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Formation, Growth, and Regeneration of Protoplasts of the Green Alga, *Uronema gigas*

Brief Report

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With 3 Figures

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Summary

Separate protoplasts were obtained by the action of snail gut juice enzymes on the cell walls of the green alga *Uronema gigas*. The cultivation of the protoplasts in mineral media caused only their enormous growth; in the presence of glucose a fibrillar network was formed on the surfaces of the growing protoplasts. Only after the addition of pectin the regeneration of the cell wall and the renewal of their morphogenesis could be observed.

The application of autolysis of the yeasts (NEČAS 1950) and especially the application of the complex of enzymes of the digestive juice of *Helix pomatia* (EDDY and WILLIAMSON 1959) enabled us to prepare a large amount of protoplasts of many lower plants. Protoplasts of fungi and moulds, deprived of their cell walls, have served as model objects, especially for investigations of the regeneration of the new cell wall for several years. The ability to restore new cell walls was demonstrated in protoplasts of most heterotrophic lower plants. The protoplasts of budding yeasts (NEČAS 1961) requiring cultivation in gels, seemed to be of an exceptional importance as far as the manner of regeneration was concerned. The formation and the regenerative ability of protoplasts of higher plants were proved in cells of tomato-fruit tissues (POJNAR, WILLISON, and COCKING 1967).

The protoplasts of algae were among the first objects in which the regeneration of cells, and especially their cell walls, was studied (*cf.* KÜSTER 1939, COCKING 1965). As previous experiments were performed with only small quantities of material we tried to obtain protoplasts of algae devoid of cell walls using snail gut juice, and to compare the regeneration of the cell

wall and the restitution of morphogenesis in them with corresponding processes in both heterotrophic unicellular plants and the tissue cells of higher plants.

In our preliminary experiments with a wide range of different species of algae we succeeded in obtaining the protoplasts in *Uronema gigas* (*Ulotrichaceae*); these were used in our investigation.

The alga was grown on the modified Bourrelli mineral nutrient medium (KNO_3 100 mg, K_2HPO_4 20 mg, $MgSO_4 \cdot 7H_2O$ 15 mg, $Ca(NO_3)_2$ 15 mg, traces of $FeCl_3$, dissolved in 1000 ml redistilled water) at laboratory temperature and under a permanent fluorescent light. Under these conditions the cells divided about every 12 hours.

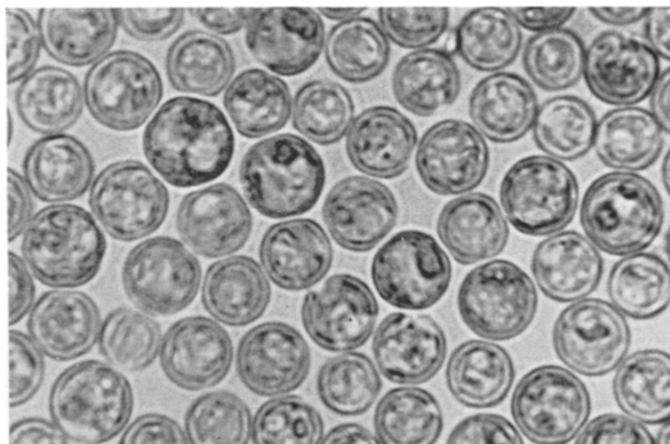

Fig. 1. The fresh protoplasts of green alga (*Uronema gigas*) released by enzymatic digestion of the cell wall. Magnification $\times$1,000

After 36–48 hours of cultivation the cells were harvested and subject to plasmolysis in 11 per cent glucose. The plasmolysed algae were then transferred into a mixture of 0,1 ml of crude liquid snail digestive juice and 1 ml of 11 per cent glucose.

Within 3–5 minutes of incubation the lysis of cell walls took place and protoplasts were released into the medium. After 20 minutes gentle shaking, the 100 per cent release of the protoplasts into the medium was completed. No remnants of the cell walls were visible apparent in the phase contrast microscope (Fig. 1).

In older cultures of algae the cell walls were only partly digested and their remnants swelled considerably. The release of the protoplasts from these partially digested cell walls into the medium could be facilitated by transferring the cells into a less hypertonic medium. After 3–4 minutes of incubation in 11 per cent glucose containing snail digestive juice, during which the cell walls were already partially digested, we transferred the algae into a 9 per

cent glucose with the snail enzyme. The protoplasts increased in volume and were released from the partially digested cell walls into the medium. As expected, the yield of living naked protoplasts was lower in the older algal cultures.

After washing protoplasts of the snail enzyme by a three-fold centrifugation (800 n.p.m.), they were resuspended in the isotonic solution of $MgSO_4$ (0,45 M). Protoplasts became spherical in shape, which resulted in the translocation of chloroplasts and other cell structures. Two methods of electron microscopy were used to observe the surfaces of the protoplasts: the

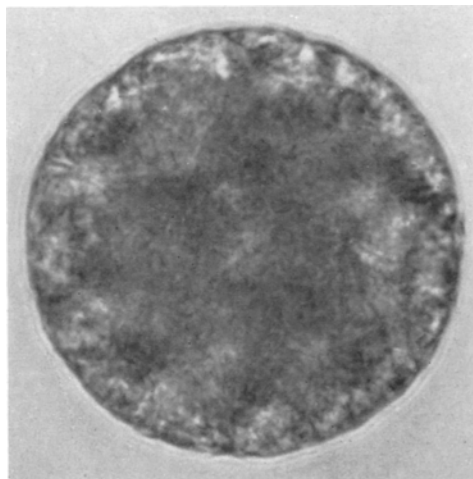

Fig. 2. The gigant protoplast growing isodiametrically after three weeks cultivation in mineral medium. Magnification ×1,000

first of ultra-thin section, the second of shadowed preparations of protoplasts lysed in distilled water. In both cases, the protoplasts have proved not to possess any remnants of the cell wall on their surfaces.

In mineral media the protoplasts grew isodiametrically. If the medium was renewed regularly, the protoplasts grew slowly into enormous sizes without new cell walls on their surface (Fig. 2). It was evidently a real growth, because karyokinesis took place. Originally mononuclear protoplasts contained a number of nuclei after several weeks of cultivation.

In the presence of glucose or another utilizable sugar the protoplasts grew in a polar pattern. On their surfaces a fibrillar network was formed which was observable in the electron microscope. No other cell wall components were formed. Here again the growth was accompanied by karyokinesis, the rate of which, however, was retarded for 2–3 days in comparison with the frequency of the original algal cells. We have not yet succeeded in obtaining a regeneration in gels of various concentrations, *i.e.*, under similar physical conditions which enabled NEČAS (1961) to observe the regeneration of yeast protoplasts.

When pectin (fruit pectin was obtained from Pektina Heřmanův Městec) was

added to the Bourrelli mineral medium, enriched with glucose and mannitol, it led to the regeneration of the protoplasts. The protoplasts were cultivated in a thin layer of liquid medium laid on a thick layer of the same medium with agar in Petri dishes. Within 3 days the basis of the cell wall was formed, clearly visible in the phase contrast microscope. The existence of this wall apparently determined the filamentous pattern of the growth of the protoplasts (Fig. 3). After 5–7 days of cultivation the formation of the cell plates could be observed. The first cells differed from the original algal cells in

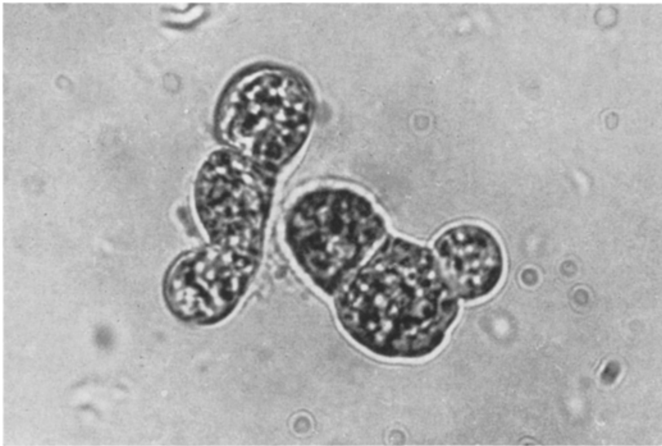

Fig. 3. The filamentous formation of regenerated protoplasts. The newly synthetized cell wall and the formation of septa are visible. Magnification ×1,000

form and sizes. In several cases entirely normal cells developed after 10–14 days of cultivation; these could be transferred into the Bourrelli mineral medium where they grew. Their morphology corresponded to that of the original algal cells.

Other aspects of the formation of the cell wall at the submicroscopic level, as well as the renewal of the morphogenesis, are being studied.

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