Studies on the Morphology and life History of *Woordruffia metabolica*, nov. sp.

By

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With 2 figures in the text and plates 20—21.

This is the first report in a series of studies being made on a large ciliate collected from a small pond near the Stanford campus over a year ago. It has been possible to culture this organism in large numbers in the laboratory since it was first collected. For reasons which will be given in the course of the paper we have placed this form in the genus *Woodruffia* described by KAHL (1931). The same species described by KAHL has been reported by HORWATH (1935). This organism differs in certain respects from the single species described by KAHL, differences which we consider are species differences.

Methods.

The following fixing fluids were used successfully with this organism: DA FANO'S, SCHAUDINN'S, B—15, saturated sublimate to which 0,1 per cent osmic acid was added.

Resting cysts were fixed in sublimate, alcohol, SCHAUDINN'S and Thymolether-alcohol. This latter mixture gave a minimum of shrinkage and distortion.

The stains used were HEIDENHAIN'S hematoxylin, HARRIS' hematoxylin, basic fuchsin, MALLORY'S triple, alum cochineal, and the FEULGEN reaction.

For preservation of form and general shape, specimens of *Woodruffia metabolica* were fixed in DA FANO'S cobalt-nitrate fluid. Subsequently they were stained in alum cochineal.

The nucleus of the division cysts stained most sharply after the use of Allen's B—15 fixing fluid.

For details of structure both whole mounts and sections were made following fixation in Schaudinn's fluid. Sections were cut at 6 u and 10 u, after dehydration with tertiary butyl alcohol.

The peculiar organization of the chromatin within the macronucleus makes it necessary to give a detailed description of its staining reactions (the following refers only to material fixed in Schaudinn's). Iron-hematoxylin (Heidenhain's): On both whole mounts and sections the macronucleus stained uniformly and had the appearance of being coarsely granular. Iron-hematoxylin (Heidenhain's) following a pre-treatment of material with HCl: By this method a startlingly different picture of the macronucleus was obtained. Two distinct bands of coarsely granular material were stained darkly by the hematoxylin and were surrounded by or embedded in a matrix of fine granules which stained a very light gray. Feulgen's reaction gave a similar picture, that is, two bands of granules which exhibit the characteristic Feulgen reaction color of reddish-violet, surrounded by a matrix of granules which remain uncolored. We assume, therefore, that the chromatin is confined to these bands. Compared with the reaction on the macronuclei of other ciliates (*Colpoda*, *Paramecium*, etc.), the Feulgen reaction on this organism always gives a very light color intensity. The presence of less chromatin substance might be indicated. Basic fuchsin (Grübler's Diamant Fuchsin) alone, or after a pre-treatment of sections or whole mounts with HCl gave the clearest staining of the chromatin granules. The result was essentially the same as with the Feulgen reaction except a deeper color intensity was obtained. Harris' hematoxylin as a stain for sections was also found useful.

The micronucleus was sharply and intensely stained with each of the above mentioned stains.

The problem of staining this organism was increased measurably by the presence in the cytoplasm of metabolic granules as well as degenerating particles of the nuclei of ingested paramecia. These latter, being chromatin, stained intensely with the Feulgen reaction in marked contrast to the lightly staining nucleus of the organism.

The use of HCl [1]) was found imperative (1) to differentiate the granular elements of the macronucleus, (2) to decrease the stainability of the metabolic granules, and (3) to stain the basal granules and fibers.

[1]) The method employing HCl will be published shortly.

KLEIN'S Silver Method: The organisms were placed on a slide in a small drop of distilled water which was allowed to evaporate at room temperature. Two-percent silver nitrate was dropped on and the slides were left in the dark from 10 to 30 minutes. They were then reduced in direct sunlight. Only small organisms could be used successfully as the larger forms invariably ruptured during evaporation of the water.

Relief methods: 0.25 per cent nigrosin was mixed with a drop of water containing the organisms. After one half an hour the organisms in a drop of this stain were transferred to a slide and the liquid allowed to evaporate. China blue (GRÜBLER'S) was also used as a relief stain, to outline the cilia.

Culture and Life History.

At first the culture of this organism seemed to offer quite a problem. Numerous trials were made using small ciliates (*Colpoda*, *Colpidium* and *Tetrahymena*) as the source of food. In every case the organisms formed resting cysts. Similar results were obtained using separately several kinds of bacteria suspended in balanced salt solution. Algae from the same pond were tried without success. While observing several specimens in a mixed culture, one *Woodruffia* was noticed swallowing a *Paramecium*. Following this observation several of the organisms were transferred to a small culture dish containing many paramecia. In 24 hours most of the paramecia were gone and the *Woodruffia* were quite numerous. Since that time we have had large numbers of them for study and observation. Care must be taken to replenish the food frequently as they form resting cysts as soon as the food is used up. Under good conditions it is not uncommon for them to divide three or four times each day. Studies are now in progress to determine the optimum conditions for their existence and the amounts of food consumed under different conditions. Since the first trials several other protozoans have been tried as food and so far nothing but *Paramecium* (both *P. multimicronucleata* and *P. bursaria*) has produced continuous growth.

The life history of this organism is similar to the life histories of other members of the Family Colpodidae. Division always occurs within a division cyst (cf. Pl. 20 Fig. 8). The cyst wall is very thin and is visible only with the high power of the microscope, and with the light cut down. After division the daughter cells rotate within the cyst wall, usually in opposite directions. Two daughter cells are normally formed in each division cyst. We have observed

only one exception to this. In this instance four organisms were present within one cyst.

Only rarely are resting cysts found in cultures containing numerous paramecia. However, in a few hours (3—4) after the food supply has been depleted all of the *Woodruffia* are forming resting cysts. At first these cysts have a thin membrane and resemble the division cysts except they are smaller in size. A thick ectocyst is gradually formed (cf. Pl. 20 Figs. 5 and 6). Rotation within the cyst may be observed for as long as 24 hours after the encystment process starts. Methods of inducing excystment in this form are being studied at the present time. It has been possible to obtain 100 per cent excystment using several different solutions.

Morphology.

There are great size variations in this organism. The free-swimming forms may be found from 85 to 400 u in length. Sample measurements of different stages in the life history are given in Table 1. These measurements are given, not as the absolute range in size, but as an indication of the size variations.

Table 1.

Sample Measurements in Microns		
Free-swimming	Division cyst (diameter)	Resting cyst (diameter)
350 × 190	125	54
305 × 178	130	46
250 × 150	92	62
262 × 116	85	39
246 × 130	130	55
185 × 108	155	46
225 × 123	125	39
162 × 100	92	62
154 × 92	108	46
85 × 46[1])	150	54

The anterior end is characterized by a short proboscis-like projection or rostrum. The free-swimming animals are relatively thin and flattened on the ventral surface.

The cystostome, a long slit-like opening about one-third the length of the animal, is on the ventral surface in the anterior third of the body. The ciliary meridians of the body all converge anteriorly at the cytostome and pre-oral suture. This short pre-oral suture is shown in Pl. 21 Fig. 17. On the left side the meridians converge at right angles to the cytostome. On the right side they converge at a much smaller angle (cf. Pl. 20 Figs. 10 and 11, Pl. 21 Fig. 13). The rows of cilia on the dorsal surface run diagonally to the posterior end of the body. Here the meridians converge around a small pore, the posterior contractile vacuole pore.

[1]) Taken from culture where all the food was gone and many resting cysts were already formed.

The rows of cilia on this organism are very numerous and the cilia themselves are short and fine. A single cilium arises from each basal granule. A typical field of cilia with their basal granules is shown in Pl. 21 Fig. 22.

The so-called "mouth parts" are differentiations of the cytopharynx. On the right side and along the outer margin of the cytopharynx is a row of long fine cilia. The basal granules of these cilia are so close together that they appear to form a line running from the anterior tip of the opening along the right side and around the posterior margin of the opening (cf. Pl. 20 Fig. 11). These cilia are clearly shown in Pl. 21 Figs. 15 and 16. Here the right side of the cytostome is on the right of the photomicrograph in each case. These cilia as shown on the photomicrographs are separate, but when viewed under a dark field on the living organism they are seen to beat in a wave-like fashion like an undulating membrane. In drying the cilia forming this membrane separate. However, in some preparations it is possible to find large areas of the membrane intact. Calkins (1930) refers to membranes which are rather easily disintegrated as "pseudomembranes". The right wall of the cytopharynx shows fine striations in silver preparations, but we have been unable to make out other cilia than the row described in the preparations we have studied.

The outer margin of the left wall of the cytopharynx is bordered with numerous short stout cilia, which on close examination turn out to be membranelles, each one being composed of two or three fused cilia. The anterior membranelles in several preparations appear to have three basal granules. These structures are shorter and thicker than the cilia on the right side, and although numerous, not as numerous as the cilia on the right side (cf. Pl. 21 Figs. 15 and 16). Pl. 20 Figs. 10 and 11 show the large basal granules for each membranelle, on the outer margin of the left wall of the cytopharynx.

The drawings [1]) in Text-Figs. 1 and 2 show the arrangement of these "mouth parts" and the pattern of the body cilia.

The macronucleus is large and oval in shape. There is one micronucleus which usually appears to be attached to the macronuclear membrane. The micronucleus is very small in comparison. In division the micronucleus behaves like the micronucleus of *Colpoda steinii* and of *Paramecium caudatum* (cf. Pl. 21 Fig. 21). Its characteristic position in the resting cyst and free-swimming form is shown in Pl. 20 Figs. 2, 5 and 9.

[1]) We are indebted to Mr. Fred Evans for making the drawings.

The macronucleus is quite interesting. The chromatin is arranged in two bands (cf. Pl. 20 Figs. 2, 6 and 9 and Pl. 21 Fig. 19). This condition is found in all stages in the life history. The chromatin

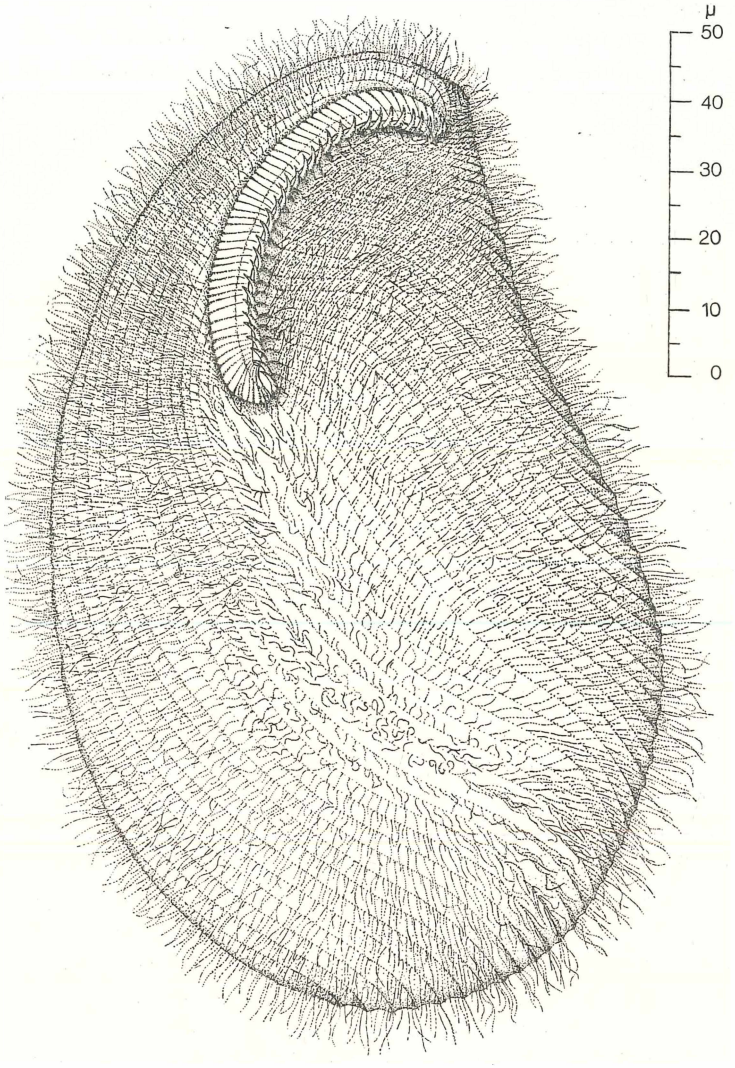

Fig. 1. Drawing of ventral view showing cytostome and body cilia.

appears to be imbedded in a material which takes a much lighter stain and which in the dividing forms is arranged as spindle-like material in the dumb-bell shaped stage of the division. This lighter

staining material may be homologous with the "Binnenkörper" described in the nuclei of many forms (cf. CALKINS, 1930).

There are numerous contractile vacuoles in this organism, but the one at the posterior end is usually the largest (cf. Pl. 20 Fig. 3). It seems probable that the other vacuoles empty into this large one. When studied in colored suspensions, it is possible to see the discharge of this large posterior vacuole, but we have never observed the discharge to the outside of the other, or accessory, vacuoles.

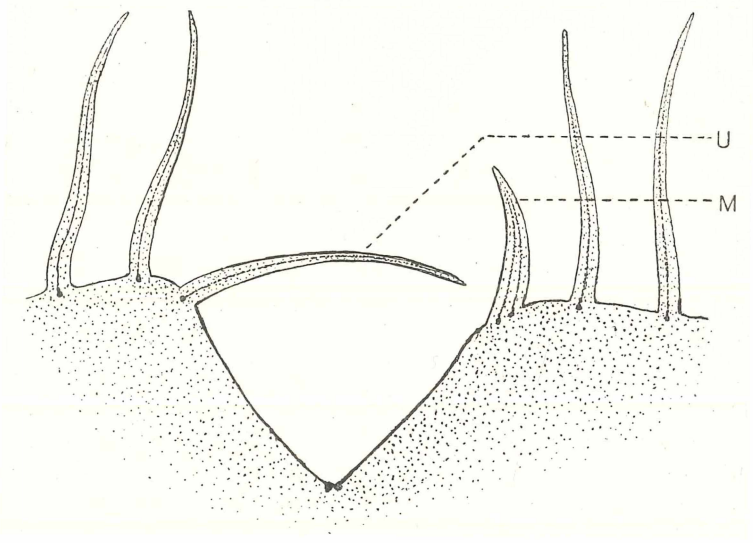

Fig. 2. Schematic section of cytostome. U, membrane in section; M, membranelle in section.

The silver stain of KLEIN gives a very definite pattern in this organism (cf. Pl. 20 Fig. 12 and Pl. 21 Fig. 18). Fibrils are shown joining the basal granules. These form the primary meridians of the body. There also appear lines between these primary meridians and cross connections from these. At the present we are of the opinion that these lines between the main meridians and their cross connections are the results of the stain being deposited on the surface of the pellicle, and are, then, pellicular markings (cf. LUND, 1933).

Behavior.

Perhaps the most obvious characteristic of this ciliate is its great metabolic movement. If placed under some gelatin or agar they writhe through it, resembling in their movements those of a flatworm. They are capable of moving through very small openings

in a manner similar to the behavior of the parasitic form, *Buxtonella sulcata*, described by Rees (1930). In swimming they rotate on their main axis, but their swimming movements are very clumsy.

In the culture dishes they tend to aggregate in one or a few clumps. This is true for all stages in the life history.

When feeding, the mouth is opened (cf. Pl. 21 Fig. 14), and they slip over a *Paramecium* very much as the finger of a glove is slipped over a finger. The small animals often have some difficulty in capturing a *Paramecium* but the average sized ones have little difficulty. When a *Paramecium* is first ingested, a large vacuole is obvious (cf. Pl. 20 Fig. 7). The *Paramecium* is visible as such in the food vacuole for only a short time (4—6 minutes) in animals which have not fed recently. In no case in the examination of stained and sectioned specimens have the trichocysts of the paramecia been observed discharged (Pl. 20 Fig. 7). Often when the food has been used up these ciliates will ingest one of their own kind. However, we have no evidence that they can exist for any length of time by feeding entirely on their own kind. Pl. 20 Fig. 4 shows a small organism in a food vacuole of a larger one.

In the formation of resting cysts the small animals almost always divide once to form two very small animals which then encyst. The larger forms always divide at least twice before encystment starts. Occasionally an organism divides once and then one of the daughter cells divides but not the other, with the resultant formation of three resting cysts from the original animal. One of these cysts, of course, is larger than the other two. In the formation of cysts, both division and resting cysts, the organisms secrete a mucin-like substance which causes them to adhere to the culture dishes.

The cytoplasm of *Woodruffia metabolica* is quite coarsely granular. When a small drop of formalin is added directly to a small culture of the organisms the cytostomes of the free-swimming forms immediately open and the contents of the endoplasm rush out, resembling volcanic eruptions in miniature. The granules so discharged, upon drying, are crystal-like in appearance.

Conjugation has not been observed in this species.

Comparison with *Woodruffia rostrata* Kahl.

Kahl's organism is somewhat smaller and it inhabits brackish water. It feeds on Oscillatoria. The pattern of the body cilia appears to be the same in both. With respect to the "mouth parts"

there are certain differences. KAHL describes the cilia on the left side of the cytostome in *W. rostrata* as being membranelle-like and, as diagrammed, each one consists of 5 or 6 cilia. In this form we find only 2 or 3 cilia fused together to form each membranelle. On the right side of the cytostome of *W. rostrata*, KAHL describes a whole field of small cilia lining that wall of the cytopharynx. In this form, in the preparations studied, we have been able to make out only one row of long fine cilia which constitute an undulating membrane or, at least, a "pseudomembrane". The cytostome is curved in this form and is longer in proportion than it is in *W. rostrata*. KAHL describes the macronucleus of *W. rostrata* as round; in this form it is oval. *W. rostrata* has one or more micronuclei; *W. metabolica* has only one. The contractile vacuole systems of both seem much alike. Both are characterized by metabolic movements.

The presence of an undulating membrane and membranelles in this form and the recent findings of TAYLOR and FURGASON (1938) that these structures are also present in *Colpoda duodenaria* seem to indicate that no hard and fast lines should be drawn between the orders Trichostomida and Hymenostomida with respect to the presence or absence of membranes in the cytostome, if the family Colpodidae is to be included in the order Trichostomida.

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Explanation of plates.

Plates 20—21.

All the figures were made from photomicrographs [1]).

Plate 20.

Fig. 1. Typical shape. Organism fixed in DA FANO's. Mag. 300 ×.

Fig. 2. Nuclear arrangement. Micronucleus and typical appearance of organization of chromatin of the macronucleus into two distinct bands which are embedded in a lightly stained matrix. Basic Fuchsin. Actual size of macronucleus 25 × 38 μ. Mag. 368 ×.

Fig. 3. Form. Showing positions of cytostome, nucleus, posterior contractile vacuole. DA FANO's. Mag. 300 ×.

Fig. 4. Section of individual which has ingested one of its own species. Prominent vacuole. Sec. 10 μ. Mag. 216 ×.

Fig. 5. Resting cyst. Macronucleus and micronucleus clearly shown. Sec. 10 μ. FEULGEN reaction after SCHAUDINN's. Mag. 576 ×.

Fig. 6. Resting cyst. Two distinct chromatin bands of the macronucleus. Sec. 10 μ. FEULGEN reaction after SCHAUDINN's. Mag. 576 ×.

Fig. 7. Individual with ingested Paramecium. Note extremely large vacuole and displacement of cytoplasm. FEULGEN reaction, following SCHAUDINN's. Actual length of organism 170 μ. Mag. 320 ×.

Fig. 8. Division cyst. Unstained. Mag. 320 ×.

Fig. 9. Section, showing the arrangement of chromatin granules of the bands of the macronucleus. (One band in focus, the second incomplete in section.) Micronucleus in typical position on the macronucleus membrane. Sec. 10 μ. Basic Fuchsin. Mag. 300 ×.

Fig. 10. Cytostome. Silver method. Mag. 880 ×.

Fig. 11. Cytostome open. Silver method. Mag. 880 ×.

Fig. 12. Details of silverline system. Left side. Mag. 2000 ×.

Plate 21.

Fig. 13. Silverline preparation of entire animal, showing meridians and position of cytostome. Mag. 320 ×.

Fig. 14. Same as fig. 13, cytostome open. Mag. 320 ×.

Fig. 15. Cytostome. China blue preparation. Mag. 800 ×.

Fig. 16. Cytostome. Long cilia of undulating membrane and short stiff cilia of the membranelles. Nigrosin preparation. Mag. 1600 ×.

Fig. 17. Silver preparation showing pre-oral suture. Mag. 1600 ×.

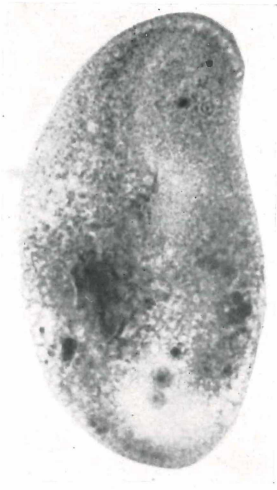
Fig. 18. Silver preparation showing details of right side of body. Mag. 2000 ×.

Figs. 19, 20. Consecutive sections of a division figure of the macronucleus. Two distinct clumps of chromatin material in fig. 19. Spindle-like arrangement of the lightly stained matrix. HARRIS' hematoxylin. Sec. 10 μ. Mag. 1080 ×.

Fig. 21. Showing division of both macro- and micronuclei. Whole mount of division cyst. FEULGEN reaction following fixation in B—15. Mag. 1080 ×.

Fig. 22. Details of body cilia. Nigrosin. Mag. 2400 ×.

[1]) We are indebted to Mr. DIETRICH BODENSTEIN for preparing the photomicrograph used in Pl. 20 Fig. 8, and to Mr. JOHN R. POINDEXTER for preparing the others.



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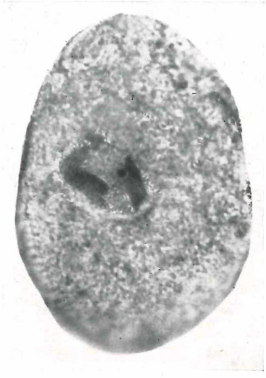

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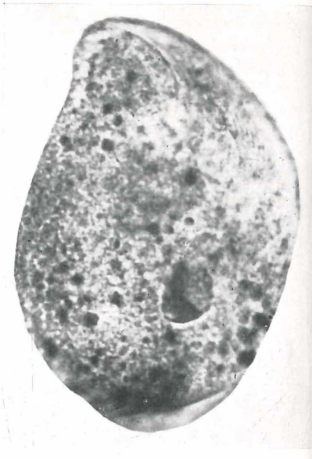

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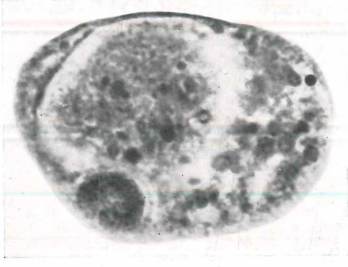

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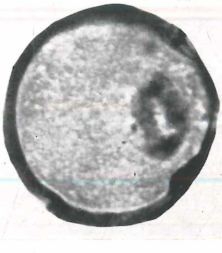

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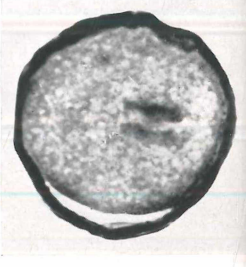

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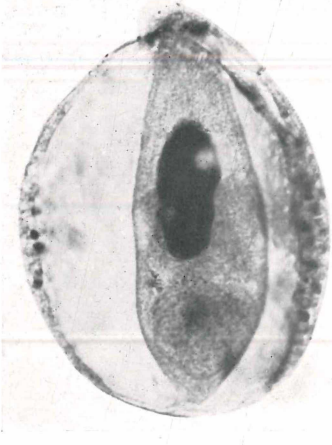

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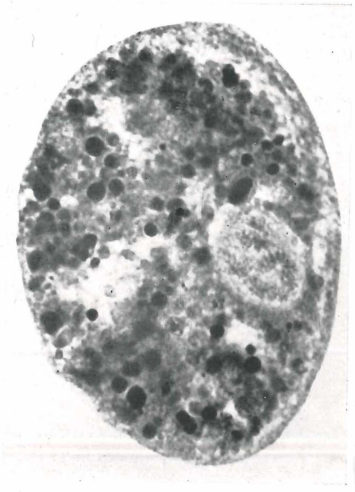
Johnson and Larson.

VERLAG VON GUSTAV FISCHER IN JENA.



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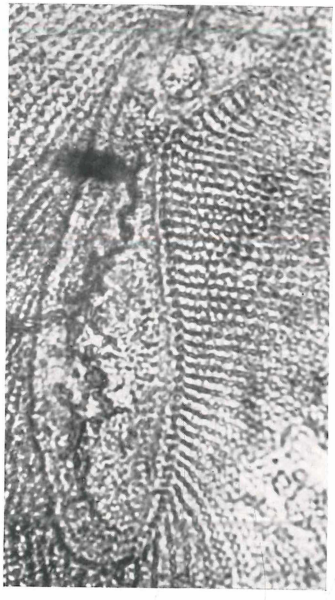

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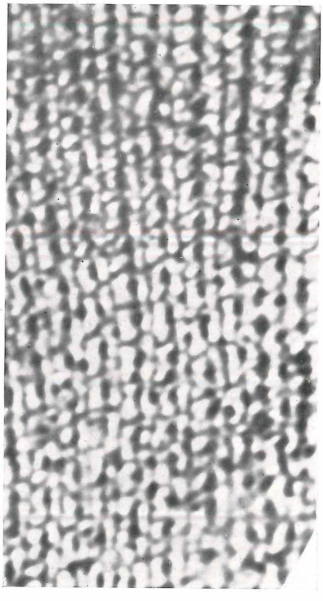

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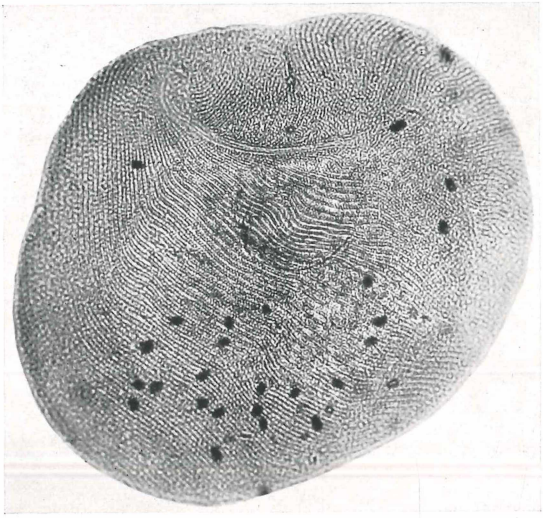

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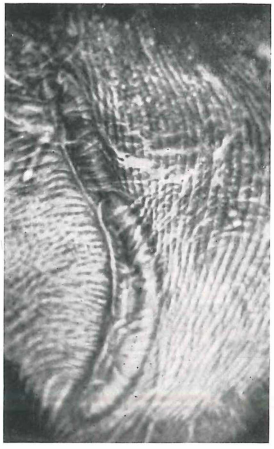

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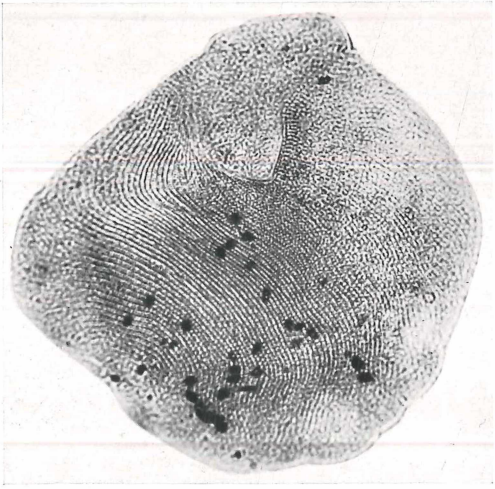

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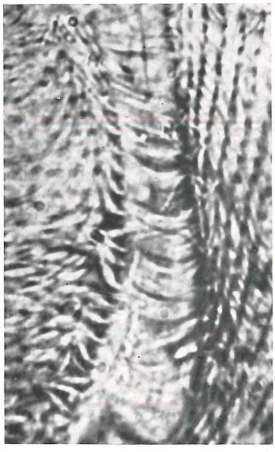

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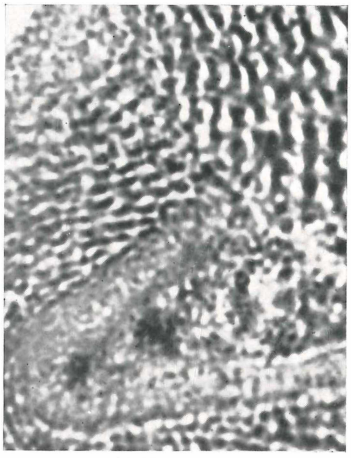

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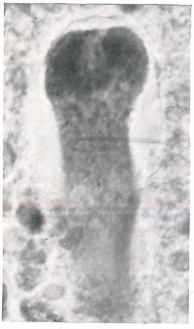

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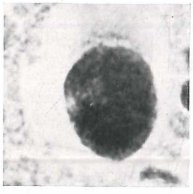

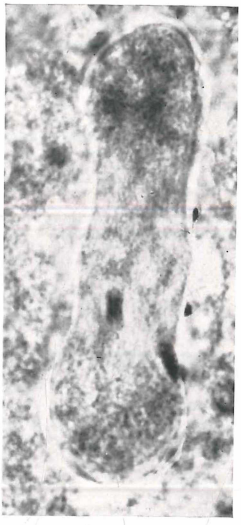

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IV FISCHER IN JENA.