The Life-history of Trypanosoma equiperdum.

By J. E. Salvin Moore, Professor of Experimental and Pathological Cytology, University of Liverpool, and Anton Breinl, Director of the Runcorn Research Laboratories, Liverpool School of Tropical Medicine.

(Communicated by Sir Rubert Boyce, F.R.S. Received March 9,—Read March 12, 1908.)

[Plates 8 and 9.]

In May, 1907, we* showed that the study of the parasite of sleeping sickness (*Trypanosoma gambiense,* Dutton), as it appears in the blood of rats artificially infected with the disease, revealed a cyclical metamorphosis, and that this cyclical metamorphosis corresponded closely to the alternating phases of presence and absence of trypanosomes in the blood. At the same time it was found that the cyclical metamorphosis in the parasites corresponded less closely, but still unmistakably corresponded, with the successive alternations of condition that characterise the clinical aspects of the malady in the host. The features of the life-cycle of the parasites of sleeping sickness as they appear in the blood during infection in rats are remarkable, and may be briefly repeated for reference. From the time of inoculation the parasites multiply in the blood through amitotic division of the nucleus,† and longi-

* Note on "The Life-history of the Parasite of Sleeping Sickness," 'Lancet,' p. 1219, May 4, 1907; "The Cytology of the Trypanosomes," Part I, 'Ann. Trop. Med. and Parasitology,' vol. 1, No. 3.

What is called the nucleus of those trypanosomes with which we are acquainted, when fixed in Flemming's fluid, or by any other appropriate method, does not appear to show any trace of chromosomes. It consists of a central sphere, intra-nuclear centrosome (Salvin Moore and Breinl, *loc. cit.*) enclosed by a mass of substance, which may be made to stain in a different manner from the sphere. When the nucleus divides, the interior sphere first elongates, then assumes a dumb-bell shape, and finally breaks nto two spheres, the outer substance collecting these new centres (intra-nuclear centrosomes), so as to form two smaller nuclei with the same structure and appearance as the first. The reasons for regarding this structure as a nucleus, *i.e.*, as equivalent in a morphological sense to the nuclei of other protozoa, and protophyte bodies, and to the nuclei of the metazoa and metaphites, are simply these: The structure in question bears a superficial resemblance to the nuclei with which biologists are familiar. It divides in the amitotic fashion, *i.e.*, as if it were a viscous drop, and owing to the existence of the intra-nuclear centrosome, and to the manner in which this body appears te the fission, the whole structure bears a close and striking resemblance to the undoubted nuclei of some unicellular organisms, such as Euglena. There is, however, this difference: the nuclei to which that of the trypanosomes bears the closest resemblance, such as those of the Euglenæ, have been found to possess chromosomes. Chromosomes have been described as appearing during the divisions of the nuclei of

tudinal fission of the trypanosomes, until a vast number of trypanosomes are produced and the infection of the blood reaches a first maximum. (See chart, p. 290.)

After such a period has been reached, the number of parasites in the blood falls until it may be impossible to detect their presence. But subsequently parasites reappear, and a second maximum is reached, and so on. The alternations of these maxima and minima in rats during an infection with *T. gambiense* are illustrated in the chart given on p. 290. In the case of man,

trypanosomes by Schaudinn, Prowazek, Minchin, and others, but we have been uniformly unable to confirm these observations, and have reached a diametrically opposite conclusion, namely, that in the case of *Trypanosoma gambiense*, *T. equinum*, *T. lewisi*, *T. brucei*, and *T. equiperdum*, chromosomes are not present, and do not exist, at any rate during those forms of division which take place in the blood of a mammal infected with these trypanosomes.

[*Footnote added April* 13, 1908.]

Minchin, in the 'Quart. Journ. Micro. Sci.,' vol. 32, Part II, describes the structure we term extra-nuclear centrosome as the kineto-nucleus, and a different structure, a small swelling or bead, at the end of the stainable portion of the flagellum, as the blepharoplast or centrosome. The reasons for regarding the body we term the extra-nuclear centrosome as a centrosome are as follows :—

The extra-nuclear centrosome appears to be derived from the intra-nuclear centrosome, and the intra-nuclear centrosome (karyosome nucleolus) appears to be a structure which is closely similar in its appearance and behaviour to the undoubted intra-nuclear centrosomic, or blepharoplastic, bodies within the nuclei of Euglena, and many protozoa. This conception is strengthened by the fact, originally observed by one of the present authors in 1894 (Moore, 'Internat. Monatschr. f. Anat. Physiol.,' vol. 11), that the true metazoan centrosome is regularly incorporated within the flagellated male gametes of these organisms. In such gametes the centrosomes become more or less definitely related to the flagella, just as the extra-nuclear centrosome is related to the flagella of the trypanosomes. In many metazoan gametes (reptiles) the flagellum abuts directly upon the centrosome. In others (some mammals) this is not so, the flagellum ending in a small bead corresponding to the blepharoplast of Minchin. In such cases the true centrosomes remain quite detached, as in some trypanosomes, for example in *T. lewisi*. The bead, when it exists on the base of the metazoan flagellum, is not a centrosome, but simply an enlargement of the proximal end of the flagellum. For this reason we do not regard the blepharoplast of Minchin as equivalent to the blepharoplastic, or centrosomic, structures of other cells, but we regard the bead in question as possibly equivalent to the swelling at the end of the flagellum found among many metazoan cells and gametes. Similarly, we regard the name kineto-nucleus, when applied to what we call the extra-nuclear centrosome, as entirely inappropriate. In the first place, in so far as this structure can be homologised with any structure known in other cells, it appears, as we have said, to have the same relationships as the centrosome. In the second place, it does not appear to have any attributes, except the capacity to divide (a capacity which, of course, is shared by all centrosomes), in common with what is understood as a nucleus. Minchin draws attention to the large size of the extra-nuclear centrosome (kineto-nucleus, nucleolus blepharoplast) in some trypanosomes, as indicating that this structure is not of the nature of a centrosome ; but we are unable to see that the dimensions of this body affect the matter in any way, for the undoubted centrosomic or blepharoplastic structures of many male plant gametes are similarly large, if not larger.

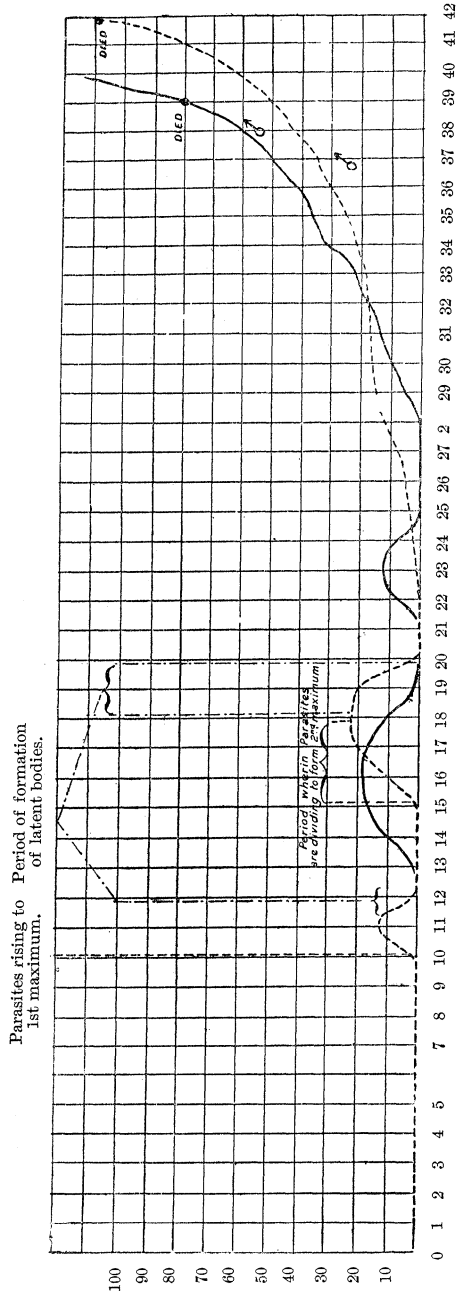


Chart of Two Male Rats inoculated with *Trypanosoma gambiense*.

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.

where the infection runs a relatively prolonged course, the maxima and minima alternate again and again. The alternation is somewhat irregular, and does not possess the definite character of the analogous periods in a

typical malarial infection. In the case of *T. gambiense* it has been found* that amitotic division of the parasites proceeds up to the first maximum, but at this period other changes are also apparent in the parasites of the blood (see Diagram I, *a*, *b*, *c*.) At this period numbers of parasites may be found in

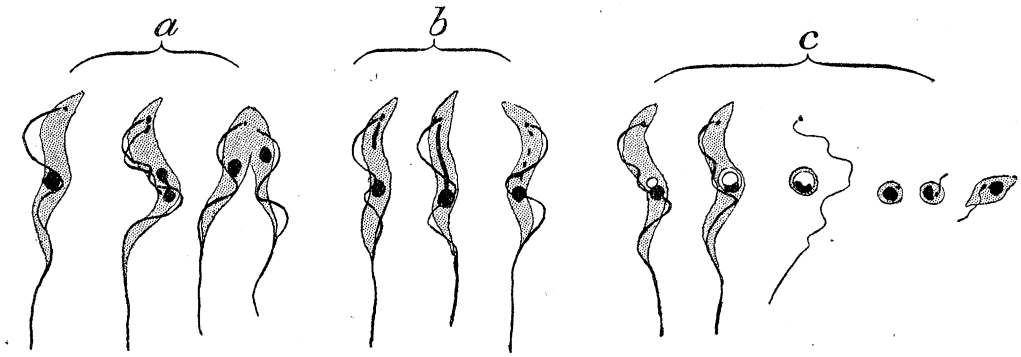


DIAGRAM I.—Showing the cyclical metamorphosis occurring in *Trypanosoma gambiense* in the blood from an infected rat. *a*. The longitudinal fission of the parasites. *b*. The interaction between the extra-nuclear centrosome and the nucleus. *c*. The formation of the latent bodies and the early stages of their redevelopment into trypanosomes.

which a thick band is seen to be growing out from the extra-nuclear centrosomes (blepharoplast) which lies about the base of the flagellum (Diagram I, *b*). This band extends down the interior of the body towards the nucleus. After a time the band enters into connection with the nucleus, and then breaks up and disappears.† At or near the maxima there is thus an interaction between the extra-nuclear centrosome and the nucleus. When the maximum has

* Salvin Moore and Breinl, *loc. cit.*

† Referring to our communication, 'Annals of Tropical Medicine and Parasitology,' *loc. cit.*, Swellengrebel ('Compt. Rend. Soc. Biol.,' vol. 64, 1908, No. 2) appears to regard the formation of the stainable band as in some manner due to a form of degeneration. We are, however, entirely unable to agree to this conception for the following reasons. The formation of the band occurs only at a particular period of the infections, and at other periods it is not induced either by the administration of substances such as atoxyl or by allowing the trypanosomes to die. A clearer demonstration of the erroneous conclusion drawn by Swellengrebel is, however, found in the facts relating to *T. equiperdum;* here a similar process regularly occurs (see this paper) and after it has occurred the trypanosomes again pass through division, this fact demonstrating that the process to which we refer can have nothing to do with degeneration and is, on the contrary, part of a cycle occurring during the ordinary course of development.

Swellengrebel appears to us to be again in error in supposing that the formation of the stainable band is connected with the production of trophic granules. That this is not the case can readily be seen by the use of stains such as that of Breinl or modifications of the iron hematoxylin method, whereby the remains of the band and the band itself stain quite differently to the trophic granules. Moreover, before any stainable band is produced the trophic granules are numerous and must have arisen from some other source.

been reached, the parasites in the blood rapidly diminish in number, and during the period of diminution large numbers of trypanosomes may be encountered in the lungs, the spleen, and the bone-marrow (but at the same time also in the blood), wherein a profound and rapid change is taking place. The nucleus becomes more compact (see Diagram I, *c*). There arises a vesicle in connection with it, and eventually a complex structure (latent body) is produced,* consisting of the nucleus and vesicle, and enclosed by a delicate covering of cytoplasm. The latent body becomes detached from the rest of the protoplasm of the cell, and the whole remaining portions of the trypanosome now rapidly degenerate and disappear, so that in a short time we find nothing but numbers of the complex spherical latent bodies remaining. These eventually become chiefly lodged in the spleen, the bone-marrow, and other organs. The process we have just described, or, at least, parts of it, have undoubtedly been seen in other trypanosomes, but not in Gambiense, by various observers, and they have generally been interpreted as a form of degeneration. That this is not necessarily so seems now, however, to have become clearly demonstrated. In the case of *T. gambiense* we were able to find, during the period when no parasites were present in the blood, that the latent bodies still persisted in the organs, and that many of these gradually grew larger by developing a cytoplasmic investment, a new extra-nuclear centrosome, and afterwards a flagellum, such forms returning eventually to the form of ordinary trypanosomes.

We have thus in the case of the sleeping sickness parasite a cyclical metamorphosis going on in the blood of the infected animal. The parasites pass through divisions until the interaction between the extra-nuclear centrosomes and the nucleus. From this period they proceed to the formation of latent bodies, and subsequently to the development from the latent bodies of ordinary trypanosomes once more.

The interaction between the nuclei and the extra-nuclear centrosome (blepharoplast) may, as we have pointed out,† suggest a novel form of sexual process, and the whole series of changes may indicate that the life-cycle of *T. gambiense* is in reality completed in the body of the rat or man, and not necessarily related to the transference of the parasites to any other form of host. We know, however, that in the form *T. gambiense* the parasites can be transmitted by the fly *Glossina palpalis*.

The observations of Schaudinn apparently indicate that in the case of *Trypanosoma noctuæ* the sexual stage (which is described by him as quite unlike the process to which we have just drawn attention) occurs in the body

* Salvin Moore and Breinl, *loc. cit.*

† Salvin Moore and Breinl, *loc. cit.*

of a mosquito. This mosquito appears thus to stand in the same relation to the owl *Athene noctua* as *Glossina palpalis* does to man. It is perfectly natural therefore to suppose that whatever cycle we may have found in the blood during infections with *T. gambiense*, the real sexual phase may occur within *Glossina palpalis* or some other fly. From the information which at present exists this line of criticism cannot be answered through observations upon infections with *T. gambiense*. On this account, and pending further investigation in relation to *T. noctuæ*, we have turned our attention to the parasites of the horse disease known as "Dourine," and caused by *T. equiperdum*. Dourine* is not necessarily transmitted through any fly or intermediate host, but by direct contact between animals that have become infected. In this case we have, then, a trypanosome the life-history of which is not necessarily complicated by transference through an intermediate host. Whatever sexual phase there may be in the life-history of this parasite must be passed through in the body of the horse.

If *T. equiperdum* be injected into rats, the parasites multiply and kill the animal in about four days after their first appearance in the blood, which occurs about three days after the inoculation.† The disease in rats thus reaches a first maximum, and the animal dies without being able to overcome the invasion even temporarily, as would appear to be the case in infections produced by *T. gambiense* in rats. The method of investigation has been as follows:—From the time the trypanosomes appear in the blood a very large number of slides have been prepared at short intervals up to death, and for a short time afterwards. Owing to the manner in which the disease is transmitted, special attention was paid to the fluids which collect in the various superficial swellings that are produced, for it naturally seemed possible that a phase of the life-history might occur in such positions in relation to the transmission of the parasites. The results of a prolonged investigation of this matter have, however, revealed nothing but the presence of ordinary trypanosomes,‡ and it is thus indicated that the transference takes place by means of the ordinary trypanosome encountered in the blood, possibly through the existence of slight abrasions on the animals that become infected, or more probably through the capacity of the trypanosomes to invade a mucous membrane, even if it is intact.

* The strain we have used was obtained through the courtesy of Geheimrath Professor Ulenhuth, in Berlin.

† The best general account of dourine is contained in the works of Laveran and Mesnil's 'Trypanosomes et Trypanosomiases,' Paris, 1904.

‡ In rabbits, when few trypanosomes appeared in the blood it is interesting to note that in the fluids from the swollen vagina there existed many more parasites than in the blood.

Preparations of the blood during early stages of infection show the trypanosomes to be increasing in numbers through rapid longitudinal fission, accompanied by amitotic division of the nucleus and the extra-nuclear centrosomes (Plate 8, figs. 1—4). As the disease advances, two series of structural changes in the parasite become apparent; one of these (figs. 6—10) consists in a gradual increase of the nuclear substance towards the side away from the extra-nuclear centrosome, until, after forming a distinct protuberance, the mass separates from the nucleus and passes away towards the free end of the flagellum in the manner represented in figs. 8, 9, and 10.

It is only possible at present to describe the existence of this process. It may be related to the formation, or rather the renewal, of the so-called "trophic granules,"* but we cannot decide this matter at the present time. The other process to which we have referred is of a totally different order. Towards the end of an infection, that is to say, on the third day after the appearance of the parasites in the blood, numbers of trypanosomes are observed, wherein the extra-nuclear centrosomes become conspicuously large (figs. 11, 12), and at the same time there exist others, in which it is seen that the extra-nuclear centrosome is budding off a large mass towards the nucleus. This mass becomes detached, and can be found in many individuals passing away towards the nucleus (figs. 12, 13, 14). There is often a distinct, but faint, suggestion of a protoplasmic thread still connecting the detached body with the extra-nuclear centrosome at the base of the flagellum (figs. 12, 13, 14). While this process is going on the intra-nuclear centrosome moves towards the extra-nuclear centrosomes, as in figs. 13, 14, and in a number of trypanosomes stages may be found in which the detached portion of the extra-nuclear centrosome is seen to pass completely through the body of the trypanosome until it becomes applied to the nucleus, as in figs. 15, 16. Some time later, the detached portion of the extra-nuclear centrosome merges with, and becomes indistinguishable from, the nuclear substance, and the trypanosomes again pass through division, as in figs. 16, 17 (Plate 9). As division sometimes begins again before the detached centrosome is fused with the nucleus, the process can have nothing to do with any sort of degeneration.

The process we have just described in the parasite of dourine is clearly analogous to the production of the stainable band in *T. gambiense*. It differs only in there being a detached mass which passes from the extra-

* It is curious to note that Laveran and Mesnil, *loc. cit.*, p. 273, are under the impression that one of the specific characters of *T. equiperdum* is constituted by the absence of protoplasmic granulations; whereas, on the contrary, we have found them in abundance. It is impossible to say upon what this difference of condition depended.

nuclear centrosome to the nucleus instead of a continuous band. As a matter of fact, the difference is really less than this, for in *T. equiperdum* the extra-nuclear centrosome not only seems to be connected with the detached portion, but also several portions may be detached, one after another. (Compare Diagrams I and II, *a*, *b*, *c*.)

The phase wherein the detachment of a portion of the extra-nuclear centrosome which passes to the nucleus takes place is of very short duration, and at the same time appears to affect the great majority of the parasites present in the blood, just in the same way that an epidemic of conjugation among infusoria will affect at the same time a whole colony. In the specimens from which the figures are given the process was in full swing at 10 A.M., but by 5 P.M. had come to an end.

At the time this process is going forward, and immediately afterwards, still further changes take place, corresponding to the changes which in *T. gambiense* precede the production of the latent bodies. But the latent bodies of dourine are very much larger than those in *T. gambiense*, and consequently their formation is proportionately less difficult to elucidate.

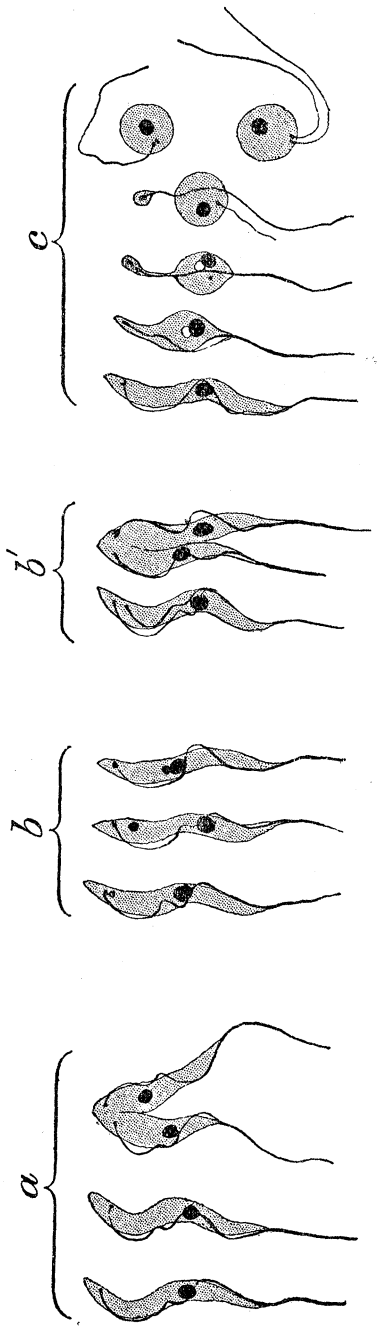


DIAGRAM II.—Showing the cyclical metamorphosis occurring in *Trypanosoma equiperdum* in the blood of an infected rat. *a*. The longitudinal fission of the parasites. *b*. The interaction between the extra-nuclear centrosome and the nucleus. *b'*. The longitudinal fission which occurs after the interaction between the extra-nuclear centrosome and the nucleus. *c*. The formation of the latent bodies and the growth of their flagellæ.

When the process of translocation of a portion of the extra-nuclear centrosome has come to an end, and the parasites have again passed through

several longitudinal fissions, a certain number of them are seen to become shorter, and when subject to the action of Breinl's stain are relatively blue, in contrast to the purple coloration of the remaining parasites (figs. 19, 20, 21, 22). In such altered forms it is seen that the extra-nuclear centrosomes become related to a protoplasmic elongation, produced perhaps by the rounding up of the protoplasm of the animal's body (figs. 20—22). This elongation becomes finally club-shaped, and the extra-nuclear centrosome, together with the flagellum, becomes detached from the rest of the cell, which is now more or less round (figs. 21—23). In many cases quite a considerable portion of protoplasm is detached along with the extra-nuclear centrosome, and the detached structure may present, at first sight, very much the appearance of a spermatozoon (figs. 23, 24).

In some cases it seems that when this process is going forward, the extra-nuclear centrosome having divided, one half of this structure passes down the stalk of the protoplasmic club and enters the rounded mass. In any case, however, at the time the extra-nuclear centrosome has become detached, a new centrosome becomes visible in the remaining round cell (figs. 23, 24, 25). From this there grows out an exceedingly fine fibre, which is much more delicate than the ordinary flagellum (figs. 23, 24, 25). The new extra-nuclear centrosome divides, and after a time a second flagellum grows out from the second extra-nuclear centrosome contained in the round form (figs. 25, 27). One peculiarity in relation to these new flagella is their great length, this often being in the proportion of 7 : 4 when contrasted with the length of the flagellum of an ordinary trypanosome.

The changes we have just described appear ordinarily to take place before the death of rats infected with *T. equiperdum*, but may be encountered also at, and for some time after, death, from which facts it might be natural to suppose that they are related to the changed condition occurring during the approach of death.

We have, however, found no evidence for this supposition. In none of the changes during the production of the round forms or latent bodies is there the least suggestion of degeneration. The growth of the new flagella and the division of the extra-nuclear centrosomes in the round forms are entirely against such a view. This latter conception is confirmed by many experiments we have made. Thus, if a rat be killed at the time the body derived from the extra-nuclear centrosome is passing towards the nucleus in large numbers of the trypanosomes, no changes analogous to those we have described take place in the blood of the dead animal. We have encountered in such cases, as time goes on, nothing but degeneration and disintegration of the trypanosomes. Again, in many cases the infection of dourine kills the

rats before the life-cycle in the parasites has reached the point at which the interaction between the extra-nuclear centrosome and the nucleus occurs, and here also it is found that after death no changes take place in the trypanosomes other than those related to their degeneration, or in any way corresponding to the formation of the latent forms.

Still further, we have at various periods of the infections abstracted blood, and watched the condition of the parasites until degeneration is becoming general, and in these cases also have encountered nothing comparable to the changes we have described in relation to the formation of the latent bodies. The change is thus related to a particular stage of the development of the trypanosomes in the blood.

It will be remembered that the formation of the latent bodies in *T. gambiense* takes place at, and immediately after, the periods of maximum number of the parasites in the blood, and the immergence from the latent bodies once more occurs a considerable time later. The infection with *T. equiperdum* in rats only progresses to a first maximum, during which the rat dies. We find also that it is only in those rats which have resisted the infection for a sufficient period that the formation of latent bodies in large numbers takes place. It should, however, be pointed out that, even on the second day after the appearance of the trypanosomes in a rat infected with dourine, a few trypanosomes with club-shaped projections, and a few latent bodies with their long, fine flagella, may occasionally be encountered. This corresponds to the occasional appearance of latent bodies during almost all the periods in an infection of *T. gambiense*.

It would be extremely interesting to ascertain what exactly happens during the successive periods of maxima and minima, which succeed one another when a horse is infected with dourine; but we have found that even at the maxima of such infections the parasites are so few in number as to render it practically impossible to utilise horses for this object.

It would seem, then: that during the infection of rats with dourine, that is to say, with a form of trypanosome which under normal circumstances is not related to two distinct hosts, there exists a life-cycle among the parasites closely analogous to that occurring during the successive positive and negative periods of infection of the same animals with *T. gambiense*.

The parasites, after introduction into a rat, multiply by longitudinal fission, accompanied by amitotic division of the nucleus. After this process, an interaction takes place between the extra-nuclear centrosome and the nucleus (sexual stage ?). Division again proceeds, and finally the trypanosomes are converted into round bodies, which correspond to the latent bodies of *T. gambiense*, but possess two long and delicate flagella.

DESCRIPTION OF FIGURES.

In both plates the coloured figures are stained with Breinl's stain, and the extra-nuclear centrosome should be purple, but not so red as the intra-nuclear centrosome.

Plate 8.

Figs. 1—5.—Stages in the longitudinal fission of *T. equiperdum*. Figs. 1—4 showing the amitotic division of the nucleus, *n*. *c*, the intra-nuclear centrosome. In figs. 2 and 3 the intra-nuclear centrosome is dividing, below are the trophic granules. Figs. 1—4 are stained with Breinl's stain, fig. 5, a late stage in the fission, shows the characteristic inequality in the size of the resulting cells.

Figs. 6—10.—Successive stages in the detachment of a portion of the nuclear substance. Figs. 7—10 stained with iron heamatoxylin. By this method the trophic granules are not shown.

Figs. 11—15.—Successive stages of the passage of a portion of the extra-nuclear centrosome to the nucleus.

Plate 9.

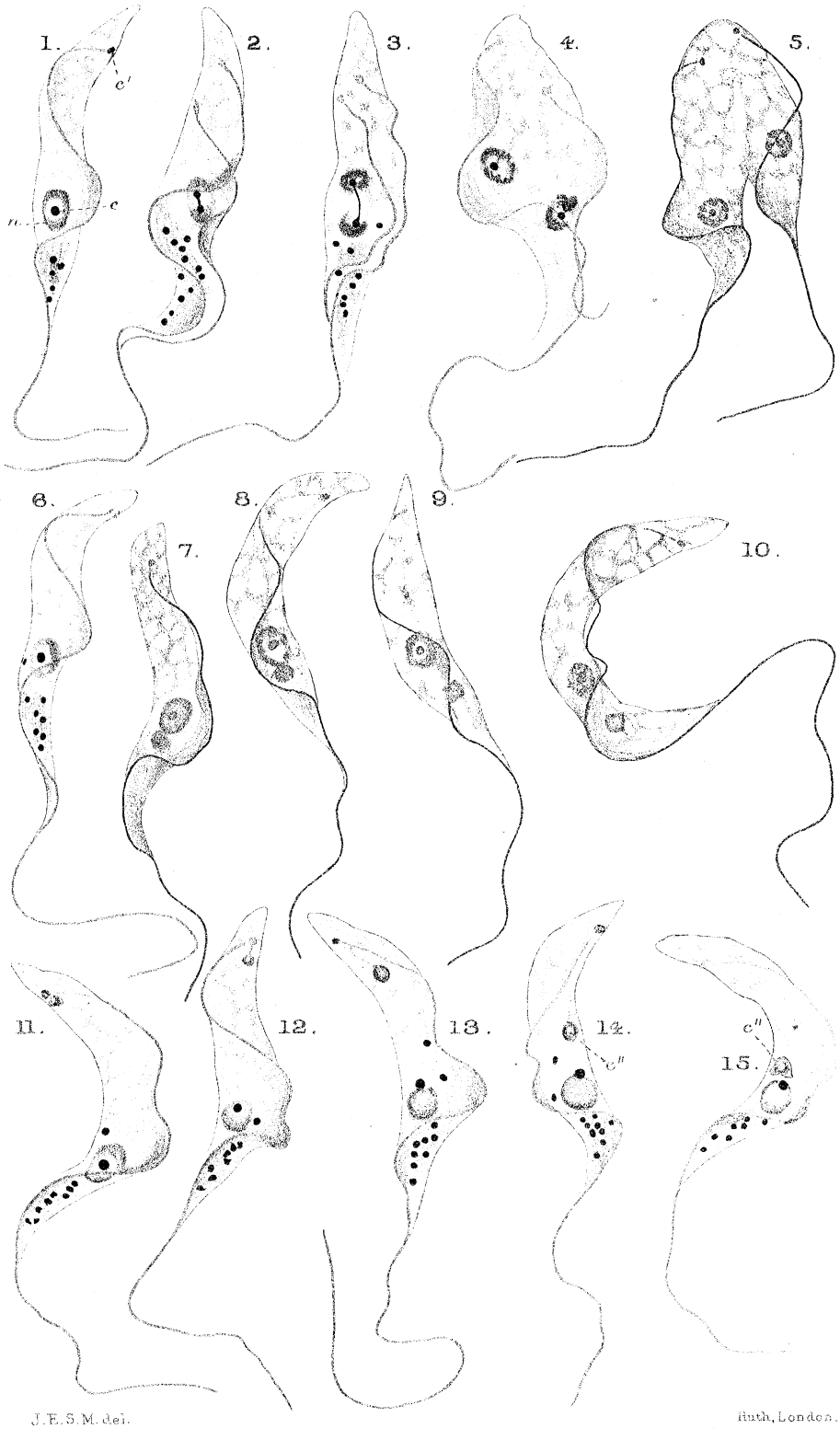
Fig. 16.—Late stage during the passage of the extra-nuclear centrosome to the nucleus. The detached mass is now practically fused with the nucleus. Compare figs. 14 and 15.

Fig. 17.—*T. equiperdum* again dividing after the fusion of a portion of the extra-nuclear centrosome with the nucleus.

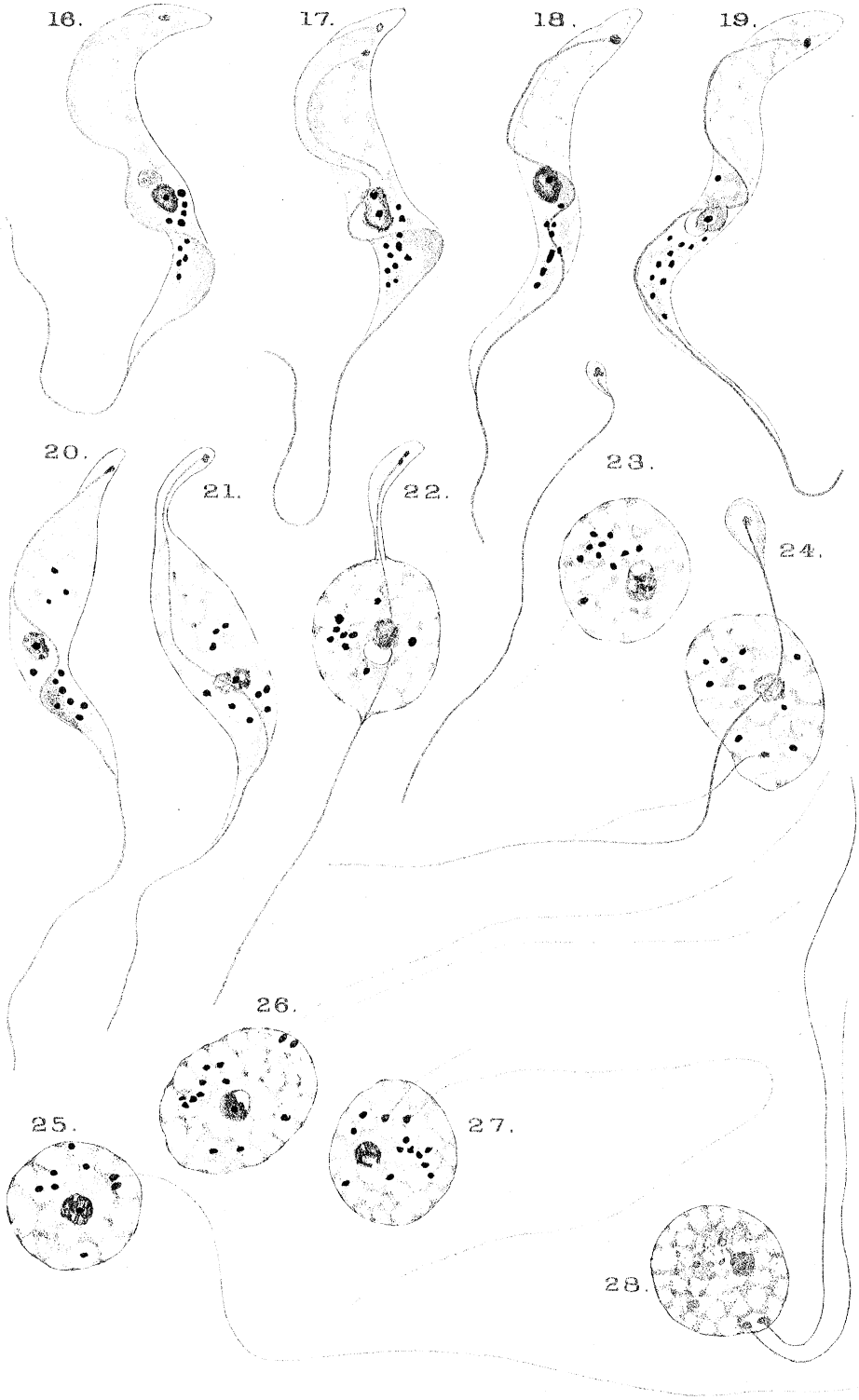
Figs. 18—22.—Stages in the development of the latent body.

Figs. 23 and 24.—Detachment of the old flagellum and appearance of a new extra-nuclear centrosome, and a new flagellum.

Figs. 25—28.—Complete latent bodies showing division of the extra-nuclear centrosome and the formation of two long delicate flagella.



J.E.S.M. del. Huth, London.


16.
17.
18.
19.
20.
21.
22.
23.
24.
25.
26.
27.
28.

1. 2. 3. 4. 5.

c'

c

n

6. 7. 8. 9. 10.

11. 12. 13. 14. 15.

c''

16.

17.

18.

19.

20.

21.

22.

23.

24.

26.

25.

27.

28.