

## Effect of methotrexate on the ultrastructure of *Allium cepa* L. and *Haemanthus albiflos* Jacq. roots cultured under anaerobic conditions

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### Abstract

Changes were found in the cell structure of the root meristems of *Haemanthus albiflos* and *Allium cepa* treated with methotrexate, an oncostatic preparation of the antimetabolite group. Root culture was conducted under anaerobic conditions to induce in this way glycolysis typical for neoplastic cells. In *Haemanthus*, where the glycolytic process runs normally, hypertrophy of the rough ER membranes was noted correlated with the presence of numerous mitochondria and dictyosomes with changed structure. In *Allium* cells, where the glycolytic process runs with the participation of alliin, methotrexate did not evoke development of ER membranes. The structure of mitochondria and dictyosomes was similar as that in the root meristem of *Haemanthus*. In both studied objects thickening of the cell wall was noted.

### INTRODUCTION

It results from the literature that methotrexate inhibits DNA synthesis both *in vivo* and *in vitro*, leading in turn to a cytostatic effect (Greenberg and Waxman 1976, Skeel et al. 1976, Skoog et al. 1976). In cytological studies performed on cancerous endometrium it was demonstrated that methotrexate reduces the pathological features of the cells manifested in vacuolisation, polymorphism and hypertrophy of nuclei as well as multinucleation (Milan 1976). It was also established that methotrexate does not act via hormonal metabolism, but directly on the cell (Dembo and Sirotnak 1976).

The performed experiments concerned the action of methotrexate on the cell structure in roots cultured under anaerobic conditions, thus brea-

thing like most tumour cells (Bernhard 1969). The experiments were performed on two species representing a typical (*Haemanthus*) and modified (*Allium*) glycolytic process. Synthesis of pyruvic acid in *Allium* occurs, namely, via enzymatic (allinase) breakdown of alliin, a component of cell sap (James 1953).

#### MATERIAL AND METHODS

Adventitious roots of *Allium cepa* L. were cultured in 250-ml vessels in tap water changed every 24 h. The bulbs with roots 2-3 cm long were transferred to  $10^{-5}$  M methotrexate (Methotrexate, Lederle, USA) for 3 and 12 h. The methotrexate concentration was applied according to literature data (Skeel et al. 1976, Skoog et al. 1976, Pinedo et al. 1976). The cut off *Haemanthus albiflos* roots were subjected to the action of  $10^{-5}$  M methotrexate for 3 h.

Anaerobic conditions stimulating glycolysis were obtained by passing gaseous nitrogen for 10 min through a vessel containing the incubation solution of methotrexate and through a desiccator. The control roots were kept in tap water under anaerobic conditions for 3 and 12 h.

The growth apices were fixed in CrAF (chromium-aceto-formalin = 0.5 : 1 : 20). The microtome sections 5  $\mu$ m thick were prepared by the paraffin method, stained with iron haematoxylin or toluidin blue. For the electron microscope 1-mm fragments of root growth apices deprived of the cap were fixed in 2 per cent  $\text{KMnO}_4$  or in glutaraldehyde after Karnovsky (1965). The roots were embedded in Epon and the sections were contrasted by Reynolds' method (1963). Observations were made in a BS-500 Tesla electron microscope.

#### RESULTS

The periblem over the initial layer was analysed in the light and the electron microscope. It was found that in control cells from *Allium* roots cultured for 3 and 12 h under anaerobic conditions numerous mitochondria and dictyosomes (Fig. 1) are present. The cytoplasm contains a large quantity of ribosomes (Fig. 20). ER is normally developed. After 3 h of action of methotrexate under anaerobic conditions numerous, sometimes swollen, polymorphic mitochondria with very well developed cristae and electron dense matrix (Figs. 6, 9, 19) were still observed as well as dictyosomes with a characteristic structure (Figs. 2, 3).

ER cisternae frequently give rise to vacuoles (Fig. 2) or undergo fragmentation to small vesicles (Figs. 4, 5) situated in close vicinity of the thickening cell wall (Figs. 4, 5). Close to the latter, frequently inclusions with dense contents were noted, derived probably from ER (Figs. 5, 18).

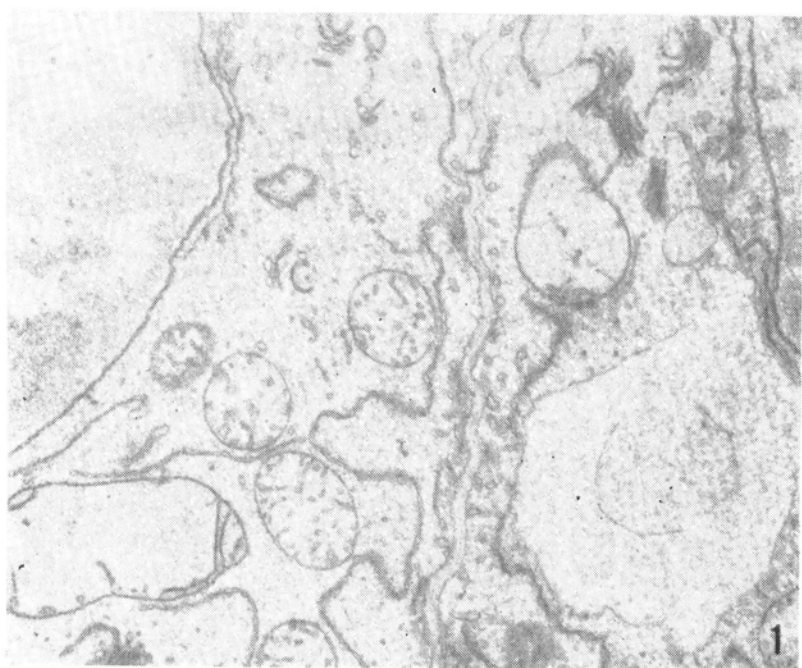
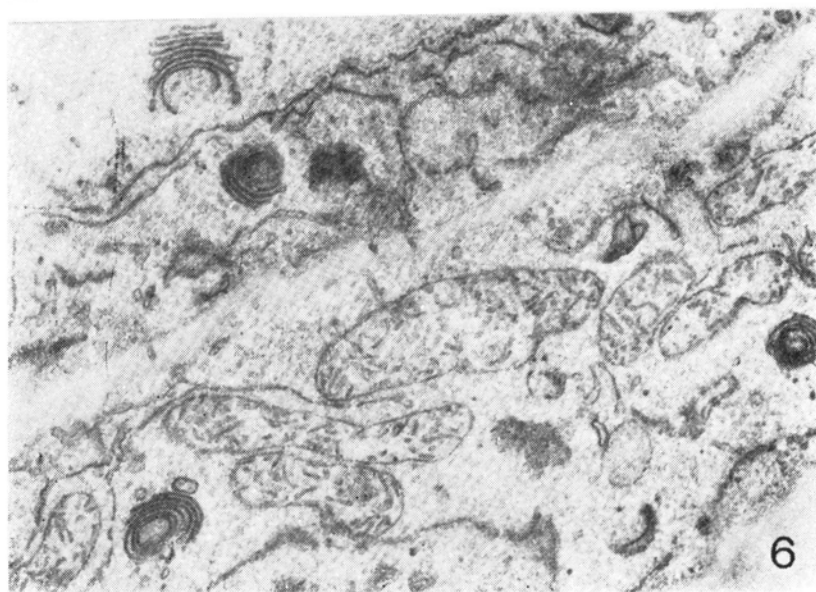
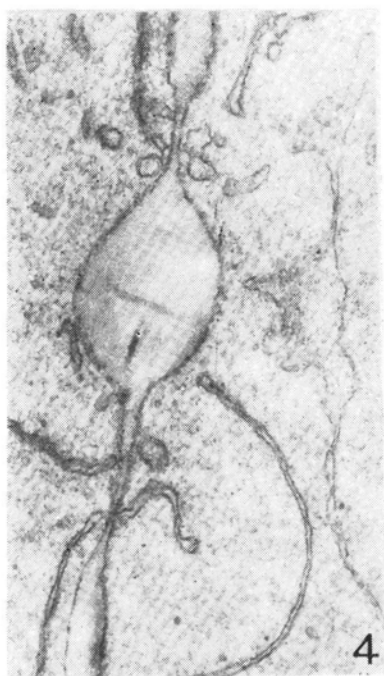


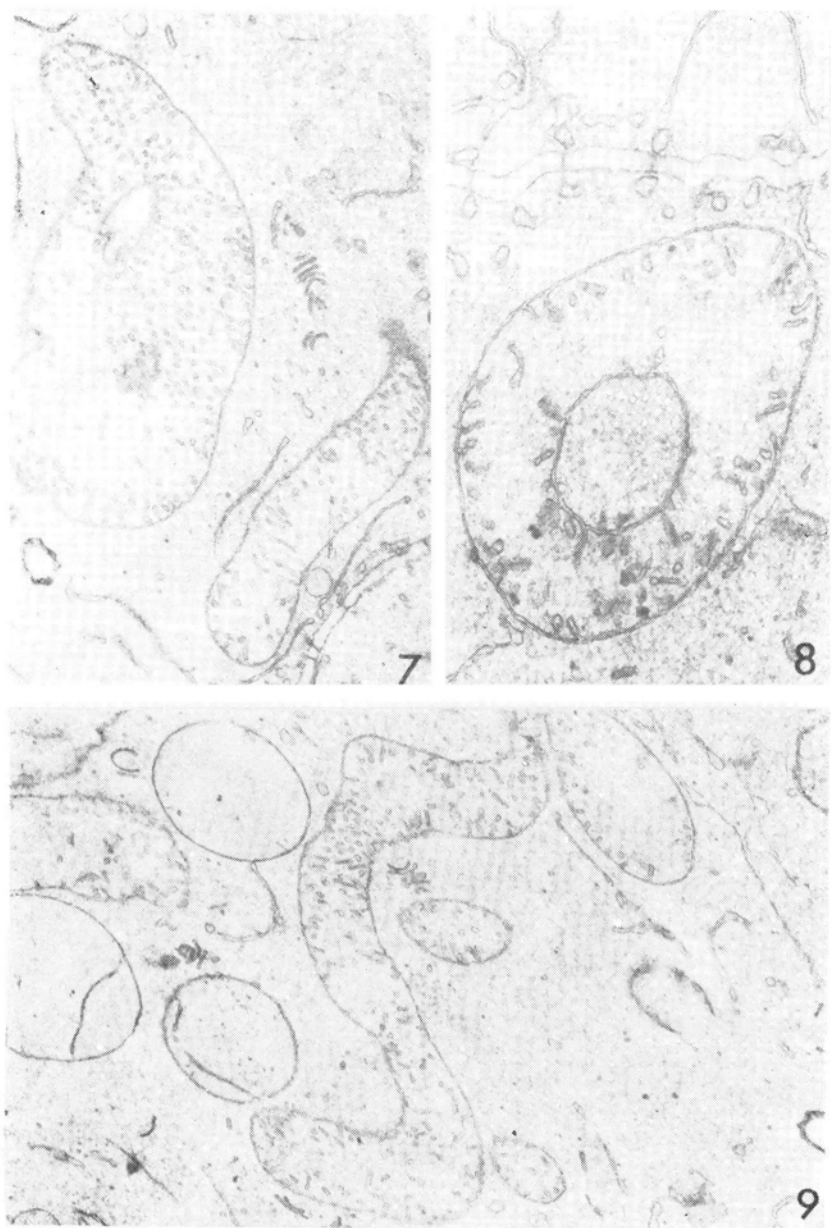
Fig. 1. Meristematic cells of root growth apices from *Allium cepa*. Anaerobic conditions for 3 h (control); numerous mitochondria and Golgi structures, 2%  $\text{KMnO}_4$ ,  $\times 20\,000$ .

Figs. 2, 3. Meristematic cells of root growth apices from *Allium cepa* treated under anaerobic conditions with methotrexate for 3 h. Dictyosomes and vacuoles arising from the ER are visible, 2%  $\text{KMnO}_4$ ,  $\times 20\,000$ .

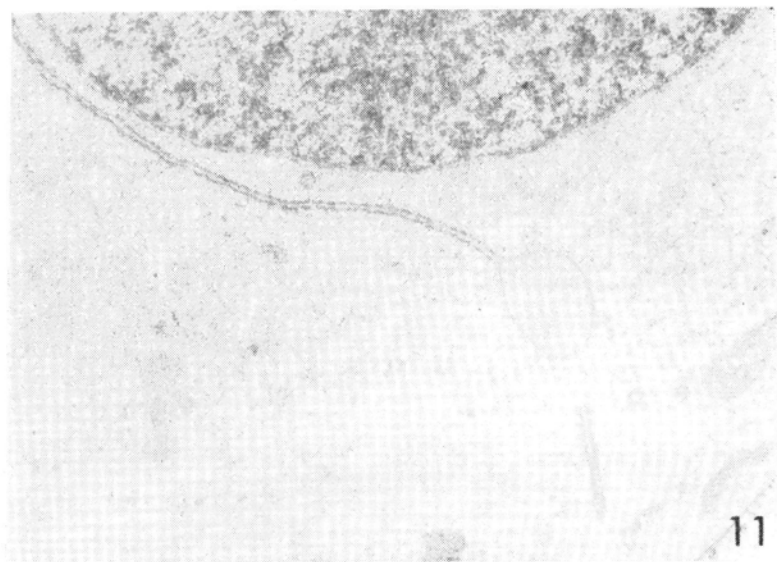
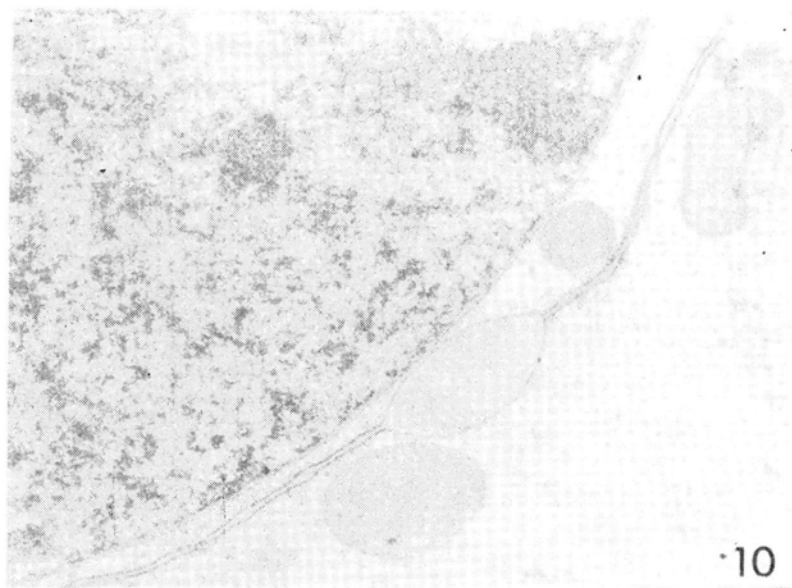


Figs. 4, 5. Meristematic cells of root growth apices from *Allium cepa* treated under anaerobic conditions with methotrexate for 3 h. Vesicle-like fragments of ER cisternae visible close to the characteristically thickened cell wall, 2%  $\text{KMnO}_4$ ,  $\times 20\,000$

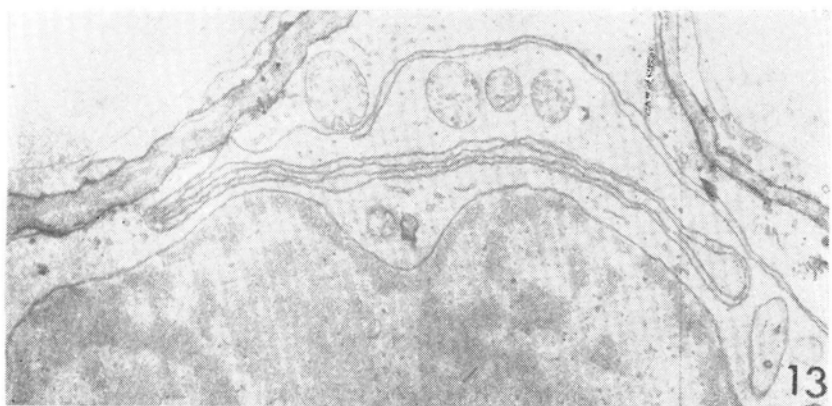
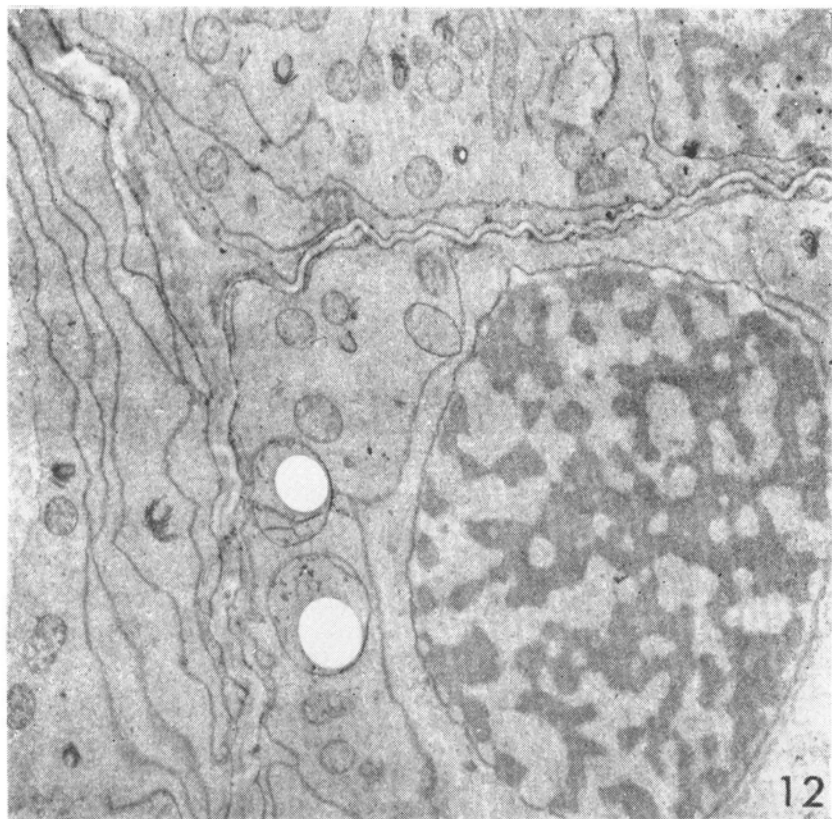
Fig. 6. Meristematic cells of root growth apices from *Allium cepa* under anaerobic conditions treated with methotrexate for 3 h. Polymorphic mitochondria and numerous Golgi structures, 2%  $\text{KMnO}_4$ ,  $\times 20\,000$



Figs. 7, 8, 9. Meristematic cells of root growth apices from *Allium cepa* treated with methotrexate under anaerobic conditions for 3 h (Fig. 9) and 12 h (Figs. 7, 8). Polymorphic mitochondria with changed structure, 2%  $\text{KMnO}_4$ ,  $\times 20\,000$

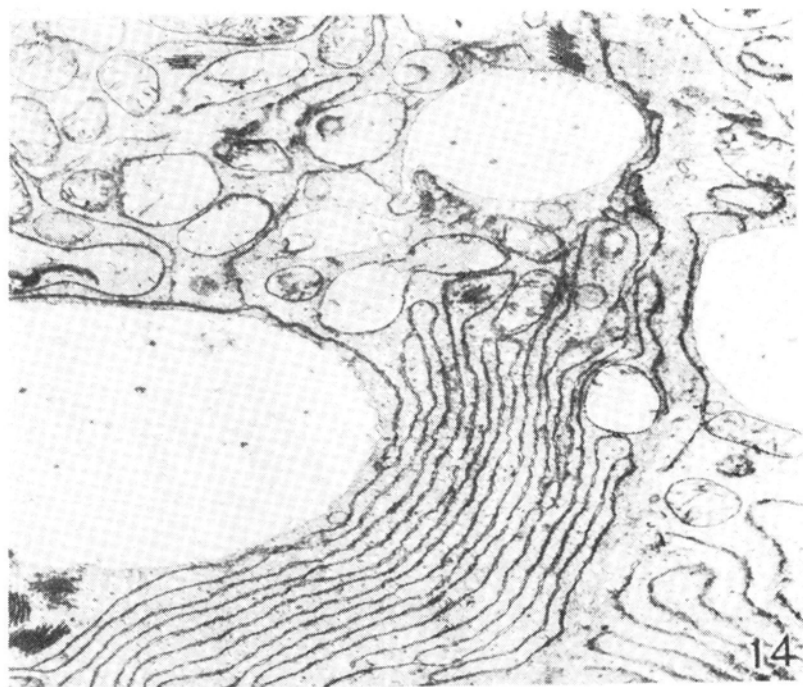


Figs. 10, 11. Meristematic cells of root growth apices from *Allium cepa* treated with methotrexate under anaerobic conditions for 12 h. Fragmentation of rough ER cisternae, GA according to Karnovsky,  $\times 20\,000$



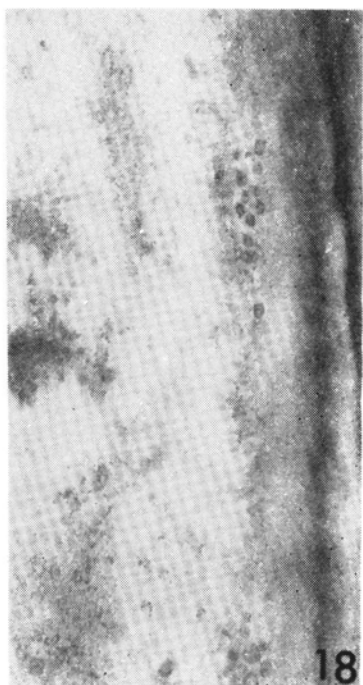
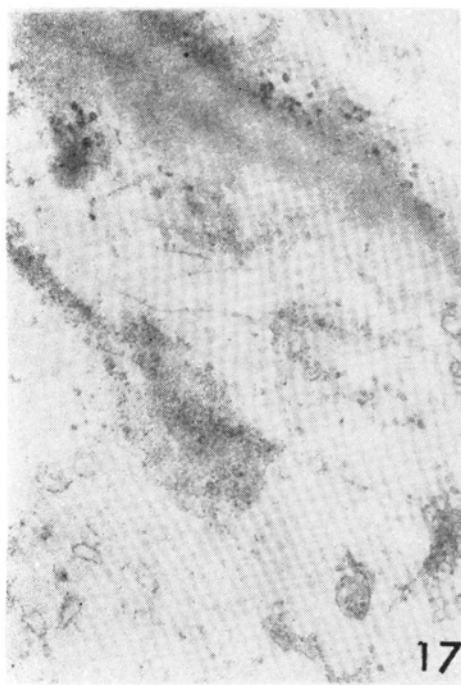
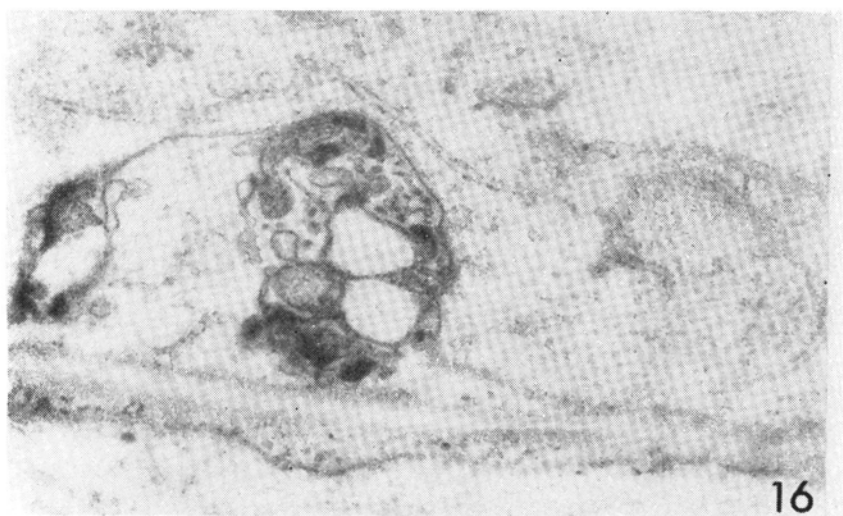
Figs. 12, 13. Meristematic cells of root growth apices from *Haemanthus albiflos*, under anaerobic conditions for 3 h (control). Developed ER membrane system, numerous mitochondria, 2%  $\text{KMnO}_4$ ,  $\times 18\,000$





Figs. 14, 15. Meristematic cells of root growth apices from *Haemanthus albiflos* treated with methotrexate for 3 h under anaerobic conditions. Numerous ER membranes in the neighbourhood of vacuoles, 2%  $\text{KMnO}_4$ ,  $\times 18\,000$





Figs. 16, 17. Meristematic cells of root growth apices from *Haemanthus albiflos* treated with methotrexate under anaerobic conditions for 3 h. Plasmalemma detached from cell wall and accumulation in aplasmatic regions of modified ER cisternae and vesicles with dense contents, GA after Karnovsky,  $\times 20\ 000$

Fig. 18. Meristematic cells of root growth apices from *Allium cepa* treated with methotrexate under anaerobic conditions for 3 h; characteristic accumulation of vesicles close to cell wall, GA after Karnovsky,  $\times 20\ 000$

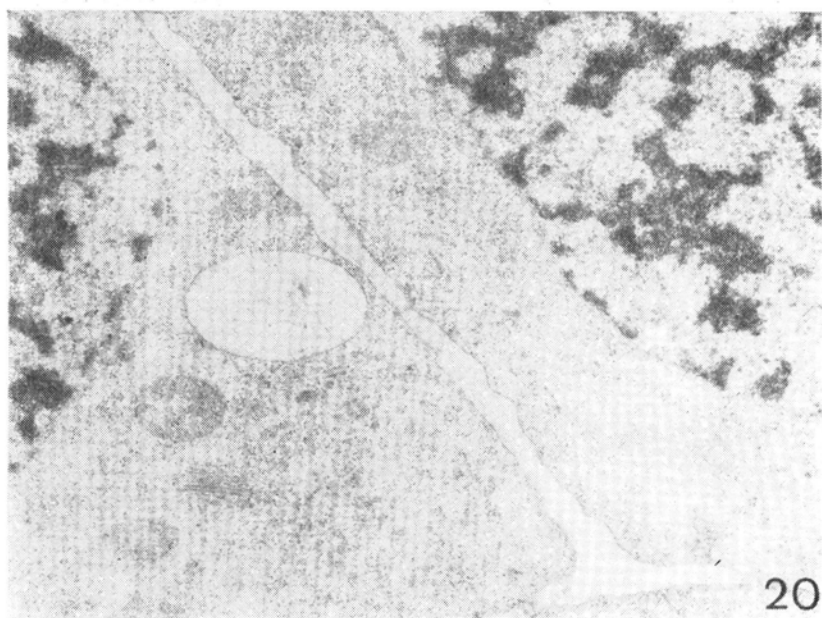
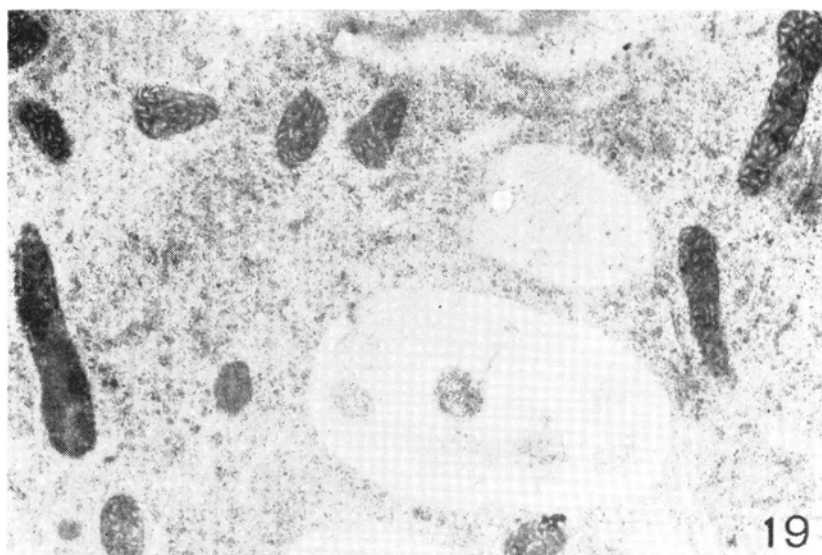


Fig. 19. Meristematic cells of root growth apices from *Allium cepa* treated with methotrexate under anaerobic conditions for 3 h, GA after Karnovsky,  $\times 13\,000$

Fig. 20. Meristematic cells of root growth apices from *Allium cepa* under anaerobic conditions (control). GA after Karnovsky,  $\times 13\,000$

Twelve-hour exposure to the action of methotrexate under anaerobic conditions caused distinct changes in the structure of mitochondria, appearing in swelling and fragmentation of cristae (Figs. 7, 8). Cisternae of rough ER, usually perinuclear, underwent vacuolisation (Figs. 10, 11).

Control meristematic cells from the root growth apexes of *Haemanthus* differ somewhat from the control *Allium* cells; after 3 h under anaerobic conditions numerous mitochondria and dictyosomes and a well developed system of ER cisternae were seen in them (Figs. 12, 13). The action of methotrexate for 3 h under anaerobic conditions caused a marked hypertrophy of rough ER membranes which usually form parallel systems (Figs. 14, 15). The cytoplasm was vacuolised (Fig. 14). Polymorphic mitochondria had well developed cristae. The cell wall was thickened in some fragments. In its vicinity frequently vesicular bodies could be seen with dense contents (Figs. 16, 17) or modified ER membranes resembling lomasomes (Fig. 16).

#### DISCUSSION

The changes in meristematic cells under the influence of methotrexate are largely dependent on the respiratory metabolism. On account of the specific course of pyruvic acid synthesis as described in *Allium* (James 1953), the action of the cytostatic results in the presence of numerous polymorphic mitochondria which after a longer action of methotrexate begin to swell. The role of mitochondria in processes of energy supply is doubtless. They usually are grouped in the intact parts of the cell where more intensive ATP uptake occurs. Active mitochondrial respiration is correlated with a high electron density of the matrix and a change in the configuration of the inner mitochondrial membrane. The observed swelling of mitochondria under a prolonged action of methotrexate accompanies states of energetic deficit.

In the meristematic tissue of *Haemanthus* roots, where the glycolytic process has a typical course, the action of methotrexate was manifested in the development of rough ER cisternae, groupings of which in the form of parallel systems was usually observed in the neighbourhood of the mitochondria, the cell nucleus or cell wall.

Cytoplasmic membranes in the cell not only play the role of semipermeable partitions (septa), but also of structures active in catabolism, integrating regions with different metabolic functions. A general feature of plasmatic membranes is the presence of a respiratory chain of high degree specialisation. Of specific character is particularly the respiratory chain localised in the ER. The oxidation substrates in this chain are probably  $\text{NADPH} + \text{H}$  arising in the cytoplasm during glycolysis. The energy of this process is utilised mainly for ion transport through

the ER membranes. The electron carriers within the ER take part in oxidative detoxication reactions (Säkevütz 1965, Omura et al. 1965).

The development of ER cisternae can be observed both in states of high and of depressed metabolic activity (Whaley et al. 1964, David 1970, Podbielkowska et al. 1975). When anabolic processes prevail, however, there occurs typical vacuolisation which has also been observed in our experiments with both plants. It has also been postulated that stimulation of glycolysis may be one of the many factors positively correlated with the hypertrophy of the ER (Podbielkowska and Kupidłowska 1976). The membranisation of protoplast observed in the present experiments in *Haemanthus* meristematic cells under the action of methotrexate may, therefore, be associated with the increased participation of glycolysis or of the microsomal respiratory chain in processes supplying energy to the cell. The formed ATP may be utilised for regulation of membrane permeability or it may play a role in detoxication of the cell.

If we compare the present results with those of the earlier experiment concerning the action of cyclophosphamide (Podbielkowska and Wałęza 1980) under analogous conditions, it would seem that methotrexate acts less specifically on cell anaerobic respiration, particularly when the glycolytic chain is modified as in onion cells.

The next step of the experiments will concern the influence of methotrexate on the respiration level, membrane permeability and ultrastructural changes in meristematic cells respiring aerobically.

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*Effekt działania metotreksatu na ultrastrukturę korzeni Allium cepa L. i Haemanthus albiflos Jacq. hodowanych w warunkach beztlenowych*

Streszczenie

W merystemach korzeni *Haemanthus albiflos* i *Allium cepa* określono zmiany w strukturze komórek poddanych działaniu metotreksatu — preparatu onkostatycznego z grupy antymetabolitów. Hodowlę korzeni prowadzono w warunkach beztlenowych, indukując w ten sposób glikolizę typową dla komórek nowotworowych. U *Haemanthus*, gdzie szlak glikolityczny przebiega normalnie, zauważono hipertrofię błon szorstkiego ER, skorelowaną z obecnością licznych mitochondriów i diktiosomów o zmienionej strukturze. W komórkach *Allium*, gdzie szlak glikolityczny przebiega z udziałem alliiny, metotreksat nie powodował rozwoju błon ER. Struktura mitochondriów i diktiosomów była podobna, jak w merystemie korzeni *Haemanthus*. W merystemach korzeni obu gatunków roślin obserwowano grubienie ściany komórkowej.