ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 49, nr 4: 363—368
1980

Result of cyclophosphamide effect on meristematic cells of anaerobic respiration

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(Received: November 21, 1979)

Abstract

Hypertrophy of rough ER under the influence of cyclophosphamide (oncostatic substance) in meristematic cells of normal and modified glycolysis pathways has been demonstrated. The organization of ergastoplasmic structures has been considered as a process of adaptation to the changed energy conditions caused by cyclophosphamide since analogous changes in protoplastic structures have been found in meristematic cells of oxygen respiration.

INTRODUCTION

Cyclophosphamide is one of oncostatic substances and belongs to a group of alcalizing agents showing characteristic cytostatic and cytotoxic effects, both *in vitro* (Cooper, Goldstein, 1976) and *in vivo* (Brock, Hohorst, 1963, 1967; Cohen, Jao, 1970), mainly by inhibiting DNA synthesis and chromosome aberrations (Stetka, Sheldon, 1976).

Biological activity of cyclophosphamide in plant cells has also been studied. The experiments were carried out on root tips of onion meristematic cells. It has been found that apart from typical mitostatic effect, cyclophosphamide caused the change of membranes in rough ER also observed in the cell energy deficit. (Podbielkowska et al., 1975; Podbielkowska, Kupidłowska, 1976).

As most tumorous cells respirate glycolytically (Bernhard, 1969), the aim of our present experiment has been to verify the results of cyclophosphamide effect upon meristematic cells of anaerobic respiration.

The experiment was performed on root tips of apical meristem of *Allium cepa* and additionally, on the roots of *Haemanthus albiflos* due

to the fact that *Allium*, so far being the main object of experiments, represents very individual metabolic pathway in anaerobic respiration. The synthesis of pyroracemic acid takes place by the enzymatic decomposition of alline contained in the onion cell liquid. The reaction is not inhibited even by high (0.5 M) concentration of glycolysis inhibitor NaF (James, 1953). In *Haemanthus* roots, glycolysis follows routine procedure.

MATERIAL AND METHODS

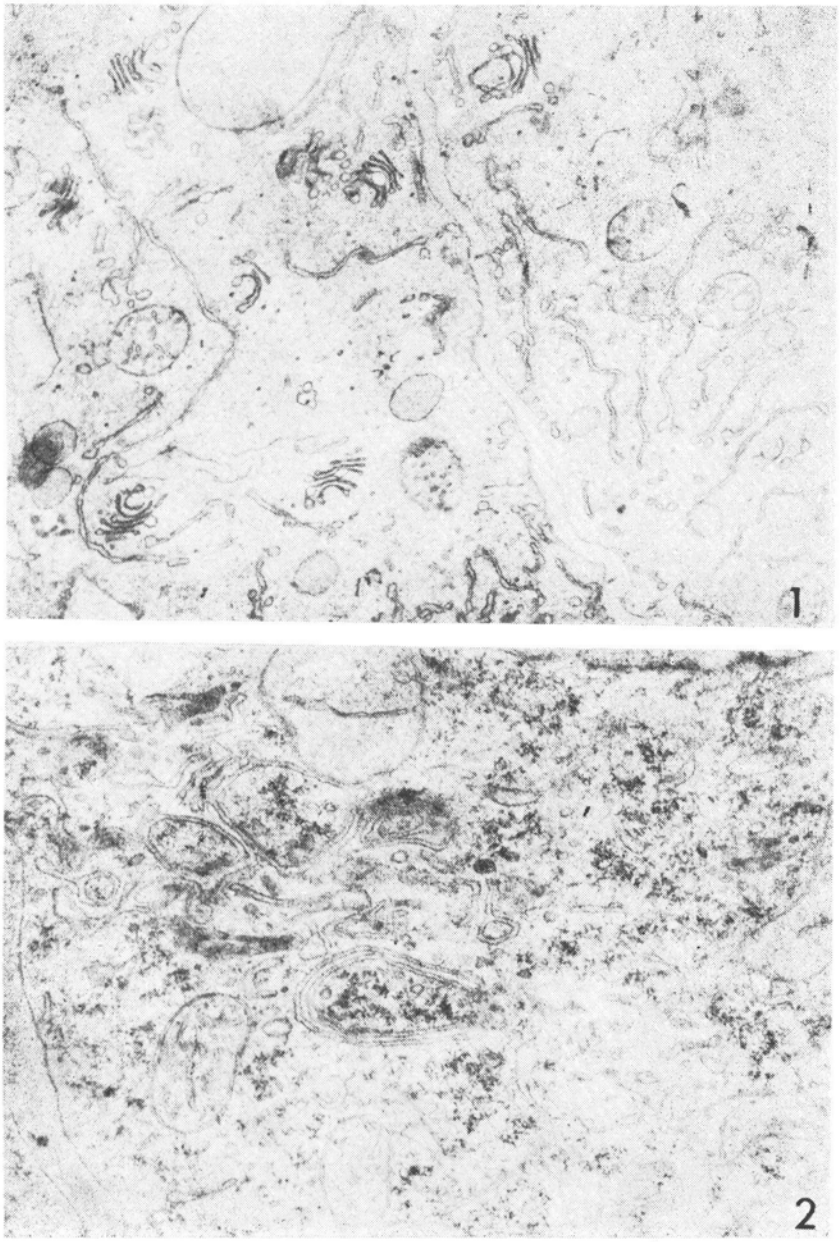
In the experiment, adventitious roots of onion of *Allium cepa* L. were run in water cultures in the 250 ml container with tap water changed every 24 h. Onion bulbs with roots 2-3 cm long were transferred to a 0.4% salution of cyclophosphamide (Cyclophosphamide Germed) for 3 and 12 h. Concentration of cyclophosphamide was determined on the basis of both, data showing the effectiveness of doses (Podlewski, Chwalibogowska-Podlewska, 1978) and our earlier tests (Podbielkowska, Nowaczek, 1979).

The second object of our experiments consisted of cut off roots of *Haemanthus albiflos* growing in the soil and treated by a 0.4% cyclophosphamide for 3 h. The factor that stimulated glycolysis was, in the experiment, anaerobic atmosphere (N_2). Anaerobic conditions were obtained by letting gasous nitrogen through vessels containing incubation solutions of cyclophosphamide for 10 minutes and then through an exiccator in which the experiment was carried out. In the case of *Allium* roots, the time of cyclophosphamide effect was 3 and 12 h and in the case of cut off *Haemanthus* roots it was 3 h. The control material was that of *Allium* and *Haemanthus* roots kept in tap water in N_2 atmosphere for 3 and 12 h.

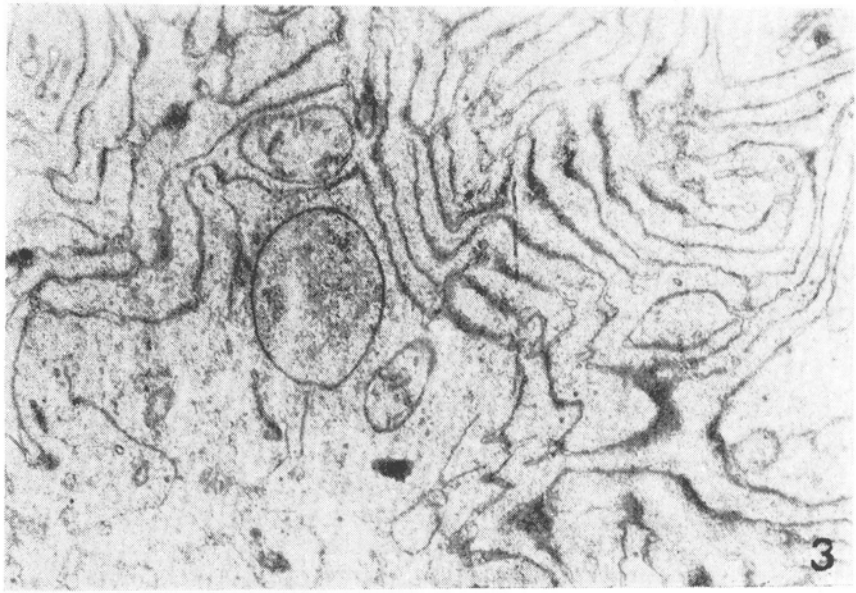
Root tips of the studied objects were fixed for the light microscope in chromium-aceto-formalin (CrAF) proportionally 0.5-1-20. Microtome sections of 5 μm thick were prepared by paraffin method stained with iron haematoxylin, or toluidine blue. Some material was fixed in a 2% $KMnO_4$, or glutaraldehyde after Karnowsky (1965) for observation in the electron microscope. The sections were embedded in epone and contrasted after Reynolds (1963). Observations were carried out by the electron microscope BS-500 Tesla.

RESULTS

On the basis of observations in both, light and electron microscopes, it has been found that in meristematic cells of root tips of *Allium* kept for 3 h in anaerobic conditions (control material), numerous, well-developed polymorphic mitochondria are observed as well as a large num-



Figs 1, 2. *Allium cepa*, anaerobic conditions fixed in 2% $KMnO_4$. Numerous mitochondria and Golgi structures. Fig. 1 — after 3 h in anaerobic conditions; Fig. 2 — after 12 h in anaerobic conditions ER cisternae in parallel and spherical systems. × ca 16000



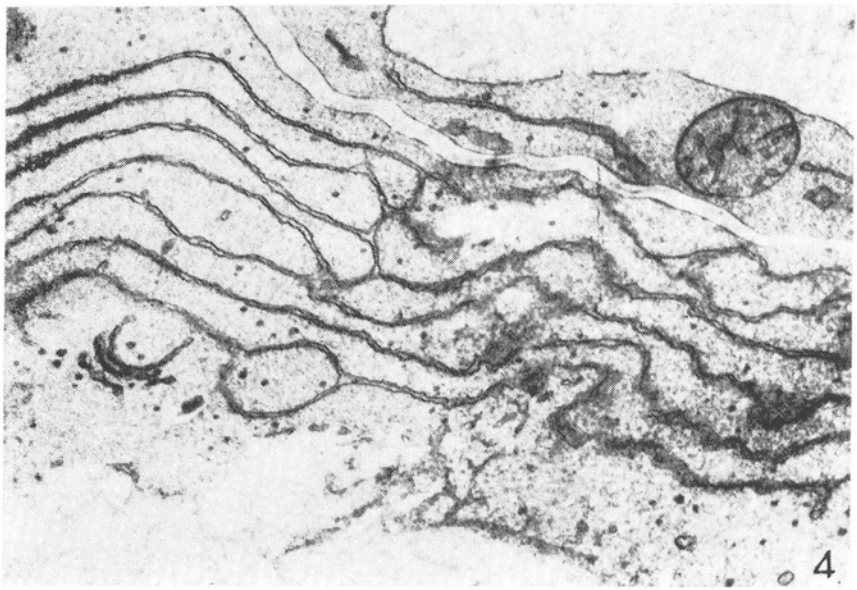
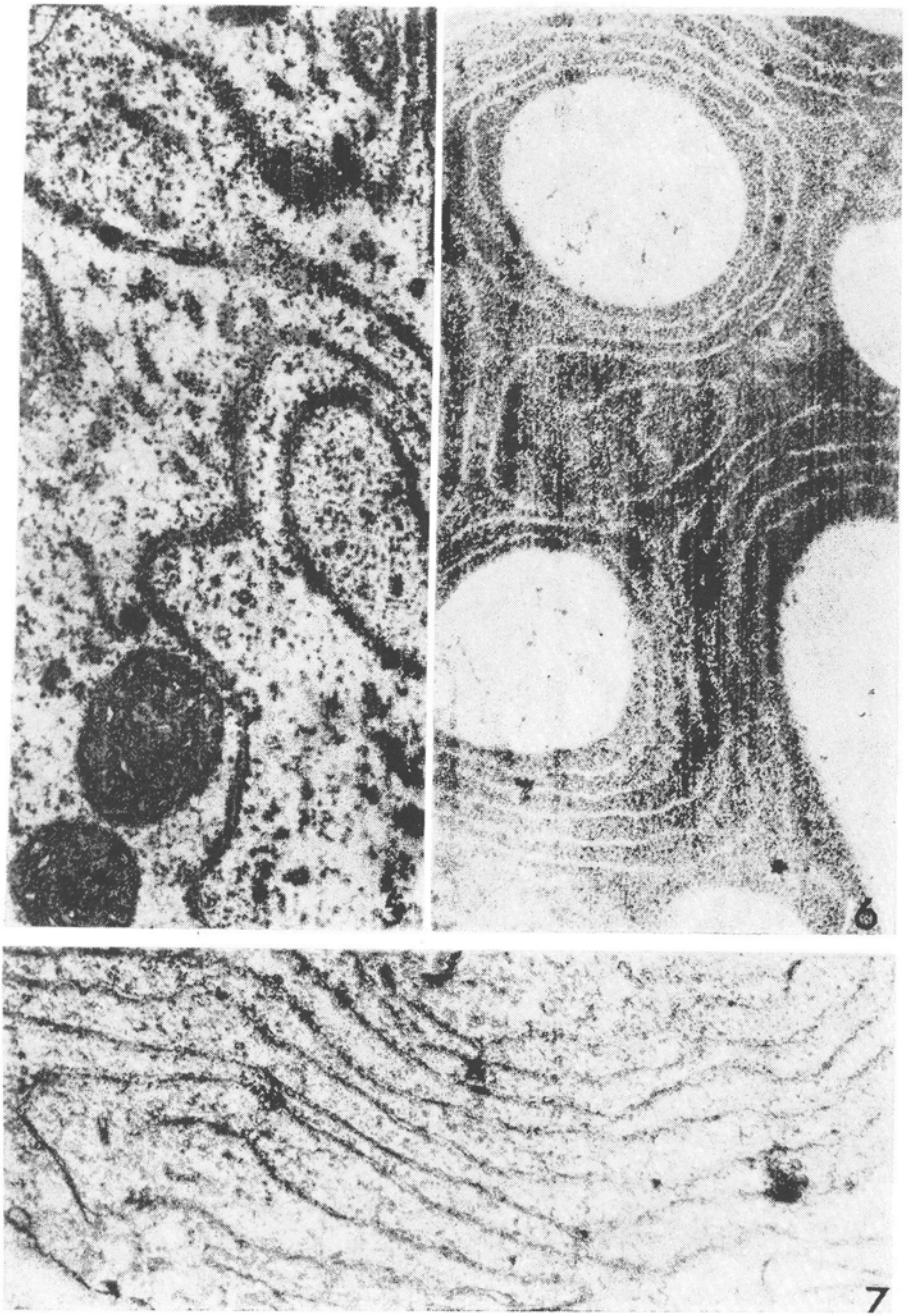


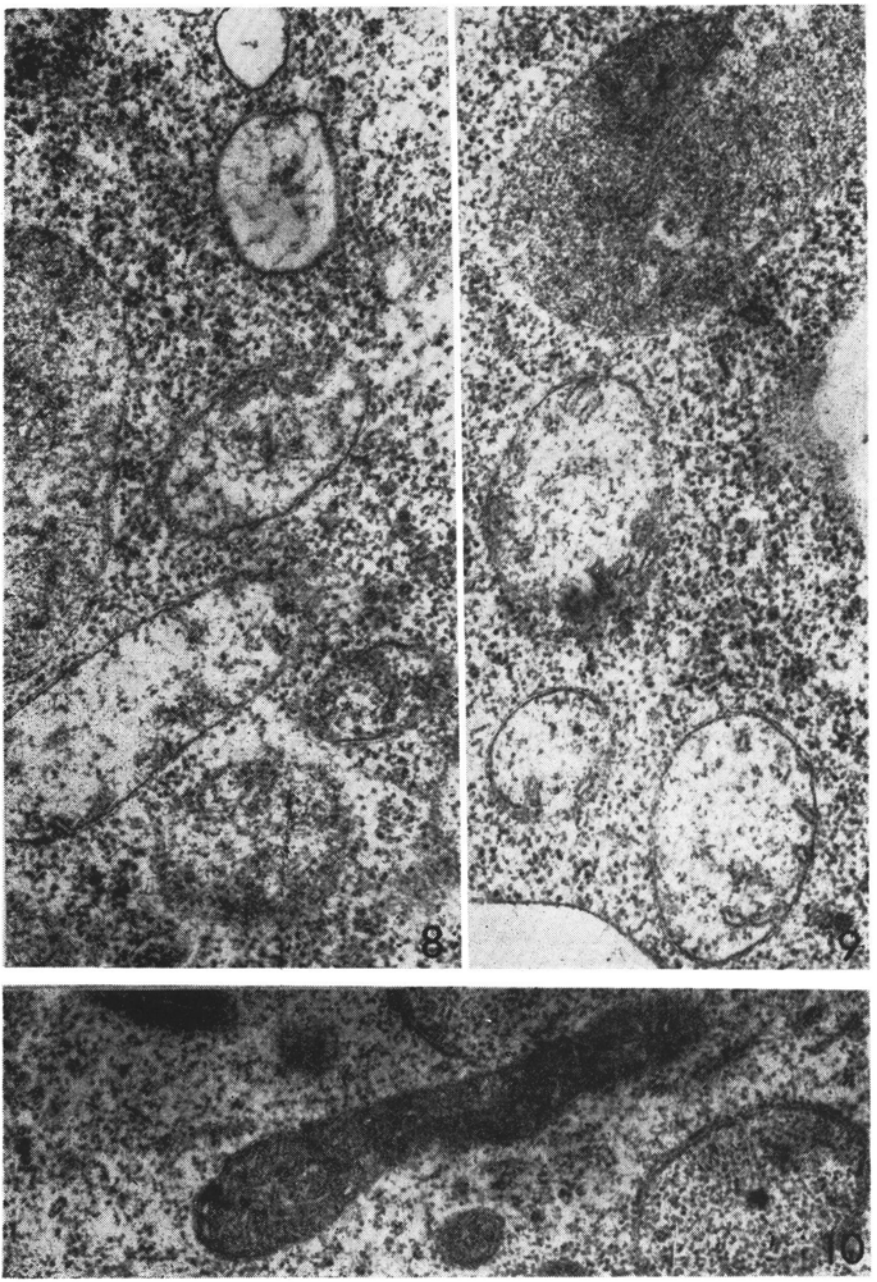
Fig. 3. As in Fig. 1 but treated with 0.4% cyclophosphamide for 3 h. Numerous ER cisternae in parallel system. × ca 20000

Fig. 4. *Haemanthus albiflos*, anaerobic conditions, treated with 0.4% cyclophosphamide for 3 h, fixed with 2% $KMnO_4$. ER cisternae in parallel system. × ca 18000

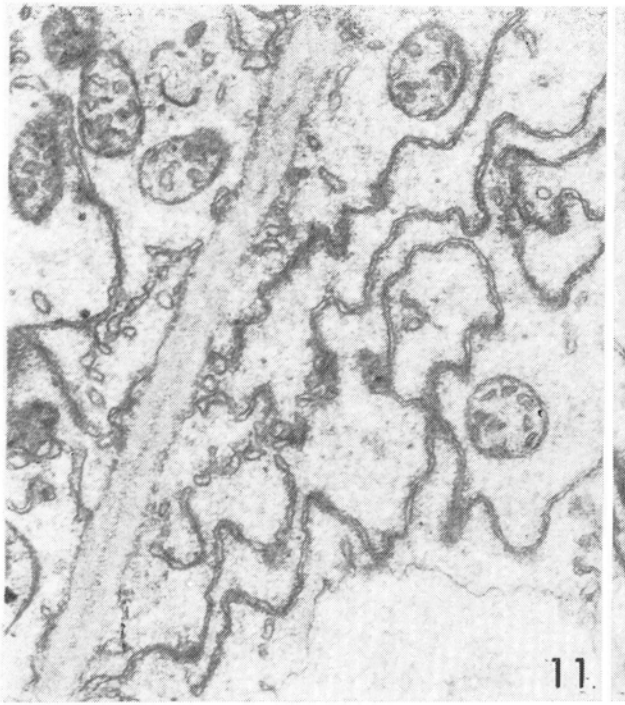


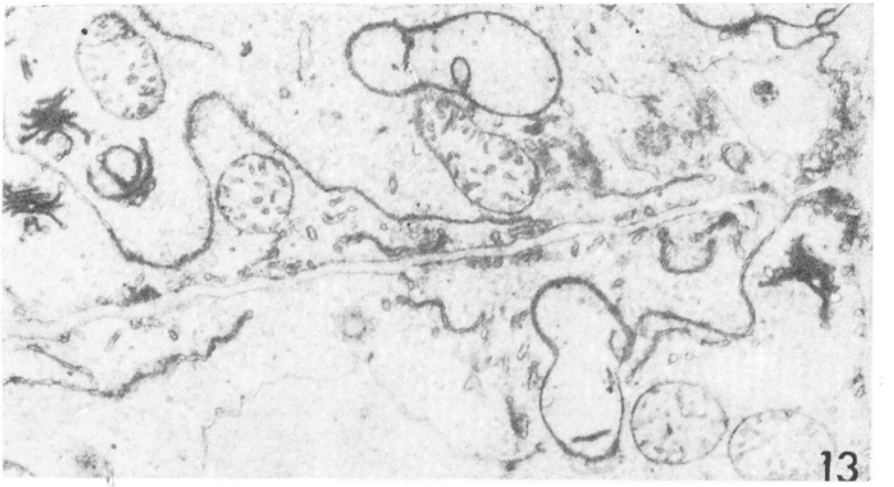
Figs 5, 6. As in Fig. 1 but treated with 0.4% cyclophosphamide for 12 h and fixed with glutaraldehyd. Numerous membranes of rough ER in neighbourhood of vacuoles. Fig. 5 — × ca 40000; Fig. 6 — × ca 26000

Fig. 7. As in Fig. 1 treated with 0.4% of cyclophosphamide for 12 h. Numerous ER cisternae in parallel system. × ca 24000



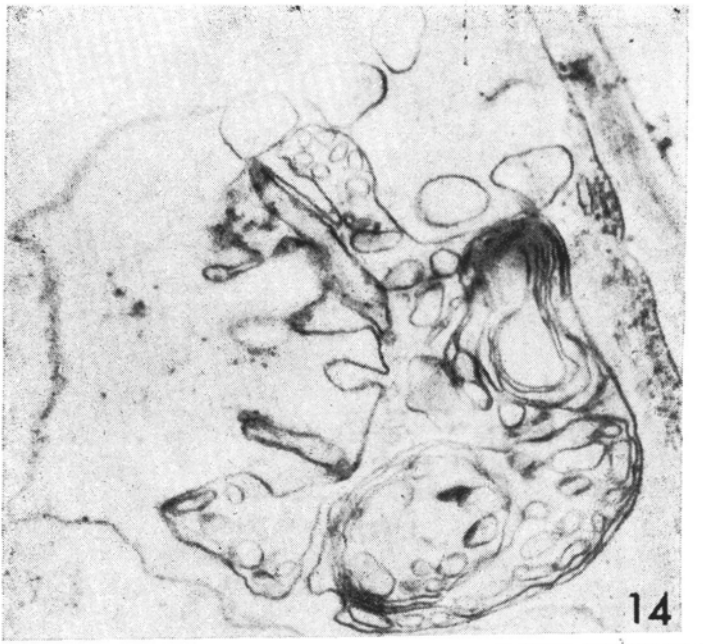
Figs 8, 9, 10. As in Fig. 1 but treated with 0.4% cyclophosphamide for 12 h and fixed with glutaraldehyd. Numerous mitochondria, ribosomes and polyribosomes. × ca 40000

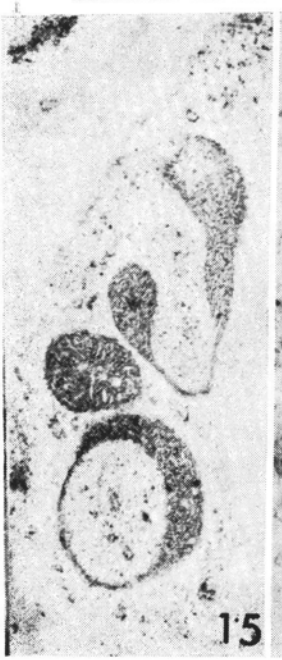


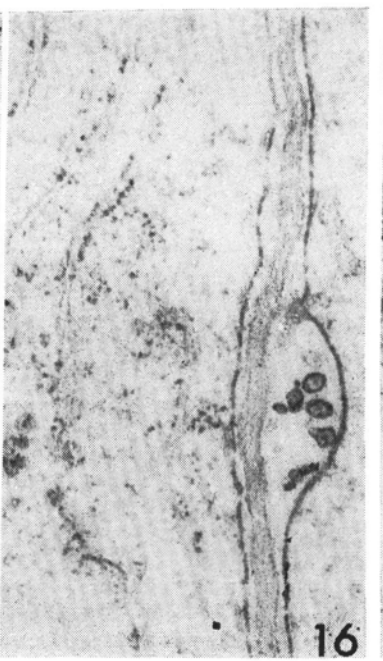


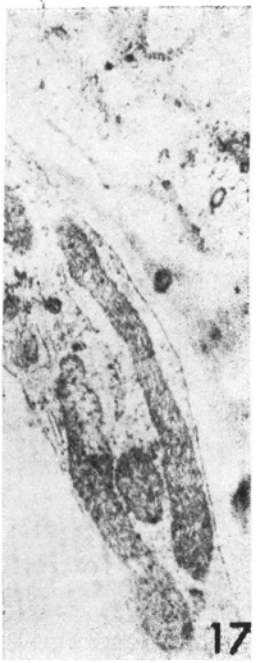
Figs 11, 12, 13. As in Fig. 4 but after 3-h anaerobic conditions. ER membranes in parallel systems, numerous dictiosomes and mitochondria. Fig. 11 and 12 — × ca 20000; Fig. 13 — × ca 18000

Plate VI









Figs 14-17. As in Fig. 4, but treated with 0.4% cyclophosphamide for 3 h and fixed with glutaraldehyd. Between the wall and plasmalemma vesicle-like distentions are visible (Fig. 16) and structures similar to mieline (Fig. 14), numerous polymorphic mitochondria (Figs 15 and 17). Figs 14, 15 and 17 — × ca 18000; Fig. 16 — × ca 24000

ber of dictiosomes with characteristic swollen ER membranes (frequently occurring) (Fig. 1). ER formed typically.

Meristematic cells of *Allium* roots growing in anaerobic conditions (control) for 12 h show much better-developed ER cisternae system (as compared with the control after 3 h in N_2 atmosphere). They form characteristic parallel or spherical configurations (Fig. 2). The number of mitochondria and dictiosomes appeared to be the same as in the control after 3 h in anaerobic conditions.

3 h effect of cyclophosphamide in anaerobic conditions causes a considerable increase in the number of ER membranes. The cisternae formed characteristic configurations of parallel systems (Fig. 3). The number and the structure of mitochondria and dictyosomes were identical to those in the control material.

The effect of cyclophosphamide in N_2 atmosphere resulted in further membranization of cytoplasm. Cisternae of rough ER (Fig. 5) formed parallel (Fig. 7) or spherical configurations (Fig. 6) and sometimes underwent fragmentation (Fig. 5). Cytoplasm full of ribosomes and polyribosomes and polymorphic mitochondria (Figs. 8-10) often vacuolized (Fig. 6).

Ultrastructure of merisematic cells in *Haemanthus* roots is quite different to that of *Allium*. In *Haemanthus* roots, after 3 h in anaerobic conditions (control), numerous polymorphic mitochondria (Fig. 13) with well-developed crista and dictyosomes of which structure proves their intensive secretory function (Fig. 12) were observed. The system of rough ER cisternae, when distinctly large, generally forms parallel configurations (Fig. 11). Near the cell wall, small vesicular structures with dense content were noticed.

Cyclophosphamide, after 3 h in anaerobic atmosphere caused the increase of ER cisternae assuming characteristic configurations. In most cases, the configurations were parallel (Fig. 4) and only very seldom spherical. Mitochondria most frequently polymorphic ones (Fig. 15, 17), with distinctly dense stroma were present in a large quantity. Vesicular forms (Fig. 16) as well as occasionally, folded spherical or fragmentated membranes similar to mieline structure were observed between plasmalemma and the cell wall (Fig. 4).

DISCUSSION

The results obtained in the experiment prove a very specific effect of cyclophosphamide on the structure of protoplast of meristematic cells of anaerobic respiration. Hypertrophy of rough ER as well as the increase in the number of polymorphic mitochondria are assumed to be the most characteristic.

In the experiment, two objects were used due to the differences in the process of glycolysis (James, 1953). It has been found that the above mentioned physiological differences are of considerable importance in structure formation of protoplast in the studied tissues. In meristematic cells of *Haemanthus*, an increased number of ER membranes organized in parallel systems has been noticed as a result of 3-h effect of anaerobic atmosphere applied in the experiment for stimulating glycolysis. In meristematic cells of *Allium*, no similar structures have been observed after the same period of time. However, the structures have been noticed after 12 h in anaerobic atmosphere, when compared with the described control. The effect of cyclophosphamide may be presented as follows. In both cases, the analysed oncostatic caused hypertrophy of rough ER which initially, after a short period of cyclophosphamide influence, formed parallel systems, most frequently grouped near very numerous polymorphic mitochondria, or very close to the cell wall.

It has been found that the longer the period of cyclophosphamide influence the more distinct ER hypertrophy. The reorganization of cisternae from parallel to spherical systems and their occasional fragmentation leading to the formation of small vascuoles system were observed.

On the basis of a large number of experiments (Whaley et al., 1964; David, 1970) it has been demonstrated that the increase of ER, both in plant and animal cells derives presumably from a more intensive production of proteins to meet the cell requirements. A marked increase in metabolism of a cell is accompanied by cisternae development of perimitochondrial and perinucleous ER but a decrease of metabolism produces diffusive ER. ER hypertrophy is a known result of the influence of toxins. These changes are not only quantitative, but also qualitative and include complex differentiation in membranes causing formation of spherical systems. Membranes become optically less dense due to local dissolution of ribosomes. The formation of mieline structures appears to be the last stage of ER degeneration. Such modification of the changed ER may be stimulated by such factors as respiratory inhibitors (David, 1970; Podbielkowska, Borys, 1975; Podbielkowska, Kupidłowska 1976), antibiotics (David, 1970), and carcinogenic factors (Weistein et al., 1975).

Earlier experiments demonstrated (Podbielkowska, Kupidłowska, 1976) that there exists a correlation between stimulation of glycolysis and protoplast membranization. Similar modification of membranes in a changed ER also occurs during disturbances in electron transport of the respiratory chain (Podbielkowska, Borys, 1975; Podbielkowska et al., 1975). Our present experiment indicated that in the cells of anaerobic respiration is a certain group of well developed rough ER still can undergo hypertrophy, induced by cyclophosphamide.

The comparison of cyclophosphamide effect on meristematic cells of oxygen and anaerobic respiration (Podbielkowska, Nowaczek, 1979) during normal and modified glycolytic pathways shows that the direction of changes caused by the influence of cyclophosphamide is always the same. Similarity of the above mentioned changes to those occurring in the cells with energy deficit (Podbielkowska, Borys, 1975; Podbielkowska, Kupidłowska, 1976) makes it possible to assume that hypertrophy of rough ER takes place in order to increase the surface active in bioenergetic process and cyclophosphamide seems to cause energy deficit. Changed rough ER, often vacuolizing, maybe responsible for the synthesis of enzymatic proteins participating in respiration processes. In order to explain more precisely the mechanism of cyclophosphamide activity, more experiments will be performed aiming to examine its influence upon physiological processes of the cell, such as respiration, permeability of membranes and content of enzymes involved in energy processes. Also the activity of oncostatic substances of different metabolism and mechanism of action will be compared.

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Wpływ cyklofosfamidu na komórki merystematyczne oddychające beztlenowo

Streszczenie

Wykazano charakterystyczny wpływ cyklofosfamidu (preparat onkostatyczny) na komórki merystematyczne oddychające beztlenowo z normalnym *(Haemanthus)* i zmodyfikowanym *(Allium)* szlakiem glikolitycznym. Wyrażał się on membranizacją protoplastu na skutek rozwoju cystern szorstkiego ER tworzącego układy paralelne, po krótkim, i sferyczne po długim czasie działania onkostatyku. Obecność licznych polimorficznych mitochondriów oraz licznych rybosomów i polirybosomów w cytoplazmie podstawowej, zwłaszcza po krótkim czasie działania cytostatyku, wskazywała na intensywniejszy metabolizm badanej tkanki. Przypuszcza się, że dłuższy czas działania cyklofosfamidu modyfikuje procesy związane z dostarczaniem energii komórce w trakcie oddychania beztlenowego, a także tlenowego (Podbielkowska, Nowaczek, 1979). Wniosek ten nasunął się ze względu na analogię przemian w strukturze protoplastu komórek, które znajdują się w deficycie energetycznym (Podbielkowska, Kupidłowska, 1976). Rozwój membran ER uznano za wyraz adaptacji komórki do zmienionych warunków. Przypuszcza się, że szorstkie ER, często ulegające wakuolizacji, produkować może specyficzne białka biorące udział w procesach oddechowych.