Mitosis and multiple fission in trichomonad flagellates / by Charles Atwood Kofoid and Olive Swezy.

Proceedings of the American Academy of Arts and Sciences.

Vol. 51. No. 6. — November, 1915.

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By Charles Atwood Kofoid and Olive Swezy.

From the Zoölogical Laboratory of the University of California.

With Eight Plates and Seven Figures in Text.

(Continued from page 3 of cover.)

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Zoological Laboratory *Berkeley, California, U. S. A.*

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Received, June 2, 1915.

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The observations and conclusions here recorded deal with the processes of binary and multiple fission and the accompanying phenomena of mitosis in trichomonad flagellates, protozoans parasitic in the digestive tract of vertebrates. The analysis is of general interest because of the light it throws on the evolution of the nuclear and extranuclear organs of the cell, especially on the kinetonucleus and

parabasal bodies of other parasitic flagellates, on the occurrence of chromosomes and of chromosome differentiation in some of the lower Protozoa, and on the simplest type of multinuclear aggregates which foreshadow the organization of the Metazoan type.

Material.

The material upon which these observations are based was obtained by the examination of more than 330 individual vertebrate hosts, including, as listed below, ten species of amphibians, three of reptiles, and two of mammals. Nearly every one of the hosts was parasitized by one or more of the species of trichomonads here discussed or by other flagellates. The host species examined were: *Amblystoma tigrinum* Green, *Diemyctylus torosus* Eschscholtz, *Aneides lugubris* (Hallowell), *Batrachoseps attenuatus* Eschscholtz, *Plethodon oregonensis* Girard, *Rana boylei* Baird (adult and larvae), *Rana pipiens* Kalm (from dealers in Chicago), *Rana draytoni* Baird and Girard, *Rana catesbiana* Shaw (from Honolulu, H. I.), *Hyla regilla* Baird and Girard, *Bufo halophilus* Baird and Girard, *Pituophis catenifer* Baird and Girard, *Python reticulatus* (Schneid.) (from Borneo), *Crotalus oregonus* Holbrook (from Guerneville and Patterson, California), *Peromyscus maniculatus gambeli* Baird, and albino mice (*Mus* sp.) from the culture stock of Professor J. A. Long. They were, unless otherwise stated, all obtained in Berkeley, or in the Coast Range of mountains within thirty miles of San Francisco from Saratoga on the south to Inverness on the north.

Technique.

As has been pointed out by Martin and Robertson (1911), the division forms are seldom found outside the mucous lining of the intestine, though the usual vegetative forms are numerous in the lumen and in the intestinal contents. Scarcity of division forms in most smears is probably due to the length of time between recurring division cycles, though a very few dividing forms may be found in nearly every host examined. Some smears have always been made from the intestinal contents, but, for the most part, they have been made from the wall itself, a small fragment of the wall being smeared over the cover-glass, which was then placed in the fixative. In every case moist film preparations were made, dried smears having

been found to be of little value for accurate morphological or cytological study.

The region of greatest infection is the junction of the large and small intestine and the upper part of the rectum. Other organs of the body have been examined including the lungs, spleen, liver, genitalia, etc., but in no instance have flagellates been found outside the digestive tract. Occasionally a few have been found in the stomach and more often in the small intestine, but their normal presence here has been uncertain in any case because of possible contamination through dissection, and scanty in every case. In the lower part of the rectum the parasites were usually not abundant.

The division cycle in these flagellates occurs at infrequent intervals and is often not observed until the stained preparations are examined, or at least until it is too late to make more smears from the same material. It is therefore important when investigating the life histories of these flagellates, to make as many preparations as the material will allow, or as can be conveniently handled, even though many of them are afterwards discarded.

Various fixing agents have been tried, Flemming's fluid and picro-mercuric being excellent, but hot Schaudinn's fluid, with a few drops of acetic acid added, has given the best results. The material to be examined was smeared over the cover glass and this was placed, film downward, on the surface of the fixing fluid, and left in that position for about five minutes when it was inverted and placed in 50% alcohol with successive changes to 100% alcohol. If the intestinal wall was not covered with sufficient fluid it was moistened slightly with normal salt solution before smearing it on the cover glass, and if the amount of liquid on the cover glass was too great it was allowed to evaporate till nearly but not quite dry before placing the smear in the fixing fluid. The material was found to adhere to the cover glass more firmly when it was not plunged beneath the surface of the fixing fluid for the first few minutes at least, but remained floating on the surface.

By far the best results in staining were obtained with iron haematoxylin. Heidenhain's method was used at first but later alcoholic solutions were substituted for the aqueous solutions, and these gave very satisfactory results and saved time. The stock solution of ½% iron haematoxylin was diluted with ten parts of 70% alcohol. For the mordant the stock solution of 4% iron alum was diluted with ten parts of 50% alcohol. The iron alum will not remain long in solution in alcohol so the solution must be renewed frequently. The preparations were left in this for 10 minutes, rinsed in 50% alcohol and placed

in the stain for the same length of time or longer. For very good chromatin staining one hour gives better results. After staining, decolorize in iron alum and wash in 50% alcohol for two hours, or in water.

Intra vitam stains such as neutral red, methylene blue N, and new methylene blue G G were used. Neutral red prepared with normal salt solution gave best results.

For examining the living flagellates the cover-glass was sealed with vaseline, after diluting the intestinal smear with normal salt solution. All of the Protozoa common in the intestine have been kept alive in this manner for about 24 hours, and in the case of *Trichomonas augusta*, which is apparently the most resistant form, individuals have been kept alive for several months without any change in the medium, or removal of the cover glass. Other species of *Trichomonas* have been kept alive for days or even a few weeks in the same manner. Hanging drop preparations were tried but were not successful, the protozoans dying within a very short time. Cultures were made by placing a bit of the intestinal contents in a hollow ground culture slide, filling the cavity with normal salt solution and sealing down the cover glass with vaseline. Trichomonads have been kept alive in these cells for six months with no apparent degeneration in the organisms. Cultures were also made by placing some of the intestinal contents in small Stender dishes with a quantity of normal salt solution, and also with sterile earth and boiled water from infusions. Most of the parasitic protozoans could be cultivated in this way for a few weeks and would then disappear. Ringer's solution was tried as a culture medium but results were not so successful as with normal salt solution. Cover glasses may be floated on the surface of these cultures and removed at any time for examination, and when the adherent flagellates are present they may be fixed and stained in the usual way.

For searching preparations and recording the location of division stages a mechanical stage with verniers has been indispensable and for the analysis of the finer structure a 100 watt Mazda lamp has been used as the source of illumination, and Zeiss apochromatic 2 mm. objective with Nos. 12 and 18 compensating oculars and Watson's new No. 20 holoscopic eyepiece to secure the desired definition and magnification.

We will now proceed to the discussion of mitosis and multiple fission in four of the representative trichomonad flagellates, *Trichomonas augusta* Alexeieff, *T. muris* Hartmann, *Eutrichomastix serpentis* (Dobell), and *Tetratrichomonas prowazeki* (Alexeieff) treating

the subject more fully in the first named form and reviewing it briefly in the others.

Trichomonas augusta Alexeieff.

This flagellate occurs abundantly in the intestine of *Diemyctylus torosus*, *Rana boylei*, *R. draytoni*, and *R. pipiens*, *Hyla regilla*, and *Bufo halophilus*. The following table indicates the proportions of the hosts which were infected:

Species	Number examined.	Number infected.
Diemyctylus torosus	25	23
Rana boylei	13	10
R. draytoni	24	22
R. pipiens	20	20
Hyla regilla	18	13
Total	100	88

The Vegetative or Premitotic Stage.

This phase is found free in the intestinal lumen or actively moving about in the mucus covering the intestinal epithelium. It is the type found most frequently in our culture slides and in sterilized inoculated media where it may be accumulated on the under surface of floating cover glasses.

The form of the body in this phase is distinctly pyriform, (Pl. 1, Fig. 4) with the larger end anterior, and its total length to tip of the axostyle 2–2.5 times it greatest diameter which is located at 0.3 of the total length from the anterior end. It is usually nearly symmetrical as it rotates in locomotion, but in some preparations (Pl. 1, Fig. 1) the ventral side is flattened and the dorsal, bearing the undulating membrane, is convex. The axostyle lies in the major axis of the body and extends from near its anterior end to, and generally beyond, the posterior limit of the cytoplasm, projecting for a distance equal to half or two-thirds of the diameter of the body.

The form of the body is frequently subject to the elongation and accumulation of the cytoplasm posteriorly, even beyond (Pl. 1, Fig. 2) the axostyle, in the form of a subspheroidal blob which may detach itself from the parent mass and slip off from the tip of the axostyle. These forms in various stages of elongation and constriction are often

abundant in fresh smears and culture slides. There is no evidence that this process of dropping off cytoplasm or plasmecdysis is pathological. It seems to bear no relation to mitosis and the detached portions which may also be detected after detachment in stained preparations, contain no nuclear structures and no peculiar chromidia or vacuoles. It may be one method of ridding the body of accumulated waste products but there is no structural evidence of this. The amount of cytoplasm thus dropped off may be nearly one half of the total mass (Pl. 1, Figs. 2, 5).

Rounded, even spheroidal forms also occur, but these are, as a rule, antecedent to, or attendant upon mitosis. The dimensions (Fig. A) vary greatly. The majority of individuals are 18–28 μ in length and 8–15 μ in width. The smallest measured was only 11 μ long, but in cultures, giant individuals 50 μ in length have been seen. If the flagella are included the length attains 70–150 μ.

Since there is a series of intergradations between the largest and the smallest forms (Fig. A) it is evident that they all belong within the range of variation, or of growth. There is not the least evidence that the stout (Fig. A, 6) and slender individuals (Fig. A, 7) are male and female respectively, an interpretation often given in the life history of flagellates to such extremes in shape, or as yet in our hands conclusive evidence that the rounded forms have any sexual significance. A rounded phase, however, antedates mitosis, and the smallest forms are the result of multiple fission and may also be produced by rapidly repeated binary fissions. In some cases the effect of this diminution of size by division or by plasmecdysis is seen (Fig. A, 7) in the retention of relatively large organelles such as cytostome, membrane, and axostyle with a relatively small mass of cytoplasm (Pl. 1, Fig. 3).

There is no evidence that either large or small forms are found exclusively or even predominantly in particular host species. Almost the whole range in form shown in Figure A could be selected from the teeming myriads of individuals in a single heavily infected host. The differences in size and proportions seem not to be of a specific or subspecific nature.

The organelles of *Trichomonas augusta* (Fig. B) are those of the typical trichomonad, to wit, nucleus (*n.*), cytostome (*cyt.*), vacuoles (*vac.*), and extranuclear motor apparatus consisting of blepharoplast (*bl.*), three anterior (*ant. fl.*) flagella and an attached posteriorly directed one, the last forming a part of the undulating membrane (*und. m.*) and projecting as a free flagellum (*post. fl.*) beyond the membrane, the chromatic basal rod (*chr. bas. r.*) or parabasal body, and

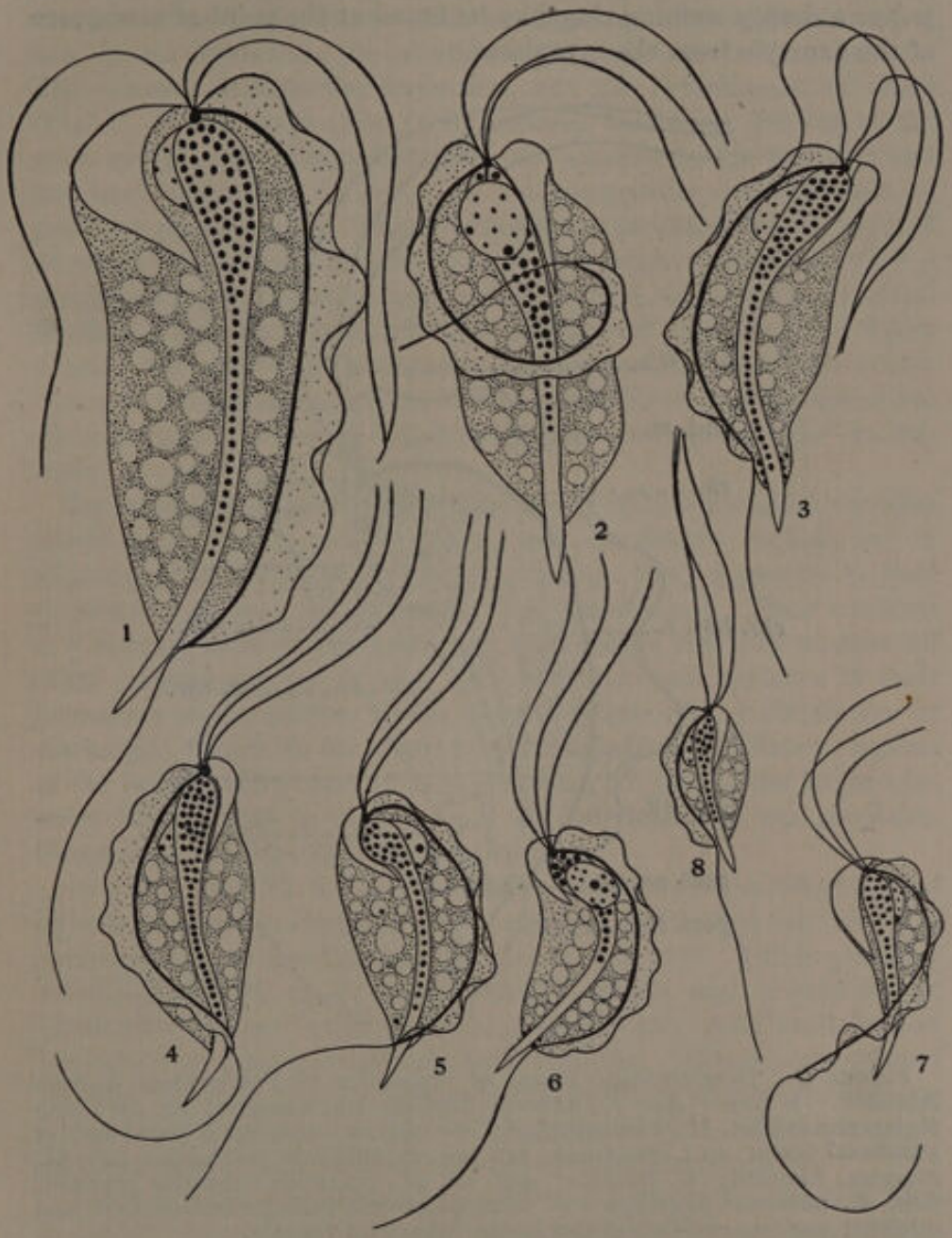

FIGURE A. Sketches from preparations showing range in size and form in typical free trophozoites of *Trichomonas augusta* Alexeieff. × 1500. In Figure A, 2 the undulating membrane is detached posteriorly from the cytoplasm. All specimens from *Diemyctylus torosus* and all but Figure 1 from the same slide and same individual host.

the axostyle (*ax.*). A pair of posterior axostylar granules (*post. ax. gr.*) or a deeply staining ring may be found at the point of emergence of the axostyle from the cytoplasm.

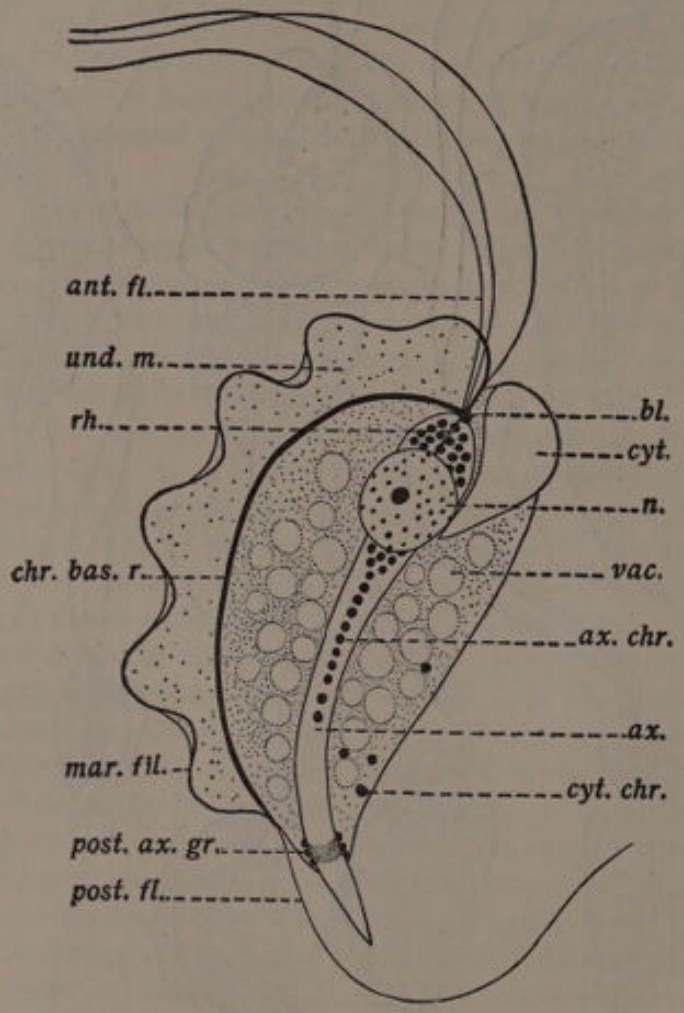

FIGURE B. Diagrammatic figure of organelles of *Trichomonas augusta* Alexeieff. × 1500. *Ant. fl.*, anterior flagella; *ax.*, axostyle; *ax. chr.*, axostylar chromidia; *bl.*, blepharoplast; *chr. bas. r.*, chromatic basal rod or parabasal body; *cyt.*, cytostome; *cyt. chr.*, cytoplasmic chromidia; *mar. fil.*, marginal filament; *n.*, nucleus; *post. ax. gr.*, posterior axostylar granules; *post. fl.*, posterior flagellum; *rh.*, rhizoplast connecting blepharoplast and nucleus; *und. m.*, undulating membrane; *vac.*, food vacuole.

The cytoplasm of this organism is peculiarly mobile and protean in its activity as Kuczynski (1914) has so well shown. This is best seen when the animal is creeping about on a substrate. In Figure C

are shown the protean changes undergone by a single active vegetative individual in the course of about fifteen minutes while entrapped in a viscous débris from the intestine but not adherent to the substrate. The changes include the formation, but not detachment, of small (Fig. C, 2) and large (Fig. C, 8) posterior blobs, the shifting of the main cytoplasmic enlargement from the anterior to the posterior end and back again (Fig. C, 5–9), and the protraction and retraction of posterior pseudopodia. These facts seem to preclude the existence of anything like a rigid pellicle, though Kuczynski (1914) describes a peripheral layer staining a light pink in Giemsa, and speaks of skeletal fibrillae which are "ganz schwach angedeutet" but does not figure them. We have found no *structural* evidence of such a pellicle except the more homogeneous texture of the surface layer, and the line about the cytostome, and none whatever of the "skeletal fibrillae" in this layer.

Scattered throughout the cytoplasm are many spheroidal vacuoles which move about, not by regular and continuous cyclosis but in adjustment to the protean changes in form. They appear to be food vacuoles or at least to be concerned in metobolism. Their contents is a homogeneous highly refractive fluid and in life they obscure all other structures more or less. We have not seen evidence of their formation at the bottom of the cytostome nor of any circulation or discharge. There is no constant differentiation in different regions of the body. They vary in diameter from 0.5 to 4 μ, the larger vacuoles being found in the rounded up individuals in the prophase. Compound vacuoles are occasionally seen.

In a few cases (Pl. **3**, Fig. 33) rod-like structures resembling bacteria have been found in elongated food vacuoles, but beyond this there has been no evidence that this species takes in solid food. Other species of *Trichomonas* such as *T. batrachorum*, *T. muris*, and several as yet undescribed species in our material, generally have solid particles and bacteria in at least some of their food vacuoles. This absence of food particles is also in sharp contrast to the condition in *Tetratrichomonas prowazeki* (Alexeieff) which actively engulfs bacteria and other organic material, and crowds its food vacuoles with such substances.

The vacuoles of individuals treated with a trace of methylene blue N are not stained at all but small spheroidal granules in the cytoplasm between the vacuoles stain (Fig. D, 2) diffusely and the animals die within an hour. In dilute Janus green the nucleus, blepharoplast, and chromatin granules in axostyle and cytoplasm stain quickly and the animal dies in a few moments.

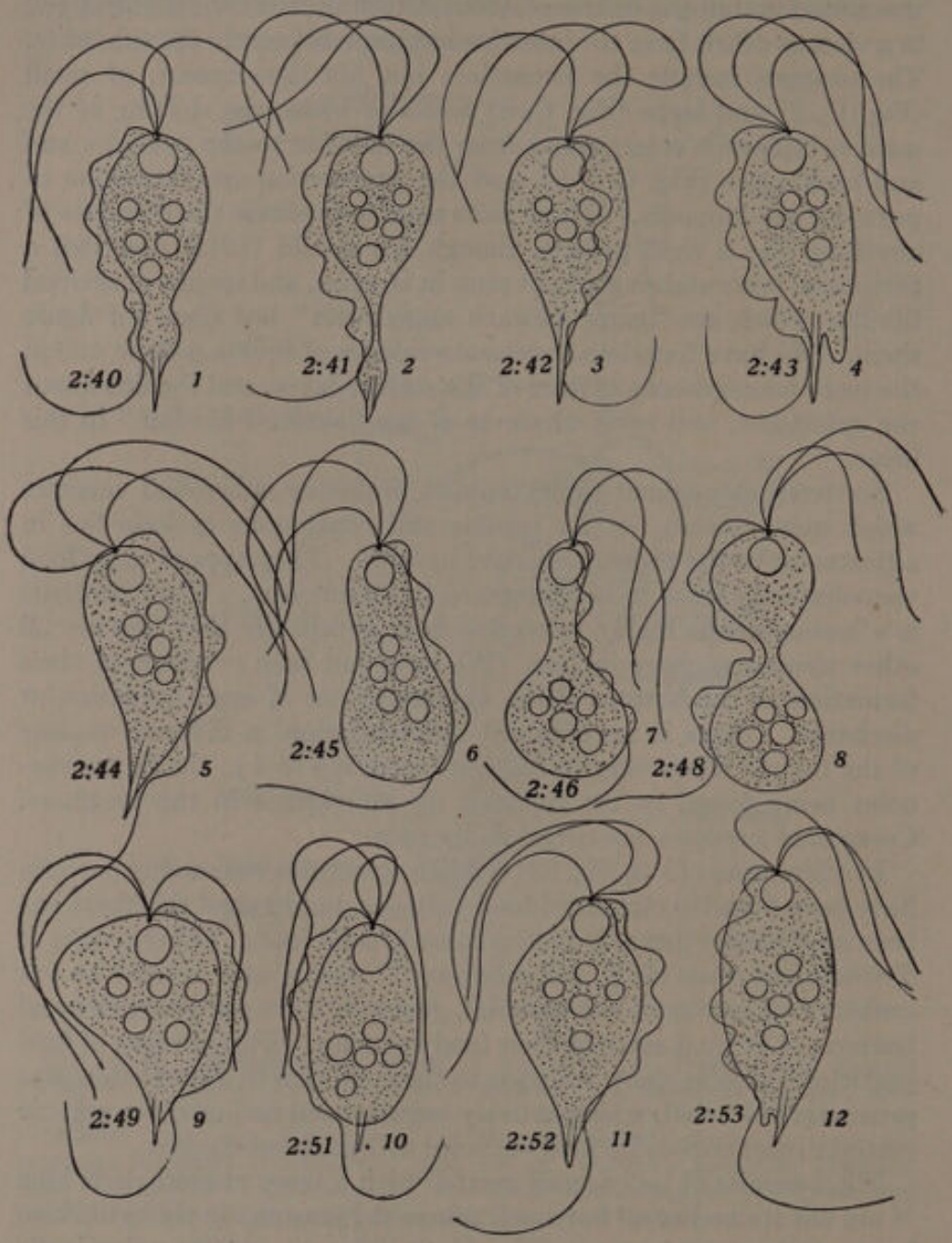

FIGURE C. Protean activity of *Trichomonas augusta* Alexeieff from *Diemyctylus torosus* between 2: 40 and 2: 45 P.M. Sketched with camera lucida and subject to some error owing to continuous movements. × 1500.

On treatment with neutral red the contents stain very differently in different individuals and sometimes differentially from different individual hosts. In some cases (Fig. D, 1) the contents of all of the vacuoles stain red at once. In others, especially in those in which the

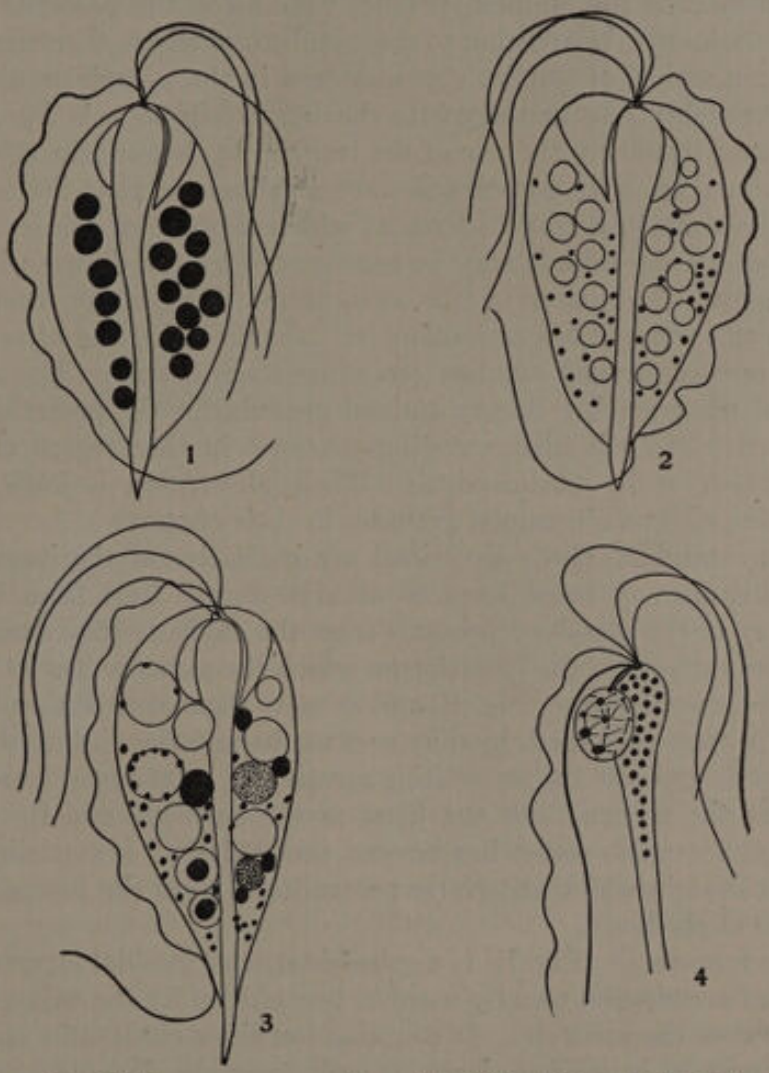

FIGURE D. *Trichomonas augusta* Alexeieff after treatment. ×1500. Stained granules shown in black.

1. After prolonged treatment with neutral red giving diffuse staining of vacuoles, probably moribund.
2. After treatment with methylene blue N showing granules in cytoplasm.
3. After treatment with neutral red. Vacuoles showing progressive changes in contents and size indicating progress in digestion.
4. Nucleus and extranuclear motor organelles persisting after maceration of cytoplasm in intestinal mucus, showing rhizoplast connecting blepharoplast and nucleus.

vacuoles are of a different size (Fig. D, 3) the staining is differentiated, the largest vacuoles are unstained, the smaller ones have a diffuse red stain or a central darker red granule, and there is a tendency for the stained granules to be most numerous posteriorly. In one case of blob formation the stained vacuoles were all in the posterior lobe of the cytoplasm. In addition to these stained particles there are others, more numerous, of smaller size scattered in the cytoplasm about the large vacuoles. By analogy with the digestive process in *Paramecium* as demonstrated by the use of neutral red by Nirenstein (1905), the indications are that the vacuoles are alkaline and that the small red granules contain a tryptic ferment which in some of the less active, rounded-up individuals may be represented by the diffuse pink stain throughout the center of the cytoplasm. The larger vacuoles in which this ferment has now dissolved take no stain, and after absorption their condensed alkaline remnants stain deeply. The aggregation of these smaller deeply stained granules in the posterior end is suggestive of their ultimate disappearance in that region either by absorption or by plasmecdysis. Their absorption is indicated by vacuoles with small stained granules in their centers.

The conditions above described are indicative of the ingestion by this organism of some form of alkaline liquid food from its host, possibly of the dissolved proteids from the chyle as they come to the mucous surfaces of the intestine on which the parasite has its habitat.

The cytostome (*cyt.*, Fig. B, and in most Figures on Plates **1–4**) is a broadly comma-shaped, hyaline area at the anterior end on the "ventral" side opposite the undulating membrane. Its concave side abuts against the nucleus and the head of the axostyle and its tapering inner end extends somewhat beyond the nucleus. It is outlined by a darker margin which anteriorly protrudes beyond the contour (Pl. **1**, Fig. 4) of the body.

The nucleus (*n.*, Fig. B) is a spheroidal or ellipsoidal structure with distinct membrane, usually more or less hidden by the enlarged anterior end of the axostyle. It contains but little chromatin and this is often massed in a single large, usually centrally located karyosome, and in several or many minute granules scattered throughout the nuclear fluid in a chromatin net (Pl. **1**, Fig. 3).

The extranuclear motor apparatus consists of a structurally continuous unit, all parts of which are more or less deeply stained with iron haematoxylin except the axostyle and the extra-cytoplasmic flagella. The parts included in this unit, the flagella, axostyle, chromatic margin and chromatic basal rod of the undulating membrane

remain intact after all of the cytoplasm macerates and disappears, as it sometimes does. Smears sometimes reveal such remnants consisting of the entire extra-nuclear motor apparatus (Fig. D, 4) still intact except for the tip of the axostyle, and attached to the nucleus by a slender fibrous rhizoplast (Fig. B, *rh.*) which passes posteriorly from the blepharoplast along the side of the axostyle. We have not found this rhizoplast in unmacerated individuals, where it does not stain deeply, but may be hidden in the dense mass about the nucleus in the stained preparations.

The center, however, both structurally and developmentally, of the extranuclear motor apparatus is the blepharoplast which is a spheroidal deeply staining granule at the anterior end of the axostyle. From it the three anterior flagella pass anteriorly out of the cytoplasm. Into the outer rim of the undulating membrane goes the chromatic margin and into its base the chromatic basal rod, both curving posteriorly on the dorsal side of the body and at their junction at the end of the undulating membrane the posterior flagellum emerges as the continuation of the chromatic margin.

The anterior flagella (*ant. fl.*, Fig. B), three in number, are of equal length and are as long or longer than the body. These flagella are habitually directed anteriorly and strike together in a lashing backward stroke which gives to the body a jerky or intermittent method of locomotion when on the substrate. When at rest and often in fixed material their outer two-thirds are reflexed.

The fourth flagellum forms the outer or chromatic margin of the undulating membrane. It runs posteriorly on the dorsal side curving spirally on to the left side in a steeper or flatter spiral (Pl. 1, Fig. 5) according to the elongation or shortening of the body. It is longer than the base of the membrane in whose margin it lies and is thrown into a series of 6–9 subequal undulations which pass without reversal ceaselessly in the posterior direction.

At the end of the membrane, which is a short distance above the point of emergence of the axostyle, the imprisoned flagellum emerges from the cytoplasm as the posterior free flagellum (*post. fl.*, Fig. B) whose length may equal or exceed that of the body. Within the undulating membrane this flagellum is stained an intense black, but outside of it the tone is the same as that of the anterior flagella. This may be due to greater bleaching of the stain in the more exposed part of the flagellum or to a chemical difference. There is no detectable difference in the diameters of the two parts.

At the point of emergence it is attached to the posterior tip of the

larger chromatic basal rod and in appearance the projecting flagellum is a continuation of both. That the posterior flagellum is, however, the projecting part of the chromatic margin only, is proved by the fact that when the latter is torn loose it carries the projecting flagellum with it as its distal part.

The undulating membrane (*und. m.*, Fig B) is a thin hyaline protoplasmic film, less granular than the general mass of cytoplasm, and apparently composed only of its outermost layer or pellicle. It, however, differs from the pellicular region elsewhere in that it does not take on amoeboid activity; always retaining, except as the animal is disintegrating, its differentiated and characteristic structure and undulating movements even throughout the protean conditions of the pre- and postmitotic phases and during the period of its own longitudinal division. In its outer margin lies the posterior flagellum differentiated as a chromatic margin and at its base the chromatic basal rod. In evolution, origin, and function this membrane is the result of the inclusion of an outgrowing posteriorly directed flagellum, whose activities raise the surface layer or pellicle above the level of the cytoplasm and result in its differentiation.

The blepharoplast also gives rise to another deeply staining outgrowth, the chromatic basal rod (*chr. bas. r.*, Fig. B) or parabasal body, lying at the base of the undulating membrane. Although this follows the spiral course of the membrane (Pl. 1, Figs. 3, 5) it is never thrown into undulations and appears to have no motor activity, though subject to modifications in curvature and in location during the postmitotic period, shifting position with the whole extranuclear motor complex. With the undulating membrane it lies opposite to the cytostome, that is, in the dorsal side of the body and gives to that side, especially in rounded-up phases and in fixed material, a greater convexity than the ventral. In this curvature the axostyle generally shares. This chromatic basal rod is of uniform caliber throughout and takes nuclear stains generally. It does not, even under extreme decolorization, break up into chromatic blocks though in its origin by outgrowth its distal end may at first form by the fusion of fine chromatic granules.

The chromatic basal rod or parabasal body is found apparently throughout the genus *Trichomonas*. It is figured in *T. lacertae* by Prowazek (1904), in *T. muris* by Wenyon (1907), in *T. batrachorum* by Dobell (1909) as a thread and row of adherent chromatin granules, in *T. eberthi* by Martin and Robertson (1911), in *T. augusta* by Alexeieff (1913), in *T. caviae*, *T. muris*, and *T. augusta* by Kuczynski

(1914), in *T. sanguisugae* by Alexeieff (1914a), and in *T. granulosa* (probably only a moribund *T. augusta*) by the same author (1914b) as a row of chromatic granules.

We believe that this slender elongated chromatic organ heretofore called the chromatic basal rod, chromatic basis, chromatic line, or *côté*, this chromatic basal rod of *Trichomonas* is really homologous with the stout deeply staining parabasals which Janicki (1911) describes in certain other flagellates such for example as those of *Devescovina* and *Parajoenia* in both of which they take their origin from the blepharoplast on the side of the axostyle next to the posteriorly directed flagellum, though they do not run posteriorly parallel to it in either case.

In the isolated and casual figure of *Trichomonas batrachorum* of Janicki (1911) an additional stout club-shaped structure attached to the blepharoplast is figured which is interpreted by him as the parabasal body. It is also figured in *Tetratrichomonas prowazeki* by Alexeieff (1910) and a comparable structure is also found in a species with slender axostyle described by Martin and Robertson (1911) as *Trichomonas gallinarum* which we here refer to the genus *Tetratrichomonas*, where, because of its four flagella, it properly belongs.

In more recent articles Alexeieff (1913, 1914a) again describes and figures it as an inconstant "chapelet de grains sidérophiles" or "côté," and calls it the parabasal body alongside the anterior end of the chromatic basal rod.

Kuczynski (1914) finds in *Trichomonas caviae* a stout deeply staining rod or a line of heavy granules adjacent to the chromatic basal line in some individuals, usually in all in a given host, but not in those from another host. He does not find it in *T. muris*, and only occasionally in *T. augusta*. He regards this as the true and only parabasal body, but not as the new chromatic basal line, or new parabasal, since he does not recognize its transformation into that structure. He is inclined accordingly to regard the parabasal body in trichomonads as an ephemeral organelle.

The homology of this structure is thus rendered somewhat perplexing by these interpretations of Janicki, Alexeieff, and Kuczynski, who distinguish not only the darkly stained chromatic basal line below the membrane but in addition a *club-shaped deeply stained* structure which they call the *parabasal body*, adjacent to and also attached to the blepharoplast. Dobell (1909) who worked over *T. batrachorum* with care does not mention or figure the parabasal of these authors. One of Alexeieff's figures (1913, Fig. VIIc) shows *Trichomonas augusta*

with apparently but a single undulating membrane, but with two nuclei, two blepharoplasts with flagella, and two chromatic basal rods, a paradesmose between the blepharoplasts, and apparently one axostyle. From *each* blepharoplast, there arises a stout "organe parabasale de Janicki" parallel to the chromatic basal rod. We interpret this as an early stage in multiple mitosis in the initial phase of a second mitosis and the two "organes parabasales" as new outgrowing parabasals indicative of a coming second mitosis. That is, they are parabasals, but so are also the chromatic basal rods of which they themselves are only the initial stages. It is apparently to this young parabasal that Alexeieff referred in an earlier paper (1909a) but regarding it (baguette recourbée) at that time as a characteristic of the *Trichomonas* of salamanders as contrasted with those of frogs, and as a part of the undulating membrane. He makes, however, no further reference to this interpretation in later papers.

In a series of preparations of this species we have traced nearly all stages of mitosis, and in hundreds of individuals find no trace of parabasals as stout as those figured by Alexeieff, but only somewhat more slender ones at this stage of outgrowth. We conclude therefore that normally there is regularly no structure so large as Janicki and Alexeieff figure as their parabasal and that this structure, large or small, is the outgrowing new chromatic basal rod (Pl. 2, Figs. 11–16) which they interpret with some adjacent chromatic granules as the sole and only parabasal. If this be true, Janicki's (1911), Alexeieff's (1913), and Kuczynski's (1914) interpretation is then only partially correct. The organ which they call the parabasal is only the first step in the origin of the new parabasal in the prophase of mitosis. The true parabasal includes both this and the organ which is regarded by them as distinct, namely the chromatic basal rod of the undulating membrane, and the inconstancy and variation noted by them is explained by the fact that they were dealing with a growing organelle.[1]

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1 In a paper received after the completion of this manuscript Janicki (1915) repeats his earlier figure of *Trichomonas augusta* showing the stout "parabasal," and adds an incomplete new one (his text figure 13) showing what we interpret to be the paradesmose, also the slender parent parabasal, and a stout daughter one. It is evident from figures and context that he has not worked over an extensive series of preparations of this genus. He regards *T. augusta* as possibly the same as *T. batrachorum* to which species he states his material to belong. We find both species, and regard them as distinct, the latter having food vacuoles with contents, a more slender axostyle, and a very general absence of both axostylar and cytoplasmic chromidia. In this species whose mitosis we have also followed, the chromatic basal rod or parabasal in our sense, is even more slender than it is in *T. augusta*. We have not, as yet, seen the

Bensen's (1909, Pl. 9) figures of *T. vaginalis* indicate an axostyle formed by a continuation of the rhizoplast through the nucleus and karyosome to the posterior end of the body. This interpretation seems improbable in the light of our own results and of the later figures of this species given by Brumpt (1913). It is probable that the structure here interpreted as rhizoplast-axostyle is in reality the chromatic basal rod lying beneath the nucleus, and that Bensen has found neither rhizoplast nor axostyle. In so far as structure and position go this latter interpretation is equally open and on comparative grounds practically certain.

It is also possible that the structure interpreted by Brumpt (1913) as the axostyle in *T. vaginalis* and *T. intestinalis* of man is in reality the chromatic basal rod or parabasal. Its stainability, position, and structure indicate this interpretation. The axostyle in *T. vaginalis* as figured by Brumpt lies on the opposite side of the nucleus from the undulating membrane, and bends about it. It is a *slender deeply staining thread*, with one line of chromidia on either side of it in the distal part of its course and is itself at times resolved in part into detached chromidia in a single or a double line. In *T. intestinalis* the same author also figures a *chromatic* axostyle which lies *between* the nucleus and the undulating membrane. In position and structure it is very much like the chromatic basal rod of *T. augusta*, a structure which would seem to be absent here if we should accept Brumpt's interpretation and call this the axostyle, unless one of the "filaments de soutien" which he mentions but does not figure, represents this basal rod. However, comparison of existing figures of these species with those of Brumpt certainly reveals the probability that neither one of these chromatic structures is the axostyle and that they are both really the chromatic basal rods. The position of the true hyaline axostyle of *T. vaginalis* may be represented by the projecting point in his figure 119, 2, and of *T. intestinalis* in a similar point in his figure 120, 1 and 3. The displacement of the undulating membrane

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initial stages of the formation of the new parabasal in this species, nor have we found the stout condition which Janicki figures as his parabasal. We find no sufficient evidence in his later paper to modify our earlier conclusion that the chromatic basal rod of *Trichomonas* is the homologue of the parabasal of the Trichonymphida as described by Janicki (1911) and that the organ which he interprets in *Trichomonas* as the parabasal is only an unusual or abnormal condition of the outgrowing new parabasal at mitosis. This interpretation of ours is supported not only by our own observations but also by the comparative absence of the parabasal of *Trichomonas* of Janicki in nearly all of the literature of this group.

far from the chromatic basal rod in his figure 119, 1, 2, and 4, is quite possible under the conditions of preparation of smears. Brumpt's interpretation of his excellent figures, as well as our own, requires further investigation before either can be finally accepted for these species.

In all species in which this chromatic basal rod is present it lies along the base of the undulating membrane. In certain imperfectly known species such as *Trichomonas hominis* Donné, *T. vaginalis* (Davaine), *T. limacis* Dujardin, *T. suis* Grube and Delafond, *T. tritonis* Alexeieff, and *T. parva* Alexeieff, it has not been adequately described, if at all. From its uniform presence in all species of trichomonads having an undulating membrane which have been adequately investigated, it seems probable that it will be found to be co-extensive with that structure in this group of flagellates. Further light on its homologies is suggested by the fact that it lies in the same undulating membrane with the intracytoplasmic part of the posterior flagellum, originates from the same blepharoplast with it, runs in the same direction, and differs from it only in its deeper location, larger caliber, and in the fact that it does not project beyond the cytoplasm. In function also it differs in the absences of undulating waves of contraction. There is thus much to favor the view that this chromatic basal rod might also be regarded as an intracytoplasmic flagellum parallel to the marginal flagellum.

However, the fact that it has no motor function militates against this view. It belongs rather to the series of intracytoplasmic chromatic structures in the Trichonymphida elaborated near the blepharoplast and nucleus and connected with the former by a fiber. Janicki (1911) called these structures the parabasal bodies. Homologous structures were found by Alexeieff (1911a) in *Heteromita lacertae* and called by him "bâtonnets sidérophiles" and also in *Monocercomonas bufonis* where the name "corps sidérophile" was used to designate them. There is a strong probability that the so-called kinetonucleus of the Trypanosomidae and related forms belongs in this same series, provided we recognize as distinct from the kinetonucleus the blepharoplast at the base of the flagellum adjacent to the kinetonucleus.

The differences in form between chromatic basal rod, *corps sidérophile*, parabasal body, and kinetonucleus are correlated to some extent at least with other structural differences. The well-developed undulating membrane in *Trichomonas* is correlated with an elongated parabasal at its base. In the absence of such a localized motor area

the parabasal or homologue is often more condensed (not, however, in the trypanosomes) and lies nearer the blepharoplast and nucleus as in *Parajoenia*.

A discussion of the grounds for including the kinetonucleus of trypanosomes in this series will appear in a later paper by the junior author. Should this homology here suggested be accepted as established by later investigations it may be desirable to apply the term parabasal to the whole series of homologous structures, reserving the special names for purposes of differentiation among them.

The function of this chromatic basal rod, or parabasal, is indicated, as Janicki (1911) has suggested, by its relationship to the incessantly active undulating membrane throughout its whole length. Its microchemical reaction, at least to a number of stains, is the same as that of the blepharoplast from which it and the motor organelles take their origin. It has, therefore, some relation to the motor activity of the undulating membrane and probably one similar to that which the blepharoplast bears to the activity of the flagella. It is stock of deeply staining chromatic substance attached immediately at the base of the region of maximum motor activity in the organism, and is connected with blepharoplast and nucleus where such substance, or allied substances, are formed. Its function is not primarily skeletal or supporting, but rather connected with the metabolism, of, and possibly also with the control of the motor activity. Analogy to the neuromotor apparatus of *Diplodinium* as described by Sharp (1914) is suggested by the structural relations.

The parabasal body in *Trichomonas* and *Tetratrichomonas* is a relatively long slender body below the undulating membrane. However, in the nearly related Trichonymphida, in which no undulating membrane occurs, the parabasal is condensed in a stout pyriform organelle which together with the rest of the extranuclear motor apparatus is developed in connection with each of the many nuclei as for example in *Stephanonympha*. Here also at mitosis the new organelle is formed by outgrowth from the blepharoplast and is attached to one daughter blepharoplast while the old parabasal is attached to the other. In this connection it should be noted that *Devescovina striata* Foa, a heteromastigote flagellate with three anterior and one trailing flagellum and a large parabasal wound spirally around the axostyle (see Janicki, 1911, Fig. 1) properly belongs in the Polymastigina near *Eutrichomastix*.

The axostyle (*ax*. Fig. B.) is a stout hyaline, club-shaped structure lying in the axis of the body. Its anterior end, for about 0.2–0.3 of

the total length, is enlarged to 1.2–3 times its diameter in the shaft which is of nearly uniform caliber, though it tapers gradually in some cases (Pl. 1, Fig. 1), may enlarge at the point of emergence (Pl. 1, Fig. 10), or may be constricted near its middle and enlarged again distally (Pl. 1, Fig. 7). Its diameter in the enlarged capitulum is 0.12–0.07 of its length, and in the shaft 0.02–0.045. Its distal end in the normal free-swimming forms (Pl. 1, Fig. 3) is exposed for about 0.2 of its length but extent of this decreases in the mitotic period (Pl. 2, Figs. 11–19) even to complete inclusion in the cytoplasm, and in cases of posterior blob formation (Pl. 1, Fig. 2, and Fig. C, 6–8) the distal end may be some distance within its margin. This end is attenuate to a sharp point, but often somewhat abruptly as in a pencil.

The axial position of the axostyle gives to it a nearly straight form in most vegetative stages. Its enlarged capitulum pushes aside the nucleus, abuts immediately upon the dorsal side of the cytostome and at its apex is attached to the blepharoplast (Pl. 1, Fig. 1) which in some instances (Pl. 1, Fig. 2) even indents this region. This axial organ, however, is very frequently curved with the convexity parallel to that of the chromatic basal rod and undulating membrane (Pl. 1, Fig. 1). This is especially noticeable in individuals which are rounding up (Pl. 1, Fig. 8). A comparison of the Figures on Plates 1 and 2 shows at once that the axostyle cannot be considered as a rigid fixed structure but rather as one subject to a high degree of mobility, or at least of flexibility.

The body of the axostyle consists of homogeneous hyaline substance which shows no internal fibrillar structure, has a more or less sharply defined periphery, and contains from 15–90 deeply staining chromatin granules or axostylar chromidia (*ax. chr.*, Fig. B). These are generally less numerous in the vegetative phase and more so prior to division. There is a very considerable variation in numbers in approximately similar stages (Pl. 2, Figs. 16, 18). These granules are 0.3–0.5 micron in diameter, generally spheroidal, and tend to be equidistant from each other in distribution as though under mutual repulsion. They are most numerous anteriorly in the capitulum of the axostyle and when but few are present (Pl. 1, Fig. 3) they are restricted to this region. As they become more numerous they extend distally to the level of emergence of the axostyle from the cytoplasm, but we have never found them beyond this level. In a number of instances one, two, or three pairs of posterior axostylar granules (*post. ax. gr.*, Fig. B) lie (Pl. 1, Figs. 1, 5, 7) in the cytoplasm or in the

membrane of the axostyle, it is difficult to determine which, near the level of emergence. These distal chromidia are not, however, of constant occurrence. The margins of the axostyle are somewhat more distinct distally where they are readily determined in all preparations. In reality a faintly chromatic band surrounds the axostyle just above its point of emergence (Pl. 1, Fig. 3). In the anterior region, owing to the greater mass of material, and to the nucleus, the outlines of the axostyle are less readily followed.

The presence in this species of these chromatic granules in the axostyle is, however, of very great assistance in clearly marking out the course of this organ in the cytoplasm. The cytoplasmic chromidia are never so numerous as to obscure or confuse the interpretation of the axostyle. Its delineation requires carefully stained iron-haematoxylin preparations, preferably made with alcoholic solutions, careful extraction of the stain to the right degree, a 100 watt Mazda lamp as the source of illumination, and the Zeiss 2 mm. apochromatic objective and compensating oculars for observation.

The axostyle is demonstrable by these means in every individual save in a very few which showed evidence of degenerative changes in that the nuclear conditions were abnormal or the cytoplasm filled with chromidia as in Alexeieff's (1910) figure of *T. batrachorum*. It was demonstrable in all individuals in the process of mitosis.

Kuczynski (1914) describes the axostyle of *Trichomonas*, including that of *T. augusta*, as being composed of two main fibrillae which meet posteriorly in the tip and anteriorly embrace the blepharoplast between their ends. These fibrillae one finds to be thickened just above the point of their emergence. From a careful inspection of his figures it appears that these "fibrillae" can be only the sheath or outer layer of the axostyle seen in optical section on either side. We find on careful search no conclusive evidence of any such fibrillae in our material though the appearances may be similar to those figured by Kuczynski. No matter what the position of the axostyle under observation one never finds these "fibrillae" in any other place than the sides of the axostyle as may be inferred from an inspection of all of our figures of this species. They do not cross the shaft obliquely or lie in any instance along the middle of the shaft as a pair of fibrillar structures might. It is true that in most of the individuals on slides the undulating membrane lies either on the right or the left margin of the figure so that were the axostyle fixed in position with reference to this membrane and the two "fibrillae" dorsal and ventral in location we should then always see them along the sides of the axostyle.

A close inspection of our figures shows, however, especially in the case of mitotic stages (Pl. 2, Figs. 11–23) and some others (Pl. 1, Fig. 2) that we must view the axostyle on other faces than those above noted, but only lateral positions of these so-called fibrillae are to be seen in these cases also. Furthermore, in the macerated extra-nuclear motor apparatus (Fig. D, 4) no trace of Kuczynski's fibrillae can be found, nor of the location of the blepharoplast between their anterior ends as he intimates. The axostyle is rather a subcylindrical rod with enlarged anterior end with no trace of fibrillar structure but with a sheath or outer layer of greater stainability, especially posteriorly, than the hyaline interior.

The function of the axostyle has been interpreted by Grassi (1888) as a "squelette interne," by Kunstler (1898) as skeletal since its "courbe régulière et tendue dénote une certaine rigidité jointe a une grande élasticité," and this view has been generally accepted by those who have since discussed it. Dobell (1909), for example, insists "that its real function is entirely skeletal" and that "it is merely an axial support." Kuczynski (1914) seems also to accept this view citing in support the view of Hartmann and Chagas (1910) that the slender axostyle in *Cercomonas parva* is a firm elastic structure not subject to contraction, that is to decrease in length with the amoeboid changes in form of the body which alone are responsible for its changes in shape. He notes, however, that the skeletal function is possible only while the two ends of the axostyle are fast to the outer membrane, and that the posterior attachment is often released with the result that the axostyle is entirely included within the cytoplasm. He concludes that it then ceases to be a "formgebendes Element" and is speedily resorbed as a result of inactivity. No previous activity is, however, attributed by him to the organ.

Kunstler (1898) has also suggested that the projecting point is an organ of fixation. Wenyon (1907) accepts this view and Kuczynski (1914) narrates an observation which leads him to champion it. He observed that the tip of the axostyle is thrust into the substrate by the activity of cytoplasm and that when thus anchored the flagella become quiet while the undulating membrane proceeds to fill the cytostome with food particles by its activity.

In our observations such attachments are not infrequent, but they seem rather to be due to the adhesive nature either of the substrate or of the organisms which under cover glass speedily become adhesive. That the protruding axostyle is especially adhesive is often seen in the string of bacteria or detritus trailing after its tip in free-swimming

forms. In moribund individuals the anterior flagella also become very adhesive and fuse in one short protoplasmic rod which continues to beat and may fuse laterally with the cytoplasm or accumulate a mass of adherent particles. The tip of the axostyle might be called an organ of fixation in this accidental or casual sense but not in a normal and essential one.

It was only after long observation that we were convinced that the true function of the axostyle is motor, but having once seen it in full motor activity, all doubt as to its function is at once dispelled. It is not merely a rigid elastic body, subject to passive curvature by the constraint of contracting cytoplasm about it. It is rather a powerful motor organ which comes into function when the animal is on a substrate, and doubtless plays an important part in the life of the organism in the mucus of the intestinal surface. As is well known the organisms penetrate the crypts and Lieberkuhn's glands, and division stages are to be sought in smears from the wall rather than in foecal contents. The intestinal surface with its coating of glandular secretion and the proteid-rich chyme which becomes ever denser as its soluble proteids diffuse through this intestinal wall, constitute the medium most favorable for the growth and division of these flagellates. The axostyle is an organ for locomotion on the intestinal surface and in the viscous medium immediately covering it.

One may watch the free-swimming stages for days and never see the motor activity of this organelle which is held in a rigid axial position thus giving emphasis to the skeletal interpretation. Nor is it to be seen in culture slides which have been made for even a short time, since individuals accumulate on the substrate as they become adhesive and moribund, and such changes in the axostyle as occur in these individuals are slow and far from characteristic. It is best seen in freshly made slides with little fluid prepared directly from the fresh mucous surface. Search of such a preparation will usually reveal some individuals in intense axostylar activity. These are usually somewhat rounded up (Pl. 1, Fig. 10) and are often of the larger size. They are on the surface of the glass with flagella and undulating membrane in full activity. In addition to this the axostyle itself keeps up a vigorous lashing from side to side, sometimes constant, sometimes intermittent, changing its position actively as a stout flagellum from one side to the other, bending and curving as it lashes about (Pl. 1, Figs. 8, 9), now parallel to the membrane and now away from it, with constant readjustments of the vacuoles among which it moves. It is impossible to say that the point of emergence from the

cytoplasm is a fixed one in the pellicle. The protruding tip, which in some cases is quite elongated (Pl. 1, Fig. 8) is also independently active at times waving back and forth in an arc of 180° or describing the surface of a cone of rotation of varying angle. In all of this activity it is strikingly suggestive of a flagellum constrained in its movements by the enveloping cytoplasm, especially at the point of emergence, and by the mass of its own substance but nevertheless independently and vigorously motile. Some progression over the substrate may occur, by reason of the rotation of the body, but the whole surface is evidently more or less adhesive. Under these conditions the axostyle is anything but an organ of fixation.

From a functional standpoint the axostyle is thus a stout, largely intracytoplasmic flagellum for locomotion in a viscid medium. Structurally it has the same connections with the blepharoplast that other flagella have. It does not stain as they do, especially within the cytoplasm. Both the chromatic margin and the chromatic basal rod stain an intense black, but the axostyle is hyaline and the least stained of all parts of the organism. It may be, however, that the axostylar chromidia within it have segregated in themselves the stainable substance which is continuous in the other structures named. It should be remembered in this connection that the chromatic basal rod also may be at times made up of chromidial blocks, as noted by Martin and Robertson (1911) for *Trichomastix gallinarum*. The tendency for the chromidia to assume an axial position in the axostyle (Pl. 1, Figs. 5, 8) is also significant in this connection as is also the tendency for the distal part of the axostyle just within the cytoplasm to have regularly grouped chromidial granules. When the axostyle protrudes a considerable distance, as it sometimes does, these granules may be seen adhering to it. Additional structural confirmation of the flagellar homology of the axostyle in the trichomonads is to be seen in the fact that the projecting parts of these structures in *Hexamitus* (= *Octomitus*) and *Lamblia* are posterior flagella comparable in appearance to that projecting from the undulating membrane in *Trichomonas*. We may conclude then that the axostyle of *Trichomonas* is an intracytoplasmic flagellum highly specialized for motor activity on the viscous mucous surface of the intestine.

The blepharoplast (*bl.*, Fig. B) is a spheroidal body lying very close to the surface near the anterior end of the body dorsal to the cytostome and at its very margin. It also abuts against and may even appear to be indented into the head of the axostyle. It is usually about 0.5 μ in diameter, varying possibly with the degree of extraction

of the stain. Kuczynski (1914) holds that it lies between the anterior ends of two "fibrillae" which constitute the axostyle. The figure cited in support (his pl. 16, fig. 101) shows in our opinion the edge of the cytostome rather than fibrillae about the blepharoplast.

In our figures the blepharoplast is large prior (Pl. 1, Fig. 4) to the formation of the intranuclear cloud and small thereafter (Pl. 1, Figs. 2, 3, 5). It is a single mass except in cases where mitosis is impending or in progress. From it spring directly without any interruption in structure or stainability the chromatic margin and the chromatic basal rod of the undulating membrane. It is also directly connected with the axostyle and the three anterior flagella.

In macerated individuals (Fig. D, 4) a delicate strand, the rhizoplast (*rh.*, Fig. B) may be seen to connect it with the nuclear membrane. This is the least chromatic part of the extranuclear apparatus and we have been able to demonstrate it only in macerating individuals. The rhizoplast described by Bensen (1909) in *T. vaginalis* at the head of a so-called axostyle, is, together with this axostyle, in reality the chromatic basal rod in our opinion, and is extranuclear.

The extranuclear apparatus, largely chromatic in nature, consisting of the blepharoplast and the organs above named which are connected with it, constitute a structurally connected unit, the extranuclear chromatic motor apparatus.

The cytoplasmic chromidia (*cyt. chr.*, Fig. B) lie in the cytoplasm between the vacuoles. In size, form and stainability they are like the axostylar chromidia. We find no evidence of their passage from the axostyle into the cytoplasm. They are not always present, being absent in many vegetative individuals (Pl. 1, Figs. 1–4) and most abundant prior to and during mitosis (Pls. 2, 3), appearing in the cytoplasm with the extranuclear chromidial cloud (Pl. 1, Figs. 5, 9).

The vegetative phases of *Trichomonas augusta* are found in the intestinal contents and in cultures. The different phases of mitosis may be found in large numbers in smears made from the intestinal wall directly. We will now proceed to follow the process of division.

This is of two distinct types, binary and multiple fission and both occur in this species and in trichomonads generally on the intestinal wall and may be followed in smears from that region. We are not at present able to relate either process to any definite phase of sexual reproduction. We find as yet no conclusive evidence that either leads to gamete formation by maturation divisions, or that either follows zygote formation or fertilization. Detection of these processes must await, it seems, the unravelling of the history of the true trichomonad cysts.

Binary Fission in Trichomonas augusta.

This occurs by mitosis in which we may distinguish phases comparable with those in metazoan cell division in the nature and results of the processes carried on in nucleus and cytoplasm, but differing somewhat in their respective chronologies, in minor details of arrangement and in relative development of the achromatic organelles, from mitosis in the metazoan cells.

Moreover, the sequence of changes in the individual parts of the organism does not proceed with the same relative rapidity in every division. The extranuclear structures especially exhibit a considerable variation in this respect. The parts are also mobile in the cytoplasm to a high degree with the result that a great diversity of pictures of the process of mitosis is here presented.

The prophase (Pl. 1, Figs. 2, 3, 5, 7–10, Pl. 2, Figs. 11–12) is that in which the chromosomes are organized out of the karyosome and chromatin network of the nucleus. The organization of the spindle is here much simpler than in the Metazoa and also progresses less rapidly than that of the chromosomes.

The first indications of mitosis appear in the development of a diffuse intranuclear chromidial cloud (Pl. 1, Figs. 2, 3, 5, 8) which fills the nucleus and is not easily removed by decolorization. Following this stage this intranuclear material disappears and the chromatin of the nucleus is aggregated in a ragged tangled chromatin thread or skein (Pl. 1, Fig. 9) lying in the now unstained nuclear sap. Surrounding the nucleus at this stage is a halo formed by a vacuole-free zone of fine granular perinuclear cytoplasm which stains more deeply here than elsewhere. At the same time the number of axostylar chromidia has considerably increased (Pl. 1, Figs. 5, 9) and the first cytoplasmic chromidia have made their appearance. This extranuclear cloud has been previously noted only by Bensen (1909) in *T. vaginalis*, though he does not connect it with chromidial formation, and by Alexeieff (1914a) casually in *T. sanguisugae*. It later disappears entirely as the chromidia in axostyle and cytoplasm increase in number.

Individuals in this stage are generally rounded up, and are often large, though mitosis is by no means confined to large cells (cf. Figs. 24 and 29). When small ones divide they also round up. The axostyle is more or less withdrawn within the cytoplasm in these rounded stages and is often much curved in fixed material, evidently

caught by the fixative during the period of vigorous lashing on the extreme limit of the outward stroke (Pl. 1, Figs. 8–10).

The first organelle to be reproduced is the chromatic margin of the undulating membrane (Fig. E and Pl. 1, Figs. 6–10) which splits from the blepharoplast distally to the tip of the posterior flagellum. In view of the fact that the new anterior flagella and the chromatic basal rod are formed by growing out and not by splitting it may be questioned whether or not the interpretation of this process as one of splitting is correct. It rests upon the fact that the two supposedly daughter marginal threads at first lie close together, show parallel undulations, and posteriorly merge in the parent thread. Differences in diameter between parent and daughter filaments which result from splitting are in the ration of 1.4: 1, and are plainly detectable with Watson's number 20 holoscopic eyepiece. In four cases (Fig. E, 6–9) splitting has progressed beyond the end of the chromatic basal rod, showing that the filament, even in the extracytoplasmic posterior flagellum also splits. No evidence of a secondary twisting together of free ends is apparent in this preparation, the distal part appearing to be the still undivided parent flagellum.

As the splitting of this filament passes the point of union of the marginal filament and basal rod (Fig. E, 6–9) one daughter filament still adheres to the end of the rod and the other detaches a minute granule from its tip as it splits off. The left filament is usually the adherent one (Fig. E, 7) but the right has been found in this position in two cases (Fig. E, 6). This chromatic marginal filament is the intracytoplasmic part of the posteriorly directed flagellum. At mitosis the new anterior flagella and the chromatic basal rod are formed by outgrowth and the axostyle and marginal filament by division. On *a priori* grounds we should expect the posterior flagellum to arise in the same manner as the anterior ones. The process is, however, clearly one of longitudinal splitting in form at least, as above shown. These facts seem to indicate that homologous derivatives of the blepharoplast may arise by different methods and the precise method of origin of these organelles is without morphological significance and possibly subject to change according to its extra- or intracytoplasmic position.

The longitudinal division of the undulating membrane below the chromatic margin progresses slowly as shown by the proximity of the daughter marginal filaments and by their common undulations in many preparations. It is accomplished, however, before the new chromatic basal rod is formed as will be seen in Figure 8 (Pl. 1) in

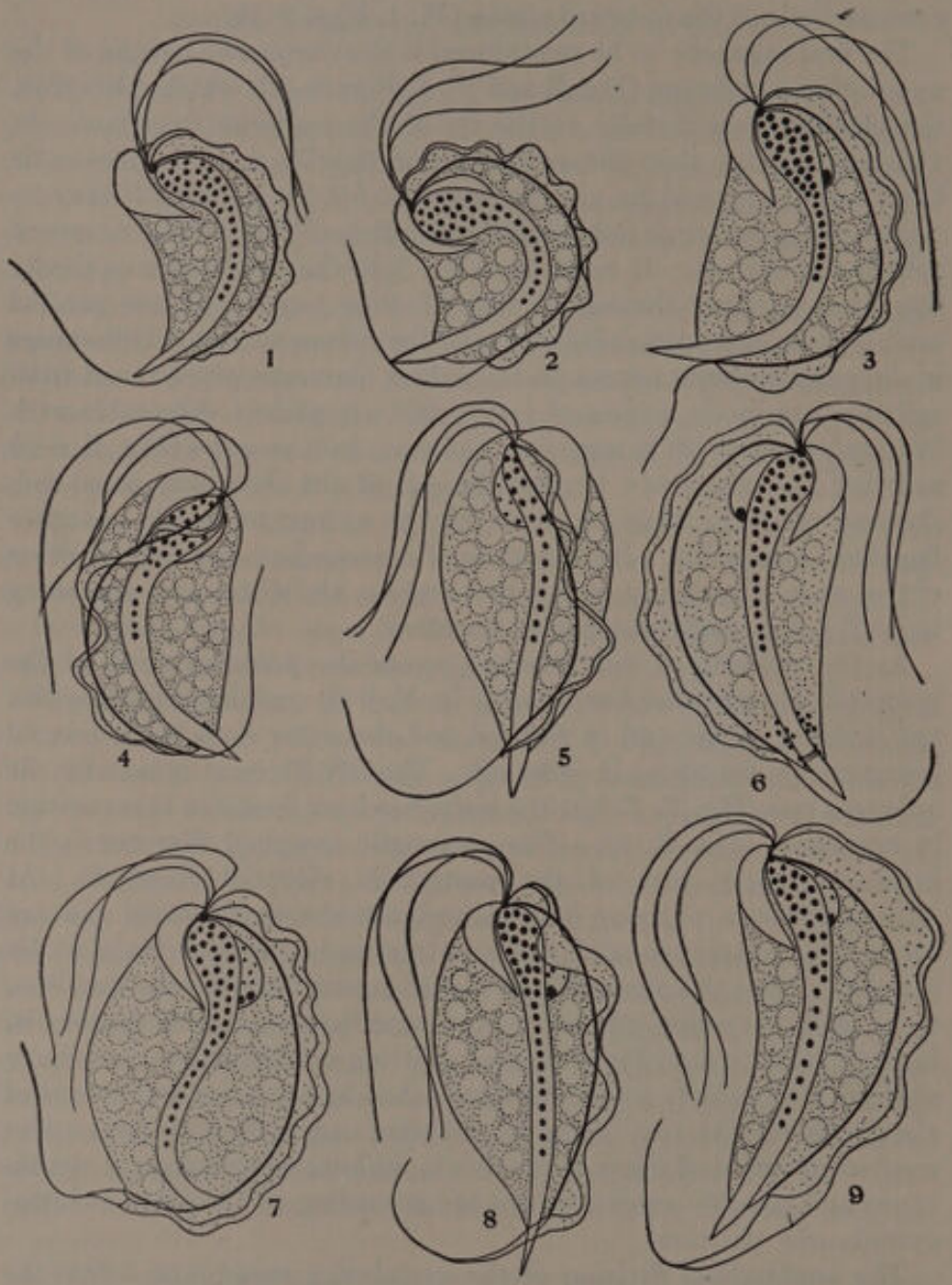

FIGURE E. Nine successive stages in the splitting of the chromatic marginal filament of the undulating membrane in *Trichomonas augusta*. × 1500. Figures 1–5 show the progress of the splitting from the blepharoplast to the point of emergence of the posterior flagellum and 6–10 the splitting of this exposed part of the organelle.

which one of the daughter membranes has been detached in making the smear. The two membranes are complete even to the posterior flagellum but the new chromatic basal rod has not as yet made its appearance.

The chromatic basal rod does not originate as does the marginal flagellum by splitting but grows out distally from the blepharoplast along the base of the divided undulating membrane (Pl. **2**, Figs. 11–18) and ultimately comes to lie (Figs. 17, 19) in the base of one of the membranes. In some cases the two rods appear to be united distally as though they too originated by splitting. If this be the case it is possible that the detachment of the short rod from the long one of Figures 11 to 18 is the result of disturbance of relations in making the preparations, but the mass of evidence is overwhelmingly in favor of the detached outgrowth of the new rod.

When the two rods or parabasals are completed, the two undulating membranes become more or less widely detached distally (Pl. **2**, Fig. 17), preserving their original connection only in the blepharoplast. With the division (Pl. **2**, Fig. 17) and the migration of the daughter blepharoplasts to the poles of the spindle (Pl. **2**, Figs. 19–23) the two undulating membrane complexes are finally detached one from the other, save by the connecting paradesmose. In some instances there is a marked disturbance of the normal relation seen in Figures 20 and 22, in that the daughter blepharoplasts (Pl. **2**, Fig. 19) or basal granules after the division of the blepharoplast into basal granule and centrosome, are more or less widely detached from polar relations to the spindle (Fig. 21). We are unable to determine whether this results from the protean activity of the organism in this period or from the disturbance in making smears. There is no evidence in the preparations of mutilation of the body in these cases of disturbed relations.

The *anterior flagella* are produced not by division but by outgrowth of new flagella from the blepharoplast. These sprouting flagella are difficult to detect and are probably overlooked in a number of our figures. They do not all arise at the same time but apparently in sequence during mitosis. The first one appears in the prophase (Pl. **1**, Figs. 7–11, Pl. **2**, Figs. 12, 15, 19). When the blepharoplast divides (Fig. 19) one daughter takes two of the old flagella and the other the one and the new flagellum. Another new flagellum is then grown out from each daughter blepharoplast making three flagella arising from each, the normal equipment of the vegetative phase. In Figure 19 a second new flagellum is sprouting from the blepharoplast at the left, but not as yet from that at the right. The full

complement of anterior flagella is in part completed rather late (Pl. 3, Fig. 33) in mitosis.

The division of the blepharoplast is difficult to detect and seems to present quite a variety of appearances and to bear no constant relation to the progress of division in other organelles. We are quite unable to find a granule at the base of each flagellum in the blepharoplast. Subdivisions of this structure seem to be concerned with mitosis rather than to indicate relations to individual flagella. Owing to the range in form which is presented it may be that the phenomena have none of the significances which we here ascribe to the stages we find. We conclude, however, that they are all referable to two steps in mitosis. The first is the division of the blepharoplast into two equal daughters which sooner or later migrate to the poles of the elongating nucleus and place themselves at the poles of the forming spindle. Each carries with it a daughter undulating membrane complex (Pl. 2, Figs. 20, 22) and its complement of the old and new anterior flagella as above described. This division and migration is always to be found as the prophase passes to the metaphase of mitosis.

The second step is the division of each blepharoplast (Pl. 2, Fig. 23) into a centrosome at the apex of the spindle to which apparently no flagella or extra-nuclear structures are attached and into the basal granule which retains connections with the undulating membrane complex, paradesmose, and the anterior flagella. We are unable to trace the connections of the rhizoplast and axostyle during this period. This second step, however, does not seem either to be of long duration, for few instances of it have been found, nor to have a definite and fixed location in the mitotic sequence. It may occur for example (Pl. 1, Fig. 8) in the early prophase prior to polar migration, and the blepharoplast appear to be made up of four granules or two groups of two each, or it may occur in the anaphase (Pl. 2, Fig. 23), and again it may occur at one pole and not at the other (Pl. 3, Fig. 24). These facts, together with its infrequency at stages such as the anaphase when it is most to be expected, lead one to doubt the universality of its occurrence. It seems, however, to be a significant process, and not an irrelevant disintegration of the blepharoplast, for the parts into which it subdivides have definite relations to structures of the organism, and relations, moreover, which are widely recognizable among Protozoa and other flagellated cells, for one granule persists in its location at the apex of the spindle, or in the comparable position on the daughter nuclei, and the other remains in the position of basal granule to the whole group of flagella.

It appears from our data that their separation is not an obligatory part of mitosis and that it may occur in many stages of the process. It is also indicated that the blepharoplast of the trichomonads is potentially, if not indeed also structurally, composed of at least two parts, centrosome and basal granule, and that it is the center from which extranuclear structural differentiation proceeds.

It is a matter of considerable comparative significance that this extranuclear blepharoplast and its adjacent parabasal body pass through the whole process of mitosis without the remotest semblance of independent mitotic behavior. Neither is in any true sense an accessory nucleus. Their behavior, especially that of the blepharoplast is wholly accessory to the division of the main nucleus. There is absolutely no basis in their behavior as recorded in their morphological changes in mitosis for regarding either or both as a kinetonucleus or as having chromatin of some subtile hereditary significance.

The importance of this conclusion will depend in part upon the correctness of our inference that the parabasal of trichomonads and the kinetonucleus of trypanosomes are homologous and that the basal granule at the base of the flagellum of trypanosomes is the homologue of the blepharoplast, at least in part, of the trichomonads. In so far as our observations are correct and the homologies suggested well-founded, to that extent is doubt cast upon the mitotic interpretation given to the so-called kinetonucleus of trypanosomes by Schaudinn (1904) and upon the Binuclearity Hypothesis resting thereon.

It is to be noted that during the later stages of mitosis the nucleus and blepharoplast seem to lose all constant relations to the axostyle and in a few cases even the blepharoplast or basal granule (Pl. **2**, Figs. 19, 21) becomes removed from the nucleus. In these latter cases the rhizoplast would either be broken or stretched out, and in the former the connection of the axostyle with blepharoplast or its parts, which are normally (Fig. D, 4) very intimate, must become very much attenuated, as for example (Pl. **2**, Fig. 17) when the head of the axostyle is 180° from the blepharoplast. In later stages of the telophase (Pl. **4**, Fig. 36) the original intimate relations of blepharoplast and axostyle are restored in such a way as to suggest the persistence of some invisible physical connection such as achromatic fibers between these organs during their detachment. No objective evidences of such organelles have been found in our preparations after closest scrutiny.

The nucleus during the prophase and all subsequent phases of mitosis retains its nuclear membrane intact. Within it the diffuse

chromidial cloud accumulates and then passes to the exterior in the extranuclear cloud. This is a period of considerable increase in the total amount of chromatin in the nucleus and in deeply staining material outside of it. Within the nucleus it aggregates at first into a ragged gradually thickening skein (Pl. 1, Fig. 9) which at least approaches the appearance of a continuous thread. This occurs prior to the division of the blepharoplast and after that of the undulating membrane. This is of brief duration and is followed by the aggregation of the chromatin into somewhat elongated chromosomes (Pl. 1, Fig. 10) resembling grains of rice in proportions. The number of these is as a rule five, of which one is large, two of medium size, and two small. During the transition from skein to chromosomes the number of masses often exceeds five, possibly as the last step in chromosome aggregation, although it is not always easy to distinguish these from following phase of division. Some instances of four masses are resolved into five on close scrutiny. In other words the evidence of a constant number of chromosomes in *Trichomonas augusta* is of the same character as that for the normal number in most metazoan cells.

Splitting of the chromosomes follows soon after their organization, in fact the numbers of individuals found in smears with five unsplit chromosomes is much less than those with split or ten chromosomes. The splitting occurs prior to the arrangement of the chromosomes in an equatorial plate (Pl. 2, Figs. 11–19) and is not synchronous. There is a little evidence that the largest chromosome is slow in dividing and that its division is unequal in an x-y fashion (Figs. 16, 23). There is, however, no evidence that we are dealing here with maturation divisions or sex chromosomes. It appears too frequently, in six out of eleven figures on Plate 2 to be a chance inequality. The direction of splitting is in each case longitudinal, though the parallel position of the sister chromosomes (Pl. 2, Fig. 15) is soon shifted to an end-to-end one resembling a telosynapsis. The evidence (Pl. 2, Figs. 15–19) points toward the assumption of the end-to-end position by the swinging of the ends at one pole apart, while those in the other remain together, rather than by a sliding of one chromosome along the other till two poles originally at opposite ends of the pair meet. Some suggestions of such sliding are, however, present in our preparations and figures (Pl. 2, Figs. 11, 17).

The precise equivalent of the metaphase of metazoan mitosis, in so far as it is represented by the splitting of the chromosomes has already been accomplished before the amphiaster is formed in *Trichomonas*

augusta. There seems to be a subsequent but temporary telosynaptic fusion of the split chromosomes at the time of the first appearance of the equatorial plate (Pl. 2, Fig. 20).

Metaphase and Amphiaster.

No arrangement in an equatorial plate occurs until the daughter blepharoplasts have migrated to the poles of the ellipsoidal nucleus (Pl. 2, Figs. 20–21). Such a plate is suggested in Figure 13, but only four pairs are here in line, and no spindle is present to contain the plate. This is only a chance grouping and not a true equatorial plate.

Amphiaster Stage.

The equatorial plate is formed apparently only after all of the chromosomes are in this "telosynaptic" relation (Pl. 2, Figs. 20, 21). In the few spindles we have found in this stage there are but five chromatin masses, rather stout (Fig. 20) or tapering (Fig. 21) towards either end, thickest at the middle, and showing there no trace of any central zone of fusion or separation where from previous conditions we may suppose "telosynaptic" fusion to have occurred. There is for this condition of the chromosomes, if it be the normal and regular sequence following that of parallel split chromosomes, but one explanation, namely that the early splitting is followed by a later "telosynaptic" fusion in the equatorial plate. The relations of the daughter chromosomes in Figures 12 to 19 support this interpretation. Coupled with this is the fact that the individuals with split chromosomes present collateral evidence in other organelles of an early phase of mitosis, whereas this evidence in Figures 20 and 20a, indicates that these are later stages. There is, however, another possible sequence, namely that in some individuals represented by Figures 20 and 21 there has been no previous splitting and the elongated chromosomes part transversely. If this be true we have two types of division of chromosomes in the one species and one of these is of the normal type by longitudinal splitting and the other by an exceptional and unique type (in non-maturation divisions) of *transverse* division. This is possible especially in view of the fact that we are dealing with parasitic organisms subject to the action of the antitoxins of their hosts and therefore to disturbances in the normal processes of growth.

Except for the spherical form of these individuals with transversely dividing chromosomes, there are no evidences of approaching moribundity. But the cells with the other type of division are also rounded up. It is thus possible, but inherently improbable, that these cells represent an unusual type of division of the chromosomes, rather than a stage of temporary telosynaptic fusion subsequent to an earlier longitudinal splitting.

A precocious longitudinal splitting or perhaps more precisely an emergence of the chromosomes from the chromatic net or spireme in the form of threads apparently split longitudinally has been described for the vegetative cells of certain higher plants by Gregorie (1906) and for *Allium* and *Vicia* by Lundegårdh (1912). Flemming long ago (1891) noted it in the somatic cells of the salamander and Dehorne (1911) in cells of annelids and trematodes, but it has not been prominent in recent cytological investigations of metazoan somatic cells. In none of these cases, however, is precocious splitting followed by a telosynaptic fusion on the equatorial plate such as we have suggested here for *Trichomonas*, though Gregoire figures a re-fusion of the split chromosomes before the equatorial plate stage.

The achromatic spindle is feebly developed. It consists of a few faint fibrils within the intact nuclear membrane which pass from the chromosomes to the centrosomes or blepharoplasts at the poles of the now somewhat elongated, broadly fusiform, or ellipsoidal nucleus. These spindle fibers are more distinct near the centrosomes and as the chromosomes part (Pl. 2, Fig. 23) faint interzonal fibers appear between them. There is no satisfying evidence of the formation of astral rays in the cytoplasm at the poles about the blepharoplasts or the centrosomes. A faint starlike structure (Pl. 2, Fig. 21) seen in one instance in the cytoplasm about one of the centrosomes is not of general occurrence.

The paradesmose is formed between the two divided blepharoplasts as they migrate to their polar positions in the spindle. Structurally it is a fiber of uniform caliber lying at all times *outside* of the nuclear membrane and is directly continuous with the blepharoplasts which it connects. It stains an intense black as do other intracytoplasmic derivatives of the blepharoplasts.

This organ has been called the axostyle (Achsenstab) by Prowazek (1904) who discovered it, since he confused it with the development of that structure. Dobell (1909) also fell into the same error of regarding the axostyle as derived from the chromatic fiber joining the daughter blepharoplasts in mitosis, saying "the axostyle is the

homologue of the central spindle, each being a centrodesmus." This homology to the central spindle is obviously a different thing from its reference to the axostyle and is in nearer conformity to the correct interpretation of this structure. Alexeieff (1914a) solves the problem by the purely objective term blepharoplastodesmose, objectionable because of its length.

A thread-like chromatic structure connecting recently divided blepharoplasts or basal granules in *Copromonas subtilis* is figured by Dobell (1908) without comment as to its significance. The blepharoplast lies adjacent to the reservoir some distance anterior to the nucleus and the paradesmose-like strand persisting between the daughter blepharoplasts after their division, lies on the wall of the vesicle much as the paradesmose of *Trichomonas* on its nuclear membrane. It later disappears as in *Trichomonas*. We regard this structure as homologous with the paradesmose of *Trichomonas*.

As noted above our researches show that this chromatic thread connects at first the dividing daughter blepharoplasts. When, however, these part each into a centrosome and basal granule at the poles of the spindle the paradesmose *connects the basal granules, and not the centrosomes*. The paradesmose thus connects here those parts of the two blepharoplasts which are attached to the extranuclear chromatic apparatus and not that which stands at the poles of the spindle. This relationship, added to the fact that it is at all times extranuclear as well as outside of the spindle, raises some doubts in our minds as to the precise homology of the paradesmose with the central spindle. The name we propose avoids the length and partial inapplicability of that proposed by Alexeieff and is in part at least descriptive of the relations of this structure.

Since the instances of the division of the blepharoplast into centrosome and basal granule seen by us are found mainly in *Trichomonas augusta* and are few in number and since the division may represent merely a precocious division of blepharoplasts, possibly followed by re-fusion (Pl. 2, Fig. 23, Pl. 3, Figs. 24–28), the resulting lack of a precise homology of the paradesmose with the central spindle should not be allowed to obscure its relationships to that structure. It is in any event a differentiation of the central spindle modified by two specializations in trichomonad mitosis, (1) the continuity of the nuclear membrane which excludes it from the typical axial position of the central spindle, and (2) the connection of the blepharoplast with the extranuclear motor complex.

The paradesmose persists for some time after the daughter nuclei

have separated (Pl. 3, Figs. 26–30) and seems to act as an elastic tether which turns the blepharoplast poles of the daughter nuclei towards one another from their original separation of 180° (Pl. 3, Figs. 26–29) when the protean activity of the organism results in a wider separation of the nuclei. In Figure 28 the paradesmose has almost its original length and position and the nuclei are but slightly turned. In Figure 29 the nuclei have parted for a distance equal to a nuclear diameter, the paradesmose is very thin and straight as though under tension and both nuclei are turned as though drawn by their blepharoplasts. In Figure 26 the nuclei are nearly two nuclear diameters apart but the blepharoplast of the nucleus to the right has become detached from its nucleus and the paradesmose has shortened up and thickened as though it had contracted.

It can no longer be detected between the blepharoplasts after the axostyle has begun to divide. It fades away without leaving any special organelle as its successor. It does not form the daughter axostyles as described by Prowazek (1904) and by Dobell (1909).

The Anaphase.

The anaphase in which the chromosomes migrate to the poles of the spindle and the nuclear membrane constricts is of some duration for a number of cells in this period have been found in our preparations. As the chromosomes approach the poles of the spindle the spindle fibres grow thicker and darker or the chromatin material of the chromosomes flows out towards the blepharoplasts until they seem to be actually in contact with it (Pl. 3, Figs. 24–28) for a time. During this period the chromosomes retain their individuality to a striking degree.

The daughter nuclei are formed by the constriction of the nuclear membrane in the equator of the broadly fusiform spindle (Pl. 3, Figs. 24, 25). Nuclei in this stage of constriction are very rarely seen in preparations. This process is therefore quickly accomplished.

The Telophase.

The telophase, in which the nuclei return to the vegetative or "resting" condition, is accomplished by the detachment of the chromosomes from the blepharoplast pole of the nuclear membrane (Pl. 3,

Figs. 29–31), their decrease in size, their rounding up, and their ultimate disappearance as separate chromatic units. Several small karyosomes may be seen in some nuclei towards the end of this process (Pl. 3, Figs. 35), but it seems normally to terminate in a nucleus with a single central karyosome (Pl. 3, Fig. 34). There is apparently some reduction in the total amount of chromatin, in the form of deeply stained masses in the nucleus during the telophase.

The division of the axostyle takes place during this process. Contrary to previously accepted accounts of the origin of this structure, it is neither derived from the centrodesmose nor by new outgrowths from the blepharoplast. It does not wholly disappear at any time during mitosis in *Trichomonas augusta* but may be located and its outlines traced in carefully stained material examined with apochromatic objective and compensating oculars. The axostylar chromidia are of great assistance in the quick location of the structure and the verification of its outlines.

The old axostyle splits longitudinally from the club-shaped anterior end to the posterior tip (Pl. 3, Figs. 31–35, Pl. 4, Fig. 36). Prior to splitting the axostylar chromidia are somewhat more numerous, and are arranged in a single row in its distal half. In several instances (Pl. 3, Figs. 31, 34) the chromidia in the distal undivided part are distinctly larger than those in the adjacent daughter axostyles suggesting their splitting *in situ* as the axostyle splits. The forms, sizes (in a few instances only), positions, and numbers of the axostylar chromidia are suggestive of their division or at least of their increase in number prior to and during division of the axostyle. In premitotic and mitotic stages prior to the division of the axostyle (Pls. 1, 2) the range in number of axostylar chromidia in 19 individuals is 18–56 and the average 33. After division (Pl. 4) the range in 12 individuals is 9–37 and the average only 23. The variation is so great and the numbers in sister axostyles at times so divergent (Pl. 4, Fig. 37) that no precise or regular method of multiplication and sharing of the chromidia thus produced between the daughter axostyles can be postulated from the evidence which, however, does indicate compensating increase prior to and during binary fission.

The position of the axostyle prior to its division especially during the metaphase-telophase is exceedingly varied (Pls. 2–4). Whereas in the premitotic phase it is axial in position with the nucleus next to its enlarged anterior end, during this later period this end becomes more or less widely separated from either daughter nucleus, lying even at the opposite pole (Pl. 3, Fig. 30). As the division of the

axostyle approaches and proceeds (Pl. 3, Figs. 31–35, Pl. 4, Fig. 36) the nuclei assume a balanced relation (Fig. 31) and move into close contact with the anterior ends of the new axostyles (Fig. 34) which they retain throughout all of protean activity which precedes the final separation of the daughter cells (Pl. 4, Figs. 36–40, Fig. F).

Previous accounts of the fate of the parent axostyle and of the origin of the new axostyles have been in a state of contradiction and confusion. According to Prowazek (1904) who first described mitosis in trichomonads (*Trichomastix lacertae*) the old axostyle disappears and the new ones form out of the organ which we have called the paradesmose which finally divides at its middle into the two new axostyles as the daughter cells are separated. According to Grassi and Foa (1904) in the division of *Joenia annectans* the paradesmose (fuso) is involved in the formation of the new axostyles as their central cores and the old axostyle (mestola) is dispersed. Evidence of the behavior of the two organs involved is insufficient in just that period in *Joenia* in which division of the axostyle and disappearance of the paradesmose takes place in *Trichomonas*. Conclusive as their evidence presented seems as to the correctness of their interpretation in *Joenia*, it is highly desirable that these critical stages be reinvestigated to see if a correlation with our results in *Trichomonas* may not be possible.

Wenyon (1907) is the only author whose conclusions regarding the origin of the axostyle in mitosis accord with ours. He states that it "divides by longitudinal division and is the last part of the animal to divide (pl. XI, fig. 3). In later stages it is seen extending through the body of the long drawn out animal from the neighborhood of one nucleus to that of the other (pl. XI, figs. 15, 21). In the final stage, two animals are attached simply by this organ, which finally gives way, leaving the characteristic pointed ends." It is unfortunate that none of his figures supports his views as to division and that his context indicates a possible confusion of paradesmose and axostyle. He notes the fiber between the daughter blepharoplasts in early mitosis (his figs. 2, 4, 10, 11) but does not distinguish it from the axostyle in later stages.

Hartmann and Prowazek (1907) state with regard to trichomonads (*Trichomastix lacertae*) that the axostyle is formed from the "Caryosom des Amphinuclolus" but, as Dobell says, there is no foundation for this statement certainly not in Prowazek's own (1904) observations, and none whatever in our own data, for we have shown that the blepharoplasts are persistent organs having nothing to do (in the

asexual phase at least) with any karyosome of a hypothetical amphinucleolus. Nor is their second conclusion that "Die *vermutlich* mit dem Centriol in Zusammenhang stehende Rippe (Achsenstab) ist eine Art von Centralspindel und geht in die Rippe des Tochterthieres über" supported by our observations.

Dobell (1909) in both *Trichomonas batrachorum* and *Trichomastix batrachorum* finds that the parent axostyle vanishes apparently by absorption and that the daughter axostyles are re-formed out of "the central spindle or centrodesmus" between the daughter blepharoplasts. His figures are inconclusive at the critical stage, however.

Prowazek and Beaurepaire Aragao (1909) state that in *Trichomonas columbarum* the axostyle disappears in greater part, and that "aus dem basalen Teil des Belpharoplastachsenstabsapparates wird durch die Teilung ein neuer Achsenstab gleichsam aus gesponnen."

MacKinnon (1910) finds that in the division of *Trichomonas trichopterae* the axostyle disappears and is replaced in the daughter cells by the thread connecting the dividing basal granules. Fuller data regarding the axostyle and the fate of the paradesmose in this form are necessary before a critical estimate of the data from this species as to the axostyle can be made.

Alexeieff (1910) states that in *Trichomastix motellae* the axostyle is resorbed at division and that the new axostyles are formed from the "fusorial band" (centrodesmose) between the daughter blepharoplast, but he figures no stages in this process.

Janicki (1910) finds in the highly differentiated trichonymphid flagellate *Lophomonas blattarum* an axial structure which he regards as homologous with the axostyle of the trichomonad flagellates. It persists till late stages of binary fission and of multiple mitosis, and the new organs are formed from the "central spindle" or paradesmose. The evidence is very convincing although there is in the phase corresponding to that of axostylar division in *Trichomonas*, a stage represented in his figures 12–14 in which more data are needed on the precise behavior of the old axial organ and of the paradesmose as it is transformed into the daughter axostyles. It is desirable that these stages be further examined.

If his account is the correct one, as the evidence in hand indicates, it is directly contradictory to that presented by us from *Trichomonas*. Three possibilities occur to us to explain this contradiction. The first is that further study will show that the axostyle of *Lophomonas* also divides as the paradesmose fades out and that Janicki has overlooked this division. The evidence at present in hand is strongly against

this view. The second is that there are among flagellates both methods of origin of the axostyle in fission. On purely *a priori* grounds that is improbable, though possible. The third is that the two structures, the axial rod of the Trichonymphidae and the axostyle of the trichomonads are not homologous at all, but are morphologically different structures of different origins. The mass of accessory evidence is against this view. Investigation of the trichonymphids on this point is needed to settle this problem.

Jollos (1911) finds that in the division of *Monocercomonas cetoniae* the old axostyle disappears and new ones arise by division of the basal granule, presumably from the central spindle between the daughter granules.

Martin and Robertson (1911) do not find in *Trichomonas* (= *Tetratrichomonas*) *gallinarum*, *Trichomonas eberthi*, and *Trichomastix gallinarum* that the paradesmose becomes the axostyles of the daughter cells. They note particularly, however, that this structure "can hardly have played the important *rôle* in nuclear division" which other workers have ascribed to it. The old axostyle according to their observations fades away "by some process of solution starting from the anterior end" and axostyles of the daughter cells are in some unexplained way re-formed.

Alexeieff (1912) in a discussion of his own results and those of Dobell (1909) and Martin and Robertson (1911) admits the possibility of several methods of origin of the axostyle and suggests that the method by which the blepharoplast gives rise to various organelles is of no consequence. The primary question, however, is whether or not the several methods are all correctly interpreted.

In a later paper (1913) he figures *Trichomonas augusta* in several stages of mitosis labeling his figure VII, *b-d*, "trois stades de division; la blépharoplastodesmose axiale on centrale qui s'étend entre les deux blépharoplastes-fils devient l'axostyle (en d) des deux individus-fils." His series is incomplete and does not show the derivation stated for the axostyle. The paradesmose in his figure *c* is chromatic, and in *d* achromatic, and the connection between the two quite hypothetical. We interpret his figure *c* as a phase of multiple fission approaching the second mitosis, and his Figure *d* as binary fission with paradesmose apparently gone and the two daughter axostyles meeting end to end, but represented as continuous, comparable with our Plate **4**, Figure 39, as to stage and arrangement of organelles. His actual data as far as figured are not, except for the continuous structures interpreted as daughter axostyles in his figure *d*, contradictory to our results.

Kuczynski (1914) after an elaborate investigation of mitosis in *T. caviae*, *T. muris*, and *T. augusta* comes to the conclusion that the new axostyles first appear after nuclear reconstruction and that they are formed by outgrowth from the "Endgranula" even while the paradesmose is still present and are therefore not formed from it as Prowazek (1904) and Dobell (1909) have stated. As to the actual method of their origin he presents no clear and convincing figures (his figures 110–112) or conclusions, but seems to regard the old axostyle and its axostylar chromidia (kapitalen Granula) as disintegrating and the new axostyles with their chromidia as forming anew (Neubildungen) by outgrowth, but from what is not clear. His figures cited as showing the early indications of the origin of new axostyles as for example his plate 12, figure 24, are far from convincing to us in the light of our own material of the same species.

We conclude from our examination of previous investigations that they are lacking in critical evidence, except in the Trichonymphidae *sensu strictu*, for the origin of the axostyle from the paradesmose, and that the objective evidence is not incompatible with our own fuller series of stages which show clearly the origin of the axostyle by the longitudinal splitting of the parent axostyle.

Behavior during Plasmotomy.

The behavior of the organism in the interval between the completion of mitosis and the termination of plasmotomy is one of ceaseless activity with repeated readjustments of position (Pl. **4**, Figs. 36–40, Fig. F). These involve almost all conceivable spatial relations of the two nuclei with their attached extranuclear complexes. At 1:55 P.M. the blepharoplasts were adjacent at one pole and the axostyles withdrawn within the cytoplasm and presumably parallel (Pl. **4**, Fig. 38). Within five minutes they had migrated 180° apart and had moved the axostyles in line (Pl. **4**, Fig. 39) with a change in form of the body from spheroidal to ellipsoidal. This process was repeated no less than four times before 2:38 P.M. Not only is there movement of each group of flagella with the adjacent nucleus through this arc of 90° but there is also some independent rotation of each individual as indicated by the relative positions, 180° apart, of the undulating membranes of the two at 2:10 and 2:13 respectively. These writhing, twisting contortions continue for four to five hours when finally (Pl. **4**, Fig. 39) tenacity of the cytoplasm is overcome by

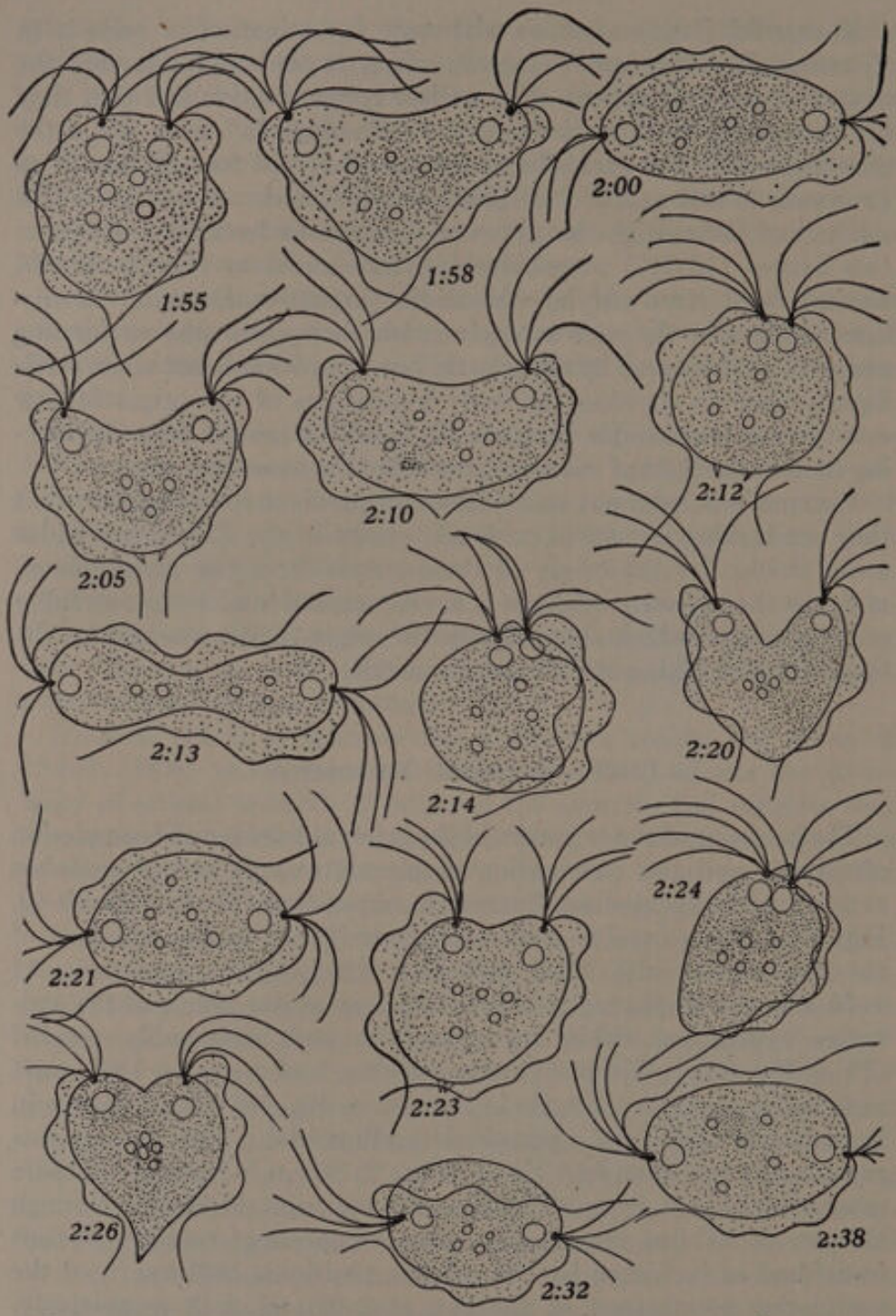

FIGURE F. Sketches by camera lucida from life of *Trichomonas augusta* after mitosis but prior to plasmotomy at intervals during 83 minutes. × 1500. Note four consecutive returns of flagella and nuclei to opposite poles.

an advantageous position of the undulating membranes and the two merozoites pull apart.

These stages might superficially simulate certain aspects of conjugation of gametes, especially such a position as that at 2:12. However, no instance of end to end conjunction with the cytostomes and flagella apposed were noted in this weird dance of monads. The necessity of caution in the search for indications of sexual reproduction in preparations is apparent in the light of this aspect of behavior of the living merozoites in the late stages of mitosis.

The Plane of Division.

The question as to the direction of the plane of division in binary fission has been raised by Martin and Robertson (1911), who find in *Trichomonas* (= *Tetratrichomonas*) *gallinarum* that the division may occur in any one of three planes, longitudinal, transverse, and oblique, the initial direction depending largely upon the direction outgrowth of the new chromatic line.

In any attempt to apply to the phases of mitosis the morphological axes of the free-swimming vegetative phase it is obvious that due regard must be given to the fairly constant relations of the organelles of that stage to its fundamental axes. The blepharoplast lies anterior to the nucleus, and the undulating membrane, marginal filament, chromatic basal rod, and axostyle are, in the main, longitudinal in position. In the mitotic phases on the other hand, the body is distinctly amoeboid and its metabolic changes induce a shifting of these organelles and of their derivatives and new formations so that these original relations to the axes are variously disarranged (Fig. F). These configurations are constantly changing as will be seen in our figures of these stages. It is therefore unsafe to conclude that the relations of organelles in a given preparation of a telophase are indicative of the plane of division, or even of the final act of plasmotomy, for it is probable from our observations on living material that final separation is wont to take place when the daughter blepharoplasts are 180° apart (Pl. 4, Fig. 39). According to these writers this would be a transverse division, but it is really only a transverse plasmotomy, the final act in fission, and not the one which reveals the true morphological relations of the plane of division.

The interpretation of the plane of division should be based on the division of the organelles which bear relations to the axes. In the

division of the blepharoplast and the migration of the daughters 90° to the poles of the intranuclear spindle we have a provision for the division of the nucleus in some one longitudinal plane. The axostyle, chromatic margin, and undulating membrane all split lengthwise, that is in some longitudinal plane, and new parabasal or chromatic basal rod grows out in such a morphological plane, as do also the flagella. On purely morphological grounds the division of the organelles is thus longitudinal. The fact that the activities of the extranuclear locomotor apparatus temporarily disarrange the earlier relations of these organelles in the mobile and less organized cytoplasm of the late mitotic phases does not in the least affect the plane of division of the organelles in question. It remains longitudinal in spite of the possibilities of varying locations of the zone of final constriction of the cytoplasm.

This matter of the interpretation of the plane of division in *Trichomonas* is one of theoretical importance since longitudinal division is regarded as a fundamental characteristic of the Euflagellata, as distinguishing them from the bacteria, especially the spirochaetes, and from the Ciliata in which division is transverse, as well as from the Dinoflagellata in which it is oblique. Exceptions to the universality of the direction of the planes of division thus acquire added interest and justly receive critical inspection. This seeming exception in the trichomonads thus disappears when we base our interpretation on morphological grounds.

The Method of Division.

The division is, as we have shown, truly mitotic though diverging notably from metazoan mitosis in the complications due to the extranuclear apparatus, the inclusion of the division center in the blepharoplast, the extranuclear position of the paradesmose, the homologue of the central spindle, in the persistence of the nuclear membrane, in the precocious splitting of the chromosomes, and in the absence of any development of astral systems. On the other hand chromosomes split lengthwise, migrate to poles of a spindle, share in the reconstruction of daughter nuclei, and the extranuclear organization divides under the leadership of an extranuclear division center, all after the general manner of the metazoan mitosis.

The interpretation of amitosis which Martin and Robertson (1911) have placed on this process in trichomonads is clearly not applicable

in the light of our data which are more complete than those at their disposal. If preparations are not adequately destained irregular pictures such as they figure may be abundant. We have found the alcoholic iron haematoxylin method particularly helpful in giving clean cut nuclear preparations. The fact that the constricting nuclear membrane in the telophase simulates amitotic conditions may be dismissed as without significance for amitosis in view of the differentiation and behavior of the chromosomes in such nuclei in trichomonads.

The theoretical importance of the critical examination of all reported cases of amitosis among the Protozoa is great, not only as to the primitiveness of this form of cell division, but also as to its normality, since all trophozoites are, in so far at least as *a priori* considerations are concerned, potential gametocytes or their ancestors. Since chromosomes are differentiated in trichomonads the significance of the occurrence or non-occurrence of amitosis is thereby increased. We believe the interpretation of division by amitosis to be without adequate foundation. Should undoubted cases of it be found, it will be desirable to know the fate of the trophozoites thus produced.

The existence of chromosomes has been somewhat problematical in the trichomonads, and their number variously interpreted. Dobell (1909) finds six chromatin masses in *Trichomastix batrachorum*, but states that "they cannot justly be called chromosomes." Martin and Robertson (1911) do not regard them as chromosomes, while Kuczinski (1914) affirms their occurrence in *Trichomonas caviae*, but regards eight as the normal number basing his interpretation on stages prior to the metaphase in which later stage he finds four plurivalent chromatin masses. We have given a different interpretation to our findings in the larger and clearer *T. augusta*. The fusion of smaller masses of chromatin or chromomeres (or the chromosomes of Kuczynski) results in the formation of chromosomes which then split longitudinally before entering the equatorial plate. We have distinguished five chromosomes rather than four, though the fifth, a small one, is often difficult to find. Many of Kuczynski's figures of the equatorial plate and adjacent stages show *five* rather than *four* chromosomes, so that we are inclined to think that this species also has five chromosomes and that the process of chromosome organization is essentially similar in his material and ours.

The fact that the number of chromosomes is five, an odd number, raises the question as to whether or not there may also be individual lines of asexual descent with four or possibly six chromosomes and

that we may have here a case of sex chromosomes among the Protozoa. The instances in which we have determined the number of chromosomes are few and though we have not succeeded in finding a critical case of four chromosomes it may be that some of Kuczynski's determinations of four are correct and that both numbers, four and five, occur in the species and possibly in the genus. If this be true the process of gametogenesis and syngamy, in these trichomonads if it occurs, acquires added interest.

Multiple fission in *Trichomonas augusta*.

We have found multiple fission to be a normal phase of widespread occurrence in the life-history of *Trichomonas augusta* and other trichomonads upon which we have worked. It occurs normally and not infrequently in individual hosts. Some hosts contain no evidence of its occurrence, others have large numbers of parasites in some phase of the process. It has been found in *Trichomonas augusta* parasitic in *Rana boylei*, *Bufo halophilus*, and *Diemyctylus torosus*, and always in the usual intestinal smears. Binary fission which is not with certainty distinguishable from the first mitosis in multiple fission also occurs in the same preparations with the late stages of the latter process.

Multiple mitosis results in the formation successively of 2–4–8 nuclei by mitosis with the accompanying multiplication of extranuclear organelles, such as cytostome, motor apparatus including blepharoplast, undulating membrane (including chromatic marginal filament, chromatin basal rod or parabasal body), paradesmose, and the axostyle. The multiplication of the latter by longitudinal division lags behind that of all other organelles so that four nuclei are often seen with two axostyles and eight with four (Pl. 4, Figs. 41, 42).

The result of multiple mitosis is the formation of a syncytium or somatella with eight nuclei each with its full complement of organelles. The nuclei lie in the periphery (Fig. G, 1) with the flagella radiating outwardly and the axostyles pointing subcentrally while the undulating membranes are on surface of the common cytoplasm with their free ends pointed more or less towards the center. Usually one or more, however, have the undulating membrane at the margin of the mass.

After multiple mitosis is completed plasmotomy ensues. This is not coincident for all merozoites but each detaches itself singly from

the somatella. In Figure G is outlined the history of one such multinucleate somatella in its later phases, with from five to one nucleus.

Plasmotomy in this case was prolonged for five hours. The first three merozoites were detached rather quickly in succession in less than ten minutes and the subsequent detachments are outlined in figure G. A merozoite with its undulating membrane at the margin of the mass is in an advantageous position for release and by its turning, twisting, and rotating movements gradually increases the outward projection of blepharoplast and flagella until the undulating membrane is in such a position (Fig. G, 2) that its movements drive the merozoite farther and farther out from the common mass, spinning out behind it around the axostyle a thinning thread of cytoplasm or plasmadesmose (Fig. G, 3) which finally parts with a jerk. The projection rounds into the common mass on the one hand and retreats upon the tip of the axostyle on the other as the merozoite swims away.

This process is again repeated by another merozoite and another (Fig. G, 3–5) until finally only two remain and these soon detach themselves (Fig. G, 6–8) from one another. The connecting cytoplasmic threads spun out between the parent and daughter mass are sometimes (Fig. G, 5) twice the normal length of the trophozoite. They bear evidence to the tenacity and viscosity of the cytoplasm at this stage.

It is obvious that the formative period of the plasmodium offers only 2–4–8-nucleate stages while the prolonged disintegrative period affords all stages with from eight to one nucleus. Some at least, and possibly all of these disintegrative stages may be distinguished from the corresponding ones of the formative period and from the final phase of binary fission by two structural characters. The first of these is the tendency of the chromatin to be massed in an unusually large central karyosome (Pl. 4, Fig. 42) and the second, the larger cytoplasmic chromidia (Pl. 4, Fig. 45). These distinctions are quickly lost by the freed merozoite. Multiple fission does not seem to give rise to individuals distinguishable from those produced by binary fission. The products of a single merogony are subequal, though occasionally considerable inequalities are seen. We are as yet unable to relate the process of multiple mitosis to gametogenesis or to syngamy.

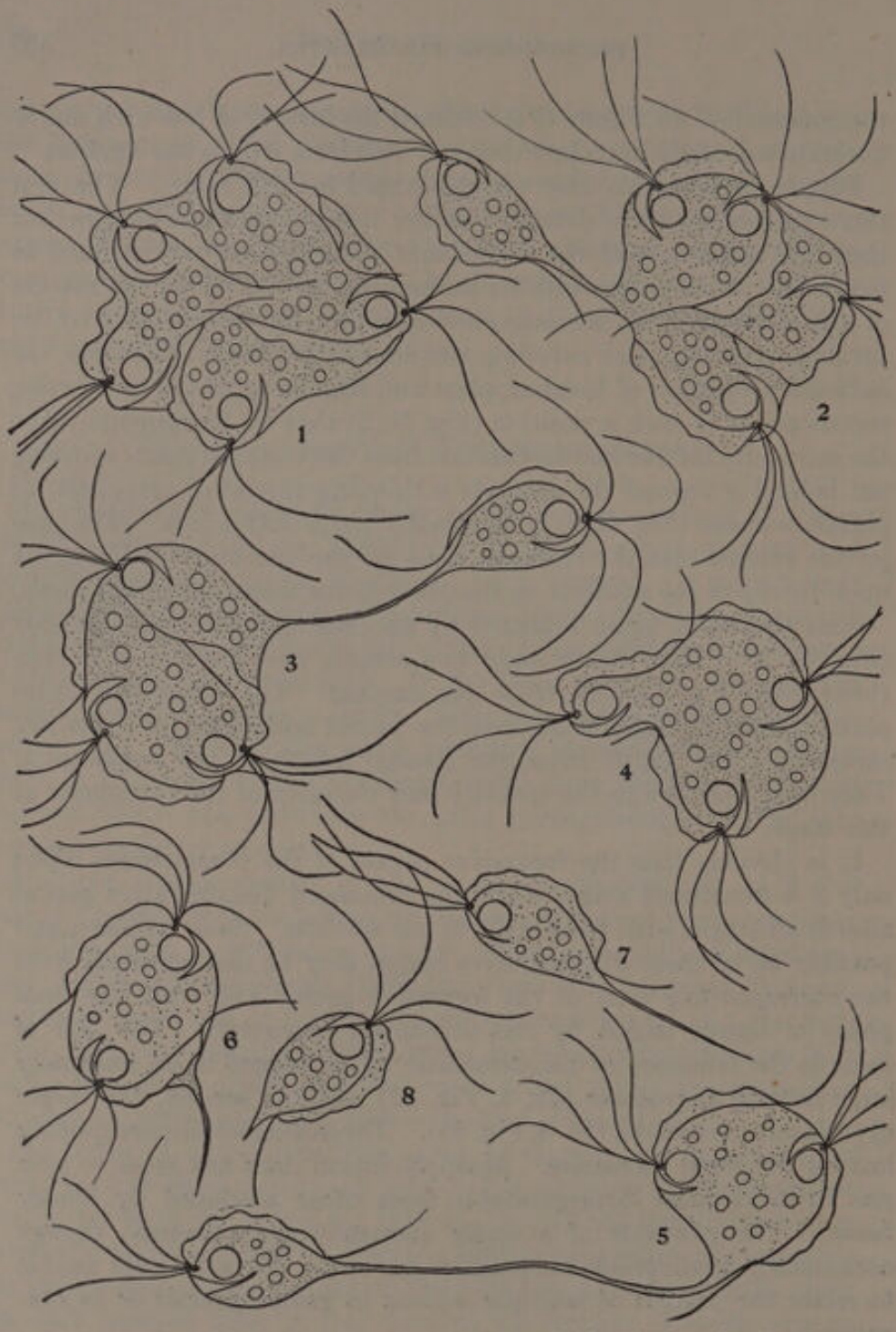

FIGURE G. Disintegrative phase of multiple fission in *Trichomonas augusta* showing detachment of the 4th to the 8th merozoite. × 1500.

1. Somatella or syncytium with five nuclei and one undulating membrane in a marginal position. 2. Fourth merozoite almost detached, two membranes marginal. 3. Fifth merozoite spinning out cytoplasmic thread, two membranes marginal. 4. Sixth merozoite emerging. 5. The same with very long cytoplasmic thread. 6. Binucleate somatella showing remnant of thread of attachment of the sixth merozoite. 7 and 8. Seventh and eighth merozoites showing slight inequality after separation.

Trichomonas muris Hartmann.

Plate 5, Figures 46–66.

The process of mitosis in this form has been very fully discussed by Kuczynski (1914) whose paper appeared while this investigation was of progress. Our results differ from his of several important particulars, especially in the discovery of the occurrence of multiple mitosis, of the origin of the axostyles by splitting, and of the number of chromosomes. In view of his detailed account only a brief summary of our conclusions will be given.

Our material has come from the albino mouse from the culture cages of Professor J. A. Long, to whom we are indebted for the material, and from *Peromyscus maniculatus gambeli* Baird from the Berkeley Hills near the University. Of these wild mice 59 were examined but only 38 were noticeably infected with this flagellate. No appreciable difference between the *Trichomonas* from the two hosts is detectable though the divergence of their lines of ancestry is possibly very great. The albino mice, on the other hand, were somewhat more heavily and more frequently infected, 42 out of 55 yielding *Trichomonas* on cursory examination. Only two instances of a cycle of mitosis in the infected mice were found. Four individuals of *Microtus californicus californicus* (Peale) examined contained no *Trichomonas*.

The Trophozoite.

This stage (Pl. 5, Figs. 46–61) is smaller and more rotund than the corresponding phase of *T. augusta*, its length being 10–15 μ and its transdiameter 7–11 μ. The undulating membrane is relatively very wide and its undulations are not so deeply incised nor so numerous. The nucleus is a trifle smaller (5 μ) than in *T. augusta* (7 μ), and is often richer in chromatin. There are no axostylar chromidia and but a single row of 8–16 large cytoplasmic chromidial granules along the inner side of the chromatic basal rod and parallel to it. These disappear at the metaphase of mitosis and reappear along the daughter chromatic basal rods after the division of the axostyle (Pl. 5, Fig. 60). The axostyle has no capitulum and projects but little posteriorly. It sometimes shows (Pl. 5, Fig. 50, 55) the posterior axostylar granules or ring at the point of emergence. It is usually curved, especially

anteriorly (Pl. 5, Fig. 46), but the nucleus is not in a constant position with respect to the concavity (Plate 5, Figs. 46, 48, 50, 52), indicating some intracytoplasmic mobility of these organelles.

Binary fission in *Trichomonas muris*.

The dense karyosome resulting from the fusion in the telophase of the chromosomes in one central mass is followed by a stage in which the chromatin is dispersed in numerous subequal spheroidal and ellipsoidal granules, with a delicate connecting achromatic net (Pl. 5, Fig. 47). As mitosis approaches a faint intranuclear cloud gives a dark homogeneous (Pl. 5, Fig. 49) appearance to the nucleus and seems to emerge thence into the cytoplasm where it is dissipated. The equidistant subequal chromatin granules, at first over twenty in number, are then arranged in a more or less connected, sometimes spiral (Pl. 5, Fig. 50) skein in which the number of separate chromatin units becomes reduced (Pl. 5, Figs. 50–52) until finally there are but five groups (Fig. 53) which present the appearance of five pairs of chromosomes in parasynapsis, or as we interpret it in the light of our results in *T. augusta*, in five chromosomes split longitudinally. These chromosomes are somewhat unequal in size. There is a pair of small ones, overlooked by Kuczynski (1914) which in the five nuclei in this stage found by us is always located near the anterior surface of the nucleus (Pl. 5, Figs. 53–55). This pair, as in *T. augusta*, also lags in division in the equatorial plate. There is a large pair and three pairs of medium size, one of which is a trifle smaller than the others. They do not divide synchronously in the equatorial plate (Pl. 5, Fig. 56).

Kuczynski (1914) concludes that there are but four of these groups and regards the granules seen in an earlier stage as the true chromosomes and these as plurivalent aggregates. However, since these are the actual masses parted in the equatorial plate (Pl. 5, Fig. 56) it seems more in keeping with the terminology of mitosis generally to regard these as the true chromosomes and the earlier subdivisions as chromomeres, whatever these may be. These pairs of chromosomes which lie with their long axes parallel and rather closely appressed, appear almost to merge into a single unit just before entering the equatorial plate (Pl. 5, Fig. 54). The indications are that they lie in this plate in an end-to-end position as in *T. augusta* (Fig. 56) parting in any event at a median transverse constriction which we assume

from the evidence from *T. augusta* represents the region of end-to-end conjunction.

The mitosis is promitotic, the nuclear membrane remaining intact, constricting at the equator (Figs. 57, 58) and forming at once about the severed daughter nuclei (Fig. 59). The chromosomes after parting increase in length and in volume and become attached to the pole of the nucleus next to the daughter blepharoplast (Fig. 58).

The behavior of the extranuclear organelles in mitosis, is similar to that in *T. augusta*. Their division is initiated by the longitudinal splitting of the chromatic margin of the undulating membrane (Pl. 5, Figs. 49, 51–53) after which the blepharoplast divides into two (Fig. 54) and each migrates to a pole of the fusiform nucleus (Figs. 55, 56). In one instance only (Fig. 56) did we find the division of a daughter blepharoplasts into two granules which we interpreted in *T. augusta* as basal granule and centrosome.

The new chromatic basal line forms at about the same stage in *T. muris* as in *T. augusta* (cf. Pl. 2, Figs. 11–17 and Pl. 5, Figs. 54, 55), though we have not determined the precise method. Kuczynski's (1914) figures also leave this in doubt. The undulating membrane splits and the two membranes part after the formation of the new chromatic basal rod (Pl. 5, Fig. 57). At the division of the blepharoplast one daughter takes one anterior flagellum and axostyle and the other the other two flagella (Pl. 5, Fig. 57) and the full complement of three from each blepharoplast is created by the outgrowth of two and one new flagellum respectively from the blepharoplasts.

The union of the distal ends of the daughter chromatic margins (Pl. 5, Fig. 52) here as in *T. augusta*, we regard as evidence of their origin by splitting, and not by one new outgrowing one alongside the old, as stated by Kuczynski (1914). It is possible that this difference in method is without morphological significance.

Between the migrating blepharoplasts is a slender deeply staining paradesmose (Pl. 5, Fig. 55–61) which does not disappear until after the daughter axostyles are formed.

The origin of the new axostyles occurs late in the telophase in *T. muris* as it does in *T. augusta*, by the longitudinal splitting of the parent structure beginning at the anterior end and progressing to the posterior tip (Pl. 5, Fig. 60). The process is evidently a rapid one as long search was necessary to find an axostyle in division. The instance found is not one of superposition of posterior ends of two daughter axostyles, for the two are plainly united, though the right one lies somewhat higher than the left.

Kuczynski (1914) finds that the old axostyle disappears and that new ones grow out, just how is not clear. We find no individuals in late telophase without one (Fig. 59) or two (Fig. 61) axostyles.

Plasmotomy is delayed some time after the completion of the full complement of new extranuclear organelles, the organism remaining in the state of an active, highly amoeboid, binucleated plasmodium, as in *T. augusta*.

Multiple mitosis in *Trichomonas muris*.

This phase of the life-history has escaped the notice of Kuczynski (1914) in this species also, as well as in the closely related *T. caviae*. It occurs in certain hosts which we have examined and not in others. Our series of the stages (Pl. 5, Figs. 62–66) is confined wholly to the disintegrative phase of the plasmodium or somatella.

We find no indications of more than eight nuclei in plasmodia but have not seen the last two pervading mitoses which result in its formation. Our disintegrative series is limited to plasmodia with 6 (Pl. 5, Fig. 62), 5 (Fig. 63), 4 (Fig. 64), 3 (Fig. 65), and 2 (Fig. 66) nuclei respectively. Some of the earlier phases (Figs. 62, 64), as in *T. augusta*, have their nuclei filled with a dark intranuclear chromidial cloud and in the later ones (Figs. 65, 66) the chromatin becomes segregated in a few large karyosomes. The plasmodia, as in *T. augusta*, disintegrate by the progressive detachment of the small merozoites one by one from the common mass.

The prolonged coherence of the two daughter cells after nuclear division is completed, thus forming temporarily a binucleate organism with two axostyles and eight flagella is significant of the probable method of evolution of the Hexamitidae, for both *Hexamitus* (= *Octomitus*) and *Lamblia* have approximately the equipment of organelles which such a temporary binucleate plasmodium of the Tetramitidae presents. This temporary phase of the trichomonad becomes the permanent one of the higher group.

Tetratrichomonas prowazeki Alexeieff.

Plate 6, Figures 67–78.

First described by Alexeieff (1909b, 1910) as *Trichomonas prowazeki* and later assigned by Parisi (1910) to the subgenus *Tetratrichomonas*, which in the following year was raised to generic status by Alexeieff

(1911a) who found it in the amphibians *Salamandra maculosa, Alytes obstetricans,* in the marine teleost *Box salpa,* and in the leech *Haemopis sanguisuga,* but notes that it is almost certain that the species as thus constituted should be dismembered.

We have found this species only in the urodele *Diemyctylus torosus* in 80 out of 96 hosts examined between March 19 and December. It was associated with *Trichomonas augusta* which is usually very abundant, but was as a rule very rare, being found abundantly only in three individuals in March. It was undergoing multiple fission in these hosts but not in the instances of light infection. This restricted and erratic distribution is suggestive of a possible greater abundance in some other host and an accidental or incidental invasion of *Diemyctylus.* In the three hosts with stages of *Tetratrichomonas* in multiple fission *Trichomonas* was entirely absent. This fact and the usual small numbers suggest but do not prove an antagonistic relationship between these parasites.

The Trophozoite.

This is a small trichomonad, generally somewhat rounded, often pyriform with the larger end posterior, less frequently anterior (Pl. 6, Fig. 68). Its length, based on Alexeieff's (1910) figure and excluding flagella and axostyle, is only 10 μ. In a later (1911d) description he gives the length as 10–14 μ and the diameter as 4–7 μ. In our material it runs somewhat larger, from 12 to nearly 25 μ, but generally less than 20 μ.

It has the usual trichomonad organelles, a spheroidal or ellipsoidal nucleus often presenting a diffuse intranuclear chromidial cloud (Pl. 6, Fig. 67), with a single (Fig. 69) or several (Fig. 68) large karyosomes. An extranuclear chromidial cloud (Fig. 72), and small sparsely present (Fig. 78) or large densely staining (Fig. 76) cytoplasmic chromidia are occasionally seen but they are not regularly present and none has been seen in the axostyle. The nucleus has four chromosomes (Fig. 70). The cytostome (Figs. 67, 68) is relatively large, and food vacuoles, either fluid filled (Fig. 75), or more generally with solid particles (Figs. 67, 70, 77), are found scattered through the cytoplasm. The food taken consists of bacteria (Fig. 77), plant cells (Fig. 67), blood corpuscles of the host, and cysts of amoeba. In one instance an individual (Fig. 69) was found with a large spheroidal cyst described as *Blastocystis enterocola* by Alexeieff (1911c) enclosed *within* its sub-

stance as shown by the continuity of the cytoplasm about it, at least upon the upper surface of the preparation, and by the presence of a slight fluid film of the food vacuole about it. We have also observed a living trophozoite actually envelope one of these cysts having a volume nearly equal to its own, by an amoeboid engulfing movement beginning at the cytostome.

Cysts of this type were originally described by Perroncito (1888) and were regarded by him as belonging to *Cercomonas* (= *Trichomonas*) *intestinalis*. Schaudinn (1903) described their formation by copulating and maturating *Trichomonas intestinalis* from man, and Prowazek (1904) also related them to this species from the rat. Later Bohne and Prowazek (1908) described autogamy in them and Bensen (1909) confirmed their results. Prowazek (1912) later again refers these cysts to *Trichomonas*, but Wenyon (1910) associated them with these flagellates only "as abnormal and degenerate forms." However, Doflein (1911) in the latest edition of his "Lehrbuch" accepts the cysts as those of *Trichomonas* but questions autogamy and reduction, and Donitz and Hartmann have included them in their wall chart of *Trichomonas*.

Dobell (1908b) was the first to expressly doubt their relation to flagellates and refer them to the yeasts or related organisms, a view supported by the fuller investigation made by Alexeieff (1911a, 1911c) who determined their life-history and named the organism *Blastocystis enterocola*.

The activities of *Tetratrichomonas prowazeki* resemble those of *Trichomonas augusta* with which it is associated and in the living condition the two are readily confused. In stained material the additional anterior flagellum, axostyle, contents of vacuoles, and absence of axostylar chromidia at once differentiate it from *Trichomonas*.

The extranuclear motor apparatus is identical in its component organelles with that in *Trichomonas* except for the presence of four instead of three anterior flagella. Alexeieff states that these are of unequal length, there being two subequal long ones and two subequal shorter ones. Our observations, Alexeieff's (1910), and Parisi's (1910) figures agree in indicating some variability in length, but we find no constant morphological differentiation in these organelles.

The undulating membrane is less developed than in *Trichomonas* having less elevation, fewer undulations and a more slender and usually relatively shorter chromatic basal rod. The axostyle is very slender, exceedingly hyaline, devoid of chromidia, and without anterior capitulum. It usually projects posteriorly (Fig. 68) as a slender

point. The blepharoplast is exceptionally large. We have not found it separating in this species into centrosome and basal granule as in *Trichomonas augusta* and *T. muris*.

Binary Fission.

No attempt has been made by us to find the full history of binary fission in this species since it differs so slightly in organization from *Trichomonas augusta* which we have described so fully. It presents certain obvious disadvantages for such study, namely the absence of axostylar chromidia, the abundance of vacuoles filled with solid food obscuring structure (Pl. 6, Figs. 67, 69), and the smaller size. We therefore present no data on binary fission in this species except to note that the four flagella are shared two and two by the daughter blepharoplasts at mitosis (Fig. 70).

Multiple Fission.

Fortunately this phase occurred in abundance in the large intestine of *Diemyctylus torosus* in March in hosts which had been scantily fed and retained in the laboratory for four to six weeks after the close of their breeding season. Slides from the intestinal wall contain stages both of the formation and of the disintegration of the plasmodium or somatella (Pl. 6, Figs. 70–78).

The formation of the 8-nucleate plasmodium results from three successive completely pervading mitoses (Pl. 6, Figs. 70–72) without plasmotomy. The nuclei at the close of the process (Fig. 71) have a single central karyosome and an intranuclear chromidial cloud. Later the karyosome disappears and fine chromatic granules are distributed throughout the nucleus (Fig. 72). The blepharoplasts are peripherally located and the undulating membranes terminate near the middle when the organism is on the substrate (Fig. 72). The axostyles are not readily found in these stages because of their small size and when present are easily confused with the elongated cytostome (Fig. 72).

The disintegrative phases are readily distinguished from those in the formative process in all cases of odd number of nuclei but not in the case of the even number when the nuclei are in the resting condition, especially from the 2- and 4-nucleate stages. Dispersal of the

merozoites occurs by the successive detachment by plasmotomy of single individuals, resulting in plasmodia with 7 (Fig. 73), 6, 5 (Fig. 74), 4 (Fig. 75), 3 (Fig. 76), and 2 nuclei. A plasmodesmose indicating recent plasmotomy is seen in some cases (Fig. 77). The later phases of this process are marked by the aggregation of the nuclear chromatin in larger karyosomes (Figs. 74, 77, 78) and the disappearance of the dense intranuclear chromidial cloud. In one case, possibly pathological (Fig. 76), the cytoplasm was crowded by many large deeply staining chromidial spheres approaching the nuclei in size. The process of multiple fission in *Tetratrichomonas* is similar in its main outline and its end result to that in *Trichomonas augusta* as previously described by us.

Eutrichomastix serpentis (Dobell).

Plates 6, 7, Figures 79–104.

Material.

This small flagellate was found in three snakes from California, to wit, in the yellow gopher or Pacific bull snake (*Pituophis catenifer* (Blainville)), in four of the five individuals examined, in the common garter snake (*Eutaenia sirtalis* (Linn.)), in one of the two examined, and in the Pacific rattlesnake (*Crotalus oregonus* (Holbrook)) in the one individual examined. It was also found in one of four individuals of *Python reticulatus* (Schneider) from Borneo examined at least twenty-four hours after death.

The parasites were found in each host in greatest abundance in the upper part of the large intestine. Smears prepared from the wall in this region contain division stages of *Eutrichomastix* in such numbers that it has been possible to work out both binary and multiple fission in this species.

Historical.

A flagellate parasite of the intestine of snakes was first described by Hammerschmidt (1844, 1845) from *Coluber* (= *Tropidonotus*) *natrix* as *Bodo colubrorum*. We have seen only the translation of his paper (1845) but from the crude figures therein reproduced it is quite impossible to determine with certainty what genus he had in hand.

He figures but a single anterior flagellum and a rather long posterior one projecting from the body. This is somewhat *Cercomonas*-like and lacks wholly the clear presentation of any *Eutrichomastix* characters, though it is quite possible that he may have been dealing with this genus but failed to distinguish its organelles.

Later Blochmann (1883) included in a paper dealing with parasitic and marine flagellates a description of a new form ascribing it to Bütschli who discovered it in the cloaca of *Lacerta agilis*. He gave it the name *Trichomastix lacertae* Bütschli apparently unaware that Vollenhoeven (1878) had previously proposed this same generic name for a hymenopteron.

This preoccupation, which no subsequent writer, except Stiles (1902) seems to have noted, necessitates a new generic name for the trichomonad. We have therefore proposed *Eutrichomastix* as preserving, at least for protozoologists, a clue to the relationships of the flagellate, and designate *Eutrichomastix lacertae* (Bütschli) as the type of the genus.

The question of the correct specific name for the organism is less readily determined since we know so little of the morphological changes incident to the life-cycle and to change from one host species to another. It seems best, as Dobell (1907) has done, to leave unutilized Hammerschmidt's name *colubrorum*, since it is indeterminable. If both Bütschli's (see Blochmann, 1883) *lacertae* and Dobell's (1907) *serpentis* are permitted to stand they should rest upon morphological distinctions rather than host habitats. Such distinctions are apparently lacking between the figures of Blochmann and of Prowazek, and those of Dobell. The range of variation in size and proportions only partially represented in our figures (Pl. **7**, Figs. 79, 81) from a single host, is such that we are loath to attribute specific values to slight differences in proportions.

Moreover, individuals upon which we have worked from hosts as widely separated in classification as *Python reticulatus* (Boidae) and *Crotalus oregonus* (Crotalidae) are not morphologically distinguishable. Nor does geographical separation seem to differentiate them since our material is recognizably similar to that of Bütschli, Prowazek, and Dobell from Europe, and forms in *Python* from Borneo examined shortly after arrival in San Francisco are not different from those in *Crotalus* from California.

The possibility that forms in snakes may be different from those in lizards, is, however, an open one, and for the present we tentatively refer the species upon which we have worked to *Eutrichomastix ser-*

pentis (Dobell) with the expectation that later work will clear up the differences between, or the identity of the species of *Eutrichomastix* in lizards and snakes.

The Trophozoite.

This small flagellate has an elongated or a stout body of variable outline, ovate, obovate, pyriform, slightly asymmetrical or more or less irregular, the latter feature resulting doubtless from its amoeboid activities on the slide as substrate. Free-swimming forms tend to be elongated and individuals resulting from recent division are typically elongated (Pl. 7, Fig. 79). The stouter forms predominate in intestinal smears. The mucus of the intestinal wall and its glands appears to be the medium in which the less active and larger stages prior to binary and multiple fission are developed. The range in form in our material from long slender to short stout (Pl. 7, Figs. 79, 81) ones is analogous to that noted above in *Trichomonas augusta*. It may also in this form represent to some extent a cyclic change from the slender type resulting from binary or multiple fission to the stout form preceding one or both of these forms of reproduction. It is possible that copulation may precede both or either of these forms of multiplication, especially the latter, but no evidence of it has been detected in the course of our examination of the material. Individuals in the metaphase of mitosis (Pl. 7, Fig. 84) and in multiple fission (Pl. 8, Fig. 99) become more or less spheroidal or ellipsoidal.

Since the whole range in form occurs in a single host (*Crotalus*) and since we can find no group of morphological characters to distinguish species within this complex we regard all of the forms as in the cycle of one species. The range is sufficiently wide to include the slender form figured by Blochmann (1883) as *Trichomastix lacertae* Bütschli from *Lacerta agilis*, the stouter one figured later by Prowazek (1904) from smears from the intestine of *Lacerta muralis*, and the stout forms figured by Dobell (1907) as *Trichomastix serpentis* from the rectal contents of *Boa constrictor*. Dobell states that his culture contained many involution forms.

The length from anterior end to the posterior tip of the axostyle in our material ranges from 8–20 μ and is usually 13–15 μ in active vegetative forms. The diameter varies greatly in different individuals, even of the same length and even in the same individual under different conditions of amoeboid activity. It ranges 4 μ in very slender

forms (Pl. 7, Fig. 79) to 10 μ in stouter ones (Pl. 7, Fig. 81), is usually 7–8 μ, and may attain 12–13 μ in metaphase and multiple fission stages.

The organs of this flagellate consist of three anteriorly directed flagella, one longer posteriorly directed one, all originating in an anteriorly located blepharoplast. Adjacent to this organ is the crescentic cytostome, and the spheroidal or ellipsoidal nucleus, and from it passes posteriorly the slender axial axostyle.

The enveloping cytoplasm has no structurally differentiated pellicle and is not separated into ectoplasm and endoplasm. It is very completely vacuolated with fluid filled vacuoles which are subject to considerable variation in size and uniformity. In some individuals they are few and large, four across the body, while in others they are small and numerous six to eight across the body, while in still others they are of mingled large and small sizes in varying proportions. They are usually small and numerous in premitotic and mitotic phases. In life and in Schaudinn-iron-haematoxylin preparations these vacuoles show no structural contents and are apparently fluid filled. Overheated slides show granular contents similar to those found in cytoplasmic vacuoles of *Trichomonas augusta* which stain with neutral red.

There is no evidence with the stains we have employed of solid food particles in these vacuoles, nor has the ingestion of such particles been observed in living individuals. The presence of the cytostome suggests its possibility but no evidence that solid particles are ever taken was seen among many living and prepared individuals under observation. No traces of deeply staining chromidia and no well-developed intra- or extranuclear chromidial clouds were detected. The dark granules of uneven size noted by Dobell (1907) do not have the appearance of chromidia. Similar granules appear in several of our figures (Pl. 7, Figs. 87, 92). The cytostome is a somewhat crescentic tract on the "ventral" side of the body near the anterior end. Its concavity lies adjacent to the nucleus and its upper end is slightly larger. There is no undulating membrane in it and no evidence of a localized pharynx leading from it deeper into the cytoplasm.

The extranuclear motor apparatus consists of the blepharoplast, flagella and axostyle, all connected and all involved with the nucleus in the process of mitosis. The blepharoplast lies rather close to the surface near the anterior end of the body, closely attached to the head of the axostyle. It is of a dense black color, has no cytoplasmic halo about it, is sometimes quadrangular, but usually spheroidal. We have been unable to resolve it into the four basal granules of the four flagella, as Martin and Robertson (1911) have for their *Trichomonas*

gallinarum and *Trichomastix gallinarum*. In one instance (Pl. 7, Fig. 80) it is divided into two granules but these are regarded by us as the initial step in the prophase of mitosis. Its diameter is 0.5–0.2 μ.

The blepharoplast gives rise to the four flagella which pass across the narrow film of cytoplasm and emerge at or near the anterior end. Three of these flagella, the anterior ones, are shorter than the fourth, and are usually directed anteriorly in a single cluster which lashes from side to side, or which may spread out and each flagellum act independently of the others in its position and movements. These flagella are about 1.5 times the length of the axostyle in length. The remaining flagellum is a trailing one but does not differ in diameter or stainability from the others Its length, however, is nearly twice as great, and in locomotion it trails posteriorly in a sweeping curve at one side of the body. Its position and relative length suggest its homology with the marginal filament and posterior flagellum of the undulating membrane of *Trichomonas* though it exhibits no trace of heavier caliber or greater stainability such as is characteristic of both the marginal filament and chromatic rod within the undulating membrane of *Trichomonas*, though not of their extension in the free posterior flagellum.

The axostyle is a slender hyaline rod, of nearly uniform caliber throughout, sometimes slightly tapering distally, sometimes slightly enlarged in that region. Its length is about twenty times its diameter and ranges from 10–15, rarely 18 μ. Anteriorly it terminates without enlargement in the blepharoplast and posteriorly it contracts rather abruptly to a sharp point. We have found no structure within it. Its shape is subject to considerable variation. It is straight or nearly so in elongated forms (Pl. 7, Fig. 80) and often much curved almost to a semicircle or bent at right angles in rounded ones (Pl. 7, Fig. 94). In life it is subject to incessant turning from side to side in a fashion difficult to follow in the combined rotation, amoeboid movement, and axostylar contortions of the organism. It appears to be the localized center in which these powerful movements occur which resemble as much as anything the labored strokes of a heavy stout flagellum. In fact this axostyle more than that of any species we have examined resembles an intracytoplasmic flagellum whose movements are impeded by the tenacious medium in which it is imbedded. Its posterior end often projects for a short distance as a naked shaft beyond the cytoplasm, even for as much as 0.3 of its length. It forms the axis along which blobs of cytoplasm may be severed by plasmectomy from the body, and dropped off at the posterior end (Pl. 7, Fig. 80).

The spheroidal or ellipsoidal nucleus generally has a single (Pl. 7, Fig. 79) large central karyosome or prior to mitosis several large ones (Fig. 81). Its membrane is intact at all times and the peripheral chromatin is not evident in decolorized nuclei. The intranuclear cloud (Pl. 7, Fig. 79) is less in evidence, as is also the extranuclear one (Fig. 85), than in *Trichomonas augusta* where cytoplasmic chromidia are abundant.

Binary Fission in *Eutrichomastix serpentis*.

Both binary and multiple fission occur here as in *Trichomonas*. Both are by divisions of the promitotic type with differentiated chromosomes, intact nuclear membrane, and intranuclear spindle with extranuclear paradesmose as described in *Trichomonas*.

The first indication of mitosis is the faint intranuclear cloud (Pl. 7, Fig. 79) followed by an increase in the amount of chromatin and its aggregation in a number of large granules which towards the end of the prophase form four chromatin masses or chromosomes (Pl. 7, Figs. 82, 83).

During this phase the blepharoplast divides, and the daughters migrate to the poles of the elongating nucleus, spinning out a deeply stained extranuclear paradesmose between them. The new blepharoplasts are not at first adherent to the nucleus and traces of faint connecting fibrils in addition to the paradesmose are found between them and outside, at least in part, of the nuclear membrane (Pl. 7, Fig. 83). Later the blepharoplasts become adherent to the poles of the now fusiform nucleus, the paradesmose forms a distinct black line on its periphery from pole to pole, and the faint delicate fibrils between the blepharoplasts become apparently wholly intranuclear (Pl. 7, Figs. 85, 86). No asters form about the poles. Each blepharoplast is attached to two flagella, the posterior trailing and one anterior flagellum to one, and the other two anterior flagella to the other. Later in the late telophase two new flagella grow out from each blepharoplast (Pl. 7, Figs. 91, 92) completing the complement of these organelles.

The metaphase (Pl. 7, Figs. 86–89) brings the chromosomes into the equatorial plate. We have found no evidence here of any precocious longitudinal splitting of the chromosomes prior to their assembling in the equatorial plate. The chromosomes are consistently unlike, There is one large one, one long one, and two subequal medium ones. One of them (Plate 7, Fig. 88) sometimes lags in division. Division in the equatorial plate is by constriction at the middle, that is by trans-

verse separation as the chromosomes are at that time located in the spindle, thus resembling this phase of the process in *Trichomonas*.

The anaphase (Pl. 7, Figs. 89, 90) is quickly accomplished, the migration of the chromosomes and their ultimate attachment to the polar blepharoplast extending into the telophase as in *Trichomonas*. During the late anaphase the nuclear membrane (Pl. 7, Fig. 90) constricts at the equator and the nucleus takes on a dumbbell shape and the chromosomes increase in size.

In the telophase (Pl. 7, Figs. 91–94) the nuclei are reconstituted. The enlarged chromosomes cluster about the blepharoplast and become attached to them by a dark chromatic thread (Fig. 91). Later they become massed together (Fig. 93) into the central karyosome. In the meantime the new cytostome has been formed (Fig. 91), and two axostyles are found in place of one (Figs. 91, 92). We have but one slide showing many phases of mitosis and it is unfortunately so decolorized that the originally small and unstained axostyle cannot be traced during mitosis. Two new axostyles appear in this organism, however, at the stage exactly corresponding to that of their formation by splitting of the parent axostyle in *Trichomonas augusta*. They are also formed prior to the disappearance of the paradesmose (Pl. 7, Fig. 93). The incomplete findings here are in accord with the interpretation of the origin of the new axostyles by splitting of the old and not anew from the paradesmose as maintained by Prowazek (1904). The deceptive nature of Prowazek's evidence may best be judged by a comparison of his figures (1904, Pl. 1, Fig. 12) with that of a corresponding stage in our material (Pl. 8, Fig. 96) in which the long-spun-out deeply stained paradesmose lies immediately over and subparallel to the new daughter axostyles. It is evident that Prowazek wholly overlooked these new organelles and mistook the continuous paradesmose for their source.

Plasmotomy finally separates the two daughter trophozoites and is accomplished after the two nuclei migrate to the poles 180° apart and thus elongate the cytoplasm. This occurs after the paradesmose disappears (Pl. 8, Figs. 95, 96, 104). During this stage the trophozoites are exceedingly active as in *Trichomonas* and assume a great variety of relations in rapid succession. For example in one instance in a preparation (Pl. 8, Fig. 97) the two trophozoites are each surrounded by a spheroidal mass of cytoplasm and still connected by a cytoplasmic bridge containing the paradesmose. The two are quite unequal in diameter. This might be interpreted as indicative of unequal division or in the absence of the paradesmose, as the copula-

tion of micro- and macrogamete. However, in the light of the intense activity of the living forms at this stage it must be regarded only as a chance phase of contraction without morphological meaning.

Multiple Mitosis in *Eutrichomastix serpentis*.

We have found evidence for the occurrence of this phase in *Eutrichomonas* from both *Crotalus* and *Pituophis*, and our series of stages is incomplete only in the disintegrative phase. The material is apparently normal in all cases and was found in but a single host in each species.

The formative phase of the plasmodium or somatella is fully represented (Pl. 8, Figs. 98–100). We find no means of distinguishing ordinary binary fission from the initial division which is followed in multiple fission by the two succeeding synchronous mitoses. Therefore any binary fission may supposedly serve as illustration of the first division in multiple mitosis (Pl. 7).

The second mitosis in multiple fission (Pl. 8, Figs. 98, 99) is synchronous in both nuclei resulting from the first mitosis. That this 4-nucleate phase is thus formed is shown by the persisting plasmodesmose (Figs. 98, 99) joining the sister nuclei. The number of chromosomes in the nuclei of the plasmodium is four (Pl. 8, Figs. 98, 99), the same as in binary fission. The plasmodium is therefore not in a diploid and the trophozoite in a haploid condition.

The derivation of the 8-nucleate plasmodium from the 4-nucleate is shown (Pl. 8, Fig. 100) by the four paradesmoses which join the sister nuclei derived from the four of the preceding stage.

It is difficult if not impossible to follow the history of the extranuclear organelles during the formative phases of the somatella. The ultimate formation of the full complement is indicated by their presence in the later disintegrative phases (Pl. 8, Fig. 103). There is some evidence that the multiplication of axostyles is delayed in multiple fission as in binary. There are apparently eight cytostomes and four axostyles in the 8-nucleate plasmodium shown in Plate 8, Figure 100, but not as yet the full complement of flagella.

We have no evidence as to the duration of any of the plasmodial stages. We do not find more than eight nuclei and the 8-nucleate plasmodium disintegrates by the successive detachment of the single component merozoites. There is no evidence of a regular plasmotomy to 4- and 2-nucleate plasmodia reversing the order of the forma-

tive steps. Plasmodia with six (Pl. 8, Fig. 102) and with 3 (Pl. 8, Fig. 103) nuclei have been found. It is impossible to distinguish binucleate plasmodia (Pl. 8, Fig. 104) of the disintegrative phase of multiple fission from late phases of binary fission.

No evidence appears to indicate that the component merozoites have any fixed relations in the somatella or any order of succession in detachment. The position of the daughter nuclei in parallel rows in one plasmodium (Pl. 8, Fig. 98) and at right angles in another (Pl. 8, Fig. 99) and the great mobility of the component merozoites noted in the multinucleate plasmodium of *Trichomonas augusta* (see text figure G) render any constant morphological relations of the component units of the somatella very improbable.

Discussion of Multiple Mitosis.

The occurrence of multinucleate forms in the life-history of trichomonads has been fragmentarily observed by several prior investigators of these flagellates though none has indicated the range and significance of the phenomenon.

Marchand (1894) noted without comment the occurrence in *Trichomonas vaginalis* of very large individuals with more than two nuclei but does not state the number. He finds in preparation a stainable thread joining the nuclei in pairs and the presence of flagella with each nucleus. He thus evidently had the formative phases of the plasmodium under observation. His single figure (1894, pl. 3, fig. 18) shows a 4-nucleate plasmodium with the paradesmose joining two of the nuclei. Another figure (his pl. 3, fig. 7) might be interpreted as a disintegrative phase in the binucleate stage with the last plasmodesmose just retreating into the common mass of cytoplasm.

Prowazek (1904) figures as a "Teilungstadien" of *Trichomonas lacertae* a single 3-nucleate plasmodium with a still persisting plasmodesmose, and the same three hours later with one merozoite nearly detached. He merely notes in passing the prolonged process of plasmotomy and the fully developed organelles of the three individuals. He presents no data as to the method of their origin or dispersal, but designates the instance as one of "gleichzeitig erfolgende Mehrfachteilung." It is evident that his own data do not justify the adjective "gleichzeitig" nor do our observations of a progressive dispersal confirm it.

Dobell (1907, Pl. 27, Fig. 14) records for *Trichomastix serpentis*

"giant forms divided abnormally commonly giving rise to three or four daughter cells." He further states that division, in living forms under observation, rarely became complete, a conclusion which our observations indicate was merely the result of the slowness of the process and its metabolic phases. That it may be regularly completed is indicated in our text figure F, of *Trichomonas augusta*. He further concludes that the whole finally fuses into a large amoeboid mass which finally dies. This may be true in moribund cultures but we do not regard this as the normal outcome of the process.

The occurrence of multiple mitosis in the Hexamitidae is definitely established by the observations of Noc (1909) and of Prowazek and Werner (1914, pl. 10, fig. 13) on *Lamblia intestinalis* in which they find and figure individuals with eight nuclei, that is, a trophozoite which has undergone two of the three synchronous mitosis, for it is obvious that the plasmodial stage of the binucleate *Lamblia* corresponding to the 8-nucleate plasmodium of *Trichomonas* will have sixteen nuclei. Noc (1909) also figures two multinucleate structures with an indeterminate number of nuclei, which he regards as multiple division stages of *Lamblia*, though the morphological basis for such reference requires further elaboration.

Our observations demonstrate the occurrence of multiple mitosis in normal fresh material from normal hosts in *Trichomonas augusta* Alexeieff, *T. muris* Hartmann, *Eutrichomonas serpentis* Dobell, and *Tetratrichomonas prowazeki* Alexeieff, as well as in certain other trichomonads an account of which will be given in later papers. The process is thus one of widespread occurrence and seemingly a wholly normal one in the Tetramitidae (*Trichomonas, Eutrichomastix, Tetratrichomonas*) in which it results in free-moving non-encysted plasmodia composed of eight merozoites which subsequently slowly detach themselves singly from the plasmodium. In some of the Hexamitidae (*Lamblia*) on the other hand, the developing plasmodium comes to be more or less encysted (Prowazek and Werner, 1914) apparently as a rule, and the method of release of merozoites is not as yet evident. Plasmodium formation by multiple fission is thus a normal process of general occurrence throughout the Polymastigina as defined by Doflein (1913).

The repeatedly offered inference that the multinucleated, multiflagellated Trichonymphida are related to the Polymastigina therefore gains added probability, for the temporary multinucleated, multiflagellated plasmodium of the Polymastigina, or more especially of the Hexamitidae, may be regarded as an evolutionary step in the

direction of permanently multinucleated multiflagellated Trichonymphida.

The occurrence in this important order of Euflagellata of a definite multinucleate stage composed of merozoites forming a common plasmodium which for a brief time at least lives an individual life of its own, is, we believe, of very great general significance and one, moreover, very largely if not wholly unrecognized. This has resulted from the dominance in developmental textbooks of the Gastraea Theory and the prevalent but mistaken use of *Pandorina* and *Volvox* as transition types between Protozoa and Metazoa. We will not develop this matter farther here, but in passing desire to emphase this multinucleate plasmodium as an initial stage in the formation of aggregates which ultimately become the Metazoa. This stage is, however, only a multinucleate, not a multicellular individual. The formation of such individuals at certain periods in the life-history of the lower Protozoa, the Mastigophora and the Sarcodina, as well as in the more highly specialized Sporozoa and Ciliata, is, in our opinion a phenomenon of fundamental evolutionary significance. We have therefore used for it the prophetic name of somatella.

The nature of the organism which forms the plasmodium or somatella is naturally a matter of great interest morphologically, phylogenetically, and physiologically. We have been unable to obtain conclusive evidence as to its relationship to other stages or its inducing causes. Binary fission occurs in trichomonads in cycles or waves, at least this stage is found abundantly only in occasional individual hosts. In like manner multiple mitosis is restricted in its occurrence. It appears to occur at certain phases of the life-history, or under certain conditions of the environment, or both. We have not found gametogenesis, nor gametes, nor any evidence that the organism giving rise to the plasmodium is a zygote. Should this ultimately prove to be the case the name somatella for the plasmodium phase will then be doubly applicable.

Addendum.

Since the completion of this manuscript we have received the paper of Janicki "Untersuchungen an parasitischen Flagellaten II." (1915) in which he adds much to our knowledge of the Trichonymphida and trichomonads. Unfortunately his material has not permitted a full analysis of all stages in mitosis or of the origin and fate

of all of the organelles. The following comments are made on his conclusions with regard to the lower flagellates related to *Trichomonas*.

The transfer of *Devescovina* to, and the inclusion of *Foaina* with the Polymastigina is in accord with our conclusion that the chromatic basal line of *Trichomonas* is the homologue of the parabasal in other forms. His inference that the parabasal of *Devescovina* divides at mitosis is not supported by evidence of actual division. It grows out independently of the old parabasal in *Trichomonas* and may be expected to do so in *Devescovina*. He still revives the old figure of *Trichomonas* showing the stout body which he calls parabasal though recognizing its rarity and temporary status, but does not reinvestigate material nor recognize the relationships of this organ to the chromatic basal rod or true parabasal. His evidence presented for the origin of the new parabasal by outgrowth in the case of *Stephanonympha* is on the other hand amply conclusive.

The extrusion of bacteria-like granules from the nucleus at the telophase in *Devescovina* is apparently analogous to the formation in *Trichomonas* of extranuclear chromidia at the prophase as we have described it.

His description of the behavior of the extranuclear organelles in mitosis in *Devescovina* differs in certain very important particulars from our conclusions in the case of *Trichomonas*. In the first place he figures (his pl. 13, figs. 10, 11) two extranuclear division centers. One of these spins out the extranuclear paradesmose (his "Spindel"). between the daughter division centers each of which consists of a centriole to which is attached a single parabasal and a group of flagella exactly as in *Trichomonas*. These daughter centers become the blepharoplasts of the daughter organisms. He states however that these centers do not arise from the blepharoplast but independently of it, no source being stated or evident in the figures. We believe this inference incorrect and that they will be found to arise from the parent blepharoplast. A second center, figured but twice, consists of a dark granule within a light halo, both of which structures are spun out in line parallel to the paradesmose. Their fate is unknown, but he suggests that they become the suspensory lamellae coming from blepharoplast past the nucleus towards the axostyle. This second division center is regarded by him as the true blepharoplast and the line between them as the "Desmose." Our interpretation of this organelle, based on *Trichomonas*, is that this second dividing center is not the blepharoplast at all but may perhaps be the remnant of the

axostyle (see his pl. 13, fig. 7) and that furthermore it may give rise by its division to the clear area with darker axial line which later disappears (his pl. 14, figs. 15, 16), out of which the new axostyles are formed. Such an alternative interpretation is in harmony both with our findings in *Trichomonas* and with his data.

In a second important particular his findings diverge from ours, namely with reference to the origin of the axostyle which in *Devescovina* is said to disappear and to be re-formed in the late telophase as a clear area connecting the daughter nuclei in the axis of which the "Spindel" or paradesmose is found. This structure later becomes V-shaped and finally parts at its apex giving rise to the two axostyles in which, however, the deeply stained axis is no longer visible. We believe his data though not his interpretation, may be harmonized with our findings. As we have suggested above, the clear area and granule functioning as a division center and regarded by him as the true blepharoplast, we interpret as the axostyle. Its position (his pl. 13, figs. 7, 10, 11) in view of the great mobility of organelles indicated in his figures of these stages is not a serious difficulty. His series of figures of this organelle is short and disconnected from later stages (his pl. 14, figs. 15–17) to which we link it. It seems probable that it passes over as above stated into the organ interpreted by Janicki as the re-forming axostyle. This organ differs from the daughter axostyle in *Trichomonas* in being continuous from one daughter nucleus to the other (his pl. 14, figs. 15, 16), a condition possibly resulting from the reduction of the parent axostyle to a small "kapital Granula" of Kuczynski. It differs also from the conditions in *Trichomonas* in the fact that it seems to include in the axial position the disappearing paradesmose. In *Trichomonas* this disappears about the time that the daughter axostyles are completed but we have not been able to trace it into any structural relation to the axostyle. An alternative explanation is open, namely that the dark axial line of his figure 16 and possibly also in 15 is derived from the dark axial lines in the dividing organelle of his figures 10 and 11 which he calls the blepharoplast, and is not (in his figures 10 and 11) the paradesmose or his "Spindel." A reinvestigation of a full series of stages is necessary to establish the correctness of our alternative interpretation that the axostyle is also derived here by the division of the ancestral organelle.

The application of the term "Spindel" to the extranuclear strand or paradesmose between daughter blepharoplasts is objectionable in view of the recurrence (in *Trichomonas*, and presumably in *Deves-*

covina where intranuclear phenomena are, as far as known, quite similar) of an intranuclear spindle on which the chromosomes are divided.

Janicki holds that the chromatic bodies seen in mitosis are not chromosomes but plurivalent chromatic strands, organized of numerous smaller granules or chromosomes. He figures four of these larger bodies in the anaphase. In form and arrangement they resemble the bodies in *Trichomonas* which we have interpreted as chromosomes.

In view of the complexity and variety of structure of the extranuclear motor apparatus in the Trichonymphida *sensu strictu* and of the divergence of results between Janicki and ourselves as to the fate of the axostyle, of the origin of the division centers at mitosis, and as to the nature and status of the chromosomes in the group of complex flagellates, it is very desirable that the process of mitosis be more fully investigated than has been possible with the limited material thus far available. The homologies of the axostyle and parabasal in *Lophomonas* and in the multinucleate trichonymphids with the simple structures in *Trichomonas* should be adequately established by a series of comparative studies in the former group and the history of organelles traced through the critical late and postmitotic periods.

Conclusions.

1. The upper part of the large intestine of most vertebrates is infected by trichomonad flagellates whose phases of mitosis and multiple fission occur at intervals and are met with in a few of the many hosts examined. These processes are carried out during periods of great amoeboid activity of these parasites in the mucus of the intestinal epithelium.

2. Mitosis is promitotic with nuclear membrane intact throughout the period of division, with nuclear separation by constriction simulating amitosis. It is, however, essentially mitotic with extranuclear division centers, intranuclear spindle fibres, chromosome organization out of a chromatin network and skein.

3. The chromosomes are definite in number, four in *Tetratrichomonas prowazeki*, and five in *Trichomonas augusta*, *T. muris*, and *Eutrichomastix serpentis*. They are differentiated in form, there being one small one, and some fairly constant size differences among the larger ones. They are differentiated in behavior, the small one

(in *T. muris*) having a particular location in the nucleus in the late prophase, and lagging on the spindle in the metaphase.

4. The chromosomes appear to be split longitudinally prior to their arrangement in the equatorial plate, and seem to slip into an end-to-end position in this plate, or to be parted by a transverse constriction.

5. The extranuclear organelles all share in the process of mitosis. The blepharoplast from which flagella, rhizoplast, chromatic margin and basal rod, and axostyle all take their origin, contains the division center. It parts into two bodies which go to the two poles of the fusiform mitotic nucleus spinning out the deeply staining always extranuclear paradesmose between them.

6. The daughter blepharoplasts may each divide in the polar position into an axial centrosome and an adjacent basal granule to which flagella, paradesmose, and parabasal are attached. These two granules subsequently reunite.

7. In its divisions the blepharoplast shows no independent mitotic phenomena. It is not a "kinetonucleus," and its behavior does not support the binuclearity hypothesis.

8. The anterior flagella are shared, two and one respectively by the daughter blepharoplasts and new outgrowths, complete the complement of each daughter organism.

9. The chromatic margin of the undulating membrane represents an intracytoplasmic posteriorly directed flagellum. It splits longitudinally to the tip of its projecting end. The undulating membrane below it also splits.

10. The chromatic basal rod is the homologue of the parabasal body of *Parajoenia* and the Trichonymphida as established by Janicki. His so-called parabasal in *Trichomonas* is in reality only the early stage in the outgrowth of a new parabasal or chromatic basal rod at mitosis, hence its rarity and transitory nature. At mitosis a new parabasal or chromatic basal rod grows out in the base of one of the new undulating membranes while the old parabasal lies in the other membrane.

11. The new axostyles of the daughter organisms are formed by the longitudinal splitting of the old axostyle from the anterior end posteriorly. They are not formed from the paradesmose (central spindle) as maintained by Dobell nor anew as claimed by Kuczynski.

12. The axostyle is not primarily a skeletal structure as usually supposed, nor an organ of fixation as described by Kunstler and Kuczynski but a locomotor organ used vigorously during the amoeboid stage in the mucous substrate.

13. During mitosis the organelles are subject to a wide variation in location due to independent shifting of axostyle and nucleus, and to a less extent to the detachment of the blepharoplast from its usual relation to the nucleus.

14. Plasmotomy is long delayed after nuclear mitosis and during this period many widely varying positions of the two daughter nuclei and their attached extranuclear organelles are rapidly assumed. Some of these may simulate copulation.

15. The plane of division is longitudinal. Its determination should be based on the fundamental morphological relations of the organelle and not, as by Martin and Robertson, on the chance relations of these structures in the amoeboid postmitotic period.

16. Multiple fission occurs in the trichomonad flagellates as a normal phase of the life-cycle and results in the formation of an 8-nucleate plasmodium or somatella. We have not been able, as yet, to relate it to a particular stage such as gametogenesis, or to the divisions of a zygote. Three rapidly succeeding synchronous mitoses give rise to 2-4-8-nucleate plasmodia which are not encysted and remain very active throughout the process. The plasmodium disintegrates into its component members by the successive detachment of single merozoites.

17. The widespread and regular occurrence of the stage of a multinucleate plasmodium among these simple protozoa is significant as an early step in the evolution of the more permanent multinucleate and multicellular aggregates which constitute the Metozoa.

May 18, 1915.

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EXPLANATION OF PLATES.

All figures have been made with camera lucida from material in smears from the intestinal wall fixed in Schaudinn's fluid and stained with Heidenhain's iron haematoxylin or with alcoholic iron haematoxylin.

PLATE 1.

Trichomonas augusta Alexeieff, from intestine of *Diemyctylus torosus* unless otherwise stated. Trophozoite and early prophase of mitosis. × 2175.

FIGURE 1. Trophozoite seen from the right side with axostyle overlying the nucleus and projecting posteriorly, and cytostome at the anterior end.

FIGURE 2. The same, with intranuclear chromidial cloud, and cytoplasm forming a posterior blob enveloping the axostyle.

FIGURE 3. Trophozoite in contracted condition with undulating membrane thrown into a spiral, intranuclear chromidial cloud, central karyosome, and well-defined posterior axostylar ring.

FIGURE 4. Axostylar chromidia grouped in the capitulum overlying the nucleus.

FIGURE 5. Cytostome much elongated, axostylar chromidia distributed, undulating membrane thrown into a spiral, and removed posteriorly from the region of the emergence of the axostyle (cf. Figs. 3, 4).

FIGURE 6. Early prophase showing first step in splitting of chromatic margin. Note small vacuoles and two large ones with (parasitic?) *Micrococcus*.

FIGURE 7. The same, blepharoplast divided, chromatic margin split to its posterior end, one new anterior flagellum growing out; axostyle constricted near the middle; unusually large (abnormal?) spheroidal chromidial (?) masses in cytoplasm, and axostyle with median constriction.

FIGURE 8. Very large trophozoite with undulating membrane, the chromatic margin and the posterior flagellum completely divided. One daughter membrane completely detached in preparation of the smear; one anterior flagellum completely grown out, many axostylar chromidia, but none in the cytoplasm; blepharoplast quadripartite.

FIGURE 9. Large trophozoite in prophase. Extranuclear chromidial cloud about the nucleus full of fine granules; few cytoplasmic chromidia present; coarse chromatin skein in nucleus.

FIGURE 10. The same, with chromatin organized in five chromosomes; one flagellum growing out.

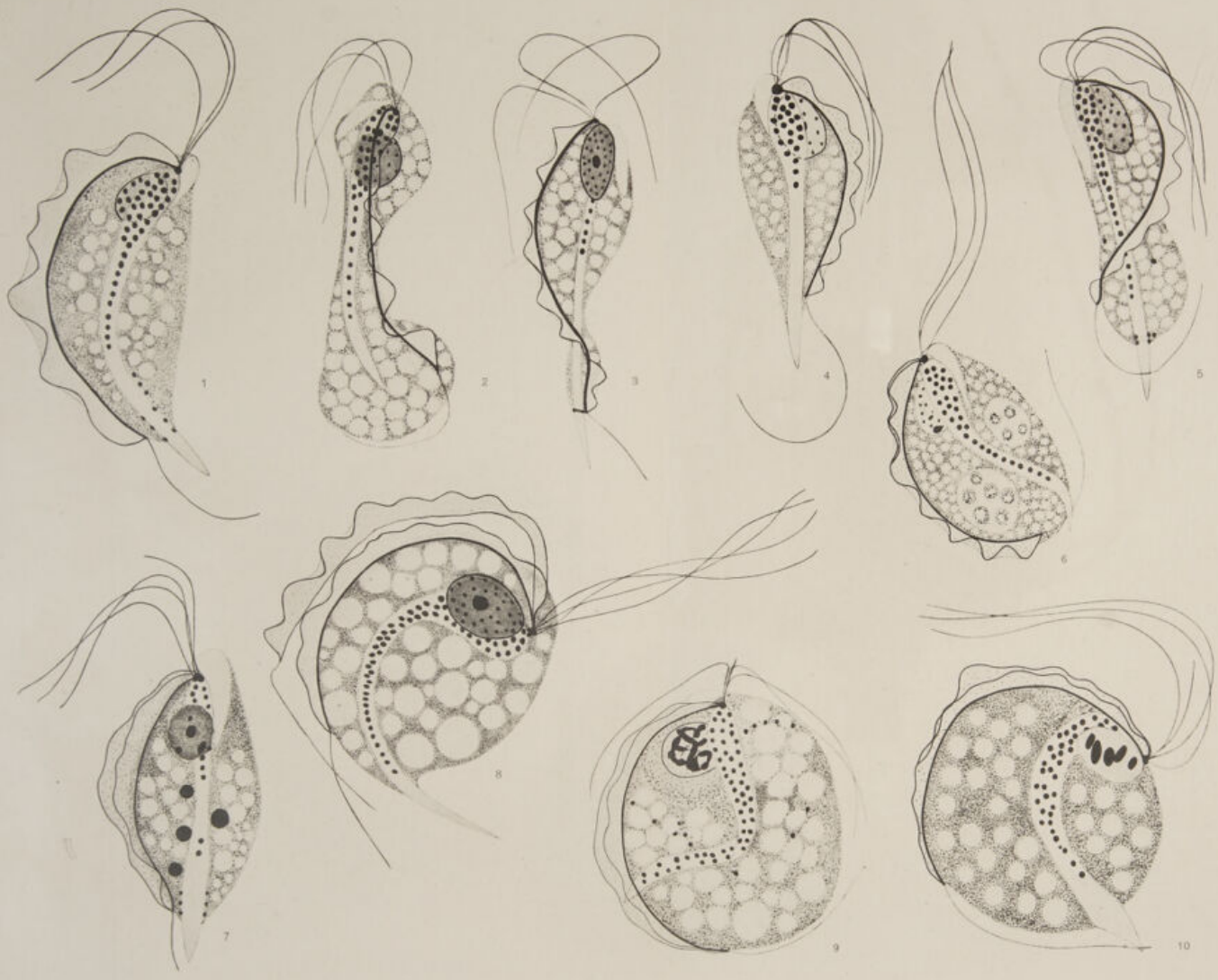

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PLATE 2.

All figures of *Trichomonas augusta* Alexeieff unless otherwise stated. Late prophase to early anaphase of mitosis. × 2175. From *Diemyctylus torosus* unless otherwise stated.

FIGURE 11. Late prophase, extranuclear chromidial cloud with small granules and cytoplasmic chromidia forming, new chromatic basal rod growing out, five split pairs of chromosomes present.

FIGURE 12. The same. Axostyle detached from blepharoplast.

FIGURE 13. The same, whole organism relatively poor in chromatin, no chromidial cloud, chromosomes nearing equatorial plate stage.

FIGURE 14. The same stage as Figures 11, and 12. Axostyle obscures nucleus, undulating membranes detached from cytoplasm.

FIGURE 15. Prophase in a small trophozoite.

FIGURE 16. The same, in a large spheroidal trophozoite showing extranuclear chromidial cloud with cytoplasmic chromidia forming, axostyle crowded with axostylar chromidia and chromosome pairs widely parted in two cases, small chromosome pair anterior.

FIGURE 17. The same with blepharoplast divided, axostyle widely detached, two pairs of chromosomes in end-to-end position, small pair anterior; undulating membranes widely separated, posterior flagellum split.

FIGURE 18. The same stage showing five pairs of chromosomes. Blepharoplasts more widely separated.

FIGURE 19. Late prophase with blepharoplasts widely separated, connected by a dark paradesmose outside of the still spheroidal nucleus; three pairs of chromosomes (on lower side) evident, two of them widely detached; undulating membrane from blepharoplast to the left is on the under side below the nucleus.

FIGURE 20. Small trophozoite at the metaphase. Blepharoplasts at poles of fusiform nucleus divided at the left and possibly at the right into lateral basal granule with attached flagella and paradesmose, and polar centrosome; capitulum of axostyle on the under surface; five undivided chromosomes on the equatorial plate; paradesmose closely applied to the outside of the nuclear membrane. From *Rana boylei*.

FIGURE 21. Same stage with left basal granules detached from the polar centrosome; faint aster about inner pole; five elongated undivided chromosomes in the equatorial plate; tip of axostyle bent under the edge of the cytoplasm.

FIGURE 22. Early anaphase. Blepharoplasts not divided; chromosomes just parted, one case (central chromosome) of unequal "x–y" division, small chromosome lagging. Note continued detachment and removal from axostyle of all nuclear and extranuclear organelles.

FIGURE 23. Later anaphase. Paradesmose in nearly same relation to x–y chromosomes as in Figure 22; blepharoplast divided into basal granule and centrosome.

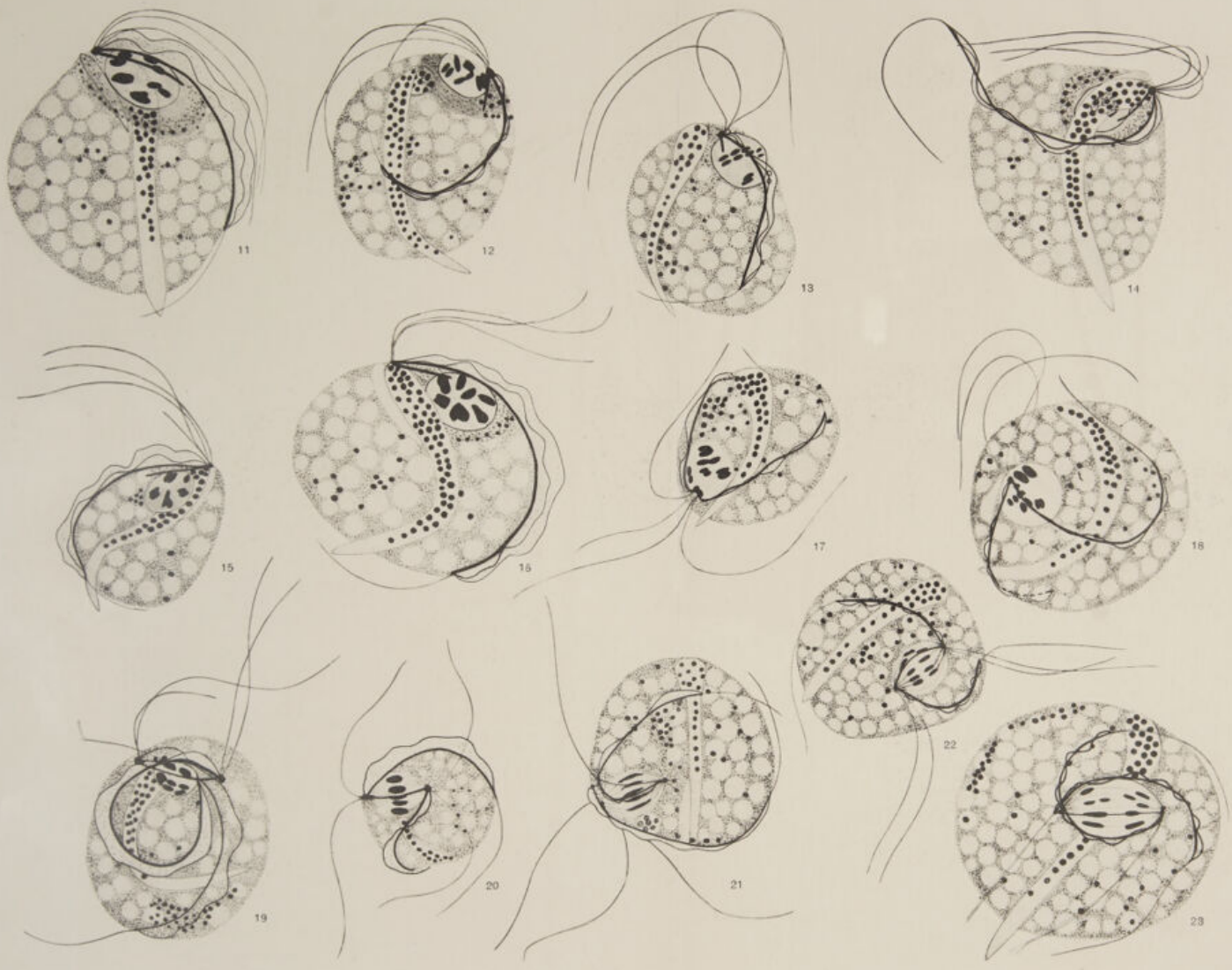

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PLATE 3.

All figures of *Trichomonas augusta* Alexeieff, in anaphase (Figs. 24, 25) and telophase (Figs. 26–35) of mitosis, including division of the axostyle (Figs. 31–35), unless otherwise stated. × 2175. All from *Diemyctylus torosus*.

FIGURE 24. Late anaphase. Chromosomes elongating and attaching themselves by stainable threads to the poles of the nucleus; blepharoplast at the left near the under surface divided into basal granule and centrosome, its undulating membrane shown in dotted lines; nucleus elongating.

FIGURE 25. Latest anaphase in *Trichomonas batrachorum* Perty without axostylar chromidia. Chromosomes fusing into several large masses, nuclear membrane constricting, blepharoplasts no longer divided, axostyle very faint.

FIGURE 26. Nuclei severed. Chromosomes still intact, grouped about polar centrosome or blepharoplast which in the lower nucleus is detached from the nuclear membrane and carries with it the extranuclear motor complex, blepharoplasts still united by the paradesmose.

FIGURE 27. Same stage but blepharoplasts in place, and paradesmose very faint.

FIGURE 28. Same stage with paradesmose taut and basal granule detached from centrosome in lower nucleus, whose motor organelles are on the under surface; chromosomes still intact.

FIGURE 29. Paradesmose taut, fading out. Blepharoplasts not divided, axostyle related (?) to one; chromosomes still evident.

FIGURE 30. Paradesmose bowed. Chromosomes detaching from blepharoplasts.

FIGURE 31. Chromosomes detaching from blepharoplasts Paradesmose no longer visible; axostyle dividing at capitulum, note enlarged axostylar chromidia.

FIGURE 32. Tip of axostyle split. Paradesmose in vertical position between blepharoplasts; lower chromatic basal rod represented by dotted line; two nuclei each with single karyosome below the lobes of the axostyle; a second cytostome on the lower surface.

FIGURE 33. Axostyle more deeply cleft, one nucleus with single karyosome close to each; the two chromatic basal rods parallel, the lower represented by a dotted line; vacuoles with rod-like (bacteria?) contents.

FIGURE 34. Axostyle more deeply cleft, second blepharoplast below and to the right. Note suggestion of division of axostylar chromidia.

FIGURE 35. Nucleus, blepharoplast and axostyle in approximately their normal relations in the trophozoite. Axostyle cleft nearly to tip.

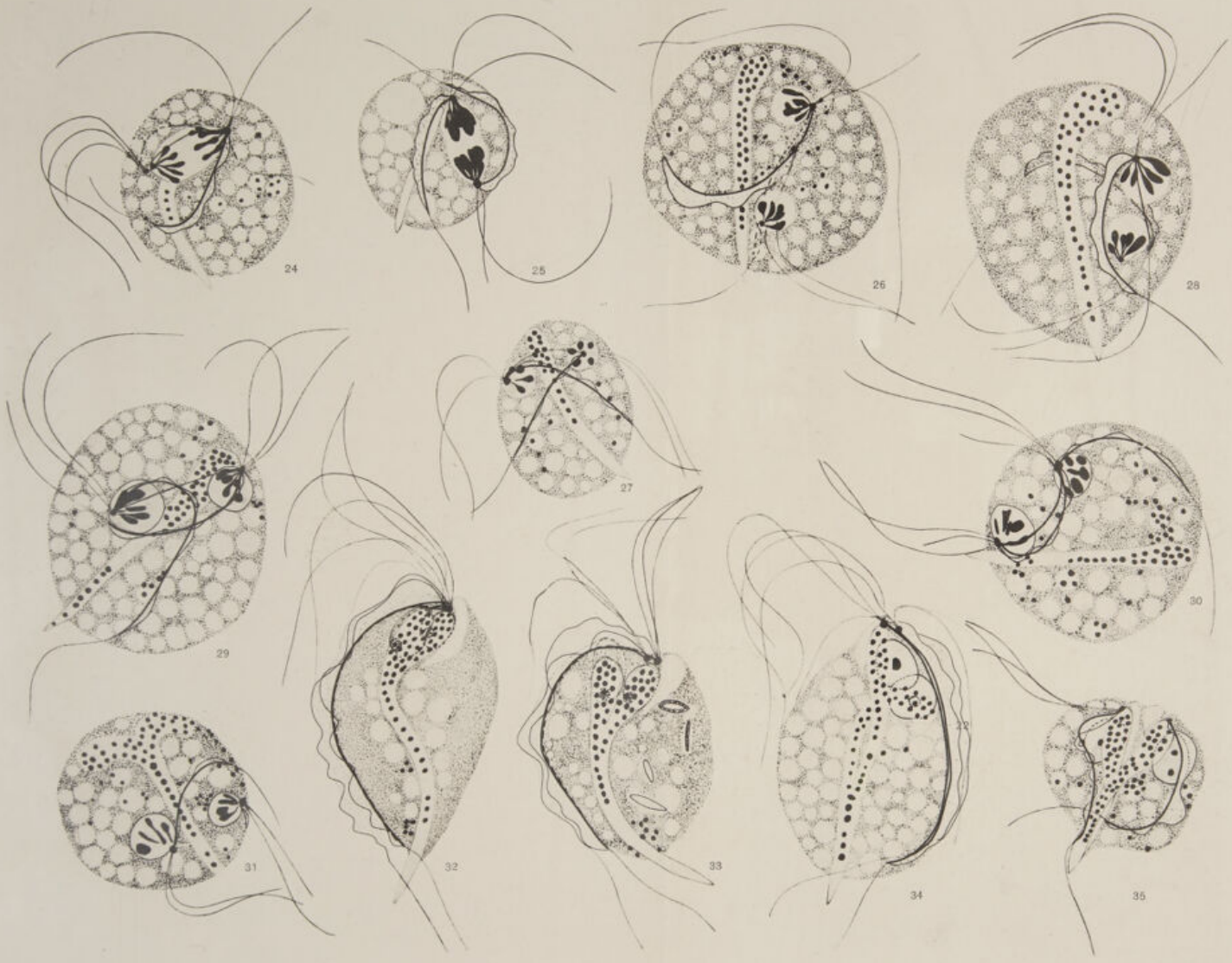

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PLATE 4.

Final stages (plasmotomy) in binary fission (Figs. 36–40), and formative (Figs. 41, 42) and disintegrative phases (Figs. 43–45) of multiple fission or formation and disintegration of the plasmodium or somatella of *Trichomonas augusta* Alexeieff and *T. batrachorum* Perty. × 2175. All from *Diemyctylus torosus* unless otherwise stated.

FIGURE 36. Daughter nuclei diverging, axostyle almost completely cleft.
FIGURE 37. Axostyles completely severed, divergence of daughter merozoites increasing.
FIGURE 38. Convergence after wider separation. Compare text figure F.
FIGURE 39. Merozoites with blepharoplasts 180° apart in advantageous position for transverse plasmotomy.
FIGURE 40. Partial convergence after the phase of Figure 39.
FIGURE 41. Plasmodium or sometella in 4-nucleate formative phase of multiple mitosis. Only two axostyles present. From *Bufo halophilus* Baird.
FIGURE 42. Plasmodium in 8-nucleate stage with four axostyles. From *Rana boylei* Baird.
FIGURE 43. Disintegrative phase of somatella, with seven nuclei. From *Rana boylei* Baird.
FIGURE 44. Same with three nuclei. From *Rana pipiens* Kalm.
FIGURE 45. Same with three nuclei in *Trichomonas batrachorum* Perty.

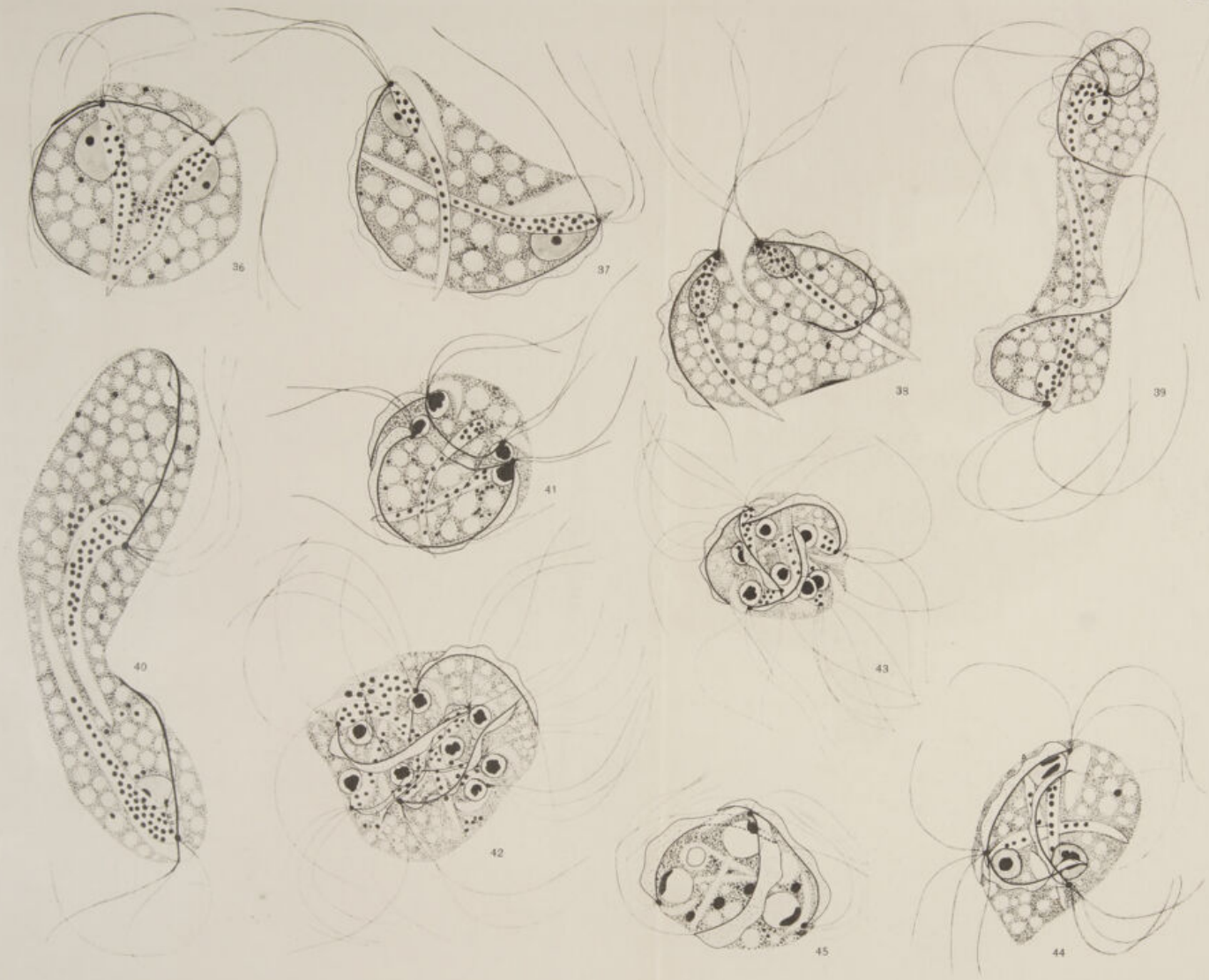

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PLATE 5.

Binary and multiple fission in *Trichomonas muris* Hartmann. × 2175. All from *Peromyscus maniculatus gambeli* Baird except Figures 48, 58–66, which are from *Mus.* sp. albino.

FIGURE 46. Normal trophozoite somewhat rounded up showing numerous karyosomes in the large nucleus, cytoplasmic chromidia along chromatic basal rod, blepharoplast at margin attached to anterior flagella, undulating membrane with chromatic margin and basal rod, much curved axostyle with distal chromatic granules.

FIGURE 47. Nucleus greatly enlarged (about 6250 diameters) showing numerous subequal ellipsoidal karyosomes and faint linin network.

FIGURE 48. Normal free swimming trophozoite.

FIGURE 49. Early prophase, chromatic margin splitting, one side detached from end of chromatic rod; blepharoplast dividing, faint intranuclear chromidial cloud emerging.

FIGURE 50. Later prophase. Blepharoplast divided, nuclear chromatin in segmented spiral skein.

FIGURE 51. The same, with ten chromomeres in the shortening skein.

FIGURE 52. Later stage of the same, with shortening skein and evidences of paired or split chromosomes emerging.

FIGURE 53. Late prophase with five pairs of split chromosomes, a small pair anteriorly located, one large, and three medium-sized pairs.

FIGURE 54. Later prophase with five massed chromosomes, the small one anterior. Blepharoplast divided; posterior chromatic ring evident on the axostyle.

FIGURE 55. Blepharoplasts diverging with straight taut paradesmose stretched between. Chromosomes showing evidence of longitudinal splitting; small chromosome anterior; motor organelles completely divided, attached to blepharoplasts, two lines of cytoplasmic chromidia.

FIGURE 56. Anaphase, chromosomes all divided but small lagging one. Division and migration not synchronous; blepharoplasts in polar position, probably divided into polar centrosomes and lateral basal granules bearing the motor apparatus.

FIGURE 57. Late anaphase. Chromosomes migrated to polar position, paradesmose applied to outside of nuclear membrane, axostyle detached.

FIGURE 58. Early telophase. Nuclei parting by equatorial constriction, chromosomes connected to blepharoplasts by chromatic prolongations.

FIGURE 59. Telophase. Nuclei separated, chromatin massed in central karyosome; short rhizoplast connecting nucleus and blepharoplast, paradesmose fading.

FIGURE 60. Axostyle splitting longitudinally. Central karyosome breaking up into a number of smaller ones; paradesmose still present.

FIGURE 61. Daughter organelles completed, two cytostomes present; paradesmose still persisting.

FIGURE 62. Disintegrative plasmodium formed by multiple fission in stage of 6-nucleate somatella. Each nucleus with axostyle, and intranuclear chromidial cloud.

FIGURE 63. Somatella with five nuclei.

FIGURE 64. Same with four nuclei.

FIGURE 65. Same with three nuclei.

FIGURE 66. Binucleate phase following either binary or multiple fission.

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PLATE 6.

Multiple fission in *Tetratrichomonas prowazeki* Alexeieff. × 2175. From *Diemyctylus torosus*.

FIGURE 67. Early prophase in rounded up trophozoite with intranuclear chromidial cloud and food vacuoles with contained plant cells. Note four anterior flagella, undulating membrane with chromatic margin and basal rod arising from the blepharoplasts, axostyle, and large cytostome.

FIGURE 68. Free-swimming trophozoite with large blepharoplast.

FIGURE 69. Trophozoite with intranuclear chromidial cloud, which has engulfed a cyst of the yeast *Blastocystis enterocoela* Alexeieff, reputed by Prowazek (1904) to be trichomonad cysts.

FIGURE 70. Binary fission or formative phase of plasmodium or somatella with two nuclei still connected by paradesmose, each with four chromosomes; food vacuoles with contents in cytoplasm.

FIGURE 71. Somatella with eight nuclei each with central karyosome and motor apparatus attached to blepharoplast.

FIGURE 72. Same, with nuclei filled with dense chromidial cloud and fine granules; elongated cytostomes and several axostyles visible.

FIGURE 73. Somatella in disintegrative phase with seven nuclei with dense intranuclear chromidial cloud.

FIGURE 74. Same with five nuclei with several karyosomes and light cloud.

FIGURE 75. Same with four nuclei and intranuclear cloud.

FIGURE 76. Same with three nuclei, with dense intranuclear cloud and very large deeply staining cytoplasmic spheres, possibly abnormal.

FIGURE 77. Same with two nuclei and persisting plasmodesmose indicating recent detachment of the sixth merozoite.

FIGURE 78. Somatella with two nuclei resulting either from binary fission or the last phase of the disintegration of an 8-nucleate plasmodium.

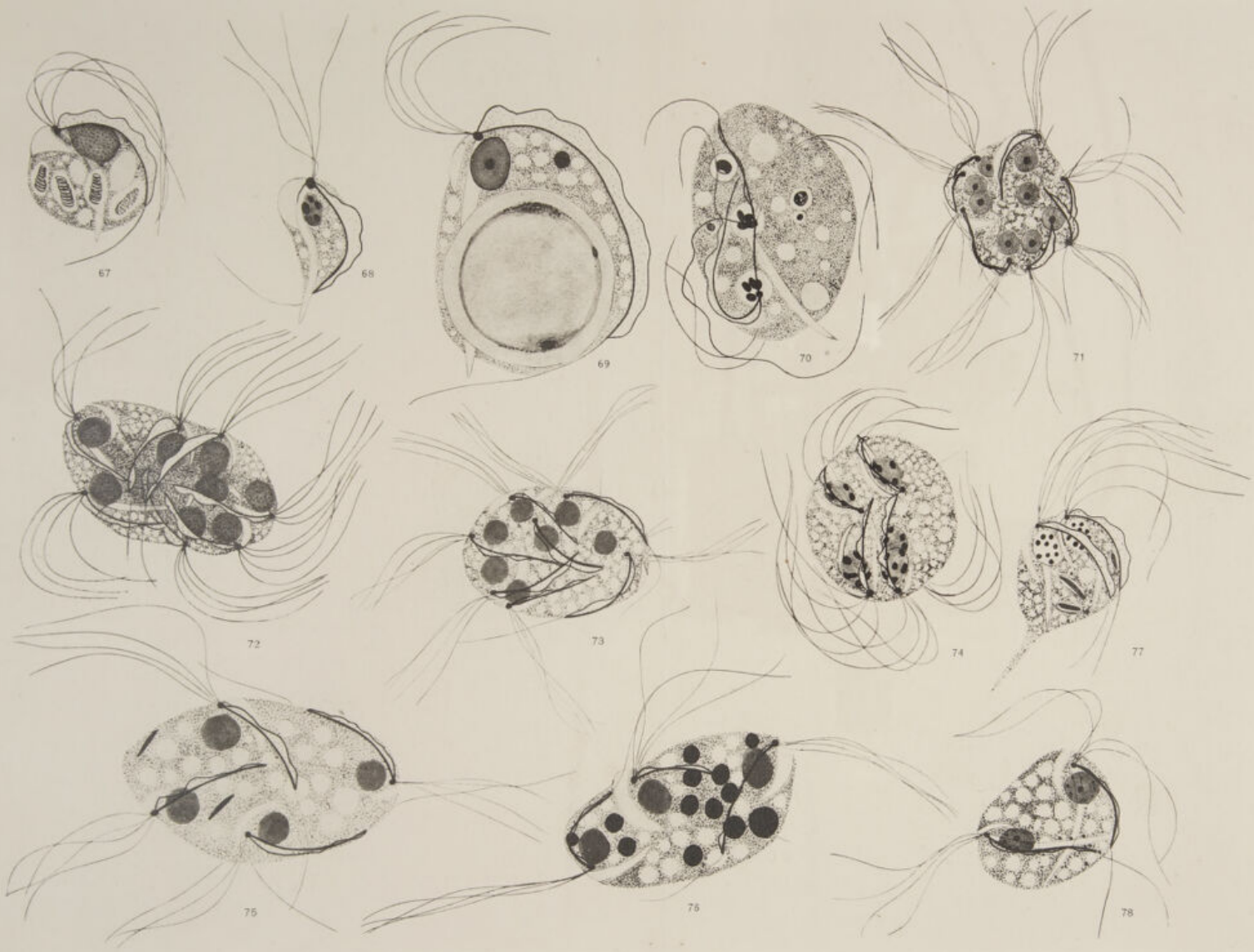

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PLATE 7.

Binary fission in *Eutrichomastix serpentis* (Dobell). × 2175. All from *Crotalus oregonus* Holbrook except Figures 81, 84, 86–88, 90, 91, and 95 which are from *Pituophis catenifer* Baird and Girard.

FIGURE 79. Free-swimming trophozoite. Note three anterior and one trailing posterior flagellum, arising from anterior blepharoplast, cytostome, axostyle, and nucleus with central karyosome.

FIGURE 80. Trophozoite in posterior blob formation or plasmectomy.

FIGURE 81. Trophozoite rounding up prior to fission. Nucleus with numerous karyosomes (skein formation?).

FIGURE 82. Late prophase. Daughter blepharoplasts migrating apart with paradesmose between; two flagella with each blepharoplast; four chromosomes in the nucleus.

FIGURE 83. Later stage showing extranuclear spindle fibers running from blepharoplasts to spheroidal nucleus.

FIGURE 84. Nucleus fusiform; two cytostomes present.

FIGURE 85. Metaphase approaching; chromosomes in equatorial plate; slight chromidial cloud about anterior end of vanishing axostyle.

FIGURE 86. The same. Note difference in shape of chromosomes.

FIGURE 87. Metaphase. Chromosomes beginning to divide by transverse constriction.

FIGURE 88. Division nearly completed. Note small lagging chromosome.

FIGURE 89. Early anaphase.

FIGURE 90. Early telophase. Constriction of nuclear membrane, massing of chromosomes; paradesmose persisting; new flagella growing out.

FIGURE 91. Telophase. Nuclei separated; full complement of flagella present; paradesmose still present.

FIGURE 92. Telophase. Chromosomes connected with blepharoplast by chromatic threads; paradesmose disappeared; one axostyle present.

FIGURE 93. Paradesmose stretched taut by diverging nuclei; two axostyles and two cytostomes present.

FIGURE 94. Two daughter cells parting, with slender plasmadesmose connecting them.

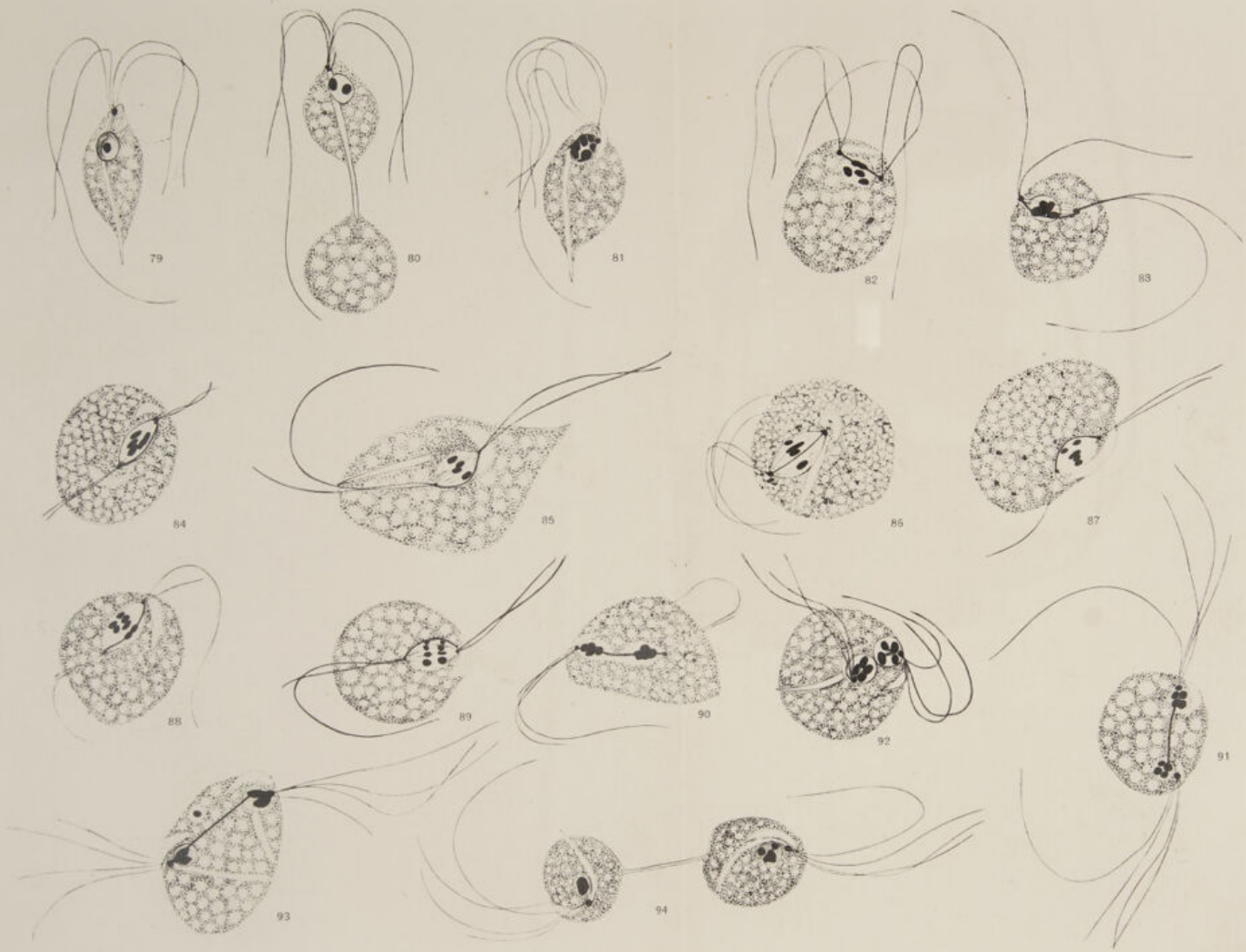

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PLATE 8.

Plasmotomy after binary fission (Figs. 95–97) and multiple fission (Figs. 98–104) in *Eutrichomastix serpentis* (Dobell). × 2175. All from *Crotalus oregonus* Holbrook except Figures 96, 98–100, which are from *Pituophis catenifer* Baird and Girard.

FIGURE 95. Late telophase. Daughter nuclei diverging, plasmodesmose taut.

FIGURE 96. Daughter nuclei 180° apart in favorable position for plasmotomy; paradesmose and axostyles parallel.

FIGURE 97. Unequal division of cytoplasm in rounded-up phase about daughter nuclei.

FIGURE 98. Multiple fission, formative phase; second synchronous division forming 4-nucleate plasmodium; two paradesmoses present.

FIGURE 99. Same stage with paradesmoses crossing.

FIGURE 100. Somatella with eight nuclei. Three axostyles visible; nuclei with intranuclear chromidial cloud.

FIGURE 101. The same, with large karyosomes forming in the nuclei, probably an earlier stage than Figure 100.

FIGURE 102. Disintegrative phase, 6-nucleate somatella.

FIGURE 103. The same, 3-nucleate sometella.

FIGURE 104. Binucleate stage, a late stage of binary fission or of the disintegrative phase of multiple fission, the seventh and eighth merozoites in the last phase of plasmotomy.

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