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Changes in cell wall structure during the protonema development on organic medium in conditions of light and darkness in *Funaria hygrometrica*

F. MŁODZIANOWSKI, A. WOŹNY and A. SZWEYKOWSKA

Institute of Boilogy, Adam Mickiewicz University, Stalingradzka 14, Poznań, Poland
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Abstract

The cell walls of a protonema of *Funaria hygrometrica* cultivated in glucose containing medium were considerably thicker in dark than in light. After a prolonged time of dark culture, a considerable reduction of the wall thicknes was observed, simultaneously with the occurrence of vesicles and plasmalemma invaginations containing fibrillar material. It is suggested that in conditions unfavourable for growth, the sugar taken up from the medium can be accumulated in cell walls, from which it can be mobilized again in conditions of starvation. The authors also think that similar mechanism and cell structures can be involved in both building and decomposition of the cell wall.

INTRODUCTION

Under light conditions, the spores of *Funaria hygrometrica* germinate in a mineral medium and develop into an abundant green protonema, consisting of long, branched filaments. The cross-walls separating the neighbouring protoplasts composing a protonema filament are thinner than the outer (longitudinal) walls, and are traversed by numerous pores with plasmodesmata (Młodzianowski 1970).

In the dark, the presence of sugar is required for the germination of spores; the developing protonema grows slowly, never produces gametophores and after 50 days of culture shows symptoms of starvation and degeneration (Woźny and Młodzianowski 1972). Monoroe (1968) observed a considerable thickening of the cell walls in a protonema of *Funaria hygrometrica* developing during 2 weeks in the dark, in the presence of glucose in the medium. A higher accumulation of saccharides in *Chlorella* under heterotrophic, dark conditions was reported by Griffiths (1965), and Budd et al. (1969) also found in this alga a thickening of the cell wall under these conditions.

In this paper, the results of some experiments and observations are reported concerning the spore germination, and the growth and cell wall structure of the protonema developing in a medium containing glucose, in conditions of light and darkness.

MATERIAL AND METHODS

The spore capsules of *Funaria hygrometrica* (L.) Sibth were surface sterilized and the spores sown on a mineral nutrient solution (S z w e y k o w s k a and H a n d s z u 1965) enriched with glucose. The germination of spores was estimated 5 days after inoculation using the criterion of K r u p a (1964), considering as germinated the spores with a protonamal protrusion equal or larger than 1/3 of the spore diameter.

The protonema cultures were grown in the dark or under light of about 1000 lux. The electron microscope observations concerned three culture variants:

30-day-old culture under light
30-day-old culture in the dark
50-day-old culture in the dark

Two methods of protonema fixation were used:

1) In 2 per cent aqueous $KMnO_4$ solution during 30 minutes and at room temperature; next washed with dist. water and dehydrated with an acetone series (at the stage of 50 per cent acetone post-stained with 1 per cent phosphotungstenic acid).

2) In a mixture of equal parts of 1.5 per cent glutareldehyde and 1.5 per cent formalin in 0.1 M phosphate buffer of pH 7 during 7 hrs., post-fixed in 1 per cent OsO_4 in the phosphate buffer during 1 hr. at a room temp.; next washed with phosphate buffer and dehydrated with ethanol series and propylene oxide.

The material was embedded in Epon 812. Ultrathin sections after the glutaraldehyde/OsO_4 fixation were contrasted with uranyl acetate and lead citrate according to R e y n o l d s (1963). Micrographs were taken with a JEM 7 A electron microscope.

RESULTS AND DISCUSSION

The germination of spores under light is about 100 per cent in a mineral as well as organic medium. In the dark, the germination is very poor or almost none in the absence of glucose. Glucose stimulates germination beginning with 0.01 per cent concentration and fully replaces light when used in a concentration range of 0.25—3 per cent (Fig. 1). The

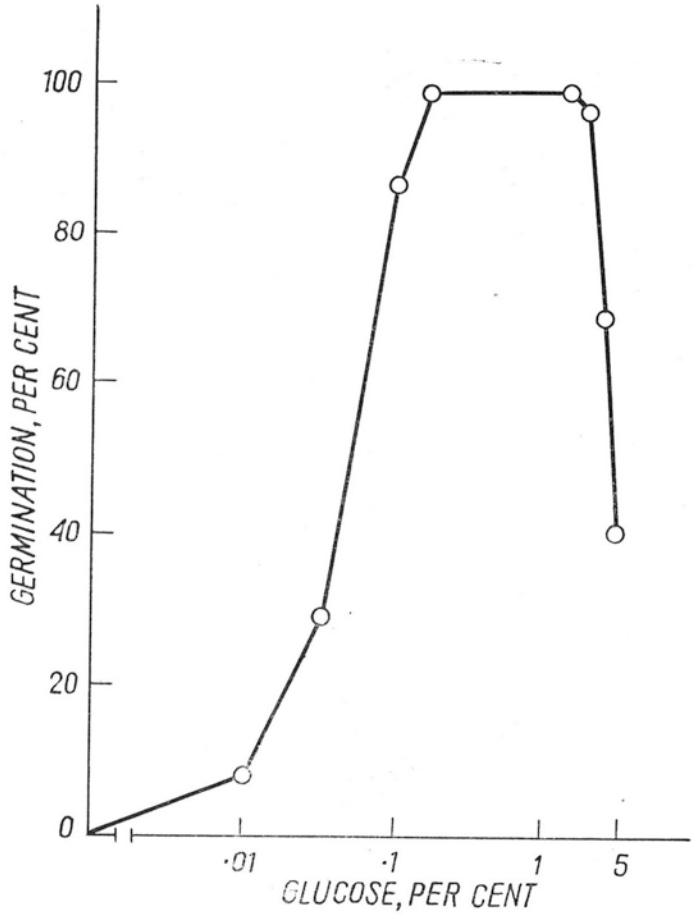


Fig. 1. The effect of glucose on spore germination in the dark.

subsequent growth of the protonema is also stimulated by glucose (Fig. 2). In the dark, the growth is relatively poor, reaches its maximum at 0.25 per cent of glucose and cannot be improved by the addition of casein hydrolysate or yeast extract. Despite of the presence of several nutritional components in the medium, the growth of *Funaria* in the darkness probably becomes soon inhibited. As shown in a cytochemical study by W o ź n y and M ł o d z i a n o w s k i (1972), the cells of the dark grown protonema after about 20 days of culture contain still appreciable amounts of RNA, proteins and enzymes, and of storage products as starch and lipids. After 50 days of growth, the protonema shows cytochemically symptoms of starvation and ageing.

The ultrastructural investigation revealed interesting changes in the thicknes of the outer cell walls occurring during 50 days of culture. The wall thickness was estimated on transverse sections of the protonema fila-

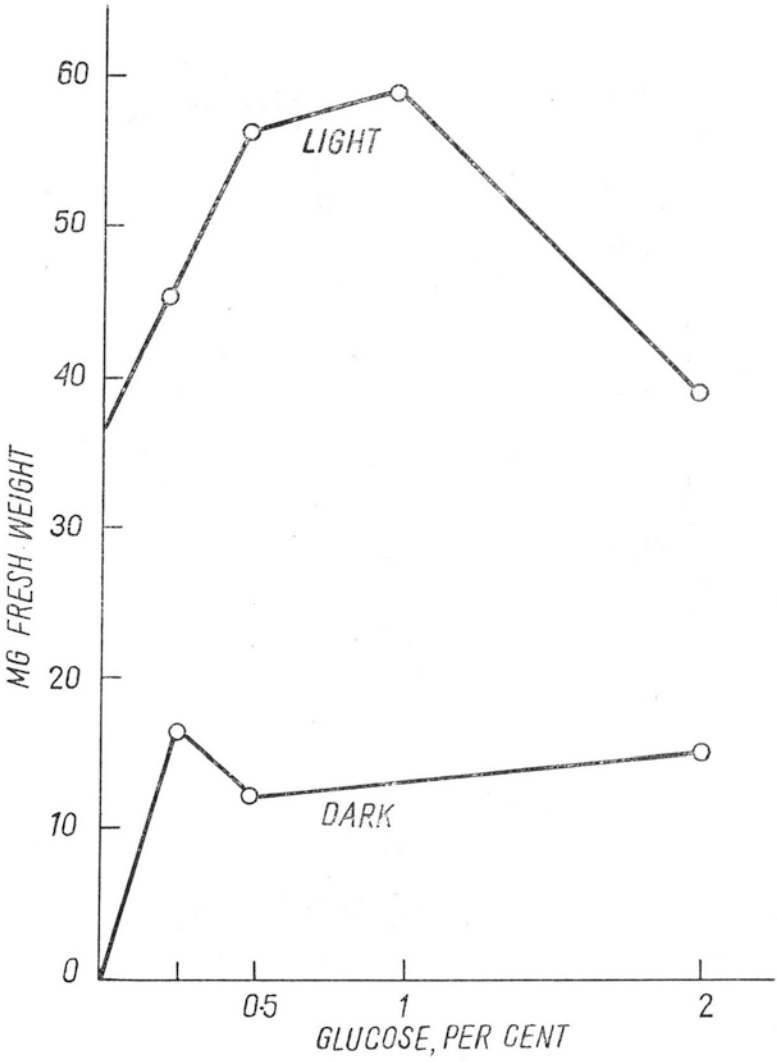
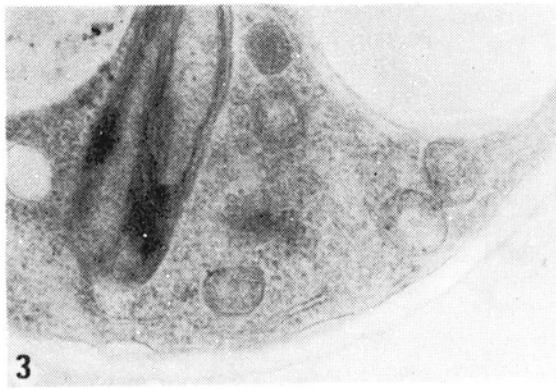
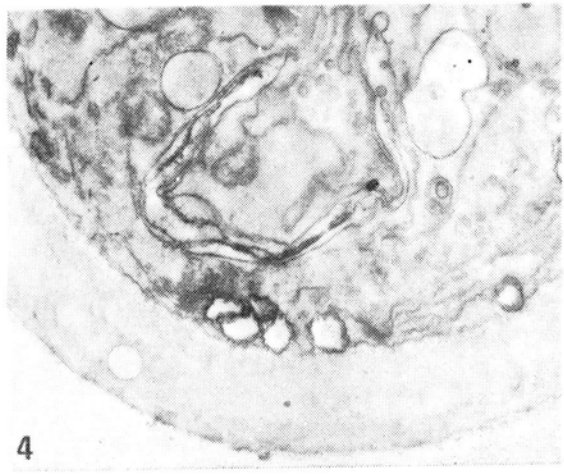
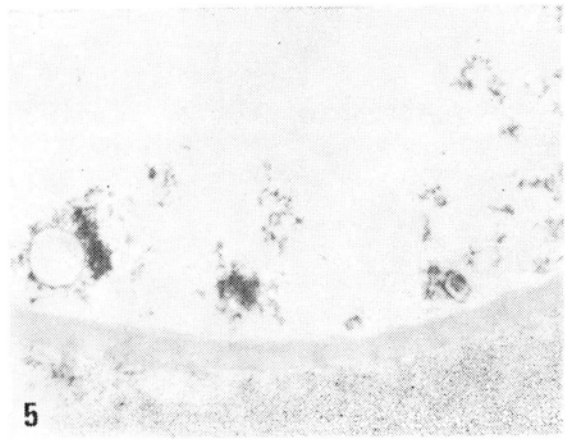


Fig. 2. The effect of glucose on the protonema growth under light (5 weeks of growth) and in the dark (10 weeks of growth)

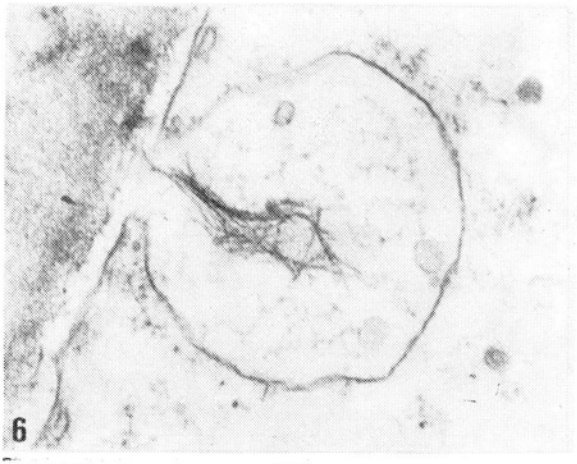
ments. In light cultures, the outer cell walls were about 0.5 μm thick (Fig. 3). In dark cultures of the same age (30 days), the wall thickness was much higher and amounted to 2 μm (Fig. 4). After a prolonged time of culture, in a 50-day-old, dark grown protonema, the thickness of the walls decreased considerably, amounting to 0.6 μm only (Fig. 5). A possible explanation of the observed changes in the cell walls during a prolonged dark culture is that the material accumulated in the thick walls may be used again by cells in unfavourable environmental conditions. The evi-

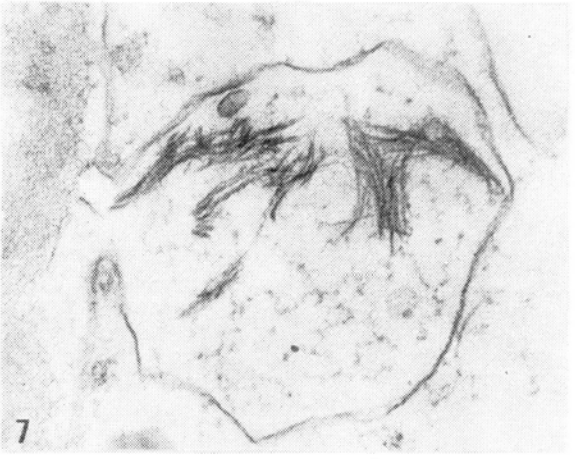






Figs. 3—5. Transverse sections of the protonema. × 7 000. Fig. 3. Light culture (30--day-old), glutaraldehyde/formalin; Fig. 4. Dark culture (30-day-old), $KMnO_4$; Fig. 5. Dark culture (50-day-old), $KMnO_4$





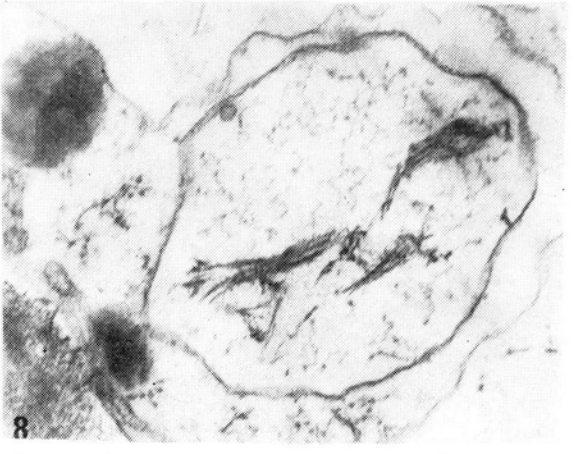


Fig. 6–7. Plasmalemma invaginations containing fibrillar material. A 50-day-old dark culture. Gludaraldehyde/formalin. × 40 000.

Fig. 8. A vesicle with a fibrillar content. A 50-day-old dark culture. Glutaraldehyde/formalin. × 40 000.

dence for such explanation provides the presence of numerous plasmalemma invaginations containing fibrillar material and of vesicles with a similar content in old, degenerating cells (Figs. 6—8). Similar structures, i.e. vesicles of various origin containing fibrillar materal were also observed in *Funaria* by Schulz and Lehmann (1970) and interpreted as structures participating in the process of the cell wall building. In conditions of our experiments, however, the abaundant occurrence of these structures, together with the observed diminution of cell wall material at the plasmalemma invaginations (Fig. 5) indicates their participation rather in cell wall decomposition than in its formation. In this way, similar structures can probably be involved in the formation as well as in the decomposition of cell walls. The results also suggest that in conditions unfavourable for intensive growth, the cells of the protonema in *Funaria hygrometrica* can accumulate the sugar taken up from the medium not only in plastids (Młodzianowski and Woźny 1972), but also in cell walls, from which it can be mobilized at the time of cell starvation.

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Zmiany w strukturze ścian komórkowych podczas rozwoju splątka Funaria hygrometrica na pożywce organicznej na świetle i w ciemności

Streszczenie

Ściany komórkowe splątka *Funaria hygrometrica* hodowanego na pożywce zawierającej glukozę są znacznie grubsze w ciemności niż na świetle. Po dłuższym czasie hodowli w ciemności obserwowano znaczną redukcję grubości ścian i równocześnie występowanie w komórkach pęcherzyków oraz inwaginacji plazmalemmy zawierających włóknisty materiał. Autorzy sądzą, że w warunkach niekorzystnych dla wzrostu cukier pobrany z pożywki może być magazynowany w ścianach komórkowych, skąd ponownie może zostać uruchomiony w warunkach głodowych. Wydaje się także, że zarówno procesy budowy, jak i rozkładu ściany komórkowej posiadają podobny mechanizm i podobne uczestniczą w nich struktury.