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STUDIES ON THE PARASITES OF THE TERMITES

BY

CHARLES ATWOOD KOFOID AND OLIVE SWEZY

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STUDIES ON THE PARASITES OF THE TERMITES I. ON *STREBLOMASTIX STRIX*, A POLYMASTIGOTE FLAGELLATE WITH A LINEAR PLASMODIAL PHASE

BY

CHARLES ATWOOD KOFOID AND OLIVE SWEZY

CONTENTS

INTRODUCTION

One of the most curious and unique faunal associations to be found among the parasitic Protozoa is the group parasitic or commensal in the intestinal tract of the social termites. These parasites are remarkable not only for the vast numbers that may be found within a single host, but also for the degree of development and specialization which distinguishes many of the species. This is especially true of the

forms belonging to the family Trichonymphidae, such as *Trichonympha* Leidy, which are among the most highly specialized members of the Protozoa.

Along with these more complex forms are others which, while simpler in structure, yet show certain peculiar morphological characteristics that distinguish them as a group apart from other intestinal flagellates. Among these we find *Pyrsonympha vertens* and *Dinenympha gracilis* described by Leidy in 1881.

Later investigators have added both to the number of genera and of species of these peculiar flagellates.

Material

The material for these studies was obtained from one species of termite which is abundant on the University campus at Berkeley. This is *Termopsis angusticollis* Walker and was identified for us by Dr. Nathan Banks of the Museum of Comparative Zoology at Cambridge, Mass. Most of this material was obtained from the decayed trunk of an oak tree in Strawberry Cañon. Many of the same species were obtained during the swarming season from the piles on Meiggs Wharf, San Francisco, by Dr. A. D. Drew of the Public Health Service.

These termites are large and show an infection of about one hundred per cent, soldiers, workers and males of the colony being infected alike. The amount of infection in a single individual is relatively enormous. The abdomen is large and nearly filled by the greatly swollen intestine. This distension is caused by the vast numbers of parasitic and commensal protozoans which fill the lumen of the intestine. When this is opened a thick milky fluid exudes. Under the lens this is found to be composed of great quantities of these small forms, thickly massed together, along with fragments of wood upon which the host, as well as some of its commensals, feeds.

In *Termopsis angusticollis* four different species of large protozoans are invariably present, sometimes about equal in number or with one predominating over the others. In addition to these there are usually present minute forms of two, sometimes three different species of flagellates, the whole forming a complex of organisms wonderful both for variety and amount. Of these forms the two largest species belonging to the family Trichonymphidae and a third species belonging to the Polymastigidae, will be reserved for discussion in later

papers. In the present paper the fourth member of this group, also a polymastigote flagellate, will be considered, with a discussion of its morphology, relationships and life history in so far as they have been determined.

Technique

The flagellates found in the termites are exceedingly delicate. Great difficulty has been experienced in keeping them alive for continuous observation under the microscope for any length of time. The use of distilled or tap water resulted in the complete dissolution of the larger flagellates in a few minutes. The smaller ones would survive a somewhat longer period. Various other culture media were tried, such as Ringers' solution, normal salt solution, malted milk and the white of egg. Of these the last one was the most successful, a few flagellates surviving in the culture at the end of twenty-four hours. These cultures were made with a hanging drop or with a greater amount of fluid in a deep culture slide.

Intra vitam stains, such as neutral red, Janus green and new methylene blue G G, were used. Of these neutral red gave the best results.

The methods of fixing and staining which have been found the most satisfactory were those outlined by us in previous work on parasitic flagellates (Kofoid and Swezy, 1915), that is, a modified Heidenhain's iron haematoxylin following fixation in hot Schaudinn's fluid. Other stains as well as various fixing agents were tried, both with smear preparations and with sectioned material. In the latter cases two methods were followed. In the first the entire abdomen of the termite was used, fixed in Schaudinn's or strong Flemming's solution. In the second the intestine was teased out in a drop of normal salt solution and then placed in the fixing fluid. These sections were stained with haematoxylin or with a modified Mallory's connective tissue stain (Yocom, 1918).

Considerable difficulty has been experienced in making good preparations of this material by the ordinary smear methods. The exceeding delicacy of the various flagellates results in distortions of the body and breaking down of its cytoplasmic organization. This is usually confined to the posterior end, leaving the anterior end, nucleus and motor organelles intact. This difficulty was partly overcome by using albumen fixative on the cover slip and diluting the contents of the

intestine with a small drop of normal salt solution before making the smear. The material thus treated may be spread with less mechanical injury and the albumen prevents the great loss of organisms that would otherwise occur when it is placed in the fixing fluid. The addition of albumen, however, necessitates quick work in making the preparations, to prevent the death of the organisms through its action rather than that of the fixing fluids.

OCCURRENCE

These flagellates are more restricted in their occurrence in the intestine of the host than are the other forms which are present with it. They are seldom found far away from the mucus of the epithelium, usually attaching themselves to it (pl. 1, fig. 9) by means of the holdfast-like anterior end of the body. They may be seen completely filling the folds of the wall of the intestine with the posterior portion projecting into the lumen of the canal.

Near the anterior part of the posterior region of the intestine, immediately behind the origin of the malpighian tubules, a slight enlargement of the intestine may be noted, with one side marked by two lines of constriction passing backward for a short distance. This forms a rounded chamber marked off from the main portion of the canal. In cross-sections this may be found completely filled with *Streblomastix*, a dense coating of the flagellates attached to the wall and others filling the remainder of the cavity. Plate 2, figure 8 shows a small portion of the wall in this region with a few only of the attached flagellates.

These flagellates occur much less frequently in other parts of the posterior and mid-regions of the intestine, but when present are always restricted to the peripheral zone with the larger flagellates occupying the remainder of the lumen. They have been found in nearly seventy per cent of the hosts examined.

MORPHOLOGY

Streblomastix is profoundly a linear organism. Elongation dominates all of its organelles in adaptation to its crowded grouping in its parasitic habitat. This elongation affects not only the body as a whole but also the nucleus, rhizoplast, and flagella, and pervades not only the normal vegetative trophozoite, but also the gigantic over-

grown and possibly abnormal phases occasionally found. The linear form of body and also of nucleus continues not only during the trophozoite phase but likewise, in so far as we have seen the stages, during both binary and multiple fission. During multiple fission itself the organism becomes a greatly elongated thread with its nuclei stretched lengthwise as a constricting thread in the axis of the body. All trace of rounding up or sphericity seems thus to have been banished utterly from both body and nucleus at all stages of its life cycle.

Size and Shape of Body

The body is ordinarily elongate fusiform, tapering subequally at the two ends. Either or both ends (pl. 1, figs. 1, 7) may be somewhat blunt but the usual form of the anterior end is a slender cone while the posterior one may have a trifle more convexity. Its length is generally from twelve to sixteen times its greatest diameter which is found near the middle of the body. The shorter individuals (pl. 1, fig. 5) may be only six times the diameter. These are evidently recent schizonts. On the other hand, giant individuals, which are possibly approaching multiple fission (pl. 2, fig. 13), may be thirty times their diameter in length, and the "plasmodial" stage of multiple fission (pl. 2, fig. 14) attains a length as much as seventy times its own diameter. Measurements of two hundred individuals gave a frequency curve with a marked left-hand skew with the mode at 40μ and the extreme range in length of from 20 to 530μ. One-half of the individuals were included between 20 and 80μ. The longest individuals included those in which multiple fission was in progress and it is probable that the others were approaching that phase.

The contour of the body is not a smooth line, for the surface is traversed by spiral ridges with furrows between, giving it the form, except for its taper, of the shaft of a Norman Romanesque column. These ridges are four in number, broadly convex and equidistant and they wind about the body from the anterior end posteriorly from the left over to the right. It is thus like a left-hand screw if the anterior end is regarded as the tip. The steepness of the spiral varies with the length of the organism, its contraction, and the phase of the life cycle. In late stages of binary (pl. 2, fig. 17) and multiple fission (pl. 2, fig. 14) much of the torsion is relaxed. In stages which may be prior to binary fission (pl. 1, fig. 7; pl. 2, fig. 10) these may be

three to five turns, and a giant individual, presumably preceding multiple fission (pl. 2, fig. 13), has eight turns. Normal vegetative individuals (pl. 1, figs. 1, 6, 8) have one to two turns only.

The grooves between the ridges mark the location of ectoplasmic lines of deeply staining material, possibly myonemes, or extensions

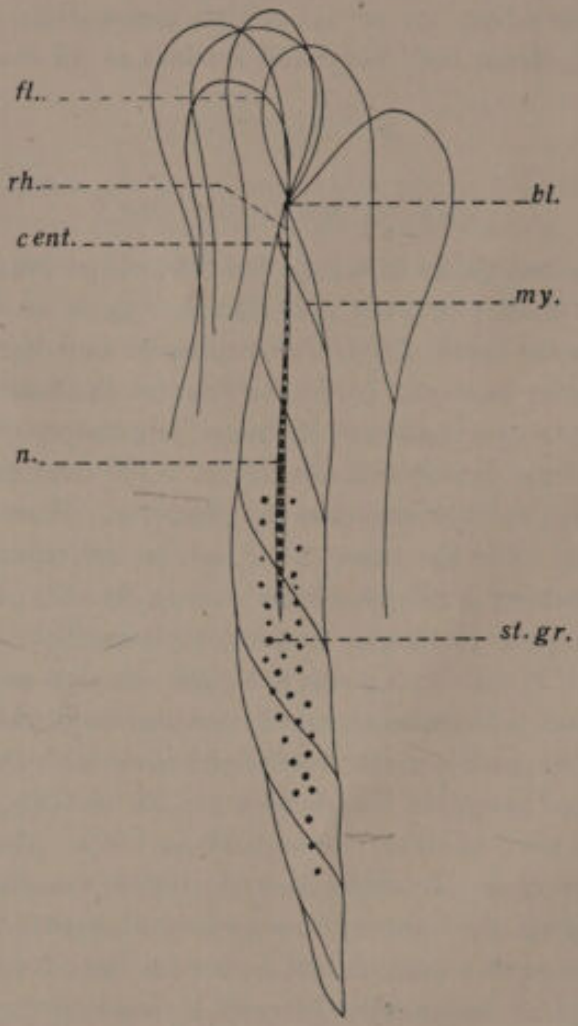

Fig. A. Semidiagrammatic figure of *Streblomastix strix* gen. nov., sp. nov. Black granules in cytoplasm show particles stained *intra vitam* with neutral red. Abbreviations: *bl.*, blepharoplast; *cent.*, centrosome; *fl.*, flagella; *my.*, myonemes; *n.*, nucleus; *rh.*, rhizoplast; *st. gr.*, stained granules. × 2000.

of the neuromotor system. They are feebly stained, if at all, in binary and multiple fission (pl. 2, figs. 14, 17, 19) and are sometimes marked by accumulations of minute granules staining black with haematoxylin. They terminate anteriorly at or near the blepharoplast and fade out posteriorly.

Cytoplasm

The cell contents are undifferentiated. No separation of ectoplasm, pellicle, and endoplasm is visible. There is no cytostome and no food particles or food vacuoles have been detected. There is no contractile vacuole visible. The only differentiated structures normally visible in the organism are the nucleus and the neuromotor apparatus. However, on treatment with neutral red (fig. A) certain granules became stained in the axial cytoplasm in the posterior two-thirds of the body, indicating an endoplasmic territory within which digestion of the absorbed food was in progress.

Neuromotor Apparatus

This neuromotor apparatus consists of a centrosome, rhizoplast, blepharoplast, myonemes, and six flagella. The centrosome (fig. A, *cent.*; pl. 1, fig. 9; pl. 2, fig. 10) is found at the anterior tip of the elongated nucleus. It appears to be a spherical granule in or on the nuclear membrane. It is temporary or evanescent and does not appear to play any visible part in either binary or multiple fission. It is quite possible that the granule thus interpreted is a mere temporary accumulation of chromatoidal substance on the rhizoplast without morphological meaning. Its position with reference to nucleus, rhizoplast and blepharoplast is similar to that of the centrosomes in *Giardia* (Kofoid and Christiansen, 1915, and Boeck, 1917) hence the designation suggested. It may be that the extrusion of the rhizoplast serves to bring the structure into view from a more intimate union with the nuclear membrane.

The rhizoplast (fig. A, *rh.*) is a slender, deeply staining thread running anteriorly from the centrosome or the anterior tip of the nucleus to the blepharoplast. Its length ordinarily is about equal to the greatest diameter of the body and the line of demarcation between the attenuate end of the nucleus and this thread is often not readily determined. There is a probability that this is more or less contractile, as is seen on a comparison of our figures. In one instance (pl. 1, fig. 7) this structure appears to be foreshortened and thickened, by contraction, onto the anterior end of the nucleus.

One remarkable feature of this structure is its capacity of being extended beyond the anterior end of the body as a long spike bearing the blepharoplast and its attached flagella at its tip. A con-

siderable increase in length to nearly three or even six times the normal seems possible (pl. 1, fig. 3). No evidence of an extension of the protoplasmic pellicle to form a sheath for this remarkable organ has been found. It may also be so foreshortened that the blepharoplast and centrosome are brought into close juxtaposition (pl. 1, fig. 2).

In view of the fact that the individuals lie closely packed against the digestive epithelium with the blepharoplast thrust against the epithelial cell of the host, it appears that this protrusible organ serves in some way as a part of a somewhat adjustable holdfast. We have no evidence that it can be or is ever thrust into the body of the cell, though such a mode of attachment seems possible.

The blepharoplast (fig. A, *bl.*)is a sphere at the anterior end of the rhizoplast about 0.5μ in diameter. The six flagella spring directly from it. It lies normally in the extreme anterior end of the body and is carried out with the extruded rhizoplast. The possibility of its being drawn out in detaching the parasite from its adhesion to the cells of the host is not precluded, but the numbers of such cases and the retention of normal symmetry of both body and rhizoplast does not support the suggestion of forceable extraction.

In one instance (pl. 1, fig. 4) a terminal blob of cytoplasm with a deeply staining terminal cap is attached to the side of the blepharoplast, and in another a considerable mass of protoplasm lies about the extruded blepharoplast. While these may be abnormalities it is possible that under certain conditions the cytoplasm assists locally in the holdfast function of the blepharoplast by forming an enlarged mass about it.

The six flagella are equal, habitually trailed posteriorly and about half as long as the body. They serve to keep up the circulation of the fluid contents of the digestive tract as they lie parallel to the closely packed bodies of the parasites (pl. 1, fig. 8) in the folds of the digestive epithelium.

The four peripheral spiral threads (fig. A, *my.*) which terminate at or near the blepharoplast must be regarded as a part of the neuromotor apparatus. Their relations to the blepharoplast and their stainability as well as the homology suggest this. Their function, if contractile (and their spiral course indicates this), appears to be to force the blepharoplast into intimate contact with the cells of the host. They persist at cytolysis and individuals are often found (pl. 1, fig. 10) in which these myonemes are frayed out as distinct lines.

These myonemes are evidently quite firm fibers, somewhat elastic and more or less rigid. They often stain very deeply especially in disintegrating individuals (pl. 1, fig. 1).

Direction of Torsion

The direction of torsion of these elements of the neuromotor apparatus is not without a deep significance. It is the same as that of the undulating membrane or attached flagellum of *Trichomonas, Trichomitus, Tetratrichomonas* and *Eutrichomastix* (Kofoid and Swezy, 1915), other polymastigotes in which torsion finds some structural expression. This same direction of torsion appears in the myonemes of *Pyrsonympha* and *Dinenympha* (Leidy, 1881). Grassi and Sandias (1893) reverse the direction of *Pyrsonympha* and of *Holomastigotes* in their figures, while Porter (1897) figures both directions. It is perhaps significant that his figures from life have the reversed direction while those from preparations, and therefore presumably accurate, have the normal leiotropic, or to be expected, direction. The reversals figured by Grassi and Sandias require confirmation before acceptance. The *Pyrsonympha* of this contribution (1893) is later designated as *Spirotrichonympha* by Grassi and Foà (1911) but without note of the differences in torsion. Zulueta (1915) figures the leiotropic direction in what appears to be Grassi's *Spirotrichonympha*. While it is quite possible that both leiotropic and dexiotropic genera or species exist, or that functional reversals of torsion occur in the individual it is even more evident that critical observations are essential to establish these diametrically opposed conditions. Pending such investigations the preponderance of the evidence favors the view that the torsion of the more primitive Trichonymphidae is leiotropic, that is from right over to left posteriorly, as it is in *Streblomastix*.

This is also the fundamental direction of the girdle and of the encircling transverse flagellum of the Dinoflagellata, and also of the attached collar-forming, ribbon-like flagellum of the Craspedomonadina (Burck, 1909). These facts are suggestive of an extensive and deep-seated leiotropism in the organization of the Mastigophora which finds expression in both externally attached flagella and internal contractile myonemes. That it may be conditioned by some equally pervasive stereometric properties of certain compounds of the living substance seems plausible.

Nucleus

The nucleus (fig. A, *n.*) shares the elongation which affects the body and appears to be pulled far anterior by the holdfast function of the blepharoplast so that in comparison with other polymastigotes its location is exceptionally far anterior. Its length is from 0.3 to 0.5 that of the body itself and its shape is fusiform but much more slender than the body, its length being fifteen to twenty-five times its diameter. It tapers about equally at both ends and appears in most of our preparations as a solid black axial strand in the anterior part of the body. Unless very strongly decolorized no internal structures can be made out. It appears to be composed of almost solid chromatin. When sufficiently decolorized (pl. 1, figs. 7, 9) a distinct nuclear membrane is evident within which a single row of black chromatin spherules, decreasing in size towards each end, can be detected. These are not uniform in size or arrangement and are about twenty-five in number. They are not unlike the chromomeres which we have found in the chromosomes of *Trichonympha*.

BINARY FISSION

The life history of *Streblomastix* presents those phases of development which we (Kofoid and Swezy, 1915 and Kofoid and Christiansen, 1915) have previously described for other polymastigotes, namely, binary and multiple fission. As yet no encystment has been detected and no indications of sexual reproduction. The differences in size which we find would doubtless some years ago have afforded a basis for the speculative designation of microgametes and macrogametes and the corresponding gametocytes as well as for the predication of sex, as Hartmann (1910) did in the case of *Trichonympha*. However, in the absence of evidence of *sexual behavior* and observed fusion of gametic nuclei, the free swing of such speculation is wisely held in abeyance.

Binary fission occurs in the trophozoite stage. There is some evidence that it is cyclic since many individuals in approximately the same stage of mitosis will be found in a single host. It is not, however, restricted wholly to such cycles since isolated cases of fission have been found and not all individuals parasitic in one host are in fission at one time. Successive infections and diverse stocks of the parasites doubtless exist in the host and may afford the occasion for this diversity.

Unfortunately our material, though extensive, has not given us all of the stages of nuclear behavior during fission so that we are unable to trace wholly, the successive phases of mitosis. We have found no clear evidence of chromosome formation, beyond the twenty-five or more spherical aggregates of chromatin in the linear nucleus. We have found no spherical stage of the nucleus, no skein, and have not detected the division of the blepharoplast which doubtless occurs, neither have we been able to find the paradesmose spun out between the daughter blepharoplasts (pl. 2, figs. 12, 13).

The process of binary fission, in so far as our partial evidence goes, takes place without any rounding-up of the elongated body. The anteriorly located blepharoplast divides, new flagella arise from one or both of the daughters, and one migrates to the opposite end of the body (pl. 2, fig. 11). In the meantime the nucleus has become greatly elongated, reaching from end to end of the body. It then constricts at the middle (pl. 2, fig. 12), finally parts there (pl. 2, fig. 19) and the two schizonts separate. To all appearances this is *transverse division*. Longitudinal division is, however, the fundamental and universal method of binary fission in the Euflagellata as compared with the Ciliata in which transverse division occurs. This seeming departure from the normal is, however, more apparent than real, for if the anterior blepharoplast divides and one daughter migrates to the posterior end we will have such an arrangement of schizonts as in *Trichomonas* after mitosis but before plasmotomy (Kofoid and Swezy, 1915, pl. 4, fig. 39). This is a temporary relation in such a metabolic form as *Trichomonas* but a more lasting one in *Streblomastix*. The mode of division is therefore still morphologically longitudinal though almost the last vestige of the appearance of that type of division has been submerged by the dominating elongation of the body in *Streblomastix*. While it is possible that there is a series of skein-chromosome changes in the nucleus which has escaped us, our present evidence indicates that these are also suppressed or hidden in the dense chromatin threads which part by simple median constriction (pl. 2, fig. 12). This parting is delayed until the posterior daughter blepharoplast is in its final position, as in other polymastigotes (Kofoid and Swezy, 1915). The frequent occurrence of stages in schizogony with the nucleus as yet undivided or dividing, but the neuromotor organelles in duplicate, indicates that both nuclear constriction and plasmotomy following thereon are prolonged processes.

MULTIPLE FISSION

Multiple fission in *Streblomastix* is a cyclic process occurring in many individuals in a single host at one time. A few only may be found in this stage, or, in some instances, at least, the majority of individuals may be in the multinucleate phase.

This condition is preceded by the growth of the schizont from the small size resulting from multiple (pl. 2, fig. 13) or binary (fig. 17) fission to a much larger or even giant stage (pl. 2, fig. 13), which may be as much as twenty-six times the length of the smallest schizont. The body may have as much as sixty times the mass of the smallest stages. The nucleus, however, does not increase proportionately, remaining, in fact, at least in many instances, almost unchanged (pl. 2, fig. 13).

At some period during this increase in size multiple fission sets in. Not all trophozoites entering upon it attain the maximum size as will be seen on a comparison of figures 15 and 16 on plate 2. It is possible that figure 16 represents only a detached section of a larger plasmodium which is fragmenting, or it may be a small trophozoite in the initial stages of multiple fission.

Contrary to the behavior of the blepharoplast-flagella complex in binary fission where it leads in division, preceding the nucleus, we find that in multiple fission nuclear division by transverse constriction is taking place prior to the division of the blepharoplast (pl. 2, figs. 14, 15). The type of nuclear division is the same as in binary fission. We have not seen stages of multiplication of the blepharoplast or of plasmotomy.

The linear form persists during the period of multiple fission and the nucleus becomes an elongated axial chromatin thread which becomes attenuate locally and parts transversely (pl. 2, figs. 13–16). The number of nuclear segments varies, probably in a 2–4–8 sequence, although irregularities in this are apparent. The largest number observed is eight. This accords with multiple fission in other polymastigotes (Kofoid and Swezy, 1915; Kofoid and Christiansen, 1915).

There is some evidence that this stage is contractile and that when foreshortened the nuclei slip by one another. This is not a common condition and probably does not represent a rounded-up condition obligatory for multiple fission but rather a passing response to stimulus resulting in contraction.

Multiple fission stages which we have seen provide for eight schizonts when the plasmodium parts by plasmotomy into its constituent zooids. From irregularities in the number of nuclei in preparations in which multiple fission is common, it seems probable that plasmotomy is an irregular dropping off of individuals or groups of individuals from the common mass as in *Trichomonas*. We have no evidence as to the presence of a centrosome during multiple fission and none as to the origin of the new blepharoplast-flagella complexes of the daughter schizonts.

ADAPTATIONS

Although seemingly simple in structure *Streblomastix* presents a series of structural adaptations which in the light of its parasitic mode of life become significant of intimate correlations with the conditions under which it exists and its habits. The entire loss of the cytostome is associated with feeding by osmosis and results in the disappearance of the bilateral asymmetry characteristic of polymastigotes such as *Trichomonas*. The absence of large food particles makes possible the elimination or reduction of cyclosis of the endoplasm and facilitates the change of form to a long and relatively very narrow spindle within which such movement would be impeded. The spiral course of the myonemes or spiral striae provides a most effective form of mechanism for an energetic thrust of the holdfast blepharoplast against or into the cells of the host. It is also a form of contraction which would disturb but slightly the closely packed grouping of the parasites. The spirally fluted surface combined with the action of the posteriorly directed flagella would give rise to vortex currents of the circumambient digestive fluids and thus provide the circulation essential to the metabolism of the parasite while at the same time permitting their segregation apart from the other organisms of the digestive tract of the host. The contractile extrusible rhizoplast-blepharoplast complex with its blob of cytoplasm affords an efficient structural holdfast. The elongation of the nucleus provides a spatially advantageous grouping for the nucleo-cytoplasmic interchanges in the absence of marked cyclosis.

The neuromotor apparatus is so arranged as to give well distributed contact with the surrounding medium by means of flagella and striae and with the host by means of the blepharoplast-rhizoplast, while this in turn is connected with the nucleus, thus establishing the structural essentials for efficient coördination of functions.

Even the reproductive phases of binary and multiple fission retain the elongated form characteristic of the trophozoite, thus permitting those stages to retain their position among the segregated parasites of their own kind. In other polymastigotes, such as *Trichomonas*, the stage of multiple fission is an amoeboid plasmodium, a rounded-up, somewhat shapeless amoeboid mass (Kofoid and Swezy, 1915). In *Streblomastix*, however, the linear form persists throughout this phase, in so far as we have observed it, although it shows greater laxity of form and less torsion (pl. 2, figs. 14, 15) than do the vegetative trophozoites. Thus in every feature of its structure and phase of its life history *Streblomastix* is intimately adapted to its peculiar parasitic mode of life notwithstanding its seeming simplicity of structure.

RELATIONSHIPS

The presence of six flagella definitely allocates *Streblomastix* in the Order Polymastigina. Its relative simplicity of structure as compared with most genera of this order is shown by the undifferentiated condition of the flagella. There is no single specialized trailer attached as an undulating membrane, and none intracytoplasmic as an axostyle.

The presence of the four longitudinal spiral ectoplasmic "myonemes" or extensions of the neuromotor apparatus is very suggestive of a relationship to the Trichonymphidae in most of which such lines arise from the blepharoplast and are the stems from which spring the many so-called cilia. This relationship will be more evident on detailed comparison. There are eight such lines in *Dinenympha gracilis* (Leidy, 1881) along the course of each of which small cilia take their origin, but there are no developed anterior flagella. Zulueta (1915) has shown that these extend posteriorly as free flagella and that they are grouped four and four on the daughter centrosomes at the poles of the spindle at mitosis. The species upon which Zulueta worked appears to be the same as that figured by Grassi and Sandias (1893, pl. 5, figs. 18–20) which Grassi and Foà (1911) later distinguish from Leidy's species as *Spirotrichonympha*. *Pyrsonympha vertens* (Leidy, 1881 and Porter, 1897) likewise has eight such lines arising from the blepharoplast with small lateral cilia arising from them. Anteriorly there is a blepharoplast from which a free slender thread extends anteriorly into the host cell not unlike the rhizoplast-blepharo-

plast of *Streblomastix* in superficial appearance, but possibly homologous with flagella and derived by modification from one or more of them. There are four such lines in *Holomastigotes* (Grassi and Sandias, 1893) giving rise to lateral cilia and pursuing a spiral course posteriorly from what is probably an anterior blepharoplast. There are, however, no large anterior flagella arising from this point.

The form most nearly allied to *Streblomastix* appears to be *Pyrsonympha* by reason of the persistence of an anterior outgrowth from the blepharoplast which may be homologized with flagella. However, it has lateral cilia arising from its spiral lines. These *Streblomastix* entirely lacks. This absence of the lateral ciliary coat justifies the exclusion of *Streblomastix* from the Trichonymphidae and renders its retention in the Polymastigina necessary. However, it may be regarded as closely related to that branch of the polymastigote stock from which the Trichonymphidae originated. *Streblomastix* thus forms a living link between the Polymastigina and the Trichonymphidae, linking the latter seemingly aberrant forms more closely and definitely than heretofore to the Flagellata as its most highly specialized order.

It is obvious that *Streblomastix* can not be allocated in the family Hexamitidae and that its relationships with the Polymastigidae are relatively remote. Even its inclusion in the Polymastigina is somewhat problematical. Its transfer to the Trichonymphidae is defensible but requires a profound modification in the definition of that order. To set forth more strongly its intermediate position we have left it in the Polymastigina and propose for it a new family, the Streblomastigidae, as follows:

Family Streblomastigidae fam. nov.

Polymastigina with spiral myonemes and anterior flagella.

Streblomastix gen. nov.

Streblomastigidae with six anterior flagella and four leiotropic myonemes. Type species *Streblomastix strix* sp. nov. from *Termopsis angusticollis* Walker.

SUMMARY

1. *Streblomastix strix* occurs as an intestinal parasite of the termite *Termopsis angusticollis* and is usually found attached to the epithelium of the intestine posterior to the malpighian tubules, segregated from the other parasites in the lumen.

2. It is a linear organism with the nucleus elongated to conform to the shape of the body. Its neuromotor apparatus consists of centrosome, blepharoplast, four myonemes and six flagella, connected with the nucleus by the rhizoplast.

3. Binary fission apparently occurs without spindle formation. The nucleus elongates and becomes constricted prior to the constriction of the protoplasmic body.

4. Multiple fission is a cyclic process occurring in many individuals in a single host at one time. It may be preceded by the formation of giant individuals. The body retains its linear formation throughout the process as in binary fission. The nucleus elongates and constricts into daughter nuclei. The greatest number observed is eight.

5. The shape of the body and the arrangement of its neuromotor apparatus show striking adaptation to the habitat in which the flagellate is found and to its parasitic mode of life.

6. *Streblomastix* forms a living link between the Polymastigina and the Trichonymphidae but without close relations in either group. We therefore propose for it a new family, Streblomastigidae, which we place in the Polymastigina.

Zoological Laboratory, University of California,
Berkeley, California.
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EXPLANATION OF PLATES

All drawings of *Streblomastix strix* were made with camera lucida from material stained with iron haematoxylin, with a magnification of 2500 unless otherwise stated.

PLATE 1

Fig. 1. Ordinary trophozoite showing few turns in torsion of the body.

Fig. 2. Contracted specimen showing centrosome and blepharoplast near together.

Fig. 3. Giant individual not fully drawn. Note elongated rhizoplast with blob of cytoplasm surrounding blepharoplast.

Fig. 4. Individual showing definite nuclear membrane and blob of protoplasm with chromatin cap attached to the blepharoplast.

Fig. 5. Body slightly contracted with little torsion.

Fig. 6. Myonemes showing heavily stained lines.

Fig. 7. Trophozoite with considerable torsion of body.

Fig. 8. Position of parasites in villi of intestine; attached to the mucous lining but not to the cells of the wall.

Fig. 9. Trophozoite with greatly elongate rhizoplast.

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PLATE 2

Fig. 10. Division form. Note length and structure of nucleus.

Fig. 11. Individual with myonemes fraying out from the protoplasmic groundwork.

Fig. 12. Binary fission with the nucleus constricting.

Fig. 13. Giant individual.

Fig. 14. Multiple fission with nucleus constricted in the formation of seven or eight schizonts.

Fig. 15. Multiple fission form preceding constriction of nucleus.

Fig. 16. Multiple fission form with three schizonts.

Fig. 17. Telophase of binary fission.

Fig. 18. Individual in process of fission.

Fig. 19. Final stage of binary fission; parting of schizonts.

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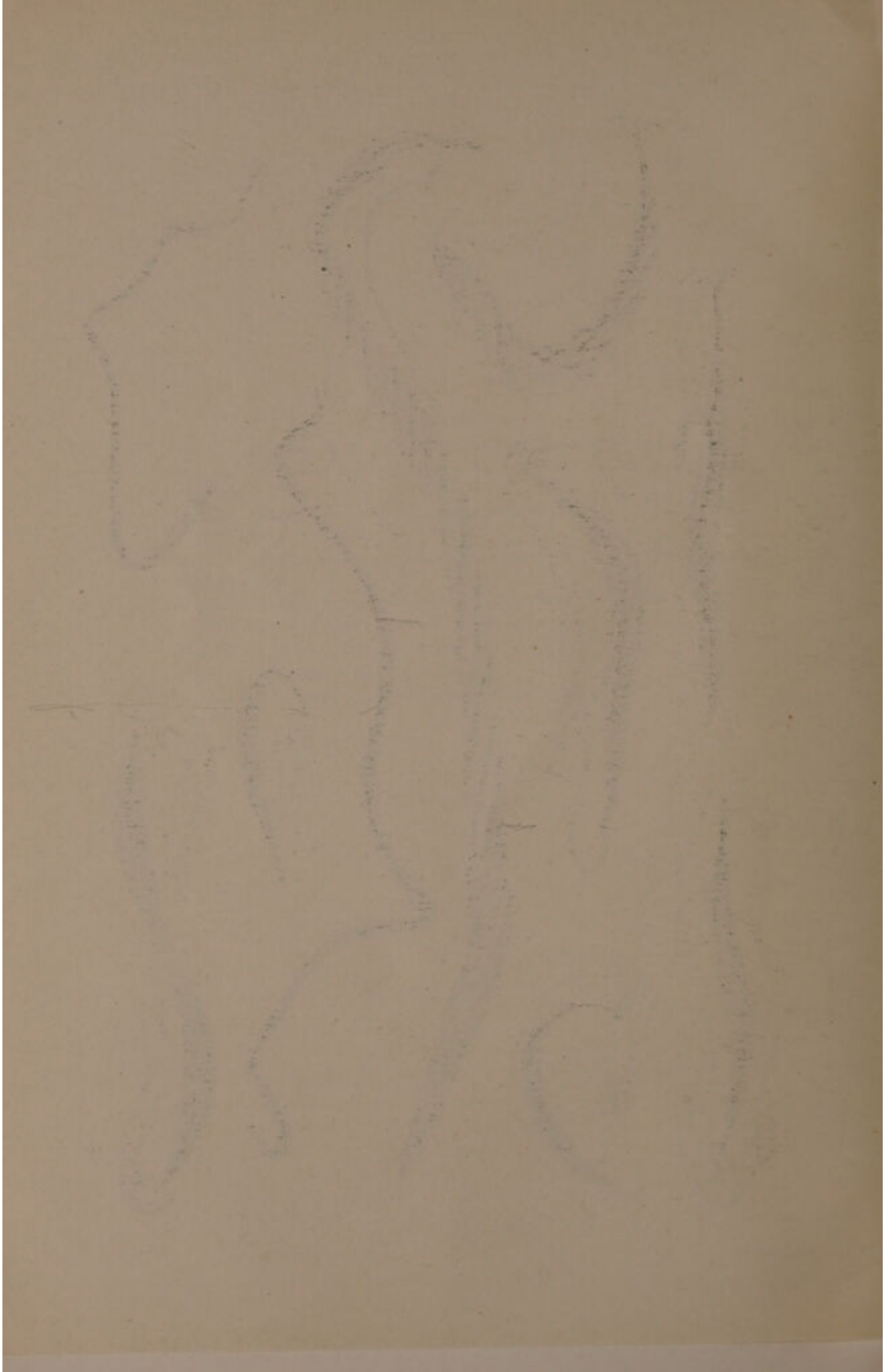

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STUDIES ON THE PARASITES OF THE TERMITES II. ON *TRICHOMITUS TERMITIDIS*, A POLYMASTIGOTE FLAGELLATE WITH A HIGHLY DEVELOPED NEUROMOTOR SYSTEM

BY
CHARLES ATWOOD KOFOID AND OLIVE SWEZY

CONTENTS

INTRODUCTION

The occurrence in polymastigote flagellates of a structurally integrated fibrillar complex consisting of centrosome, rhizoplast, blepharoplast, axostyle, undulating membrane, parabasal body, and flagella in *Trichomonas* was described by us (1915) and the complex designated as an extra-nuclear motor apparatus. Its analogy to the neuromotor apparatus of the ciliate *Diplodinium* was noted.

In the following year the more highly specialized and intimately integrated fibrillar apparatus of the binucleate diplozooic *Giardia* was definitely designated (Kofoid and Christiansen, 1916) as the neuromotor apparatus. In a paper read before the Second Pan-American Scientific Congress at Washington, January 7, 1916, the senior author extended the neuromotor conception to the flagellates generally to include the centrosome-blepharoplast and its external and internal fibrillar derivatives and connections under the name of the neuromotor apparatus.

It is the purpose of this paper to describe the neuromotor apparatus or system of one of the simpler trichomonads in which there is no axostyle but in which there occurs in response to the parasitic habit an exceptionally massive development and structural continuity of the several elements of this coördinating organ system. The organism also presents a prophetic prolongation of the period of existence of the paradesmose and of the incipient stage of mitosis,—features which are strongly suggestive of a tendency which, if continued, might well culminate in the evolution of the diplozooic flagellates, such as *Giardia*. The potency of the biochemical environment of parasites in bringing to expression latent possibilities of the organization of the living substance is once again demonstrated in this flagellate of those extraordinarily parasitized insects, the termites.

OCCURRENCE

This flagellate has been found abundantly in *Termopsis angusticollis* Walk., a large termite commonly found in decayed oak trees on the University campus. The flagellate infests the posterior and midregions of the intestinal tract of the termite with only a slight infection or none at all of the anterior region. It is found in the lumen of the canal with no attachment to the wall. Associated with it is a large *Trichonympha*, and in cross-section of the entire intestinal tract it is found that these two flagellates, with the latter usually predominating, completely fill the lumen of the canal.

Almost every individual of this species of termite which has been examined has been found to harbor these flagellates. The number in a single host may vary greatly as it or the trichonymph may be the dominant form. In some instances the latter species may be rare with *Trichomitus termitidis* present in vast quantities. These are sporadic cases, however, with no indications of a rhythmical cycle

that is seasonal in its occurrence, as shown by examinations of the host which have been made throughout the year.

Trichomitus greatly resembles its near relative *Trichomonas* in its activities. It is, however, difficult to keep these flagellates alive in cultures, hence observations on the active forms have been limited. The extreme fragility of the cytoplasmic body as contrasted with the stout, persistent parabasal body, is particularly striking in preparations of living material. A few seconds usually suffices, in ordinary tap water, for the dissolution of the protoplasm, leaving the neuromotor system still intact.

Nutrition in *Trichomitus* is holozoic. It, like *Trichonympha*, is evidently only a commensal, or at least is not truly parasitic, i.e., living on the tissues or fluids of the host. The food particles found within the cytoplasm consist principally of woody fibers upon which the termite feeds.

MORPHOLOGY

The morphology of this species of *Trichomitus* is of especial significance not only in view of the distinctness with which the neuromotor organ system is developed and integrated but also in the unquestionable certainty with which the relationship of the centrosome to the blepharoplast is established, as will be shown later. The relatively large size of the organisms (75 to 150μ) and their abundance have made possible an analysis of these structures not obtainable with the smaller trichomonads of our earlier studies (Kofoid and Swezy, 1915).

Shape and Size of Body

The body of *Trichomitus termitidis* is exceedingly amoeboid and protean in life, having neither constancy of form nor resistance to deformation on contact with other organisms or objects. Its periplast is unusually thin and delicate and in the larger forms especially is not infrequently ruptured in the making of smear preparations. It has nevertheless a certain characteristic range of forms within which, in free movement, it is seen or preserved on fixation. These vary from the asymmetrical pyriform contour, with the large end anterior and the posterior tapering to a blind point (pl. 3, fig. 1), to the ellipsoidal (pl. 3, fig. 14) or subspheroidal shape (pl. 3, fig. 5), with the slightly greater diameter posterior to the center.

The factors conducing to these changes in form are the stages of general contraction of the body, the mass of food vacuoles which is usually greater in the more rounded forms, and the proportional length of the parabasal body and undulating membrane. As a result of multiple fission (pl. 4, figs. 28, 30) one of the daughters receives the ancestral parabasal and two of its associated flagella, both of which are disproportionally large for the cytoplasmic mass of this schizont. Regulative resorbtion, in (pl. 4, fig. 31) or out of a cyst, or rapid cytoplasmic growth, would be necessary to readjust the volumetric relation of the neuromotor system and the cytoplasmic mass.

The range in size in this species is very considerable (fig. A, 1–7). The smallest schizonts we have recorded (pl. 4, fig. 32) are but 16μ in length while the largest exceed 200μ. These giant individuals are probably approaching multiple fission. They rarely survive the smearing operation intact. Not infrequently the nucleus and its attached neuromotor system of such giant individuals will be found intact and still active after the loss of the cytoplasm indicating that this stage is particularly susceptible to destruction under normal conditions in the host. Most of the individuals seen range from 75 to 125μ in length.

The organs of *Trichomitus* (fig. A, 5) consist of the cytostome (*cyt.*), nucleus (*n.*), food vacuoles (*f. vac.*), and the neuromotor organ system. We will now consider these organs with the cytoplasm in detail.

Cytoplasm

The cytoplasm of *Trichomitus termitidis* is reasonably labile, finely granular, and somewhat alveolar in structure. This lability may be the cause of such abnormal proportions (such as are seen in plate 4, figure 30) rather than the inheritance of the ancestral parabasal suggested above. The dropping off by plasmotomy of the labile cytoplasm has been observed by us in *Trichomonas augusta* (Kofoid and Swezy, 1915). No contractile vacuole is present but food vacuoles (fig. A, 5, *f. vac.*) are found everywhere within the body except about the nucleus. These contain fragments of cellulose from the digestive tract of the termite or coccoid bodies, possibly bacterial (pl. 3, fig. 5). Defecation of undigested fragments has not been seen.

Upon treatment with neutral red a large number of food particles or metaplasmic droplets stain deeply. They lie scattered throughout the cytoplasm and are larger near the center of the body (fig. A, 8).

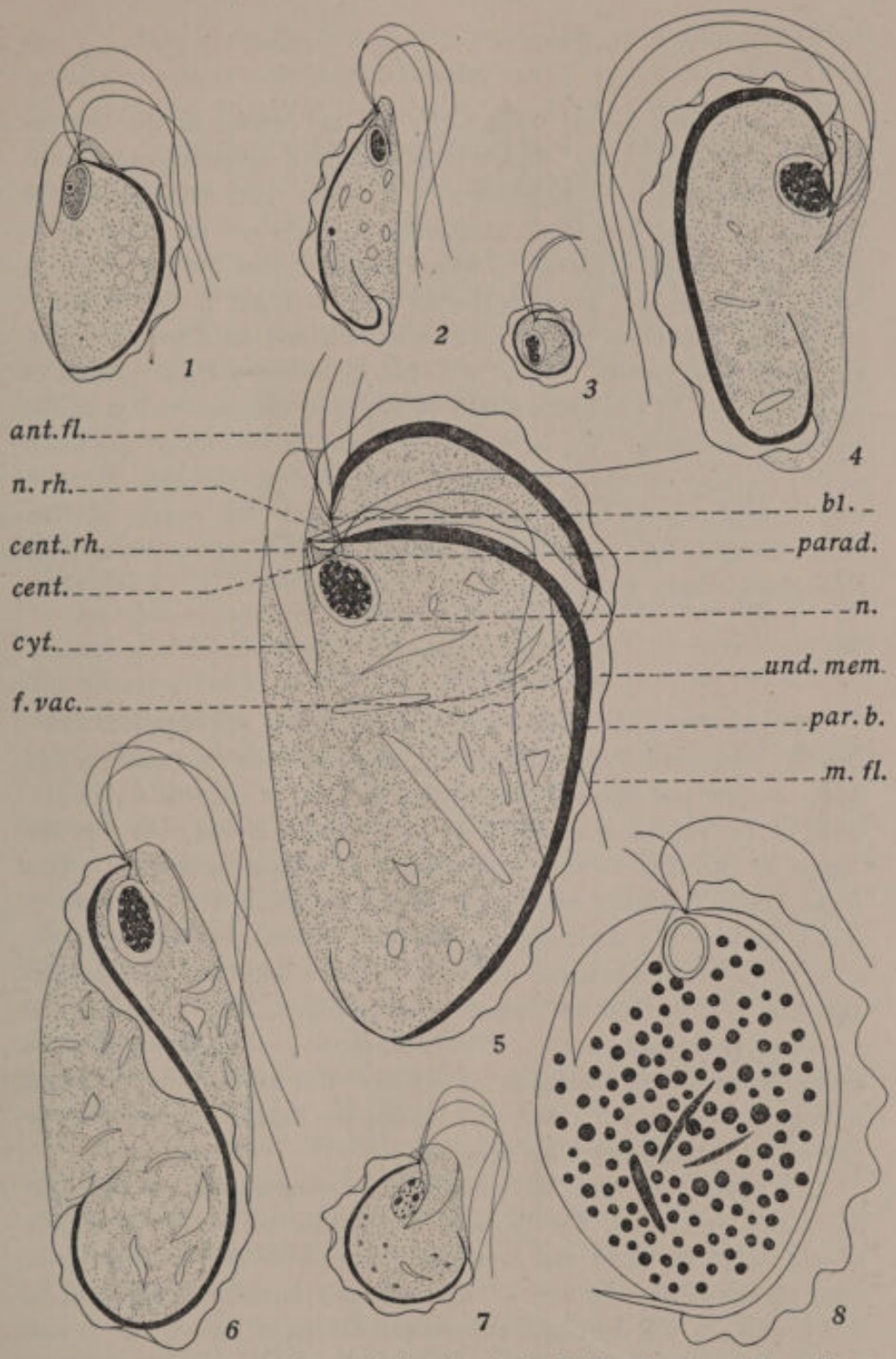

Fig. A. A series showing variations in size in *Trichomitus termitidis*. Figure 8 is drawn from individual stained with neutral red.

Abbreviations: *ant. fl.*, anteror flagella; *bl.*, blepharoplast; *cent.*, centrosome; *cent. rh.*, centrosome-rhizoplast; *cyt.*, cytostome; *f. vac.*, food vacuole; *m. fl.*, marginal flagellum; *n.*, nucleus; *n. rh.*, nuclear-rhizoplast; *par. b.*, parabasal body; *parad.*, paradesmose; *und. mem.*, undulating membrane. × 700.

Cytostome

The cytostome lies on the anterior ventral surface at the extreme anterior end of the body. It is a large elongated asymmetrical pocket, slender pyriform in outline but curved on its right side against the nucleus. Its length is about 0.3 that of the body and its width 0.3 to 0.2 its own length. It leads into the cytoplasm near the center of the body. Its large size, great flexibility and its slight projection anteriorly in a prominent lip all indicate its efficiency as a food-grasping and enveloping organ. We have found it during the later stages of binary fission, in the plasmodium of multiple fission, but not in the encysted condition. We have not been able to determine the exact mode of origin of new cytosomes. Its location immediately adjacent to the blepharoplast and nucleus necessitates a high degree of elasticity, integrity and resistance on the part of these organelles and the rhizoplasts arising from them.

Neuromotor System

The use of the term organ system to designate the complex, structurally integrated apparatus which links together the nucleus and motor organs and plays such a distinctive rôle at mitosis, seems justified by the canons of comparative morphology, unless it be that the dogma of the Cell Theory blights such morphological license. This organ system includes the blepharoplast (fig. A, 5, *bl.*) from which spring directly the three anterior flagella (*ant. fl.*), the attached, posteriorly directed undulating membrane (*und. mem.*) with its marginal flagellum (*m. fl.*), and the deeper lying parabasal body (*par. b.*), and a nuclear rhizoplast (*n. rh.*). The centrosome (*cent.*) lies within the centroblepharoplast, emerging at mitosis with its own independent centrosomal rhizoplasts (*cent. rh.*) joining the ends of the paradesmose (*parad.*) to the parent blepharoplast.

It is noteworthy from the standpoint of comparative cytology that the motor organelles, flagella, and undulating membrane terminate in and originate from the centroblepharoplast. The nucleus never loses its connection, by one or more rhizoplasts, with this structure, which also plays a dominant rôle in the drama of mitosis for at this time there springs from it the centrosome, which later divides, forming the paradesmose and its connecting rhizoplasts. To it also is attached the enormously large parabasal body, a reservoir of chromatoidal sub-

stance. This centroblepharoplast is thus most truly a morphological center intimately associated with the motor organs of this cell and their activities during the vegetative phase. At mitosis it dominates not only the extra-nuclear neuromotor system but also the polarization and subsequent movements of the chromosomes within the nucleus (fig. B, and pl. 3, figs. 5–21) as well. It will be difficult to find in any metazoan cell so continuous and complete a control, so pervasive an influence upon cell activities by the cell organ there known as the centrosome, as we find by the centroblepharoplast in *Trichomitus*.

The *centroblepharoplast*, during the vegetative phase of *Trichomitus* (fig. A, 6), is a minute granule about a micron in diameter anterior to the nucleus and attached to the anterior end of a single nuclear rhizoplast (fig. A, 5, *n. rh.*; fig. A, 2). This rhizoplast is a delicate thread easily overlooked. The centroblepharoplast itself is imbedded in the end of the deeply staining parabasal body, and may likewise readily escape detection.

In view of its later history it seems advisable to designate this granule at this period as the centroblepharoplast, since from it emerges the parent centrosome at mitosis. There is, however, no duplicity of structure evident, and there is no granule at any time at the point where the nuclear rhizoplast passing from the centroblepharoplast (fig. B, 1) and later from the blepharoplast proper (fig. B, 5) meets the nuclear membrane. After the centrosome withdraws from the larger granule (fig. B; pl. 3, figs. 6–21), the latter becomes a blepharoplast in the restricted sense of a basal granule from which the flagella originate, having no other function in mitosis, whereas the centrosome emerging from it divides and its daughters form the paradesmose between them, assume a polar position thereon and move to the nucleus (fig. B; pl. 3, fig. 21).

In our investigations of mitosis in the trichomonads (1915, pl. 2, figs. 21, 23; pl. 3, figs. 24, 29) there appeared to be a separation of the polar centrosome-blepharoplast into two granules, one of which, the centrosome, remained in the polar position on the nucleus, and the other, the blepharoplast, usually with the flagella attached, was removed a short distance therefrom. The conditions which we have found in *Trichomitus* where there is a general, more complete and perfectly distinct separation of these two organelles is thus the full accomplishment of the segregation imperfectly realized in *Trichomonas*.

The *flagella* are four in number, the three undifferentiated, equal, anterior ones (fig. A, 5, *ant. fl.*) and the attached posteriorly directed

one included within the undulating membrane as its marginal fiber (*m. fl.*) and carried out beyond the projecting tip of the parabasal body as a bit of free flagellum. The anterior flagella usually exceed the body in length. The posteriorly directed location in our figures is merely for spatial accommodation, an anterior direction being usual in life.

The *undulating membrane* (fig. A, 5, *und. mem.*) is attached to the left side of the body (fig. A, 1) in a sweeping C- or S-shaped curve reaching to the posterior end of the body (fig. A, 4, 6; pl. 3, figs. 1, 14). It exceeds the length of the body two to three times in some small schizonts (pl. 4, fig. 30). The coiling into the S-shaped forms appears to be an accommodation of the somewhat rigid but elastic parabasal, when longer than the body, to its location within the cytoplasm. The membrane always follows the course of the parabasal and remains adherent to it upon cytolysis (pl. 3, fig. 11). In one case (pl. 3, fig. 4) a detached membrane consisting only of the marginal flagellum and the fold of the protoplasmic pellicle running from the parabasal around the flagellum, was found free in a smear preparation. The membrane and flagellum are thrown into twelve to twenty subequal, subequidistant waves of contraction which fade out in the distalmost end.

The parabasal body (fig. A, 5, *par. b.*) is a rigid, elastic, deeply staining, chromatoidal rod lying at the base of the undulating membrane in the peripheral plasma of the body. Its C- or S-shaped course appears to determine the direction of that membrane. Its length usually exceeds that of the body by 10 to 25% and its diameter, 2 to 3.5μ, is greatest somewhat anterior to its middle. From this region it tapers gradually toward either end, terminating anteriorly at the centroblepharoplast (fig. A, 6), or in mitosis at the blepharoplast proper (fig. A, 5), and posteriorly at its junction with the marginal flagellum which projects beyond their union for a short distance as a free lash. It stains densely with haematoxylin and constitutes the dominating feature of the organism in all preparations and in life. It shows in stained sections (pl. 3, figs. 3 and 3*a*) a differentiated structure consisting of an outer deeply staining shell less than a fifth of its diameter in thickness and a less deeply stained core. This core is traversed by wedgelike discs of the cortical substance, which arise principally on the concave face and fade away towards the opposite side.

We have elsewhere (Kofoid and Swezy, 1915; Kofoid, 1917) interpreted the parabasal body as a reservoir of substances utilized by the neuromotor system in motor activities. It is obvious on observation

that locomotion by *Trichomitus* in the midst of the seething mass of parasites in the digestive tract of *Termopsis*, involves not a little expenditure of energy. It is also conceivable that the conditions of life therein are subject to marked variations incident not only to the food and feeding of the host but also to the varying constituents of the enormous mass of parasitic associates and their changing metabolism due to phases of their reproductive activity. Biochemical changes of no small import are consequently a feature of this creature's environment. That some of these are peculiarly fatal to *Trichomitus* is evident from the unusual numbers of moribund or cytolized individuals, each represented by a more or less decadent nucleus and its attached neuromotor apparatus, which may be found in most smear preparations.

Considerable changes in extent and volume of this structure are apparent upon an inspection of our figures, and even more so in our preparations. These are indicative of changes resulting from metabolism, or multiple fission, or both. In addition to the storage or reservoir function it is apparent that the parabasal in *Trichomitus* serves also as a somewhat rigid organ of attachment for the undulating membrane.

The other organs of the neuromotor system, the paradesmose and its rhizoplasts will be discussed in connection with mitosis. The paradesmose is a more or less temporary organ in most trichomonads, but in *Trichomitus* the organism appears to pass a much greater part of its existence in what is comparable to the prophase stage of trichomonad mitosis, so that the paradesmose is actually present, suspended by rhizoplasts from the blepharoplasts (fig. A, 5), and the whole neuromotor system is in some phase of duplication in many of the individuals which we have seen. The stage with a single centroblepharoplast and rhizoplast (fig. A, 1, 2, 6) is relatively much less common in this species than in other trichomonads. This prolongation of the prophase is the first step towards diplozoic organization such as we find realized in *Giardia*, where nuclear division is added to that of the duplication of the neuromotor system with the resulting formation of a coördinating system for multicellular organization.

Nucleus

The nucleus (fig. A, *n.*) is a symmetrical ellipsoidal structure, or even ovoidal or pyriform, with the wider end posterior (fig. B, 1). The longer axis is two to three times the shorter one in length. It

lies in or near and parallel to the major axis of the body on the left side of the cytostome within a short distance of the anterior end of the body. It shows distinctly a peripheral clear zone which is somewhat regularly chambered (fig. A, 1, 4, 5; pl. 3, fig. 9) as we have found it also in *Trichonympha*. This zone surrounds the dark, dense and often seemingly undifferentiated central chromatin mass. On heavy destaining this central mass is at times resolved into fairly uniform rounded granules (pl. 3, fig. 2) which appear to have some special relation to the elements of the chambered zone, indicating the possibility of a persistent organization of the nucleus. In some instances resting nuclei (fig. B, 1; pl. 3, fig. 5) show large deeply staining granules resembling nucleoli but these are as a rule absent. It is quite possible that these may be end knobs of emerging chromosomes.

The size of the nucleus ranges from 10 to 20μ in length whereas that of the body ranges from 16 to 200μ or even more. Although larger individuals have larger nuclei the increase in the volume of the cytoplasm is many-fold greater than is the increase of the nucleus in these giant forms.

One of the most significant and striking features of the life history of *Trichomitus* in the digestive tract of *Termopsis* is the repeated and seemingly constant occurrence of large numbers of isolated neuromotor systems with the nucleus attached but no enveloping cytoplasm. It is evident that the delicate pellicle is easily destroyed, and the labile cytoplasm escapes. Such an isolated structure in late prophase with duplicated neuromotor systems but degenerate nucleus is seen in plate 3, figure 11. These occurrences afford indisputable evidence of the organic continuity and structural integration of the neuromotor system of *Trichomitus* and of its direct and efficient physical connection with the nucleus. One of us (Swezy, 1915*a*) has noted a similar phenomenon in *Hexamitus*, a diplozooic polymastigote.

Still more significant is the fact that such isolated systems are still capable of flagellar activity and locomotion after the destructive process of cytolysis of the cytoplasm. They continue to move for some time in smears of the contents of the digestive tract mounted in tap water. The very large number of isolated systems found in some smears is indicative of a considerable period of persistence of the isolated neuromotor system and nucleus after the loss of cytoplasm. It is obvious that grave limitations on such activities must arise as a result of the loss of the cytoplasm. The nucleo-cytoplasmic reactions are suspended, nutrition is impeded if not wholly suspended, and rapid exhaustion is accelerated by the loss.

MITOSIS

Owing to this prolongation of the prophase of mitosis in *Trichomitus termitidis* an exceptional opportunity is afforded for a detailed study of the behavior of the neuromotor system during mitosis. This is made possible by reason of the fact that the centroblepharoplast is the center of the neuromotor system and the point of origin of structures and processes playing the main rôle in mitosis.

The phases of mitosis recognizable in the division of *Trichomitus* are those of the metazoan cell, but, as shown in our (1915) discussion on mitosis in trichomonads, considerably differentiated by the association of the extra-nuclear organelles of the cell in the protozoan from that in the usually simpler metazoan unit. In *Trichomitus* these differences, in consequence of the massive development of the neuromotor system, are even more developed than in the other trichomonads. They consist mainly in the sharp separation of centrosome and blepharoplast and the excessive prolongation of the prophase.

The *resting stage* of *Trichomitus* has a single nuclear rhizoplast running from the centroblepharoplast to the anterior end of the nucleus where it is attached to the membrane without evidence of enlargement into a centrosome on the nuclear membrane. This rhizoplast is often very short (fig. A, 1) and is never very long. The nucleus has in this period a dense, coarsely and uniformly granular central chromatin mass in which no polarization or evidence of chromosome formation is present.

The *prophase* (fig. B; pl. 3, figs. 1, 2, 5–15) is a prolonged one and in it the duplication of the entire neuromotor system takes place by division and outgrowth from the centroblepharoplast. The initial step is the splitting of the centroblepharoplast and the nuclear rhizoplast (fig. B, 1). It appears in some cases (pl. 3, fig. 1) that the rhizoplast may divide at the nuclear membrane first and split distally towards the centroblepharoplast as though these were directly upon the nuclear membrane at the anterior end of the nucleus. At the same time the centroblepharoplast separates into its constituent centrosome and blepharoplast, with the latter immediately dividing, one granule taking a single flagellum, the new parabasal body and one of the two nuclear rhizoplasts (fig. B, 1). The granule remaining attached to the old parabasal body takes the remainder of the flagella and the second nuclear rhizoplast. As these two blepharoplasts separate a thread is drawn out from each attaching them to the centro-

some (fig. B, 1). This is followed by a division of the centrosome. As the two new centrosomes move apart a darkly staining line or bar is drawn out between them, the paradesmose (fig. A, 5, *parad.*; fig. B).

The rhizoplasts connecting the centrosomes with the blepharoplasts gradually elongate with the paradesmose also increasing in both length and thickness. The latter structure with its connected centrosomes moves down until it comes to rest upon the nuclear membrane (fig. B). As a result of this the centrosome-rhizoplasts come to have the same length as the nuclear rhizoplasts. All four of these rhizoplasts are exceedingly delicate, particularly the nuclear ones and the latter especially long escaped our notice. Since the two are rather close together (fig. B, 5) their distinctiveness may be easily overlooked. The centrosomes (fig. A, 5, *cent.*) are minute knobs on the ends of the paradesmose, which is a stout, heavy, sometimes granular, deeply staining bar. They are not always visible as expansions of the bar and are never seen detached from it.

In the meantime the new undulating membrane and parabasal body have reached sizes equal to the ancestral ones (pl. 3, fig. 11; fig. B, 2–6). The parabasal body first appears as a slender dark thread growing posteriorly subparallel to the old parabasal in the peripheral cytoplasm (pl. 3, fig. 5). With the beginning of the formation of the paradesmose (fig. B, 2) the new flagella have all formed by outgrowth from the daughter blepharoplasts, two anterior ones and a posteriorly directed one as a marginal filament from the blepharoplast attached to the new parabasal, and only one anterior one from that attached to the old parabasal. At first (fig. B, 2) the new undulating membrane is very narrow but it soon attains full structural size and functional efficiency (pl. 3, fig. 11). With the completion of these structures by growth the duplication of the neuromotor system is accomplished.

Up to this time the changes visible within the nuclear membrane have been very slight. The chromatin granules or chromomeres grow larger and darker, and evidences of polarization appear in the linear grouping of the granules (fig. B, 6; pl. 3, figs. 6–10) which culminates in the emergence of linear V-shaped chromosomes. During this process a deeply staining cone-shaped extension of the central chromatin mass projects anteriorly until it comes in contact with the paradesmose (fig. B, 6; pl. 3, figs. 6–8). As this disappears the V-shaped chromosomes become more evident in the central mass, and

gradually spread out below the paradesmose as though hung across a string suspended from its ends (pl. 3, figs. 10, 12, 13).

The number of chromosomes is rather obscure since the loops are at all times rather closely entangled. It appears to be twelve or thereabouts. In the earlier phases each is composed (pl. 3, fig. 10) of a line of distinct granules, like chromomeres, but these fade out as the metaphase approaches.

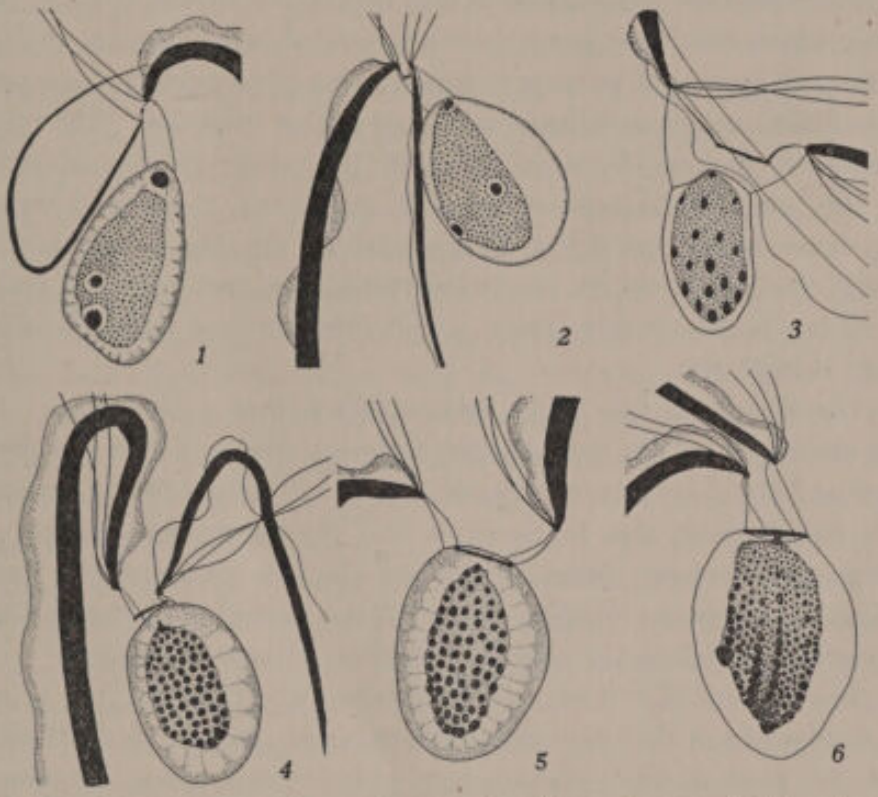

Fig. B. Development of paradesmose in *Trichomitus termitidis*. 1. Outgrowth of new parabasal body after separation of centrosome and blepharoplast and division of latter. 2. Centrosome divided and paradesmose forming between them; new flagella and undulating membrane formed. 3. Elongation of paradesmose as it moves down to the nucleus with the lengthening of the centrosome-rhizoplasts. 4. Later stage of same, condensing chromatin in nucleus with cone-shaped projection starting towards the nuclear membrane and paradesmose. 5. Paradesmose attached to nuclear membrane. 6. Attachment of central chromatin mass to paradesmose and formation of spindle; beginning of formation of chromosomes. × 1575.

The *metaphase* is obscured by the fact that there appears to be no arrangement of the chromosomes in an equatorial plate and no amphiaster phase of the nucleus. The nuclear structures appear to conform their arrangement to the stout bar-shaped paradesmose and not the latter mould itself to the configuration of the nucleus as in *Trichomonas* and *Eutrichomastix* (Kofoid and Swezy, 1915).

The loops or V-shaped chromosomes are gradually drawn by the angle of the V towards the two ends of the paradesmose (pl. 3, figs. 15, 17, 18). It is possible that each original loop is split lengthwise during this movement but the evidence for it is by no means clear. During this process the loops shorten, thicken, and stain more deeply, so that when they have finally parted (pl. 3, figs. 16, 20, 21) they form chrysanthemum-shaped rosettes.

The *anaphase* is brief and is also dominated by the stout paradesmose, which continues to produce a one-sided, asymmetrical grouping of the two groups of parting chromosomes and to modify the constriction of the nuclear membrane so that it is also one-sided. The nuclear membrane remains intact throughout the whole process of mitosis. In the late anaphase constriction is completed, the nuclei separate and move apart (pl. 4, fig. 22), stretching out the paradesmose between them as a result of the uncoördinated activities of the two daughter neuromotor systems, which are attached to the nuclei by their rhizoplasts.

The *telophase* (pl. 4, fig. 36) ensues before plasmotomy. In it the chromatin of the massed chromosomes rounds up in the central mass and the clear zone reappears and the ellipsoidal form is resumed. The paradesmose also fades away, and the centrosome merges with the daughter blepharoplast which, by the shortening of the nuclear rhizoplast, comes to lie closer to the nuclear membrane, thus bringing the schizont back to the nuclear condition prior to mitosis.

The process of mitosis in *Trichomitus* is similar to that in other trichomonads in that the nuclear membrane remains intact throughout the process, the extra-nuclear paradesmose arises between the daughter centrosomes, and the duplication of the neuromotor system proceeds from the centroblepharoplast and takes place prior to division of the chromosomes. It differs in having a distinct separation of centrosome and blepharoplast for a long period, in having a rigid bar-shaped paradesmose, and in the large size and greater elongation of the chromosomes. This higher specialization is conditioned by crowded conditions of parasitic life in association with other parasites of relatively complex organization. The conditions of locomotion in this association and the excessive amount of stimulation consequent thereon are causes conducive to the extraordinary development of the neuromotor apparatus in *Trichomitus* and the resulting modifications in mitosis.

BINARY AND MULTIPLE FISSION

Both of these processes take place frequently in *Trichomitus*. There is much evidence of a high death rate in this species within the digestive tract of its host. This is compensated for by rapid multiplication. For this also there is abundant evidence in our material.

The distinction between stages of binary and multiple fission is not readily made in all cases in early stages. The earliest phases of both are obviously the same in mitotic phenomenon. Binucleate plasmodia may lead on to further division when prophases appear in their nuclei (pl. 4, fig. 23). When, however, no later prophase phenomena are evident and the organism is not unusually large (pl. 4, fig. 36) binary fission only may be expected. It also occurs in the small cysts (pl. 4, fig. 26) and small free forms (pl. 4, fig. 29).

Multiple fission, on the other hand, occurs in large individuals and leads to the formation of, presumably, eight-celled plasmodia.

One such large plasmodium with six constituent zooids is seen in plate 4, fig. 28. It is possibly in plasmotomy and has lost two of its members. Not all multiple fission plasmodia are as large as this. Smaller ones, in which the first division has been completed and the second initiated, are frequently found (pl. 4, figs. 23, 25, 35).

The process is one of three repeated divisions prior to plasmotomy with the formation of an eight-nucleate somatella and its subsequent disintegration into its constituent zooids by their detachment singly or in groups. These somatella or plasmodial stages are exceedingly mobile and the constituent individuals shift about without seeming order of arrangement. The uncoördinated movements of the powerful neuromotor apparatus of the individual zooids finally result in their separation. The connecting paradesmoses are lost long before this separation.

No trace of unequivocal sexual phenomena has been detected. Large and small individuals simulating macrogametes and microgametes and gametocytes are present. Binucleate individuals without evidence of recent division occur (pl. 4, fig. 36), simulating zygotes, and similar associations are found in cysts (pl. 4, fig. 26). There is, however, no evidence of maturation divisions leading to gamete formation, no sexual behavior detected, and no evidence of the fusion of gametic nuclei. In the absence of such evidence any conclusions as to the possibility of sexual reproduction in this organism must be held in abeyance.

ENCYSTMENT

Associated with the vegetative and fission stages of *Trichomitus* in a few hosts but not all, we have found many small individuals (pl. 4, figs. 24, 29–33) in which binary fission is occurring and in which there is a tendency for the body to round up into a spheroidal or ellipsoidal mass. These small sizes may result from rapid fission without compensating growth or from plasmotomy of part of the cytoplasm. In these same hosts occur also numerous ellipsoidal cysts about 13 by 20μ with a deeply stained network with thickenings at the nodes spread over the surface (pl. 4, figs. 26, 27). The cyst wall is double and the network is due to the accumulations of some stainable substance between the walls. Within the cyst is a single individual (pl. 4, fig. 31) with a very long parabasal and undulating membrane making nearly two complete coils, such as might result from the coiling up of an individual with abnormally large neuromotor system (pl. 4, fig. 30). In other cases two such individuals (pl. 4, fig. 26) are found within the cyst. This might result from encystment after or during mitosis but prior to plasmotomy. Such cysts may facilitate the carrying over of infection from one individual host to another. They have all the indications of being resistant stages.

RELATIONSHIPS

This is a species of *Trichomitus,* a genus founded by one of us (Swezy, 1915*b*) for the reception of a minute and simple trichomonad from amphibians. In its vegetative phases the form here described has the morphological features of *Trichomitus parvus* Swezy, namely, three anterior flagella, undulating membrane, parabasal, and no axostyle. It differs greatly in size, in the massive development of the parabasal, and at mitosis in the distinct separation of centrosome and blepharoplast. Binary and multiple fission were followed in the species from amphibians but no trace of such separation was detected.

Such a difference as this might justify generic separation but it might be impracticable to apply it in future diagnosis of any species of the genus which may come to light. The difference is, however, of such morphological import as to justify subgeneric separation. We accordingly assign it to

Trichomitopsis subgen. nov.

Trichomitus with centrosome separated from blepharoplast at mitosis. Type species *Trichomitus termitidis* sp. nov. from *Termopsis angusticollis* Walker.

SUMMARY

1. *Trichomitus termitidis* sp. nov. occurs in the intestinal tract of *Termopsis angusticollis*. It is apparently not pathological to its host, is never attached to the wall and feeds on the débris of the intestinal contents.

2. It has a highly developed neuromotor system with parabasal body, undulating membrane, centroblepharoplast and flagella attached by a rhizoplast to the nucleus.

3. Binary fission occurs frequently. Mitosis is marked by the development of a large paradesmose following the separation of the centrosome from the blepharoplast. One schizont retains the old parabasal body and membrane, while new ones are formed for the other.

4. Multiple fission results in the formation of an eight-zooid somatella followed by plasmotomy.

5. Owing to the great differences in the process of mitosis between *Trichomitus parvus* and the new species, *T. termitidis*, subgeneric distinction is given to the latter, as we assign it to the new subgenus *Trichomitopsis*.

Zoological Laboratory, University of California,
Berkeley, California.
Transmitted September 6, 1918.

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There is no mention of association of this flagellate with a typical Trichomonas but Cleveland (1925) says that Kofoid has informed him that such an association always occurs - Cutler finds this answer, and includes the view that Trichomitus is a Trichomonas in which an undulating membrane is not visible. He employs name Trichomitus termopsidis. Kirby (1931) in his description of Trichomonas

EXPLANATION OF PLATES

All figures of *Trichomitus termitidis* sp. nov., from material stained with iron alum haematoxylin. Magnification 625, unless otherwise stated.

PLATE 3

Fig. 1. Trophozoite in early prophase of division with nuclear rhizoplast divided.

Fig. 2. Isolated neuromotor apparatus of the same stage with nucleus attached.

Fig. 3. Sagittal section of the parabasal showing the outer deeply staining shell and the inner core. × 1250.

Fig. 3*a*. Cross-section of the same. × 1250.

Fig. 4. Isolated undulating membrane with darkly staining marginal flagellum. × 1250.

Fig. 5. Early prophase with outgrowing of new parabasal body.

Fig. 6–10. Early prophase stages showing gradual condensation of chromatin into definite chromosomes. New parabasals, undulating membranes and flagella complete.

Fig. 11. Isolated neuromotor system in prophase stage of division.

Figs. 12, 13. Spindle forming with chromosomes attached to it by the angle of the V. × 1250.

Fig. 14. Prophase. Note relative lengths of parabasal bodies.

Figs. 15, 17. Separation of chromosomes into two groups. × 1250.

Figs. 16, 18–20. Anaphase with paradesmose elongating. × 1250.

Fig. 21. Beginning of constriction of the nuclear membrane. × 1250.

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PLATE 4

Fig. 22. Late anaphase, showing formation of new cytostomes. Note length of paradesmose.

Fig. 23. Multiple fission with beginning of the third division of nucleus.

Fig. 24. Small individual apparently about to encyst.

Fig. 25. Multiple fission with nuclei in preparation for second division.

Fig. 26. Encysted form which has divided. Note structure of cyst wall. × 1250.

Fig. 27. Encysted form showing outer surface of cyst only. × 1250.

Fig. 28. Somatella of six zooids formed by multiple fission. Full complement of flagella, parabasals, undulating membranes and nuclei.

Fig. 29. Dividing individuals just escaped from cyst.

Fig. 30. Product of multiple fission. Note relatively enormous length of parabasal.

Fig. 31. Encysted form with single nucleus.

Fig. 32. Individual following excystment.

Fig. 33. Small form dividing. Note relative lengths of parabasals.

Fig. 34. Late anaphase of binary fission.

Fig. 35. Second division of nucleus in multiple fission.

Fig. 36. Telophase of binary fission.

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STUDIES ON THE PARASITES OF THE TERMITES III. ON *TRICHONYMPHA CAMPANULA* SP. NOV.

BY

CHARLES ATWOOD KOFOID AND OLIVE SWEZY

CONTENTS

INTRODUCTION

Among the instances of parasitism by the Protozoa in the alimentary tracts of the Metazoa, none exceeds that found in the termites as to the relative volume of the invading parasites, the diversity of organization found among them, and the degree of specialization which they attain. In the vanguard of this evolutionary development stands the genus *Trichonympha* discovered by that pioneer American investigator, Dr. Joseph Leidy, who in 1877 and 1881 first revealed this teeming menagerie of termite parasites to scientific view.

The extent of specialization attained by *Trichonympha* and its allies has obscured their relationships, allied them at first with the ciliates, and obliterated their true affinities with the flagellates. The purpose of the present paper is in part to place the flagellate origin and relationships of the Trichonymphidae on a firm cytological foundation. It is also our aim to set forth the most complicated neuromotor system thus far known among the Protozoa, to analyse its elements, relate them to the elaborate motor activities of the individual, and trace their behavior during the mitosis of this highly specialized cell.

The degree of structural complexity, the extent of the coördinated mechanism, the number of its constituent elements, and the striking similarity of their interrelations in *Trichonympha* to those obtaining in multicellular organisms, is illuminating as to the biological significance of cellular organization. This organism is a single cell, with one nucleus, yet it has attained a degree of structural complexity and functional diversity in respect to one organ system comparable almost with that of its host and surpassing that of many of the lower Metazoa. The multicellular state is plainly not an essential condition for evolutionary specialization and functional efficiency, except as it places limits on the size of organisms and on developmental processes arising therefrom. The differentiation of organs within the confines of a single cell is here accomplished with a perfection comparable with that where the organ is a complex of diverse cells instead of one of many parts within a single cell. The organism and not its cells is the fundamental basis of differentiation.

OCCURRENCE AND ACTIVITIES

The posterior and mid-regions of the intestinal tract of *Termopsis angusticollis* Walker are usually found to be greatly distended, often filling the entire cavity of the body. It is in these regions that *Trichonympha campanula* is found in great abundance, filling the entire lumen of the canal but never attached to its walls. When other flagellates, as *Streblomastix strix* and two other smaller forms, are present with it in any numbers, these are found occupying the region near the walls, with *Trichonympha* filling the central portion of the canal.

The intestinal contents resemble a thick milky fluid, the great consistency of which is due to the vast numbers of protozoans which it contains, along with minute débris of woody particles. Through this mass the trichonymphs move with considerable rapidity, using the mobile anterior portion of the body to clear a pathway. This is done by quick sidewise movements, such as those shown in figure C, 2, bending the anterior cone first to one side and then to the other without halting in its progress. Its path is usually straight ahead with little or no rotation of the body on its longitudinal axis.

The group of long anterior flagella seems to be its main propelling organelle. These are thrown forward and sidewise somewhat as other flagellates use their flagella, with, however, less of the forward motion than is usual with anteriorly attached flagella. The flagella or cilia on the remainder of the body are uniformly directed posteriorly. These are found to have a characteristic motion, both during locomotion of the organism and when it is at "rest." Waves of contraction pass constantly from the anterior end backward to the tips of the flagella, affecting all on any given plane alike. The motion of the group of longer, anterior flagella, particularly when they are directed posteriorly, as is frequently the case, may coincide with these waves of contraction, which then include all the ciliary covering of the body. These waves continue during observation on the slide until the rounding up of the body in the culture fluid prior to dissolution.

These flagellates are extremely susceptible to environmental changes. When placed in culture slides with tap water, distilled water, normal salt solution, or Ringer's fluid, the results have been disastrous in a very short time, varying from a few minutes to half an hour. The body rounds up with considerable distension, becomes transparent with an almost complete loss of organelles, the nucleus only remaining visible until the final dissolution of the body.

The flagella continue moving as long as they are visible at the beginning of this process but soon disappear.

Dilute egg albumen has been found to be the most satisfactory culture medium but even with this these flagellates have been kept alive only a few hours, with the rounding-up process soon visible in a majority of the forms.

As has been earlier noted for trichonomad flagellates (Kofoid and Swezy, 1915), binary fission in *Trichonympha campanula* is cyclic in its occurrence, being found abundantly in occasional individual hosts.

In order to secure all stages of the division cycle, preparations were made every day continuously for thirty days, the number of individual hosts used each time varying from three to ten or more. For several days also these preparations were made at two-hour intervals during the day. In this way we have been able to secure a fairly complete series of figures of all stages of the mitotic process. A few isolated division forms may be present in almost any host, but in general it has been found that where more than these occur in a single host, that from one-third to one-half or even more of the individuals of a single parasitic species observed, will present some signs of division and usually of a single stage of mitosis. This is especially true of the early prophase stages which, on the whole, have been most abundant in our material. A few slides have been found, however, on which almost all stages of the division process may be found. This seems to be the exception and not the rule.

MORPHOLOGY

Every observer of the parasites of the digestive tract of the termites is amazed, on his first glance at its seething contents, at the locomotor activities displayed by its constituent organisms. Foremost among these in constancy, agility, and variety of its movements is *Trichonympha*. It is signficant that Leidy (1877) used the specific name *agilis* for the first trichonymph discovered. This capacity for motor activities is based on structural features of corresponding complexity. Hence it is that any discussion of the morphology of this animal is mainly occupied with the neuromotor system. The only other differentiated structure found in the body is the nucleus. There is no apparent mouth, no excretory system, no food-taking organs. The food vacuoles are temporary in a seemingly undifferentiated endoplasm. The structural specialization of this parasite thus affects mainly one organ system only, the neuromotor system.

Shape and Size

The shape of the body is companulate with the posterior end broadly rounded (fig. B), almost spheroidal, while anteriorly it is a tapering, slightly convex cone of 20°. The anterior half of the body

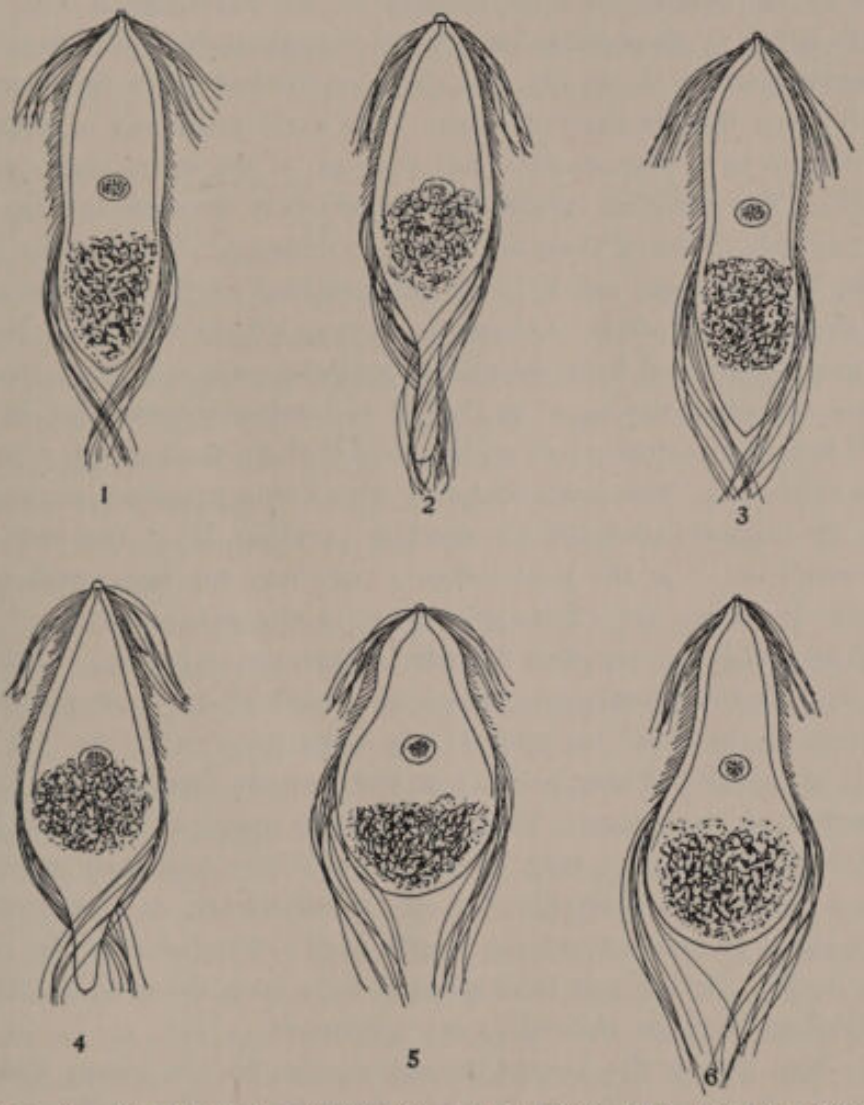

Fig. A. *Trichonympha campanula*, drawn from life, showing metabolic changes in the posterior portion of the body of the normal trophozoite. × 186.

has a transdiameter of about one-half or less than that of the posterior part. It is contracted to a slender acuminate point at the anterior end which is surmounted by a rounded, transparent, caplike structure (fig. B, *oper.*) which we call the operculum. This covers a shallow depression at the base of which is a small pit surrounded by a darkly staining ring (fig. C, 5, 9). The base of this pitlike depression may

be protruded a short distance (fig. C, 1, 2, 8) or may be deeply withdrawn (fig. C, 5). The membrane forming the caplike covering is thin, remarkably transparent, and does not stain with any of the reagents used in the preparations of the material.

The body is radially symmetrical with graceful outlines. All trace of the characteristic asymmetry of the flagellates is lost. Its length is two or three times its greatest transdiameter which is in the posterior third of the body. The shape of the body may vary considerably from the campanulate form. The early prophases of division are marked by a characteristic rounding up of the entire body (pl. 7, fig. 31). The variation in shape in the ordinary trophozoite stage are less marked. Some of these are shown in figure A. The anterior half of the body is more stable in its outlines than is the posterior half and shows fewer metabolic changes. It may become thickened, losing the graceful curved lines, becoming conical in shape (fig. A, 4), or with a transdiameter equal to that of the posterior part (fig. A, 1).

It is in the posterior half of the body that the most striking variations are found. This is covered only with a thin periplast in contrast with the thick ectoplasm of the anterior part (fig. B). As a result of this condition it is the most delicate part and the one which most frequently shows the effects of injury in the manipulation of the material and its preparation for microscopical examination. In addition to this individuals are sometimes found which show metabolic changes in the posterior part of the organism, which are not the result of injury but may possibly be due to some chemical changes in the surrounding medium. These changes are shown in figures A, 2–4.

The posterior part may be drawn out into a slender cylinder with a consequent shortening of its transdiameter, and may sometimes equal or even exceed the length of the remainder of the body (fig. A, 2). Individuals thus affected have been observed in living material and appear normal in other respects.

In size this is the largest known species in the genus *Trichonympha*. In general length it varies from 250 to 460μ and in width from 110 to 200μ. The average length is about 350μ. A frequency curve plotted from 121 measurements had its mode at 360μ and a slight right-hand skew. *Trichonympha agilis*, as figured by Leidy (1881) and Porter (1897), varies in length from 60 to about 100μ, and for *T. hertwigi* Hartmann (1910) gives the length as between 260 and 330μ. The latter species equals in length many individuals of *T. campanula*, but falls short of the larger specimens, as well as having

a more slender body, its width varying from 40 to 60μ. In shape *T. campanula* differs but little from *T. agilis*, the latter having a somewhat broader transdiameter anteriorly with a noticeable constriction near the middle of the body or slightly anterior to it. This constriction is entirely absent in *T. campanula*. Its divergence from *T. hertwigi* is still more pronounced, varying greatly in proportions from any of the three distinct forms which Hartmann (1910) has described under that name, being more slender anteriorly and broader posteriorly.

Neuromotor System

Included in this system is the entire set of fibers concerned in movement of all parts of the body, both in the ectoplasm and the endoplasm, the external coat of cilia, the three zones of flagella, and the centroblepharoplast from which the other fibrils radiate, take their origin, or with which they have some more or less direct connection. In view of its many elements, their diversification into various groups, and their structural coördination we apply the conception of an organ system to their complex and designate it as the neuromotor system.

This system consists of two distinct parts, lying respectively in the ectoplasm and endoplasm, and differing in their staining reactions and in their relationships to the process of mitosis. The first part is composed of the flagella, their basal granules and connections, the anastomosing sheet of oblique fibers, the centroblepharoplast and paradesmose. It appears to be more highly specialized as conductile organelles, although the flagella may be sensory and are certainly contractile. This part of the system shares in mitosis, forms the polar centrosomes, the structures radiating therefrom, and the paradesmose. These organs lie in or project from the ectoplasm. The other part consists of two antagonistic sets of fibers, the outer circular and inner longitudinal myonemes. These lie against if not in the endoplasm, and take no direct part in mitosis. Their connections with the centroblepharoplast are problematical. They are primarily contractile.

The use of the term neuromotor to designate the system is based on morphological grounds and observations on the behavior of the animal. It responds to stimuli, contracts, and moves as it might be expected to do with such a structurally coördinated mechanism. It must, however, be evident that the distinction between strictly neural

and strictly motor functions can not be sharply drawn and that the two functions are in all probability not wholly separated and carried on by distinct organs, but are rather in most, if not all parts of the system, served to some extent by the same structures. It is possible that the basal ciliary lines (*b. cil.*, fig. B) are wholly conductile rather than motor, and that the centroblepharoplast has little if any motor function. It is likewise not improbable that the longitudinal and transverse myonemes are mainly motor and that the flagella and cilia are sensory, conductile, and motor, while the enveloping undifferentiated cytoplasm in which all these structures lie, except cilia and flagella, has doubtless preserved some of its primitive neuromotor capacities. The oblique fibers are by their staining reaction allied to the neural system and function rather than to the motor. These overlapping conditions, however, do not preclude the use of the term neuromotor system to designate the complex integrated fibrillar structure of *Trichonympha*.

The discussion of this system will for convenience include also that of the surrounding cytoplasmic structures, such as the surface ridges, and the alveolar layer, although these are not strictly parts of the system.

The neuromotor system (fig. B) may be divided into two very unequal parts according to its location in the two fundamental subdivisions of the cytoplasm, the ectoplasmic and endoplasmic. The ectoplasmic portion consists of the anteriorly located centroblepharoplast (*centrobleph.*) from which spring the anterior flagella (*ant. fl.*) and from which radiate posteriorly the longitudinal basal ciliary lines (*b. cil.*) which give rise laterally to the lateral cilia (*lat. cil.*) and posteriorly to the posterior cilia (*post. cil.*). These lines are in the axes of the longitudinal ridges (*surf. rdg.*) which cover the surface above the equator of the posterior region. From the centroblepharoplast arise also the spirally directed, opposing sets of oblique fibers (*obl. f.*).

One set of fibers, the circular transverse myonemes (*tr. my.*), lies in the innermost zone of the ectoplasm, or outermost zone of endoplasm. Their course is such that their connection, if any exists, with the centroblepharoplast can not be traced. In the peripheral layer of endoplasm the stout longitudinal myonemes run posteriorly from the centroblepharoplast to the margin of the thick ectoplasmic zone. No trace of any other part of the neuromotor apparatus can be found within the labile endoplasm. The juxtaposition of nucleus and cen-

troblepharoplast at mitosis is suggestive of some structural relation such as is represented by the nuclear rhizoplast in the Polymastigina, but no rhizoplast has as yet been found by us in *Trichonympha*.

ECTOPLASMIC STRUCTURES

A superficial examination of *T. campanula* reveals the fact that the anterior two-thirds of the body is marked off by a thick ectoplasm. This is thickest anteriorly and as its extreme posterior limit becomes thin, disappearing distally in the frail pellicle of the posterior region of the body (fig. B; pl. 5, fig. 6). Under the low powers of the microscope this appears as a nearly clear zone in the living flagellate, distinctly marked off from the granular endoplasm. Higher magnifications bring out the fact that it is divided into three distinct zones which are traversed by fine lines and that one layer or zone contains alveoli closely massed together. These are, (1) the outer projecting ridge, (2) the alveolar layer, and (3) the inner ectoplasmic layer (fig. B; pl. 12, fig. 80). In stained material the structure of the ectoplasmic region can be more clearly differentiated, and reveals a high degree of complexity. These regions will now be described, beginning with the outer zone and proceeding inward.

Surface Ridges: The outer surface of the body is raised in relatively high, narrow, longitudinal ridges (fig. B, *surf. rdg.*) which are best observed in a transverse section (pl. 5, fig. 4; pl. 12, fig. 80). The cilia or flagella which cover the surface of the body spring from the crest of each ridge. These ridges extend from the anterior end posteriorly to the equator. The spaces separating them become narrower anteriorly and the ridges fewer in number, finally converging around the base of the operculum-like structure at the extreme anterior tip of the body (pl. 5, fig. 1). Posteriorly they fade out at the point where the differentiated ectoplasm and flagella disappear, giving place to the thin periplast of that region of the body. The crests, or tops, of the ridges are narrow or knifelike. The base of each ridge is usually somewhat compressed (pl. 5, fig. 4). Farther posteriorly the base becomes broader (pl. 12, fig. 80) and the ridges disappear so that the entire surface becomes smooth. The ridges have been well illustrated by Porter (1897, p. 2, fig. 17) for *T. agilis*, cross-sections of the body being nearly identical in that species and *T. campanula*. Their course is longitudinal, not spiral, and is not changed to a spiral on contraction.

Locomotor Organelles: The surface of the anterior two-thirds or more of the body is covered with cilia or flagella. Both terms are not inappropriate here, since three distinct lengths of this hairlike covering is found in this species, in three distinct zones or locations. At the anterior end of the body is a zone of long, threadlike flagella (fig. B, *ant. fl.;* pl. 5, fig. 1), that may have a length equal to one-third that of the body. These arise from the narrow portion immediately behind the operculum, directly from the centroblepharoplast or its immediate branches. Posterior to this group and extending backward for slightly more than half the length of the body is a thick covering of short, cilia-like hairs (fig. B, *cil.*; pl. 5, fig. 1) of uniform length. These have a length of one-fourth to one-fifth or less of the anterior flagella. In apparent texture and thickness they seem to be similar to the longer flagella of both the anterior and posterior regions, differing from them only in length.

The third zone of long flagella is found between the distal limits of the short cilia and the posterior end of the ectoplasmic differentiation of the surface of the body (fig. B, *post. fl.*; pl. 5, fig. 1). These are considerably longer than the flagella of the anterior zone, often having a length equal to that of the entire body (fig. A). They extend posteriorly, trailing after the body when in motion, and often intersecting (fig. A, 1) or twisting around in a loose spiral (figs. A, 2, 4).

The anterior group of flagella seems to be the chief means of locomotion. When the organism is at rest constant vibratory waves pass through the entire coating of cilia and flagella, beginning at the anterior end and passing posteriorly to the tip of the longest flagella, but dying out in intensity distally. These vibrations continue in the cilia and posterior flagella when the flagellate is in motion but the rate of movement of the anterior flagella is greatly accelerated. They are thrown out in longer and stronger vibrations, resulting in a rapid movement forward of the body.

Flagella intermediate in length between these three groups are not infrequent (pl. 5, fig. 3) near the margin of the areas. In *T. agilis*, as figured by both Leidy (1881) and Porter (1897), the entire area of short cilia is replaced by flagella intermediate in length between the short anterior group and the longer posterior flagella. This results in a thick coat of long flagella for nearly the entire body in that species.

Each flagellum or cilium in *T. campanula* arises from a minute basal granule below the ridges of the surface of the body (pl. 12,

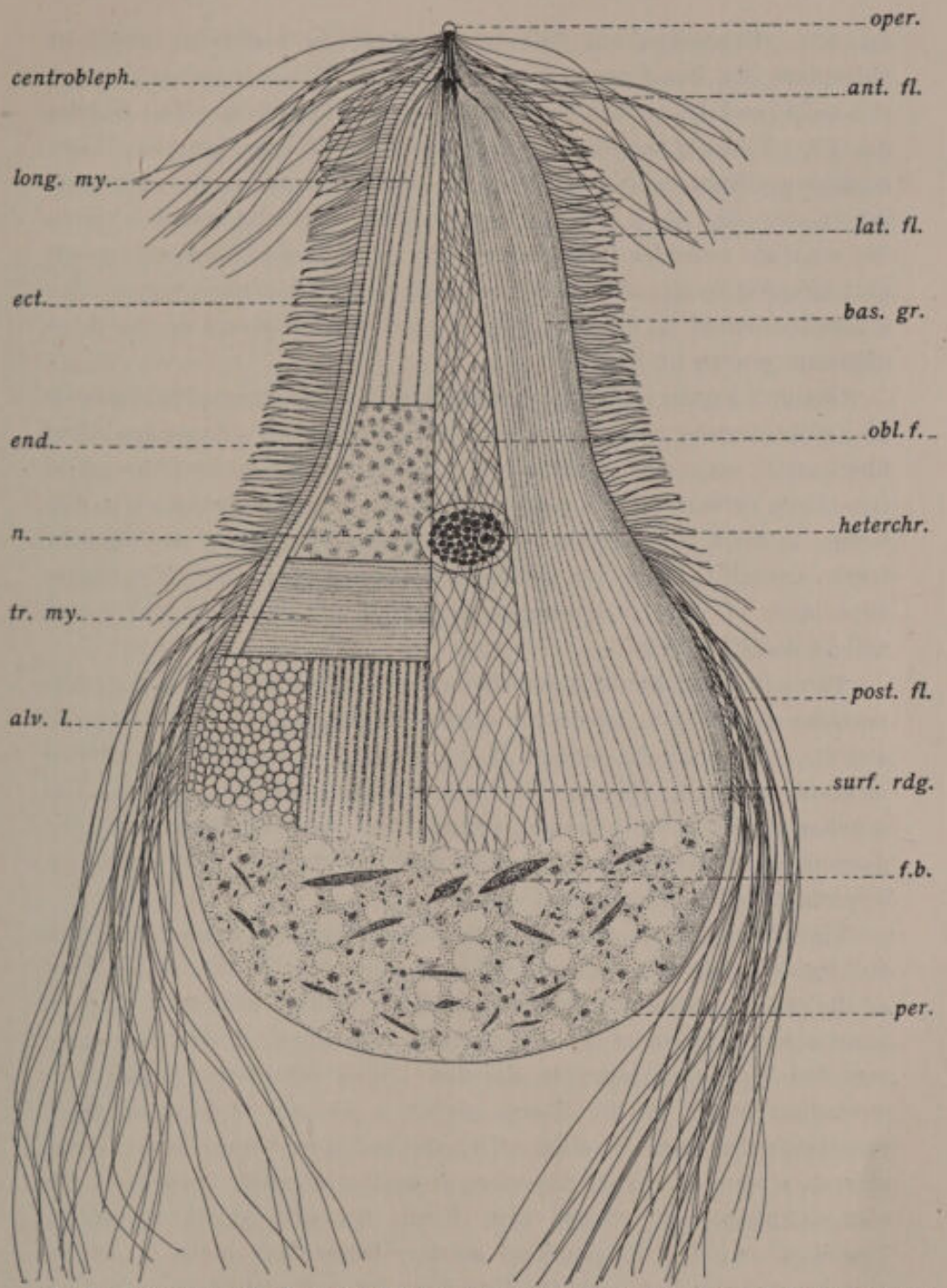

Fig. B. Diagrammatic figure of *Trichonympha campanula*. Sections of the body show the structures found at different levels. Surface ridges form the outer layer with their rows of flagella; beneath are successively the oblique fibers, alveolar layer and transverse myonemes. In the endoplasm are the longitudinal myonemes.

Abbreviations: *alv. l.*, alveolar layer; *ant. fl.*, anterior zone of flagella; *bas. gr.*, basal granules; *centrobleph.*, centroblepharoplast; *ect.*, ectoplasm; *end.*, endoplasm; *f. b.*, food bodies; *heterchr.*, heterochromosome; *lat. fl.*, lateral zone of flagella; *long. my.*, longitudinal myonemes; *n.*, nucleus; *obl. f.*, oblique fibers; *oper.*, operulum; *per.*, periplast; *post. fl.*, posterior zone of flagella; *surf. rdg.*, surface ridges; *tr. my.*, transverse myonemes. × 600.

fig. 80). Extending out from each granule is a slender thread or rhizoplast, the basal part of the flagellum. This passes up through the ridge and leaves the crest as the single flagellum. The flagella are placed closely together so that the rows of basal granules form continuous lines extending from the anterior end of the organism posteriorly (fig. B, *b. gr.*). No connection could be found between the separate granules in the same rows or in successive rows, except that afforded by the oblique fibers which will be described below. No differences could be detected between the basal portions of the three different groups of flagella.

Oblique Fibers: The most superficial examination of this species of *Trichonympha* reveals a wonderful development of myoneme-like fibers which cross and intercross in an intricate pattern over the entire two-thirds or more of the surface of the body. A closer examination brings to light three different sets of these fibers lying at different levels, and all more or less visible in the living organism. The outermost layer of these is composed of oblique myoneme-like fibers and will be described first.

These lie immediately below the surface ridges and cover the same portions of the body as do the other ectoplasmic structures (fig. B, *obl. f.*). On plate 12, figures 79, 84, and 85, these fibers are shown in dividing forms with the other ectoplasmic structures omitted. The number of fibers or separate strands is somewhat reduced in the drawing to obtain clearness, both in these figures and in others which appear elsewhere on the plates.

The oblique fibers arise from the darkly staining masses, the centroblepharoplast (fig. B, *centrobleph.*; pl. 6, figs. 7, 10), at the base of the short, narrowed anterior part of the body. Figure 7, plate 6, gives a vertical view of this region in an individual which had become rounded up, preparatory to division. This condition results in a spreading apart of the fibers, giving a clearer picture of these structures than may be obtained in the ordinary trophozoite. Thick strands stream out in all directions from the irregular borders of the centroblepharoplast, which soon break up into small threadlike branches. At first longitudinal as they leave their place of origin, this direction is lost with the first branching, the threads extending obliquely and crossing and intercrossing with one another in a complex, anastomosing network. Each intersection of two branches seems to anastomose completely so that the course of a single branch is soon lost. In addition to this network of fibers, very slender, minute

branches are being continuously given off which pass to the surface along the ridges (pl. 6, fig. 9). The basal granules of the flagella seem to be connected with these minute branches, as in optical section the flagella are seen to be continuous with the branches that are given off by the oblique fibers. In cross-sections of the body this connection could not be followed, owing apparently to decolorization in the staining methods used.

The structure of these fibers is scarcely granular and presents a more nearly homogenous appearance than is the case with either the transverse or the longitudinal myonemes. In the living organism they may be seen as very slender refractive lines. In preparations stained with iron haematoxylin they are very distinct as greyish lines, darker in the anterior region. Owing to their affinity for this stain a considerable degree of decolorization is required before they lose the black color. With Mallory's connective-tissue stain these fibers usually, though not invariably, show a clear red color similar to that found in the neuromotor apparatus of ciliates.

CENTROBLEPHAROPLAST: Intimately related to these fibers is another structure at the anterior end of the body which we have called the centroblepharoplast, an organelle homologous with the blepharoplast or centroblepharoplast found in other flagellates (Kofoid and Swezy, 1915), and suggestively like the motorium in ciliates (Sharp, 1914, Yocom, 1918), although that organ has no proven relations to mitosis as has this structure. Owing to the staining reactions and apparent structure of the oblique fibers first described, it seems probable that they are, of all the neuromotor apparatus, most intimately associated with the centroblepharoplast as well as with the flagella.

The anterior end of the body becomes narrow, sometimes with a slightly constricted appearance (pl. 6, fig. 6), but usually subconical in outline (pl. 6, figs. 1, 2). The ectoplasmic zone is here much thicker than in other regions of the body. In the center of the terminal cone is a slender cone-shaped structure composed of several strands of darkly staining material surrounding a central core which does not stain (fig. B, *core*; pl. 6, figs. 1, 6). This reaches the tip of the cone, where it may terminate in two ways. The first of these presents a ringlike appearance in vertical view, with the central core of endoplasm showing in the center as a light area (pl. 6, figs. 3, 7). This is a circular band around the core, to which the radiating strands of the oblique fibers are attached at their anterior ends. The second method is generally found in division stages and shows the

sides with their darkly staining strands drawn out beyond the central core and terminating in a point (pl. 6, fig. 10) with a complete obliteration of the core. Some curious modifications of this are sometimes seen in which a considerable amount of the darkly staining material has accumulated at the tip and is thrown out sideways into hornlike processes (pl. 6, fig. 9; pl. 8, fig. 34; fig. C, 6).

This axial structure extends backward for a short distance from the apex of the cone, tubular in shape or slightly larger posteriorly (pl. 5, figs. 1, 6). Near its base the separate strands, which are usually quite distinct, become enlarged into broad, irregular, darkly staining masses (fig. C) which appear to fray out around their distal margins. These may sometimes be separated (pl. 6, figs. 9, 13) into ropelike and brushlike masses, or an almost continuous band may be formed around the base of the tubular part of the neuromotor apparatus (pl. 6, fig. 7). Distally these masses break up into the oblique fibers which have been described above. The two structures seem to be continuous and composed of the same material. They connect the centroblepharoplast with the external motor organs, the flagella, by their intimate association with their basal granules.

Alveolar Layer: Closely filling the same regions traversed by the oblique fibers is a layer of alveoli (fig. B, *alv.*; pl. 7, fig. 24). This is continuous in extent with the other ectoplasmic structures, but along the posterior border it may sometimes seem to merge into the larger endoplasmic alveoli which often fill the posterior portion of the body (pl. 5, fig. 6).

In cross-section the alveoli seem fairly regular (pl. 5, fig. 4), and are placed closely together. In surface view they appear less regular with larger alveoli in the posterior region. In the rounded dividing forms this layer is often very striking, the alevoli having the appearance of clear globules much larger than those usually present in the normal vegetative forms (pl. 7, fig. 24; pl. 12, fig. 76), possibly as a result of pressure. The intra-alveolar spaces are traversed by branches from the oblique fibers (pl. 6, fig. 9) and by the basal fibrils of the flagella, the basal granules lying in the zone immediately beneath the alveoli, which is also occupied by the major portion of the oblique fibers (pl. 5, fig. 4). The zone lying between the alveolar layer and the surface is also traversed by these basal fibrils which give to that region a striated appearance (pl. 5, fig. 5). Optical sections from the posterior border of the ectoplasm in a dividing trichonymph (pl. 12, fig. 80) show the basal granules lying close

to the inner border of the alveoli. The outer zone between the alveoli and the surface ridges has here almost disappeared, the alveoli abutting on the bases of the ridges, from which they are rather widely separated anteriorly (pl. 5, fig. 4).

A distinct alveolar zone lying in the ectoplasm is a ciliate rather than a flagellate characteristic. It is not, however, entirely unknown

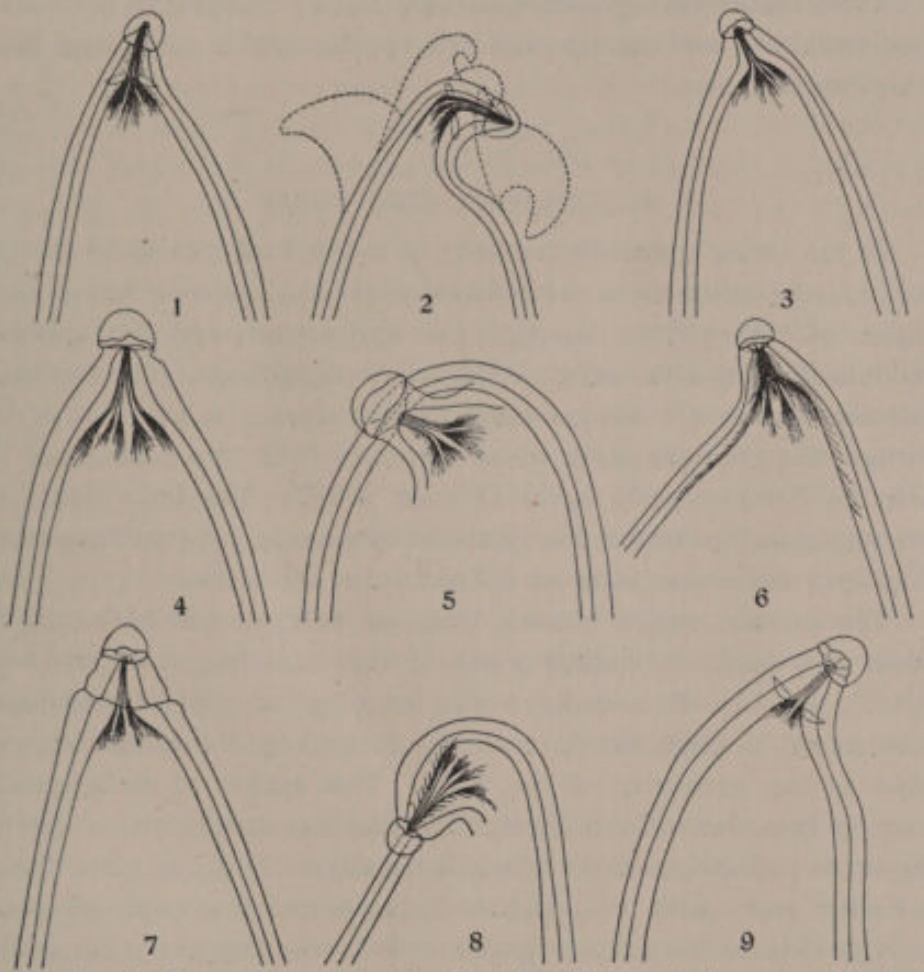

Fig. C. *Trichonympha campanula.* Sketches of the anterior end of the body to show its great mobility. Figure 2 illustrates the successive movements used in moving forward through the thick intestinal contents. × 200.

in the latter group. In the subgenus *Pachydinium* of the genus *Gymnodinium*, among the dinoflagellates, the different species are provided with a differentiated ectoplasm the outer layer of which consists of rather large alveoli (Kofoid and Swezy, 1919*d*).

Transverse Myonemes: The innermost layer of ectoplasm, beneath the alveolar and oblique fiber zone, is occupied by the transverse myonemes (fig. B, *tr. my.*). These differ in structure from the oblique

fibers, being granular in appearance as are the longitudinal ones. They are circular, passing around the body in nearly transverse planes, and may be found throughout the entire region of thickened ectoplasm. Each myoneme is a slender, granular band, not more than one or two microns in thickness. These lie in parallel rows which give a finely striate appearance to the innermost borders of the ectoplasm.

These transverse myonemes are only faintly outlined in the stained specimens and are usually obscured by the wealth of oblique fibers which overlie them.

ENDOPLASMIC STRUCTURES

In the living organism the body is marked off into three distinct regions, the outer clear ectoplasmic zone, the anterior endoplasmic region of rather dense homogeneous appearance, and the posterior endoplasm filled with coarse alveoli and food particles. The two endoplasmic regions are always more or less clearly differentiated, but without the granular layer which separates these two portions of the body in *Trichonympha agilis* (Porter, 1897). The transition from one region to the other is distinguished by a change of structure rather than by a delimiting layer of differentiated ectoplasm.

The anterior region extends from the anterior end backward for about two-thirds or slightly more of the total length of the body (pl. 5, fig. 6). Proximally a slender plug or core of endoplasm extends out through the narrow neck formed by the centroblepharoplast to the periphery of the body. This region of endoplasm is coarsely granular without distinct alveoli. The distal portion, extending up to and around the nucleus, is usually more dense, often taking a darker stain with iron haematoxylin, showing greater metabolic activity than in the anterior portion. It is also frequently filled with minute flecks of a dark color (pl. 5, fig. 6; pl. 7, fig. 23). These are often present in the posterior region of endoplasm and are possibly remnants of bacteria which have been ingested with other food particles. The possibility of their being chromidia is not excluded, however.

The posterior portion of endoplasm is filled with large alveolar spaces with the interstices occupied by coarsely granular plasma (pl. 5, fig. 6; pl. 7, fig. 23). This part of the body usually contains an abundance of food particles. As most of the termites examined were obtained from decayed wood, this was the only foreign material found in the intestine. It also seems to form at least a part of the

food of the trichonymphs, since few specimens were noted which did not contain small particles of wood in the endoplasm. The entire posterior portion of the body is often found densely filled with this material which is light in color in the unstained preparations. The particles seem to be confined exclusively to the posterior region of the endoplasm.

Longitudinal Myonemes: The longitudinal myonemes are found in the outer layer of endoplasm (fig. B, *long. my.*), a short distance below the inner layer of ectoplasm containing the transverse myonemes. These extend from the base of the tubular portion of the neuromotor apparatus posteriorly to near the end of the differentiated ectoplasmic region (fig. B, *long. my.*). They are subparallel for most of their length, spreading apart posteriorly and converging anteriorly until they meet in the region of the lobes of the centroblepharoplast. The connection between these myonemes and this portion of the neuromotor system is one difficult to determine. The myonemes stain only faintly or not at all in the ordinary smear preparations. In sections they may be seen as dark granular masses near the outer border of endoplasm. It is in the living flagellates that they are best observed. Here they may be seen as slender bands of homogeneous appearance, somewhat refractive, and extending in an anteroposterior direction and vibrating with the movements of the body. In stained material they appear as strands of rather coarse granules which are somewhat denser and slightly darker than the surrounding endoplasm. Their structure seems to be entirely changed by the processes of fixing and staining.

The longitudinal myonemes seem to be the chief organelles concerned in flexions of the anterior end of the body. This is extremely mobile, turning easily from side to side (fig. C, 2), sometimes reaching backward upon itself until it touches the posterior portion of the body. With such movements in the living organism somewhat slowed down, these strands may be seen to sway slightly with each movement.

Nucleus: The nucleus is a rotund ellipsoid lying in the middle third of the body. Its position varies from one-third of the total length of the body from the posterior end (pl. 7, fig. 23) to about the mid-region (pl. 5, fig. 6). In a cross-section of the body at its level it lies near the center of the plane (pl. 5, fig. 4). A thin, distinct nuclear membrane separates it from the surrounding plasma. In its internal structure it presents some unusual features which differentiate it from other flagellate nuclei.

In the "resting" nucleus four parts may be distinguished (pl. 6, fig. 11). The center is occupied by a linin reticulum closely filled by a mass of chromatin granules. These are sometimes large and closely massed together, or they may be small and show a definite linear formation (pl. 6, fig. 14). In the predivision stages the latter arrangement becomes the common one and results in the formation of the chromosomes (pl. 6, figs. 14–18). Near the outer border of this central mass and often slightly imbedded within it, is a small vesicle surrounded by a very thin membrane. This contains a single small, coiled or twisted rod of chromatin (fig. B, *heterochr.*; pl. 6, figs. 11, 14, 16), surrounded by a clear area which does not stain. The position of this vesicle varies somewhat in different individuals. It is most frequently found near the end of the longer axis (pl. 6, figs. 11, 14, 19), but may lie at the side of the nucleus near the end of its shorter axis (pl. 6, fig. 16). Its size also varies somewhat as well as that of the chromatin rod contained within it which we have designated heterochromosome for convenience. In plate 6, figure 11, the vesicle is relatively small and nearly filled by the chromatin rod. In figure 16 of the same plate, both the vesicle and the chromatin rod are relatively large.

Outside of these two nuclear regions is a zone of large, clear alveoli (pl. 6, fig. 11). The walls of the alveoli seem, in some cases, to be continuous with the linin reticulum of the central area, but otherwise the two regions are distinct. The alveoli are rounded outwardly and pressed close together on their inner faces. They are filled with a clear fluid which does not stain. Outside of this alveolar zone and separating it from the outer membrane is a granular area, the rather coarse granules of which stain lightly with iron haematoxylin. This granular portion may have a width equal to half that of the alveolar zone (pl. 6, fig. 11), or it may narrow down to a thin line (pl. 6, fig. 14). In many individuals both the granular and alveolar regions may be almost indistinguishable, the central chromatin mass nearly filling the entire nuclear spaces.

In its structure the nucleus of this flagellate recalls that of *Gyrodinium corallinum* (Kofoid and Swezy, 1919*d*, pl. 10, fig. 117); the latter, however, has been observed only in the living condition. In this it presents a similar alveolar zone surrounding a central mass of granules. The presence of a small vesicle with its chromatin rod, or heterochromosome, has not been observed in this species.

GENERAL DISCUSSION

The entire absence of a cytostome in this flagellate has proven a source of some difficulty in explaining its methods of food taking. It is distinctly holozoic in its mode of nutrition, as the abundance of food particles found in the endoplasm testify. These often fill the posterior region and consist of particles of wood and bacteria, and even encysted forms of *Trichomitus termitidis*. The anterior region of endoplasm has, in all individuals observed, been entirely free from food bodies or vacuoles, with the exception of small, darkly staining rodlets which may be bacteria or possibly chromidia. The particles of wood found in the posterior region of endoplasm are often relatively huge and may be contained in a distinct food vacuole, but are usually found lying free in the plasma, without evident vacuoles.

The method of ingestion of these particles is a complete mystery. Leidy (1881), in his account of these flagellates, called attention to the presence of food bodies and the lack of any visible channel for their entrance into the body. Kent (1884), in his studies on the forms from the Tasmanian ants, decided that there was an oral aperture at one side of the body a short distance from the apical extremity. From this he traced a narrow oesophageal tract which opened into the digestive cavity at the posterior region of the body. He further states that in a medium of thinly diluted milk both the pharynx and digestive tract were frequently found filled with the milk corpuscles.

Porter (1897) attempted to confirm these observations of Kent's, both in the living animals and by means of sections of the body, but was unable to find any trace of an oral aperture. He does, however, offer another solution to this problem, that is, that the food particles are drawn to the posterior part of the body by the cilia and there ingested through the thin pellicle. Unfortunately the evidences for this are unconvincing.

Other investigators working on these forms, e.g. Hartmann and Grassi, have been equally unsuccessful in solving this mystery, nor has our own work afforded any light upon the subject. It is manifestly impossible to consider that food may be taken in at any point of the surface covered by the highly differentiated ectoplasm possessed by this flagellate. That the food is taken in at the extreme posterior portion of the body seems to be in direct contradiction to all known methods of feeding among Protozoa or elsewhere. There remains, then, the anterior end of the body to consider.

The extreme anterior tip of the cone-shaped end or head of the body reveals, in surface view (pl. 6, figs. 7, 12), a central core of endoplasm surrounded by a dark ring at the base of a pitlike depression. This central core of endoplasm extends backward through the tubular part of this darkly staining structure, the centroblepharoplast complex (pl. 5, fig. 6), and connects with the endoplasm of the body. As shown in figure 3, plate 5, this structure presents the requisites for functioning as a cytopharynx leading into the body. Its size as compared with that of the ingested food particles, would not militate against such a supposition, since the great flexibility of these parts might also be correlated with a considerable degree of elasticity permitting distension. That the centrosome should form part of the mouth structures, however, seems hardly plausible, but scarcely less so than that its food should be taken in at the posterior end of the body. The fact that the operculum appears always to be intact and to cover over the anterior tip of the body militates against this interpretation.

An analogous condition, in case the core is the gullet of *Trichonympha*, is found in *Diplodinium* (Sharp, 1914), where a ring of neuromotor material and connections surrounds the gullet. There is no evidence, however, that this ring has the remotest relation to any centrosome of this ciliate. Ciliates are, moreover, not mononucleate as is *Trichonympha*.

Porter (1897) has described for *Trichonympha agilis* a peculiarity in the structure of the anterior part of the body, which might afford some basis for the view of Kent that a cytostome existed in this region. He described the cone-shaped end or "nipple," as he terms it, as separated from the remainder of the body by a deep constriction, the central axial rod forming the only means of union between the two parts of the body. This appearance is shown in our own material also (pl. 5, fig. 5) but Porter's explanation of the structure of the body at this point does not agree with the actual conditions as we find them. Our own interpretation follows.

At the base of the tubular portion of the centroblepharoplast and abutting upon its lobes, is a clear area that forms a ring completely surrounding the tube. This is not traversed by the fibers that give to the remainder of the ectoplasm a striate appearance, nor is it granular in its composition. In some individuals this lack of myonemes and fibrils may be seen to extend to the outer surface which then shows a zone devoid of flagella covering this region. Usually the outer zones

are completely filled by the wealth of fibrils which crowd this part of the ectoplasm. In no case, however, have the lines bordering the different layers of the ectoplasm, which here is unusually thick, been found to be broken, or otherwise to present any indications of a constriction in the surface of the body at this place. Were this the case, flexions of the head or cone, such as are common in many individuals on every slide, would betray the lack of continuity on one side at least, and no instance of this has been observed, though the flagellates have been thrown into every conceivable attitude in making smear preparations. The surface lines or ridges on the cone also seem to be continuous with those of the body without a break in their continuity. We find no evidence of an anterolateral cytostome.

What purpose this circular vacuole may subserve, finds no explanation in observations we have been able to make. It is almost or quite obliterated in many individuals and always disappears at the time of division. Its conspicuousness when present in stained material, with its complete lack of structural differentiation in the midst of a highly differentiated zone, would suggest that it is a fluid-filled vacuole of non-stainable substance but gives no further aid in explaining it. That it might function as a cytostome seems impossible.

Evidences of the complex ectoplasmic and neuromotor structures which we have described above, are to be found in the figures of Porter (1897) for *Trichonympha agilis*, and in those of Hartmann (1910) for two, at least, of the species he assigns to *T. hertwigi*. In the former the alveolar zone of ectoplasm with the surface ridges, and something of the fibrillar system with its centroblepharoplast, are shown. In two species which Hartmann has figured, the centroblepharoplast and suggestions of the complex of myonemes may be found. The complete minute structure has in no case been worked out heretofore, neither has the presence of an integrated system been noted nor its relation to mitosis demonstrated.

BINARY FISSION

Binary fission and mitosis in *Trichonympha campanula* present some interesting phases, both in regard to cytoplasmic structures and mitotic phenomena, the latter bearing some striking resemblances to certain stages in the mitosis of the metazoan germ cells. These processes will be discussed separately, beginning with the division of the centroblepharoplast and related ectoplasmic structures.

Division of the Neuromotor Apparatus and Ectoplasmic Structures

The onset of division is marked by certain nuclear changes that will be discussed later. The first evidences of it in the grosser structures of the organism are found in the change from a bell-shape to a spherical contour in the body as a whole (pl. 7, figs. 24, 31). With this change the nucleus migrates anteriorly until it comes to lie immediately below the centroblepharoplast (fig. 31). No trace of a connecting rhizoplast has been found. This change in the form of the body results in a spreading apart of the myonemes and fibrils of the ectoplasmic layers, with an apparent enlargement of the alveoli, so that these structures are more easily studied in this stage than in the more usual but contracted form of the normal vegetative trophozoite.

The next step in the division process is found in the partition into two parts of the lobes at the base of the tubular portion of the neuromotor system, the centroblepharoplast. With this occurs a separation of the entire ectoplasmic layer into two parts, with a small, spindle-shaped portion of endoplasm appearing in the chasm thus made (pl. 7, fig. 30; pl. 8, fig. 35). At about the same time or slightly earlier, the operculum-like, transparent cap with the cup-shaped depression which it covered, disappears, and the tip of the tubular neuromotor apparatus reaches quite to or near the outer surface of the anterior end of the body.

The splitting or division of the tubular part of the neuromotor apparatus or centroblepharoplast proceeds from the base anteriorly to the tip (pl. 8, figs. 33, 34), and with the splitting each half draws together its parted edges until they meet and each moiety forms a new tube. The attachment to each other at the tips may persist for some time, as such stages are more common than are the intermediate stages shown in plate 8, figure 33. As the two halves of the tube separate, strands of darkly staining material are found joining the two inner surfaces near their bases (pl. 8, fig. 34). This is the paradesmose which functions in the formation of the spindle, and is apparently drawn out from the material of the centroblepharoplast itself. With the final separation of the tips of the new daughter tubes the paradesmose remains as the only connecting link between the two structures (pl. 8, fig. 36).

As the two parts of the centroblepharoplast separate, the alveoli frequently form a rosette at the base of each (pl. 8, fig. 38; pl. 9, fig. 42), presenting, especially in a focus showing the oblique fibers, striking resemblances to well developed asters in optical section. With the further separation, the angle formed by the splitting ectoplasmic structures becomes greater and extends farther out towards the periphery of the ectoplasmic region (pl. 8, fig. 38). This region is usually conspicuous since the flagella as well as surface ridges, myonemes and alveoli have been drawn aside, leaving only the granular endoplasm beneath the thin ectoplasmic layer. The paradesmose becomes thicker and broader, generally forming a heavy band that persists throughout the entire process of division, and may occasionally be found for some time after the final separation of the daughter nuclei (pl. 11, fig. 75).

No other changes in the ectoplasmic structures during these stages have been detected. The staining reactions of the neuromotor apparatus seem to vary slightly as the different phases follow each other. At the beginning of its division it often presents a greater affinity for iron haematoxylin than during the later phases. This, however, may possibly be due to changes in the preparation of the material, as decolorization of slides containing mitotic figures was carried on with the view of obtaining the best possible results for the chromosomes, without regard to the remainder of the cell.

In the final stages of division, when the complete separation of the divided organelles has taken place, an enveloping movement of the ectoplasmic zone may be seen. This begins with a gradual creeping out of the margins of the divided area (pl. 12, fig. 76), partly as a result of the spreading of structures already formed and partly as new outgrowths over the undifferentiated intermediate zones. As the two daughter organisms separate, pulling out a long protoplasmic bridge between them, the rounding-up of the body which results aids in closing the gap between the margins of ectoplasm (pl. 12, fig. 79). This process may, however, be hastened or retarded in some individuals, and is not always synchronous in the two daughter organisms. This possibly results from contractions of the intermediate zones or of the old differentiated zones. In plate 12, figure 85, an almost complete union of the borders of the ectoplasm has taken place in one individual of the dividing body, while the other still shows a rather broad gap between the margins of ectoplasm. In figure 84 of the same plate the final separation of the two daughter organisms has

taken place with only a comparatively small amount of new ectoplasm formed in one individual.

The behavior of the neuromotor apparatus during mitosis is significant in its intimate association with the nucleus. The entire extra-nuclear mechanism which consists of polar centrosome, the astral rays attached thereto and the paradesmose which is stretched out between them as an extranuclear band of large size, is all a direct transformation of the most intimately connected parts of the neuromotor apparatus, the centroblepharoplast, the oblique anastomosing fibrils, the lines of basal granules and the attached flagella and cilia. The longitudinal and transverse myonemes simply part at the zone of bipartition of the ectoplasmic territory, without forming an integral part, with structural modifications, of the nuclear figure of mitosis. The comprehensive fashion in which the sum total of the neuromotor system, excluding myonemes, forms the extra-nuclear mechanism of mitosis, is instructive in the matter of the unity of the system, and its integration into an organic complex which survives the shock and readjustments contingent upon mitosis without dedifferentiation and reorganization. This is in marked contrast with the extent of such dedifferentiation and reorganization in the multinucleate Ciliata, such as *Euplotes* (Yocom, 1918).

Another feature of cytological significance is the derivation of the astral rays of the nuclear spindle from what are structurally distinctly fibrillar organs, the anastomosing oblique fibers. The ciliary lines which are more granular in appearance in stained material, more homogeneous and distinctly fibrillar in life, also form radiating lines in semicircle from the poles of the paradesmose. The resemblance to the aster of dividing metazoan cells is so striking that one is inclined to regard them as homologous structures. If so, Chambers' conclusions (1917) that the asters are sol phases of the surrounding cytoplasmic gel present considerable difficulties, as does likewise the fact that these fibers in *Trichonympha* lie in one superficial plane in the surface of the organism, while the asters of the Metazoa are infiltrated through the mass of the cytoplasm in three dimensions to a much wider extent. The latter difficulty is not insurmountable since it is a logical consequence of the structural specialization of the cell which is the whole undivided trichonymph. It may also be true that with so universal a phenomenon as mitosis pervading all types of living substance, we should expect to find the bipolar organization which appears in the nuclear figure utilizing not one but many diverse

structural units in its make-up, according to the nature of the cell in which it occurs—even so diverse as the neuromotor system of a flagellate and the sol phase of reversible cytoplasm. It is the organization rather than the state of the substance that is significant.

Mitosis

Evidences of the approach of division may be looked for in the nucleus before any changes may be apparent in either the ectoplasmic structures or the external form of the body. The changes that take place in the nucleus relate to the formation of the chromosomes, and are significant both from the standpoint of their later history and of the question of the continuity of the chromosomes. Their development will now be taken up in detail.

Prophase: The structure of the vegetative nucleus shown in figures 11 and 14 of plate 6, has already been described. The central masses of chromatin seem to lie at the intersections of the linin reticulum, in some cases (fig. 11) the individual masses being large enough to completely fill the interstices also. In the early prophase the outer alveoli disappear, leaving a clear space with a granular region near the membrane (pl. 6, fig. 15). This granular material later becomes diffused through the entire intranuclear spaces and persists throughout mitosis. The chromatin moves out from the rounded particles along the lines of the reticulum, sometimes before the alveolar zone has disappeared (fig. 14). This movement becomes more evident with the change in the outer region of the nucleus, and a slight expansion takes place, with the chromatin filling a larger area than is usual in the vegetative stage.

The outpushing of chromatin from the central masses is not equal in all directions. This may be due to a lack of continuity in the linin reticulum or to its breaking up. The latter seems the more probable explanation, as threadlike ends are frequently seen in these stages (fig. 17). This breaking-up appearance begins at one side of the nucleus while the remainder still shows a close reticulum thickly encrusted with chromatin (pl. 6, fig. 15; pl. 7, fig. 26). As the breaking up proceeds further the rounded masses gradually disappear, the chromatin apparently moving out along the threads which assume a thicker, more compact appearance.

As these threads become differentiated some evidences may be found of a longitudinal split in each one (pl. 7, figs. 25, 27, 28). This may occur in some threads while the remainder are still emerging

from the undifferentiated mass of chromatin-encrusted network, hence the number of threads originally formed can not be determined. The separation of the two parts thus formed seems to take place immediately, since in slightly later stages the number of threads appears much greater, with no evidences of the splitting of a single thread (pl. 6, figs. 20–22; pl. 7, figs. 23, 29). In these stages also many of the threads or chromosomes are arranged in pairs which are nearly equal in size and length. In a later stage, but still one which precedes the rounding up of the body, this pairing of the chromosomes becomes more pronounced (pl. 6, fig. 18). The threads thus sorted out in pairs are evidently the products of the splitting of the original threads or chromosomes.

During the time these changes in the central nuclear mass are taking place, the small vesicle with its single coiled, chromatin thread or chromosome remains intact, with no apparent change beyond an enlargement of the vesicle itself. This forms a large clear area with the chromosome contained within it loosely coiled or V-shaped (pl. 7, fig. 29; pl. 6, figs. 16, 20). In common with the remainder of the chromatin of the nucleus, it seems to increase somewhat in bulk during the early prophase, though this is not invariably the case (pl. 9, fig. 46). The vesicle disappears before the end of the prophase, leaving the single chromosome lying in a clear space apart and detached from the remainder of the chromatin threads (pl. 6, fig. 22; pl. 7, fig. 29). This isolation is apparently retained throughout the subsequent stages, though this can be satisfactorily demonstrated only when the nucleus is oriented so that it is seen near the lateral margin (pl. 9, figs. 46, 47). In other positions its relations are obscured by the great number of chromosomes.

The occurrence of a definite, continuous spireme stage is not certain, the short chromatin threads or chromosomes apparently being formed directly from the breaking up of the reticulum (pl. 6, figs. 14–22), before the body of the flagellate has begun to round up or give other evidences of the approach of division (pl. 7, fig. 23). The exact number of the threads thus formed seems to be fifty-two. In the stages represented in figures 18 to 22, plate 6, these could not be counted, but in the later stages, represented by figures 44, 47 and 49, plate 9, with the chromosomes more fully organized, this could be done with a considerable degree of accuracy, and the number of chromosomes given is based on counts made on fifteen different individuals.

Each chromosome consists of a long thread composed of chromomeres closely strung together (pl. 6, figs. 20–22; pl. 9, fig. 44). In earlier stages these appear diffuse but later become more compact, at the same time drawing together at the ends to form a loop (pl. 9, figs. 46–49). These loops may appear coiled together (fig. 46) or may preserve a V-shape. Both of these appearances may be seen in the same nucleus (fig. 49).

Soon after the appearance of definite chromosomes the flagellate begins to round up, with an anterior migration of the nucleus (pl. 7, fig. 31). This is followed by the splitting of the centroblepharoplast and the separation of the two halves which remain connected by the darkly staining paradesmose (pl. 8, fig. 33).

The nucleus at this stage is found a short distance below the base of the dividing neuromotor system (pl. 7, fig. 30). The chronological relation of the changes occurring in the nucleus and those of the neuromotor apparatus vary considerably. The stages shown in figures 20 to 22, plate 6, usually occur before the centroblepharoplast divides, yet occasionally the paradesmose may be fully formed before definite chromosomes appear. The formation of the spindle fibers immediately below the paradesmose does not occur until the nucleus approaches the paradesmose with the nuclear membrane apparently touching it. The spindle fibers are stretched between the dark masses or centroblepharoplasts at either end of the paradesmose (pl. 9, figs. 41, 43), but inside the nuclear membrane. When these are fully formed the nuclear membrane is drawn out to a spindle shape with the ends reaching the ends of the paradesmose (pl. 10, figs. 55–59). The latter structure remains outside of the membrane but closely pressed against it, usually partly imbedded within a fold, which in many views gives it the appearance of occupying the center of the spindle and chromosomes (pl. 10, fig. 55). In reality, however, the chromosomes and spindle fibers are at all times completely separated from it by the nuclear membrane. Its position thus approaches that of the centrodesmose or central spindle of the metazoan cell. Since it is outside of the nuclear membrane it is a paradesmose.

With the beginning of the formation of spindle fibers or somewhat earlier, another change takes place in the chromosomes, the loops straightening out so that the chromosomes come to lie parallel to the paradesmose. This process may be followed in figures 56 to 59, plate 10, with the chromosomes in various stages of unbending. The completion of this gives the equatorial plate phase (pl. 10, fig. 59),

with the chromosomes still joined by an end to end union in the equatorial plane. The behavior of the heterochromosome is not always easy to determine in this stage. In figure 56, plate 10, it is found lying near one end of the mass of chromosomes. Its attachment to distinct spindle fibers could not be demonstrated. In figure 59, plate 10, it has moved nearer the equatorial plane but is still outside the main mass of chromosomes.

Metaphase: The equatorial plate is wide and usually heavily stained, with the chromosomes closely massed together. It is apparently of shorter duration than either the prophase or later stages. With the elongation of the nucleus the chromosomes separate at the middle point, i.e., at the apex of the looped thread or V of the chromosome before it became attached to the spindle (pl. 10, fig. 60; pl. 11, fig. 62).

During the later prophase the small chromatin thread or heterochromosome is often obscured, particularly in the formation of the equatorial plate. With the separation of the chromosomes in the metaphase, however, this again becomes prominent. It is found that the vesicle has disappeared and the single thread has divided (pl. 10, fig. 61). The separation of the two new heterochromosomes in the metaphase lags somewhat behind that of the other chromosomes, hence these usually may be seen between the two groups as they pass towards the poles (pl. 11, figs. 62, 64). The attachment of these chromosomes to spindle fibers in these stages, as in the earlier ones, has not been determined.

Anaphase: The separation of the chromosomes after the final parting seems to take place by reason of an elongation of the entire nucleus in the equatorial region, rather than by a shortening of the spindle fibers, since the chromosomes have in no case been found closely attached to the poles. The spindle fibers remain approximately the same length throughout the anaphase until they disappear in the telophase (pl. 11, figs. 61–65, 73, 74). The elongation of the nucleus in the equatorial region is accompanied by a constriction of the nuclear membrane. It is usually drawn out into a long, slender strand before the final break occurs which separates the two daughter nuclei (pl. 11, figs. 71, 72). At the same time the paradesmose also lengthens as the centroblepharoplast complexes of the newly forming daughter cells move farther apart (pl. 11, figs. 68, 72, 75).

The connecting nuclear thread thus formed soon breaks and the elongated portion of each daughter nucleus is gradually withdrawn

(pl. 11, figs. 70, 73, 74), the nuclei becoming rounded or nearly so, with a separation from the paradesmose and the centroblepharoplast (fig. 75). The paradesmose loses its staining reactions and soon fades out, apparently being resorbed, either in the cytoplasm or by the centroblepharoplast complexes. The chromosomes in the meanwhile undergo few or no changes, retaining a position at some distance from the poles after the disappearance of the spindle fibers, which may not occur until the nucleus has begun to round up.

Telophase: The reorganization of the nucleus may take place before the constriction of the cytoplasmic body (pl. 12, figs. 76, 79), or it may be delayed until after separation of the two daughter cells (fig. 84). The heterochromosome may be found at this period lying near the ends of the chromosomes opposite to their point of attachment to the spindle fibers (pl. 11, figs. 73–75).

A vesicle is formed about this (pl. 12, fig. 83), and the other chromosomes become aggregated in the central part of the nucleus. These gradually lose their parallel positions and become mingled in a coarse network (figs. 83, 78, 81) which resembles in many respects a similar stage of the prophase nucleus (pl. 6, fig. 16). The nucleus may begin its migration away from the centroblepharoplast to the posterior region of the body even before final constriction of the cell (pl. 12, fig. 76), though this is usually delayed until the daughter flagellates begin to assume their elongate, campanulate form.

Discussion of Mitosis

Observations on the division of the members of this unique genus have been scanty heretofore. Foà (1904) describes and figures some stages of this process in two species which she designates as *Trichonympha agilis* forma *minore* and *T. agilis* forma *maggiore*. The stages she has figured are strikingly similar to the same stages found in our own material.

In both of these species division is preceded by a rounding up of the body and an anterior migration of the nucleus, followed by division of the anterior, narrowed portion of the body, the "tubolo," and the ectoplasmic zone of flagella. As these structures separate they spin out between them a stout band, the external spindle (*fuso esterno*). The internal spindle (*fuso interno*) is formed by fibers arising from the ends of this, passing through the nuclear membrane and attaching themselves to the chromosomes. The remaining steps

in the process, so far as Foà has described them, are identical with those of *Trichonympha campanula*. She does not, however, give further details of the process, the formation of the chromosomes or their number. She did not record division of the chromosomes in the small form but figures longitudinal splitting in those of the larger species.

In the nearly allied form, *Holomastigotes* (*Trichonympha hertwigi*) Hartmann (1910, pl. 28, figs. 23–29) has figured certain stages of the prophase nucleus, leading up to the formation of the chromosomes, which are nearly identical with phases found in our own material (pl. 6, figs. 14–16, 20–22). He also figures division of the structure which we call the centroblepharoplast and the anterior tip of the body, processes which also closely parallel those found in our own material. Further details of the mitotic phenomena he did not record.

The work of earlier investigators of these flagellates, such as Leidy (1881) and Porter (1897), also fails to give any clue to the details of the division processes.

The abundance of division stages in our own material has given us an unusual opportunity of determining the flagellate type of mitosis in *Trichonympha* and to follow out the details of the mitotic phenomena. The relatively large size of the nucleus of *Trichonympha* and the structures connected with its division, renders it a favorable object for study. In the foregoing outline of these various processes, certain points have been omitted or briefly touched upon, which will be discussed more fully in the following paragraphs, along with an explanation of some of the terms used in this paper.

The union of blepharoplast and centrosome in one structure which may or may not become separated at the time of division, is a condition quite common throughout the flagellates generally. The occasional separation of these in trichomonad flagellates (Kofoid and Swezy, 1915), becomes a permanent condition in the mitosis of *Trichomitus termitidis* (Kofoid and Swezy, 1919*b*). This relationship makes the term centroblepharoplast an appropriate one for this structure. The application of it to the more complex organelle of *Trichonympha* is also based on these same relations. The enormous increase in the number of flagella in this organism necessitates a coördinating mechanism related to each flagellum individually. This is found in the intricate system of fibrils radiating from the central mass at the anterior cone-shaped portion of the body, which thus

becomes a huge blepharoplast complex. At the time of division the entire structure divides into two parts, taking the rôle of centrosomes in the succeeding mitotic figures, while continuing its intimate relations with the flagella. The term centroblepharoplast thus designates the dual functions of this organelle complex.

Equally distinctive and typical of the phenomena of mitosis in flagellates is the formation of a paradesmose conecting the divided centroblepharoplasts. In *Trichomonas* (Kofoid and Swezy, 1915) at the occasional separation of the blepharoplasts and centrosomes this structure appears to be connected with the blepharoplasts and not with the centrosomes. In *Trichomitus* (Kofoid and Swezy, 1918*b*), however, the paradesmose is found connecting the centrosomes to which the blepharoplasts are attached by a slender rhizoplast. In this form these structures have a longer lease of life, the majority of individuals noted showing the prophase stage with the completion of the formation of the paradesmose. In both these flagellates, as in *Trichonympha*, it subserves the same function in mitosis with the same relative position *outside* the nuclear membrane.

The heavy band connecting the two parts of the divided centroblepharoplast in *Trichonympha*, to which Foà (1904) has given the name external spindle (*fuso esterno*), we consider homologous with the paradesmose of the trichomonad and other flagellates, and have so designated it. We have given a fuller discussion of this subject in an earlier paper (1919*c*).

The similarity of the paradesmose and the "sphere" of *Noctiluca* points to an homology between them, which thus links what has been considered a peculiar type of mitosis (Calkins, 1899) in the latter form with conditions found among other flagellates. Whether the nuclear membrane dissolves at the points of contact with the paradesmose in *Trichonympha*, as in *Noctiluca*, has not been definitely ascertained, but no evidences to support such a conclusion have been found. The ultimate fate of the paradesmose in both *Noctiluca* and *Trichonympha* seems to be the same, that is, it fades out in the middle and is absorbed or drawn up into the central mass of the centrosomes.

In the small chromatin rod, isolated from the remaining chromatin contents of the nucleus, we have a structure that is unique among the Protozoa and finds its nearest counterpart in the Metazoa in the "sex" chromosomes of the germ cells. Its resemblances to the latter are particularly striking during the different phases of division.

Its significance is problematical. No evidences of sex or sexual behavior have thus far been found in these flagellates. Hartmann (1910), it is true, has described both male and female forms in his *Trichonympha hertwigi*. His observations, however, do not bear out this assumption. No critical evidences of conjugation were found by him, neither in the behavior of conjugating gametes nor in the nuclear changes which precede this process. Indeed, it is evident to anyone familiar with taxonomic conditions in the Protozoa, that his male and female forms belong to different genera, as has already been pointed out by Grassi (1911). In his "junge, männliche, and weibliche" forms he has confused three distinct species and even genera, with a fourth species added to this confusion in the "gametes" which are minute oval flagellates such as are frequently abundant in the intestinal contents of many termites. His elaborate life cycle of this form is thus seen to be without adequate foundation, the product of an overwrought creative imagination.

This lack of evidence of sexual behavior in these organisms renders doubly difficult any explanation of the significance of the function of this peculiar structure. It remains distinct throughout both the vegetative and division cycles, dividing in the metaphase, its position that of a lagging chromosome in the anaphase, with one part going to each daughter nucleus. At some stages it bears a strong resemblance to the chromatoid body described by Wilson (1913) in the sperm cells of *Pentatoma* and some other insects. Its further behavior, however, clearly distinguishes it from that body, which is cytoplasmic in origin and does not divide. In *Trichonympha* this body has never been found outside of the nucleus and behaves as do other chromosomes at the time of division. As a convenient designation for this body and one which leaves its specific function still open to investigation, we have used the term heterochromosome, since that word, though originally used for the sex chromosome, has come to be applied to other forms of chromosomes as well (Wilson, 1911). The possibility is still open that further investigations may find undoubted evidences of sexual behavior in these flagellates.

Certain other aspects of mitosis in *Trichonympha* show striking resemblances to the mode of procedure found in the division of metazoan germ cells. The most remarkable of these is found in the "pairing" of the chromosomes with a reduction of their number from fifty-two to twenty-six (pl. 9, figs. 44–51*a*). This pseudosynapsis, however, may be explained on grounds other than that of sexual behavior and our interpretation of it follows.

The formation of distinct chromosomes takes place in the nucleus some time before any signs of division may be detected in the remaining structures of the body (pl. 7, fig. 23). The nuclei shown in figures 14 to 22, plate 6, and figures 25 and 26, plate 7, show different steps in this process. The number of threads or chromosomes that result has not been made out clearly at this stage. With

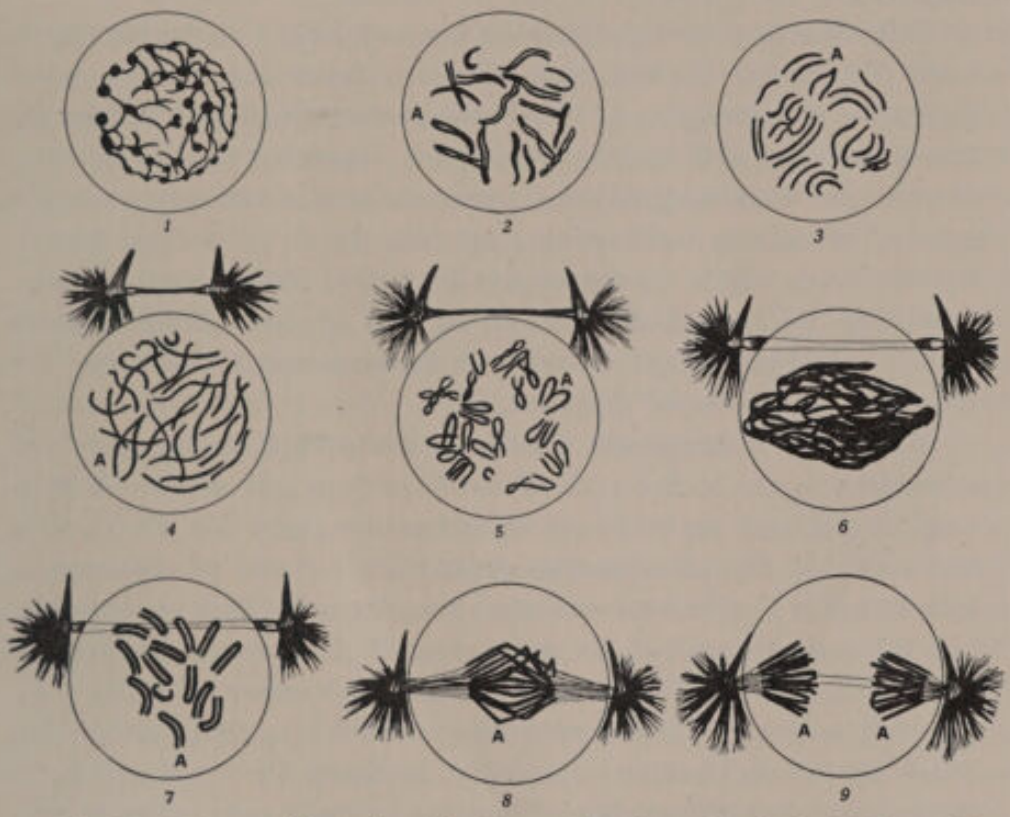

Fig. D. Diagram illustrating the phases of nuclear mitosis in *Trichonympha campanula*. One-half the number of chromosomes is shown. 1–7. Prophase. 1. Vegetative phase of chromatin-encrusted network. 2. Splitting of the chromosomes. 3. Separation of chromosomes resulting from splitting but paired arrangement noticeable. 4. Paradesmose formed between daughter centroblepharoplasts. 5. Formation of loops; nucleus approaching elongated paradesmose. 6. Tangled stage in which pseudosynapsis occurs. 7. Number of chromosomes reduced one-half. 8. Metaphase; looped chromosomes unfolding on the spindle. 9. Late anaphase; paradesmose still connecting centroblepharoplasts. Chromosome marked A is splitting in figure 2, appears as two chromosomes in 3 to 5, is reunited in 7 and 8, and separated into two distinct chromosomes in 9. The small coiled chromosome is the heterochromosome.

their *first* appearance, however, signs of splits in the threads may be detected (pl. 7, fig. 25; text fig. D). In the following stages these threads become clearer and their number may be ascertained. The nucleus is then found to contain fifty-two chromosomes arranged somewhat in pairs (pl. 9, fig. 44; text fig. D), that is, the end of one

chromosome will be found near or attached to the end of another, though the opposite ends may be widely separated. This is shown more clearly in the diagrammatic scheme in figure D, where one-half only of the actual number of chromosomes has been drawn in each nucleus. Each of these groups of two chromosomes is probably formed by the splitting of a single thread in the earlier stage (fig. D, 2).

Following this, each chromosome becomes looped or doubled upon itself (fig. D, 5). In this stage also it is found that there is some suggestion of a grouping of the chromosomes in pairs (pl. 9, figs. 46, 49), though the ends may be more widely separated than in the previous stage. Following this the chromosomes condense into a tangled mass of threads or contraction stage (fig. D, 6; pl. 9, figs. 42, 43, 45, 50), from which emerge twenty-six looped or V-shaped chromosomes (fig. D, 7; pl. 9, fig. 51*a*; pl. 10, figs. 52, 53). What happens in this contracted condition can only be conjectured, since thus far the details have escaped detection.

When the chromosomes divide in the metaphase the point of separation occurs at the apex of the V or loop. If division here is longitudinal, and the evidences of the earlier stages (fig. D) confirm this view, the line of separation must be at one end of the original split found in the chromosomes when first formed. This would postulate the supposition that the two halves of the original chromosome are reunited at the time the change in the number of units from fifty-two to twenty-six occurs. To illustrate this, let us follow the course of the chromosomes marked A in figure D. In figure 2 the chromosome A is a single thread that has partially split. This is completed in figure 4 but the ends of the chromosomes are still connected. In figure 3 this connection has apparently been lost, for the ends are separated by a considerable space. The course of these two threads is lost in the contraction stage in figure 6 but they emerge again in figure 7 as a single split chromosome which parts "transversely" on the spindle in the following figure, the two threads having apparently become united end to end into one, in the stage represented by figure 6. The seemingly transverse division is in reality the final step in longitudinal splitting of the original chromosome.

The only difficulty in this explanation lies in the lack of a visible mechanism by means of which two threads which are more or less widely separated from each other, are reunited, with their relative positions those of the original split. This difficulty is, however, not

so formidable as would seem at first glance. A single chromosome is evidently composed of a ground substance, framework, or thread of linin in which the chromomeres are imbedded and which gives such great consistency to the entire structure. This does not stain and its presence is difficult to demonstrate satisfactorily except as it is outlined by the stained chromatin.

At the time of division of the chromosomes in *Trichonympha* (fig. D, 2; pl. 7, fig. 25), a physical continuity may still be retained between the two threads thus formed, by the incomplete division of this framework. This invisible link, whose existence is suggested by the behavior of the ends of the chromosomes, would serve to keep one end of each chromosome near the corresponding end of its fellow, and would explain the apparent pairing of chromosomes in these stages (pl. 9, figs. 46–49). These stages, however, have not been observed in the living cell and it is possible that the spreading apart of such pairs may be due to the manipulations of the operator in making smears in the preparation of the material. It is conceivable, however, that considerable separation or strain may occur in the chromosomes of the normal cell without reaching the point of complete separation.

Given this physical continuity the drawing together of the two threads and their condensation into two shorter parallel threads joined at the apex, becomes a simple matter. Such precocious splitting and separation and their subsequent union before going on the spindle, have been found to occur in the chromosomes of the trichomonad flagellates (Kofoid and Swezy, 1915) and in *Giardia* (Boeck, 1917), a procedure which would seem to support an explanation similar to that given above. In view of our present knowledge of cytology, the alternative explanation would be that we have here a synapsis of chromosomes occurring in the ordinary vegetative division cycles, since it is highly improbable, even were these flagellates found to be sexually differentiated, that all the division cycles found within a single year, would be only those of gametes and not the ordinary trophozoite division, where such a reduction in the number of the chromosomes would not be expected to occur. The possibility that the behavior of pregametic chromosomes in the Metazoa is a specialization of a more widely prevalent phase of mitosis in the Protozoa is not precluded.

Evidences for the precocious splitting of the chromosomes immediately following the end of a division period, have been carefully searched for, but thus far have not been found. At what period in

the "resting" phase of the nucleus this occurs has not been determined. The abundance of such stages in what appear to be normal vegetative forms, without other signs of the approach of division, would suggest that it occurs very early in the between-division periods of the life of the organism.

That we may have here a continuity of chromosomes from one division period to the next is suggested by certain appearances of the resting phases of the nucleus. This is best seen in figures 14, 15, and 17 of plate 6, and figures 26 to 28 of plate 7. These are the nuclear figures which are most frequently met with in the ordinary trophozoite. They present a broken, ragged network with distinctly marked ends of chromatin threads scattered through it. Occupying the nodes of the network or sometimes at the ends of the threads, are chromatin granules. The process of changing from this condition to that of the distinctly marked chromosomes seems to consist in an outmoving of the contents of the granules along the threads to which they are attached (pl. 6, figs. 17, 19). On the completion of this, the fully formed chromosomes become apparent, with an entire absence of large chromatin granules. Differentiation of individual chromosomes other than the heterochromosomes has not been detected.

In the late prophase of division the chromosomes retain distinct outlines for a considerable period. With the disappearance of the spindle fibers the chromosomes move out to near the center of the nucleus, with the threads lying parallel or nearly so (pl. 12, figs. 77, 82). Without apparently losing their individuality, these separate threads begin to change their position (fig. 78), and form a loose network by the interweaving of the separate strands (figs. 78, 81, 83). Later granules appear at the intersection of the threads which then may become thinner and we have the same nuclear structure as that shown in figures 27 and 28 of plate 7, and figures 14 and 15 of plate 6. Distinct chromomeres cannot be detected in these stages of the nucleus, but they appear later following the formation of distinct chromosomes.

The type of division of the chromosomes is apparently transverse in the later stages, but is in reality longitudinal, as shown by the longitudinal splitting of the single threads (fig. D; pl. 7, fig. 25). The contraction of the two, visibly separated halves of the chromosomes (pl. 9, fig. 49) into single short, thick threads with a V-shape (fig. 51*a*), produces the units which are unfolded on the spindle as long, single threads (pl. 10, figs. 56, 59). These part in the middle,

at the apex of the original V, and thus finally complete the original splitting. Division of the chromosomes is thus in *Trichonympha*, as in other flagellates, a fundamentally longitudinal process.

In the division of the protoplasmic body the same longitudinal type of division also holds true. In the rounding up of the body the anteroposterior relations are somewhat obscured. The beginning of the division process is found to be a longitudinal splitting of the centroblepharoplast and the cone-shaped anterior portion of the body (pl. 8, figs. 33–36). This is followed by a splitting of the entire ectoplasmic surface of the body in the same plane, which is fundamentally longitudinal (pl. 9, fig. 51). This relation is maintained through the various stages of division to the early telophase (pl. 12, fig. 76). At this point, however, the activity of the motor organelles of the two attached daughter cells becomes operative in different directions, resulting in a change in the orientation of the two parts. With the continued opposing activities of the flagella the daughter flagellates are found attached to each other at the posterior regions only, giving an apparent transverse direction to the plane of division (pl. 12, fig. 79). This is apparent only and not the real direction which is fundamentally longitudinal. The morphological plane in which the chromosomes finally part at the metaphase coincides with that in which the highly organized neuromotor system is divided in plasmotomy. The plane of division of the chromomeres and chromosomes, and of the organized structures of the cytoplasm whose behavior at mitosis can be determined, is thus one and the same morphologically longitudinal plane.

RELATIONSHIPS

The relationships of these peculiar and highly evolved organisms has proven a source of some confusion. On the one hand ciliate affinities have been claimed for them, and, on the other, they have been listed as flagellates. Stein (1878), with the meager description given in Leidy's first paper in 1877 as his only basis of classification, correctly placed them among the flagellates. In this he has been followed by most later taxonomists. Leidy, however, with his fuller account of their structure in 1881, considered them intermediate between the gregarines and ciliates but more nearly related to the former. Kent (1882) followed this by placing them among the holotrichous ciliates in the faimly Trichonymphidae, a family he

formed for these parasites of the termites and included in it *Pyrsonema* and *Dinenympha*.

The flagellate affinities of *Trichonympha* were recognized by Grassi and Sandias (1893) who placed it in the family Lophomonadidae in the Flagellata. Bütschli (1889), however, reverts to the classification of Kent and recognized them as belonging to the ciliates. Senn (1900), with some doubt as to their actual position, placed them in the order Trichonymphida as an appendix to the Flagellata, but later (1911) allocated them in the Euflagellata. Hickson (1903) in Lankester's *Treatise on Zoology*, added the family Trichonymphidae as an appendix to the Ciliata. Doflein (1911) followed Senn in his classification of these puzzling forms, and, as had been earlier done by Bütschli, attributed the formation of the family Trichonymphidae to Leidy, overlooking the fact that Leidy nowhere attempted to classify the forms he described, while the family in question had been formed by Kent for these parasites of the termites.

The first complete systematic review of this subject is that given by Grassi (1911), in which he presents some changes in the previous taxonomic groupings. He formed a new order, the Hypermastigina, closely following the order Polymastigina in the Flagellata. This contained a single family, the Lophomonadidae Grassi. In this family he placed all the forms possessing many flagella, as *Trichonympha*, *Lophomonas*, and *Joenia*. Other changes that were made in the taxonomic position of other members of this group of parasites will be noted in a later paper discussing this subject.

In the genus *Trichonympha* he recognized two species, *T. agilis* Leidy and *T. minor* Grassi. The species described by Hartmann in 1910 as *T. hertwigi*, he rejects as defined by the describer, dividing the forms he has figured among three different genera, that is, the "young form" is referred to *Pyrsonympha*, and two new genera are created for the others. The "male" (Hartmann, 1910, pl. 28) he placed in the genus *Holomastigotoides* and the "female" (pl. 30) in the genus *Pseudotrichonympha*.

Poche, in 1913, added still further to the confusion already existing in this group by creating a new order, the Trichonymphida, which he placed in the Euflagellata. This contained four famliies, Dinenymphidae, Devescovinidae, Calonymphidae, and Trichonymphidae. The family Devescovinidae he formed for a single genus, *Devescovina* Foà, having but four flagella and in nowise related to the remainder of this group of many-flagellated organisms. This genus we consider

belongs in the order Polymastigina near the genus *Trichomonas*, to which its axostyle, parabasal body and four flagella ally it.

In a later paper we propose to give a fuller discussion of the systematic relations of the parasites of the termites and will here confine our attention to the genus *Trichonympha*, with the order and family to which it belongs.

The question of flagellate or ciliate relationships of *Trichonympha* is one the solution of which finds little difficulty in the light of facts concerning its morphology and division presented in this paper. We have already discussed some aspects of its relationships elsewhere (1919c) and will here only point out a few additional considerations. The relation between the motor organelles and the centroblepharoplast is distinctly flagellate in character. The nearest approach to this relationship among the ciliates is perhaps that found in the ciliate *Euplotes* (Yocom, 1918). Here the ciliary components of part of the cirri and the oral membranelles are bound together by their connection with the motorium. The latter structure, however, plays an entirely different rôle during division than does the centroblepharoplast of the flagellates. In *Euplotes* the motorium is passive or apparently disappears at the time of division and is formed anew in the daughter cells. In no case does it play an active part in mitosis. In the flagellates, on the other hand, this structure becomes the dominant, guiding figure in mitosis, dividing and acting as centrosomes in the formation of the mitotic spindle.

The occurrence of a highly differentiated ectoplasm is here correlated with the development of the complex myonemes, which in turn are probably directly correlated with the great increase in the number of flagella. A comparable degree of ectoplasmic differentiation is found in one other group of flagellates, the members of the subgenus *Pachydinium* in the genus *Gymnodinium* among the dinoflagellates (Kofoid and Swezy, 1919*d*), but without myonemes or flagella.

It is thus seen that in its morphology as well as division, *Trichonympha* possesses distinctly flagellate characteristics with none that are exclusively ciliate in character. The complexity of its structures is the result of a high degree of specialization and parallel evolution, and in no way connects it with the ciliates.

The possibility of these flagellates forming a connecting link between the Flagellata and the Ciliata, is also one for which we can find no adequate basis. Thus far no near relatives of *Trichonympha* have been found as free-living forms. They are confined exclusively

to the rôle of parasites or commensals of the termites and related insects. This fact in itself would throw them outside the line of evolution along which the present group of the presumably much earlier evolved, free-living ciliates were developed.

For these reasons we accept the allocation given by Grassi for these peculiar organisms, placing them among the true flagellates and, as shown by a study of the various stages of division as well as morphology, not far removed from certain members of the Polymastigina.

The utilization of the order Hypermastigina, proposed by Grassi (1911) for this group of flagellates possessing numerous flagella, is more appropriate as a descriptive term than either Trichonymphida Poche or Lophomonadina Lankester. It is further desirable as at once connoting its relation to the order Polymastigina near which it stands in the Flagellata.

For the family designation of the group to which *Trichonympha* belongs, we retain the term proposed by Kent (1882), Trichonymphidae. The remaining constituent members of this family we need not further specify at this time.

The genus *Trichonympha* contains three species previously described: *T. agilis* Leidy, from American termites; *T. Leidyi* Kent, from Tasmanian termites; and *T. minor* Grassi, from Italian termites. No figures have been given for the last two species, the description of both being imperfect without dimensions or other exact data. To these we add a fourth species, *T. campanula*.

Key to the Genus *Trichonympha*

Hypermastigina with flagella of various lengths disposed over about two-thirds of the surface of the body; nucleus submedian; specialized ectoplasm; complex neuromotor system; holozoic nutrition.

1. Large, 250μ–460μ 2
— Small, 100μ or less 3
2. Flagella of three distinct lengths, posterior endoplasm not separated from anterior region by distinct line ..... **campanula** sp. nov.
3. Flagella short, almost cilia-like, elongate or pyriform body ..... **leidyi** Kent
— Distinct line separating two regions of endoplasm, body constricted at the point of separation, cilia long ..... **agilis** Leidy
— Small form, flagella short, lines separating two regions of endoplasm long, crossing each other behind nucleus ..... **minor** Grassi

SUMMARY

1. This organism has a highly specialized flagellate type of structure with a highly developed neuromotor system, the centro blepharoplast connected by a complex system of oblique fibers with the numerous flagella which cover two-thirds of the surface of the body. Besides these fibers the ectoplasm contains an alveolar layer and one of transverse myonemes. Immediately below it in the endoplasm are the longitudinal myonemes.

2. The nucleus is submedian in position and part of its chromatin contents is permanently separated as a "heterochromosome" contained within a small vesicle.

3. Nutrition is holozoic but its method of feeding is unknown. No cytostome is present. The endoplasm is divided into two regions, anterior and posterior. The latter region, which is covered only by a thin pellicle and not the thick ectoplasm of the remainder of the body, is usually filled with food particles. These are entirely absent from the anterior region of endoplasm.

4. The body rounds up at the time of division and the centroblepharoplast divides, forming a paradesmose, the entire ectoplasm splitting into two parts with it. These act as the centrosome in the succeeding mitotic figures, the spindle fibers arising from the ends of the paradesmose or the centroblepharoplasts.

5. Precocious splitting of the chromosomes takes place previous to the prophase, forming fifty-two, V-shaped threads. In a pseudotelosynapsis this number is reduced to twenty-six. These part on the spindle along the line of the original split.

6. Division of the chromosomes as well as the body is fundamentally longitudinal.

7. *Trichonympha campanula* is fundamentally flagellate in its morphology and method of division and is in nowise related to the ciliates. It shows a high degree of specialization and development of its neuromotor system which is the most complex one thus far described among the Protozoa.

8. It is a member of the family Trichonymphidae Kent in the order Hypermastigina Grassi. This order stands near the Polymastigina, to the members of which *Trichonympha* is nearly related both morphologically and in its development.

Zoological Laboratory, University of California,
Berkeley, California.
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EXPLANATION OF PLATES

All figures of *Trichonympha campanula* sp. nov. from *Termopsis angusticollis* Walker, stained with iron haematoxylin and drawn with camera lucida. Magnification 800, unless otherwise stated.

PLATE 5

Fig. 1. Normal trophozoite showing the three zones of flagella, surface ridges of the body, and centroblepharoplast. × 300.

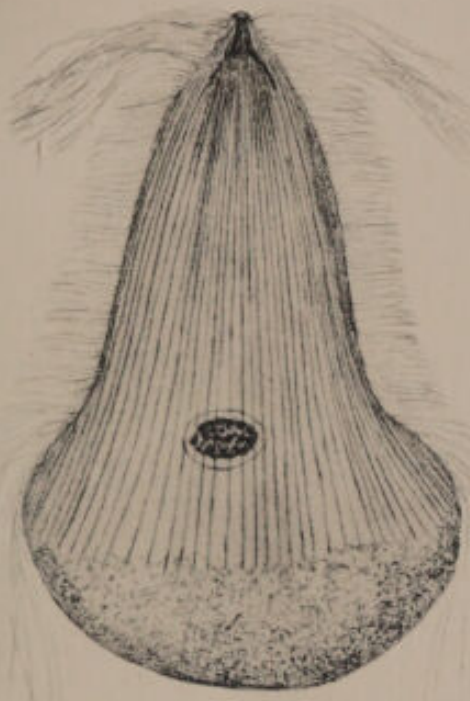
Fig. 2. Optical section of anterior part of the body, showing the differentiations of ectoplasm and endoplasm with the longitudinal myonemes in the latter, and part of the centroblepharoplast. × 300.

Fig. 3. Surface view of anterior end showing surface ridges, the cuplike depression with end of centroblepharoplast at its base and its covering operculum. Internally the ectoplasm and endoplasm may be seen with the vacuoles near the base of the centroblepharoplast.

Fig. 4. Semidiagrammatic cross-section of body in the region of the nucleus, showing origin of flagella from crests of surface ridges, ectoplasm, endoplasm, and the longitudinal myonemes in the latter. × 300.

Fig. 5. Optical section of anterior portion of the body showing striate appearance of ectoplasm, with its different layers, and the circular vacuole at the base of the centroblepharoplast. × 300.

Fig. 6. Optical section of entire body showing extent of differentiated ectoplasm and the two regions of endoplasm. Dark granules are probably remnants of ingested bacteria. × 300.



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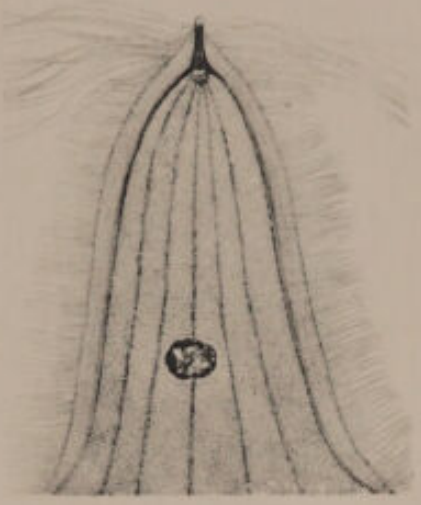

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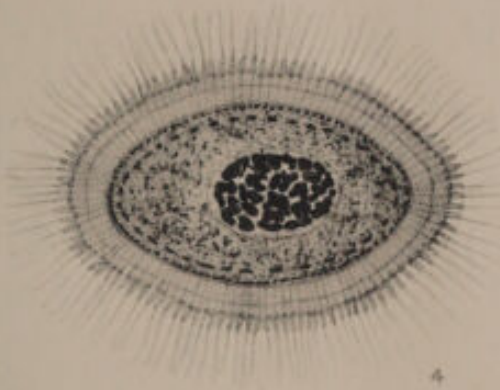

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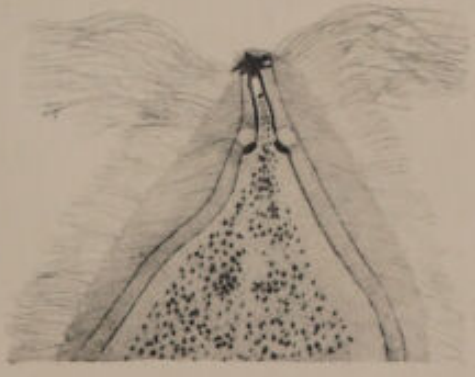

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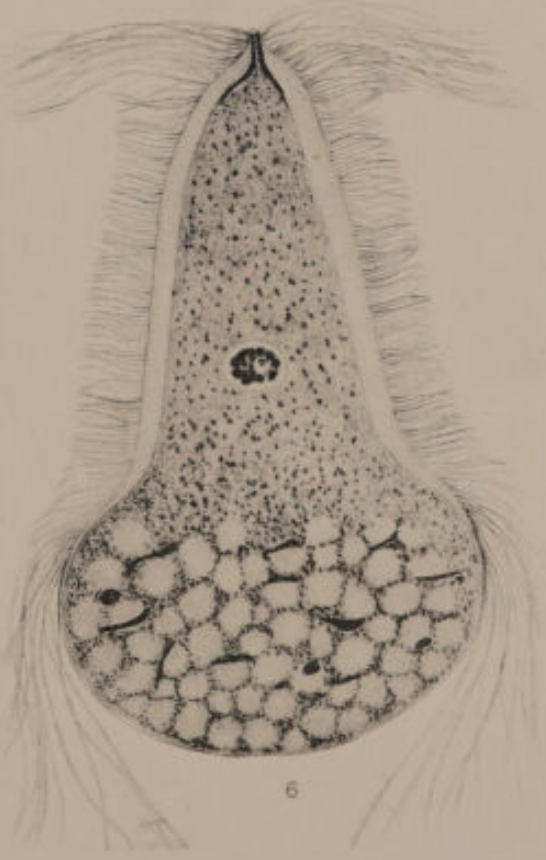

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PLATE 6

Fig. 7. Vertical view of centroblepharoplast complex in rounded-up form. Cuplike depression and operculum have disappeared. Oblique fibers radiating out from lobes of centroblepharoplast.

Fig. 8. Cross-section of distorted individual showing relative extent of endoplasm and ectoplasm. × 300.

Fig. 9. Lateral view of anterior end showing centroblepharoplast complex, oblique fibers and alveolar layer.

Fig. 10. Centroblepharoplast complex of individual in early prophase. Operculum and depression have disappeared, end of centroblepharoplast drawn out to a point.

Fig. 11. Vegetative nucleus showing the central region of chromatin granules, the heterochromosome and its vesicle, the alveolar zone and the outer, granular region.

Fig. 12. Cross-section of anterior end of body showing the centroblepharoplast, myonemes and surface ridges.

Fig. 13. Vertical view of centroblepharoplast complex of the early prophase stage.

Fig. 14. Vegetative nucleus showing the chromatin-encrusted network. Other parts as in figure 11.

Figs. 15–22. Nuclei of the early prophase stages in individuals which present no other sign of division.

Fig. 15. Early prophase; alveolar zone has disappeared, chromatin moving out from the granules along the threads of the network.

Fig. 16. Later stage of the same. Note size of heterochromosome and its vesicle.

Fig. 17. Formation of chromosomes; vesicle surrounding heterochromosome has disappeared.

Fig. 18. Appearance of distinct, paired chromosomes.

Figs. 19–22. Various figures of fully formed chromosomes.

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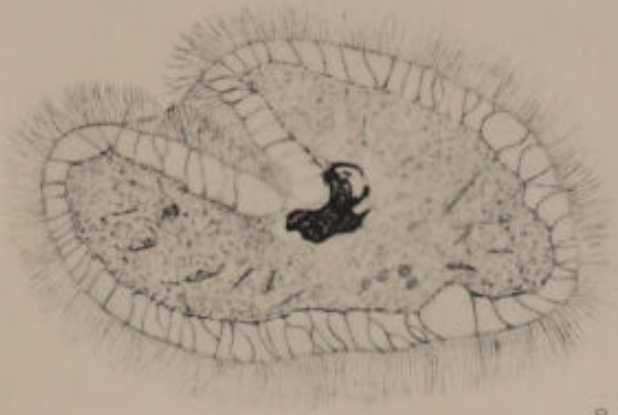

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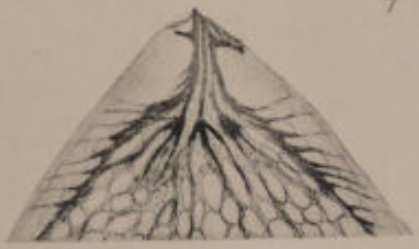

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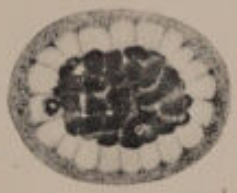

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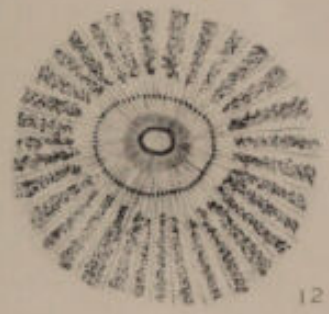

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PLATE 7

Fig. 23. Chromosomes appearing in the nucleus before other signs of division can be detected in the body. Figures 25–29 were drawn from similar individuals. × 300.

Fig. 24. Rounding up of the body preparatory to division; part of the centroblepharoplast complex and alveolar layer shown. × 300.

Fig. 25.—Longitudinal division of the chromosomes.

Fig. 26.—Breaking up of the chromatin-encrusted network beginning at one side of the nucleus.

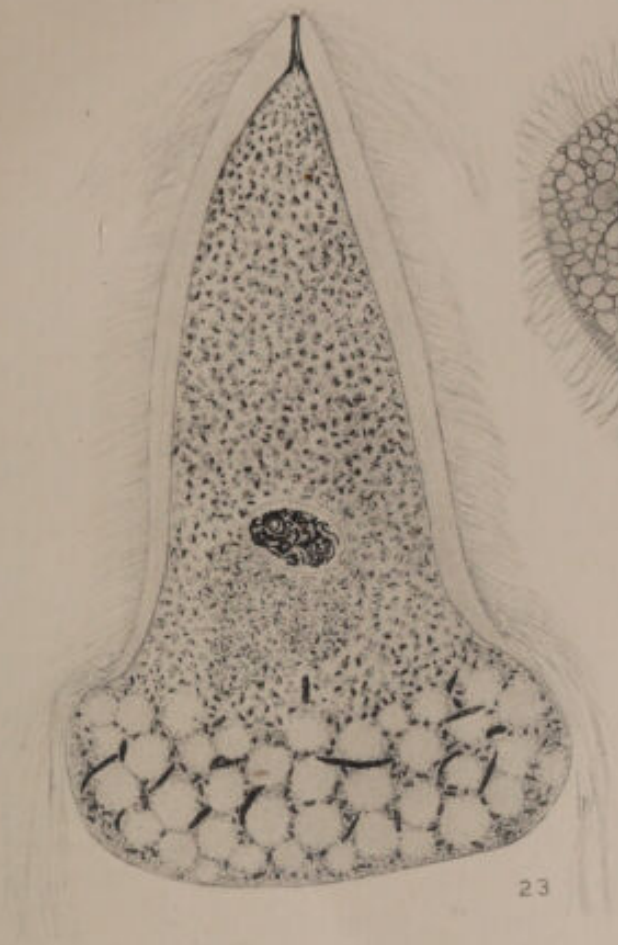
Fig. 27. Network thickly encrusted with chromatin showing free ends.

Fig. 28. Later stage of the same with the threads beginning to split.

Fig. 29. Chromosome formation nearly completed.

Fig. 30. Beginning of division of the centroblepharoplast complex. Note the spindle-shaped areas of endoplasm appearing between split.

Fig. 31. Optical section of rounded-up individual of the prophase period. × 300.



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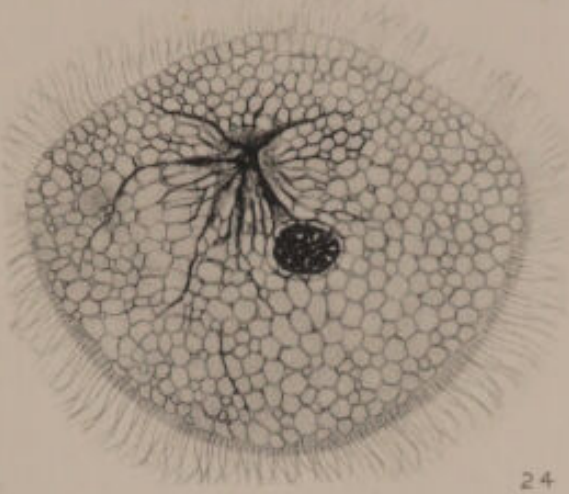

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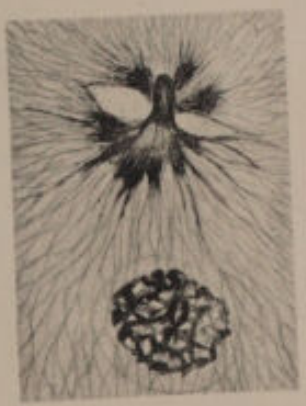

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PLATE 8

Fig. 32. Prophase with division of the centroblepharoplast completed in the tubular part but no split yet appearing in the ectoplasmic structures. An unusual figure. × 300.

Fig. 33. Splitting of the centroblepharoplast and the formation of the paradesmose between the bases as they separate.

Fig. 34. A later stage of the same with the halves forming new tubes connected at the tip. Note development of spines at tip.

Fig. 35. An earlier stage of the same showing the split beginning at the base, with the ectoplasmic structures drawing apart.

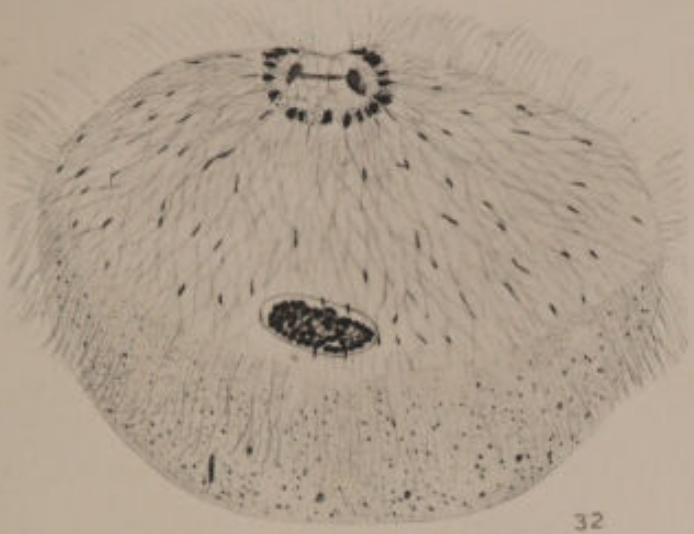
Fig. 36. Splitting completed; paradesmose elongates as the new centroblepharoplasts move apart.

Fig. 37. Vertical view of an early stage of splitting of centroblepharoplast.

Fig. 38. Vertical view of individual in prophase with the divided centroblepharoplasts connected by paradesmose. Part of oblique fibers and alveolar layer shown. Note aster arrangement of alveoli around centroblepharoplasts. × 300.

Fig. 39. Prophase nucleus showing the breaking up of the chromatin-encrusted network.

Fig. 40. Paradesmose, centroblepharoplasts and their related fibers in the prophase, with the nucleus elongating preparatory to formation of mitotic spindle.



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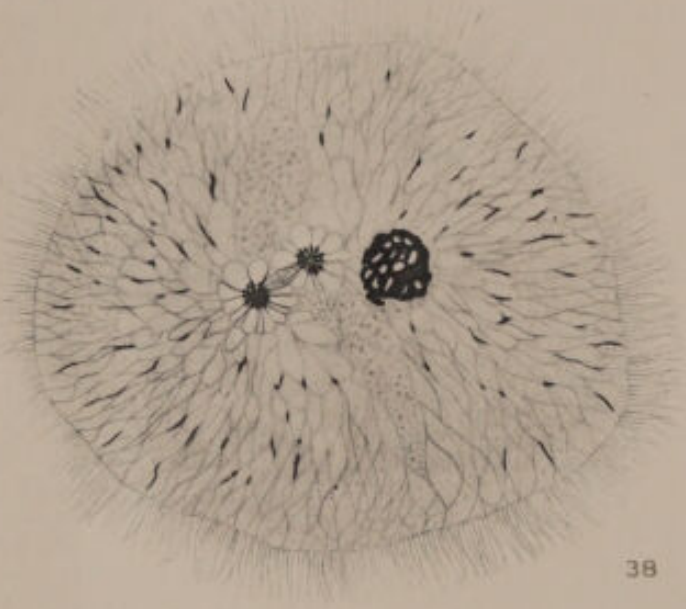

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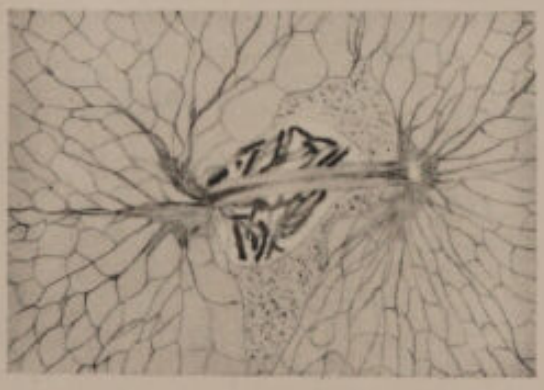

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PLATE 9

Figs. 41, 42. Two views of the same figure showing different structures. 41. The paradesmose, centroblepharoplast and oblique fibers. Immediately below and intermingled with these are the structures shown in 42: prophase nucleus and alveolar layer.

Fig. 43. Nucleus becoming closely attached to the paradesmose.

Fig. 44. Nucleus showing fifty-two chromosomes with marked chromomere structure. × 1250.

Fig. 45. Prophase nucleus in the tangled-skein stage.

Fig. 46. Prophase nucleus with looped chromosomes.

Fig. 47. Same stage. In both figures heterochromosome remains isolated.

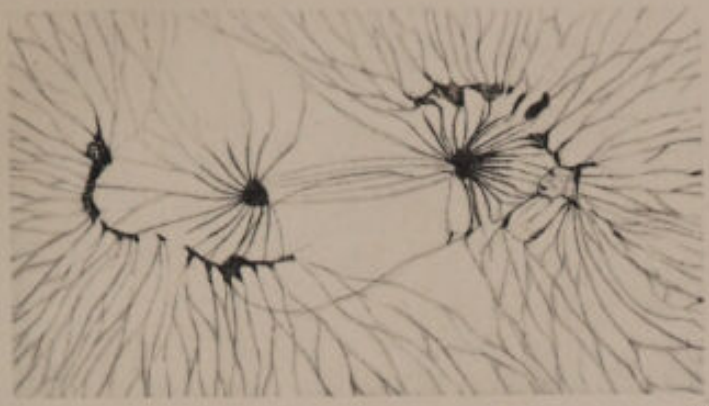
Fig. 48. Beginning of the tangled-skein stage (?); chromosomes at one side of nucleus stain deeper than those on the other.

Fig. 49. Marked tendency of chromosomes to assume paired arrangement.

Fig. 50. Tangled-skein stage.

Fig. 51. Individual in prophase showing completion of division of ectoplasmic structures. Only a few of the surface ridges are indicated. × 300.

Fig. 51*a*. Enlarged view of nucleus of last figure. Chromosome number reduced to 26.



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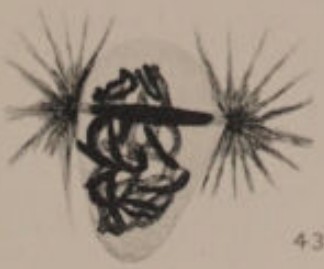

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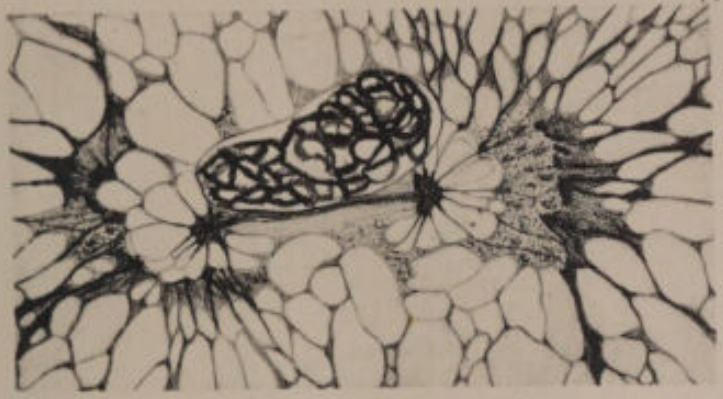

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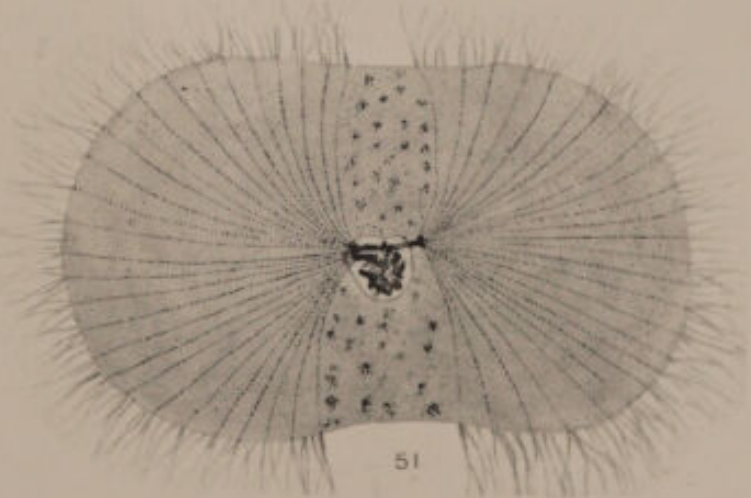

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PLATE 10

Nuclei on this and the following plates have the reduced number of chromosomes. In some figures the paradesmose is shown in position, i.e., above the nucleus. In others it is omitted or only indicated by faint lines to secure clearness.

Fig. 52. Late prophase; nucleus elongated and attached to the paradesmose.

Fig. 53. Same stage; tubular part of paradesmose omitted.

Fig. 54. Chromosome arrangement previous to spindle formation.

Fig. 55. Spindle fibers extending from centroblepharoplasts through nuclear membrane and attached to chromosomes.

Fig. 56. Showing process of unfolding of chromosomes in the metaphase. Heterochromosome distinct. Vertical view, with paradesmose uppermost and outside nuclear membrane.

Fig. 57. Lateral view of same stage with paradesmose above the nucleus.

Fig. 58. Same stage viewed from below, i.e., the interior of the body. Paradesmose is on opposite side of nucleus.

Fig. 59. Metaphase; paradesmose partly imbedded within the nucleus.

Fig. 60. Final division of the chromosomes; heterochromosome distinct and still undivided. Entire nucleus elongating.

Fig. 61. Early anaphase; heterochromosome divided.

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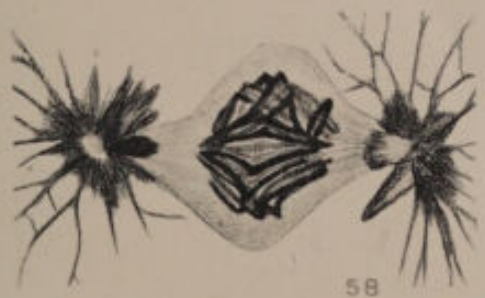

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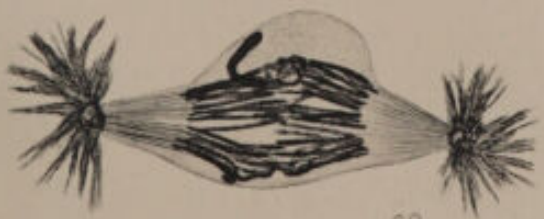

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PLATE 11

Fig. 62. Anaphase; heterochromosome divided without apparent attachment to spindle fibers.

Fig. 63. Unusual appearance of chromatin granules strung along the spindle fibers. × 1250.

Fig. 64. Anaphase; heterochromosome dividing.

Fig. 65. Telophase of nucleus; constriction of the nuclear membrane. × 1250.

Fig. 66. Daughter nucleus with division completed.

Fig. 67. Telophase with nuclear constriction advancing. Spindle fibers disappearing at one pole, still intact at the other. × 1250.

Fig. 68. Telophase showing surface ridges of the body. Centroblepharoplasts still connected by the paradesmose. × 300.

Fig. 69. Telophase of nucleus.

Fig. 70. Daughter nucleus after constriction and prior to rounding up.

Fig. 71. Greatly elongated nuclear band still connecting daughter nuclei. × 300.

Fig. 72. Completion of nuclear division with paradesmose still persisting.

Figs. 73, 74. Sister nuclei recently divided.

Fig. 75. Completion of nuclear division. Paradesmose beginning to fade out.

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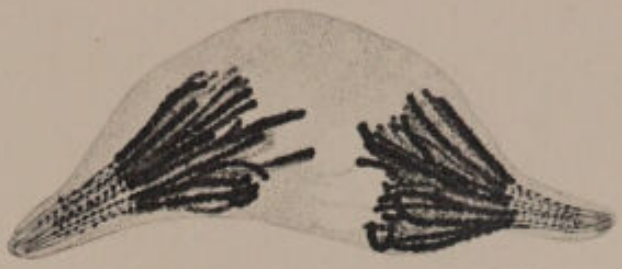

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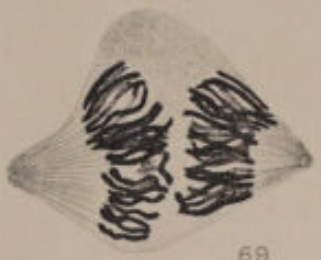

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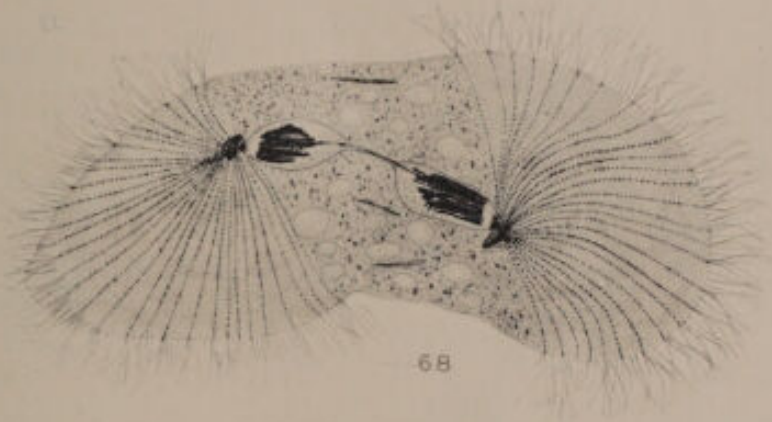

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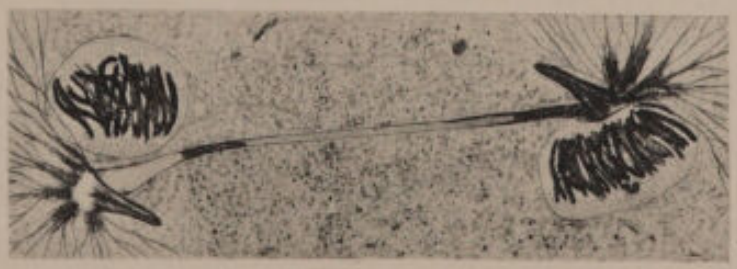

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PLATE 12

Fig. 76. Late telophase; nuclei have become reorganized; alveolar layer shown. × 300.

Fig. 77. Nucleus at the beginning of reorganization.

Fig. 78. Later stage showing chromosomes forming a coarse network.

Fig. 79. Plasmotomy; all ectoplasmic structure except oblique fibers are omitted. × 300.

Fig. 80. Optical section of individual shown in figure 76 at the point marked by the arrow. Note basal granules and basal portion of flagella.

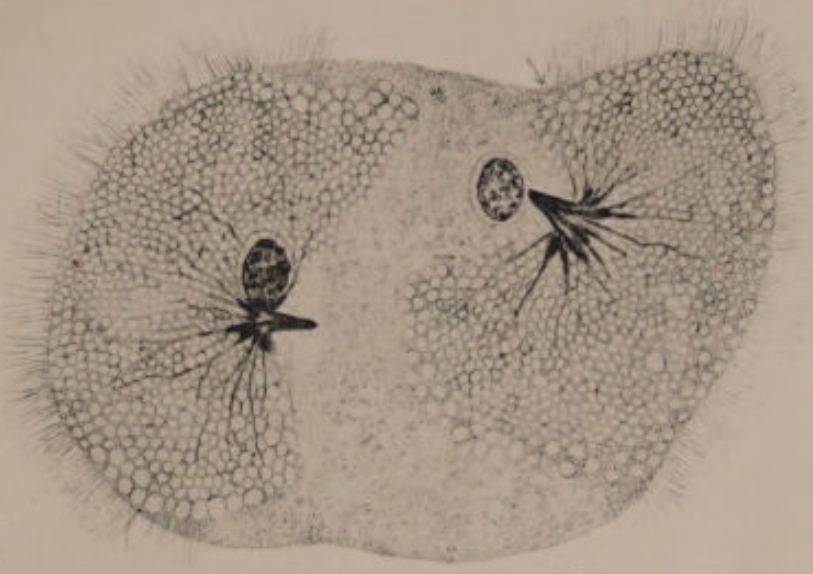
Fig. 81. Reorganization process of nucleus.

Fig. 82. Early stage of the same process.

Fig. 83. Later stage showing the vesicle forming around the heterochromosome.

Fig. 84. After completion of plasmotomy. Nuclear reorganization not yet begun. × 300.

Fig. 85. Somatella prior to plasmotomy. Note lack of synchronism in development of ectoplasmic structures. × 300.



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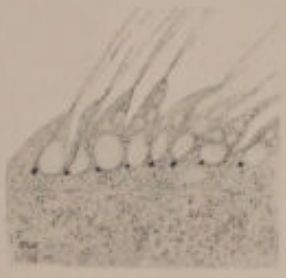

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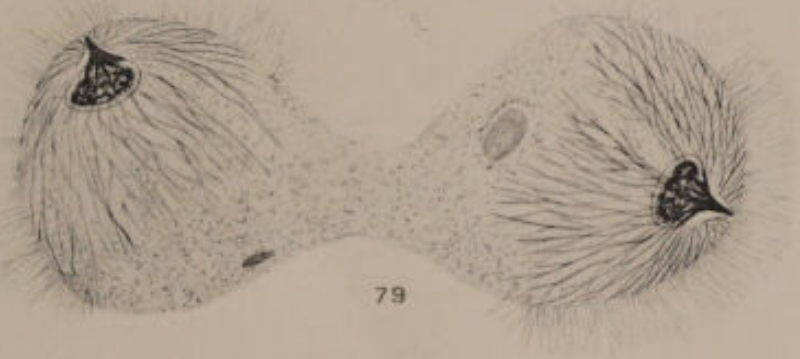

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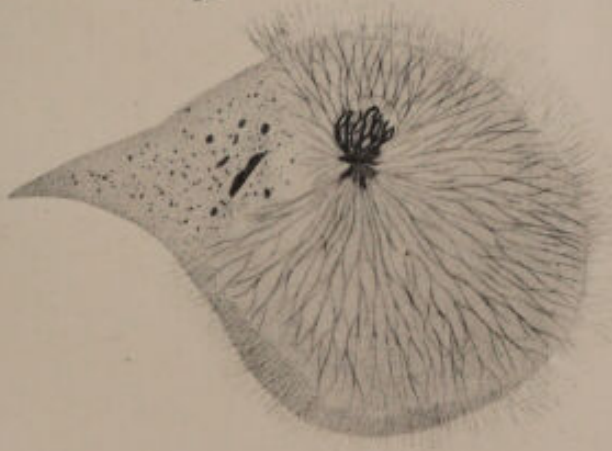

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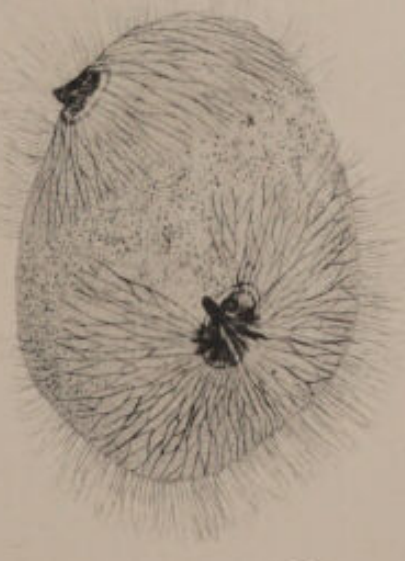

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STUDIES ON THE PARASITES OF THE TERMITES IV. ON *LEIDYOPSIS SPHAERICA* GEN. NOV., SP. NOV.

BY

CHARLES ATWOOD KOFOID AND OLIVE SWEZY

CONTENTS

INTRODUCTION

In our previous studies on the parasites of the termite, *Termopsis angusticollis* Walker, we have described three of the curious and highly interesting protozoans in this faunal complex. These are the flagellates *Streblomastix strix* (1919*a*), *Trichomitus termitidis* (1919*b*), and *Trichonympha campanula* (1919*c*). A fourth member of this group remains to be noticed. This flagellate is closely akin to *Trichonympha campanula* yet presents some striking differences which, in our opinion, give it a position generically distinct from that species. We therefore propose for it the name *Leidyopsis sphaerica* gen. nov., sp. nov., naming it in honor of the pioneer investigator of this group, the American naturalist Dr. Joseph Leidy.

It is less abundant than are the other members of this association of termite parasites. It is found in the lumen of the intestinal tract and is in no case attached to the walls. In this location, as also in its activities, it resembles *Trichonympha campanula*. The mass of flagella attached to the anterior portion of the body stream backward, partly clothing its rotundity. In the living animal the action of the flagella is similar to that of its larger relative. Waves of contraction pass from the proximal to the distal ends of the flagella without cessation. Owing to the differences in the shape of the body its anterior end is slightly less mobile than is the case in *Trichonympha campanula*. Its rate of progression is also somewhat slower, as though impeded by the rotundity of the body in moving through the seething mass of organisms found in the intestinal tract.

MORPHOLOGY

In its morphology *Leidyopsis* presents a type of structure which is as highly differentiated as that found in *Trichonympha*. It differs from it mainly in the extent and distribution of the specialized structures as compared with the remainder of the body. These differences will be pointed out more specifically below.

The outstanding feature of its morphology, next to its abundant supply of flagella, is its neuromotor system, which is of the trichonymphid type (Kofoid and Swezy, 1918*c*). Correlated with this are the ectoplasmic differentiations, while the remainder of the body presents no structural features which mark an advance over that of the simpler flagellates. The evolutionary development of organelles

in *Leidyopsis* is thus marked only in its neuromotor system, while the remainder of the body still shows a low degree of development. Its mode of nutrition is holozoic, as shown by the food particles in the endoplasm, yet no cytostome or other organelles for food taking are present.

Shape and Size

In size *Leidyopsis sphaerica* presents less variation than has been found in the three other flagellates previously described from the same habitat. This is probably due, however, to the paucity of our

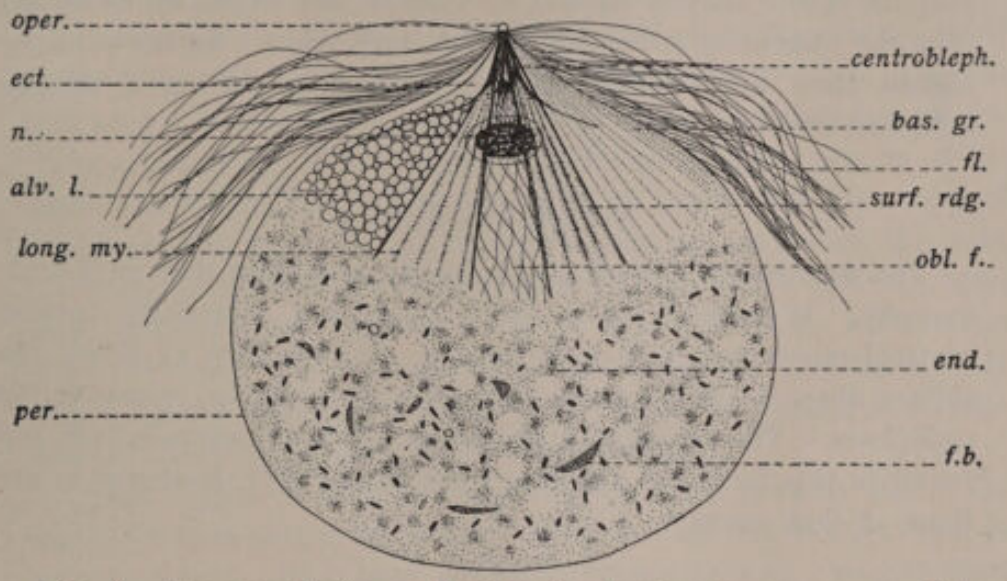

Fig. A. Diagrammatic figure of *Leidyopsis sphaerica* gen. nov., sp. nov. Ectoplasm drawn in sections to show structure of its different parts.

Abbreviations: *alv. l.*, alveolar layer; *bas. gr.*, basal granules; *centrobleph.*, centroblepharoplast; *ect.*, ectoplasm; *end.*, endoplasm; *f. b.*, food bodies; *fl.*, flagella; *long. my.*, longitudinal myonemes; *n.*, nucleus; *obl. f.*, oblique fibers; *oper.*, operculum; *per.*, periplast; *sur. rdg.*, surface ridges. × 400.

material, as a larger number of individuals would present room for greater variations. It is nearly spherical in shape, its length only a few microns greater than its width, due to the cone-shaped projection of the anterior end of the body. The length varies from 165 to 190μ and the width 160 to 185μ. Figure 1 of plate 13 is that of an individual 182μ in length and 176μ in breadth. These proportions vary considerably, particularly in stained material where the body becomes flattened and relative proportions almost entirely lost. Measurements are of value only when made from the living organism.

The body is perfectly symmetrical in outline, a condition found in only a few protozoans. The shape is that of a sphere with the

anterior end drawn out into a short cone-shaped projection. This is terminated by a small, transparent, caplike structure, the operculum (fig. A, *oper.*), which covers a cup-shaped depression (pl. 13, fig. 10). This is similar to the structure of the anterior end of *Trichonympha campanula.* No evidences have been found which would indicate the function of this peculiar formation. It is the only part of the surface of the body bearing any resemblance to a cytostomal opening into the endoplasm, yet we have no evidence that it is used for that purpose.

The surface of the anterior and sometimes even of the middle portions of the body is marked by longitudinal ridges which extend from the operculum posteriorly (fig. A, *surf. rdg.*). In the posterior regions these are entirely lacking, the surface having a smooth, unbroken outline.

Neuromotor System

The neuromotor system of *Leidyopsis* resembles that of *Trichonympha.* It consists of a highly developed, anteriorly located centroblepharoplast, numerous flagella, and two sets of fibers, the oblique fibers in the ectoplasm and the longitudinal myonemes in the endoplasm. The transverse myonemes of *Trichonympha* are not found in this form and the others are not so well developed as are those of that genus.

Ectoplasmic Structures

The division of the body into ectoplasm and endoplasm is clearly marked only in a narrow zone surrounding the cone-shaped anterior end of the body. Here it presents the three distinct layers so striking in the ectoplasm of *Trichonympha,* but these soon fade out, though the myonemes and surface ridges can be traced farther posteriorly, the latter usually having a length of one-third to one-half that of the body. Anteriorly the ectoplasm is thick around the base of the cone, becoming thin posteriorly until it merges into the thin periplast of the middle and posterior regions of the body (fig. A). It is composed of an outer layer of surface ridges (fig. A, *surf. rdg.*), an alveolar layer (*alv. l.*), and a narrow, inner ectoplasmic layer. These are traversed by the oblique fibers and also contain the basal granules of the flagella. These separate structures will now be discussed in detail.

Surface Ridges: The outer layer of ectoplasm is raised in narrow longitudinal ridges (fig. A, *surf. rdg.*) from the crests of which spring the flagella. The ridges extend from the base of the operculum at the anterior end posteriorly for about one-third to one-half the length of the body. Anteriorly they are very narrow and placed close together, the ends forming an irregular, wavy line around the base of the operculum (pl. 13, fig. 10). Posteriorly they spread out in fan-shaped or radiating lines, increasing in number by the interposition of new ridges. Their course is longitudinal without spiral twisting, and when the limits of the differentiated ectoplasm are reached they disappear in the thin periplast of that region.

Locomotor Organelles: These are confined to the anterior third of the body, and consist of long flagella, approximately equal in length and arising in longitudinal rows from the crests of the surface ridges. They are most numerous anteriorly on the cone-shaped portion of the body, where they form a dense mass which retains the dark color of iron haematoxylin. A single flagellum alone does not show the stain nor do the ends which stream out from this mass. Collectively, however, they are usually stained so darkly as to obscure the nucleus and other structures beneath them. The spreading apart of the surface ridges distally results in a distinct thinning of the coating of flagella behind the narrowed anterior end of the body.

Each flagellum arises from a minute basal granule below the surface ridges, passes up through the ridge and leaves the crests as a single thread. Each basal granule is connected with a minute fibril from the oblique fibers, apparently in the same manner as in *Trichonympha campanula*. These fibrils and basal portions of the flagella give a finely striate appearance to the layers of ectoplasm through which they pass.

The flagella stream outward and backward over the surface of the body and in the living flagellate are in constant motion.

Oblique Fibers: These are not so prominent in *Leidyopsis* as in *Trichonympha*, owing partly to their smaller extent, and partly to the difficulty of differentiating them from the mass of dark flagella. When the flagella have been sufficiently destained to allow observation of the structures beneath them, the latter have also completely lost their color, and only the most careful focusing enables the observer to distinguish between the flagella and the equally slender oblique fibers immediately beneath.

The oblique fibers form an integral part of the centroblepharoplast

complex. They arise from its basal lobes as branches which break up into the minute, threadlike oblique fibers. These follow an oblique course posteriorly, continually giving off branches, part of which go to the basal granules of the flagella and the remainder cross and intercross in an irregular anastomosing network through the ectoplasm of the body (fig. A, *ob. f.*). Near the distal limits of the ectoplasmic zone these fibers fade out and disappear.

In their structure they are homogenous and not granular, and their width is about equal to that of the flagella. The smaller branches leading out to the basal granules are more slender. At the anterior end, near the basal lobes of the centroblepharoplast, they take a darker stain than is the case farther posteriorly, though this may be largely due to their slightly greater thickness in the posterior region.

Centroblepharoplast: The oblique fibers are intimately related to the centroblepharoplast. This organelle in *Leidyopsis* differs but little if any from that of *Trichonympha*. It forms a tubular or rod-shaped structure in the pointed anterior end of the body (fig. A, *centrobleph.;* pl. 13, figs. 1, 3), from the base of which stream out the oblique fibers.

The tubular part of the centroblepharoplast is composed of a number of rods or strands which extend to the base of the cuplike depression at the anterior end of the cone-shaped portion of the body. Here the ends are joined together by a circular band of darkly staining material, the center of which is occupied by the core of endoplasm (pl. 13, figs. 2, 10, 13). The tube extends posteriorly to near the base of the cone where it expands into a collar-like structure composed of several large, irregular lobes, which may sometimes be united into a solid band encircling the base of the tube (pl. 13, fig. 3). Distally these lobes fray out into the oblique fibers.

The center of the tube is occupied by a slender core of endoplasm continuous with the endoplasm of the body. This extends up to the base of the small, cup-shaped depression, where it may be seen as a light area within a dark ring (pl. 13, fig. 10).

The tubular part of the centroblepharoplast complex may present some modifications, such as the formation of short, spinelike processes extending out from the tip (fig. 3) or along its sides (fig. 1). At the time of division the cuplike depression and operculum disappear, leaving the tip of the centroblepharoplast complex exposed at the surface. The entire structure takes a deep stain with iron haematoxylin, making it a conspicuous part of the body in stained preparations.

Alveolar Layer: This is closely associated with the oblique fibers, occupying the same region of ectoplasm. It is difficult to demonstrate and sometimes appears to be entirely lacking. This, however, seems to be due to the small extent of the ectoplasmic layer which it covers in those cases. In only a few specimens does it extend as far posteriorly as do the other ectoplasmic structures. It seems to be the first part to disappear in the thinning of the ectoplasm, which takes place a short distance behind the base of the cone.

The alveoli are most prominent at the time of division and appear in surface view as small, colorless spheres, varying considerably in size, and not closely packed together (pl. 14, fig. 24). In the ordinary trophozoite the arrangement is more compact, with the individual alveoli of smaller size.

Endoplasmic Structures

The endoplasm may be divided into two regions, anterior and posterior, but with no definite boundary line separating them. The distinction between the two portions is rather less pronounced in this form than in the different species of *Trichonympha*.

The anterior part is relatively small, extending but a short distance behind the nucleus (pl. 13, fig. 1). It is granular, without alveoli or food inclusions. The part immediately surrounding the nucleus is usually more dense than the remaining portion, evidently the result of greater metabolic activity.

The posterior region of endoplasm is coarsely vacuolate in structure and is often abundantly filled with food bodies (fig. A, *f. b.*). These are sometimes found enclosed in food vacuoles but more frequently are found lying free in the plasma. They consist of particles of wood, small flagellates, bacteria, or other small bodies that may be present in the intestinal contents. The method of ingestion of these is entirely unknown. No cytostome is present unless it is possible for the cup-shaped depression at the anterior tip of the body to assume that function. Neither the feeding reactions nor defecation of *Leidyopsis* have been observed.

Longitudinal Myonemes: These myonemes are found in the outer zone of endoplasm, immediately beneath the ectoplasm of the anterior part of the body, and coextensive with it. They are granular in structure and form straight, longitudinal lines radiating out from the region of the centroblepharoplast (fig. A, *long. my.*). Their connection with that structure, if any exists, could not be clearly detected.

Near the distal limits of the ectoplasmic differentiation the longitudinal myonemes fade out in the endoplasm, without showing distinct attachment areas. Their function here, as in *Trichonympha*, seems to be concerned with the mobility of the anterior tip of the body. The globular form of *Leidyopsis* as compared with the elongate one of *Trichonympha* allows for much less activity, yet the same type of sidewise movements, though greatly restricted, may be observed in the living, active flagellate.

Nucleus: The nucleus is found in the anterior part of the body, a short distance posterior to the centroblepharoplast (fig. A, *n.*). It is a rotund ellipsoid, with its longer axis, which lies in the transverse plane of the body, exceeding its shorter axis by nearly a third of its own length. No rhizoplast could be detected between it and the centroblepharoplast.

The structure of the nucleus is like that of *Trichonympha campanula*. The membrane is thin and overlies a narrow granular zone, beneath which is an alveolar zone. These alveoli are rounded outwardly where they abut upon the granular zone, with the remaining facets closely pressed together. The alveoli are fairly uniform in size, yet in stages which show distinct chromosome changes prior to the prophase of division, great modifications may often be noticed. The alveoli may be fewer in number and larger in size. This may be the result of a breaking down of the walls between several adjacent alveoli, since in the early prophase their disappearance is usually complete.

The remainder of the nucleus inside the alveolar zone is occupied by an irregular linin network in which loose ends may often be detected. The network is usually encrusted with chromatin, with large granules of the same substance at the nodes. The disposition of the chromatin, however, varies considerably in different individuals and is probably conditioned by the chromosome cycle, which here apparently follows a course similar to that previously outlined for *Trichonympha campanula* (Kofoid and Swezy, 1919*c*). The nuclei shown in figures 5 to 8 on plate 13, give different phases of nuclear structures in individuals which do not yet give other indications of the approach of division. In these nuclei the network progressively breaks up, the chromatin moving out from the granules along the threads which become thicker and split lengthwise (fig. 6). From these threads the definitive chromosomes are produced.

The nucleus of *Leidyopsis* is further distinguished by the presence of a small, coiled chromatin rod similar to the heterochromosome of *Trichonympha campanula*. This is isolated in a small, clear vesicle at one side of the central chromatin mass and partially imbedded within it.

BINARY FISSION

The process of binary fission in *Leidyopsis sphaerica* shows a close similarity to the various phases of binary fission in *Trichonympha campanula*. It is characterized by the longitudinal division of the centroblepharoplast complex and of the ectoplasmic structures, the formation of a paradesmose, precocious, longitudinal splitting of the chromosomes and pseudosynapsis. Owing to the scanty numbers of these flagellates which have been present in any one host, we cannot present the full details of the different stages. The close similarity between the phases that we have secured and those of the other species, however, would seem to suggest that the remaining stages of *Leidyopsis* are also similar. In the following discussion, therefore, the different phases are interpreted in the light of our fuller knowledge of the mitotic phenomena of *Trichonympha*.

Division of Ectoplasmic Structures

The first evidences of division in the extranuclear structures of the body are found in the centroblepharoplast. This structure divides, beginning at the base and splitting longitudinally to the tip. The basal masses become separated into two equal parts (pl. 13, figs. 12, 13), taking with them their attached fibers and motor organelles. This divides the entire ectoplasmic layer, leaving a constantly increasing strip of endoplasm between them as they move apart. As the two halves of the centroblepharoplast separate, a broad band, the paradesmose, is formed between them (pl. 13, figs. 15, 16). This is attached directly to the bases, leaving the tubular portions of the centroblepharoplasts standing out from it at right angles, or nearly so. In figures 15 and 16 these parts are omitted, as the structures are viewed from the posterior end of the organism and show the relations of the paradesmose and the basal lobes of the centroblepharoplasts. Here, as in *Trichonympha*, with the final separation of the tips of the centroblepharoplast, each half develops into a tube similar to the original structure. This seems to take place immediately after separation of the halves.

The further course of division of the ectoplasmic structures shows a continued separation of the two portions until they come to lie on opposite ends of the organism, connected by the spindle and paradesmose (pl. 14, fig. 17). The relatively large portion of the body which is not covered with ectoplasmic structures and flagella, renders this separation very conspicuous in these stages. The completion of these structures for each daughter cell is partly the result of new outgrowths and partly the readjustments of those derived from the parent cell. At the time plasmotomy occurs these may be completed or may still be in the process of formation (pl. 14, figs. 24, 25).

Mitosis

The first evidences of the approach of mitosis may be looked for in the nucleus. How early these appear cannot be stated, but, as in the case of *Trichonympha*, the relative abundance of individuals showing nuclear organization leading to the appearance of distinct chromosomes, would suggest that it begins soon after the completion of a previous division period.

Prophase: The chromatin of the nucleus may be disposed in large granules (pl. 13, fig. 2) or in smaller granules with the network connecting them thickly encrusted with chromatin (fig. 4). In the change from one condition to the other there seems to be a reduction in the amount of chromatin. The nucleus, however, becomes enlarged hence the reduction may be more apparent than real. The chromatin of the granules continues to move out along the network until the threads of the latter have become greatly thickened and the granules have disappeared (pl. 13, fig. 5). This produces a coarse, heavy network in which many free ends may be detected (fig. 12). These are most frequently found at one side of the nucleus, with the opposite side staining more densely and presenting an unbroken outline. A further disintegration of the network is shown in figure 9 of plate 13. Here its component parts are forming threads which begin to take on the appearance of chromosomes on one side of the nucleus, while the other still retains its network formation.

The threads thus formed split longitudinally (pl. 13, fig. 6), while at the same time becoming thicker, with the chromatin arranged in distinct chromosomes. The outer alveolar zone of the nucleus disappears, though this may frequently occur at an earlier stage. The chromosomes, which at first are straight or irregularly twisted or bent

(pl. 13, figs. 7, 8, 11), gradually assume a V-shaped form, and the cloudy appearance surrounding them disappears, leaving them distinct against a clear background (fig. 16).

These different phases of nuclear organization are almost identical with those previously described for *Trichonympha campanula*. There is some suggestion in figure 16 of plate 13 that the chromosomes are arranged in pairs, as in *Trichonympha*. This point, with an exact count of the number of chromosomes, could not be as clearly made out here as in the other species, owing to the small number of individuals under observation. The number of chromosomes seems to be slightly less than that of *Trichonympha*, though this cannot be stated with certainty. In the prophase this seems to be forty-eight (pl. 13, fig. 15), with a reduction to twenty-four in the later stages (pl. 14, figs. 18–22). The process of pseudosynapsis by means of which this reduction takes place, cannot be figured from our material but is evidently similar to the same process in *Trichonympha*.

METAPHASE: This stage also has been lacking in our material. The appearance of the following anaphase (pl. 14, fig. 17) would suggest in part the probable mode of procedure. In the late prophase the nucleus had taken its place close against the paradesmose (pl. 13, figs. 15, 16), and elongated until its length is equal to that of the paradesmose. Spindle fibers are formed from the ends of the paradesmose or bases of the centroblepharoplasts, and to these the chromosomes become attached, with a single fiber from each pole attached to the end of the chromosome lying nearest it. The nuclear membrane remains intact throughout the entire process of mitosis.

ANAPHASE: A slight shortening of the spindle fibers assists in the separation of the chromosomes, but apparently this is more dependent upon a lengthening of the entire nucleus in the equatorial plane than upon any other factor. The spindle fibers show but little contraction up to the time of their disappearance in the late telophase, and the chromosomes are not drawn to the poles (pl. 14, fig. 23). The nucleus becomes greatly elongated and constricted in the middle until the two halves are connected by a slender line of nuclear material. The paradesmose also increases in length.

As the chromosomes separate the heterochromosome may usually be found near the ends of the two groups (pl. 14, fig. 19). As in *Trichonympha* it is the lagging chromosome and is apparently the last one to divide. In a later stage this assumes a coiled shape preparatory to the formation of the vesicle by which it is later enclosed (fig. 20).

Its course in the prophase has not been followed but it apparently retains its isolated position throughout.

TELOPHASE: As the two centroblepharoplasts with their related structures move towards opposite ends of the cell, the slender band connecting the two halves of the nucleus becomes ruptured, while the paradesmose fades out and begins to disappear at its middle portion (pl. 14, fig. 21). The daughter nuclei at this time are spindle-shaped both ends drawn out to a slender point, with the chromosome lying in a roughly subparallel band near the center. The point of the nucleus opposite the poles is withdrawn (fig. 20), the spindle fibers disappear, and the nucleus loses its connection with the centroblepharoplast (fig. 18) and begins to round up. This part of the process is usually completed before the chromosomes begin to undergo reorganization (pl. 14, figs. 22, 23, 25). Plasmotomy may occur before this takes place (fig. 25) or it may be delayed until reorganization of the nucleus and the reformation of the neuromotor system of each daughter cell has been completed (fig. 24).

RELATIONSHIPS

The close similarity of the various phases of division, combined with the striking resemblances in their morphological characters, indicate at once a close relationship between *Leidyopsis* and *Trichonympha*. These resemblances are found in the differentiations of the ectoplasm, the neuromotor system, and the interrelations of its various parts, the lack of a distinct cytostome, and the division of the endoplasm into a uniformly granular anterior portion and a posterior part filled with coarse alveoli and food particles, indicative of holozoic nutrition.

The differences between these two genera are few, but are important from a taxonomic standpoint. The most striking one is found in the number and arrangement of the flagella. In *Trichonympha* these cover two-thirds or more of the surface of the body and are divided into three distinct groups, an anterior, middle and posterior group. The anterior group is composed of long flagella, the middle or lateral group, which is the largest in extent, of short, cilia-like flagella, and the posterior group of flagella, which are twice or even three times the length of those in the anterior group. In *Leidyopsis* the middle and posterior groups of flagella are lacking, leaving only the group of long flagella at the anterior end of the body.

In view of the taxonomic importance attached to the number of flagella in other orders of the Flagellata, where these organelles serve as the chief features of classificatory value, these differences in their flagellar coating seem to create a generic distinction between the two forms and not a specific one only. We have therefore proposed a new genus in the order of Trichonymphidae for this form with the following characters:

Leidyopsis gen. nov.: Trichonymphidae with ectoplasmic differentiations found only on the anterior third of the body, the remainder of which is covered with a thin pellicle; neuromotor system consisting of one anterior group of long flagella, oblique fibers, longitudinal myonemes, basal granules and centroblepharoplast. One species only, the type, *Leidyopsis sphaerica* sp. nov. from intestine of *Termopsis angusticollis* Walker from Berkeley, California.

SUMMARY

1. *Leidyopsis sphaerica* is a flagellate of the *Trichonympha* type, with the same structural characteristics but with a lower type of evolutionary development.

2. It is characterized by the presence of a neuromotor system consisting of a highly developed centroblepharoplast, oblique fibers, basal granules and related flagella, restricted to a single anterior zone, a differentiated ectoplasm on the anterior third of the body, surface ridges from the crests of which spring the flagella, and an alveolar layer. Longitudinal myonemes are found in the endoplasm.

3. The nucleus is anterior in position and is distinguished by the presence of a heterochromosome contained within a small vesicle.

4. Nutrition is holozoic but its methods of feeding are unknown.

5. Division is of the trichonymphid type. The centroblepharoplast, with its connecting motor organelles and ectoplasmic structures, divides longitudinally with the formation of an extra-nuclear paradesmose between them as the two parts separate.

6. Division of the chromosomes is longitudinal, and takes place prior to the appearance of division in other structures of the body. About forty-eight chromosomes are formed. This number is reduced in pseudosynapsis to twenty-four.

7. The nuclear membrane remains intact throughout mitosis, with the spindle fibers arising from the ends of the paradesmose and centroblepharoplast and passing through it.

8. *Leidyopsis* is a member of the family Trichonymphidae Kent, in the order Hypermastigina Grassi. It stands close to the genus *Trichonympha*, to which it is related both morphologically and in its division processes.

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Zoological Laboratory, University of California,
Berkeley, California.

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EXPLANATION OF PLATES

All figures of *Leidyopsis sphaerica* gen. nov., sp. nov., from *Termopsis angusticollis* Walker, stained with iron haematoxylin and drawn with camera lucida. Magnification 800 unless otherwise stated.

PLATE 13

Fig. 1. Optical section of trophozoite showing thickness and extent of ectoplasm, centroblepharoplast and nucleus with the two portions of endoplasm. × 300.

Fig. 2. Vertical view of anterior end showing centroblepharoplast with central endoplasmic core, oblique fibers and nucleus slightly misplaced.

Fig. 3. Centroblepharoplast with a few of the radiating oblique fibers.

Fig. 4. Nucleus of the vegetative trophozoite; note the inner region filled with a coarse network with granules interspersed along its nodes, the surrounding alveolar zone and the outer granular region beneath the nuclear membrane.

Fig. 5. Nucleus showing the heterochromosome and its vesicle. Alveolar region has disappeared.

Fig. 6. Early prophase nucleus showing formation and splitting of the chromosomes.

Fig. 7. Early prophase with the alveolar region breaking up.

Fig. 8. Same stage as in figure 7.

Fig. 9. Another phase of the breaking up of the chromatin network.

Fig. 10. Anterior portion of the body, showing the cuplike depression with its covering opereulum, the tip of the centroblepharoplast, and the surface ridges.

Fig. 11. Nucleus showing the full number of chromosomes.

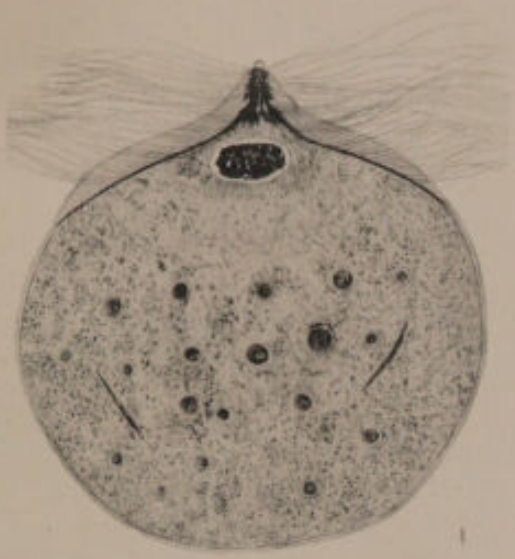
Fig. 12. Beginning of the splitting of the centroblepharoplast and ectoplasmic structures.

Fig. 13. A slightly later stage of same process as shown in figure 12.

Fig. 14. Separation of the centroblepharoplast with the completion of the paradesmose connecting them.

Fig. 15. Same stage as in figure 14.

Fig. 16. Prophase of nucleus and the first appearance of the spindle fibers. Dark areas at the end of fibers are parts of the centroblepharoplasts.



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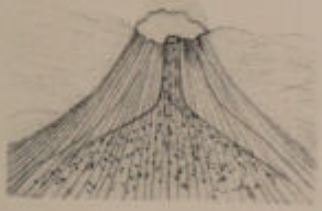

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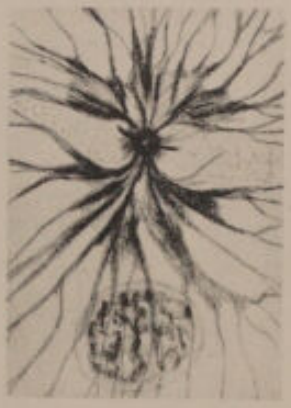

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PLATE 14

Fig. 17. Anaphase; note flagella and other ectoplasmic structures attached to the centroblepharoplasts. × 300.

Fig. 18. Telophase; paradesmose has been absorbed, the spindle fibers and connection of nucleus and centroblepharoplast are lost, but nuclear reorganization has not yet begun.

Fig. 19. Enlarged nucleus of figure 17. One lagging heterochromosome may be seen.

Fig. 20. Telophase of nucleus; spindle fibers still present as well as attachment to centroblepharoplast. Heterochromosome may be found near ends of the other chromosomes. × 1250.

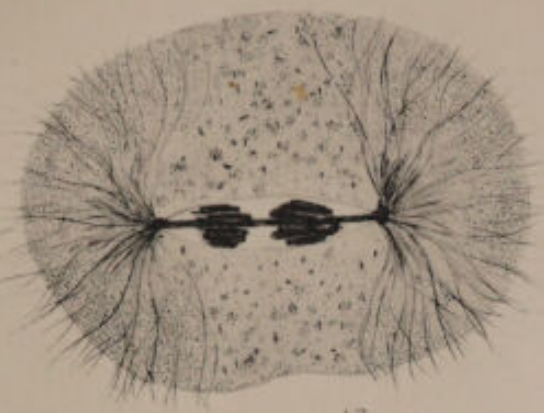
Fig. 21. Telophase; remains of paradesmose are seen above nuclei; flagella are omitted and the oblique fibers shown with their attachment to the centroblepharoplasts.

Fig. 22. Telophase of nucleus. It has rounded up and the chromosomes are losing their parallel position preparatory to forming a network.

Fig. 23. Telophase: beginning of constriction of the body. Note different relative positions of nuclei.

Fig. 24. Telophase; nuclear reorganization nearly complete as well as the formation of the new ectoplasmic structures. The alveolar zone, oblique fibers and flagella are shown.

Fig. 25. A daughter flagellate, after plastmotomy, which here has taken place before the reorganization of the nucleus.



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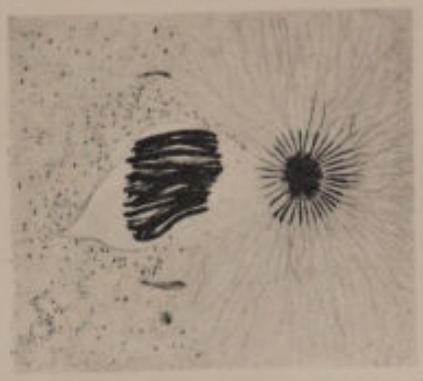

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