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COMPARATIVE LIGHT AND ELECTRON MICROSCOPICAL STUDIES ON THE ARGYROPHILIC STRUCTURES OF *EUGLENA VIRIDIS*

W. FOISSNER

INSTITUTE OF ZOOLOGY, UNIVERSITY OF SALZBURG, SALZBURG, AUSTRIA

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Abstract

(1) A method for the electron microscopical investigation of Algae is described. (2) The silverlines of *Euglena viridis* are oriented in the same helical manner as the pellicular stripes and are linked by very fine argyrophilic structures. Accordingly, the silverlines are continuous over the whole cell, even in its anterior and posterior parts, where the silverlines and pellicular stripes are reduced in number. (3) Electron micrographs of silvered *Euglena viridis* clearly show that the silverlines are located in the posterior part of the pellicular stripes, just underneath the plasmalemma. At the same site, material of fibro-granular appearance is present in specimens which have been prepared in the conventional way. This kind of silver deposition could be observed in dry- and wet-silvered *Euglena viridis* alike. (4) The argyrosomes are not identical with the muciferous bodies. In ultrathin sections, they appear as subpellicular vacuoles with electron-dense content. (5) The electron micrographs of the pyrenoids show a strictly localized silver deposition at the lamellae of the chloroplasts which penetrate the pyrenoids. (6) In the anterior and posterior regions of *Euglena viridis*, there are more microtubuli below the pellicular stripes than in the central region of the cell. This is interpreted as an effect of the reduced number of pellicular stripes in these regions. The shape of the pellicular stripes is correlated with the state of movement of the cells. (7) The argyrophilic structures were not affected by colchicine, but in many specimens they were partially or completely destroyed by cytochalasin B. It is therefore suggested that the silverlines of *Euglena* are cytochalasin sensitive filaments. Rupture of the pellicle by mechanical pressure showed similar results: in many specimens, the silverlines appeared broken into pieces or transformed into a fine network. (8) The structure and function of the silverline system of euglenoid flagellates is discussed. It is supposed that the silverlines are filaments which may have a neuroid function.

Introduction

It was two years after the discovery of the silverline system in ciliates by KLEIN [29] that JIROVEC [25] succeeded in demonstrating, by the dry silvering method, silverlines in certain euglenophyceans. The argyrophilic lines regularly followed the pellicular stripes. The line system staining with opal blue proved to be identical with that demonstrable with silver impregnation [27], comp. also [9]. Soon thereafter, JIROVEC's [25] findings were confirmed by KLEIN [30], who discovered a continuous silverline network in *Gonium* sp. and *Volvox* sp. This finding, however, has not been confirmed by others since then. JIROVEC [25, 27] and KLEIN [30, 32] considered the sylver-

line systems of flagellates to be a fibrillar pellicular structure conveying impulse. However, they could not exclude a supporting function. KLEIN [30] and DEFLANDRE [12] called attention to the fact that the silverline system of euglenophyceans is covered by a rigid pellicle, therefore, a supporting function cannot be its primary task.

HALL [20] and JIROVEC [27], the first authors who used the wet silvering method of CHATTON and LWOFF [8] in studying euglenophyceans, emphasized that by this procedure other cell organelles may also be impregnated, therefore, the pictures should be cautiously interpreted. CHADEFAUD [4] has mentioned that in some cases the silverlines appear not as fibrils but as impregnated pellicular stripes. KÜSTER [33] extended this statement to all euglenophyceans. He is convinced that the impregnated pellicular stripes share nothing with the silverlines described in *Gonium* sp. and *Volvox* sp. Later CHADEFAUD and ARLET [6] refused the fibrillar nature of the silverlines and noted true fissures in the pellicle, a view which has been taken over by HOLLANDE [22] and by PRINGSHEIM [40]. The silverlines must be built up of rows of linearly arranged argyrophilic granules which are more or less connected with one another. Furthermore, in *Peranema trichophorum*, the argyrophilic substance must lie in the fissures of the pellicle [5]. CHADEFAUD [5] has suggested that, in fact, the silverlines might not be pellicle elements. CHADEFAUD and ARLET [6] were the first to recognize that the pellicular stripes are not argyrophilic, they only contain argyrophilic granules called argyrosomes by them.

Recently POCHMANN [39] has demonstrated a gradually variable impregnability of the silverlines in *Phacus pyrum* and *Lepocinclis* sp. and emphasized the fragility of the argyrophilic lines. He raised the question whether there are in the area of gyri mucous substances, cytoplasm fragments or enzymes which, being reductants, might bring about artefacts. More recently DE HALLER [21] has suggested on the basis of electron microscope studies that the cisterns of the endoplasmic reticulum beneath the pellicular stripes are responsible for building up the silverlines.

This brief historical review shows that we have little information about the structure, location and function of the silverlines. At least part of the problems have been solved in the present study.

Material and methods

Masses of *Euglena viridis* (determined according to [28, 40, 21] were found in a sewage highly contaminated by kitchen sewage near Linz. For light and electron microscopy, cells were taken with a spatula from the surface of the sample and poured with the fixative into a centrifuge tube.

(1) *Light microscope preparations.* Argyrophilic structures were looked for by the use of a dry [14] and a wet [11] silvering method.

(2) *Electron microscope preparations.*

a) Three-minute fixation according to CHAMPY (prescriptions, *v.* [11, 17]). The fixative was removed by washing for 60 min with twice changed Da Fano mixture [11, 17]. The preparations were dehydrated in an alcohol series and in propylene oxide (30 min) and embedded in EPON 812.

b) Wet-silvered *Euglena viridis* was carried up to 50% alcohol in an alcohol series, then removed by a razor blade from the slide, together with the gelatine layer. Then, dehydration in alcohol and propylene oxide (30 min) was continued. The silvered specimens in gelatine layer were embedded in EPON 812.

c) After silver reduction, dry-silvered *Euglena viridis* cells were overlaid by distilled water for 5 min. The cells, on the top of the protein layer, were separated from the slide with a razor blade, dehydrated in an alcohol serie transferred into propylene oxide (for 30 min) and embedded in EPON 812.

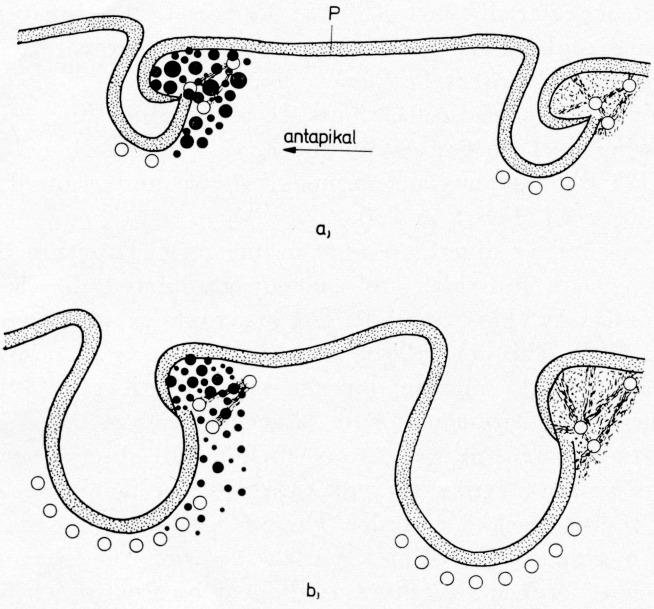


Fig. 1a, b. Location of the silverlines and the pellicular microtubuli in the middle, apical and antapical regions of *Euglena viridis*. A schematic drawing. The silverlines lie in the posterior (antapical) part of the pellicular stripes, tightly beneath the three-layered plasmalemma (P).

All electron microscope preparation procedures except silvering were carried out in a centrifuge tube. In preparing thin sections we used a diamant knife in a Reichert OMU-2 microtome. The thin sections were transferred to carbonized pioloform-filmed slides. The preparations were counterstained in an ethanolic (50%) uranyl acetate solution and Reynold's lead citrate [41] for 15 min each. A Zeiss EM 9S apparatus was available for electron microscopy.

(3) *Experimental methods*

a) Treatment with aqueous colchicine solution (5%) for 10 min or 2 h. After treatment the cells were dry-silvered.

b) Treatment with 50 μg/ml cytochalasin B solution for 10 min, 1 h or 2 h. For this purpose, cytochalasin B was dissolved in DMSO, 1 mg in 0.5 ml, and diluted with distilled water up to 5 ml.

c) Mechanical destruction of cells by pressure. One drop containing many *Euglena viridis* cells were pressed between the slide and a coverslip until the majority of the cells had burst. Then the preparations were dry-silvered at intervals of 10 sec, 2 min and 10 min.

Results

Light microscopic studies

Figs 2 and 3 show the silverline system in a partially and a fully contracted *Euglena viridis*. The silverlines run in a spiral form and, like the pellicular stripes, are separated from each other by a distance of 1 μm. In relaxed cell regions, the silverlines are somewhat farther from each other than in contracted cell areas; the silverlines are bound together by transversally running silverlines at irregular distances (Fig. 2, 3 arrows). The transversal lines show very weak argyrophilia and are only seen in the best preparations. KLEIN [30] described similar silverlines in *Euglena* sp.

In the centre of the cells, there are argyrophilic lines, some 40—50 in number. Owing to the fusion of silverlines, this number is reduced to its half (comp. [25, 34]) at the apex and antapex, where uninterrupted, Y-like fusioning figures are seen (Figs 2 and 3).

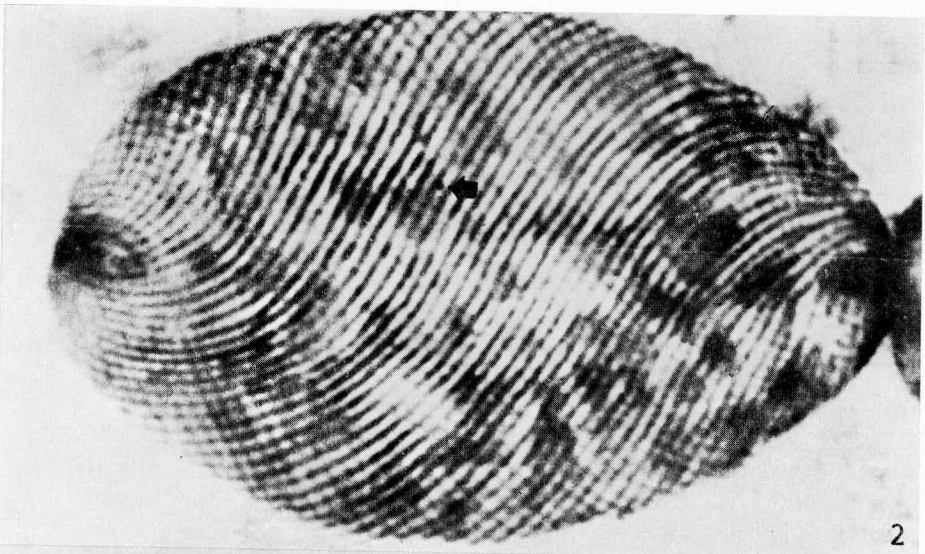
The dry-silvered silverlines appear uneven in structure. They are quite fine in some places and thick and coarsely granulated elsewhere. Sometimes they lose continuity (Figs 2 and 3). The argyrophilic substance is often drop-like (comp. [30]). This irregularly variable picture is certainly due to artefacts. Presumably, the uneven impregnability led KLEIN [30] to supposing the existence of a two-component silverline system in euglenophyceans. However, JIROVEC [25] as well as myself failed to observe such a structure. Furthermore, unlike CHADEFAUD and ARLET [6], we have never seen silverlines built up of tightly packed granules. There is no doubt that the silverlines are continuous in structure (Figs 2 and 3) as also shown in the electron micrographs. The existence of broken silverlines as claimed by POCHMANN [39] is unlikely because the silverlines follow the cell in all of its movements without showing breakage (Figs 2 and 3). The argyrosomes of *Euglena viridis* are of little size and occur sparsely. They lie as a rule tightly beside the silverlines (Fig. 2).

Fig. 2. The silverline system of a partially contracted *Euglena viridis*. The spirally running silverlines, which turn down at the edge of the cell, appear distinctly thick and are reduced in number by fusion in the antapical region (right side). Arrow points to an argyrosome. Dry silver impregnation. ×2000

Fig. 3. Antapical view of a fully contracted *Euglena viridis*. The numerical reduction of silverlines ensues as a result of further fusions of two silverlines each time. The silverlines are connected by very fine argyrophilic lines with each other (arrow). Dry silver impregnation. ×2000

Fig. 4. Part of the silverline system of a non-contracted *Euglena viridis*. The irregular thickness of the silverlines is especially emphasized by this method of preparation. Arrow points to an argyrosome. Wet silver impregnation. ×2000

Fig. 5. Cross-section of the pellicle in the middle region of *Euglena viridis*. The pellicular stripes are variable in height, width and shape and are strictly correlated with the state of cell movement. The microtubuli (arrows) running tightly beneath the three-layered pellicle are partially recognizable. Note the fibrogranular substance between the three outer microtubuli and the pellicle (thick arrow, comp. *Fig. 1a*) Method *a*, ×56,000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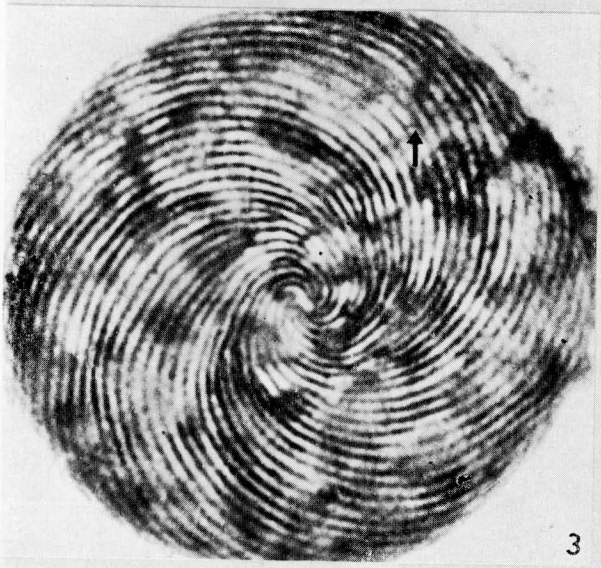
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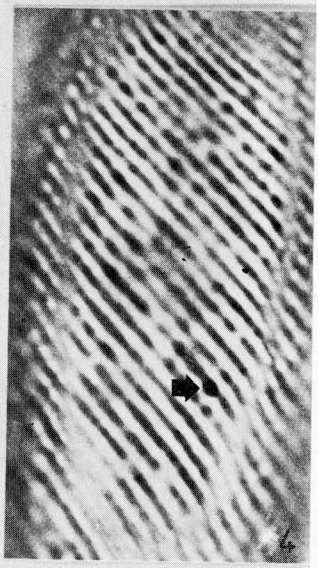
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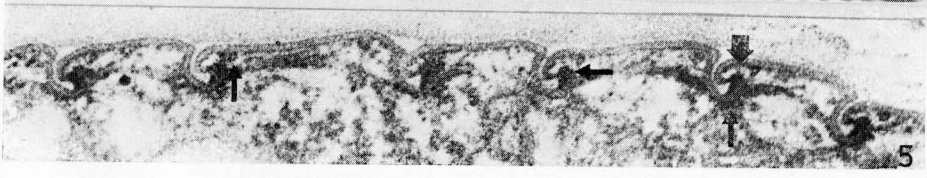
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Wet-silvered preparations showed essentially the same picture (comp. Figs 2 and 3 with Fig. 4 and see ref. [27]), except for the fine transversal silverlines found between the spirally running silverlines. These could not be observed. Furthermore, silverlines dissolved in small granules and discontinuity of them occurred more frequently by this impregnation. The argyrosomes, on the other hand, appeared more pronounced in wet-silvered preparations (Fig. 4, arrow). Weak argyrophilia often occurred around the paramylon granules as well.

Electron microscope studies

E. viridis as prepared according to method a. In preparations fixed by CHAMPY's method, the ultrastructure remained as clear as after simple OsO_4 fixation. Even the pellicular microtubules were distinctly visible.

The fine structure of the pellicular stripes essentially corresponds to that of other *Euglena* species [3, 35, 36, 37]. As described by DE HALLER [21], they are limited towards the environment by a triple membrane the middle of which appears less contrasted. This middle zone disappears in the area that is overlapped by stripes (Figs 1*a*, *b*, 5), and it was not clear whether the outer zones of this membrane, too, end here or, whether they bind the single stripes together as it is generally supposed. There lies more medially in the stripes a thin fibrogranular zone transversally to the length of stripes. Supposedly [34], these are formed by the teeth. Tightly nearby, or sometimes deeper, many, more or less spherical vacuoles are seen, which may correspond to channels of the endoplasmic reticulum [1, 18, 43] or, more probably, muciferous bodies [34] (Figs 12, 15 M). The vacuoles are limited by a single membrane and appear empty.

The sections from the apical and antapical areas have proved that the numerical reduction of pellicular stripes, in accordance with LEEDALE's suggestion [34], is due to fusion of stripes (Fig. 7). The observation that the silverlines in the area of fusioning stripes run without being interrupted ac-

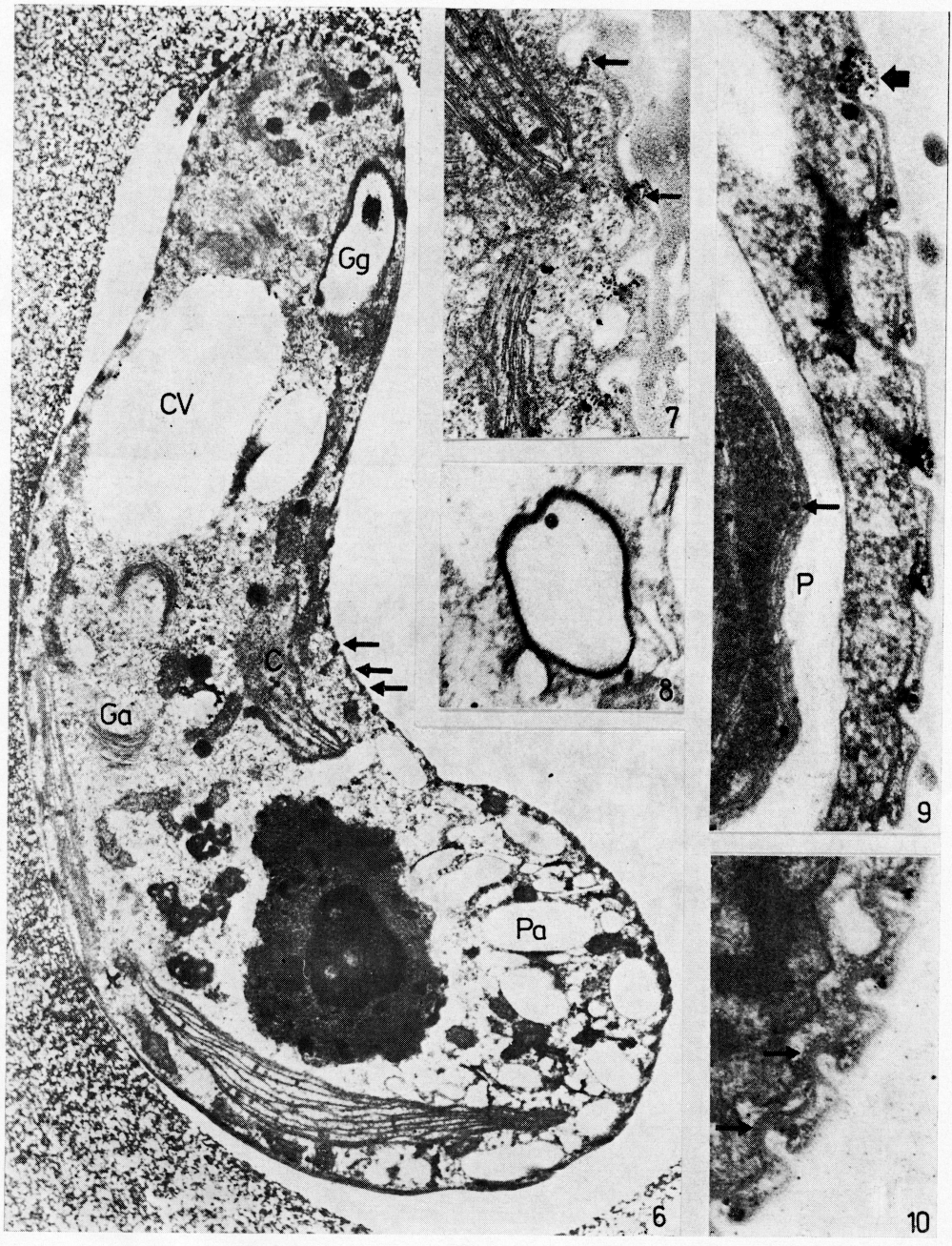
Fig. 6. Longitudinal section of a silvered *Euglena viridis*. The silverlines run in the posterior part of the pellicular stripes. The nucleus with a large and many small nucleoli, paramylon grains (Pa), chloroplasts (C), the Golgi apparatus, the ciliary cavity (Gg) and the contractile vacuole (CV) are to be recognized. Method *b*, ×9000

Fig. 7. Cross-section of the pellicle in the antapical fusion zone of pellicular stripes and silverlines. The silverlines (arrow) at pellicular stripes in fusion have not fused yet. Method *b*, ×48,000

Fig. 8. A very fine silver precipitate is visible on the membrane enclosing paramylon grains. Method *b*, ×48,000

Fig. 9. This cross-section clearly shows that the silverlines (thick arrow) lie in the posterior part of the pellicular stripes, in the zone of microtubuli. Note the sporadic silver aggregates on the chloroplast lamellae of the pyrenoids (P). Method *b*, ×48,000

Fig. 10. Cross-section in the apical area of *Euglena viridis*. Microtubuli have markedly increased in number. The silverlines lie beneath the pellicle. Method *b*, ×48,000


Gg
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cords well with this suggestion. However, all these are inconsistent with the data published by GUTTMAN and ZIEGLER [19], who believe that the apparent reduction in the number of pellicular stripes is due to simple growing down by crossing stripes.

An analysis of many cells being in different states of moving has shown that the height, the width, and the shape of the pellicular stripes must be correlated with the state of cell movement. The stripes in weakly contracted or non-contracted regions are wide and flat (about 450×100 nm) and only slightly depressed at the middle (Figs 5, 9), whereas those in contracted cells are depressed and higher (about 300×300 nm) and more narrowed at the middle (Figs 10, 11). Pellicular stripes, even those lying in closely adjacent regions, on the other hand, are often very different in height, width and shape (Figs 5, 12). This suggests that individual stripes can change their shape independently. These observations clearly show that when studying the function mechanism of metabolic movements we should taken into account the configurational changes of the pellicular stripes as already suggested by GUTTMAN and ZIEGLER [19], who investigated configurational changes by raster electron microscopy.

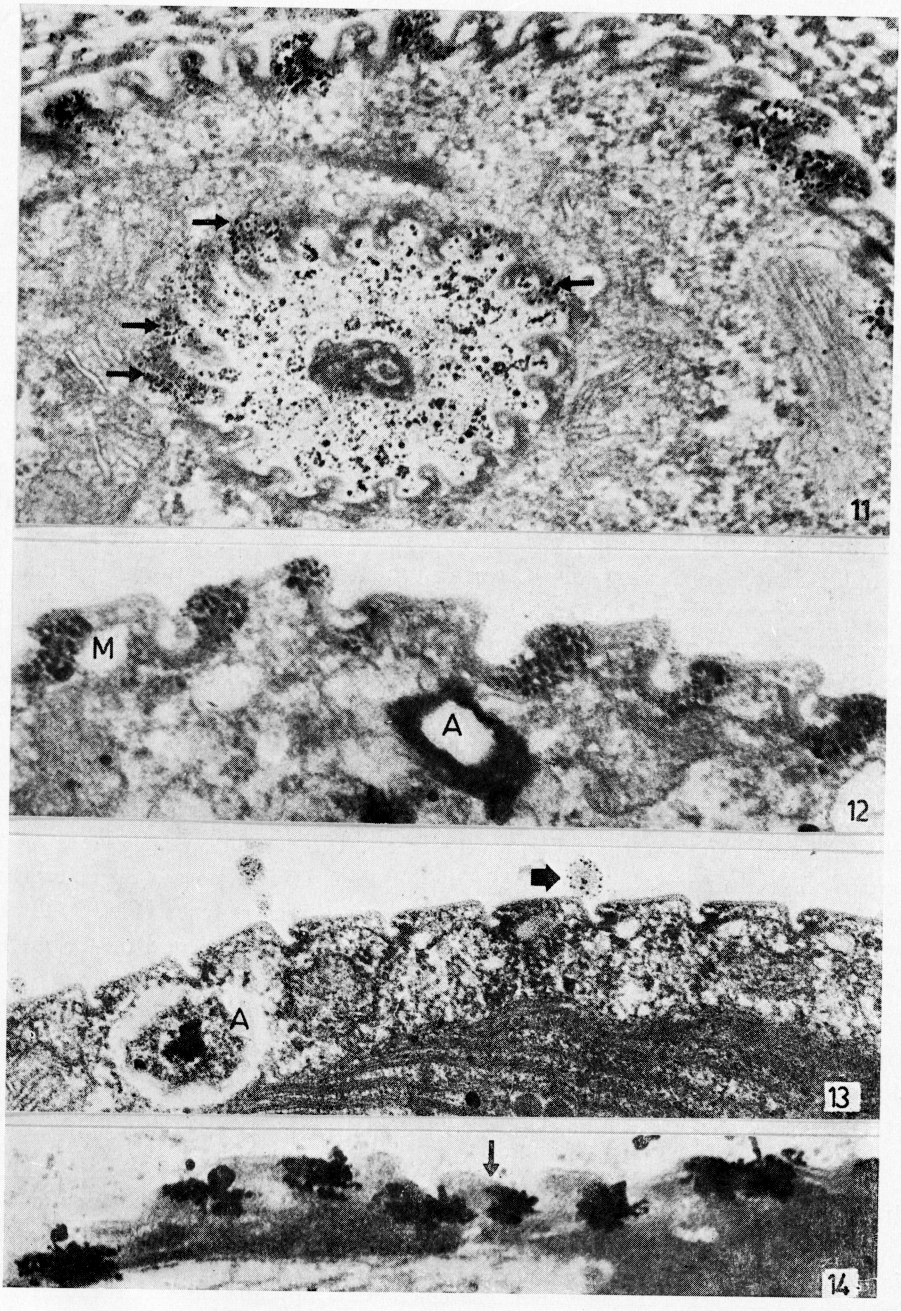
The pellicular microtubuli, except those in the area of the channel, had been described as evenly arranged, *e.g.*, [3, 35, 36, 37]. Surprisingly, I have found that the microtubuli in different cell regions are differently arranged. Figs 1*a* and 5 show the arrangement of microtubuli in the central region, where no fusion of pellicular stripes occurs. A very similar arrangement was described as typical by DE HALLER [21] in *Euglena viridis* and by MIGNOT [36] and ARNOTT and WALNE [1] in *Euglena gracilis*. Around the microtubuli, there lies a more or less contrastable fibrogranular substance. Especially the three outer microtubuli are bound together with one another and with the pellicle by well-defined bridges (Figs 1*a*, *b*, 5; comp. [36]). Figs 1*b* and 10 show cross sections in the apical and antapical regions of a cell. It is clear that unlike the 2 or 3 microtubuli in the central part of the cell (Fig. 5, arrows) 8 or 9 are seen in these regions (Fig. 10, arrows). I attribute the increased number of microtubuli beneath the pellicular stripes to that here the pellicular stripes are reduced

Fig. 11. Cross-section in the channel region. Owing to the irregular silver impregnation, only few pellicular stripes appear impregnated. The channel is filled by gelatine in which many small silver aggregates are embedded. Nevertheless, silverlines at some pellicular stripes have been impregnated even here (see, *e.g.*, arrows). Method *b*, ×48,000

Fig. 12. This picture impressively shows cross-sectioned silverlines, muciferous bodies (M) and an argyrosome (A). A pellicular stripe carries small silver aggregates on both sides. Method *b*, ×56,000

Fig. 13. Numerous silver aggregates are seen only in the centre, at the large argyrosome (A) lying tightly beneath the pellicle. Note the droplet-like structures (arrow) with very fine-granulated silver precipitates outside the pellicle. It may correspond to muciferous bodies. Method *b*, ×44,000

Fig. 14. Cross-section through a dry-silvered *Euglena viridis*. Silver aggregates occur only beneath the pellicle. Method *c*, ×44,000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M
A
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in number, whereas it is only farther that the microtubuli are reduced by fusion.

E. viridis prepared according to method b. The structural state of the samples is markedly affected during preparation. Consequently, a great number of cells should be examined to establish the location of the argyrophilic structures.

(a) *Silverlines.* As shown in Figs 7, 9, 10, 12, 13 and 15 the silverlines lie tightly beneath the plasmalemma, *viz.*, in the area of microtubuli where the fibrogranular substance is to be found. In serial sections, the silverlines appear as continuous structures consisting of round silver aggregates 5—50 nm in diameter. In exactly cross-sectioned pellicular stripes, they are more or less distinctly spherical, 80—140 nm in diameter, and indistinctly limited towards the cytoplasm. The zone of silver deposition extends in general from the outer apex of the pellicular stripe to the microtubuli situated between two stripes each (Figs 6, 9, 10, 12, 15). The same is seen in obliquely (Fig. 15) and longitudinally sectioned stripes. In the apical and antapical regions, where the pellicular stripes are markedly tapering, the whole stripes may be filled by silver aggregates (Fig. 11). This may be attributed to an insufficiently fine impregnation. Silverlines were demonstrated in the region of channel (Fig. 11), they probable end in the basal bodies of cilia. Very rarely, we found pellicular stripes which produced silver deposition on both sides (Figs 12, 14, arrow). We cannot decide whether these deposits corresponded to real silverlines or to accidental silver depositions. The second alternative seems to be more probable because in light microscope preparations of *Euglena viridis* the distance between silverlines proved to be very stable. It is clearly seen in the Figures that among the numerous pellicular stripes possessing silverlines several stripes may occur which do not elicit silver aggregates (Figs 9, 11, 13). This phenomenon may be attributed to an incorrect silver impregnation, not to lacking silverlines.

(b) *The argyrosomes.* The argyrosomes of *Euglena granulata* were identified by ARNOTT and WALNE [1] on the basis of comparative light and electron microscopic investigations. The argyrosomes in this species are pores opening externally; they contain a highly osmophilic core. However, these authors

Fig. 15. Section through the apical region. Obliquely and cross-sectioned silverlines and muciferous bodies (M). Note the silver deposition on both ends of a pellicular stripe (arrow). The eye spot (A) shows no argyrophilia. Method *b*, ×53,600

Fig. 16. Part of the silverline system of an *Euglena viridis* after one hour treatment with colchicine. No changes in the silverlines. The chloroplasts, appearing as dark spots, are highly argyrophilic. Dry silver impregnation. ×3000

Fig. 17. Part of the silverline system of an *Euglena viridis* treated for 1 h with cytochalasin B. Granular dissociation of the silverlines. Dry silver impregnation. ×3000

Fig. 18. Part of the silverline system of an *Euglena viridis* treated with cytochalasin B for 2 h. The silverlines are fully destroyed. Only irregularly distributed coarse-granular silver aggregates are seen. Dry silver impregnation. ×3000


A
M
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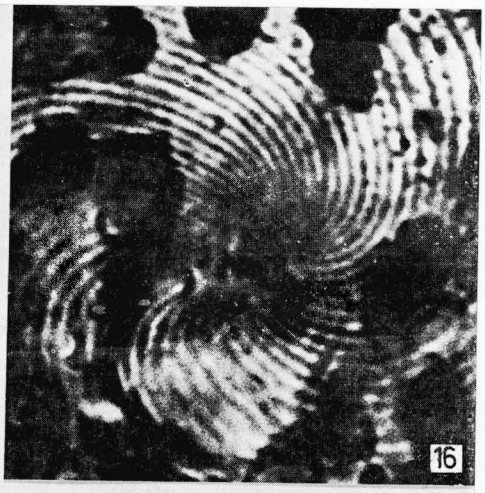
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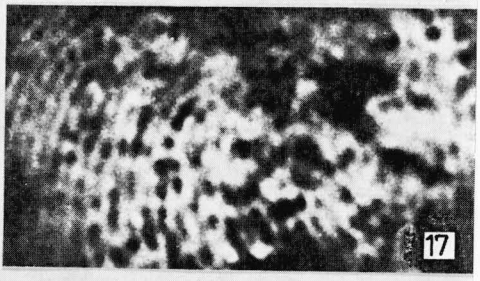
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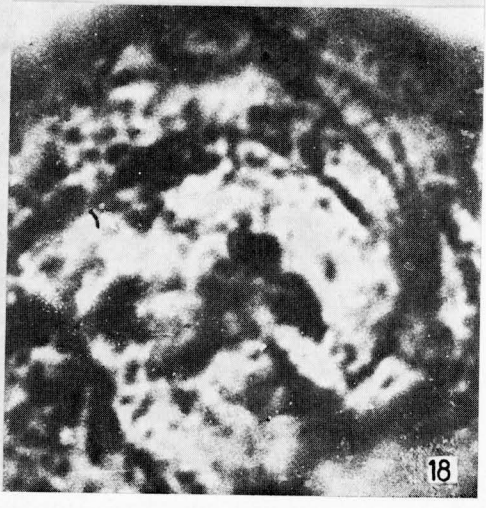
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did not examine silvered specimens, therefore, could not localize the site of silver deposition.

The argyrosomes of the *Euglena viridis* strain examined by us are few in number and less distinct than those of *Euglena granulata* and *Peranema trichphorum*. Supposedly, Figs 12 and 13 (A) show cross-sections through argyrosomes. Irregular spherical bodies are seen with distinctly denser silver deposition. They lie at the middle of two pellicular stripes tightly beneath the pellicle. No connection with the pellicle could be demonstrated. The size and the location of these argyrophilic bodies suggest that they are not identical with the muciferous bodies. DE HALLER [21] has described subpellicular vacuoles with electron-dense core, of the same appearance, in *Euglena viridis*. I suppose that muciferous bodies may also be slightly argyrophilic, for sometimes many small droplets with fine silver deposition occurred (Fig. 13, arrow) in the immediate vicinity of the pellicle. These droplets may represent muciferous bodies.

(c) *The pyrenoids and the paramylon granules.* In the pyrenoids, where light-microscopically no argyrophilia was seen, very fine, strictly localized silver deposition was demonstrated (Fig. 9, arrow). Silver aggregates occurred between the broad stroma bundles lying most frequently on the chloroplast lamellae, outside the pyrenoid.

Sometimes there was another argyrophilic zone demonstrable around the paramylon granules. The silver was deposited on the outer side of the membrane surrounding the paramylon grains (Fig. 8).

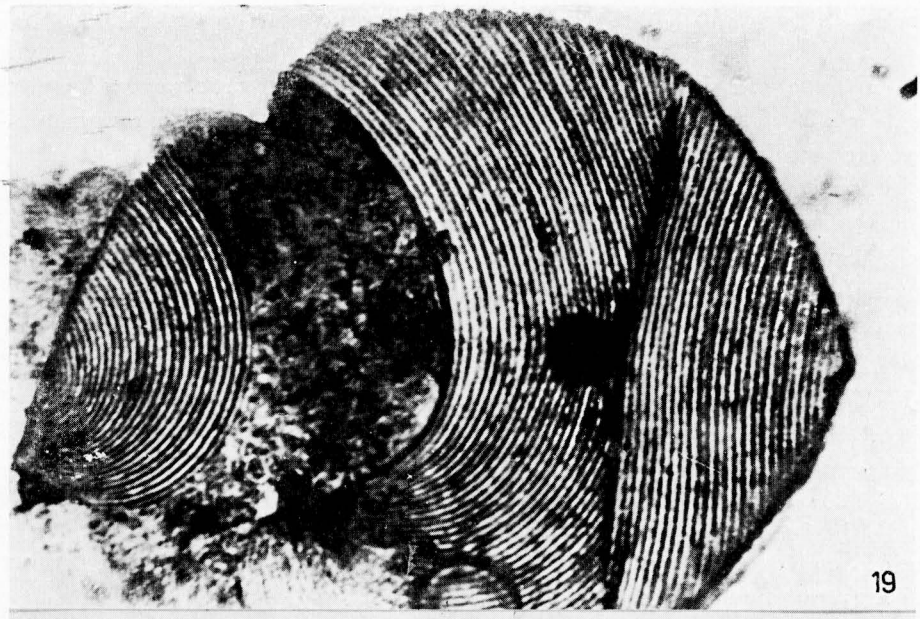
(d) *Cell organelles without specific argyrophilia.* There was no specific argyrophilia at any other cell organelle (*e.g.*, nucleus, chloroplasts, Golgi apparatus, stigma) (Figs 6, 15). The silver aggregates in these organelles were very small and irregularly arranged.

(e) *Euglena viridis prepared according to method c.* The same results were obtained as with method *b*, except that the state of the specimens prepared with method *c* was much worse. Fig. 14 clearly shows the silverlines immediately beneath the plasmalemma. It is therefore evident that the same structures are impregnated by both methods.

Fig. 19. Dry silver impregnation of an *Euglena viridis* 5 sec after squatching. No changes in silverlines of the disrupted cell. ×2000

Fig. 20. Dry silver impregnation of an *Euglena viridis* 30 sec after squatching. The silverlines have been partially disrupted into pieces (see, *e.g.*, arrow). ×2000

Fig. 21. Dry silver impregnated *Euglena viridis* 10 sec after squatching. The cell has been disrupted and at the site of splitting (arrow) the silverlines have disintegrated to form a very fine meshed network. ×3000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Experimental studies

Effect of colchicine. Colchicine affected neither the structure nor the movements of *Euglena viridis*. Only chloroplasts with an increased argyrophilia were frequently seen (Fig. 16).

Effect of cytochalasin B. After two-hour treatment, cyto-chalasin B caused in silverlines a partial destruction in 50%, and total destruction in 5%, of the specimens. The specimens with destroyed silverlines ceased euglenoid moving. The first changes, *viz.*, granular disintegration of silverlines in many specimens appeared one hour after cytochalasin B had been added (Fig. 17). Later the silverlines disappeared, leaving behind an irregularly distributed silver precipitate (Fig. 18). In about 50% of the specimens, the silverlines remained apparently intact by the end of the second hour of cytochalasin treatment, suggesting the existence of a distinct individuality in cytochalasin sensitivity.

Effect of mechanical pressure. Owing to pressure, the pellicle bursts and the cell contents flow out. When cells were silvered immediately after burst, the argyrophilic substance appeared unchanged in the majority of the specimens (Fig. 19). Latticed disintegration (Fig. 21) or breaking into pieces (Fig. 20) of silverlines at the site of disruption was seen in some 10% of the disrupted specimens. If the preparations were silvered 5 to 10 min after burst, only rudiments of silverlines were observed, owing to postmortal changes. The silverline system in the surviving *Euglena viridis* specimens appeared normal.

Discussion

Location, structure and function of the silverline system in euglenophyceans

The present investigations have made a number of the hypotheses mentioned in the introduction clear. It is now obvious that the silverline system in euglenoid flagellates is identical neither with pellicular splits [5, 6, 22, 40] nor with the pellicular stripes [4, 33]. Mucous substances [39] and the canaliculi of the endoplasmic reticulum [18, 21] can also be excluded. Only the hypotheses of JIROVEC [25] and KLEIN [30], postulating a fibrillar nature of silverlines and the POCHMANN's [39] generally formulated hypothesis suggesting that the impregnable structure corresponds to cytoplasmic elements or enzymes, have remained to be discussed. A strict definition of silverlines as pellicular structures should first of all exclude their being cytoplasmic elements. However, it cannot be excluded that enzymes are impregnated even though there is no information as to their nature and it is unlikely that such

a stable and regular arrangement exists without any morphological basis. SOMMER and BLUM [43] succeeded in inducing pellicular phosphatase activity near the silverlines, but the impregnable substance cannot correspond to the phosphatase, latter being located outside the plasmalemma.

The present results fully agree with JIROVEC's [25] and KLEIN's [30] view, *i.e.*, with the fibrillar nature of the silverlines of euglenophyceans. This hypothesis is supported by the following observations: (*i*) fibrogranular substance has been demonstrated in the place of the silver deposition (Fig. 5, comp. [36]); (*ii*) cytochalasin B does, colchicine does not, destroy the argyrophilic lines. SILVERMAN and HIKIDA [42] have shown that the euglenoid movement and the pellicular microtubuli are influenced by colchicine but very slightly, whereas cytochalasin B is known to distroy microfilaments first of all [10]; (*iii*) on mechanical destruction of the cells, at least part of the specimens showed a reticular disintegration of silverlines, a phenomenon typical of cytochalasin B-sensitive filaments [24]; (*iv*) the structurally and morphologically very similar silverline system of ciliates (*v.* below) is a fibrillar structure of the cortex [13, 15, 16, 17].

No essentially new information has been obtained as regards the function of the silverline system. It can nevertheless be excluded that it is a supporting [27, 30] or elastic structure [5]. Such differentiations would be clearly visible by conventional electron microscopy. As already emphasized by KLEIN [31] and JIROVEC [27], the close connection of the silverlines with the basal bodies as well as their morphological arrangement is suggestive of a neuroid function. POCHMANN [39], on the other hand, believes that euglenophyceans, having no cilia, do not need an impulse-conveying system. Similarly, JAHN and BOVEE [23] consider the existence in euglenophyceans of a "nervous system" very unlikely. However, unequivocal observations have proved that the euglenoid movement is strictly controlled by environmental factors [38] and the euglenophyceans are able to control their metabolic activities [23]. We believe that the question put by JAHN and BOVEE [23], *viz.* "where such a coordinative, initiative system morphologically resides or is morphologically distributed, and how it operates is only theoretical and still a mystery", might be answered by supposing that the silverline system is this co-ordinative system. There is no doubt that, owing to its morphology and location (Figs 2, 10, 12), the silverline system is predestined for a co-ordinating function. The fact that, perhaps through the destruction of the co-ordinating silverlines, cytochalasin B caused an inhibition of the euglenoid movement points to the same direction.

Comparison of the silverline system of euglenophyceans with those of ciliates

Silverline systems have been discovered, besides ciliates and euglenophyceans, in all the other groups of *Protista, viz.*, in *Pyrosomida* and *Trypanosoma* by JIROVEC [26], in *Dinoflagellata* by BIECHELER [2] and CHATTON [7] and in *Heliozoa, Amoeba, Sporozoa* and *Oscillatoria* by KLEIN [31] and FOISSNER [14]. The silverline systems are very similar to each other in their light-microscopic structure, though, they are variable in shape.

As to the silverline systems of euglenophyceans and ciliates, the differences are very slight. Among the many common features the following deserve mentioning: (*i*) deposition tightly below the pellicle; (*ii*) more or less fibrillar character; (*iii*) similar size; (*iv*) connection with locomotor organelles; (*v*) silverlines form a continuum in the cell.

POCHMANN [39] disagrees with the similarity between ciliate and euglenophycean silverline systems because of differences in dividing processes and lack of multiplication by copulation. However, his arguments are not convincing because *(a)* there are no observations on the division of the silverline system in euglenophyceans available, *(b)* a negative marker like lack of copulation cannot be accepted as proof.

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WILHELM FOISSNER, Zoologisches Institut der Universität Salzburg, Akademiestrasse 26, A-5020 Salzburg, Austria