The morphology of the glycogen reserves in *Polyplastron.*

By

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(With 1 figure in the text and Plate 13.)

Introduction.

The intestinal protozoa such as *Nyctotherus*, *Balantidium*, and the free-living protozoa leading an anaerobic or partially anaerobic life are particularly rich in glycogen reserves (Reichenow, 1929). The Ophryoscolecidae, ciliates from the stomach of cattle, show a very high degree of specialisation in their glycogen reserves, equal to their high degree of specialisation in locomotor and other organelles. The so-called skeletal plates of the Ophryoscolecidae have recently been shown to be largely made up of glycogen or of a glycogen-like substance (Schulze, 1924, 1927). Usuelli (1930) showed that this is a storage product resulting from the digestion of starch by these ciliates. This interpretation of the skeletal plates, however, is not universally held. Sharp (1914) described the general morphology of the skeletal plates of *Epidinium* (his *Diplodinium*) and strongly supported the idea that they are purely skeletal in function. Strelkow (1930) described the morphology of the carbohydrate material of the plates of an allied group, the Cycloposthiidae, ciliates from horses. Both he and Dogiel (1923, 1927) consider the iodine-staining material of the skeletal plates to be a type of cellulose, „Ophryoscolecin", which is not storage product, but a permanent skeletal product.

This paper is a report on the morphology of the glycogen reserves in the skeletal plates and in the general cytoplasm of *Polyplastron* as shown by a new staining method. In connection with

an investigation of the GOLGI apparatus of the Ophryoscolecidae (MACLENNAN, 1933) it was found that Sudan III dissolved in the embedding paraffin or in xylene stained the skeletal plates and certain cytoplasmic granules a deep red, providing that the sections had not been immersed in water or lower alcohols. These plates and the granules could be restained with chlor-zinc-iodide and under such conditions they gave the characteristic glycogen reaction. The material is insoluble in xylene, turpentine, and other fat solvents, and, so far as could be determined, is absolutely unchanged by immersion of the sections in these solvents for times up to one week. The staining reaction is therefore not due to fats along with the glycogen. This method is simple and permanent, and gives preparations with very clear cytological detail in which GOLGI granules, fibrils etc., according to the fixation and staining methods used, can be very clearly distinguished. The simplest method used was to fix in CHAMPY'S fluid at 35—40° C, embed in paraffin melting at 58° C saturated with Sudan III, and then to section and mount the ciliates in the usual manner. The glycogen granules and platelets are smoothly rounded as in living ciliates, not roughly granular as they appear in iodine preparations.

This work was started under the direction of Prof. C. A. KOFOID at the University of California at Berkeley, and I wish to express my gratitude for many suggestions received in connection with this problem.

Description.

Skeletal plates: There are five skeletal plates in *Polyplastron*, the two main plates lying under the right side and the three minor plates lying beneath the left side (Text-Fig. 1). These plates are composed of small, irregularly prismatic platelets, separated from each other but enclosed in a single membrane. These platelets give typical glycogen staining reactions. The platelets are separated from each other by a clear, homogeneous matrix apparently of a protein nature. The platelets correspond to the clear spindles and the matrix to the lamellae described by SHARP (1914) in *Epidinium* (his *Diplodinium*). Longitudinal and transverse sections of *Polyplastron* show clearly that the plates are separated from the cuticle by a considerable thickness of ectoplasm. The apparent molding of the cuticle over the platelets as seen in living specimens and in glycerine mounts is obviously an illusion due to the refractivity of the glycogen.

The two main plates are broad and thin at the anterior end (Pl. 13 Fig. 8), become narrower and thicker toward the middle (Pl. 13 Fig. 9), and taper off posteriorly (Pl. 13 Fig. 10). The anterior parts of the two plates lie near the cuticle, but they sink in posteriorly and lie close against the boundary layer. The anterior end of the plate is convex, the middle concave and the posterior part is again convex. The decrease in the width of the plate is due to a lessening of the number of rows of platelets from thirteen or fourteen at the anterior end to three or four at the posterior end.

The three minor plates are short and spine-like and are only three or four platelets wide at the anterior end (Text-Fig. 1). The

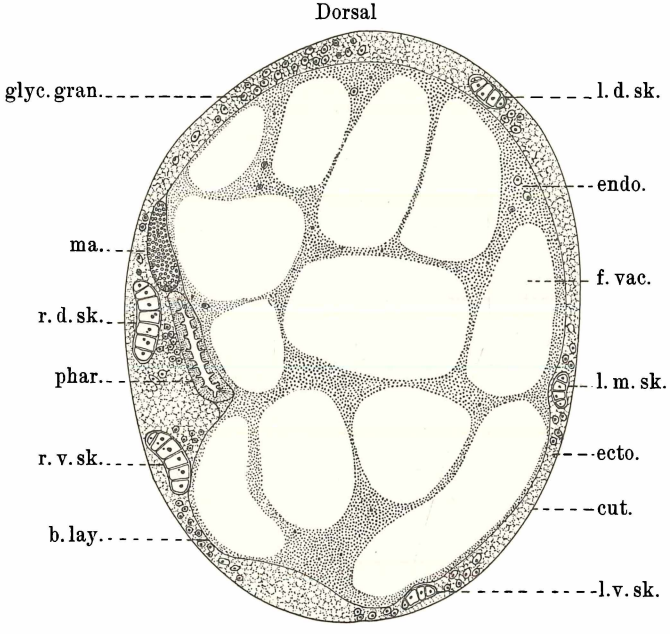

Fig. 1. Cross-section of *Polyplastron multivesiculatum,* through the anterior third of the body, to show the relationship of the skeletal plates to the other structures. b lay boundary layer; cut cuticle; ecto ectoplasm; endo endoplasm; f vac food vacuole containing starch granules; glyc gr glycogen granule; l d sk left dorsal skeletal plate; l v sk left ventral skeletal plate; ma macronucleus; l m sk left median skeletal plate; phar pharynx; r d sk right dorsal skeletal plate; r v sk right ventral skeletal plate.

narrow bands which DOGIEL (1927) describes as connecting the anterior ends of these plates could not be found clearly in the Sudan III preparations and only occasionally in whole mounts stained with chlor-zinc-iodide. Since there is almost always an accumulation

of free glycogen granules in this region of the ectoplasm, it is possible that these bands are merely occasional linear aggregations of these granules, rather than a permanent structure.

Platelets: The platelets are rather irregular, prismatic bits of glycogen, 2 to 4 μ in height, and 1 to 2 μ in diameter at the base in the anterior part of the plate. The platelets in the middle are of approximately the same diameter as in the anterior parts but range in height from 4 to 7 μ. The platelets decrease somewhat in size posteriorly.

One or two small granules staining a deep blackish-red color lie in each platelet, surrounded by the red glycogen forming the major part of the platelet. The granules are usually spherical but are sometimes rod-like.

The platelets are entirely separate structures morphologically and show no anastomosing strands between them such as STRELKOW (1929) described in *Cycloposthium*. STRELKOW noted the absence of these strands in *Polyplastron*. There are no alveoli in the peripheral ends of the platelets such as STRELKOW (1929) describes in *Cycloposthium* and *Ophryoscolex*. The platelets are usually very clearly outlined by the clear matrix between them, but occasionally they are so closely crowded that the boundaries can be made out only with difficulty.

Cytoplasmic granules: The ectoplasm is rather variable as a glycogen depot. Any well-fed specimen shows many glycogen granules in this region while poorly fed specimens show few or no glycogen granules here. These granules are particularly abundant in the operculum and in the ventral lobe. In preparations stained with chlor-zinc-iodide the glycogen is shown in rough, irregular granules, unlike any of the granules found in living specimens or in specimens stained with Sudan III.

The glycogen granules found free in the ectoplasm are essentially similar in structure to the individual platelets of the skeletal plate, although they show a great variety in their outlines. Small dark center granules with no envelope of more lightly staining material are common in the endoplasm and a few occur in the ectoplasm. Elongate granules with a lightly staining envelope are also common. Occasionally elongate granules with a dumb-bell center granule or with two granules are found. Sometimes especially large granules with two center granules show a constriction around the equator.

The shape and size of the granules and platelets were confirmed by studies on live material. Due to the refractivity of the glycogen, the central granules could not be observed.

Discussion.

The material described in the skeletal plates and in the cytoplasmic granules gives the typical glycogen reactions with iodine, and is soluble in water as pointed out by SCHULZE (1924), USUELLI (1931), and others. In the present paper it is interpreted as glycogen or paraglycogen in accordance with the views of the above investigators. It does not appear to be a distinct material as claimed by DOGIEL (1923) and STRELKOW (1931) and thus there is no necessity for giving it the name Ophryoscolecin.

The relative rigidity of the skeletal plates, demonstrated clearly by SHARP (1914), DOGIEL (1923) and STRELKOW (1931), indicates that the original interpretation of their function as supporting structures as well as storage structures is correct. This supporting function is obvious in such forms as *Ostracodinium*, *Epidinium*, *Ophryoscolex*, etc., in which the plates extend nearly the full length of the body and also more or less completely surround the oral zone. The skeletal function of the plates in *Polyplastron* and its relative *Elytroplastron* is far less obvious than in the first named genera from a consideration of the relationship of the plates to the other structures. In *Polyplastron*, the skeletal function is only partially fulfilled, the storage function being relatively more important.

Both the platelets and granules are more complex in structure than has heretofore been described in the storage of glycogen, since in *Polyplastron* the glycogen is in granules or plates of a definite shape, size, and internal structure. The skeletal plates are made up of platelets comparable in form and composition to the glycogen granules. The skeletal plates are therefore not an entirely new structure appearing in the evolution of the Ophryoscolecidae, but rather a compound structure originating from aggregations of the scattered granules.

It has been shown previously (MACLENNAN, 1933) that the glycogen granules and platelets in the Ophryoscolecidae show no close relationship to the GOLGI material, or chondriome. The glycogen granules and vacuome show some correlation in distribution, since both are usually concentrated in the opercular zone and in the anal zone. However, the skeletal plates, which are the prime storage centers of glycogen, and which have a unit of structure similar to

the separate granules, are entirely free of vacuome. This shows clearly that the glycogen granules and platelets are independent of the regular cytoplasmic constituents in the Ophryoscolecidae.

Since the center granules have been observed only in the Sudan III material and cannot be found in living material or in the material stained with iodine reagents, it might be assumed that they are artifacts. The most logical type of artifact to suspect in this case is one in which a uniformly distributed fluid or solid lying in a cytoplasmic vacuole is condensed and shrunk by the action of the fixation and staining processes so that the larger uniform granules are changed to small condensed granules in more or less empty vacuoles. Fry (1932) has demonstrated the possible activity of such factors in producing centrioles from homogenous or vacuolar cytoplasm. Although such a structure is far removed from the center granules of a glycogen vacuole, the same colloidal conditions might be present in both. It might also be objected that the center granules results from a partial solution of a large homogeneous granule leaving untouched, because of shortness of time or some similar factor, a portion in the center. Both of these objections can be met very simply and adequately. There is never any sign of an empty space between the glycogen and the surrounding cytoplasm, nor between the center granule and the more lightly staining envelope. Furthermore, naked center granules are often found, and in all cases the cytoplasm adheres closely to them, never leaving a clear vacuole around them. These observations clearly disprove the idea that these center granules are artifacts due to contraction or solution on fixation and staining. The uniformity in size and shape of the center granules even under diverse conditions of fixation, staining, and sectioning, refutes the idea that they are artifacts due to partial solution of a large, homogeneous granule. Therefore, although the details of structure of the glycogen granules and platelets cannot be made out in live ciliates, their relatively complex structure is accurately shown in preparations stained with Sudan III.

The different shapes of glycogen granules (Pl. 13 Figs. 1—5) when arranged in a series show a marked resemblance to a growth and division cycle. In this cycle the central granules remains relatively unchanged in size, while the less deeply staining material varies greatly in quantity. When the lightly stained envelope is at a maximum size, the center granule enlarges slightly and divides, followed by a division of the envelope. If this interpretation of the various shapes of the granules is sustained by further work, it will

be seen that the glycogen masses in these ciliates are not merely inactive storage masses, but are definite, organized centers of glycogen synthesis during feeding and of glycogen dispersal during starvation.

Summary.

1. The glycogen granules and platelets in the Ophryoscolecidae may be stained with Sudan III, a method cytologically far superior to the usual iodine methods.

2. Glycogen is distributed in *Polyplastron multivesiculatum*, a ciliate found in the stomach of sheep, in small ectoplasmic granules which are most numerous in the opercular and anal regions, and also in the skeletal plates.

3. The skeletal plates are made up of small, roughly prismatic blocks of glycogen, in each of which is a small central granule.

4. The scattered glycogen granules are also made up of an outer portion and a dense central granule. Evidence is presented showing that these granules undergo a regular division cycle.

5. The glycogen granules in *Polyplastron* show no relationship to the GOLGI material, chondriome, or vacuome, but appear to be relatively independent, self-perpetuating cytoplasmic granules.

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Explanation of Plate.

Plate 13.

All drawings were made with a Leitz binocular microscope and camera lucida, 15 × oculars, $^1/_{16}$ apochromat objective, giving a magnification of 2700 ×. Figs. 1—5 were enlarged three times. All sections were cut 7 μ in thickness.

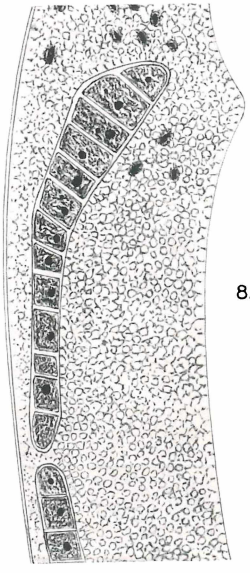
Figs. 1—5. Glycogen granules from the ectoplasm, semidiagrammatic. Champy fixation followed by Sudan III in paraffin.

Fig. 6. Tangential section of right dorsal skeletal plate. Champy fixation followed by Sudan III in paraffin.

Fig. 7. Dorsal view of portion of right skeletal plate, from a whole mount fixed in Schaudinn's fluid and stained with chlor-zinc-iodide.

Figs. 8—10. Cross-sections of right dorsal skeletal plate from a complete series of twenty-two sections. Champy fixation followed by Sudan III in paraffin. Fig. 8, section six, near the anterior end of the plates; Fig. 9, section ten, mid-portion of the plate; Fig. 10 section sixteen, posterior end of the plate.

Fig. 11. Longitudinal section of right dorsal skeletal plate. Champy fixation followed by Sudan III in paraffin.



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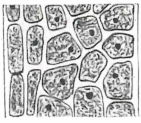

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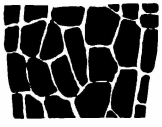

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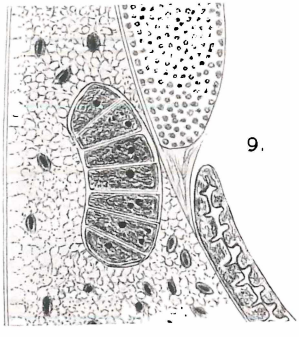

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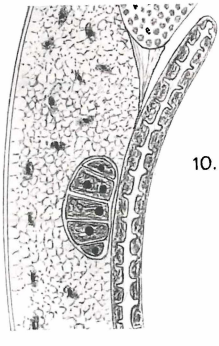

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Mac Lennan.

VERLAG VON GUSTAV FISCHER IN JENA.