The Zoological Laboratory of The Johns Hopkins University and the Mount Desert Island Biological Laboratory [1]).

A New Type of Cytoplasmic Structure in the Flagellate *Chilomonas paramecium.*

By

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(With plate 4.)

It has long since been demonstrated that *Chilomonas paramecium* contains a contractile vacuole, a well differentiated nucleus, numerous granules in a basket-like structure around the gullet, and usually many starch granules (Bütschli, 1878; Prowazek, 1903; Nägler, 1912; Pascher, 1913; Belar, 1916; Kepner and Edwards, 1916).

Hall (1930) asserts that in addition to these it contains about 20 "osmophilic granules" scattered thru the body and "occasionally 2 or 3 [contractile] vacuoles instead of a single one". He confirmed Nassanow (1924) in his contention that the contractile vacuole stains black with osmic acid but he does not agree with him in the conclusion that this proves that it constitutes "the Golgi apparatus".

Several years ago one of us, in observations on the process of digestion of *Chilomonas paramecium* in *Amoeba proteus*, discovered that in addition to the structures mentioned it contains two prominent ellipsoidal bodies which are located between the nucleus and the anterior end of the body and that it has about 50 small spherical granules scattered thru the cytoplasm (Fig. 1). We shall call the former ellipsoids and the latter spherules.

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[1]) We are greatly indebted to Dr. P. L. Johnson and Dr. Wm. F. Hahnert for assistance.

The results obtained in these observations in so far as they concern the structure of *Chilomonas paramecium* can be briefly summarized as follows:

The two ellipsoids can be seen in normal, living specimens, especially if they have been without food for some time, but they can be much more clearly seen if they are stained with any one of a number of different dyes. They become brilliant violet or purple in neutral red, violet in cresyl echt violet, dark blue in methylene blue, brilliant blue in nile blue sulfate, orange red in trypan blue, lavender in bismark brown, blue in thionin, faint light blue in alizarin rot, lavender in brilliant cresyl blue, and violet in janus green. They do not stain in any of the other vital dyes in Conn's list (1929) or in a considerable number of other so called vital dyes tested and they do not stain with iodine, sudan III, or scharlach R. but they become greyish in osmic acid. They are readily soluble in water, disappearing in 30 $\pm$ seconds. They are doubtless the structures which Hall considered to be accessory contractile vacuoles.

The spherules vary greatly in size in given individuals and in different individuals. The diameter of the largest ones is fully equal to one-third that of the nucleus; the smallest ones are barely visable under a magnification of 1200 diameters. They become brilliant red in neutral red, orange in trypan blue, blue in nile blue, yellowish red in bismark brown, violet in cresyl echt violet, and dark blue in methylene blue. They do not stain in any of the other vital dyes tested. They do not stain with sudan III or scharlach R. but they become black in osmic acid. They are doubtless the bodies designated "osmophilic granules" by Hall.

We have recently obtained the following additional results concerning the structure of *Chilomonas paramecium.*

The spherules are not optically active. They are insoluble in water. In chilomonads which are in the food vacuoles of *Amoeba proteus* they disappear in about 20 minutes if the specimens in the vacuole burst so as to discharge the spherules into the fluid in the vacuole and in about $2^1/_2$ hours if the specimens do not burst.

In chilomonads grown in solution of $NaC_2H_3O_2$, K_2HPO_4, $MgSO_4$ and NH_4Cl in water, the spherules are much larger and probably somewhat more abundant than in those grown in solution containing complex organic compounds (Fig. 3).

The ellipsoids are strongly optically active (Fig. 5). This property is not destroyed by fixation with mercuric chloride or cadmium acetate but it is destroyed by fixation with hot water, absolute

alcohol, formalin, osmic acid, or picric acid. In some of these solutions the ellipsoids probably dissolve but in others they do not. In formalin for example they remain normal in size.

If neutral red is added in excess to a solution of mercuric chloride containing chilomonads, the ellipsoids do not stain but the spherules become dark red. This shows that the spherules and the ellipsoids differ markedly in composition. In *Chlilomonas* in the food vacuoles of *Amoeba proteus* stained with neutral red, the ellipsoids lose their optical activity in 5 to 6 minutes after movement ceases but they usually remain purple for several hours and they are among the last of the structures in *Chilomonas* to disappear. The staining properties and the optical properties are therefore not specifically correlated.

When *Chilomonas* is about to divide, both of the ellipsoids divide crosswise near the middle (Fig. 6); then as fission continues two of the four bodies thus formed migrate into each of the two new individuals.

The ellipsoids vary greatly in size and considerably in form. In some individuals they cannot be seen; in others their maximal diameter equals one-fourth the length of the organism. The size is not correlated with the amount of fat or starch present but it seems to be correlated with the composition of the culture fluid. In the acetate culture fluid given above there are relativly many more individuals in which no ellipsoids can be seen than in culture fluids containing wheat or other complex organic mixtures [1]).

The fact that these bodies become grey in osmic acid and blue in nile blue sulfate indicates that they contain lipids and this contention is supported by the fact that their optical properties are not destroyed by fixation in mercuric chloride and cadmium acetate, since it is known that heavy metal salts generally tend to preserve the physical structure of lipids. The facts that thay are soluble in water and that they become purple in neutral red and blue in nile blue indicate that the lipid is a fatty acid. They probably consist of a protein stroma impregnated with fatty acid, and the decrease

[1]) In a number of observations made later the following was found. In wild, well fed cultures the ellipsoids can be seen in about four of every five specimens. In sterile cultures in acetate culture fluid the number in which the ellipsoids can be seen rapidly decreases, so that at the end of six days they can be seen in only about one of every fifteen specimens.

In experiments made after this paper went to press it was found that if specimens are put into culture fluid without sulfur the fat content increases greatly and the ellipsoids disappear.

in size and the loss of optical activity and staining properties is probably due to the loss of the fatty acid.

We have found similar bodies in *Cryptomonas ovata* and *C. erosa.*

The facts that there are always two ellipsoids in an individual and that they divide during cell division indicate that they have some specific biological function but we have no suggestions as to what it is. It may possibly be that these bodies are vestigal eyespots, for nearly all of the closely related organisms have eyespots and many of them have two.

Summary.

There are two prominent ellipsoidal bodies in *Chilomonas paramecium*, located near the anterior end.

They are strongly optically active and they stain readily with various vital dyes.

Their optical activity is destroyed by fixation in hot water, absolute alcohol, formalin, osmic acid and picric acid but not by mercuric chloride or cadmium acetate.

They become grey in osmic acid, blue in nile blue sulphate and purple in neutral red and they are readily soluble in water.

They probably consist of a protein stroma impregnated with fatty acid.

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Plate of figures.

Plate 4.

Camera drawings and photomicrograph of *Chilomonas paramecium*.

Fig. 1. A well-fed, living specimen stained with nile blue sulphate. Starch and fat not stained.

Fig. 2. A well fed specimen stained with Lugol solution and sudan III. No ellipsoids were observed.

Fig. 3. A starved specimen dried on the slide and stained with Wright stain. Ellipsoids not stained.

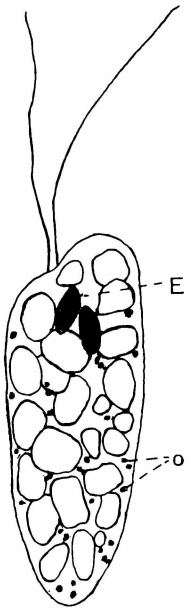
Fig. 4. A living chilomonad grown in acetate culture fluid and stained with neutral red. There were numerous starch and fat globules, not represented. Note that some of the spherules were very large.

Fig. 5. Photograph of a living chilomonad taken between crossed Nicols and a gypsum plate.

Fig. 6. Outlines of the structures in the photograph.

e, ellipsoids; o, spherules (HALL's osmiophilic granules); n, nucleus; s, starch; f, fat; g, gullet with numerous granules in wall; c, contractile vacuole; mm, projected scale.

Note that the ellipsoids produced highly illuminated spots in the photograph, indicating strong optical activity, and that there were a considerable number of other light spots scattered through the body. These are due to the presence of slightly optically active starch grains. Note also the line across the ellipsoids indicating division.



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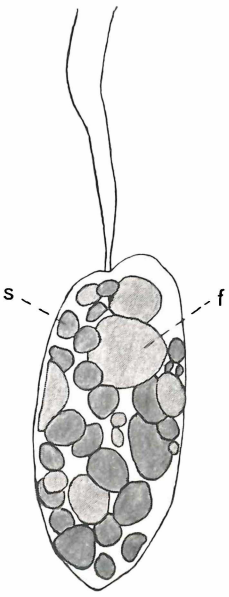

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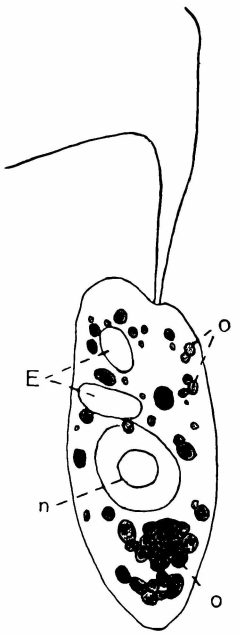

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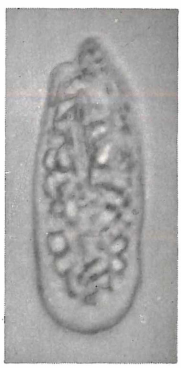

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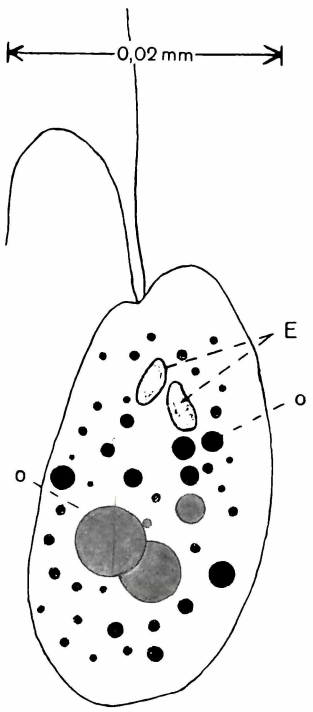

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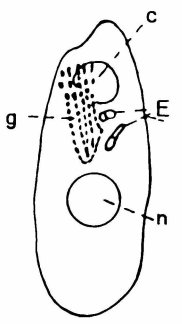

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Mast and Doyle.

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