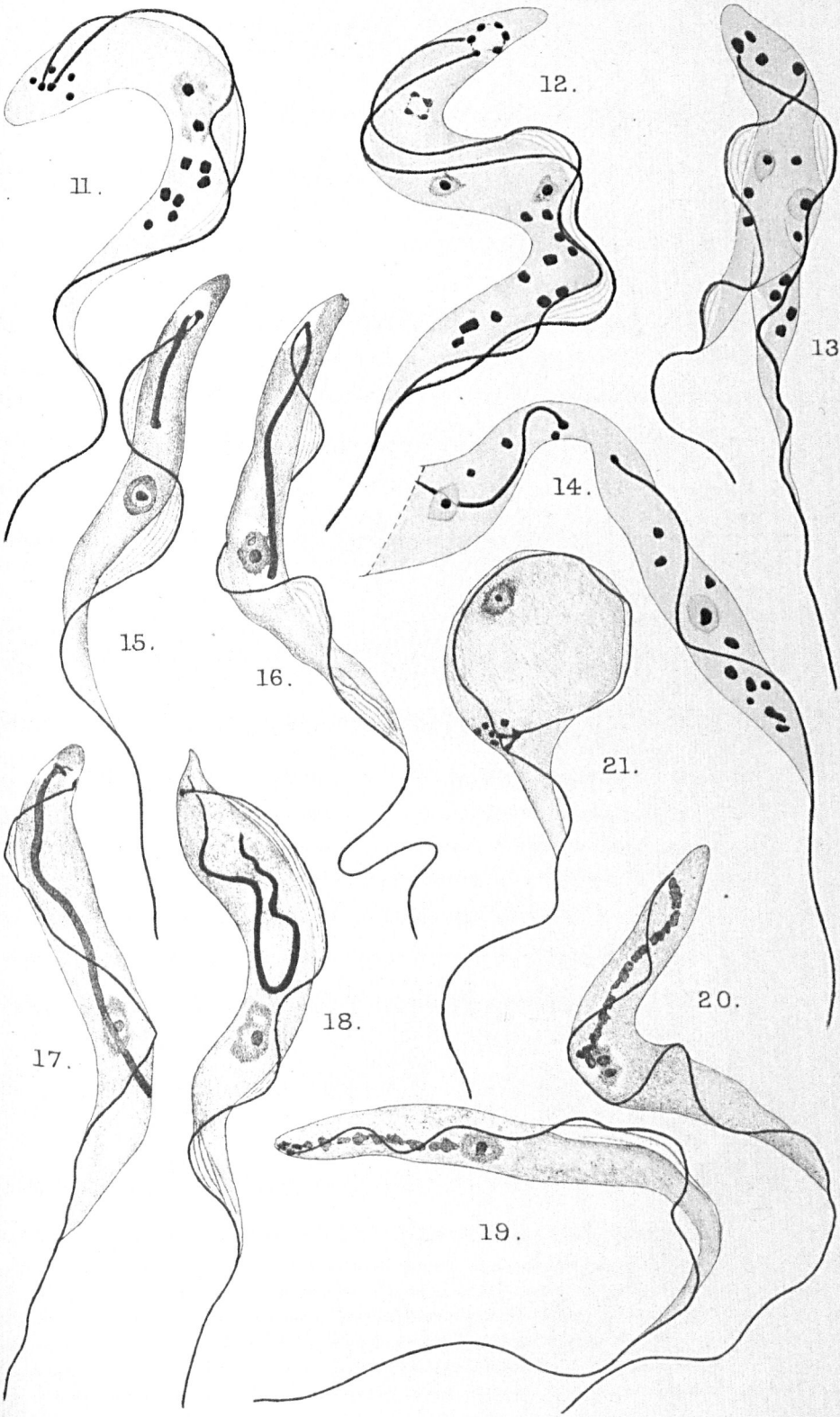

PLATE XL

Stages in the Metamorphosis of Trypanosoma gambiense in the Organs

Figs. 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, stained with a modification of Heidenhain.

Figs. 25 and 33 stained with Breinl.

Fig. 22.—Trypanosome during the decrease of the parasites in the blood of a rat, showing alteration in the nucleus marked by the formation of a vacuole.

Fig. 23.—The same.

Fig. 24.—One of the common forms in the lung at this period, showing the same changes in the nucleus.

Fig. 26.—Low power view of trypanosome at this period, showing the detachment of the latent body from the parent cell.

Fig. 27.—Latent bodies. The nucleus is attached to a vacuole and both are surrounded by a thin film of protoplasm. The nucleus contains a single intra-nuclear centrosome.

Fig. 28.—Latent bodies. To the left the intra-nuclear centrosome is shown divided. To the right stages in the division of this body and the extrusion of one daughter element from the nucleus.

Fig. 29.—Latent bodies showing origin of a new flagellum from the intra-nuclear centrosome.

Figs. 30, 31.—Later stages in the formation of small trypanosomes from the latent bodies.

Fig. 32.—Latent body from the spleen of a rat infected with *Trypanosoma brucei*, showing nucleus, vacuole, and the origin of the flagellum from the intra-nuclear centrosome. (Compare fig. 29.)

Fig. 33.—Trypanosome showing early stages in division of the nucleus.

Fig. 34.—Trypanosome drawn to show the Schaumplasma structure of the protoplasm.

Figs. 34 to 36.—The same.

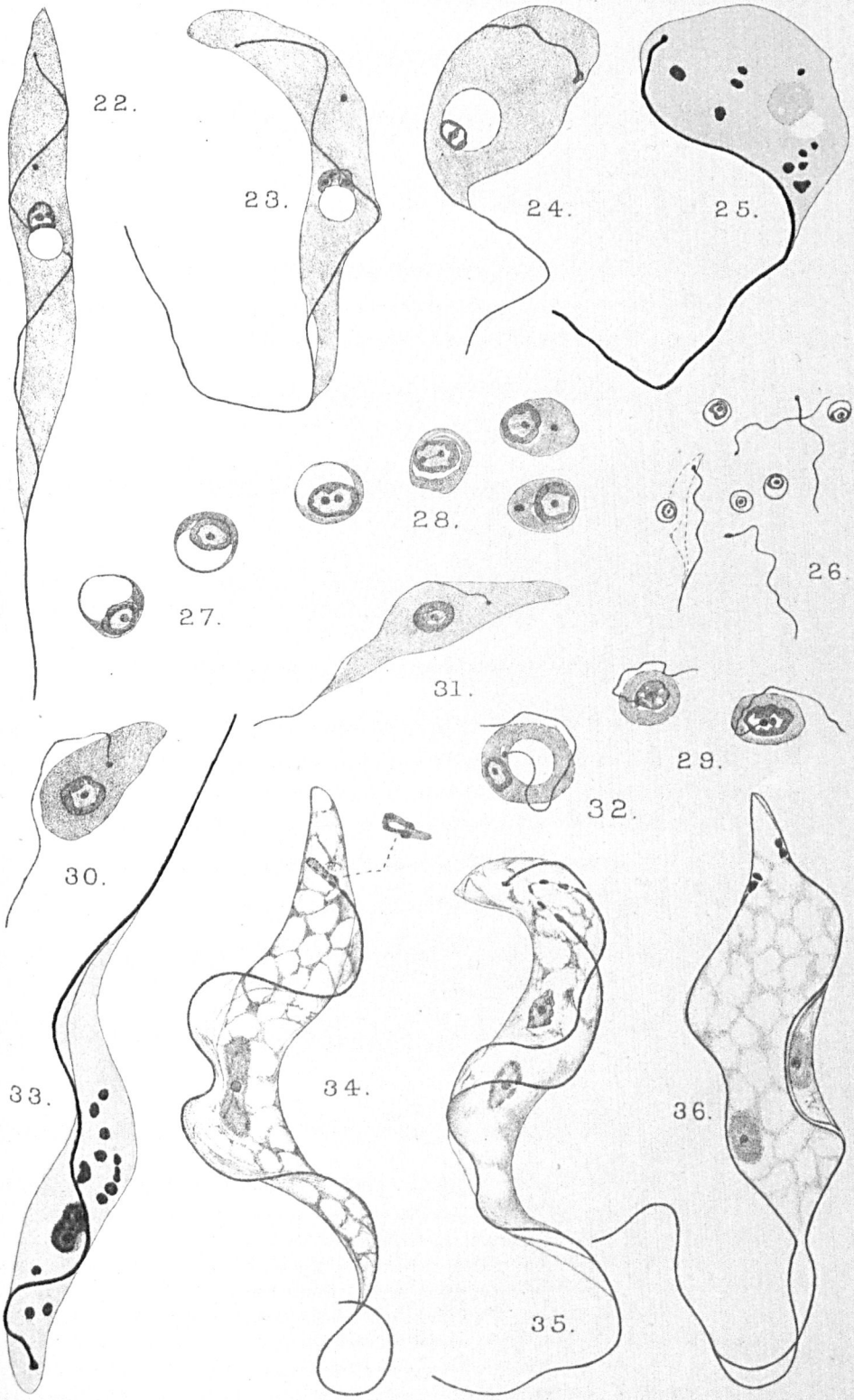

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PLATE XLI

Trypanosoma gambiense and Trypanosoma brucei

Figs. 37-41, *T. gambiense.*
Figs. 42-46, *T. brucei.*
Figs. 37, 38, 40, 41, 42, 43, 45, 46, Breinl's stain.
Fig. 44, Heidenhain.

Fig. 37.—Trypanosome in the blood of a rat after treatment with atoxyl, showing rounding up of the cell body.

Fig. 38.—Further stage in this process, flagellum is still attached. A slight modification of a membrane is apparent round the periphery of the cell.

Fig. 39.—The same, flagellum not visible.

Fig. 40.—Later stage in the formation of the cyst. The membrane more apparent.

Fig. 41.—Trypanosomes killed by atoxyl in the blood.

Fig. 42.—*Trypanosoma brucei* in resting condition, showing structure of the nucleus and relation of the schaumplasm.

Fig. 43.—The same, showing the division of the extra-nuclear centrosome.

Figs. 44 to 46.—Later stages of division.

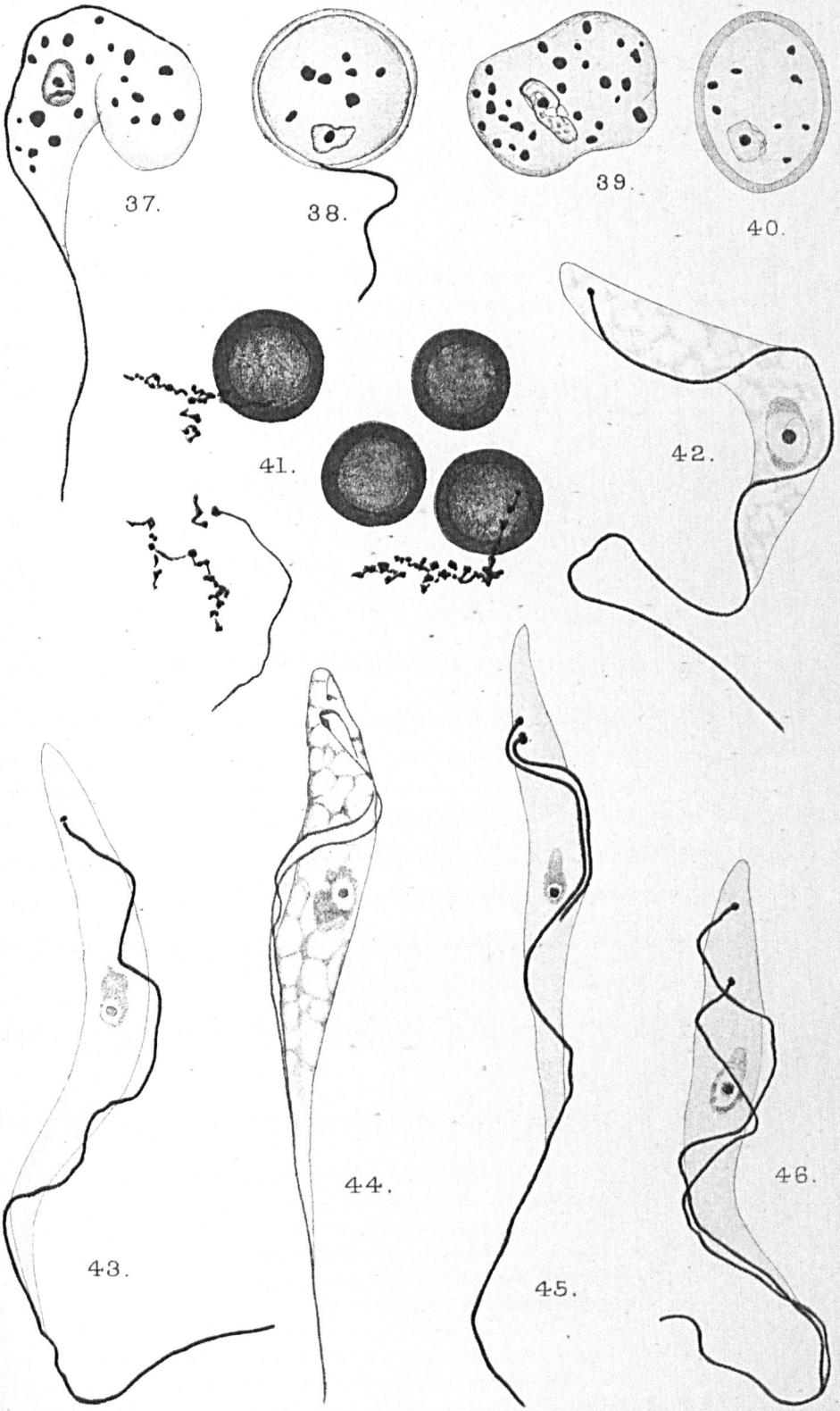

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PLATE XLII

Trybanosoma equinum

Figs. 47, 49, 50, 51, 52, 53, 54, stained with Breinl.
Fig. 48 stained with modification of Heidenhain.

Fig. 47.—Resting stage of the trypanosome.

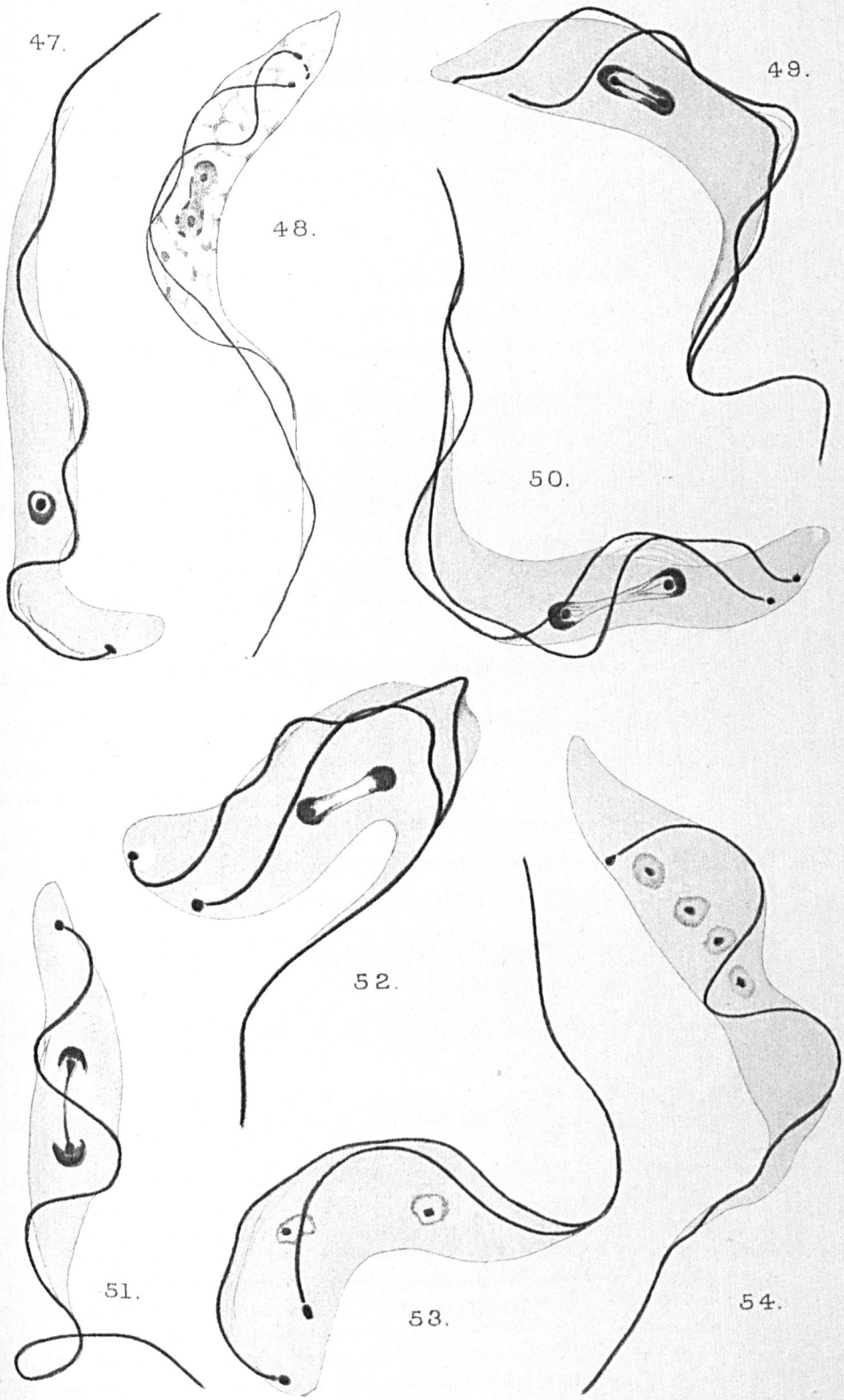
Fig. 48.—Stage showing formation of the new flagellum and division of the nucleus.

Fig. 49.—Trypanosome showing details of the division of the intra-nuclear centrosome and the nuclear substance.

Figs. 50, 51.—Trypanosomes showing later stages of the same process.

Figs. 52, 53.—Trypanosomes showing still later stages in the division of the nucleus and the characters of the intra-nuclear centrosomes.

Fig. 54.—Trypanosome wherein the nucleus has divided into four constituents, although there is only one flagellum.



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