SALVIN-MOORE

THE LIFE HISTORY OF *TRYPANOSOMA LEWISI*

BY

J. E. SALVIN-MOORE

PROFESSOR OF EXPERIMENTAL AND PATHOLOGICAL CYTOLOGY, UNIVERSITY OF LIVERPOOL

ANTON BREINL

DIRECTOR OF THE RUNCORN RESEARCH LABORATORIES, LIVERPOOL SCHOOL OF TROPICAL MEDICINE

AND

EDWARD HINDLE

DEMONSTRATOR IN THE RUNCORN RESEARCH LABORATORIES, LIVERPOOL SCHOOL OF TROPICAL MEDICINE

(*Received for publication* 5 *May*, 1908)

I

The Trypanosomes, so far as at present known, are universally parasitic organisms inhabiting the blood and the body fluids of a variety of animals. In certain cases, the presence of trypanosomes produces the most marked pathological results. In others, the parasites are apparently quite harmless. Moreover, the same trypanosomes which are pathogenetic with respect to one animal, are often non-pathogenetic in the case of others. There is a tendency at present to attempt to draw a distinction in a classificatory sense between the so-called pathogenetic, and non-pathogenetic forms of trypanosomes. But even from the facts just referred to, it would seem to be clear that any such method of grouping can have but little real significance, and is more likely to entirely mislead enquiry than to throw any fresh light upon the singular and, morphologically speaking, closely knit group of organisms which the trypanosomes undoubtedly constitute.

There appear, as a matter of fact, to be two main groups of problems connected with the trypanosomes at the present time. The first is constituted by our ignorance of the complete features of the developmental cycle of even any well-known and characteristic representatives of the group. The second by the present impossibility of determining to what group of non-parasitic

protozoa or protophytes the trypanosomes belong. There seems, however, to be little doubt that much of our present ignorance and confusion concerning the morphology and the life cycle of the trypanosomes has been due largely to an accident of technique, due in fact to the circumstance that the presence of trypanosomes can very readily be demonstrated by drying and staining the blood in which they are contained. It has so happened that by this method in its various forms, not only is the presence of trypanosomes demonstrated, but the preparations produced in this way are often extremely sharp, and beautiful to look at. It has consequently been only after a prolonged investigation of the effects produced upon such organisms, and on other forms of cells, by the process of drying, and by a careful comparison of the results obtained by this and other methods of fixation, that it has begun to be realised that dried preparations of cells, except for the purposes of demonstrating the presence of parasites, are generally as misleading as they are beautiful.

We have referred to this matter in our former papers, and we may say that all our further acquaintance with trypanosome morphology indicates clearly that the process of drying is entirely destructive of the finer cytological details, and consequently that it is altogether inapplicable to investigations wherein a true conception of the normal features of trypanosomes, or indeed of any cells, is necessary. In consequence of these considerations, we have entirely abandoned the use of dried preparations, and have relied here, as in our former work, upon modifications of the various methods of fixation in common use among Cytologists, together with such modifications of the various staining methods as have been found necessary during the course of the work. The features of the developmental cycle or life history of *T. lewisi*, although remarkable, in reality only assume their true proportion when considered in conjunction with the facts relating to the development of other trypanosomes that have now been studied.

For the sake of convenience, and for purposes of reference in subsequent portions of the present work, we shall in the first place briefly recapitulate our observations upon the developmental cycle in the case of *T. gambiense* and *T. equiperdum*. Before doing so, however, it is desirable to indicate the objects which we originally had in view in selecting the three forms, *T. gambiense*, *T. equiperdum* and *T. lewisi*. Our primary intention with regard to *T. gambiense* was to ascertain what morphological results could be obtained with this form through the application of ordinary cytological methods in place of the usual drying process. But the subsequent results of this investigation were to reveal the existence of a life cycle among the parasites in the blood, which is definitely related to the alternating phases of presence and absence of trypanosomes in the circulation of infected rats.* The appearance of a cyclical metamorphosis among the trypanosomes in the blood indicated that the general conception of a special phase of their life history being definitely related to transference to another host (as is the case according to Schaudin with *T. noctuae*) might not be correct. Since, however, it is known that *T. gambiense* can be transmitted by the bites of tsetse flies, it was obvious that apart from investigations upon the transmitting insects, no ultimate conclusions could be arrived at with regard to this matter in the case of *T. gambiense*.

There existed, however, in the disease Dourine, a trypanosome which under normal and natural conditions is not transmitted by any fly, or biting animal, but simply through contact. It was clear, therefore, that in this instance we had a trypanosome life-history which was not normally complicated by the passage of the parasite through any intermediate host. Whatever life cycle *T. equiperdum* may possess, this cycle must be completed, and can be studied in the body of a single host. The acquisition of a knowledge of the facts relating to the life history of *T. equiperdum*, the parasite of Dourine, was therefore of the first importance as a means of affording comparative material during a consideration of the significance of the features of the life cycle of *T. gambiense* in the blood.

These two series of investigations in the case of *T. gambiense* and *T. equiperdum* having been undertaken, and both being related to parasites which produce violent and fatal maladies, it seemed further desirable to extend the investigation to some particular trypanosome which under normal circumstances belonged to the so-called non-pathogenetic forms. For this reason we selected *T. lewisi*. There was, however, another important point to be considered. *T. lewisi* can be transmitted from infected to non-

* Salvin-Moore and Breinl. Annals of Trop. Med. & Parasitology, Vol. I, No. 3, 1907. Lancet, May 4, 1907.

infected rats by means of the rat louse, and consequently we had in this form of trypanosome a convenient object for investigating the changes which might take place among the trypanosomes when in the body of the louse, which here forms an intermediate host.

Passing now to the features of the developmental cycle in the parasites *T. gambiense* and *T. lewisi* in the blood, we find in the case of *T. gambiense** injected into rats that the disease is marked by alternating phases of presence and absence of parasites in the peripheral circulation. If numerous preparations be made of the blood at short intervals during the whole course of the infection, it is found that at the time the parasites are increasing in number in the blood, rapid multiplication is going forward by means of longitudinal fission. Such fission is accompanied by amitotic division of the nucleus and the intra-nuclear centrosome (nucleolus, karyosome), as well as by amitotic division of the extra-nuclear centrosome (blepharoplast), and lastly by the development of a new flagellum, and the final splitting of the original trypanosome into two separate flagellated cells, each containing a nucleus, an intra-nuclear centrosome, an extra-nuclear centrosome, and a flagellum.

Apart from the form of multiplication to which we have referred, no other form of reproduction takes place during the increase in the number of the parasites in the blood, and when we reach a point at or near the maximum number of trypanosomes in the circulation, the parasites cease to divide. At such periods it is found that in large numbers of them a stainable band develops from the extra-nuclear centrosome. This band extends, and finally becomes connected with the nucleus. It then breaks up and disappears. Subsequent to the development of the band, whether the trypanosomes again divide longitudinally, as in the case of *T. lewisi*, has not been ascertained. As we pass to those parts of the infection where the number of the parasites in the blood is falling, it is found that further rapid changes are taking place among the trypanosomes. The nuclei become more compact, vesicles appear in relation to them, and the nuclei, together with the vesicles, become separated from the outer portion of the cell, and enclosed by a delicate layer of cytoplasm. The remainder of such cells now disintegrates, and the composite body, consisting of the nucleus, the vesicle, and a covering of

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

cytoplasm, becomes set free in the blood. These *latent bodies*, as we have termed them, are eventually carried out of the peripheral circulation, and are subsequently to be found in large numbers in the spleen, the bone-marrow, and other organs. The process just described goes on until there may be no parasites to be found at all in the peripheral blood, but the latent bodies do not disappear, and after a time some of them grow larger in size, develop a new extra-nuclear centrosome (apparently from the division of the intra-nuclear centrosome), become flagellated, and finally gradually transform themselves into trypanosomes again. When this process has been completed, a similar cycle is passed through in relation to each alternating period of presence, and absence of the parasites in the blood, which the infected animal may present. It should be noted, however, that the cycle does not necessarily go forward at the same rate in all the trypanosomes present in an infected rat. All through the alternating periods there may be found a few trypanosomes at almost every stage of the cycle.

Turning now to the development of *T. equiperdum*,* it is found that in horses the infection presents the same sort of alternation of presence and absence of parasites in the blood that occurs during the infection of rats with *T. gambiense*, but in relation to the study of *T. equiperdum* in horses a difficulty presents itself. In such infections the parasites are so few, that it is practically impossible to obtain a sufficient number of them at the various periods of the curve of the infection for any adequate study.

The same is the case when rabbits are infected with Dourine. We have therefore utilised rats, wherein the parasites multiply very rapidly, the features of the disease being as follows:—

After injection no parasites appear until about the third day. They then multiply with extreme rapidity, and kill the animal in about four days after their first appearance in the blood. In rats, therefore, there is during an infection of Dourine only one developmental period, which is completed at, or about, the time of death of the infected animal. From the time of their first appearance the parasites multiply by longitudinal division, the features of this process being the same as those occurring during the multiplication

* Salvin-Moore & Breinl, "On the Life History of *Trypanosoma equiperdum*," Proc. Roy. Soc., 1908, Vol. 80.

of the trypanosomes with an infection of *T. gambiense*. After the multiplication has proceeded for some time, two normal changes occur. The first is constituted by the budding off of a mass from the nucleus, the mass eventually disappearing altogether. The second obviously corresponds to the formation of the stainable band at a similar period of an infection with *T. gambiense*. In the case of *T. equiperdum*, this process proceeds by the production of a large bud, originating from the extra-nuclear centrosome. The bud rapidly increases in size, becomes detached, and passes towards the nucleus, with which it finally becomes definitely associated. Afterwards the trypanosomes again pass through divisions, and subsequently enter upon another change. They become altered in shape. The extra-nuclear centrosome becomes related to a long neck of protoplasm. A vesicle appears in relation to the nucleus. The extra-nuclear centrosome, together with the protoplasmic neck and the flagellum, becomes detached, and a large round body remains wherein a new extra-nuclear centrosome is apparent. From this a fresh and exceedingly delicate flagellum grows out. The extra-nuclear centrosome divides, and a second flagellum is produced. These large round double flagellated forms obviously correspond to the latent bodies of *T. gambiense*, but owing to the fact that the disease invariably kills the rats at or about this period, we have as yet been unable to follow completely the transformation of the large latent bodies into trypanosomes once more. The latent bodies seem, however, in the first place to divide up and to produce smaller forms, which latter probably correspond to those occurring in the life cycle of *T. lewisi*, as we shall see.

T. lewisi is frequently found in the blood of wild rats in all parts of the world, but is rarely found in the blood of tame white rats. It is usually non-pathogenetic, and is relatively a large parasite. The morphology of the organism, and the various forms which it assumes in the blood, have been studied by several authors independently during recent years.* When a rat has become infected with *T. lewisi*,

* Laveran and Mesnil. Ann. de l'Institut Pasteur, Vol. XV, No. 9, p. 673.
Rabinowitsch and Kempner. Zeitsch. für Hygiene und Infectionskrankheiten, Vol. XXX, p. 251.
Wasielewski and Senn. Zeitsch. für Hygiene und Infectionskrankheiten, Vol. XXXIII, p. 240.
Ward, J. MacNeal. Life History of *Trypanosoma lewisi* and *Trypanosoma brucei*. Journ. Infect. Diseases, Vol. I, No. 1.
Prowazek. Studien über Säugethiertrypanosomen. Arb. a. d. Kaiserl. Gesundheitsamte, Vol. XXII, No. 2.

the parasites may be found in the blood in various forms at all periods of infection. Their development does not appear to occur in successive phases related to alternating presence and absence of trypanosomes in the blood, as is the case with *T. gambiense* and *T. equiperdum*. The elucidation of the developmental relationship of the various forms which thus exist together in the blood would at first sight seem to present a certain amount of difficulty, but in reality this difficulty is not so great as it appears. Thus the various authors who have already considered the subject are fairly well agreed with regard to the relationship in a developmental sense of the various forms one with another. Medium-sized parasites, such as those represented in fig. 1, certainly give rise by growth to the large pointed types represented in figs. 11-18, so also these latter unquestionably pass into the still larger round and regular multi-nucleated masses, such as those represented in fig. 23. Such masses may again in turn be found in all stages of breaking up into smaller bodies, and this process of dissociation certainly produces the characteristic rosettes, and other forms of temporary association commonly met with (figs. 26-30). Longitudinal division among the medium-sized forms such as those represented in fig. 1, appears to be a rare occurrence, but that it does take place is indicated by such types as those represented in figs. 2-5, wherein the nucleus or the extra-nuclear centrosome, or both, have become divided, and a second flagellum has arisen. We have, however, ourselves not encountered the late phases of division in such forms of *T. lewisi*, and have consequently only been able to figure the early stages of the process. The forms such as those represented in fig. 1 appear to certainly arise from, and to merge into, forms such as those represented in figs. 37-38, and these undoubtedly in turn have arisen from the products of the dissociation of the large multi-nucleated masses. Thus we appear to have a cycle of development in the blood, which, starting from any particular type such as those represented in fig. 1, passes through the phases represented in figs. 12-19 into the large forms represented in figs. 24-25. From this stage the cycle continues through stages such as those represented in figs. 30-38, and finally through growth and division the individual derivatives of the multi-nucleated masses pass back to the formation of types such as those originally chosen, as the starting point, and represented in fig. 1.

That such a progressive development in the case of each individual really represents the course of the cycle in the blood, receives complete confirmation from the study of the various morphological changes which take place at the successive periods of the cycle; for these changes, as we shall see, correspond closely to the analogous changes which occur during the development of *T. equiperdum* and *T. gambiense*, that is to say, in forms where the successive stages are passed through approximately simultaneously by the majority of the parasites during the course of infection.

The study of the morphology of *T. lewisi* may perhaps most simply be illustrated by taking in the first place examples such as those represented in fig. 1. In this condition the cell is long and pointed at both ends. The extra-nuclear centrosome, which is large, lies at a considerable distance from the pointed extremity of the cell. The extra-nuclear centrosome stains very deeply with many forms of coloration, and can be seen during life as a highly refractive body. In various stained preparations the extra-nuclear centrosome appears to be always related to a vacuole, or space in the surrounding cytoplasm, and the flagellum may present various appearances in relation both to the vacuole and the extra-nuclear centrosome. The flagellum, which is a long stainable band, extends in a curved course over the whole length of the body, and projects at the opposite end as a whip-lash. It is enclosed in a thin expansion of the cytoplasm, forming the so-called undulating membrane. The flagellum generally ends in a small body, or bead, near the extra-nuclear centrosome (fig. 1), but this is not always the case, for at times it certainly appears to run directly on to the extra-nuclear centrosome. When the flagellum is detached from the latter body, there can frequently be seen passing from the bead or thickening at the end of the flagellum, fine unstained strands which connect the bead with the extra-nuclear centrosome. The bead upon the end of the flagellum corresponds closely in appearance to the similar beads which are often found at the ends of the flagella among metazoan gametes, and such beads are in like manner often connected with the centrosomes by fine slightly staining strands. It would thus appear that so far as these structures among the trypanosomes can be directly homologised, the flagellum and its end-bead, together with the extra-nuclear centrosome, would correspond to the flagellum, bead, and centrosomes of many forms of

metazoan gametes. For this reason and others, to which we shall refer subsequently, we regard it as extremely misleading to name the end-bead a blepharoplast, and the extra-nuclear centrosome, a kineto-nucleus, for the end-bead (blepharoplast) does not present the relationships of a centrosome, or blepharoplast; while the extra-nuclear centrosome (kineto-nucleus) does so. Moreover, the extra-nuclear centrosome does not, so far as we are aware, present anything in common with a nucleus, except its capacity to divide, and in this connection such a capacity amounts to nothing, for the capacity to divide is one which is, of course, shared by every known centrosome. The extra-nuclear centrosome is generally in the form of a thick rod, often slightly curved, and sometimes presenting the appearance of being divided in the middle. The division of the blepharoplast does not appear, however, to take place through any transverse separation, which such appearances might suggest.

The nucleus in *T. lewisi* lies relatively very near the end of the body from which the flagellum projects. In consists of an outer less stainable area, and a large inner much more darkly staining globe, the *intra-nuclear centrosome* (karyosome nucleolus). The outer portion of the nucleus is often very distinctly bounded, and in such phases of the development as those represented in figs. 14-19 might certainly be said to possess a membrane. During the phase of the development we are now considering, the cells do not present any very definite granules in the cytoplasm, which is seen, both under examination during life with a dark ground illumination, and after proper fixation, to consist of a fine protoplasmic foam bounded on the outside by a denser and homogeneous layer.

Having thus briefly described the features of *T. lewisi* when in such a stage as that represented in fig. 1, it will be most convenient in proceeding to describe the passage of such forms through the phases of the cycle we have already outlined, and to consider the various divisional and other phenomena as they occur in relation to this cycle. The form of trypanosome represented in fig. 1 passes by simple growth into the large forms represented in figs. 11-18, and all the intermediate stages can be readily found stretching from the morphological condition represented in fig. 1 to that represented in fig. 17. Among such trypanosomes as those represented in figs. 8-17, two stages of metamorphosis are found to

occur. The first consists in the unequal budding of the intra-nuclear centrosome, so as to form what appears as a small nucleus, which becomes pushed off towards the free portion of the flagellum. This little mass, which consists of a small portion of the intra-nuclear centrosome and a small portion of the outer nuclear substance, appears subsequently to simply disappear. The process we have just described undoubtedly corresponds to the similar production of a degenerating nuclear bud in *T. equiperdum.* The second metamorphosis is constituted by the production of a body originating from the extra-nuclear centrosome. A portion of the substance of the extra-nuclear centrosome appears to pass round the adjacent vacuole, and to collect into a small mass on the side of the vacuole which faces the nucleus. This soon becomes completely detached, and passes away toward the nucleus through the cytoplasm. During its development the detached body becomes larger, and the outer portion of it stains less densely, but it is often possible to see a darkly staining bead at the centre of the growing mass (figs. 8-10). The body thus detached from the extra-nuclear centrosome may be found in all stages of transit from its original position to a close approximation to the nucleus (figs. 14-16). Having reached this latter position, it appears to remain for some time unchanged. The process here described in *T. lewisi* obviously corresponds to the similar detachment of a portion of the extra-nuclear centrosome, and its subsequent passage to the nucleus, which we have described in *T. equiperdum.* It also undoubtedly corresponds to the formation of the stainable band stretching between the extra-nuclear centrosome and the nucleus during the life cycle of *T. gambiense.* The nucleus itself usually at this period begins to show signs of division. Such division which is represented in figs. 18-19 takes place in a typical amitotic fashion; the intra-nuclear centrosome dividing like a drop as in *T. gambiense* and *T. equiperdum,* and the outer nuclear substance collecting round the two derivatives in the same manner.

As in *T. gambiense, T. equiperdum,* and *T. equinum,* so also in *T. lewisi* we have been absolutely unable to observe anything during the division of the nucler, or during any other periods, which in the remotest degree suggests the presence of chromosomes. During division of *T. lewisi* the intra-nuclear centrosome first elongates, then becomes dumb-bell shaped, and finally assumes the form of two large

globes widely separated from one another, and connected by a generally curved and tapering mass of substance which seems to have been simply drawn out between them. At the same time the outer nuclear substance collects about the diverging daughter elements, and finally separates along with them into two smaller masses, which, together with the new intra-nuclear centrosomes, eventually reproduce two complete and round nuclei, exactly like the parent nucleus only smaller. The process of nuclear division just described may be rapidly repeated; and at the same time the original trypanosome loses its characteristic form, and become rounded up so as to produce the well-known multi-nucleated masses such as those represented in figs. 12-25. In some cases, however, when the nucleus, in a specimen such as that represented in fig. 19 has divided, the trypanosomes may become longitudinally split as in fig. 39, and in these cases the nuclei may at the same time travel towards the extra-nuclear centrosome so as to occupy the position represented in fig. 39. The ends of these division products may become detached from one another, and a very curious appearance result, represented in fig. 30. We think that the features of this form of division at the period we are discussing deserves particular attention, for the appearances produced when it occurs are indistinguishable from the figures given by Prowazek, and interpreted by him as conjugation.

We have, however, found nothing in relation to the nuclei, or any other structure in such cells, when in this condition, to suggest that the forms in question can be interpreted as conjugation. When such forms are produced, their future history appears to be this: Either the nuclei divide further, and the separation remains incomplete, the final product being one of the irregular multi-nucleated forms, or the fission is completed and the daughter cells, each after further nuclear divisions, produce fresh multi-nucleated masses.

We have referred to this process because of its obvious bearing upon the interpretation to be put upon the identical figures given by Prowazek. Our observations indicate that it is relatively a rare method of procedure, the more normal processes being the multiplication of the nuclei and the rounding up of the trypanosome to produce eventually the multi-nucleated masses. During this period, i.e. the time and after the body becomes detached from the extra-nuclear centrosome the history of this latter

structure in *T. lewisi* is difficult to follow. It certainly often enters into close contact with the nucleus before division as in figs. 15, 16, but it is frequently discernible after the nuclei have divided, as in fig. 20. In some of the resulting forms, moreover, when two, three, or four nuclei have been produced, the extra-nuclear-centrosomic derivative may sometimes still be observed lying between the nuclei, and apparently in close association with them. Whether the substance of the extra-nuclear-centrosomic derivative is directly absorbed by the nuclei, or merely disappears in the cytoplasm, the body in question sooner or later vanishes, and cannot be observed any further. The division of the nuclei of *T. lewisi* in the form we have just described is accompanied by the fission of the extra-nuclear centrosome, the fission of this latter body being generally accompanied by a movement towards the nucleus. It sometimes happens, however, that not only does the extra-nuclear centrosome move towards the nucleus, but the nucleus itself also moves towards the extra-nuclear centrosome. The advent of division of the extra-nuclear centrosome is marked by the development of the rod-like form into a flat disc, which perhaps through its thinness stains less darkly than the extra-nuclear centrosome when in a condition of rest (figs. 40-42.) The next phase is constituted by the collection of the staining material on opposite sides of the disc, and finally by the production in this way of two curved rod-like bodies on each side of the disc (figs. 41, 42). These new rod-like bodies constitute the new extra-nuclear centrosomes. They now rapidly diverge; it may be widely, showing at first a faint connection, which appears to be the remaining substance of the disc that has been simply drawn out. This connection rapidly disappears, the resulting extra-nuclear centrosomes having then the same appearance as those in the parent form; but they are naturally smaller. During the nuclear division at this period which result in the production of the multi-nucleated masses, the division of the extra-nuclear centroseme does not, so far as we have been able to see, result directly in a division of the flagellum or the body attached to its proximal end. During such phases in *T. lewisi*, the original flagellum and its bead remain unaffected, and apparently do nothing. When the extra-nuclear centrosome has divided, as in fig. 44, it is often seen that a small body is closely attached to it, appearing as if it had been separated from the extra-nuclear

centrosome. These little granules lie in the position from which new flagella finally arise, and it is consequently suggested that in *T. lewisi* the flagellum originates from a small fragment of the extra-nuclear centrosome, which becomes detached after the extra-nuclear centrosome has divided. This view of the method of procedure is further enforced by the fact that after the dissociation of the flagellum from the extra-nuclear centrosomes (which take place during the division of the latter bodies, see figs. 41-46), the original flagellum and its bead appears to be left, and is certainly finally shed in a degenerative condition, in the same way as the flagellum is cast off during the formation of the latent bodies in *T. gambiense* and *T. equiperdum*. From the vicinity of each of the new extra-nuclear centrosomes, and apparently from the granules budded off from these bodies, new and delicate flagella arise, and the multi-nucleated mass may assume in consequence appearances such as those represented in figs. 41-46.

It will be seen that the features of the phase we have now described, that is, the production of the large pointed forms, the passage of an extra-nuclear-centrosomic derivative to the nuclei, the subsequent division of the nuclei, the formation of new extra-nuclear centrosomes, the degeneration and disappearance of the old flagellum and the formation of new flagella in association with new extra-nuclear centrosomes, certainly correspond in a biological sense with the phases we have considered and described in relation to the production of the latent bodies in *T. gambiense* and *T. equiperdum*. It would seem, indeed, that the multiplication of the nuclei in the large multi-nucleated masses of *T. lewisi* correspond to the division in *T. equiperdum* after the passage of the extra-nuclear-centrosomic derivative to the nucleus. The subsequent history of the multi-nucleated forms is equally interesting in this comparative aspect. The nuclei and the extra-nuclear centrosomes may become multi-nucleated till there are 10, 15, or more of each in a single mass. The flagella become distributed on the periphery of such masses, and the mass finally separates by forming either a mulberry-like aggregate of round flagellated forms, or the fission proceeds in a slightly different manner, and a curious group of somewhat elongated forms may be produced, as fig. 20. In all these resulting forms, whether elongated or round, the morphological conditions are quite different from those of the

characteristic trypanosome form. The nuclei occupy a more or less central position. The body of the cell is short (fig. 28), or actually round (fig. 31), and the long delicate flagellum is quite free. When such forms are elongated, as in fig. 27, the flagellum and the extra-nuclear centrosomes lie together on one side of the nucleus, and the flagellum passes away from the vicinity of the extra-nuclear centrosome in an opposite direction to that of the nucleus. These forms arising from the ultimate breaking up of the multi-nucleated masses are thus seen to possess all the morphological characteristics of the latent bodies, which are produced after the corresponding cycle of internal changes in the case of *T. gambiense* and *T. equiperdum*. The derivatives of the multi-nucleated masses in *T. lewisi* thus correspond to the latent bodies of *T. gambiense* and *T. equiperdum*. In *T. lewisi*, however, the subsequent history of the latent bodies is far more easy to follow than in any case which we have hitherto been acquainted. *T. lewisi* in this respect constituting an admirable example for the further study of this important phase, which is less easy to follow in the development of the pathogenetic forms to which we have referred.

The changes which succeed in the small flagellated forms or latent bodies of *T. lewisi* are essentially similar to the transformation of the latent bodies of *T. gambiense* into the ordinary trypanosome form. The latent body elongates, the flagellum at first passing directly away from the surface of the body and in a direction opposite to that in which the nucleus lies in respect to the extra-nuclear centrosome (fig. 31). After a time the extra-nuclear centrosome migrates to one end of the cell body (fig. 32), and the flagellum is apparently drawn over the surface of the body after it. This portion of the flagellum which remains attached to the cell forms, as it were, the 'Anlagen' of the future undulating membrane. The further development is simple, the body elongating and enlarging into the ordinary trypanosome shape, as in figs. 33-38. When the form of small trypanosome, such as that represented in fig. 38, has been assumed, the cells again enter into division, as may be seen in figs. 34-37, and through the process of growth and further fissions gradually pass back again to the forms with which this description started (fig. 1).

In briefly considering the foregoing observations upon the life history of *T. lewisi*, the most striking biological feature which emerges is the obvious similarity that exists between the successive phases

presented by *T. lewisi*, and the homologous phases occurring in the life cycles of *T. gambiense* and *T. equiperdum*. In each of these three cases the 'trypanosome form' multiplies through fission until an interaction takes place between the extra-nuclear centrosome and the nucleus. This interaction may be succeeded again by simple fission, as in *T. equiperdum*. Possibly this is also the case in *T. gambiense*, while in *T. lewisi* it is followed by a series of rapid nuclear divisions resulting in the formation of the characteristic multi-nucleated masses. These differences, however, appear to be mere specific differences, of quite minor importance, which simply help to characterise in a specific sense the particular parasites we have considered. The divisions following the interaction between the extra-nuclear centrosome and the nucleus are, however, succeeded by a complete change of form, and by the assumption of the peculiar morphology of the round flagellated 'latent body.' The fact that in *T. equiperdum* the latent bodies possess two flagella has probably a profound morphological significance, but it seems to be inappropriate at the present time, and in view of future work, to enter into a consideration of its actual significance. The details of the structure of the latent bodies, and their passage into the trypanosome form, is a matter which, although apparently simple in *T. lewisi*, is one which must receive further study. It is, for example, not at present clear in what way the two flagella of the latent body of *T. equiperdum* behave during this process. It becomes clear, when we consider the observations on the three forms to which we have referred, that during the life cycle in the blood the different phases in this cycle may become prolonged or shortened relatively with respect to one another.

Thus the stage in *T. gambiense*, where one or two fissions possibly follow the interaction between the extra-nuclear centrosome and the nucleus is prolonged in the case of *T. equiperdum* into a period where certainly several divisions take place, and this same period is again prolonged and rendered specifically characteristic in the case of *T. lewisi* by the production of the large multi-nucleated masses.

In this connection it seems also to be a very striking fact that, whereas in the pathogenetic forms *T. gambiense* and *T. equiperdum* the phases of the life cycle as they appear among the trypanosomes do so nearly simultaneously among all the parasites existing in the blood at a particular time, and thus mark successively

the stages of the infection, in the non-pathogenetic form *T. lewisi* all the stages of the life cycle may be present and represented by different parasites which are found in the blood at the same time. This difference is perhaps what might have been expected. Such forms as *T. lewisi* are usually present in the animals they inhabit in large numbers for weeks, or even months; whereas among the pathogenetic varieties the parasites are numerous in the blood for only relatively short periods, the phases of the life cycle being here apparently adapted to the varying conditions of the host.

In this way we find that the parasites in such forms either multiply without limit, and by their action rapidly kill the host, or they periodically disappear from the altered blood in the form of latent bodies, and only reappear, it may be, after a very considerable time.

We have referred, in dealing with *T. gambiense* and *T. equiperdum*, to the fact that we have been quite unable to make anything of the arbitrary distinction which has come into vogue since the publications of Schaudinn between the so-called males, females, and any different forms. These seem to us to be either mere varieties of size, or, where morphological distinction is obtained, examples that have been taken from different parts of the life cycle.

The same results in relation to this matter have been enforced by the study of *T. lewisi*. Moreover, the terms male and female have, biologically speaking, always a strict and obvious reference to two varieties of cells which conjugate, or gametes, and to use terms of this type in reference to mere varieties of size, or to the morphological characters of different phases in a life cycle where no ordinary conjugation has hitherto been found, seems to us to be in the highest degree misleading and erroneous.

PLATE II

T. lewisi

1-6.—Stages of rest and division of the nucleus, the extra-nuclear centrosome, and the development of new flagella.

7.—Stage in the division of the nucleus.

8.—Formation of a small nuclear body which is thrown off from the nucleus and production of a large mass from the extra-nuclear centrosome.

9, 10, 11.—Further stages in the development of the body derived from the extra-nuclear centrosome, and its passage towards the nucleus.

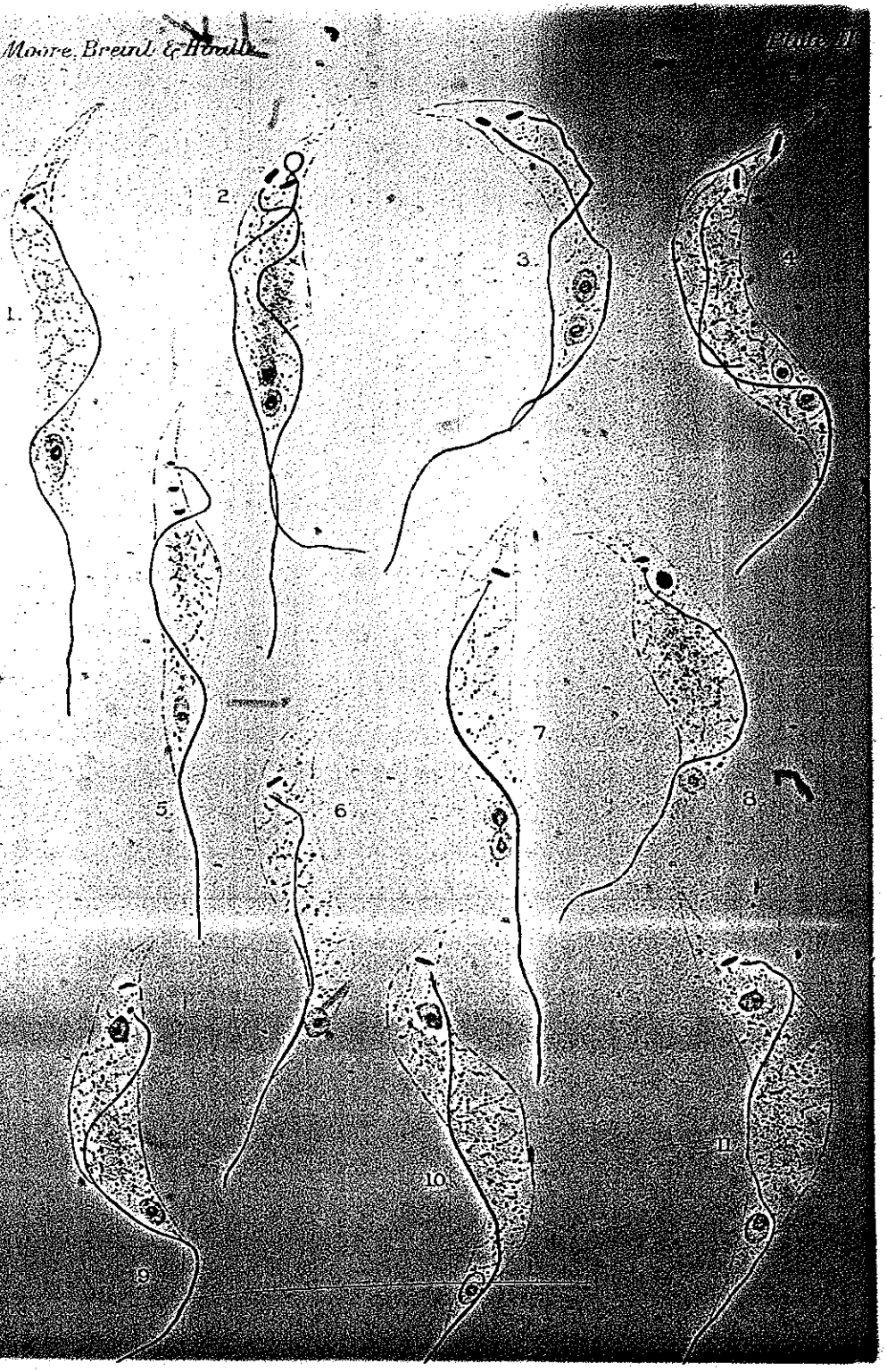


PLATE III

T. lewisi

12, 13, 14, 15, 16, 17.—Stages in the passage of the body developed from the extra-nuclear centrosome towards the nucleus. Figs 12 and 17 show the degeneration of the small mass detached from the nucleus.

18, 19.—Stages of the division of the nucleus.

20.—Partly rounded form with two extra-nuclear centrosomes, and the body derived from the extra-nuclear centrosome.

21.—Rounded mass produced after the division of the nucleus, and extra-nuclear centrosome.

22.—Division of a nucleus in a large rounded form.

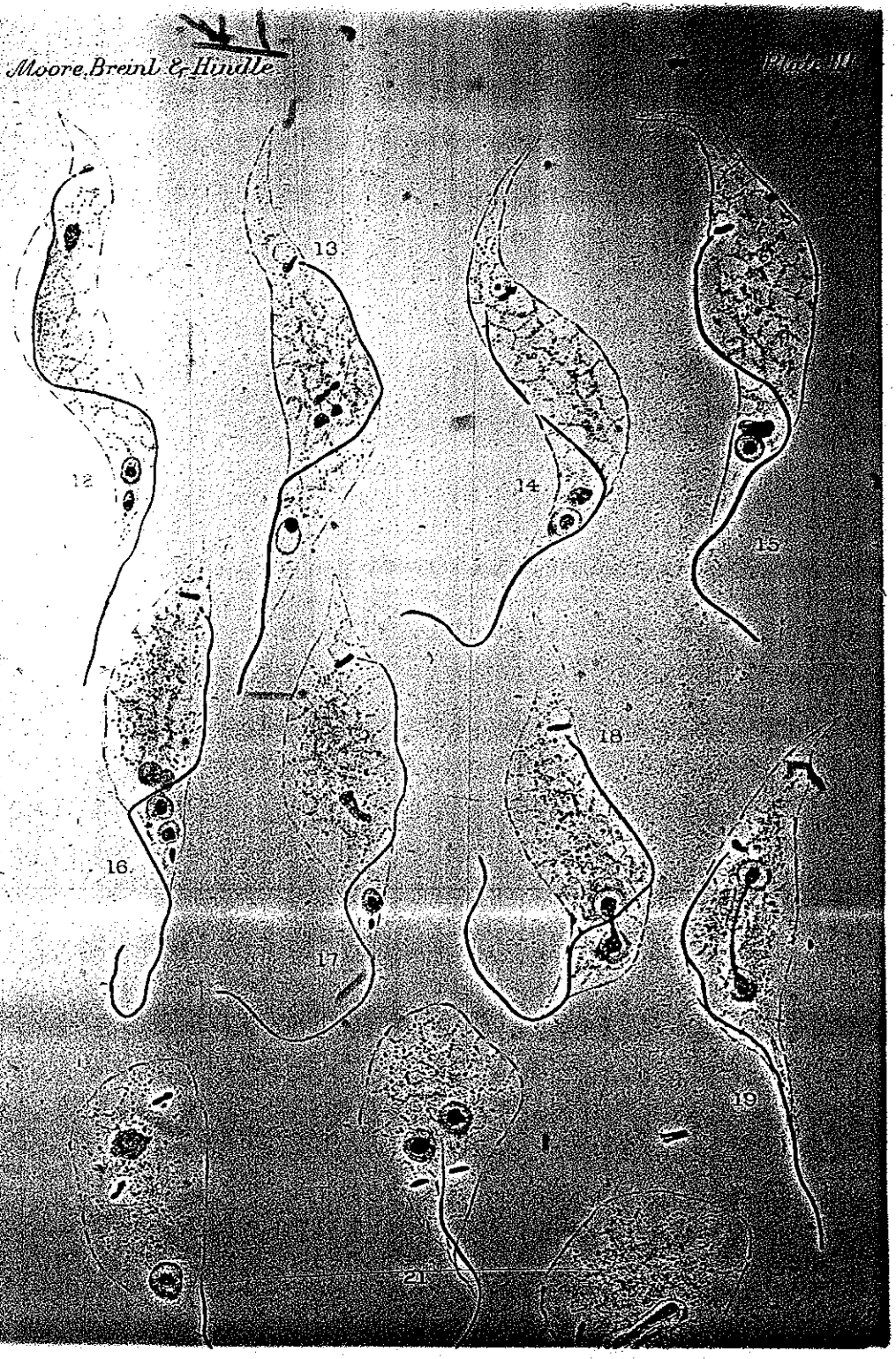


PLATE IV

T. lewisi

23.—Large form showing two nuclei and two extra-nuclear centrosomes.

24, 25.—Large multi-nucleated masses, fig. 24 showing division of one of the nuclei.

26, 27, 28, 29, 30.—Breaking up of the large multi-nucleated masses into forms equal to the latent bodies of *T. gambiense* and *T. equiperdum.*

31, 32, 33, 34, 35, 36, 37, 38.—Stages of transformation of the latent bodies into ordinary trypanosomes.

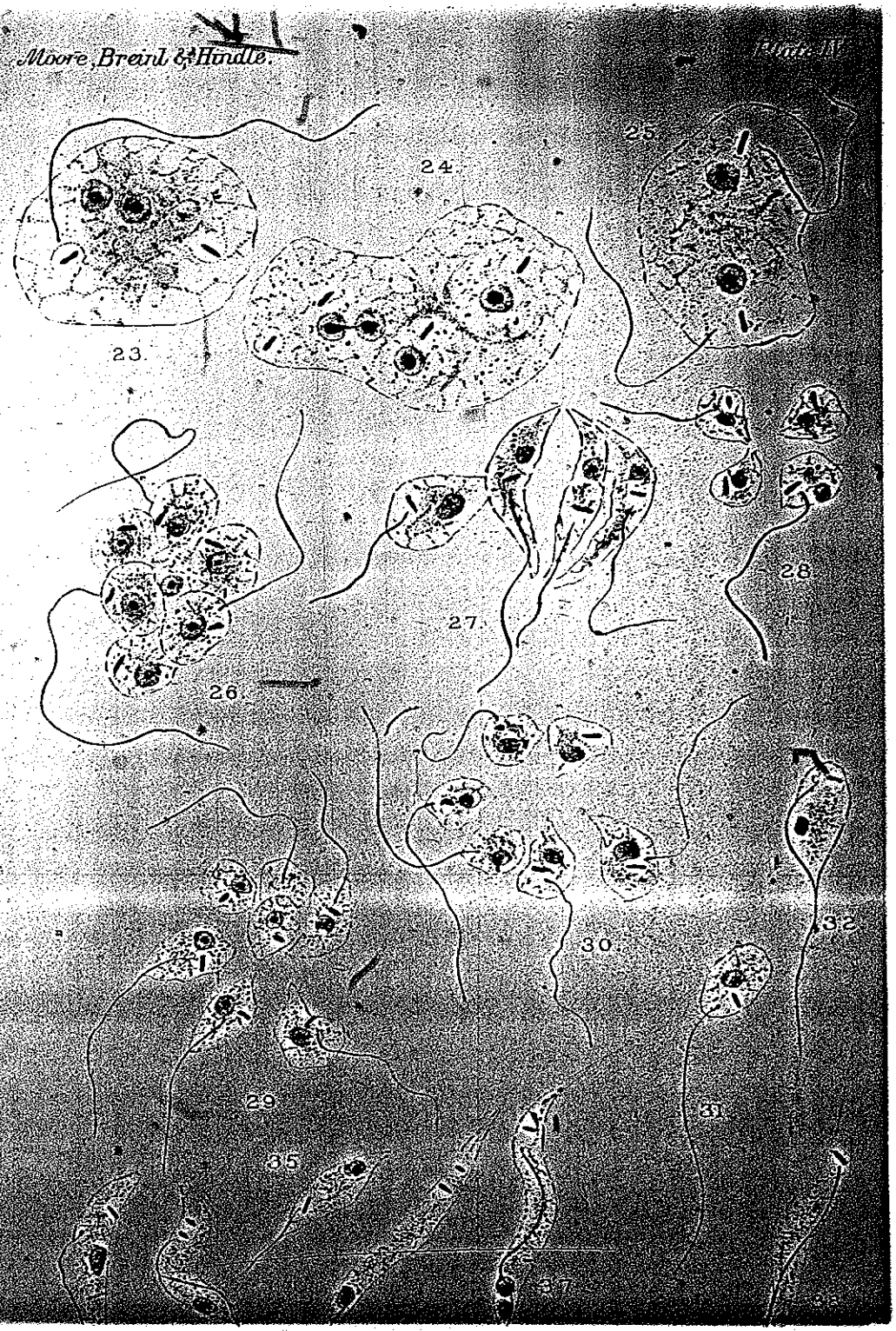


PLATE V

T. lewisi

39.—Division which at first sight suggests an act of conjugation.

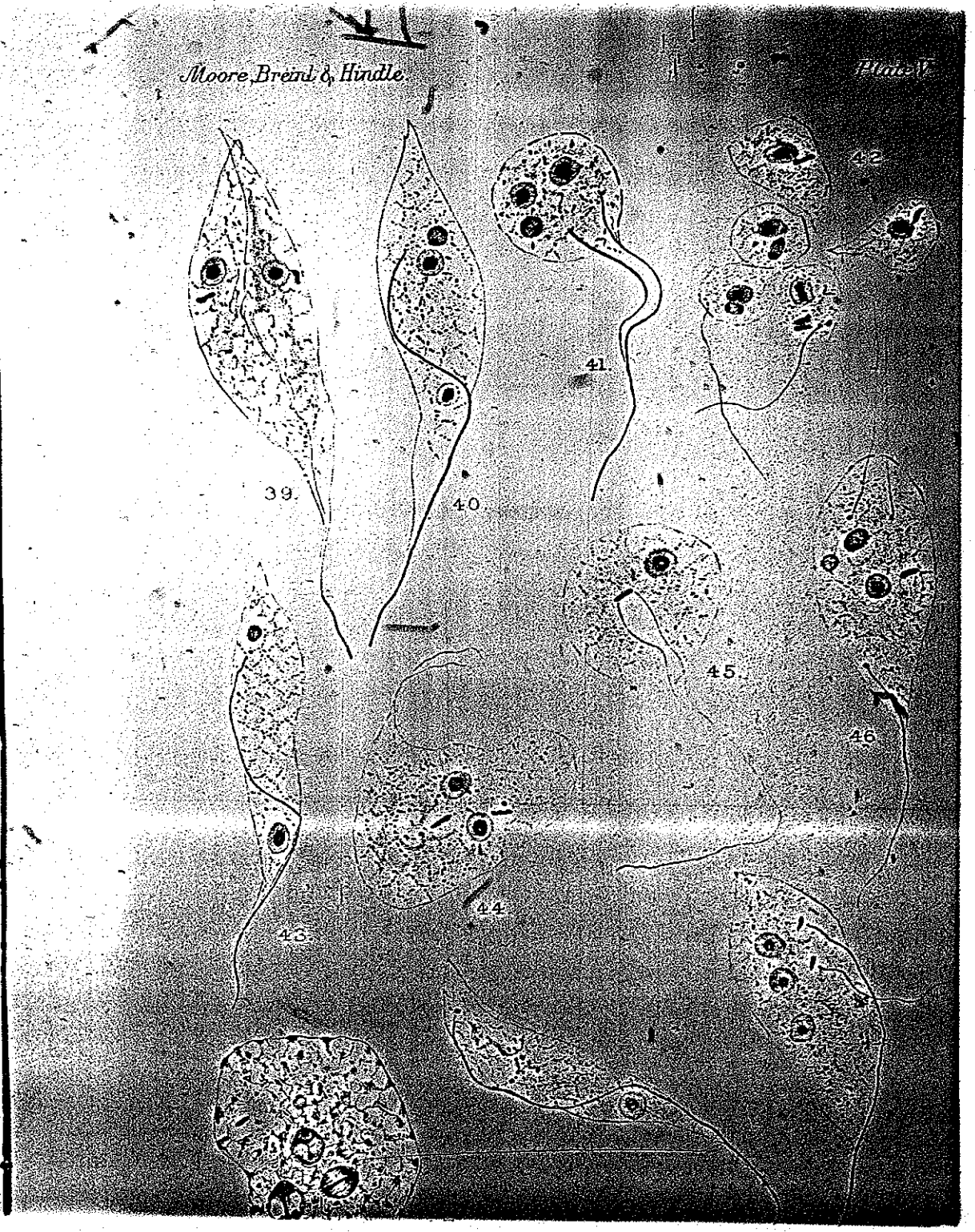
40, 41, 42, 43.—Forms showing details of the division of the extra-nuclear centrosome.

44.—Form showing detachment of small bodies from the extra-nuclear centrosome.

45, 46.—Details of the origin of the flagella.

47.—Division of the nuclei in a multi-nucleated mass.

48, 49.—Unusual division of the nuclei.



Volume II March, 1908 No. 1

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY

THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Editor

PROFESSOR RONALD ROSS, MAJOR I.M.S. (RET.), D.P.H., F.R.C.S., D.Sc., LL.D., F.R.S., C.B.

In Collaboration with

J. W. W. STEPHENS, M.D. CANTAB., D.P.H.

R. NEWSTEAD, A.L.S., F.E.S.

J. L. TODD, B.A., M.D., C.M. McGill

H. WOLFERSTAN THOMAS, M.D., C.M. McGill

ANTON BREINL, M.U.D.

PROFESSOR SIR ROBERT BOYCE, M.B., F.R.S.