ELECTRON MICROSCOPIC SUDIES ON THE GONIDIOGENESIS OF BOTRYDIUM GRANULATUM (L) GREV. (XANTHOPHYCEAE)

by

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Introduction

The heterosyphonal alga *Botrydium granulatum* is a typical polenergidal organism which – presumably on account of its "amphibious" way of life – reacts with intense internal structural transformation to environmental changes, mainly to the water supply of the soil. This transformation agrees with the different ways of reproduction characteristic of the ontogenetic cycle and can be provoked experimentally, too. Its coenoblast is of a comparatively primitive organization, and precisely for its simple structure the morphological changes which accompany of the fructification cells and which can be observed under the electron microscope may be of cytological interest and can be partly generalized.

Botrydium granulatum may reproduce itself in a vegetative way, as well as either sexually or asexually. The gametae and the copulation were described only by Rosenberg, M. (1930) and Moevus, J. (1940); however, the reliability of their light microscopic observations is questioned by comprehensive algological studies. (Schussnig, B. 1953, 1960; Fott, B. 1959). The various types of akineta (ciste) formation caused by dessiccation, i.e. of vegetative reproduction were described and illustrated – partly erroneously – Rostafinski, J. and Voronin, M. (1877), and more exactly by Miller, V. (1927) and Vischer, W. (1937). The present authors (1969) have carried out electron microscopic studies on akineta formation.

The most frequent and best known way of reproduction of *Botrydium granulatum* is the asexual type. The "zoospores" or gonidia have been mentioned by all of the authors cited above; their electron microscopic description is from Falk, H. (1967), who did not, however, treat the question of genesis. The different phases of gonidiogenesis were observed by the present authors (1969). Further details of gonidion organization are described in the present paper.

Material and Method

Immediately before starting the experiment, *Botrydium granulatum* entities in vegetative state was collected from its natural habitat in the flood area of

the Danube. The plants were placed into tap water and 0.9M sucrose solution in Petri dishes, so as to be covered by the liquid. The dishes were kept in darkness at a temperature below 20°C. After light microscopic examination samples were taken from the various stages of gonidiogenesis and fixed for electron microscopic observations. The material was fixed in 0.75%, 1% and 2% $KMnO_4$ buffered with veronal-acetate, and in 4% GA+O, then dehydrated and embedded in araldite and durcupane, respectively. Finally, it was stained with uranylacetate and leadcitrate, according to Reynolds (1963). An electron microscope type KEM—1 (German Democratic Republic) was used.

Results and Discussion

Prior to transformation the vegetative plant can be described as follows (Nagy, J. 1966): The different organells are arranged in a more or less laminate pattern between the central vacuole and the cell wall and so there are zones composed partly of chromatophores and mitochondria, partly of nuclei and mitochondria, as well as of minor storing vacuoles; a rich network of endoplasmic reticulum can be found between them. The plant, 2 mm in diameter, has most of its volume (4.15 mm^3) filled up by the central vacuole (3.73 mm^3) and the 0.42 mm^3 cytoplasm contains cca. 2000 nuclei.

During the development of the fructification cells a profound change takes place in this arrangement of the organells. Accompanied by a lively movement of the cytoplasm an essentially different structure is formed, which can be equally well characterized. In this stage every nucleus is surrounded by a certain amount of cytoplasm containing 3—4 chromatophores, some mitochondria, ER and storing vacuoles. During transformation the entire volume of the coenoblast increases by cca. 30%, mainly due to the water uptake of the central vacuole and partly to the swelling of the cell wall. According to Wells, K. (1965) the mechanical action of this turgor may contribute to the induction of the reproduction process. The large oil vacuoles are breaking up into fragments and are moving conspicuously in the streaming cytoplasm. The full gonidiogenesis takes about 8—10 hours, and most of this time is used for the rearrangement of the organells. At the beginning the organells can move freely in every direction, but this movement is subsequently more and more inhibited by the cytoplasmic vacuoles which appearing gradually in the cytoplasm and become larger. We consider the vacuoles which develop independently of the central vacuole as ER dilatations, in agreement with the findings of Bracker, C. E. and Williams, C. M. (1966) on the development of the ascospore (Fig. 1). Due to the gradual fusion of initially small dilatations there are ducts being formed (Fig. 2), which gradually become wider and finally the earlier coherent cytoplasm layer is changed into a reticular network of cytoplasm strands.

Presumably the large central vacuole remains until the reticular structure is formed, and only after the formation of the cytoplasmic strands will this characteristic structure cease to exist. With the appearance and the fusion of further vesicles the division continues in the cytoplasm disorderly placed next to the cell wall. Actually the division goes on until the uninuclear fructification

cells – the gonidia develop. With the help of its flagella the newly organized gonidium gets detached from that part of the cytoplasm where the gonidium formation is still in an earlier stage (Fig. 5). Meanwhile the chromatophores divide in these parts of the cytoplasm (Fig. 3) and the flagella develop. There is no real cell wall to be found on the gonidium moving with flagella (Fig 5, 6, 7), but the intensive formation of Golgi vesicles can already be observed (Fig 8). The nucleus elongates towards the flagella in a characteristic way (Fig. 5). The flagella were found to be of the same thickness. In agreement with F a l k, H. we think that there is no difference between the diameter and the internal structure of the two flagella. They are sometimes covered by fine plasma extensions (Fig. 6).

With the help of their flagella the gonidia cannot move longer than for 30–45 minutes. When this time is over, the formation and thickening of the cell wall begin on the gonidia having lost their flagella, with an intense activity of the dictyosomes. The correlation between cell wall formation and Golgi vesicles was confirmed also by M a n t o n, I. (1964) on "zoospores".

We have observed that the formation of gonidia is not always accompanied by the development of the flagella, which seems to need light in certain phases of gonidiogenesis. Under natural condition the first phase of this process takes place at night and the gonidia with flagella begin to move at 5–6 o'clock in the morning. In the case of gonidiogenesis provoked under artificial conditions the plants must be illuminated after a certain period 8–9 hours after beginning of the process-, or else the development of the flagella may not take place. Even in this case the cytoplasm undergoes a fragmentation down to the gonidion units – the so called "Pflasterstein" stage – but then the moving stage is passed over, and the development and thickening of the cell wall begin immediatelly after the fragmentation on the gonidia which remain together (Figs 4, 9).

Having lost its flagella and limited by a thickening cell wall, the motionless spore (Figs 11, 12) accumulates very much reserve material without causing any change in the number and the internal structure of the different organells. The tubular structure of the mitochondria, the dimension of the chromatophores and the dictyosomes remain unchanged. Protein storage seems to take place by means of ribosomes, since they can be found in a conspicuous quantity in the cytoplasm, in the nucleolus and along the internal nucleus membrane (Fig. 10).

Summary

Electron microscopic studies have been carried out on the main morfological phases of gonidiogenesis on *Botrydium granulatum*. The dilatation of the endoplasmic reticulum was found to play the main role in the fragmentation of cytoplasm. The gonidia which move with flagella have no cell walls; cell wall synthesis begins only later in motionless gonidia. The Golgi vesicles contribute intensely to this synthesis. The thick-walled aplanogonidia contain not only fat and oil vacuoles but also a conspicuous amount of ribosomes.

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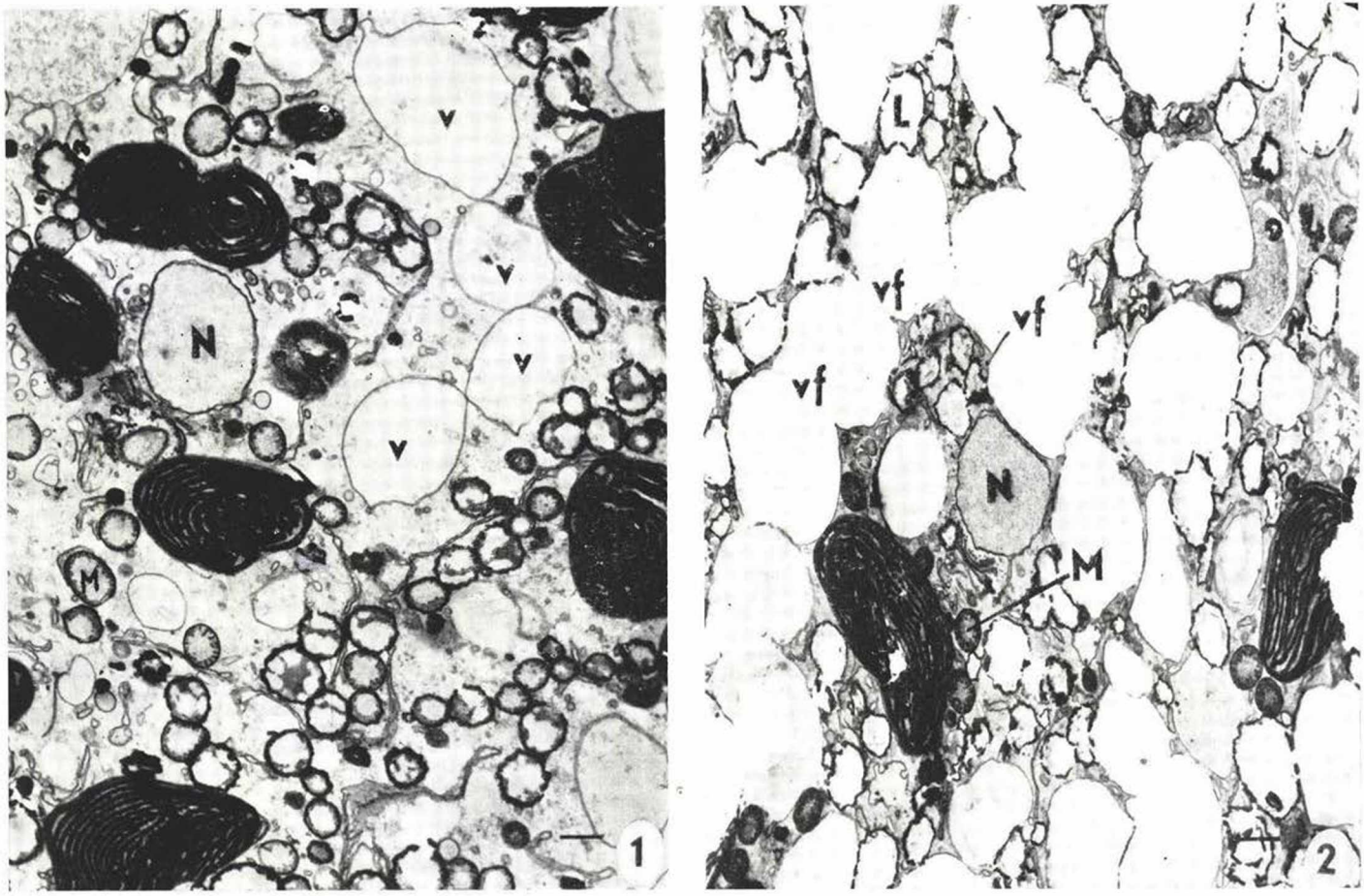


Fig. 1 – 2. Development and fusion of vacuoles. V = vacoule, Vf = vacuole fusion, N = nucleus. 5500x

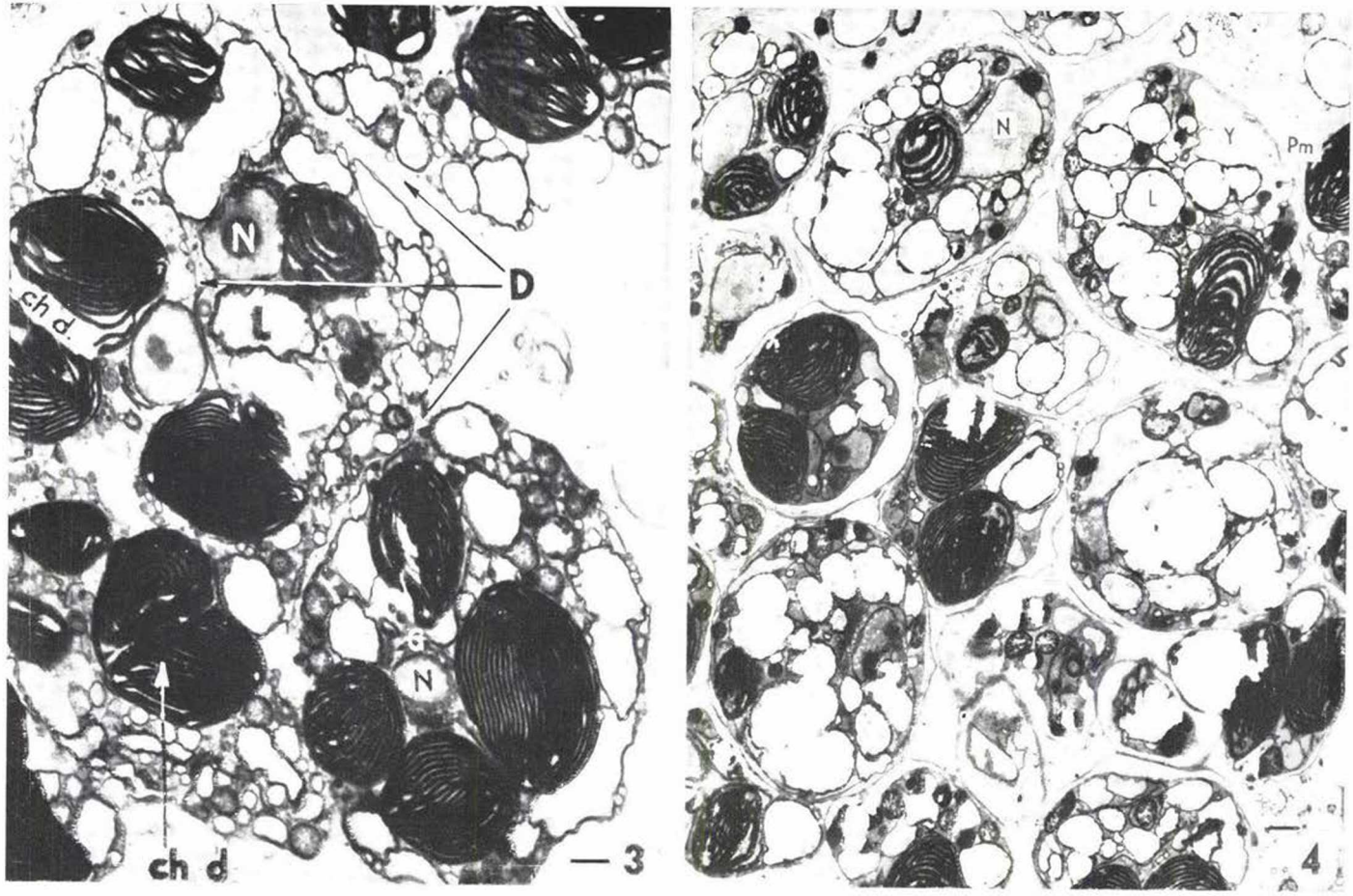


Fig. 3. Chromatophore division (ch, d), development and detachment of gonidia. D = dilatation, l = lipid bodies, M = mitochondrion. 5500x
Fig. 4. „Pflasterstein" stage with aplanogonidia. 3000x

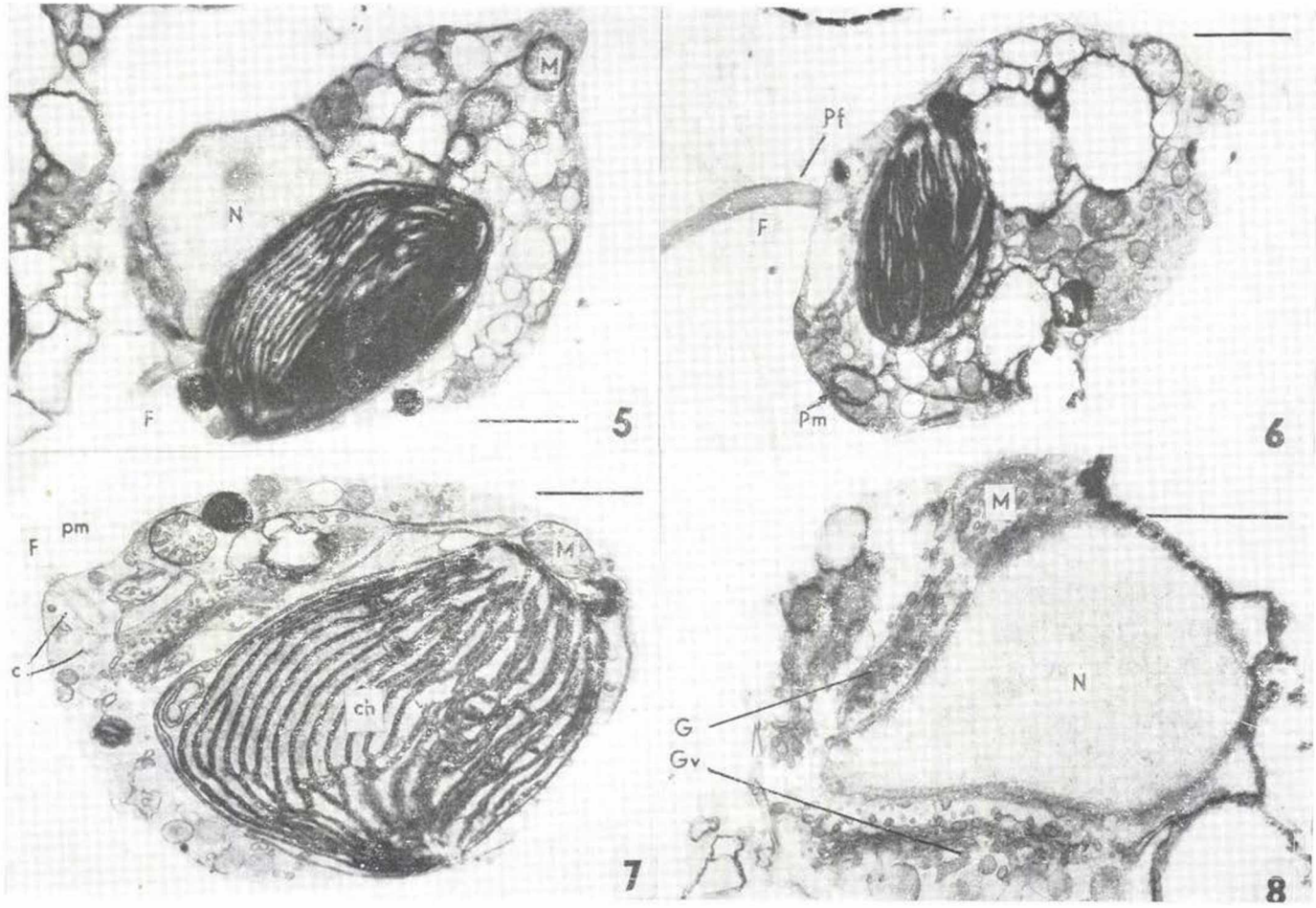
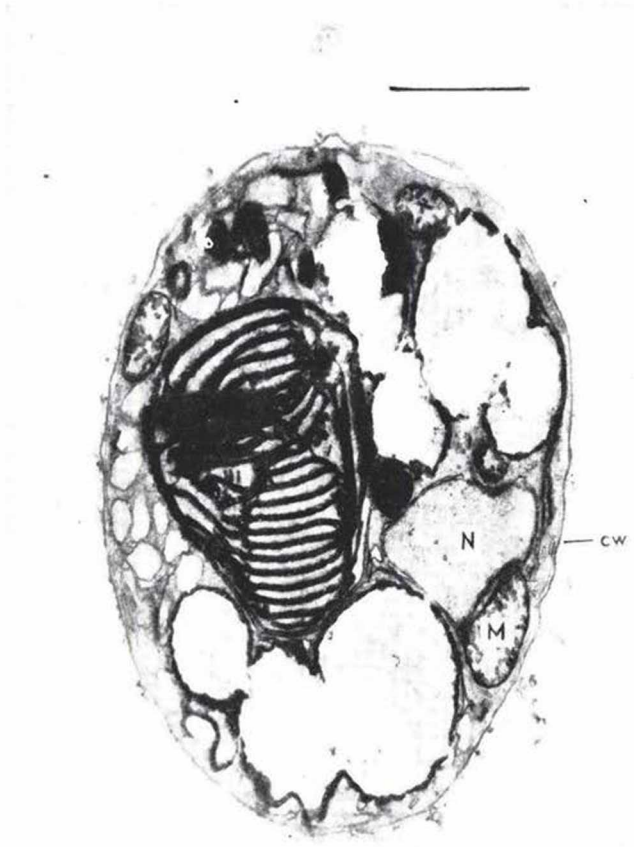


Fig. 5. Detaching planogonidium with elongated nucleus. F = flagellum. 13 000x
Figs. 6 – 7. Naked planogonidia limited by plasma membrane. pm = plasma membrane, pf = plasma fibrills, ch = chromatophore, c = centriole. Fig. 6 13 000x, Fig. 7 15 000x
Fig. 8. Active dictyosomes next to the elongated nucleus. G = Golgi app. G = Golgi vesicles. 30 000x



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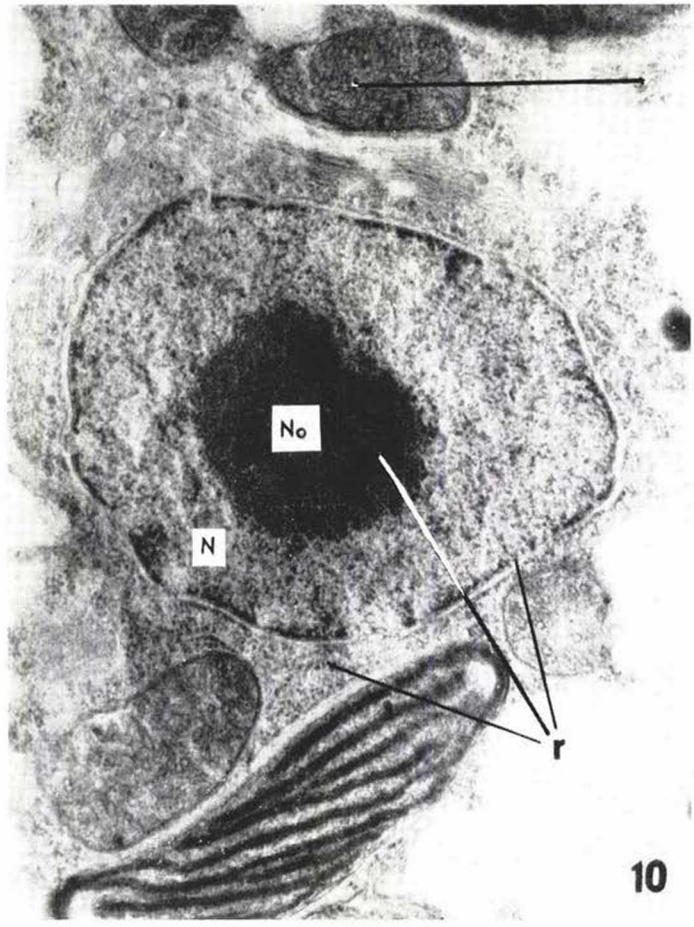

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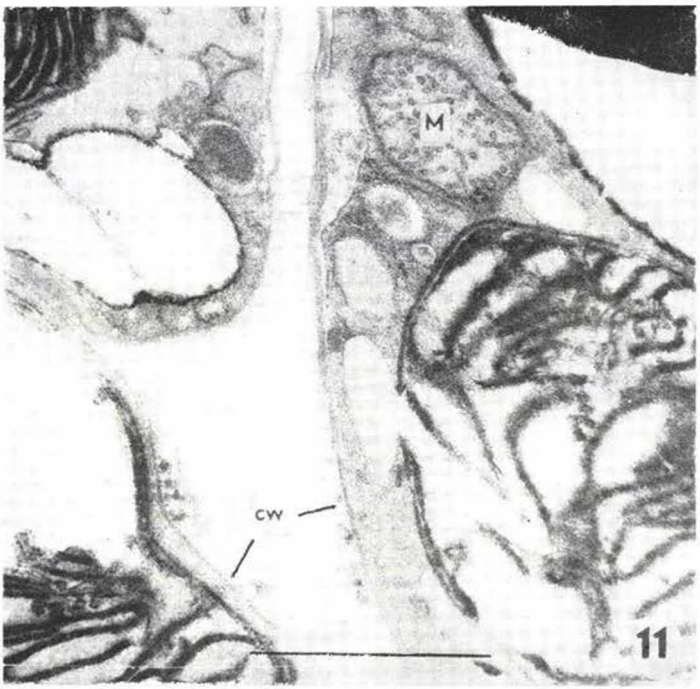
Fig. 9. Motionless gonidion in an early stage of cell wall development. cw = cell wall. 18 000x
Fig. 10. Accumulation of ribosomes in the nucleolus, on the internal cell membrane and in the cytoplasm. No = nucleolus, r = ribosome 38 000x



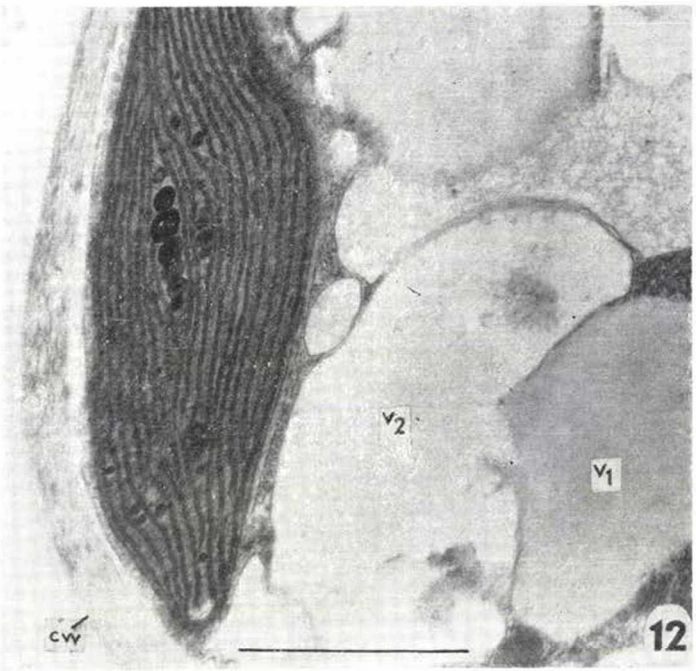


Fig. 11. Aflanogonidia with thickening cell wall. 30 000x
Fig. 12. Thick-walled gonidion (spore) with storing vacuoles. V_1 = vacuole containing carbohydrate, V_2 = vacuole containing lipid. 30 000 x