

Resumen por el autor, D. H. Wenrich.

La estructura y división de *Trichomonas muris* (Hartmann).

Este flagelado que habita en el ciego del ratón mide 12 a 16 micras de longitud por 5 a 10 micras de espesor, y posee los siguientes orgánulos: Núcleo, citostoma y blefaroplasto con las estructuras que en él se insertan—los tres flagelos anteriores, el flagelo posterior que corre a lo largo del margen de la membrana ondulante, el bastón basal cromático en la base de dicha membrana, el axostilo, las filas externa e interna de gránulos cromáticos y el cuerpo parabasal. Esta última estructura es el cuerpo parabasal de Janicki ('11) pero aparece solamente con ciertos métodos técnicos. En la división pueden reconocerse estados comparables a la profase, metafase, anafase y telofase de las células de los metazoarios. Durante la profase se forman seis cromosomas dobles (hendidos longitudinalmente), mientras que el cariosoma desaparece gradualmente como en el caso del nucleolo de los metazoarios.

El nuevo bastón basilar cromático se origina como una hilera de pequeños gránulos que se inserta por uno de sus extremos en el blefaroplasto. La nueva membrana ondulante y el flagelo posterior se desarrollan al mismo tiempo que el bastón cromático basal. Un pequeño blefaroplasto nace por gemación del primitivo, y ambos permanecen reunidos por una paradesmosis durante la división. La membrana nuclear persiste durante la mitosis. El comportamiento de los cromosomas durante la metafase y anafase es semejante a los de las células de los metazoarios. El axostilo primitivo degenera, formándose uno nuevo a expensas de cada blefaroplasto. El borde interno del bastón cromático basal produce por gemación una nueva fila de gránulos cromáticos. La división de la célula se retrasa hasta que las dos series de orgánulos están completas. El núcleo y el cuerpo celular son las únicas partes que se dividen ecuacionalmente, mientras que todas las demás partes necesarias aparecen como crecimientos de las estructuras primitivas correspondientes.

# THE STRUCTURE AND DIVISION OF TRICHOMONAS MURIS (HARTMANN)

D. H. WENRICH

*Department of Zoology, University of Pennsylvania*

ONE TEXT FIGURE AND FOUR PLATES (THIRTY-SIX FIGURES)

## CONTENTS

Introduction.....	119
Materials and methods.....	120
A. Materials.....	120
B. Methods.....	121
The vegetative individuals.....	122
A. Form.....	122
B. Size.....	123
C. Organization of the cell.....	125
D. Differences due to different fixatives.....	130
E. Encystment.....	133
Division.....	134
A. Prophase.....	134
1. The nucleus.....	134
2. Chromatic basal rod.....	137
3. The new undulating membrane and chromatic margin.....	137
4. The blepharoplast.....	138
5. Other structures.....	139
B. Metaphase.....	140
C. Anaphase.....	141
D. Telophase.....	141
Summary of the more important results.....	145
Literature cited.....	147
Explanation of plates.....	148

## INTRODUCTION

The structure and division processes of various species of *Trichomonas* have received the attention of several investigators, but there is much disagreement among them regarding details of structure in the vegetative condition and events during division even in the same species. Such differences are very noticeable, for example, in the most extensive accounts in recent years, one

by Kofoid and Swezy ('15) and two by Kuczynski ('14, '18). The structure and division of *Trichomonas muris* were described in these papers as well as in the earlier one of Wenyon ('07). Since I have been able to secure some material which seems to be especially favorable for the study of cell structure and division in this species, and since my findings are not in entire agreement with any of the authors mentioned above, it would seem to be worth while to place on record my observations. I have begun an investigation of the various intestinal protozoa of rats and mice, but the present account will be limited to the one species.

#### MATERIALS AND METHODS

##### *A. Materials*

*Trichomonas muris* (Hartmann) is found chiefly in the coecum of mice and to a less extent in the large intestine. Only rarely has it been found in the small intestine and then only at the lower end.

The first material from the coecum of a mouse (*Mus musculus*) in which I found the division stages numerous was secured in December, 1916. Slides made from this material have proved to be the most valuable in the collection, and many of my figures have been made from them. Since that time 102 additional mice have been examined, of which fifty-one were wild and fifty-one were albinos. Of the wild mice, nine were *Peromyscus leucopus* and the others were the house mouse, *Mus musculus*. Only two of the *Peromyscus* and only five of the forty-two house mice showed infection with *Trichomonas muris*, while fifteen of the fifty-one white mice were found to harbor this species. Young mice showed less tendency to infection than adults and the degree of infection was extremely variable. It ranged from occasional specimens to cases when the entire contents of the coecum appeared to consist of *Trichomonas* and a few bacteria. In these latter cases division stages were common in the mass of coecal contents as well as near the mucous membrane.

*B. Methods*

Aside from preparations of living flagellates in fresh coecal contents mixed with salt solution, cover-glass preparations fixed and stained in various ways and mounted in balsam were employed. Coecal material, usually from the region adjoining the mucous lining, was mixed with a little salt solution and smeared out thin on clean cover-glasses. With few exceptions these smears were fixed without allowing them to dry, although occasionally some were dried and subsequently stained with some modification of the Romanowsky method. These dried smears do not give satisfactory preparations and have not been used as the basis of the observations here recorded.

For the wet smears the following fixatives, usually heated to about 40°C., have been tried: Schaudinn's sublimate and alcohol, with and without the addition of acetic acid; Worcester's formol-sublimate-acetic; Flemming's stronger and weaker solutions; Perenyi's chrom-nitric acid; Carnoy's alcohol-chloroform-acetic; Bouin's picro-formol-acetic; Allen's ('16) modification of Bouin's (B 15); sublimate-acetic, and picro-mercuric. The most satisfactory of these have proved to be Schaudinn's, Bouin's, Allen's, and Flemming's, in about the order named. Some other fixatives were used in special experiments which will be described elsewhere.

For staining, Delafield's, Heidenhain's iron alum-haematoxylin, and safranin (after Flemming's) have been tried, but most of the smears have been stained with Heidenhain's haematoxylin, which has always given the most satisfactory results. Alcoholic solutions of haematin, haematoxylin, and iron-alum, according to the methods described by Dobell ('14) and Kofoid and Swezy ('15), were tried, but did not give results as satisfactory as the twenty-four-hour staining in iron alum-haematoxylin, so their use was not continued. Various counterstains were tried, but none of them appeared to add to the value of the preparations, and were not generally employed.

## THE VEGETATIVE INDIVIDUALS

*A. Form*

Both in the living and the fixed condition the body of this species of *Trichomonas* is rather fusiform, with a length of from one and a half to two times the greatest width. There is some tendency for the so-called dorsal side to be more convex than the opposite, somewhat flattened, ventral side. In the free-living condition the region of greatest width is usually near the middle, but the flexibility of the pellicle permits a variety of shapes, especially when the animals are creeping or forcing their way through the coecal debris. Then the body may change shape rapidly and some of the variations are to be seen in the fixed material. For example, figure 7 shows an animal with the anterior end much more pointed than the one in figure 8. Figures 8 and 17 show animals with the greatest width at the posterior end instead of in the middle, as is more common.

Adverse conditions, such as lowered temperature, changes in the constitution of the surrounding fluid, or desiccation, often lead to considerable changes in form, the most common modification being the rounded-up condition (figs. 11, 13, and 15). The rounded form also seems to be characteristically assumed during the process of division (figs. 20 to 30). When confined in cramped quarters the form changes are exceedingly various.

In free-swimming animals the undulating membrane is spirally arranged on the surface of the body, and they rotate on the long axis, without any appreciable changes in diameter. On the other hand, fixed and stained individuals often give the impression of being flattened and of lying on one side with the undulating membrane at one edge, as seen, for example, in figures 1 to 4. In those specimens showing the spiral arrangement of the undulating membrane and accompanying structures, it is seen that the direction of the spiral is from the left over to the right, as shown in figures 5 and 17 and in text figure A.

*B. Size*

Wenyon ('07) called attention to the great variation in size in the *Trichomonas* of mice giving the length as from 3 to 20  $\mu$ . Kofoed and Swezy ('15) emphasized a similar variation in size for *T. augusta*. In both cases the authors raise the point that differences in size alone do not furnish sufficient criteria for the separation of species. I have found two species in mice which do differ as to size, and it may be that the range of sizes observed by Wenyon had a greater significance than he supposed. Careful study and measurement of the flagellates found in mouse no. 1, for example, revealed a larger species which I take to be *T. muris*, ranging in length from 8 to 20 $\mu$ , with an average of

TABLE 1

*Table showing results of measurements, from certain host mice and for certain fixatives*

MOUSE NUMBER	FIXATIVE	NUMBER OF INDIVIDUALS MEASURED	AVERAGE LENGTH, MICRONS	RANGE, MICRONS
1	Schaudinn's	100	13.1	10-16
19	Schaudinn's	50	12.7	10-16
24	Schaudinn's	100	12.8	10-16
24	Allen's	100	15.7	11-22

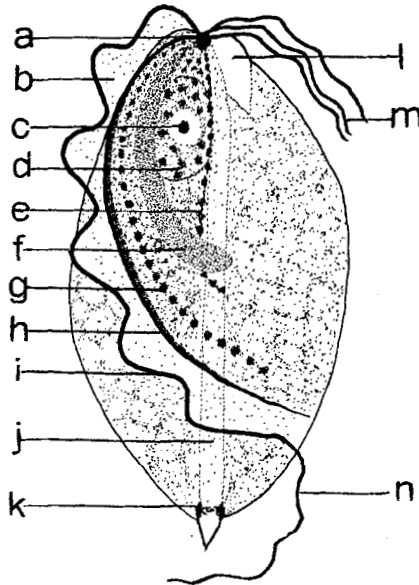
12.9 $\mu$ , and a smaller species ranging in length from 6 to 9 $\mu$ , with an average of 7.2 $\mu$ . The smaller species may be *T. parva* Alexeieff, and can be differentiated on morphological grounds other than size, and shows only three chromosomes in division. In other mice pure infections of each species were found as well as other mixed infections.

Measurements have been made from several series of slides, and the results indicate slight racial differences for the different hosts as well as differences due to various methods of fixation. Table 1 indicates some of these differences.

The results for different fixatives is strikingly illustrated by mouse no. 24, where the average length for animals fixed with Allen's fluid (15.7 $\mu$ ) is 22.6 per cent more than the average length for those fixed in Schaudinn's fluid (12.8 $\mu$ ). It is prob-

able that Schaudinn's fluid causes shrinkage, and possibly Allen's fluid may cause swelling.

In making these measurements great care has been taken to secure an unbiased selection of individuals for measurement. Only individuals which appeared normal and had the axostyle approximately straight were measured. All such individuals in



Text figure A Vegetative individual of *T. muris*, partly diagrammatic; *a*, blepharoplast; *b*, undulating membrane; *c*, caryosome; *d*, nucleus; *e*, inner row of chromatic granules; *f*, parabasal body; *g*, outer row of chromatic granules; *h*, chromatic basal rod; *i*, posterior flagellum as chromatic margin of undulating membrane; *j*, axostyle; *k*, chromatic ring at point of emergence of axostyle; *l*, cytostome; *m*, anterior free flagella; *n*, posterior free flagellum.

any one field of the microscope were measured. Successive fields were treated the same way, duplication of fields being prevented by the use of a mechanical stage. All measurements were made by the aid of an eye-piece micrometer which had been calibrated for the set of lenses used. The measurements obtained agree very well in a general way with those of Kuczynski ('14) and Kofoid and Swezy ('15).

*C. Organization of the cell*

The organelles of this species of *Trichomonas* are those typical of the genus and are indicated in text figure A. They include besides the nucleus (*d*) and cytostome (*l*) the series of structures attached to the blepharoplast (*a*), consisting of the three anterior free flagella (*m*), the long posterior flagellum running as the chromatic margin (*i*) of the undulating membrane (*b*) and continuing posteriorly as a free flagellum (*n*), the chromatic basal rod (*h*) at the base of the undulating membrane, the axostyle (*j*), the parabasal body (*f*) and the inner (*e*) and outer (*g*) rows of chromatic granules.

The protoplasm is enclosed in a cell membrane or pellicle, which, as previously noted, is flexible enough to permit variations in form. These form variations may be classed as 'euglenoid' in type. Pseudopodia formation has been described by several authors, for example, by Kuczynski ('14) and by Kofoid and Swezy ('15), but I have seen such apparent pseudopodia only under conditions which appeared to be either degenerative or precystic, and therefore I do not regard this phenomenon as normal for the active individual. The protoplasmic projection shown in figure 16 is probably the result of mechanical injury in making the smear, and is not a pseudopodium.

The protoplasm itself is rather fluid in nature, as is indicated by the rapidity with which form changes occur. It appears to be somewhat vacuolated, although not to the extent seen in some other species, such as *T. augusta*, as figured by Kofoid and Swezy (15), or *T. mirabilis*, as figured by Kuczynski ('18). The appearance or non-appearance of vacuoles seems to vary somewhat from host to host and from cell to cell. Variations from one fixative to another are discussed further on.

The nucleus (text fig. A, *d*) lies in the anterior third of the body dorsal to and usually a little to the left of the axostyle which occupies the position of the principal axis. It is usually oval or broadly elliptical in shape, being approximately 4 to 5 $\mu$  long and 2.5 to 3 $\mu$  wide. At the periphery is a delicate nuclear membrane or caryotheca, which is sometimes difficult to see.



Within the membrane the chromatin occurs as small granules scattered upon a fibrous network, and as a caryosome (*c*) of relatively large size which is usually surrounded by a clear area. The exact number of the small chromatin granules had not been determined, but in the early prophases they are reduced to six, which are paired or split.

The clear area about the caryosome is sometimes large with a diameter as much as one-half to two-thirds that of the nucleus (figs. 1, 2, 7, 8, etc.). In other instances it is much smaller (fig. 13). In figure 1 the caryosome appears to be double, but this condition is rare. Careful focusing usually discloses fine fibrous connections between the caryosome and the network at the periphery of the clear area (figs. 7, 8, 10, 12). There is no apparent constancy in the position of the caryosome, since it is found at either extremity of the nucleus or in any intermediate position.

Occasionally there is seen a nucleus like that shown in figure 9, but such nuclei often are accompanied by signs of degeneration, and the condition is regarded as abnormal.

I have not been able to make out a rhizoplast connecting the nucleus with the blepharoplast as described by Kofoid and Swezy ('15).

The cytostome is an opening at the anterior margin of the body on the side of the major axis opposite the nucleus. This side is usually considered as ventral. The cytostome is not so large as that described and figured for *T. augusta*. There appears to be a short cavity leading into the interior along the ventral side of the axostyle.

The blepharoplast is a deeply staining granule, or possibly a pair of granules at the anterior end of the major axis of the body. To it a series of other organelles are attached, as already mentioned. The nature of this focus of organization is difficult to determine. By some authors it is regarded as homologous with the similarly named structure in some of the simpler flagellates, such as the haemoflagellates, and by others it is assumed that in the *Trichonomads* it is composite, being composed of a number of granules equal to the number of flagella attached. Martin

and Robertson ('11) thus describe it for *Trichomonas* (*Tetratrichomonas*?) *gallinarum*. Kofoed and Swezy ('15) believe it is composed of two parts, one of which is a centrosome and the other the basal granule for the flagella. In the material that I have studied this structure frequently appears to be double, that is, composed of two approximately equal parts, and the posterior flagellum is attached to the anterior moiety, while the chromatic basal rod is connected with the posterior one. Such a condition was also described by Wenyon ('07). Since the three anterior flagella take the stain so slightly, it is difficult to determine what their relation is to the blepharoplast components.

Because the three anterior flagella do not stain very deeply, they are difficult to make out. This difficulty is often increased by the presence of spirochaetes of similar caliber and staining power and by the flagella taking a position in contact with, or under, the body. In the drawings they have been omitted when not plainly seen. In all cases in which they could be clearly discerned, they appeared to be of equal length, wavy, and about one-half the length of the body, although sometimes shorter. Figures 2 to 5 and 7 to 15 show the flagella in their typical condition.

Hartmann ('10), Wenyon ('07), and Kuczynski ('14, '18) figure these flagella just as I have found them, but the figures for this species given by Kofoed and Swezy ('15) have the anterior flagella as long as or longer than the body of the animal. On account of this and other differences, one may be led to suppose that the latter authors were dealing with a different species.

The posteriorly directed flagellum running as the chromatic margin of the undulating membrane is very much longer than the others, extending the length of the body, making six to eight undulations in its course and projecting posteriorly as a free flagellum as long as the anterior flagella. This posterior part is similar to the anterior flagella in caliber and staining power, but the intracytoplasmic portion appears to be much thicker and takes the stain intensely. There is some variation in stainability depending on the fixative employed, as will be noted elsewhere.

As has been observed by other authors, the undulating membrane seems occasionally to be broken, allowing the entire flagellum to become free. Individuals with the posterior flagellum free are not rare in fixed and stained preparations (fig. 15).

The chromatic basal rod takes origin in the blepharoplast, or possibly the posterior portion of it, and extends along the surface of the body at the base of the undulating membrane. It, together with the undulating membrane, takes a spiral course on the living animals, as in figures 5 and 17, as previously noted, passing posteriorly from the left over to the right. It ends free in the cytoplasm. It appears to be a body of some rigidity because changes in its position are usually accompanied by corresponding changes in the form of the body. As described by Wenyon ('07), it may project from the body as a stiff thread. It is broadest near the middle, tapering to a slender distal terminus, and to a less slender proximal or anterior end attached to the blepharoplast. Near the anterior end it often exhibits a bend, which may even be S-shaped, which suggests a high degree of flexibility of that region (fig. 14, e.g.).

Most observers have represented this structure as a homogeneous rod. In this species I have been considerably puzzled about its organization, for frequently it appears to have embedded within it a row of granules on the inner side, similar to the row which lies close to it, but deeper in the cytoplasm (figs. 11 and 14). At other times the additional row seems to be just in contact with the rod (figs. 2, 10, 13, 21), and again the row may be adjacent to but not in contact with the rod (fig. 8). These observations indicate that new rows of granules take their origin from the basal rod and migrate inward, possibly replacing, during division, the one that is always found close to and parallel with the rod.

This outer row of chromatic granules close to the chromatic basal rod is very characteristic of this species and extends from 60 per cent to 90 per cent of the length of the rod out from the blepharoplast. It is figured by Hartmann ('10), Wenyon ('07), Kuczynski ('14, '18), and Kofoed and Swezy ('15). Another row of similar granules is found deeper in the cytoplasm, and

close to the axostyle on its dorsal side. It is easily seen in the region posterior to the nucleus, but its anterior extension is frequently obscured (figs. 1, 2, 4, 7, 9, etc.). In some cases it is traceable forward outside the nucleus up to the blepharoplast. Posterior to the nucleus this row is nearly parallel to the longer, more peripheral one. The inner row of granules is mentioned and figured by Wenyon ('07) and by Kuczynski ('14), but seems to be absent from the form described by Kofoed and Swezy ('15) under the name of *T. muris*.

In the region between the nucleus and the blepharoplast there are often additional granules similar to those in the two rows (figs. 7, 10, 14). The presence of these extra granules often makes it difficult to determine the anterior limit of the nucleus, on account of their resemblance to the granules of chromatin within the nucleus and the faintness of the nuclear membrane.

The axostyle is a hyaline cylindrical rod attached to the blepharoplast and it traverses the major axis to project slightly at the posterior end, where it tapers rapidly to a sharp point. At the point of emergence there is the ring of deeply staining substance (text fig. A, *k*) mentioned by Kofoed and Swezy. In the region of the nucleus the axostyle is frequently somewhat curved around that body which appears to lie slightly to the left of it. The axostyle seems narrower in the region near the blepharoplast than elsewhere. I have never seen any cases of a capitulum in this species such as Kuczynski ('18) mentions.

The flexibility of the axostyle is indicated by the frequent occurrence in fixed material of a decided bend at the most flexible region just posterior to the nucleus (figs. 2, 3, 11, 13, 15), but I have never seen this structure used as an organ of locomotion, as maintained by Kofoed and Swezy ('15) for *T. augusta*.

There are no chromatic granules in the axostyle except in new ones growing out from the blepharoplast in the telophase of division. However, the deeper row of granules often appears to be in contact with the axostyle in the region immediately anterior to the nucleus (figs. 1, 2, 3, 7, 8, etc.).

The parabasal body is a cylindrical curved rod, of a diameter comparable to that of the axostyle, connected by a narrow attachment to the blepharoplast and lying dorsal to and to the right of the nucleus. Its texture is apparently different from that of any other structure in the cell and its staining reaction with haematoxylin is different from the other structures. While it appears to be homogeneous, its texture is of a looser, more spongy nature than that of the structures so far mentioned. Its appearance compares well with the figures of it given by Janicki ('11). It is quite variable in length, as indicated by figures 3, 4, 5, 6, and 16, but when it is longer it often has a constriction (figs. 4, 5), or a thinner place (fig. 16), marking off two regions. One wonders if the distal portion may not become detached and serve some function in metabolism.

I have never seen any indication of a central core or thread as described by Cutler ('19) for the parabasal of *Ditrichomonas termitis*. On the contrary, in an animal which was either rounding up for encystment or else had started to degenerate (fig. 6), the parabasal appeared as a granular peripheral case enclosing a non-staining area.

Since Kofoed and Swezy ('15) employed mainly Schaudinn's fluid which seems to dissolve out the parabasal, this elusive organelle was apparently overlooked by them, and they applied the term 'parabasal' to the chromatic basal rod. The homology of the above-described parabasal in *Trichomonas muris* with the similar structures figured by Janicki ('11) for *Devescovina*, *Parajoenia*, *Stephanonympha*, and *Trichomonas* and by Cutler ('19) for *Ditrichomonas termitis* seems to me to be justifiable, but a homology between the chromatic basal rod and these parabasals of Janicki, as claimed by Swezy ('16), would, in my opinion, be open to some question.

#### *D. Differences due to different fixatives*

It will be profitable, I think, to consider at some length some differences of appearance in the organization of *Trichomonas muris* which are correlated with the use of different fixatives. The conditions found in the series of slides from mouse no. 24

illustrates this point. In this case the entire set of instruments, reagents, glassware, microscope, etc., were placed in a warm room at 37°C. a number of hours before the mouse was killed. The mouse was taken into the same warm room, killed, opened, and the coecal contents examined. The coecum was found to be swarming with *Trichomonas*, so fixations were made with Allen's, Bouin's, Carnoy's, Schaudinn's, sublimate-acetic and weak Flemming's fluids. After fixing for half an hour at 37°C., the subsequent washing and further treatment were carried out at room temperature, and all the slides were stained at the same time and in the same way with the same stock solutions of iron alum and haematoxylin. The chemical differences in the different fixatives would therefore appear to be the variable factors in this experiment, so that differences in appearance can, I think, be attributed to different effects of the fixatives on the organisms. In any smear of this kind, of course, there are always thicker and thinner areas, and the intensity of the stain varies with the thickness of the film on the cover-glass. It is therefore possible to compare for a wide range of intensities of the stain.

The general cytoplasm may first be considered. Figure 1 indicates the results from fixation with Carnoy's fluid. Little vacuolization is indicated, and such vacuoles as there are do not show any stainable contents. Figure 2 is from a smear fixed in sublimate-acetic, and here not only are the vacuoles well defined, but the contents have taken the stain. Some few individuals on this smear did not show the vacuole contents stained, but the great majority did. The smears of this series fixed in Schaudinn's fluid showed an occasional individual with vacuole contents stained. In the other series which were fixed with Schaudinn's fluid vacuole contents did not usually take the stain. Figure 3 is from a smear fixed in weak Flemming's fluid, and the structure of the protoplasm is much like that in figure 1.

The various organelles may next be considered. Schaudinn's fluid and sublimate-acetic gave somewhat similar results except for the protoplasmic vacuoles already mentioned. The nucleus, blepharoplast, posterior flagellum, chromatic basal rod, and specific granules are all sharply differentiated, although in the

sublimate-acetic slides the chromatic basal rod was not so intensely stained as in those fixed in Schaudinn's. Similar results were obtained by the use of Allen's and Bouin's fluids, except all structures appeared swollen in comparison with those prepared with other fixatives. Also the free flagella were better stained after the last two fixatives named than after the first two. In the case of Carnoy's fluid (fig. 1) the results varied considerably with the stain. In the animals showing an average intensity of the stain, the nucleus was very black, often failing to show any structure, while the chromatic basal rod and the chromatic margin of the membrane failed to stain. In contrast, the two rows of chromatic granules were stained very deeply. In the specimen drawn (fig. 1) the chromatic margin was not so strongly stained as is indicated and the nucleus was lighter than in the majority of individuals. The blepharoplast was also faintly stained on these slides, while the free flagella and the axostyle were fairly well defined in most cases. After weak Flemming's fluid all the structures were rather indistinctly differentiated by the stain, and yet these slides were the only ones in which the parabasal body appeared.

I did not find the parabasal body until after reading the paper by Cutler ('19), who describes its occurrence in *Ditrichomonas termitis*. According to Cutler, this structure was not constant in material prepared with the usual fixatives, but by employing Flemming's without acetic acid and other fixatives which contained neither acetic acid nor corrosive sublimate, he was able to demonstrate it consistently. Following his suggestion, I employed on the same lot of material from mouse no. 29 Allen's, Bouin's, and Flemming's fluids each without acetic; also 1 per cent chromic acid containing 1 per cent urea and several strengths of formalin, together with unmodified Schaudinn's and Allen's fluids as controls. The latter two fluids gave the best general fixation, but the Flemming's without acetic and the 1 per cent chromic acid both brought out the parabasal in some individuals when subsequently stained with iron-alum haematoxylin. Since Janicki ('11) found the parabasal in *T. baetrachorum* which had been fixed with an 'osmic acid mixture,' I was led to scrutin-

ize all of my slides which had been fixed with Flemming's fluid, with the result that I detected this structure in *T. batrachorum* and *T. augusta* from the leopard frog and in some slides of *T. muris* fixed with weak Flemming's. Later I found the same structure in *T. caviae* in material fixed with weak Flemming's and Flemming's without actic. The parabasal was most clearly differentiated in the slides of *T. muris* fixed in weak Flemming. Since in the weak Flemming the amount of osmic is reduced and since, further, the parabasal appeared in slides fixed with 1 per cent chromic acid, it would seem that the chromic acid is as much if not more responsible for bringing out this structure than is the osmic acid. Also, my experience does not parallel that of Cutler ('18) in the case of formol, since none of my formol-fixed preparations showed the structure.

In the slides fixed with weak Flemming from mouse no. 24 a great majority of the flagellates showed the parabasal plainly, while in a few it was difficult or impossible to make it out. In the slides from mouse no. 29 fixed with 1 per cent chromic and with Flemming's without acetic only a small percentage of the flagellates exhibited the parabasal. There thus appear to be individual variations with the same technique as well as differences due to differences in technique. Kuczynski ('14) found the parabasal in only four out of more than fifty guinea-pigs and in none of the mice, although over a hundred were examined.

The above results point to the necessity of employing a variety of methods of technique, since reliance upon a single method might readily lead to erroneous conclusions.

### *E. Encystment*

Encystment in *Trichomonas* has been much disputed, there being few observations of a conclusive nature showing the existence of cysts. Wenyon ('07) called attention to the existence in the faeces of the mouse of large numbers of rounded-up individuals which he stated could live for a week or more outside the host if kept moist. Some others, which were much contracted and rounded up, he thought were encysted, and he figures such a specimen in his figure 35, plate 11. I have seen many of the



rounded-up kind, especially in material from hosts which had been dead several hours. I have also seen in some hosts considerable numbers of the contracted forms in the coecal contents. In figure 36 I have represented one of these, and it is very similar to the one figured by Wenyon. In figure 35 there is shown one which is apparently in the process of changing to the rounded and contracted condition. I am inclined to the belief that these animals are preparing to encyst, since there is no sign of degeneration except the apparent disappearance of the free flagella.

#### DIVISION

All authors who have studied carefully the division of any of the species of *Trichomonas* agree that the process is complicated and appears to take a relatively long time for its accomplishment. Kuczynski ('14) gives eight hours as the time for *T. augusta*. It is also generally agreed that the flagellates remain active during the entire process, the flagella and undulating membrane continuing to vibrate even in the rounded-up condition which is characteristically assumed during part of the time. The extensive activities of the post mitotic phase have been well described and illustrated for *T. augusta* by Kofoid and Swezy ('15).

Since it is possible to recognize in the division of the nucleus stages comparable to those of mitosis in metazoan cells, it will be convenient to refer to these stages under the conventional terms, prophase, metaphase, anaphase, and telophase.

#### *A. Prophase*

1. *The nucleus.* The first changes in the nucleus which indicate the approach of mitosis result in the formation of the prophase chromosomes out of the scattered chromatin granules of the 'resting' nucleus. There are always six of these chromosomes, and each one consists of a pair of closely associated moieties. The parts are often somewhat elongated and the two components lie side by side. These prophase elements remain connected with each other and with the caryosome, until the end of the prophase stage, by the fine strands of non-chromatin

reticulum of the nucleus (figs. 10 to 17 and 21). Occasionally the six elements become arranged in the form of a chain, recalling the chains of split chromomeres sometimes seen in metazoan prophases (fig. 15). In cases where the fixation has not been good, the two parts of each element appear to be fused together, so that the nucleus seems to have six single granules in it in addition to the caryosome. This condition seems to be more prevalent in the later than in the earlier prophases (fig. 19). Since in the earliest stage in which the prophase chromosomes can be distinguished they are already double, it has been impossible to determine whether or not the doubling is the result of antecedent splitting.

In the earlier stages the six chromosomes are always outside the clear area surrounding the caryosome, but later the boundary of the clear area disappears, and the caryosome then seems to be more directly connected with adjoining chromosomes by the non-chromatic reticulum (fig. 15). In cases where the chromosomes appear to be single, due to fusion, and where the pericaryosomal space can no longer be defined there seem to be seven chromosomes instead of six, since the caryosome is not always easily distinguishable from the chromosomes. In all such cases, however, careful study has resolved the group of seven into six chromosomes and one caryosome. During the progress of the prophase changes the caryosome gradually loses its staining power just as do nucleoli of metazoan cells, and at the metaphase no trace of it is visible. Figure 22 shows a very late prophase or early metaphase with the spindle partly formed and a faintly defined vestige of the caryosome.

*Number of prophase chromosomes.* Wenyon ('07) reports the number of prophase chromosomes as six and says that they early divide into two, giving six pairs of granules. In his figure 2, plate 11, for example, he shows six pairs of granules besides a caryosome. My results are thus in agreement with his. Kuczynski ('14) describes eight prophase and four metaphase chromosomes and again insists on these numbers in his later paper ('18). In this later paper, however, he admits (p. 128) that "Over 70 per cent of the observed prophase nuclei of the Tri-

chomonads named (*T. muris*, *T. augusta*, *T. Caviae*, and *T. batrachorum*) contain seven sharply outlined chromosomes although in many cases, of which a number have been pictured (e.g., plate I, figs. 16, 17; plate II, fig. 20; plate IV, fig. 57; plate VII, fig. 96), the probability is great that the position of the chromosomes interferes with the certain recognition of an eighth. Chromosome-groups of only six, of uncertain separation, occur much more seldom." In all the figures mentioned in the quotation (except in fig. 96), and in some others not mentioned, the groups can be resolved into six split prophase chromosomes and one caryosome. In the few cases where Kuczynski thinks he finds eight, I am inclined to the belief that he may have counted as separate chromosomes the two parts of one which had become rather widely separated; then, with the caryosome, the number eight is obtained.

Kofoid and Swezy ('15) give five as the chromosome number both for the prophases and the metaphase for *T. muris* and *T. augusta*. If the form which they called *T. muris* is the same species as the one I have been studying, the difference in chromosome number needs to be accounted for. I will merely refer to the great difficulty in elucidating these small details in such minute organisms, even when the technique has been good, and to the further possibility that the form studied by them was of a different species.

As for other species, since Kuczynski finds and figures conditions in *T. caviae* so similar to those in *T. muris*, I am inclined to believe that there are six chromosomes in *T. caviae*. Dobell ('09) found six chromatin bodies in *T. batrachorum*, but hesitated to call them chromosomes. Martin and Robertson ('11), on the other hand, described for *T. eberthi* eight prophase and four metaphase chromatin units, although they prefer not to call them chromosomes. It can hardly be argued that all species of *Trichomonas* should have the same number of chromosomes, but since Dobell and Wenyon have both found six and since the numbers in the species studied by Kuczynski are probably six instead of eight, the situation in *T. eberthi* might bear reinvestigation.

I have not found stages with the so-called 'nuclear cloud' as described by Kofoid and Swezy ('15) as shown in their figure 49, nor have I seen the spirene stage shown in their figure 50. My figure 9 shows a condition somewhat similar to their figures 46 and 47, but I think such nuclei are abnormal, particularly since they are so much larger than usual and often accompany other evidences of degeneration.

2. *Chromatic basal rod.* Coincident with the intranuclear changes of the early prophase, the new chromatic basal rod makes its appearance. Usually it appears some time before the blepharoplast has divided and is very difficult to recognize in its earliest stages. Figure 11 shows the earliest stage in which I have been able to find this structure, and here it will be seen to consist of a row of very fine granules closely connected together and joined to the blepharoplast. Figure 10 shows a stage which seems to be a little later, judging by the nuclear changes, and here also the new chromatic basal rod is a row of granules, but much longer than the one in figure 11. I was unable to trace it past the nucleus and up to the blepharoplast.

The new rod is always in a characteristic position, dorsal to, and to the right of, the nucleus (figs. 10 to 17). Although relatively slender at first, it gradually increases in size until by the time the blepharoplast divides it is easily recognizable. After the division of the blepharoplast the new rod does not always maintain its position near the surface of the body. In figure 19, for example, the new blepharoplast is at the upper surface, while the new rod extends from it around the nucleus, deep into the protoplasm to the lower surface.

3. *The new undulating membrane and chromatic margin.* As the new chromatic basal rod grows, irregular thickenings appear along its length, as indicated in figures 12 and 15. A little later one can see the new chromatic margin of the new undulating membrane closely applied to the new rod (figs. 19 to 21). In its first recognizable condition this chromatic margin is of much smaller caliber than the old one, its undulations are low and in length it cannot be traced beyond the distal end of the new rod (figs. 20 to 26). In figure 19 it was possible to trace the new

chromatic margin along only apart of the course of the new rod, although presumably it extended the whole distance. In the part which could be made out, however, it remained close to the rod, and hence transversed the deeper protoplasm along with the latter organelle. This deeper position would hardly be expected if the new chromatic margin, or posterior flagellum, had been split off from the peripherally placed old one.

I have not been able to see evidence of a splitting of the undulating membrane and the chromatic margin, as described by Kofoid and Swezy ('15), although I have searched long and diligently for such evidence. My evidence indicates that the new chromatic margin grows out along the new chromatic basal rod as a new structure just as the other flagella grow out as new structures. In figure 18 I have drawn an individual which appeared to have the old chromatic margin double for the anterior half of its length. The two portions appear to be of equal caliber. The nucleus could not be made out distinctly and there are other indications of degeneration, so that I regard this individual as abnormal, especially since I have carefully examined such large numbers in all stages of division without ever finding any other specimen that indicated a splitting of the membrane.

Wenyon ('07), Martin and Robertson ('11), and Kuczynski ('14, '18) also find the new posterior flagellum growing out as a new structure, although Dobell ('09) describes the splitting of the undulating membrane in *T. batrachorum*. I am inclined to agree with Kuczynski that Dobell, and Kofoid and Swezy have been misled by the secondary filament in the undulating membrane of *T. augusta* and *T. batrachorum*, and I am quite convinced that splitting of the undulating membrane does not normally occur in *T. muris*.

4. *The blepharoplast.* After the new chromatic basal rod has been formed, the new blepharoplast appears, connected to the old one by the paradesmose (Kofoid and Swezy, '15). Figure 17 shows a relatively early prophase with the new rod attached to a small granule, which in turn is connected with the old blepharoplast. In my opinion, this small granule is the new

blepharoplast. In nearly all cases the new blepharoplast is smaller than the one attached to the old chromatic margin, and it would not be unexpected if it should begin as a small bud from the main or mother blepharoplast. The daughter blepharoplast continues to separate from the old, until the two are on opposite sides of the nucleus. The paradesmose connecting them remains on the outside of the nuclear membrane which appears to persist during division (fig. 23). Figure 22 shows the two blepharoplasts in place and the spindle forming in the nucleus, while the chromosomes are not quite completely aligned in the equatorial plate.

5. *Other structures.* On account of the poor stainability of the anterior flagella and on account of their frequent position on or close to the cell to which they belong, and on account of the presence oftentimes of large numbers of slender bacilli and wavy spirochaetes, the behavior of these structures in division has been difficult to follow. I am convinced, however, that the accounts of other authors are correct to the effect that one or two of these flagella accompany the new blepharoplast, while the other two or one remain with the old or parent blepharoplast (figs. 25 and 26). New flagella to make the full number appear to be formed as new outgrowths from the blepharoplasts (fig. 32).

Late in the prophase the axostyle becomes separated from the blepharoplast and begins to degenerate (figs. 20 to 22). New axostyles grow out from the blepharoplasts in the telophase, as will be described later.

I have not been able to detect any peculiarities in the behavior of the parabasal body during the prophases. I have drawn figure 16 to show that there cannot possibly be any confusion between the parabasal and the outgrowing new chromatic basal rod. The parabasal is unusually long in this specimen and there is a thin region over the nucleus which suggests that the distal end may possibly become detached. This idea is also suggested by figures 4 and 5, where there is a constriction; but in these latter cases there is no evidence of approaching division.

I have already suggested that new long rows of chromatic granules grow out from the chromatic basal rod. On the other

hand, there is some evidence of division of these granules, as seen, for instance, in figure 8. Here the long row seems to be double in the distal part and the two rows appear to lie close together. The distance between them is foreshortened, however, in this position. The duplication in connection with the short row behind the nucleus is difficult to interpret, and I am not sure that division of the granules is indicated.

### *B. Metaphase*

Figures 22 to 26 show a series which includes a very late prophase or early metaphase (fig. 22), metaphases, and early anaphases, which indicate very well the behavior of the chromosomes in these stages. In figure 22 the chromosomes are still similar to those of the earlier prophases, the two parts of each being closely approximated with their long axes parallel. Although the fibers of the forming spindle have already become attached to the chromosomes, the latter have not as yet lined up into a definite plate. It appears from these figures that whatever directive influence the spindle fibers may have in the separation of the chromosomes, it is exercised for some of them before the plate has become established. All the figures with an equatorial plate show the two parts of some of the chromosomes already drawn out so that they are in contact only at their ends, while others are just in the process of being separated. Since I have seen a great many animals in the stage indicated by figures 23, 24, and 25 and none showing stages between them and figure 22, I judge that some of the chromosomes are separated during the formation of the metaphase plate.

As seen in the figures mentioned, the number of chromosomes in the metaphase is definitely six, the number found in the prophases. Martin and Robertson ('11) and Kuczynski ('14, '18), as previously noted, believe that eight(?) prophase chromosomes are reduced to four multiple elements in the metaphase. I think I have demonstrated the probability that the prophase number in Kuczynski's figures is six, and the tendency for the metaphase chromosomes to clump probably accounts for the apparent number, four. Kofoed and Swezy ('15) do not show

any metaphase figures for *T. muris*, and even in their extensive figures for *T. augusta* they have nothing corresponding with my figure 22. They therefore missed the evidence showing that the process of separation at the metaphase and anaphase corresponds to the details as seen in the corresponding stages in metazoan mitoses, except for the precocious separation toward the two poles before the equatorial plate is completely formed. Their figures 20 and 21 for *T. augusta* in which the metaphase chromosomes are seen as single elements elongated in the direction of the spindle axis possibly show conditions in which the constriction between the separating chromosomes has been eliminated by contraction of the chromatin in the process of fixation.

### *C. Anaphase*

Figures 26 and 27 illustrate anaphases. I have not seen so many anaphases as I have metaphases, and presume that this phase is of shorter duration. During this stage the chromosomes appear to become elongated (fig. 26) and constricted (fig. 27). Figure 27 shows the smallest chromosome as having divided precociously and the daughter elements are much nearer the poles than those of the other chromosomes.

### *D. Telophase*

After the chromosomes have been completely separated and the two daughter groups have arrived at positions some distance apart, the nucleus which has been elongating during the anaphase (fig. 27) becomes constricted in the middle (figs. 28 and 29), thus forming the two daughter nuclei. The nuclear membrane persists throughout this process. In the early telophases the chromosomes begin to change their appearance, becoming less dense and more granular. The constriction which first appeared in the anaphase becomes more pronounced and each of the former chromosomes appears to be made up of two rounded or slightly elongated parts in contact at the ends (fig. 31). These eventually give rise to the scattered granules seen in the resting nucleus and the new caryosome becomes established surrounded by its



characteristic clear area. I have not been able to make out the precise method of origin for the caryosome.

The entire number of six chromosomes can usually be seen when a polar view of the telophase group can be had, such as is shown for the lower nucleus in figure 30. The complete number is also seen in the side views of figure 31. Kuczynski ('14) likewise shows six in a similar stage in his figure 66 of *T. muris*. His figures 64 to 67 and 69 also show well the constriction of the anaphase and early telophase chromosomes that I have mentioned.

Since the telophase chromosomes appear to resolve themselves each into two chromomeres, and since the earliest prophase chromosomes which can be recognized as such are already double, one naturally wonders if the two parts of a prophase chromosome may not be represented by the two telophase chromomeres. Since the two telophase chromomeres are arranged end to end, while the two parts of a prophase chromosome are arranged side by side, and since the number of chromatin granules in the resting nucleus is rather large and indefinite, the direct relationship suggested is improbable.

While the two daughter nuclei are becoming reorganized into typical resting nuclei, complete sets of other organelles are being established for the two new individuals. The origins of most of these organelles have been discussed in connection with the prophase. The chromatic basal rod and the flagella merely complete a development initiated at the earlier phase. The new axostyles, however, apparently do not begin to grow out until the telophase. There is a suggestion of a new axostyle growing out from the old blepharoplast in the early stage shown in figure 29, but in figures 32, 33, and 34 the new axostyles are distinctly seen. In figure 34 it will be noted that the new axostyle growing out from the older, larger blepharoplast is longer than the other one, as might be expected. It will also be seen from these figures that there is a row of chromatic granules along the new axostyles. These appear to be imbedded in the axostyles and are probably intimately concerned in the formation of these organelles. These granules must go to the surface later or disappear, for they do not occur within the adult

axostyle. It is possible that the chromatic granules seen in the adult along the axostyle from the blepharoplast to behind the nucleus (figs. 3, 7, and 8) are the same as the ones which appear to be concerned in the formation of the new axostyles.

It is probable that the degeneration of the axostyle in the late prophases accounts for the rounding up of these animals at about that stage in the division process.

Kuczynski ('14, '18) saw and figured the degeneration of the old axostyle and the growing out of the new ones from the blepharoplasts, and Martin and Robertson ('11) report the same thing for *T. eberthi*. Wenyon states that the axostyle ('pointed organ') divides by longitudinal division, but offers no evidence in support of this statement. Kofoid and Swezy ('15) show one figure (fig. 60) which they interpret as showing division of the axostyle in *T. muris*. But the figure is also open to the interpretation as a partial superposition of two independently formed elements, and since it is the only one they could find after prolonged search, the evidence is not very conclusive. Since the evidence of the degeneration of the old axostyle and the origin of new ones as outgrowths from the blepharoplasts is so conclusive in my material and in the results reported by Kuczynski, the origin of this structure by splitting may be regarded as extremely doubtful, at least for *T. muris*. Dobell ('09) and others were undoubtedly in error in believing that the new axostyles developed from the paradesmose. This structure retains its connection with the two blepharoplasts for some time after the division of the nucleus (figs. 31, 34), but eventually disappears.

Kofoid and Swezy state for *T. muris* that the long row of granules disappears during metaphase and reappears in the telophase. I have been able to find them at practically all stages of division but, as previously noted (p. 128), there is evidence that the old row may be replaced by a new one which is budded off from the ventral (inner) side of the chromatic basal rod. Just how the new chromatic basal comes to have an associated row of granules has not been determined.

I have not been able to find stages showing any division of the parabasal body. In all the anaphases and later stages (figs. 26 and 28) there appears to be a parabasal for each blepharoplast. Whether the old one disappears and two new ones grow out, or whether the old one remains and one new one grows out, or whether some other mode of origin may prevail has not been determined. One point should be noted, however, namely, that in these anaphases and telophases the parabasal attached to the daughter blepharoplast is always smaller than the one connected with the old blepharoplast. Considering a possible analogy with the chromatic basal rod, this fact might be interpreted as indicating that the old parabasal persists and a new one grows out from the new blepharoplast. The one attached to the old blepharoplast is not so long as the longest ones seen in the non-dividing and earlier prophase stages (figs. 3, 4, 5, 16), but is comparable in length to the portion proximal to the constriction as seen in figures 4 and 5 or proximal to the fainter area in figure 16. The suggestion already made that the portion distal to the constriction may become detached will be recalled.

The origin of all the new structures has been discussed, except the cytostome. This structure is not much in evidence during the metaphases and anaphase, but two cytostomes appear in the telophase. It is possible that the old one, like the axostyle, disappears, and two new ones are formed. Before division of the cell body, all the organelles in the two sets apparently become developed to a condition corresponding to that of the original set.

No cases have been found showing the constriction of the cell body in my fixed and stained slides, but I have frequently observed this process in the living animals. It takes place rapidly and the two separating individuals always appear to be of equal size and completely developed. The long interval between the division of the nucleus and the division of the cell body doubtless serves to allow the new organelles to attain complete development before the daughter cells separate.

I think it is worth while to point out that according to the evidence which I have presented there appear to be only two

parts of this complicated flagellate that divide equationally. They are, 1) the nucleus, including the chromosomes, and, 2) the cell body. The blepharoplast, chromatic basal rod, posterior flagellum, and possibly also the parabasal body and one or two of the anterior flagella of the parent appear to be retained by one of the new daughter individuals, while the other daughter is supplied by new outgrowths, including a new small blepharoplast budded off from the parent one. The old axostyle, and possibly also the old cytostome, disappear and a new one is formed for each new cell. New chromatic granules appear to have a different origin, as previously described. This behavior is paralleled by that of the Infusoria, exemplified by *Paramecium*, which remains active during the process of division. Some of the cilia and one of the contractile vacuoles are taken by each daughter cell, and new ones are formed to make the complete set of organelles. Part of this development in *Paramecium* takes place after the separation of the daughter cells, whereas in *Trichomonas* development of the new organelles appears to be completed before the daughters separate. Since in both cases the daughter cells come to resemble each other completely, their hereditary potentialities must be equally descended from the parent. An equational division of the nuclear material would therefore be sufficient to insure equality between the daughter cells, granting that the nuclear material constitutes the physical basis of heredity.

#### SUMMARY OF THE MORE IMPORTANT RESULTS

1. *Trichomonas muris* (Hartmann) from the coecum of the mouse measures 10 to 16 $\mu$  long by 5 to 10 $\mu$  wide, but varies in size slightly from host to host and to a larger extent as a result of the use of different fixatives.

2. Different fixatives also give rise to different staining reactions of the protoplasmic vacuoles, nuclei, and other organelles.

3. The anterior free flagella are short, not more than half the length of the body, and stain faintly with iron-alum haematoxylin stain. The posterior flagellum stains intensely as the chromatic margin of the undulating membrane, but its posterior

free extension is similar to the anterior flagella in length and staining capacity.

4. The chromatic basal rod is thicker in the middle and tapers toward both ends. It appears to give origin to the outer row of chromatic granules by a kind of budding process.

5. There is a deeper row of chromatic granules near the axostyle extending from behind the nucleus up to the blepharoplast.

6. There is a parabasal body similar to the one described by Janicki and Kuczynski. It has a position dorsal and to the right of the nucleus. It varies in appearance and occurrence from host to host, from flagellate to flagellate, and from one fixative to another. It has appeared after the use of weak Flemming's, Flemming's without acetic, and 1 per cent chromic acid solutions.

7. In the prophase of division the chromatin becomes organized into six double (split ?) prophase chromosomes and the caryosome gradually disappears. A new chromatic basal rod grows out from the blepharoplast and appears first as a row of fine granules. It is connected with the small new blepharoplast which a little later becomes budded off from the main, or parent, one. The two blepharoplasts are connected by a paradesmose.

8. Before the metaphase has been reached the axostyle becomes detached from the blepharoplast and begins to disintegrate.

9. In the metaphase six definite chromosomes are found, but the two parts of each tend to separate in the late prophase while the equatorial plate is forming.

10. In the anaphase the chromosomes become granular and each divided into two equal parts by a transverse constriction. The body of the nucleus divides by simple constriction, the nuclear membrane persisting through the process.

11. In the telophase six chromosomes, each doubled by the transverse constriction, can be seen. These become organized into the 'resting' nucleus. A new axostyle grows out from each blepharoplast. The origins of the new parabasal bodies and cytostomes were not definitely made out.

12. The two sets of organelles retain the common protoplasmic body until development is complete, and then the cell body divides rapidly.

13. The only parts of the cell to divide equationally are,  
a) the nucleus, including the chromosomes, and, b) the cell body.

## LITERATURE CITED

- ALLEN, EZRA 1916 Studies on cell division in the albino rat (*Mus norvegicus*, var. *alba*). II. Experiments on technique, etc. *Anat. Rec.*, vol. 10.
- CUTLER, D. WARD 1919 Observations on the protozoa parasitic in the hind gut of *Archotermopsis wroughtoni* Desm. Part I. *Ditrichomonas* (*Trichomonas*) *termitis* Imms. *Quart. Jour. Mic. Sci.*, vol. 63.
- DOBELL, CLIFFORD C. 1909 Researches on the intestinal protozoa of frogs and toads. *Quart. Jour. Mic. Sci.*, vol. 53.
- 1914 Cytological studies on three species of amoeba, etc. *Arch. f. Protist.*, Bd. 34.
- HARTMANN UND KISSKALT 1910 *Praktikum der bakteriologie und protozoologie*. II Teil. Protozoologie, von M. Hartmann. 2te Aufl. Jena.
- JANICKI, C. 1911 Zur Kenntniss der Parabasalapparat bei parasitischen Flagellaten. *Biol. Cent.*, Bd. 31.
- KOFOID, C. A., AND SWEZY, OLIVE 1915 Mitosis and multiple fission in trichomonad flagellates. *Proc. Amer. Acad. of A. and S.*, vol. 51.
- KUCZYNSKI, M. 1914 Untersuchungen an Trichomonaden. *Arch. f. Protist.*, Bd. 33.
- 1918 Ueber die Teilungsvorgänge verschiedener Trichomonaden und ihre Organisation im allgemeinen. *Arch. f. Protist.*, Bd. 39.
- MARTIN, C. H., AND ROBERTSON, MURIEL 1911 Further observations on the coecal parasites of fowls with some references to the rectal fauna of other vertebrates. Part I. *Quart. Jour. Mic. Sci.*, vol. 57.
- SWEZY, OLIVE 1916 The kinetonucleus of flagellates and the binuclear theory of Hartmann. *Univ. of Cal. Publ. in Zool.*, vol. 16.
- WENYON, C. M. 1907 Observations on the protozoa in the intestine of mice. *Arch. f. Protist.*, Suppl., Bd. 1.

## EXPLANATION OF PLATES

The drawings have all been outlined with the aid of a camera lucida, using a Spencer 1.8 mm, oil-immersion objective and a Zeiss no. 12 compensating ocular. The draw-tube was set to make a magnification of 4000 at the level of the table where the tracing was done. In reproduction the magnification has been reduced to 3000. Details of structure were completed with ink while the object remained under observation, then each drawing has been checked two or three times by subsequent comparison with the object. All figures from material stained with iron-alum haematoxylin. Fixation will be indicated for each figure. For these fixing fluids the following abbreviations will be used: Allen's for Allen's 'B-15,' Carn. for Carnoy's fluid, Schaud. for Schaudinn's fluid, sub.-acet. for sublimate-acetic, and wk. Flem. for the weaker fluid of Flemming.

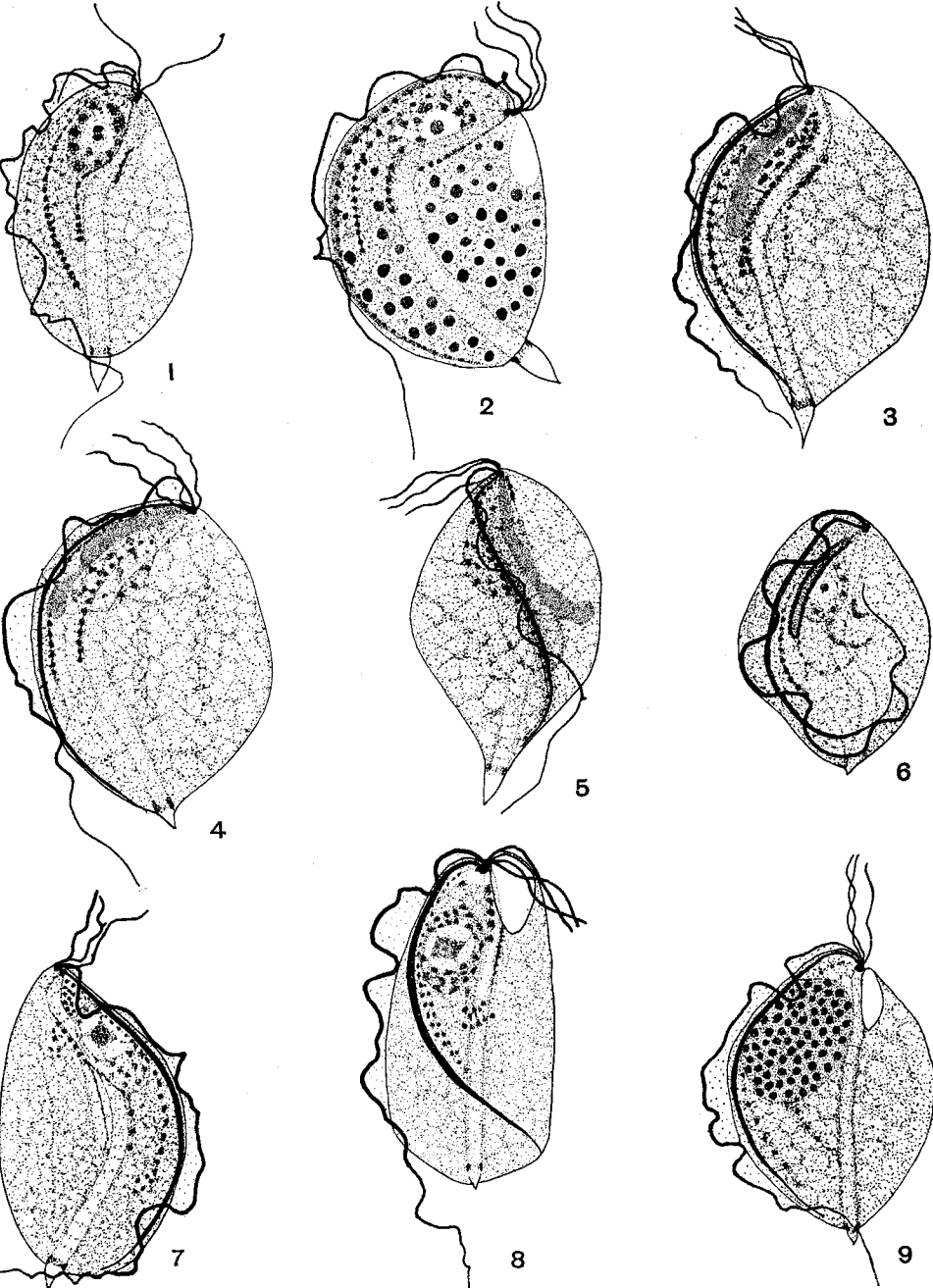
### PLATE 1

#### EXPLANATION OF FIGURES

1 to 3 Vegetative individuals showing some differences due to use of different fixatives; fig. 1, Carn., chromatic basal rod not stained; fig. 2, sub.-acet., vacuole contents stained; fig. 3, wk. Flem., parabasal body stained.

4 to 6 Wk. Flem., parabasal body stained, constricted in figs. 4 and 5, undergoing change in fig. 6.

7 to 9 Schaud., vegetative individuals; fig. 7, view from left side; fig. 8, possible division of rows of chromatic granules; fig. 9, hypertrophied (abnormal ?) nucleus.



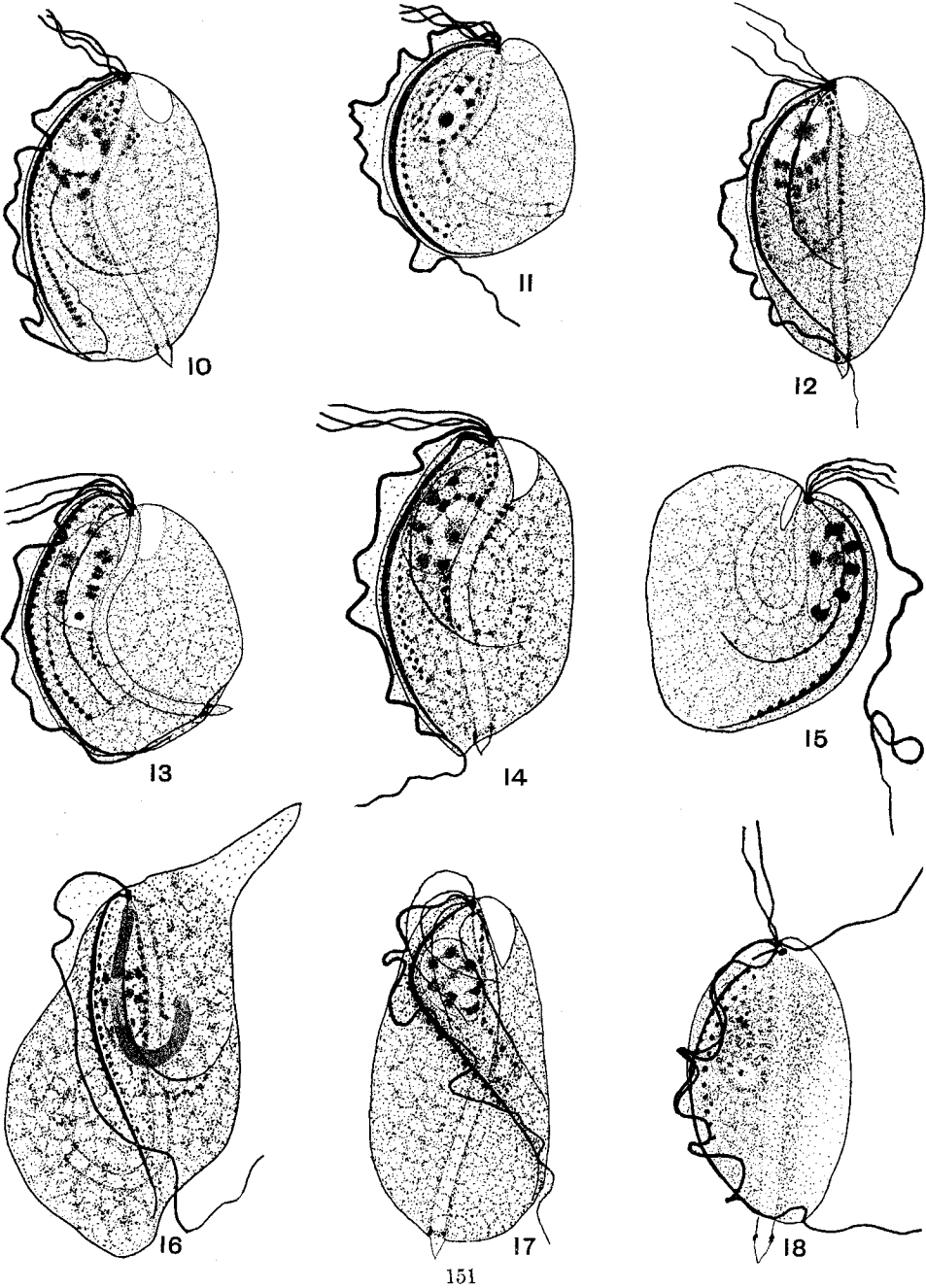


## PLATE 2

### EXPLANATION OF FIGURES

Figs. 10 to 15, 17, 18, Schaud.; fig. 16, wk. Flem.

10 to 17 Prophases; fig. 18, apparent division of chromatic margin of undulating membrane. Figs. 10 and 11, early granular stage in the formation of the new chromatic basal rod. Figs. 12 to 15 and 17, six double split chromosomes besides the caryosome which gradually loses its staining capacity. Fig. 16, new chromatic basal rod together with the parabasal body. Fig. 17, budding of a small new blepharoplast to which the new chromatic basal rod is attached.



## PLATE 3

### EXPLANATION OF FIGURES

Figs. 19 to 21, 25, Schaud; figs. 22 to 24, 27, Allen's; fig. 26, wk. Flem. Figs. 19 to 21, late prophase; figs. 22 to 25, metaphases; figs. 26 and 27, anaphases.

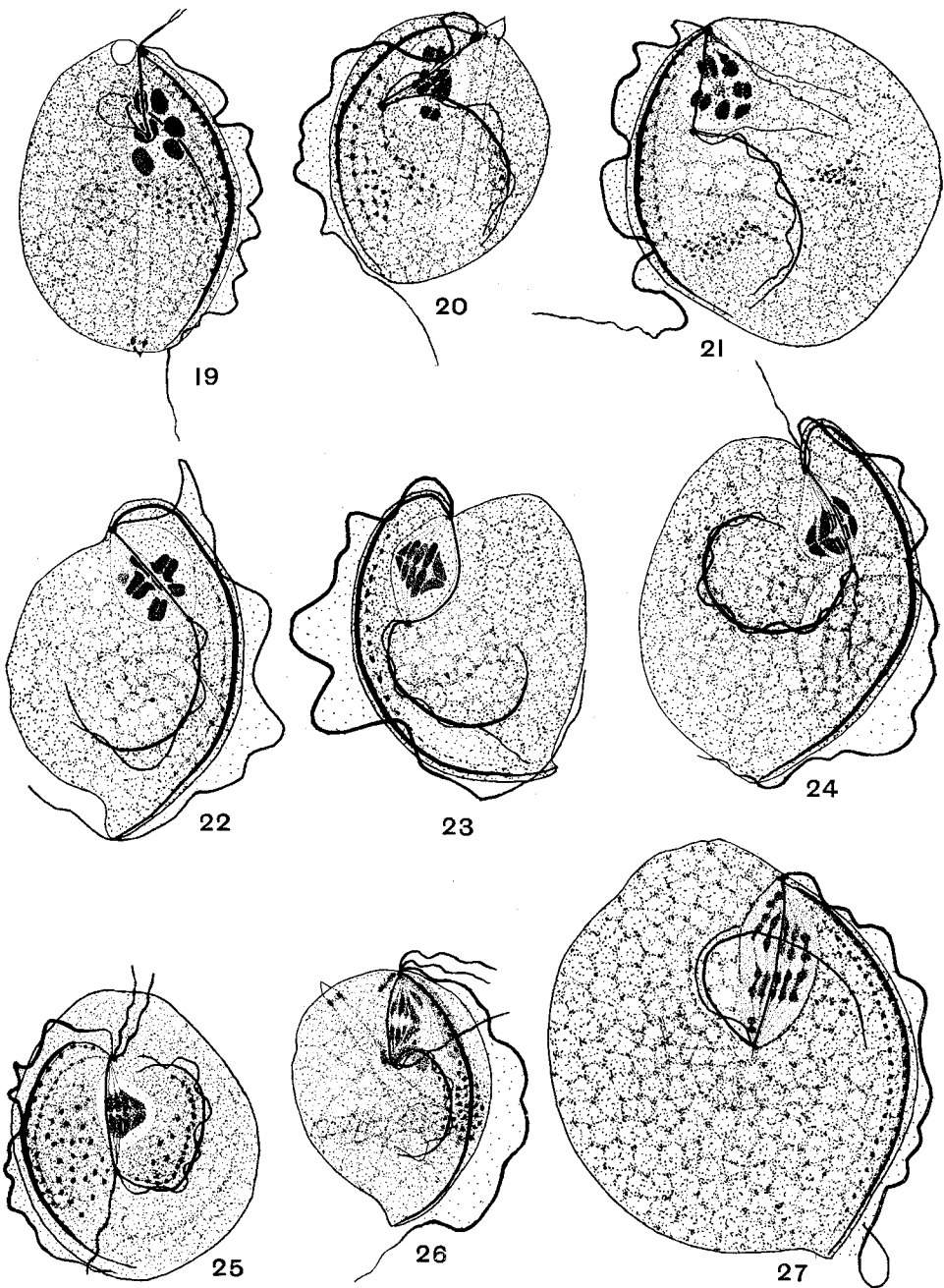
19 Paradesmose between blepharoplasts; new chromatic basal rod and new chromatic margin penetrating deep into the cytoplasm; chromosome moieties fused, giving the appearance of six single elements.

20 Blepharoplasts 180 apart; axostyle detached and beginning to degenerate.

22 Beginning of the spindle and beginning of separation of daughter chromosomes; remnant of caryosome seen.

26 New parabasal body attached to the daughter blepharoplast.

27 Constriction in the anaphase chromosomes; a small chromosome has divided precociously.



## PLATE 4

### EXPLANATION OF FIGURES

Fig. 28, wk. Flem.; figs. 29 to 32 and 36, Schaud.; figs. 33 to 35, Allen's.

28 Early telophase: delayed separation of chromosomes; two parabasal bodies.

29 Constriction of nuclear membrane; degenerating axostyle.

30 to 34 Telophases. Fig. 30, side view of one, and polar view of the other daughter nucleus. Fig. 31, constriction in daughter chromosomes; paradesmose intact. Figs. 32 to 34, formation of 'resting' nuclei and outgrowth of new axostyles.

35 and 36 Precystic changes.

