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Electron Micrographs of the Pellicle of a Species of *Euglena*^{1, 2}

By JAMES F. REGER AND H. W. BEAMS

INTRODUCTION

Electron microscope studies on whole mounts of *Euglena* demonstrated that the pellicle was composed of alternate grooves and ridges which were orientated in a spiral and longitudinal direction with respect to the long axis of the animal (Saxe 1947; Groupe 1947). Wolkin and Palade (1953) have presented electron micrographs of sectioned *Euglena* and made brief reference to the pellicle. It is the purpose of this paper to present further details on the structure of the *Euglena* pellicle as seen in thinly sectioned material under the electron microscope.

MATERIAL AND METHODS

The material for this study was taken from a mixed culture of *Euglena* which contained over 50% *E. gracilis*. The animals were concentrated by centrifugation and fixed in two percent osmium tetroxide, or in one percent buffered osmium tetroxide (pH 7.25) after the method of Palade (1952). They were then washed in water, dehydrated and embedded in a mixture consisting of equal parts of N-butyl methacrylate and methyl methacrylate. Polymerization was accomplished in an oven at 48° C for 8 to 12 hours. Sections were cut at 0.2 of a micron with an adapted Spencer rotary microtome No. 820. The electron microscope used was an RCA model EMU type. Magnification of the electron micrographs is indicated by the 1 micron scale drawn on each figure.

OBSERVATIONS

Pellicle

Whole mount preparations show the pellicle as a series of alternating grooves and ridges (fig. 1). That these are oriented in a longitudinal and spiral direction is evident in the top portion of figure 2. In this section the most anterior ridge (R) is complete, the second one has been partially sectioned and part of it re-

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moved. However, the remaining part is marked by a relatively thin band at (Y). The subsequent ridges have been removed by sectioning leaving only the cut, curved edges to mark their position. A similarly arranged section is seen in figure 6, except that here the knife passed near the periphery instead of through the deeper portion of the animal. An accordion-like structure is apparent and in comparing positions (C) and (G) the difference between compressed and open grooves may be seen. It will also be noted in this picture that faint but nevertheless definite zones extend laterally from the edges of the grooves (H). The significance of these is unknown. However, it is conceivable that they may represent some slime-like secretion produced by the animal which aids in its locomotion or that it simply represents an outline of the animal in the surrounding media before a possible contraction or shrinkage of the animal occurred.

Figure 3 is a section through the animal showing the pellicle cut crosswise. Here the alternating grooves and ridges are particularly striking. Two membranes appear to make up the pellicle, an outer ridged one of about 500 Å in thickness and an underlying relatively smooth one of about 300 Å in thickness. The latter membrane may also be seen in the pellicle on the right side of figure 2 and in figure 7. In addition, the accordion-like shape of the pellicle is seen in the oblique sections of figures 4, 7 and 8 where E represents the surface of a ridge and D the groove.

Chloroplasts

The chloroplasts (figs. 3C and 5) consist of an outer limiting membrane, best seen in figure 3L and a series of internal laminations of about 500 Å in width. The matrix between the lamellae appears more or less structureless.

Mitochondria

Mitochondria (fig. 3M) appear to be rod-like bodies of variable size with an outer membrane, internal vacuolization, with some evidence that "internal cristae" exist (fig. 3X). It may be that a difference in fixation explains the difference in internal appearance of the mitochondria herein described from those observed by Wolkin and Palade (1953). There also occasionally appear in these bodies, which we have interpreted as mitochondria, dense granules of unknown significance (figs. 2 and 3).

Paramylon

Other dense appearing bodies are observed within the cytoplasm of the animal, see especially (P), figures 2 and 3. These may be

Paramylon bodies, they seem to be disk-like in shape and nothing of their internal structure can be determined.

DISCUSSION

Pellicle

We have observed that in *E. gracilis* there exists a corrugated pellicle of alternating grooves and ridges. In observing the movements of the animal the pellicle appears plastic, alternately extending and contracting with movement of the animal. Such an accordion-like structure, with its folded surface, allows for expansion and thus lends itself well to changes in shape of the animal. Whether or not the source of pellicle movement resides within the pellicle itself or whether it only passively extends and contracts, we do not know. The significance of the underlying membrane seen in figure 3 might have some bearing on this subject.

SUMMARY

1. Sectioned preparations of *E. gracilis* were examined with the electron microscope by using osmium tetroxide fixation, methacrylate embedding, and thin sectioning methods.

2. The pellicle was observed and evidence was presented for the fact that it is ridged and grooved alternately, exhibiting spiral and longitudinal orientation with respect to the long axis of the animal.

3. Thickness of the pellicle was found to be 500 Å and a thinner underlying membrane of 300 Å was observed.

4. Bodies identified as chloroplasts, mitochondria and paramylon were observed and their structure described.

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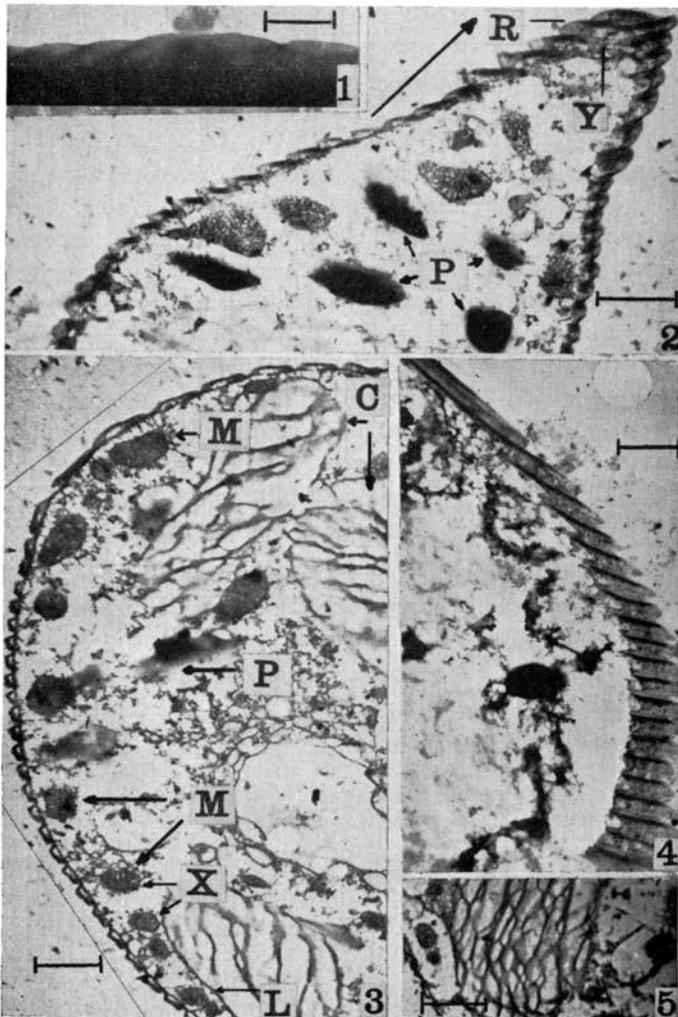


PLATE I.

Explanation of figures

Electron micrographs

(Two percent osmium tetroxide fixation)

1. Surface view of a portion of the Euglena pellicle.
2. Section of anterior portion of Euglena with arrow indicating longitudinal axis. (R) ridge, (P) paramylon, (Y) partially sectioned area of ridge.
3. Posterior portion of Euglena showing corrugated pellicle sectioned almost perpendicular to its surface. (C) chloroplasts, (M) mitochondria, (P) paramylon bodies, (L) limiting membrane, and mitochondria with internal laminations (X).
4. Oblique section through pellicle showing distinct alternating areas of different electron densities.
5. Section of part of a chloroplast.

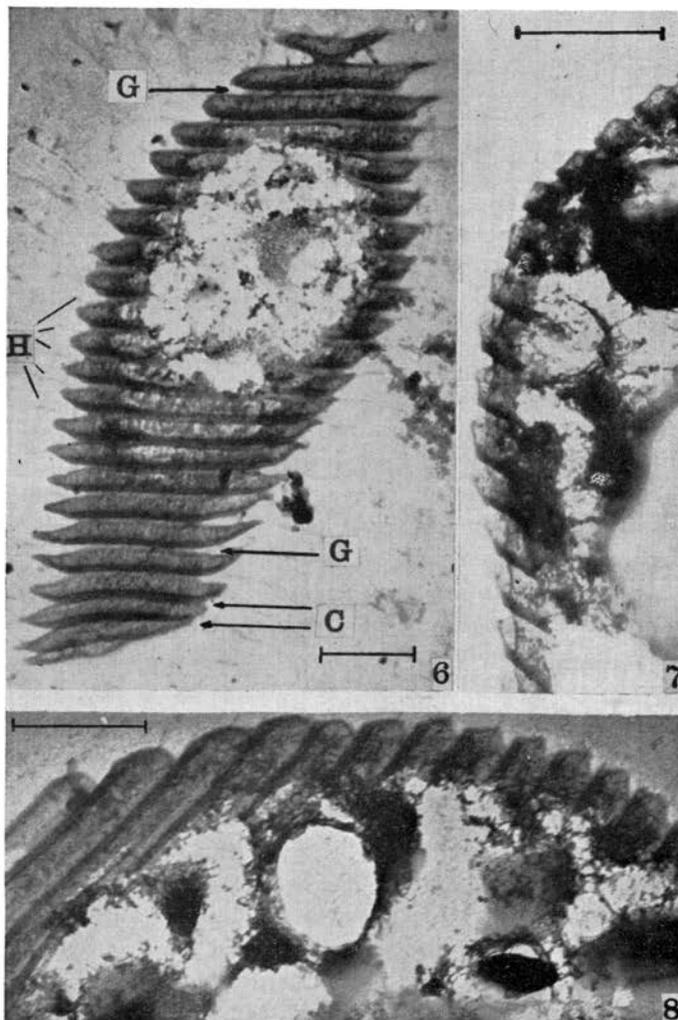


PLATE II.

Explanation of figures
Electron Micrographs

6. A section with much of pellicle sectioned almost parallel to its surface. (G) gap, (C) closed groove, and (H) unknown structure. 2% osmium tetroxide fixation.
7. Oblique section of pellicle. 1% buffered osmium tetroxide fixation.
8. Oblique section of pellicle. 2% osmium tetroxide fixation.

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