vard as far as the fourteenth magnitude. This scale intersects the Mount Wilson photovisual scale at the sixth and the twelfth magnitudes, but at other points there are important differences, some of which are obviously due to color. Further, the *Yerkes Actinometry* contains photovisual magnitudes as far as 7.5. The Mount Wilson results agree satisfactorily with these, and there is also good accordance with the visual magnitudes of Müller and Kempf. Beyond this no results have as yet been published, although others are in preparation. A detailed comparison with all these various investigations will be included in forthcoming papers in the *Astrophysical Journal*,[4] which will also give fuller details and a summary of the numerical results for the Mount Wilson scales. The complete discussion will appear as Volume 3 of *Papers of the Mount Wilson Solar Observatory*.[5]

[1] *Mt. Wilson Contr.* No. 80; *Astrophys. J.*, 39, 307 (1914).
[2] *Ibid; Mt. Wilson Contr.* No. 70; *Astrophys. J.*, 38, 241 (1913).
[3] *Potsdam Publ.* No. 67.
[4] *Mt. Wilson Contr.* Nos. 97, 98, 102; *Astrophys. J.* (In press.)
[5] *Publications of the Carnegie Institution of Washington.*

MITOSIS IN TRICHOMONAS

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The process of cell-division in the simpler Protozoa is significant in its relation to the evolution of nuclear and extra-nuclear structures. The behavior of the extra-nuclear organelles in unicellular organisms during the process of binary fission is significant as to the origin and relationships of such structures as flagella, blepharoplast, and axostyle. One of the distinctions between the Ciliata and the Flagellata has been held to be the direction of the plane of division, transverse in the former, longitudinal in the latter; therefore all reported cases of transverse division in Flagellata should be critically inspected.

Observations on mitosis in *Trichomonas augusta*, a flagellate parasitic in the digestive tract of amphibians (*Diemyctylus torosus Rana boylei*, and *Bufo halophilus* from California, and *Rana pipiens* from Chicago) enable us to make a fuller correlation of mitosis in the Flagellata with that in the Metazoa, to correct or supplement the observations of others regarding mitosis in trichomonad flagellates, and to establish on ample morphological grounds the essentially longitudinal nature of their division. Our conclusions have been verified in all essential features, in

eleven species of seven genera parasitic in vertebrates. The conclusions are based on hundreds of preparations made by the wet Schaudinn iron-haematoxylin, or by the Giemsa method, and controlled by observations on living forms in sealed culture slides, or on mixed pure cultures in sterilized media.

The vegetative phase of *Trichomonas augusta* (fig. 1) presents an elongated pyriform body with a hyaline axial rod, the axostyle (*ax.*), enlarged anteriorly and projecting posteriorly in a sharp point. A pale spheroidal nucleus (*n.*) lies in the anterior end. It contains one or more deeply staining karyosomes and a faint chromatin network.

The spheroidal deeply staining blepharoplast (*bl.*, figs. 1–3) lies close to the anterior surface attached to the anterior end of the axostyle. From it pass anteriorly the three long lightly staining anterior flagella (*ant. fl.*), and posteriorly the undulating membrane (*und. m.*) consisting of a waving protoplasmic film in whose margin lies a deeply staining chromatic thread or margin (*chr. m.*), and at whose base is a heavier basal chromatic rod (*bas. chr. r.*). Posteriorly the two chromatic structures unite at the point of emergence in the lightly staining posterior flagellum (*post. fl.*).

From the blepharoplast the axostyle (*ax.*), a large hyaline club-shaped flexible and mobile structure, passes posteriorly in an axial position. It is enlarged anteriorly near the nucleus and contains a varying number of fairly uniform axostylar chromidia (*ax. chr.*). In macerated specimens (fig. 9) the nucleus is seen to be attached to the head of the axostyle by a persistent and but slightly chromatic strand or rhizoplast (*rh.*). These extra-nuclear structures together with the nucleus survive as a coherent unit the maceration of the surrounding cytoplasm.

The prophase of the process of mitosis is initiated in rather large, somewhat spheroidal individuals (fig. 2), by the apparent splitting of the wavy chromatic margin of the undulating membrane distally from the blepharoplast. At the same time the homogeneous intra-nuclear chromidial cloud, which diffusely fills the nucleus as mitosis approaches, emerges from it and forms the extra-nuclear cloud (*ex. chr. cl.*), a diffusely staining halo about the nucleus, in which minute chromidia soon appear (fig. 2), spread into the surrounding cytoplasm, and increase in the axostyle throughout its length.

As the nucleus clears up there emerges in it a distinct (fig. 2), often clearly continuous chromatin skein, which speedily breaks up, finally into five chromatin masses, or chromosomes, each of which splits longitudinally into two chromosomes (fig. 3) within the intact nuclear membrane, prior to any arrangement in an equatorial plate. There are

differences in these chromosomes observable in most, if not all, of the mitotic figures (figs. 3–6). There are two of medium size, one large one, and two small ones, one of which lags (fig. 4) in the metaphase. After splitting the sister chromosomes swing into an end-to-end position (figs. 3, 4) as they move into the equatorial plate.

The blepharoplast has in the meantime (fig. 3) divided into two, to
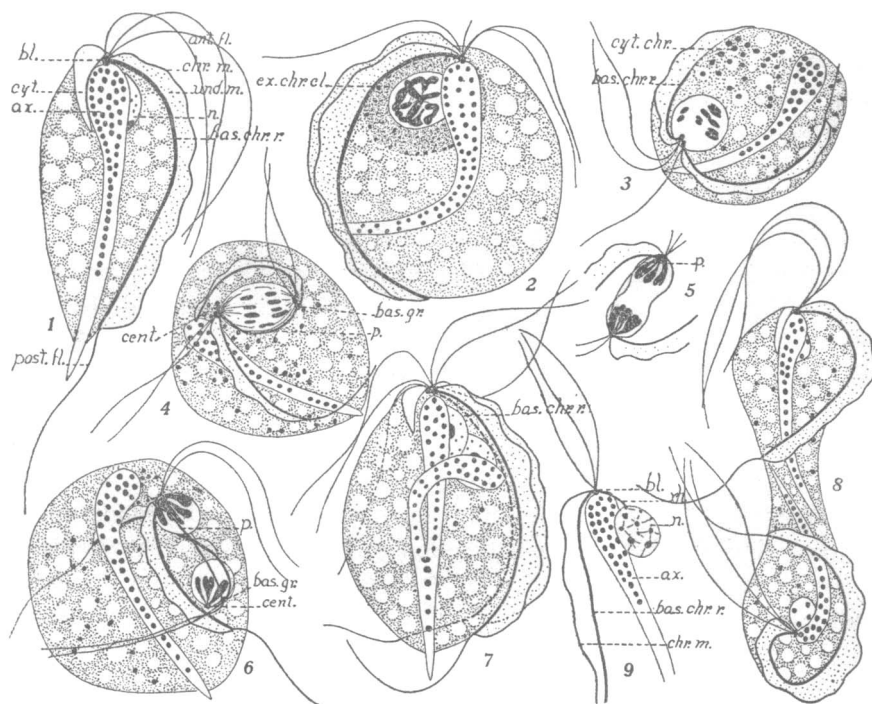
Mitosis in *Trichomonas augusta* Alexeieff. × 1500; *ant. fl.*, anterior flagella; *ax.*, axostyle; *ax. chr.*, axostylar chromidia; *bas. chr. r.*, basal chromatic rod; *bas. gr.*, basal granule; *bl.*, blepharoplast; *cent.*, centrosome; *chr. m.*, chromatic margin; *cyt.*, cytostome; *cyt. gr.*, cytoplasmic granule; *ex. chr. cl.*, extra-nuclear chromidial cloud; *n.*, nucleus; *p.*, paradesmose; *post. fl.*, posterior flagellum; *rh.*, rhizoplast; *und. m.*, undulating membrane.

Fig. 1. Active motile form prior to division.

Fig. 2. Prophase, with chromatin skein, split border and sprouting flagellum.

Fig. 3. Later prophase with five pairs of split chromosomes.

Fig. 4. Late metaphase with blepharoplasts at poles, each divided into centrosome and basal granule.

Fig. 5. Late anaphase with blepharoplast not divided.

Fig. 6. Early telophase. Nuclei still connected by paradesmose.

Fig. 7. Late telophase. Paradesmose seen end to end and therefore foreshortened, axostyle splitting.

Fig. 8. Mitosis completed, cytoplasmic division approaching.

Fig. 9. Nucleus and extra-nuclear motor apparatus after maceration.

one of which are attached two of the anterior flagella, while the other takes the remaining one and a new anterior flagellum which grows out from the blepharoplast. We have not found the blepharoplast to consist of four basal granules at the roots of the flagella as indicated by Martin and Robertson[3] for *Trichomonas gallinarum*.

The new chromatic basal rod grows out from the blepharoplast (figs. 3, 6), apparently independently of the old, as a new structure in the membrane below one of the chromatic marginal filaments (fig. 3) and distally extends in a new posterior flagellum, thus completing the division of the undulating membrane.

The metaphase is approximated in figure 4 in which, however, the nuclear membrane remains intact, as in fact it does throughout mitosis. The two blepharoplasts have migrated to the two poles of the pointed ellipsoidal nucleus, and each has divided into a centrosome (*cent.*), at the apex of the spindle, and the adjacent basal granule (*bas. gr.*) to which the flagella remain attached. In some instances (fig. 5) this division of the blepharoplast is not apparent. No astral rays are evident. Within the nuclear membrane faint spindle fibres connect the parting chromosomes to the centrosomes at the poles. Connecting the two blepharoplasts as they migrate to the polar position (figs. 3–6) is a heavy chromatic thread which lies *outside* of the nuclear membrane. This we name the paradesmose, though in origin it may seem to be homologous to the central spindle of the metazoan mitotic figure. It is this structure which according to Dobell[1] gives rise to the new axostyles of the daughter cells. It later disappears (fig. 7) without giving rise to any structures. As the split chromosomes swing in to the equatorial plate they assume the end-to-end position like that described by Montgomery[5] for the spermatocytes of *Euschistus*. Spindle fibres play no part in their splitting. Slight inequality in an "x"-"y" relation between the daughter chromosomes is generally evident in the case of the large chromosome. There is no evidence, however, that any of the divisions here described are maturation divisions, or that their end-to-end position is a telosynapsis.

The anaphase is accomplished by the movement of the chromosomes to the poles of the spindle into contact with the polar centrosomes and by the constriction of the nucleus into a dumbbell shape (figs. 5–6).

The telophase (fig. 6) results from the final constriction and separation of the daughter nuclei which immediately assume a spheroidal form and move apart drawing out the paradesmose between their basal granules. This is perfectly distinct as an extra-nuclear chromatic thread joining the two blepharoplasts or the two basal granules after each blepharoplast parts into centrosome and basal granule (figs. 4–6). The parades-

mose later fades out after the karyosomes and chromatin network reappear within the daughter nuclei (fig. 7).

At the close of the telophase (fig. 7) division of the axostyle proceeds by longitudinal splitting from the anterior end posteriorly. There is a suggestion that axostylar chromidia are also divided, at least in the distal region (fig. 7). Actual division of individual chromidia has not been seen though in some dividing axostyles (fig. 7) their relative dimensions in parent and daughter axostyles strongly suggest their division. The numbers of chromidia vary so that they do not afford critical evidence for use on this point.

The proofs of division of the axostyle in our material are ample though their detection requires well-prepared slides and persistent search. Dobell[1] and Prowazek[2] describe the origin of these axostyles from the "centrodesmose" and the former concludes that it is therefore the homologue of the central spindle, and Doflein in his recent *Lehrbucher der Protozoenkunde* accepts this origin. This strand throughout all our material is everywhere extra-nuclear and we therefore propose for it the name paradesmose since it cannot occupy the position of the central spindle or "centrodesmose." Kuczynski[4] has shown that Dobell's and Prowazek's conclusions are invalid since the paradesmose persists *till after the new axostyles appear*. This author, however, falls into the error of concluding that the daughter axostyles arise as new outgrowths from certain end granules of the old axostyle originally connected with the blepharoplast. He finds that the old axostyle fades out and the daughter axostyles arise as new outgrowths. In our material it has always been possible to detect in all individuals which have passed the anaphase of mitosis one of three conditions, either (1) the single undivided axostyle or (2) this axostyle in process of splitting longitudinally, or (3) two complete daughter axostyles. The presence of the axostylar chromidia has been of great assistance in following the longitudinal splitting of this organ in *Trichomonas augusta*. A similar splitting of the axostyle occurs in *Lamblia muris* and in two or more species of *Hexamitus*.

Wenyon[6] states that the axostyle of *Trichomonas intestinalis* of the mouse divides longitudinally after the division of the nucleus and blepharoplast. His figure, however, does not support his statement. It shows two axostyles rather than a dividing one.

Shortly after the axostyle splits the two nuclei and axostyles move farther apart, the body elongates, the anterior ends of the two daughters come to lie at opposite poles (fig. 7) and the cytoplasmic central bridge progressively narrows down until the two daughter organisms separate. The final plane of constriction is transverse to the main axis of each cell and of the common elongating mass.

An inspection of our figures and still more of our preparations reveals a great variety of positions of the organelles of the cell. Observations on living cells under high magnifications in culture slides convince us that this variability in position is normal and not due to artefacts.

These conditions have led Martin and Robertson[3] to state that division in *Trichomonas gallinarum* may occur in any one of three planes and that "these divisions may be termed respectively longitudinal, transverse, and oblique."

During the period of mitosis the body is somewhat rounded up, and is constantly in more or less violent motion. In these activities not only the flagella and undulating membranes share but also the axostyle. This bends at right angles (fig. 2), curves (fig. 6), and twists about very energetically almost as a flagellum lashing about in the viscid cytoplasm. Its activity is accompanied by a considerable mobility of the cytoplasm with the result that the positions of the organelles, of the nuclei, of the blepharoplasts with their accompanying flagella and undulating membrane, and even of the axostyle itself, are subject to incessant readjustments in their relations. They may even become somewhat widely detached from one another (figs. 4–7). These conditions which Martin and Robertson[3] have interpreted as indicating other types of division than longitudinal are thus due to the protean activity of the organisms. When, however, we seek to define the plane of division on morphological grounds and accept the premitotic cell (fig. 1) as indicating the normal orientation of the organelles within the body, we find that, if the daughter blepharoplasts migrate equally 90°, as they normally do in mitosis, the nucleus of *Trichomonas* would be divided in a plane which in the premitotic arrangement is approximately longitudinal. The chromatic margin of the undulating membrane, the membrane itself, and the axostyle all split lengthwise, that is, longitudinally. *On morphological grounds the division of this flagellate is therefore solely longitudinal.* The interpretation of any other plane rests purely on variable and temporary positions of organelles which become movable during the *later phases* of mitosis.

This process of division has been interpreted, with emphasis, in *Trichomonas gallinarum* by these same investigators[3] as purely amitotic. The differences between their figures and our own are not very great, and it is quite possible that under certain pathological conditions nuclear appearances simulating amitosis may occur. We are inclined to the view that division in this species also is mitotic and that Martin and Robertson's[3] figures are not wholly incompatible with this interpretation. A division in which chromatin masses (chromosomes) of definite number and regular forms are differentiated, split longitudinally, and

move to the poles of a spindle at each of which a centrosome formed by the division and polar migration of the parent organelle is found, is essentially mitotic rather than amitotic.

The conclusions drawn are:

(1) Cell division in trichomonad flagellates is a true mitosis with differentiated chromosomes (five in *Trichomonas augusta*), which split longitudinally prior to their location in the equatorial plate.

(2) The nuclear membrane persists throughout mitosis. The blepharoplast which is connected to the flagella, rhizoplast, chromatic margin and basal rod of the undulating membrane, and to the axostyle, after division usually gives rise by division to a polar centrosome and an adjacent basal granule which alone retains connection with the extra-nuclear apparatus including the paradesmose. These two fuse again to form the daughter blepharoplasts.

(3) The paradesmose between the migrating blepharoplasts is extra-nuclear at all times, disappears after nuclear division, and does not give rise to the axostyle. Since this connecting strand lies outside of the nuclear membrane and connects basal granules and not centrosomes, it should not be called centrodesmose or central spindle, but rather the paradesmose.

(4) The axostyle splits longitudinally and thus forms the two daughter axostyles. These do not grow out as new structures in *Trichomonas augusta*. It and the chromatic basal rod (homologous with the para-basal chromatic structures of Janicki[7]) may be regarded as derivatives of the blepharoplast, homologous with flagella but intra-cytoplasmic.

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