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Ultrastructure of cells after reversible dark-induced blocking of mitotic divisions in antheridial filaments of *Chara vulgaris* L.

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Abstract

As compared with the control plants cultured under photoperiodic L : D = =14 : 10 conditions (Kwiatkowska, Maszewski, 1978), the ultrastructure of nuclei in cells blocked by a 5 day exposure to continuous darkness is characterized by homogenous arrangement. This homogeneity is maintained in all generations of antheridial filaments irrespective of cell length, which in the controls, being directly correlated with particular type of nuclear structure, may serve as a precise indicator of a given stage of interphase. From similarities in both the spatial distribution and content of condensed chromatin it is concluded that the block of the cell cycle is imposed at the beginning of the G_2 phase. On comparing these cells with the early G_2 period (stage VII) in the control plants, marked changes in the structure of nucleoli were found. They decrease in size by half owing to the complete decline of granular component. The area occupied by endoplasmic reticulum undergoes a 50% reduction. The decrease in the activity of Golgi apparatus expressed by a drop in number of smooth vesicles surrounding a single dictyosome is found to parallel the limited rate of cell growth. The number of coated vesicles and cisterns of dictyosome slightly increases. Mitochondria show typical condensed configuration with dense matrices and swollen cristae, while in the control orthodox forms are prevailing. The mean size of mitochondria is smaller, but their number exceeds that of the control plants. The surface area of mitochondrial profiles is found to remain constant proportion of the cytoplasm section, e.g., about 3%. Dark-cultured antheridial filaments show absolute decline of lipid droplets. No differences were found in structure of plastids and vacuols, as well as in number of ribosomes in cytoplasm surface unit.

INTRODUCTION

The course of the cell cycle in antheridial filaments of *Chara vulgaris* is greatly influenced by changes in photoperiodic conditions. As

compared with the control plants grown under natural day-night alternating environment, continuous light promotes mitotic activity. Extended period of total darkness was found, furthermore, to prolong cell division cycle, leading, in consequence, to gradual decrease in mitotic index (Maszewski, 1977). In plants grown totally in the dark for 3 days, complete cessation of cell divisions was shown in all the earlier spermatocyte generations (i.e., the 2-, 4-, 8-, and 16-celled antheridial filaments) and a significant drop was observed in the mitotic activity of the 32-celled generation. The latter filaments become entirely blocked after 5-day dark-treatment. The arrest of the mitotic cycle was found to be reversible; all stages of spermatids resumed cell division within some 18 hrs after backshift to light (Maszewski, 1980).

Autoradiographic studies of the mitotic cycle duration and its component parts: S, G_2, and M phases, have shown that the light factor affects mostly the G_2 period. The reduction or prolongation of the cycle induced by changes in light conditions was found related to modifications of the G_2 phase, whereas S period was shown to remain approximately constant (Maszewski, 1977). Moreover, investigations suggest that the arrest of mitotic divisions after an extended dark treatment takes place also at the G_2 stage. Blocked cells incorporate ^{3}H-thymidine neither during the exposure to continuous darkness nor after the backshift of plants to light conditions, i.e., at the period preceding resumption of mitosis (Maszewski, 1979). This same conclusion has been reached by interferometric measurements. The dry mass content of nuclei in blocked cells was found nearly equal to that of control cells with replicated DNA levels (Maszewski, Kwiatkowska, in prep.).

Cells which lost their ability to continue mitotic cycle are characterized by a sharp decrease in the transcriptional activity, as measured by ^{3}H-adenine incorporation, and, as evidenced by a drop in uptake of many labelled amino acids by the reduced intensity of protein synthesis (Maszewski, 1981).

Ultrastructural studies of antheridial filaments which became unable of entering mitotic divisions in response to a 5-day dark treatment were performed now in seeking morphological alterations connected with the functional state of arrested cells. On the basis of previous studies (Kwiatkowska, Maszewski, 1978) estimating a structural evolution of nuclei in relation to successive stages of the cell cycle in *Chara* cultured under control conditions it was possible at present to localize the arrest point precisely at the early G_2 phase.

MATERIAL AND METHODS

Antheridial filaments obtained from axenic oospore-grown *Chara vulgaris* L. cultured in Forsberg's (1965) mineral medium were used for testing. Nessler tubes with plants were placed in darkness for 5 days and throughout sterilely aerated.

Apical parts of *Chara* thalli carrying antheridia were fixed in 3% glutaraldehyde in 0.0125 M Sörensen phosphate buffer, pH 7.3, at room temperature for 2 hrs. The fixation has been performed together with a control series cultured under photoperiodic L : D = 14 : 10 conditions which served as material for earlier investigations (Kwiatkowska, Maszewski, 1978). Fixed antheridia were isolated and squeezed gently out onto slides in drops of agar, according to Kwiatkowska and Maszewski (1976). Selected agar dice with antheridial filaments were postfixed in veronal buffered (0.0125 M; pH 7.3) 1% OsO_4 solution, dehydrated in an ethanol series and embedded in Epon 812 in flat plastic moulds. This method combined with light microscopic examination of cell number within individual filaments enabled a precise identification of developmental stages prior to microtome sectioning. Ultrathin sections were double stained in uranyl acetate followed by Reynold's lead citrate.

Morphometric measurements were made on micrographs taken from longitudinal sections of whole cells, under 16,000 times magnification. The Golgi vesicles were measured using ocular micrometer under a magnifying glass.

RESULTS

1. Ultrastructure of nuclei

Interphase nuclei of the 32-celled antheridial filaments in control plants cultured under natural photoperiodic conditions display a considerable heterogeneity of contours, sizes, and spatial organization of chromatin. Basing on both nuclear shape and structure 10 types of cells corresponding to successive periods of interphase were discerned, their exact locations in the course of the cell cycle being indicated by mean cell length within a given population and referring them to cell sizes of DNA synthesizing filaments (Kwiatkowska, Maszewski, 1978). During S phase which begins in early telophase a gradual chromatin decondensation takes place, the process reaching maximum in cells of type IV and V; this is accompanied by the enlargement of the nuclear surface resulting from numerous invaginations of the envelope (Fig. 1).

The final stage of S phase (type VI), i.e., the period characterized by a spot ^{3}H-thymidine labelling pattern over late replicating heterochromatin regions is strongly marked by an increased chromatin decondensation arranged in the form of a network and a simultaneous dispersion of perinucleolar chromocenters. The most critical period in interphase is early G_2 phase typified by the appearance of large and irregular clusters of dense chromatin distributed at random between wide areas of dispersed chromatin (type VII). By the end of the first half of the G_2 phase (type VIII) a renewed total reorganization of nuclear structure occurs, and the highest degree of chromatin dispersion is at that time to be observed. At later stages (types IX and X) the progressively increased process of chromatin condensation precedes the arrangement of mitotic chromosomes.

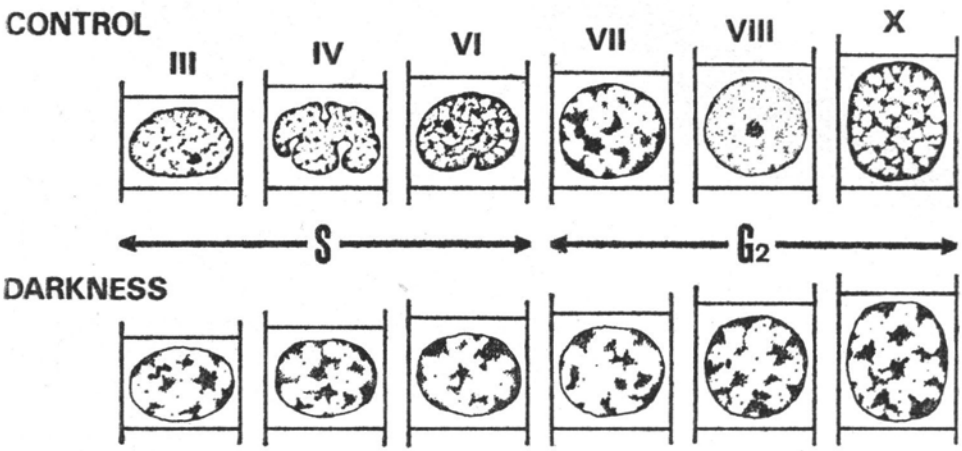
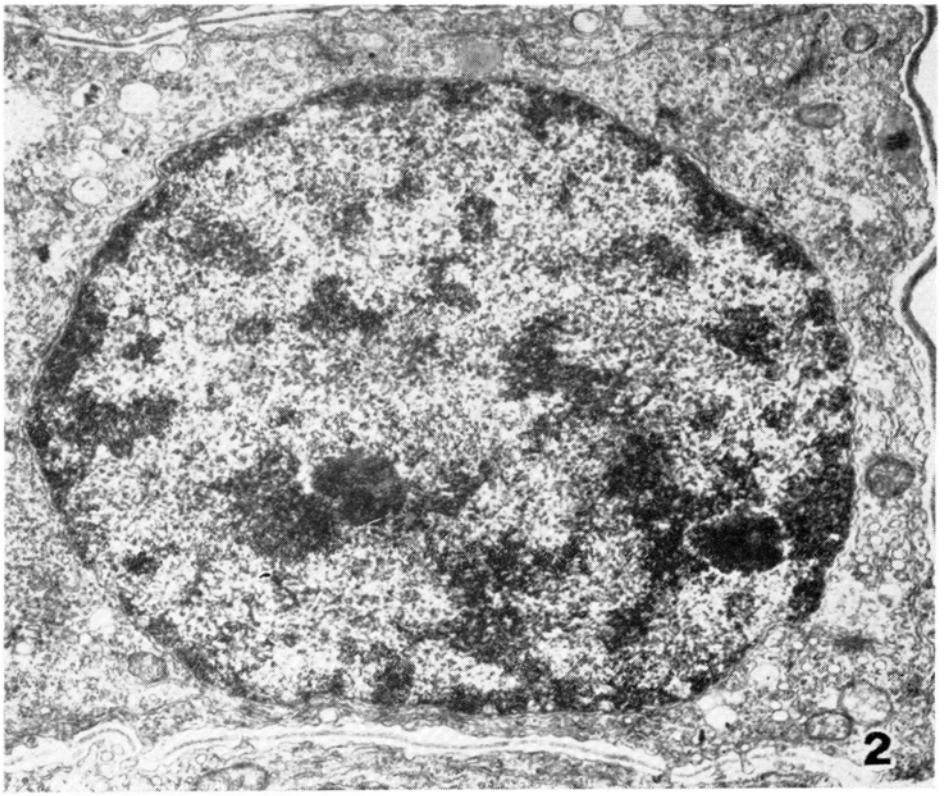
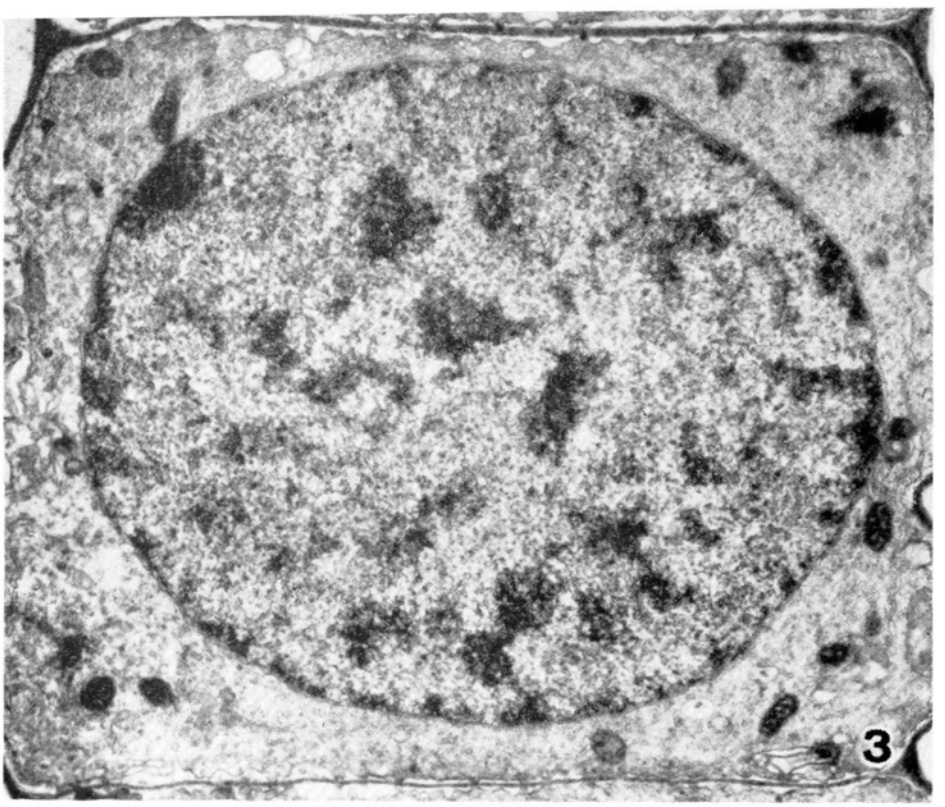


Fig. 1. Ultrastructure of the 32-celled antheridial filaments of *Chara vulgaris* during successive stages of interphase (according to Kwiatkowska, Maszewski, 1978) and the structure of nuclei in equally sized cells after a 5-day dark treatment. Some stages are omitted in the scheme.

The homegeneity of both nuclear shapes and morphology is the main change in overall appearance of cells inhibited by a 5-day dark treatment, as compared with the control material. All nuclei, irrespective of cell lengths, show large and variably sized electron dense masses of condensed chromatin distributed irregularly between wide regions of dispersed chromatin (Figs. 1, 3, 4, 5). Substantial amounts of condensed chromatin are localized at nuclear periphery in attachement to the envelope and in close association with nucleoli (Fig. 3).

The structural organization of nuclei in earlier generations, i.e., in the 2-, 4-, 8-, and 16-celled antheridial filaments after a 5-day dark

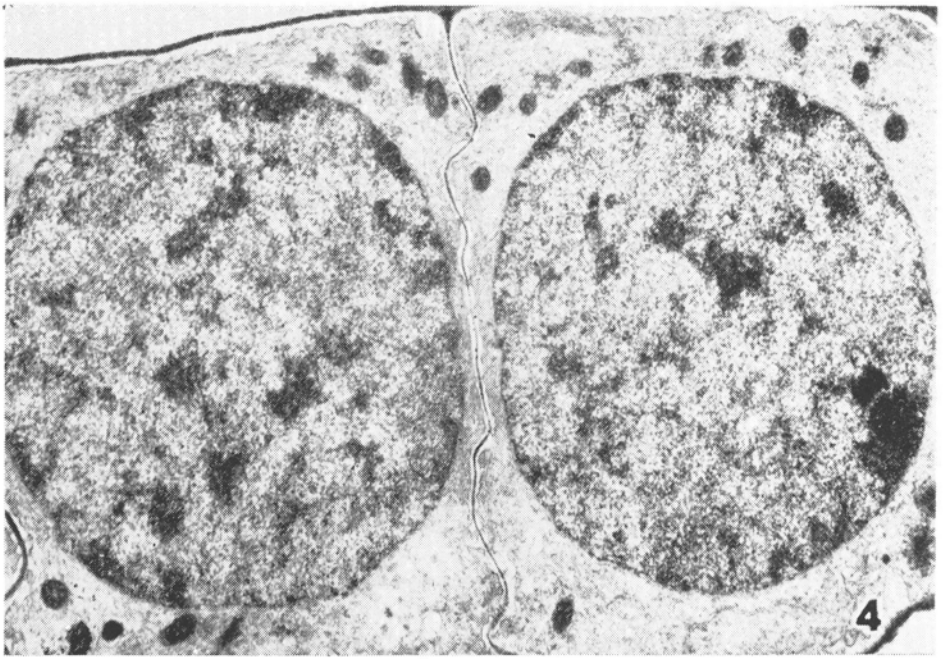


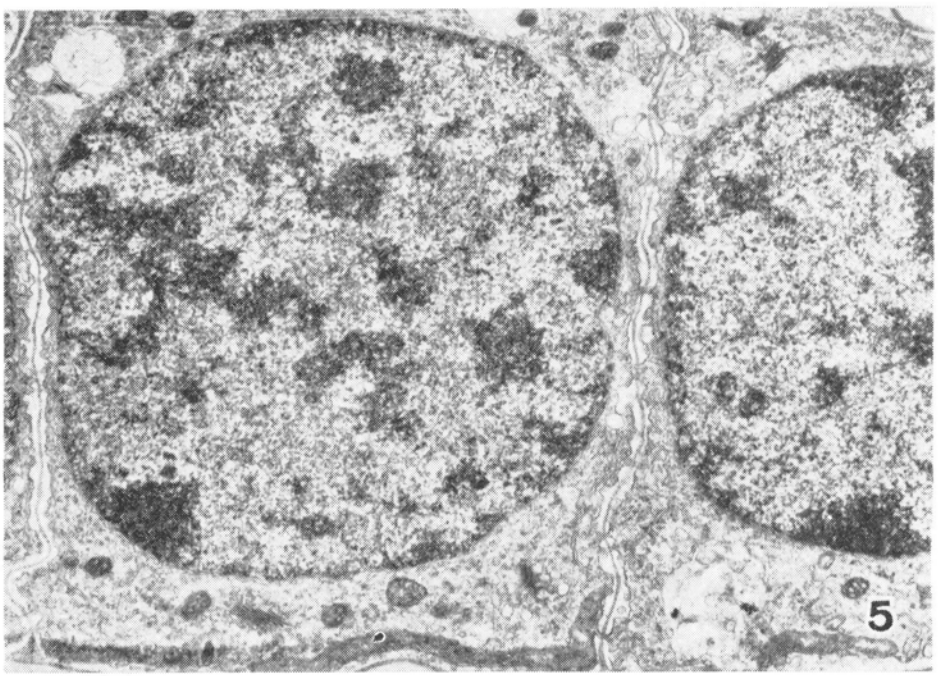


Cells in the 32-celled generation of antheridial filaments of *Chara vulgaris* L: (× 11,000)

Fig. 2. Control (L : D = 14 : 10), early G_2 phase — nuclei of type VII.

Fig. 3. Dark treated cell of size equal to control (compare Fig. 2).

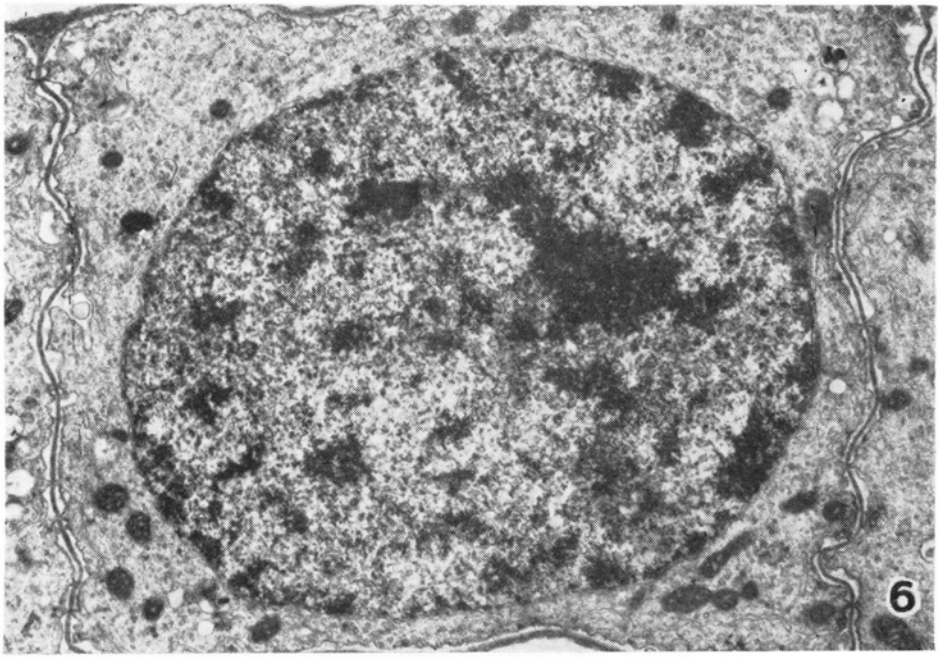


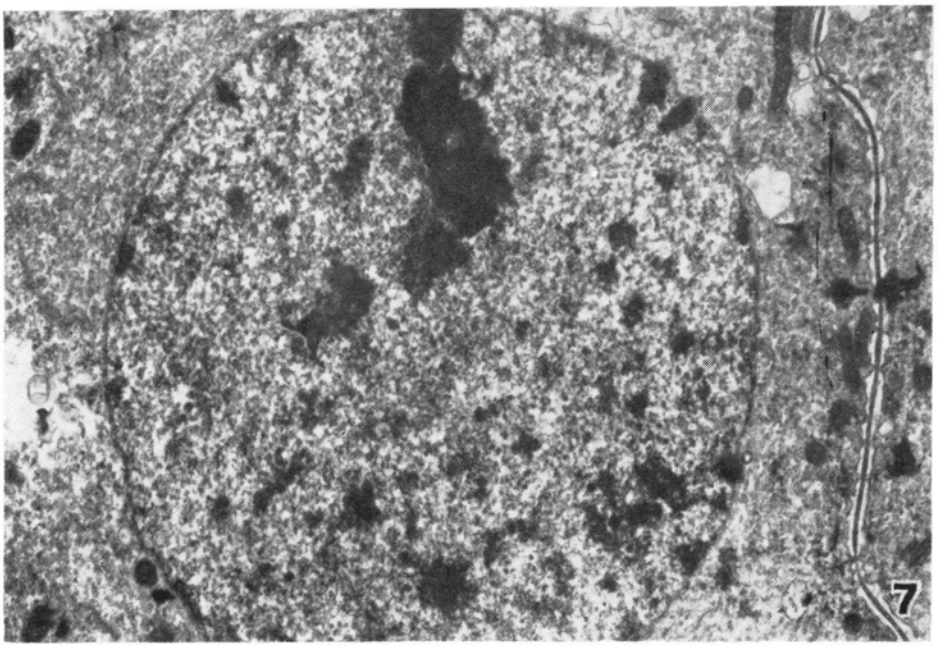


Antheridial filaments of the 32-celled generation after dark treatment (× 9,100)

Fig. 4. Cell of size equal to S phase control cells.

Fig. 5. Cell of size equal to G_2 phase control cells.

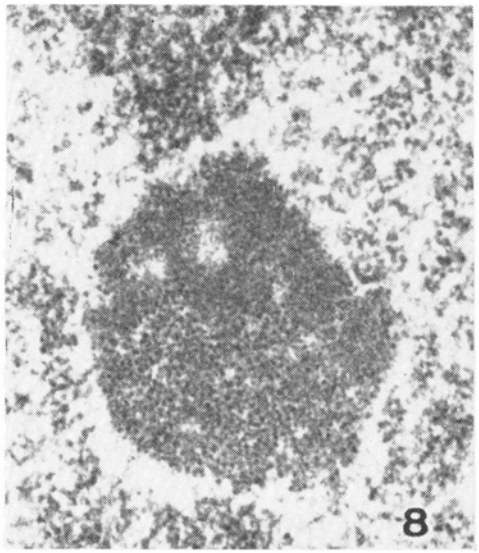


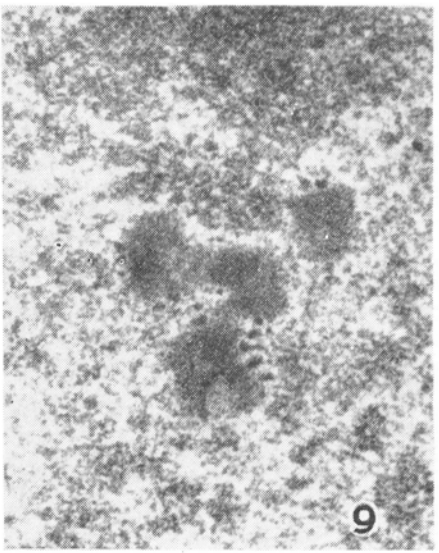


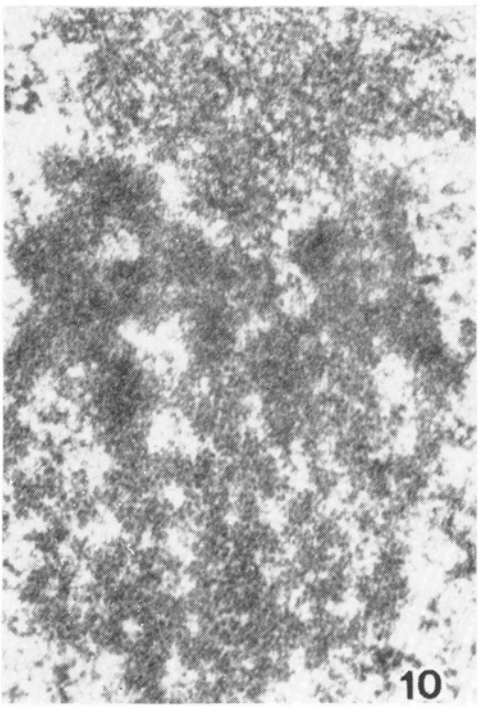
Dark inhibited cells of the earlier generations of antheridial filaments. (× 9,100)

Fig. 6. 16-celled generation.

Fig. 7. 8-celled generation.







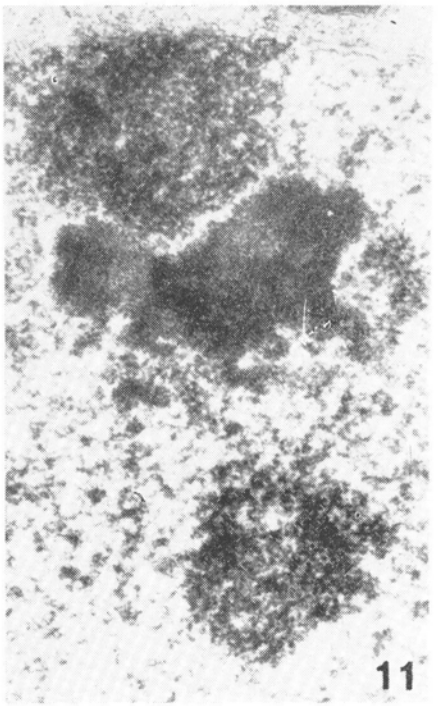
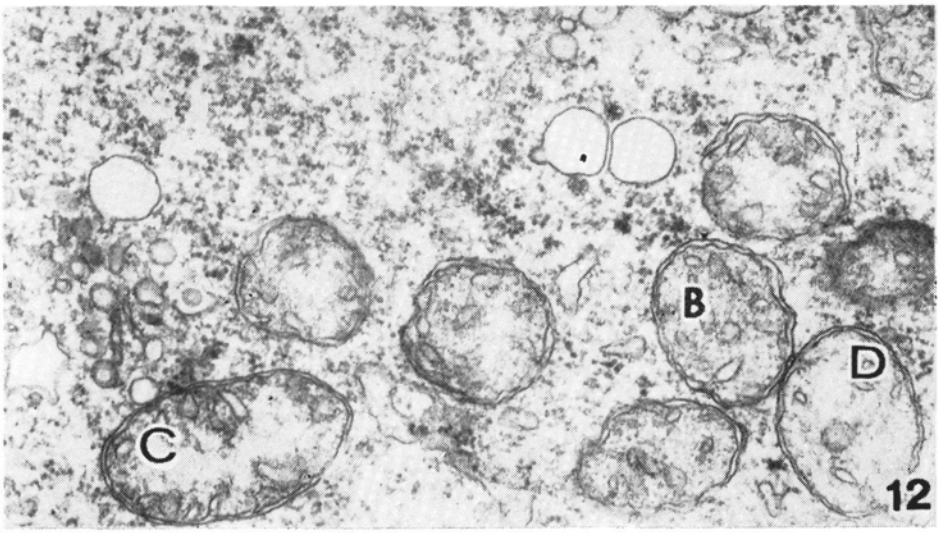
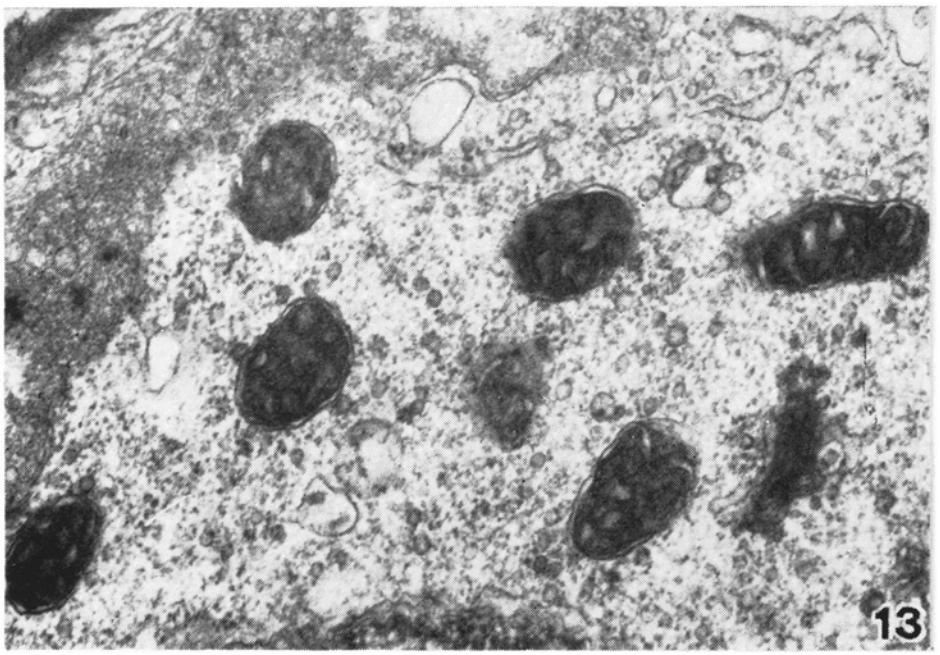


Fig. 8 and 9. Nucleolus in the 32-celled antheridial filament (× 36,000): Fig. 8. Control material. Fig. 9. Dark treated material.

Figs. 10 and 11. Nucleolus in the 4-cells antheridial filament (× 36,000): Fig. 10. Control material. Fig. 11. Dark treated material: ch — chromatin, f — fibrilar component, g — granular component.





Mitochondria of the 32-celled antheridial filaments of *Chara vulgaris* (× 36,000):
Fig. 12. Control material; mitochondria of type B, C, and D are visible.
Fig. 13. Dark treated material, condensed mitochondria.

treatment closely resembles that described above for the 32-celled stage (Figs 6, 7).

From among all the structural types of nuclei discerned within control material, early G_2 phase nuclei of type VII approximate to the highest degree those after a long exposure to total darkness. Comparable observations indicate similarities in regularities of nuclear shapes, in structural organization and spatial distribution of irregular clusters of condensed chromatin, as well as in the nature of nucleolar association with enlarged chromocenters (compare Fig. 1 and Figs. 2, 3). According to morphological features it seems thus reasonable to assume that the "arrest point" of the nuclear mitotic cycle is imposed at the beginning of the G_2 phase, i.e., at the stage characterized by the unique spatial organization of chromatin which can not be attributed to any other period of interphase.

2. Content of condensed chromatin

In parallel with the structural changes quantitative alterations of the content of condensed chromatin were found to accompany successive stages of interphase nuclei in *Chara*. Morphometric analyses of the 32-celled generation of antheridial filaments in control plants (K w i a t k o w s k a, M a s z e w s k i, 1978) indicated that there were two periods of intensified chromatin decondensation. One of these takes place in the middle S phase (type V), the other in the middle G_2 phase (type VIII). The periods of increased chromatin condensation were found to occur immediately before and after mitosis and in the mid-interphase, i.e., at the end of the S phase.

Structural homogeneity of nuclei in antheridial filaments after the extended dark treatment and their apparent similarity to control nuclei of type VII made it possible to confine comparable studies of the content of condensed chromatin merely to control cells of the early G_2 population and to the dark inhibited cells which were equal in size to untreated controls at stage VII. The content of condensed chromatin was established at nearly the same level in both analysed cell classes, e.g., 41.1% in the control material cf. 45.0% within nuclei of the dark grown plants. This slight increase in cells kept totally in darkness has been found on the border of statistical significance (Table 1).

3. Ultrastructure of nucleoli

The mean area occupied by nucleolar profiles in the 32-celled antheridial filaments during early G_2 phase was estimated by morphometric analysis to comprise 1.33 per cent of the nuclear section. In the compar-

Table 1

Morphometric analysis of the 32-celled antheridial filaments after a 5-day dark treatment (D) and in the compared population of control cells in stage VII, i.e., at early G_2 phase (C)

	C		D	
	μm²	%	μm²	%
Cell section area including:	860.6±22.4*	100	808.6±30.5	100
cytoplams	312.2±12.5	36.3±1.5	290.9±13.1	36.0±1.6
nucleus	548.4±18.5	63.7±2.1	517.7±19.7	64.0±2.4
The relative area occupied by condensed chromatin and nucleolus as a per cent of nuclear profile				
Nucleus	100		100	
condensed chromatin	41.08±0.97		44.98±1.56	
nucleolus	1.33±0.28		0.64±0.20	
The relative area occupied by cytoplasmic structures as a per cent of the surface area of cytoplasm				
Cytoplasm region	100		100	
mitochondria	3.0±0.3		3.5±0.5	
dictyosomes	2.1±0.4		2.7±0.5	
proplastids	2.0±0.8		2.1±0.6	
vacuoles	5.0±0.9		5.4±1.3	
lipid droplets	0.4±0.1		0	
endopl. reticulum	9.5±0.5		4.3±0.8	
ground cytoplasm	78.0±3.6		82.0±4.0	
Number of free ribosomes per 0.25 μm²	39.7±1.3		40.4±1.2	

* ±SD.

ed population of cells cultured for 5 days in darkness which in respect to cell length were of equal size to control filaments at the stage VII, nucleolar dimensions underwent reduction and their relative area diminished by half averaging 0.64 per cent. Ultrastructural analysis of nucleoli showed a considerable reduction of granular component and revealed a marked contrast of the dense filamentous regions maintaining their ring- or ribbon-shaped arrangement in the background of surrounding nucleoplasm (compare Fig. 8 and 9). The apparent lack of granular components within nucleoli of earlier generations of antheridial filaments has also been observed (compare Fig. 10 and 11).

4. Ultrastructure of the cytoplasm region

The ultrastructural characteristics throughout the region of cytoplasm within dark treated cells shows no evident changes in comparison with the control material, apart from mitochondria which were visibly

altered (page 177). The differences concerning all the cytoplasm structures are restricted to quantitative changes which might be evaluated merely by employing morphometric measurements. For comparison, control cells of the 32-celled antheridial filaments in early G_2 stage (type VII) and equally sized population of dark treated cells were chosen. Results are given in Table 1.

In both analysed populations the relative area occupied by vacuoles and proplastids comprises a constant per cent of the cytoplasm area in the section. Culture light conditions do not influence the ribosomal population density per surface unit of the ground cytoplasm.

The main differences between the two classes lies in the relative areas occupied by endoplasmic reticulum; its average surface in cross sections of dark treated cells was found to be only less than 50 per cent of the control value. Moreover, cells subjected to extended dark period were totally deprived of lipid droplets. Only comparatively minor variations were observed in the relative areas of mitochondrial profiles and of dictyosomes. In parallel with these quantitative changes, exposure to darkness brought about visible alterations in the appearance of both structures which will be discussed below in details.

a) Golgi apparatus

Cytological characteristics throughout the Golgi complexes in both populations under comparable studies (control cells of type VII and dark inhibited cells) are shown in Table 2.

Table 2

Golgi complexes in the 32-celled antheridial filaments in stage VII within control plants (C) and after dark-induced retention of mitotic divisions (D)

Mean values	C	D
Number of dictyosomes per 1 cell section	2.52±0.22*	2.91±0.20
Number of cisterns within a dictyosome	5.37±0.40	7.40±0.46
Number of smooth vesicles surrounding a dictyosome	8.75±0.88	6.55±0.49
Diameter of smooth vesicle (nm)	111.31±6.01	78.50±4.42
Number of coated vesicles surrounding a dictyosome	1.85±0.18	2.85±0.22

* ± SD.

The number of dictyosomes found in one central cell section in plants given long exposure to darkness was found to be 2.91, and thus, to exceed slightly the value characteristic of the equally sized population of control cells (this difference was on border of statistical significance).

The structure of dictyosomes shows no evident changes after dark treatment; marked differences were estimated, however, in number of cisterns composing a single dictyosome. They amounted to 5.37 in the control material, and increased in number up to 7.40 after exposure to darkness, in spite of ranging from 5 to 12 in both classes of investigated cells. Dictyosomes composed of numerous cisterns assume a characteristic shape of oblique stacks.

The activity of Golgi complexes expressed by a number and size of vesicles surrounding a single dictyosome is strongly inhibited in darkness. As compared with the control material, Golgi vesicles decline in number and decrease in diameter by about 25 and 30 per cent, respectively. In contrast to Golgi vesicles, the number of coated vesicles within dark treated cells is markedly increased which leads to a significant change in the ratios derived from both the above types. The average ratio of smooth vesicles : coated vesicles is 4.7 : 1 under the control conditions, while after a 5 day exposure to darkness their relative proportion was calculated to be 2.3 : 1.

b) Mitochondria

The 32-celled generation of antheridial filaments under the control light-dark conditions is characterized by a marked heterogeneity of mitochondria. Basing on both the degree of matrix density as well as the structural features, 4 types of mitochondria were discerned (Kwiatkowska, in prep.), as follows: (i) type A, with matrices much denser than the surrounding cytoplasm, (ii) type B, with matrices of equal contrast to the cytoplasm, (iii) type C, with dense peripheric zones and DNA-containing electron transparent centres, and (iv) type D mitochondria with translucent matrices owing to which these organelles might be easily distinguished as more electron transparent than the enclosing cytoplasm. Mitochondria of type A are the smallest in size and they may be termed therefore as semicondensed in state. Mitochondria denoted as types B and C were slightly greater and indicated an orthodox structure. The maximum size of mitochondria was found in type D.

Within the control early G_2 phase cells (stage VII) mitochondria of types A and B are most numerous, comprising 37.1 and 47.0 per cent of the whole population, respectively. Each of both C and D types averaged 7.97 per cent of the population composed of 251 mitochondria.

In cells given prolonged exposure to darkness mitochondria were found to be the only cytoplasmic organelles characterized by conspicious changes in conformation. They are typified by the presence of a very dark matrices which distinguish them as most contrasty structures against the background of the cytoplasm (Fig. 13). Incidently, in the control material exclusively mitochondria of type A were slightly denser than the cytoplasm. The outer and inner compartment of an mitochondrion is swollen and appears as electron transparent against the background of a dark matrix. So, mitochondria present in the dark inhibited cells are typical for the state of condensed conformation. Such organelles were found to occur in all the generations of antheridial filaments after a 5-day treatment in darkness (Figs 3, 4, 5, 6, 7).

The number of mitochondria in the early G_2 cells of the control plants was on average some 30 per cent smaller than that within equally sized population in dark grown plants (Table 3). The sizes and shapes of mitochondrial profiles vary significantly which in both experimental series is largely due to differences in lengths of the organelles. The analysed populations of mitochondria in control and dark treated cells were divided into three classes differing in ratios of length to breadth of the profiles. Circular profiles of mitochondria, elongated, i.e., more than twice as long as broad mitochondrial profiles, and transitional structures were discerned. Within each of these groups, both mean lengths and breadths of mitochondria analysed in the dark treated cells (Table 3) are smaller than the adequate parameters evaluated for the control

Table 3

Mitochondria of the 32-celled antheridial filaments of *Chara vulgaris* in stage VII of interphase in control (C) and after dark-induced retention of mitotic divisions (D)

	C	D
Number of mitochondria in 1 cell section	6.28±0.23*	9.06±0.30
Total number of analysed mitochondria	238	625
Frequency of mitochondrial profiles in relation to contours:		
round	15.1%	6.2%
transitory	71.5%	73.3%
elongated	13.4%	20.5%
Sizes of mitochondrial profiles:		
breadth, μm	0.36±0.01	0.30±0.01
length, μm	0.57±0.01	0.47±0.01
surface area, μm^2	0.154	0.108
total surface area of mitochondrial profiles in 1 cell section	0.966	0.980

* ±SD.

material. The mean profile area of a single mitochondrion in cells exposed to darkness is reduced by nearly 30 per cent compared to the control. After dark exposition, the total length of all mitochondria present at one cell section is enhanced by increasing numbers of these organelles, the total surface area occupied by mitochondrial profiles being increased, however, to only a minor degree, comparatively.

In both analysed series transitional class of mitochondria is most numerous. The percentage of round profiles decline, while there was an increase in numbers of elongated mitochondria. The ratio of both these extreme categories is thus considerably altered and suggests that the increase in number of mitochondria after a prolonged exposure to total darkness is not related to the enhanced process of fragmentation.

DISCUSSION

The present electron-microscopic observations of antheridial filaments which ceased mitotic divisions in response to a 5-day exposure to total darkness support the conclusion based on previous ^{3}H-thymidine labelling studies (Maszewski, 1981) and interferometric analyses of the dry mass contents (Maszewski, Kwiatkowska, in prep.) that the block of the cell cycle is imposed in the G_2 phase. Both nuclear structures and shapes were found quite different than structural types distinguished in successive stages of S period, the late being characterized by a reticulate organization of chromatin and by numerous invaginations of nuclear membrane (Fig. 1). Likewise in the control G_2, nuclear shapes in cells given prolonged exposure to darkness are regular, round or ovate, depending upon cell length.

Ultrastructural similarity of nuclear morphology constitutes the most striking feature observed in dark inhibited cells, as compared with the control plants. The apparent absence of heterogeneity as regards the spatial arrangement of chromatin in antheridial filaments of *Chara*, irrespective of generation and cellular dimensions, suggests that the arrest of the mitotic cycle is set in one only, definite stage of interphase. The evident convergence of nuclear structure in both the dark inhibited cells and control cells of type VII from early G_2 phase seems to indicate that the block of the mitotic cycle is imposed at this very stage of interphase. It may be assumed therefore that the morphological features of nuclei serve as indicator of the period of interphase not merely within actively proliferating populations (Kuroiwa, Tanaka, 1971; Klueva et al., 1974; Lafontaine, Lord, 1974; Nagl, 1977), but also in cells which became blocked in the progress of the cell cycle. The same conclusion has been reached by preliminary

observations of hydroxyurea-treated cells of antheridial filaments in *Chara* (Kwiatkowska, Maszewski, unpubl.). The nuclear arrangement was found at that time to correspond precisely to structural features of early S phase chromatin, i.e., to the stage which arrest some 90 per cent of the 32-celled filaments if the inhibitor applied in proper concentration and during an adequate period of incubation (Bilecka, 1979).

The retention of mitotic divisions in antheridial filaments at early G_2 phase after a 5-day exposure to darkness seems thus to correspond precisely with the location of the hypothetical "G_2 arrest point" which had been proposed by Prescott (1976) in the schematic course of the cell cycle. In agreement with the postulated model compiled in most part from studies of animal cells, the critical point of retention at early G_2 phase is followed by successive periods of RNA and protein synthesis which are related to initiation of mitosis. Also in plant materials the synthesis of proteins which condition the onset of cell division was found to occur during early G_2 phase (González-Fernández et al., 1974). During the mitotic cycle of the 32-celled generation of antheridial filaments the increase in transcriptional activity correlated with a maximum chromatin dispersion and an enhanced ^{3}H-AMD binding was found to occur at stage VIII (i.e., in the second part of the first half of G_2) following stage VII, the latter being suspected to act as the arrest point of the G_2 phase. Both, stage VII and stage VIII are followed by stages IX and X which just precedes prophase (Kwiatkowska, Maszewski, 1979a).

Ultrastructural similarity of all nuclei in cells given prolonged period of darkness is evidenced by an apparent lack of cell-size-related changes in transcriptional activity (Maszewski, 1980), whereas in the control conditions, periods of the decreased as well as increased RNA synthesis were clearly observed (Kwiatkowska, Maszewski 1979a). Moreover, the transcriptional activity of dark treated cells is on average some 50 per cent lower compared to control cells, even to those involved in S-G_2 transition, i.e., cells characterized by the lowest intensity of RNA synthesis (Maszewski, 1981). No parallel increase in the content of condensed chromatin was found, however, in nuclei of plants receiving prolonged period of darkness. On the other hand, a dependent relationship between the amount of condensed chromatin and transcriptional activity was found during interphase of the 32-celled antheridial filaments under photoperiodic growth (Kwiatkowska, Maszewski, 1979a). A reasonable assumption is that the decrease in transcriptional activity following exposure to darkness is limited mainly to preribosomal RNA synthesis. This conclusion is supported by nucleolar size measurements which were found to undergo reduction in darkness by half owing to a complete lack of granular component. Numerous

investigations have shown that the amount of granular components within nucleoli is related to intensities of rRNA synthesis both in animal cells (e.g., Simard, Bernhard, 1967) and plants (e.g., Havelange, Bernier, 1974; De Barsy et al., 1974; Olszewska, 1976). More recently, these observations were supported by correlated biochemical, autoradiographic, and ultrastructural studies on the influence of elevated temperature on *Zea mays* root embryonic cells which showed a decrease in the preribosomal RNA fraction concurrently with a reduction of granular component within the nucleolus (Fransolet et al., 1979).

The lowering of rRNA synthesis in dark treated cells did not influence parallel changes in ribosome numbers falling to the surface unit of the cytoplasm. Thus, it may be suspected that the slowing of cell growth in darkness occurs simultaneously, but it seems also probable, however, that a low rate of cell growth is still maintained. Cells differing in size are found within populations of dark inhibited antheridial filaments. Besides those which are shorter or equal in length to control cells at stage VII, cells of mitotic size or even longer are to be found, as well (Maszewski, 1977, 1979). The latter group is suspected to be held in early G_2 phase already at the very beginning of exposure to total darkness. Thus, in environment conditions applied in the present experiments the parameter of cell length has lost the attribute of a precise indicator of the interphase stage which is characteristic of the control cells (Olszewska, Godlewski, 1972). Quite similar process has also been found after hydroxyurea-induced blocking of mitotic divisions in antheridial filaments of *Chara* (Bilecka, 1979). A certain dissociation of nuclear cycle, "division-division" cycle and cell growth cycle (Mitchison, 1978) is evident in this phenomenon. Furthermore, the process of cell growth has probably an unballanced nature (ref. Mitchison, l.c.), which is indicated by alterations in quantitative ratios of particular cell structures, some of them being increased in numbers (e.g., dictyosomes, mitochondria), while others undergoing reduction (e.g., endoplasmic reticulum), as compared with the control material.

The decreased rate of cell growth in antheridial filaments of dark cultured *Chara vulgaris* is shown indirectly by changes in the activity of dictyosomes expressed by the number and sizes of smooth vesicles. The enhanced activity of Golgi apparatus in control plants coincides with the period of increased synthesis of cell wall components, i.e., during cytokinesis and nuclear division (by formation of the cell wall between the two daughter cells), as well as with the middle G_2 phase stages characterized by an intense cellular elongation. The decrease of the dictyosome activity associates S phase (Kwiatkowska, Maszewski, 1979b) which is typified by a slow rate of cell growth (Go-

dlewski, Olszewska, 1973). Both, numbers and diameters of smooth vesicles in dark treated cells was found reduced by some 25 per cent which decreased the total volume of these vesicles by about half, as compared with the activity of Golgi complexes within control cells at stage VII. On the other hand, no parallel drop in numbers of coated vesicles was found in plants exposed to darkness. In control conditions there was no relationship between the number of coated vesicles surrounding a single dictyosome and the intensity of cell wall growth, as well (Kwiatkowska, Maszewski, 1979b). Instead, the number of coated vesicles was found closely related to the degree of chromatin condensation; this however, will be the subject of further studies. Moreover, the mean number of cisterns composing a single dictyosome in cells exposed to darkness is on average some 30 per cent greater than in controls. Some authors (ref. Whaley, 1975) interprete such process as slowing of the rate of membrane flow which consists in limited activity of vesicle production and a maintained formation of new membranes. Parallel increase in number of dictyosomes may also take place within the cell. The surface area occupied by the membranes of rough endoplasmic reticulum undergoes reduction by about 50 per cent. It is probable that the latter process is connected with a decreased incorporation of labelled amino acids (Maszewski, 1980).

One of the most striking changes in the antheridial filaments of *Chara* exposed to continuous dark treatment is the condensation of mitochondria. This reflects, presumably, changes in functional state of the organelles in relation to alterations in cellular metabolism. Combined biochemical and ultrastructural analyses of isolated mitochondrial fractions indicated that from among five functional states distinguished by Chance and Williams (1955), condensed configuration is attributed to mitochondria being in state II and III (Hackenbrock, 1968; Hackenbrock et al., 1971; Muscatello et al., 1972). The electron dense image of mitochondrial structure found within different cellular systems is frequently interpreted as an expression of their high activity of oxidative phosphorylation (e.g., Młodzianowski, Idzikowska, 1978; Rohr, 1978). An analogous opinion in relation to condensed mitochondria of antheridial filaments of *Chara* is valid in view of Weber's (1968; and ref. Gumińska, 1971) studies indicating the presence of a highly positive correlation between the rate of cellular growth and concentration of the key enzymes for glycolysis. The above-mentioned experiments have shown a close relationship between the glycolytic pathway of energy production and the enhanced synthesis of nucleic acids and proteins within different types of cells. Competitive system of ATP production — the compound designated a

main regulator of activity of glycolysis enzymes (ref. Gumińska, 1971) — takes place within mitochondria.

According to the oscillatory system of domination of oxidative phosphorylation or glycolysis, a condensed configuration of mitochondria in *Chara* after dark induced retention of cellular growth and divisions may reflect an enhanced functional activity of mitochondria in carrying out oxidative phosphorylation (state III) as a main source of energy supply under dark conditions. Care must be taken, however, in judging the functional state of mitochondria on the ground of configuration (ref. Michejda, 1973). After a long exposure to darkness, antheridial filaments of *Chara* are totally deprived of lipid droplets which may indicate depletion of cellular reserves of energy. Therefore, the condensed structure of mitochondria would reflect, probably, effects of starvation induced by a low substrate level and a high concentration of ADP, and thus, in respect of functional activity to represent the transition of mitochondria into metabolic state II (e.g., Muscatello et al., 1972). Such possibility is supported by ultrastructural studies of mitochondria at fertilization in sea urchin (Innis et al., 1976). It was found that the condensed conformational state of mitochondria prior to fertilization is correlated with the enhanced ADP : ATP ratio and is not related to the high level of oxidative phosphorylation. The latter was shown to increase just after fertilization, at the period characterized by an orthodox structure of mitochondria.

Another cause of changes in mitochondrial configuration may be due to modifications in ion transport (ref. Michejda, 1973). The influence of the light factor at the level of ionic relations is documented by numerous observations performed in part on internodes of *Chara* (e.g. Lucas, 1975; Smith, Raven, 1974; Lucas et al., 1977). This problem needs further studies, however.

Another question is the apparent increase in number of mitochondria (by about 30 per cent) in cells subjected to darkness, as compared with equally sized cells at stage VII. Although the total area of all mitochondrial profiles in one cell section appears to be nearly equal to the control values, the dark induced rise in number of all organelles is not suspected, however, to be the result of enhanced mitochondrial fragmentation which may occur under changes of physiological conditions (e.g., Kwiatkowska, 1970). The transition from orthodox to condensed configuration is accompanied by the decrease in mitochondrial volumes (Muscatello et al., 1972). Consequently, if the total volume of condensed mitochondria equals that of control orthodox (in most part) organelles the global volume of mitochondria must have been virtually increased. Moreover, mitochondrial fragmentation would induce increases in numbers of round profiles as well as decreases of elongated

structures, while an opposite process resulting in the increase of the longest mitochondrial profiles was found to occur. It is suspected that the increase in numbers of mitochondria within cells which ceased mitotic divisions in response to a 5-day dark treatment must be attributed to continuation of mitochondrial division cycles in spite of nuclear cycle arrest in G_2 phase. Similar conclusion was drawn from studies of mitochondrial reproduction in nonproliferating cells of *Tetrahymena* (Elliot, Bak, 1964; Lloyd et al., 1971; Gleason et al., 1974) and *Paramecium* (ref. Perasso, Beisson, 1978). These results together with the studies on mitochondrial cycle in control *Chara* plants (Kwiatkowska, in prep.) suggest that there is no fixed temporal relationship between the growth and division of mitochondria and the nuclear events of the cell cycle, as well as between the number of mitochondria and a gene dosage of nuclear origin.

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Ultrastruktura komórek nici spermatogenicznych Chara vulgaris L. *po odwracalnym zablokowaniu ich podziałów działaniem ciemności*

Streszczenie

Ultrastruktura jądra komórek, których cykl podziałowy uległ zablokowaniu wsutek 5-dobowego działania ciemności, cechuje jednorodność organizacji strukturalnej w porównaniu z kontrolą (L:D = 14:10). Jednorodność ta występu-

je we wszystkich stadiach nici, niezależnie od długości komórki, która w kontroli stanowi wyznacznik fazy interfazy i jest skorelowana z określoną strukturą jądra. Na podstawie podobieństwa przestrzennej organizacji chromatyny zwartej i jej ilości przypuszcza się, że zablokowanie cyklu następuje we wczesnej fazie G_2. W porównaniu z wczesną fazą G_2 w kontroli (stadium VII), istotne zmiany wykazuje struktura jąderek: zmniejszają się ich rozmiary o połowę, zanika składnik ziarnisty. Stwierdzono redukcję ilości retikulum endoplazmatycznego o ok. 50%. Wykazano, skorelowane z ograniczeniem tempa wzrostu komórek zmniejszenie aktywności diktiosomów wyrażonej liczbą i wielkością otaczających je gładkich pęcherzyków. Liczba opłaszczonych pęcherzyków (coated vesicles) nieznacznie wzrasta. Wzrasta także liczba cystern w diktiosomie. Mitochondria wykazują typową konfigurację skondensowaną — z gęstą matriks i rozdętymi cristae, natomiast w kontroli dominują mitochondria ortodoksyjne. Rozmiary mitochondriów są mniejsze, a ich liczba jest większa niż w kontroli. Zajmują one ± stały % powierzchni przekroju cytoplazmy, tj. ok. 3%. Stwierdzono całkowity zanik kul lipidowych. Struktura plastydów, wakuol i liczba rybosomów przypadających na jednostkę powierzchni cytoplazmy nie ulega zmianie.