# ULTRASTRUCTURAL, AUTORADIOGRAPHIC AND ELECTROPHORETIC EXAMINATIONS OF *CHARA TOMENTOSA* SPERMIOGENESIS

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ABSTRACT

Ultrastructure of a spermatid nucleus changes many times during spermiogenesis. Condensed chromatin forms irregular clusters during phases I-II, a continuous ring adjacent to a nuclear envelope during phases III-V and a network occupying the whole nucleus during phase VI. In advanced spermiogenesis dense chromatin disappears and short randomly positioned fibrils arise, then long parallel ones are found (phase VIII) which during phase IX form a lamellar structure. In mature spermatozoids (phase X) chromatin becomes extremely condensed.

$^3$H-arginine and $^3$H-lysine incorporation into spermatids during 2-min incubation is intensive during phases I-V, decreases during phases VI, VII and becomes very low during phases VIII-IX.

Capillary electrophoresis has shown that during *Chara tomentosa* spermiogenesis replacement of histones with basic proteins whose mobility is comparable to that of salmon protamines takes place. At the beginning of spermiogenesis core and linker histones are found in spermatids. During early spermiogenesis protamine-like proteins appear and their amount increases in late spermiogenesis when core histones are still present. In mature spermatozoids only protamine-like proteins represented by 3 fractions: 9.1 kDa, 9.6 kDa, 11.2 kDa are found. Disappearance of linker histones following their modification precedes disappearance of core histones.

The results indicate that dynamic rearrangement of chromatin ultrastructure and aminoacid incorporation rate during spermiogenesis are reflected in basic nuclear protein changes.

KEY WORDS: *Chara tomentosa*, spermiogenesis, histones, protamine-like proteins, ultrastructure, autoradiography, capillar electrophoresis.

## INTRODUCTION

Higher algae from *Charophyta* form spermatozoids as a result of structural and biochemical rearrangements of nuclear chromatin during spermiogenesis. Cytological changes have been described in several species of *Chara: C. fibrosa* (Pickett-Heaps 1968), *C. contraria* (Cocucci, Caceres 1976), *C. vulgaris* (Robert 1979; Kwiatkowska 1996; Duncan et al. 1997; Kwiatkowska, Popłońska 2002) and in *Nitella missouriensis* (Turner 1968) while biochemical analyses are scarce. In Robert's paper (1984) only a general electrophoretic characteristic of basic nuclear proteins from mature *C. vulgaris* spermatozoids can be found. To our knowledge in literature dealing with *Charophyta* no information concerning dynamic changes of nuclear proteins during successive spermiogenesis stages is available (apart from a summary of preliminary studies by Kaźmierczak 2000).

A complex analysis of *Chara tomentosa* spermiogenesis is the aim of the present paper. Basic protein changes during 4 successive stages of spermatid differentiation were examined. These changes were correlated with simultaneous, precise observations of nuclear ultrastructure rearrangements during spermiogenesis. Such studies have not been carried out in *C. tomentosa*. Moreover, in order to visualize spermatid metabolic activity during the consecutive spermiogenesis phases autoradiography with $^3$H-lysine and $^3$H-arginine was carried out. *C. tomentosa* was chosen because of its bigger, as compared to other *Chara* species, antheridia giving more material for biochemical studies. Additionally, a segmental structure of thallus makes estimation of spermiogenesis phases possible on the basis of the distance between successive nodes from the thallus apex and microscopic analysis of randomly chosen antheridia from each node.

The results indicate that changes in nuclear proteins during spermiogenesis are reflected in dynamic rearrangements of nuclear chromatin ultrastructure and in aminoacid incorporation rate.

## MATERIAL AND METHODS

Apical parts of thallus of *Chara tomentosa* were obtained from plants taken from the Powidzkie lake situated close to the village Powidz near Konin (Poland).

Antheridia were taken from III-V node pleuridia counting from the apical buds. Before the onset of the experiment, the plants were cultivated for a few days in tanks containing water from natural environment at the photoperiod similar to natural, i.e. L : D = 14 : 10.

*Electron microscopy*

Selected *C. tomentosa* plants were fixed in Sörensen phosphate – buffered (0.125 M, pH 7.2) – 3% glutaraldehyde for 3 h at 4°C supplemented with 0.007 M $CaCl_2$. The antheridia were isolated and squashed in a drop of heated 2% agar in Sörensen phosphate – buffer and postfixed in 1% $OsO_4$ in 0.125 M veronal buffer, for 1 h. After dehydration in alcohol series the material was embedded in Spurr's medium. Ultrathin sections were double stained with uranyl acetate and lead citrate according to Reynolds. The sections were examined and photographed in a JEOL JEM-1010 transmission electron microscope.

*Autoradiographic studies*

The apical parts (with antheridia at different spermiogenesis stages) of *C. tomentosa* were incubated in the mixture of radioactive precursors of proteins – $^3$H-lysine (79 Ci/mM – Amersham) and $^3$H-arginine (58 Ci/mM – Amersham) at the concentration 25 µCi/ml (concentrations of both $^3$H-lysine and $^3$H-arginine were 12.5 µCi each) for 2 min and fixed in 2% glutaraldehyde with cacodylate – buffer (pH 7.3) for 2 h at 4°C. In order to obtain squash preparation antheridial filaments were placed on gelatinised slides. Such preparations were covered with liquid photographic emulsion made by Amersham and exposed for approximately 3 weeks in darkness at 4°C. The autoradiograms were stained with 1% hematein and embedded in Canadian balsam.

Statistical significance was determined by Student's t-test at $p<0.05$.

*Extraction of basic proteins*

Extraction of basic proteins from antheridial filaments were carried out according to combined methods of Reynolds and Wolfe (1978) and Spiker et al. (1983). Samples with the antheridia were homogenised in cold mortar in grinding medium (0.25 M sucrose; 10 mM Tris-HCl, pH 8.0; 5 mM 2-mercaptoetanol; 5 mM $MgCl_2$ and 0.1 mM PMSF, 12 mM $NaHSO_3$ added immediately prior to use, to retard proteolysis). The homogenate was centrifuged at 1000×g for 10 min. The pellet was then washed in the same medium with 0.03% Triton added by stirring using the magnetic stirrer for 60 min and centrifuged at 1000×g for 10 min. Then the washing and centrifugation were repeated in grinding solution until the supernatant was clear. The pellets were suspended in grinding medium by homogenisation and centrifuged at 12 000×g for 10 min. The basic proteins were extracted from the pellet which contained only antheridial filaments by treatment with 0.8 N $H_2SO_4$ for 18 h at 4°C. The resulting solution was centrifuged at 12 000×g for 10 min to remove insoluble material and the proteins were precipitated from the solution by adding 4 vol. of methanol at -20°C.

*Capillary electrophoresis – CE-SDS*

Separation of SDS-protein complexes was carried out using the CE-SDS Protein Kit by Bio-Focus 3000 capillary electrophoresis system (BioRad), equipped with a UV detector (220 nm) and uncoated fused silica capillary (50 µm ID × 375 µm OD × and 24 cm long). Dried basic proteins were solubilised in 1 mL of sample buffer (diluted with water 1:1) containing 25 µL 2-mercaptoethanol and the mixture was heated at 100°C for 10 min. Complete mixing of the solution was accomplished by vortexing at 5-min intervals. The samples were cooled and microcentrifuged for 2 min at 1000 xg. The samples were injected electrophoretically (10kV for 5 sec) and electrophoresis was performed at 15 kV and polarity "-" to "+" at 20°C for 15 min. Protamines (from salmon, Sigma), mixture of core histones and histone H1 (from calf thymus, Sigma) were used as a standard for qualification of the extracted basic proteins. Protein molecular weight markers, histones and protamines standards were run before electrophoresis of the basic protein extracts. Molecular weights of the basic proteins in extracts were estimated using the Bio-Size software (Bio-Rad Laboratories Munich, Germany). The electropherograms were recorded and analysed with BioFocus Integration software 5.0, based on Microsoft Windows. For determination of molecular masses were used two sets of protein markers: Bio-Rad Markers (Bio-Rad Laboratories Munich, Germany) and Sigma Marker (Sigma) low range.

## RESULTS

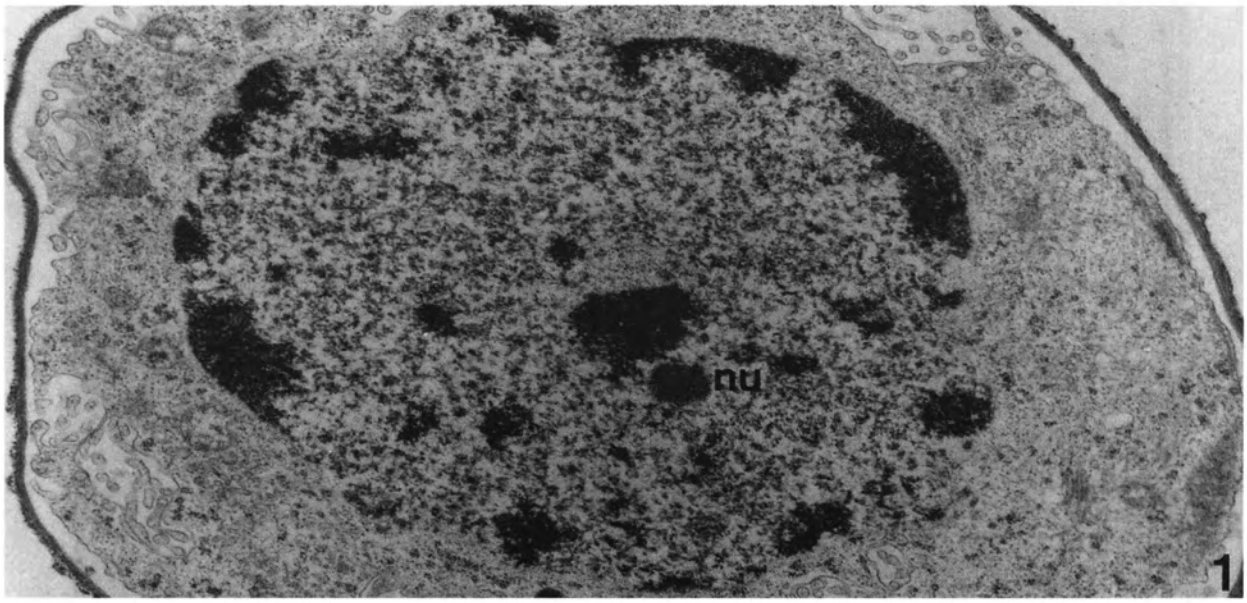
*Changes in nuclear ultrastructure in consecutive spermiogenesis phases*

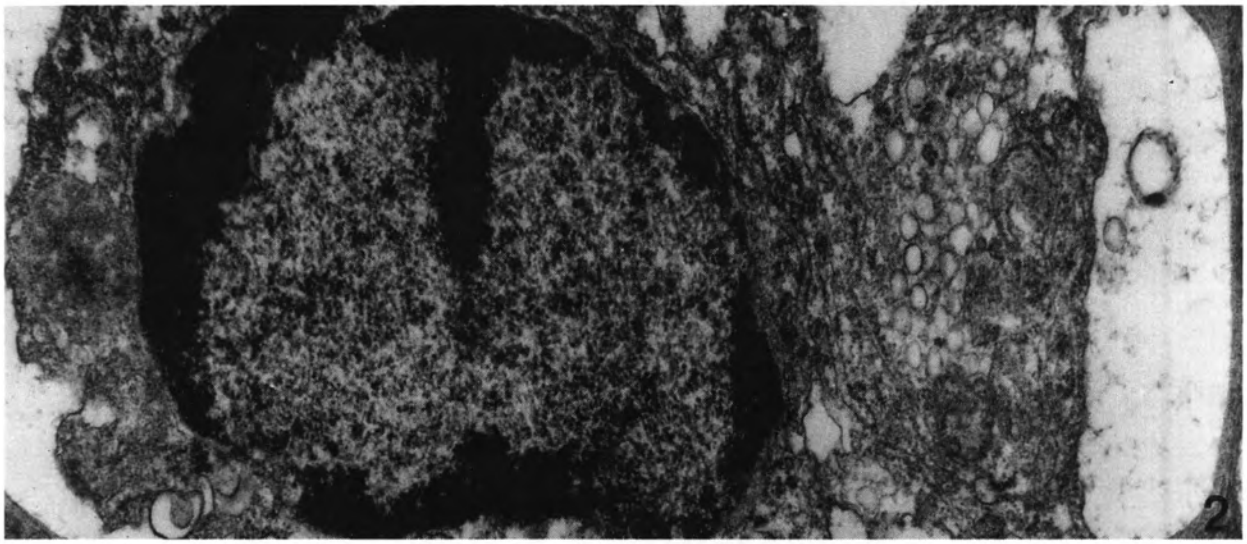
On the basis of changes in the position, shape and structure of a nucleus during *C. tomentosa* spermiogenesis, 10 phases were described similarly as in autoradiographic examinations as well as in cytochemical analyses of the basic nuclear proteins (Popłońska 2002) and in ultrastructural studies of *C. vulgaris* (Kwiatkowska, Popłońska 2002).

At the beginning of spermiogenesis (phases I-II) the structure of the nucleus is typical of meristematic cells. It differs only in having very little nucleoli (Fig. 1) which later (phase IV) disappear completely.

During phase I the big nucleus fills ca 2/3 of a cell and occupies the central position, condensed chromatin forms irregular clusters (Fig. 1). Starting from phase II the cell becomes polarised and the nucleus at first unchanged in ultrastructure is moved towards a side wall of a spermatid. Starting from phase III dense chromatin forms a ring adjacent to the nuclear envelope. Simultaneously the nucleus diminishes. Gradually (phases III and IV) loose chromatin surface decreases (Fig. 2). A protoplast sticks out from a cell side wall and in a space which appears later (during phase V), 2 flagellae surrounding a spermatid are seen.

During phase V the cell is significantly polarised and the nucleus adheres to plasmalemma over a great surface separated from it by the microtubular manchette (Fig. 3b) which accompanies the nucleus till the end of spermiogenesis elongating together with it. The nucleus with a dense layer of condensed chromatin is characterised by a small number of pores in a nuclear envelope and by thickening of an area occupied by loose chromatin. A thick substance the same as in RER cisternae found in the nuclear envelope in-



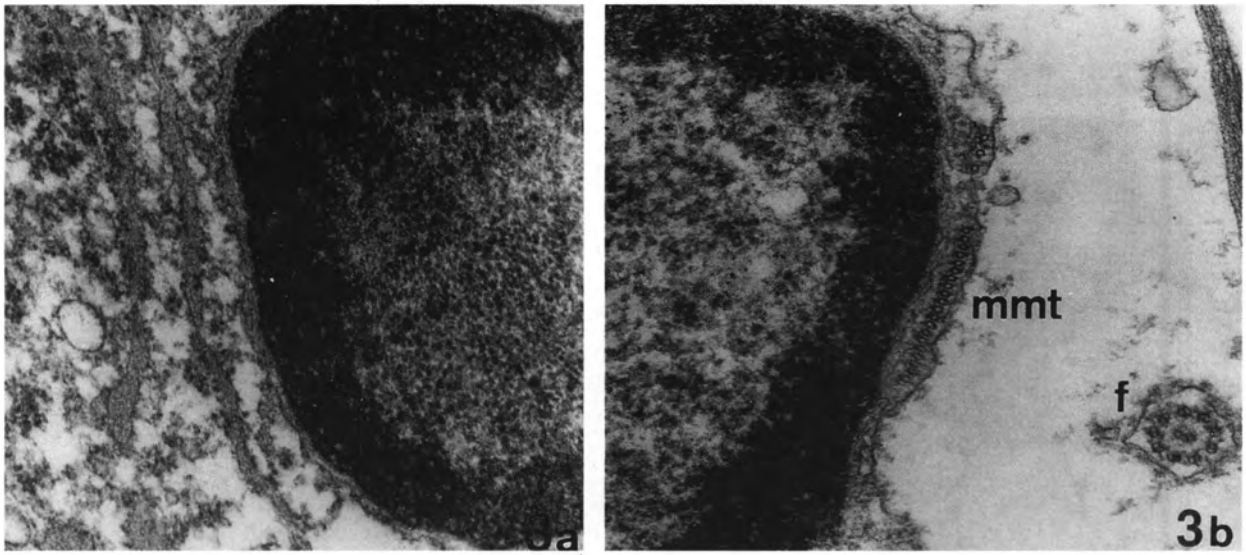
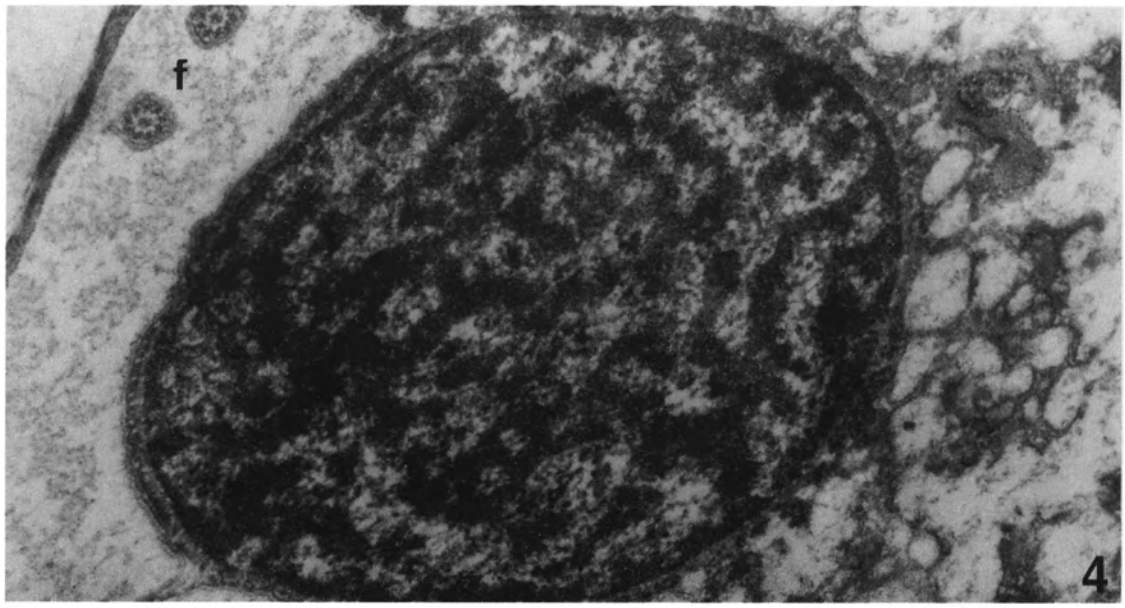
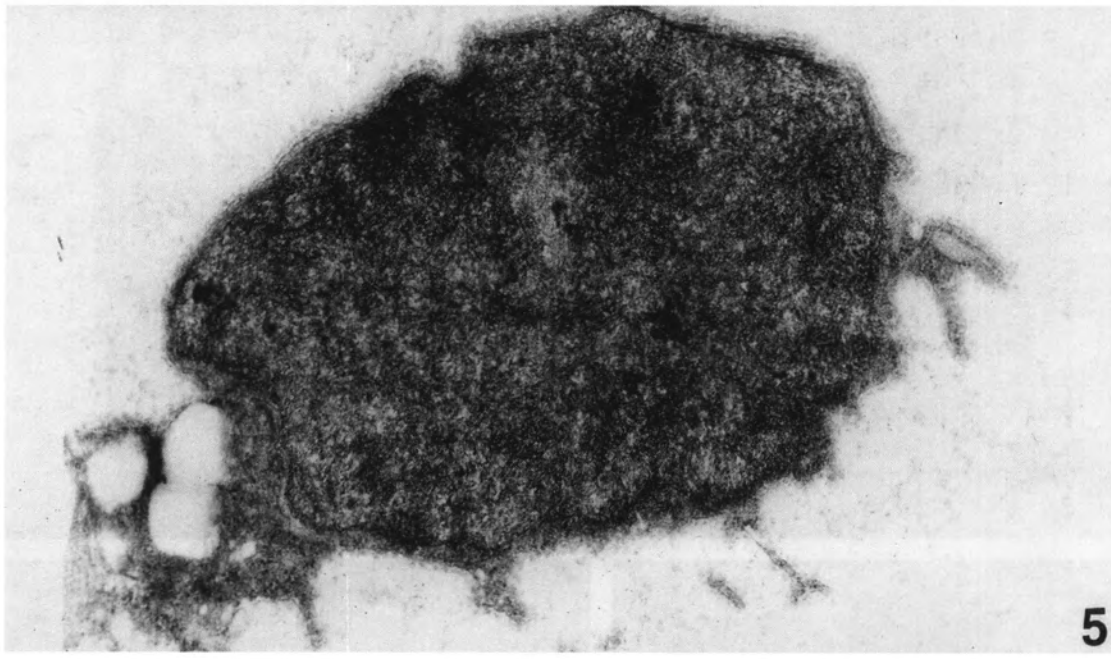




Fig. 1. *C. tomentosa* spermiogenesis phase I. Nucleus centrally positioned, dense chromatin in irregular clusters, ~×18 000.
Fig. 2. Spermiogenesis phase IV. Nucleus moved to a side wall, dense chromatin mainly adjacent to a nuclear envelope, ~×18 900.
Fig. 3a, b. Spermiogenesis phase V. a – intermembranous space of a nuclear envelope and ER filled with fine granular substance, b – microtubular manchette between a nuclear envelope and plasmalemma, ~×37 000.

Abbreviations to Figs 1-9
nu – nucleoli, microtubular manchette – m mt, f – flagella, S – plastid with starch.



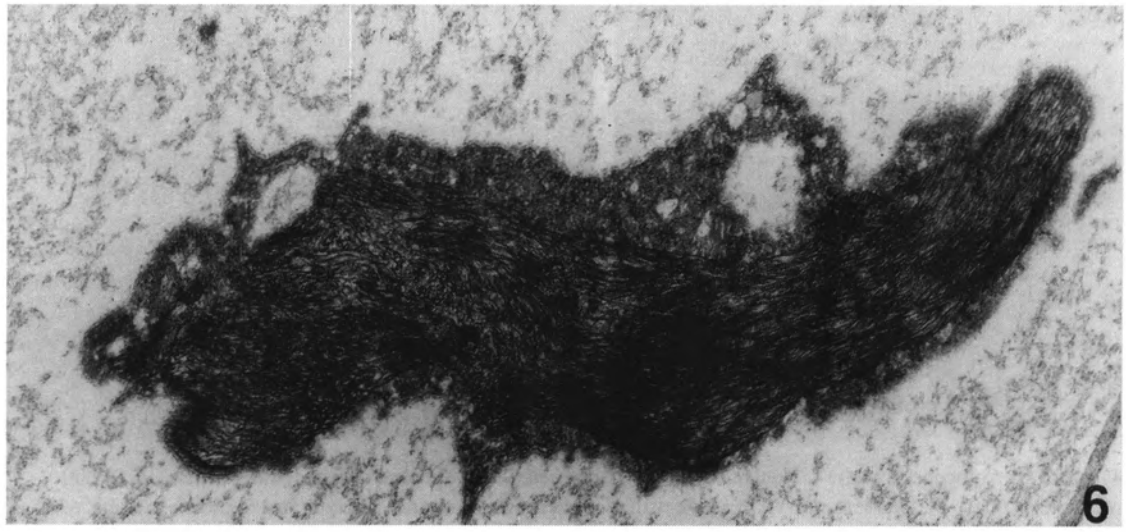




Fig. 4. Spermiogenesis phase VI. Dense chromatin forms a network, ~×33 000.
Fig. 5. Spermiogenesis phase VII. No dense chromatin. Short fibrils in bundles visible, ~×33 000.
Fig. 6. Spermiogenesis phase VIII. Fibrils mostly oriented parallelly to the long axis of a nucleus, ~×25 000.

termembranous space is also characteristic of this phase (Fig. 3a).

During phase VI there is a marked reorganisation of condensed chromatin which becomes a network (Fig. 4). Spermatids change visibly – cytoplasm shrinks and 2 flagellae appear. Spermatids contain plastids with big starch grains.

In the course of transition from phase VI to phase VII dense chromatin disappears gradually. The whole nucleus is uniformly grey (Fig. 5). Randomly positioned short fibrils forming boundless become more distinct in the nucleus. Clusters of dark homogenous substance are also found. This is a transitional form of the nucleus before it becomes typically generative.

During phase VIII in the crescent nucleus chromatin forms thin, long fibrils parallel to the long axis of the nucleus (Fig. 6).

In phase IX, when the nucleus forms 2 coils, thin fibrils aggregate into lamellar structures. They are best seen in cross-sections of the nucleus Fig. 7).

During phase X lamellae aggregate to form a compact, electron-dense mass. Thickening of a nuclear structure first appears around condensation centres and spreads outwards (Fig. 8). In a homogenous, very dark nucleus (Fig. 9) sometimes very thin spirals can be seen (Fig. 8).

In the mature spermatid a nucleus forms 2.5 coils. At the anterior there are mitochondria adhering to 2 flagellae at the posterior there are plastids with starch and mitochondria. The microtubular manchette runs along a spermatozoid adhering to the nucleus and plastids and in the anterior part of a spermatozoid to mitochondria.

Differentiation of spermatozoids in *C. tomentosa* is basically like that in *C. vulgaris* (Kwiatkowska 1996; Kwiatkowska, Popłońska 2002).

### *$^3$H-arginine and $^3$H-lysine incorporation into spermatids at consecutive spermiogenesis stages*

The analysis of a mean number of silver grains per spermatid ±SE shows that $^3$H-lysine and $^3$H-arginine incorporation during 2-min incubation is similar during phases I-V although cytoplasm and nucleus area gradually diminishes (Fig. 10). The statistically significant decrease in labelling is observed in phases VI and VII. Labelling in phases VIII-IX is very scarce (Fig. 10).

TABLE 1. Molecular masses (kDa) of protamine, histone, sperm nucleic acid proteins standards and basic proteins isolated from *Chara tomentosa* antheridial filaments identified by CE-SDS.

| | | Basic proteins in selected stage of antheridial maturity | | | | |
|---|---|---|---|---|---|---|
| Protein | | standards | early | medium | late | terminal |
| Protamines | 1 | 9.1 | | 9.1 | 8.7 | 9.1 |
| | 2 | 9.6 | | 9.5 | | 9.6 |
| | 3 | 11.1 | | | | 11.2 |
| Core histones | H4 | 12.5 | 12.5 | 12.4 | 12.4 | |
| | H2A | 14.2 | 14.1 | 14.1 | 14.1 | |
| | H2B | 15.0 | 14.7 | 14.7 | 14.7 | |
| | H3 | 16.5 | 15.5 | 15.5 | 15.5 | |
| H1 histone | H1a | 35.0 | 35.0 | 37.0 | 35.0 | |
| | H1b | 40.0 | 40.0 | 40.0 | 40.0 | |
| | H1c | | | 44.0 | | |

TABLE 2. Percentage participation of basic protein extracted from antheridial filaments of *C. tomentosa* in relation to all identified fractions.

| | | Basic proteins in selected stage of antheridial maturity | | | |
|---|---|---|---|---|---|
| Protein | | early | medium | late | terminal |
| Basic proteins | ChPlP1 | | 5.5 | 10.0 | 8.2 |
| | ChPlP2 | | 5.5 | | 77.3 |
| | ChPlP3 | | | | 9.3 |
| Core histones | H4 | 14.5 | 27.8 | 25.3 | |
| | H2A | 16.8 | 13.4 | 29.6 | |
| | H2B | 19.0 | 16.2 | 12.5 | |
| | H3 | 21.2 | 8.3 | 17.2 | |
| H1 histone | H1a | 12.2 | 6.0 | 1.9 | |
| | H1b | 10.4 | 4.1 | 3.5 | |
| | H1c | | 13.2 | | |

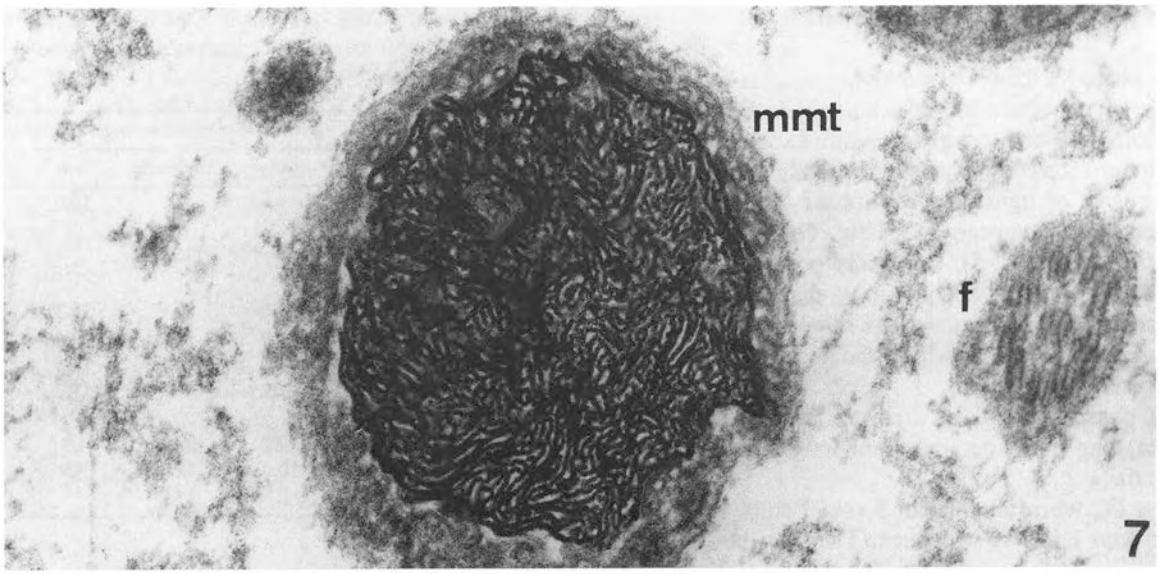
### *Capillary electrophoresis*

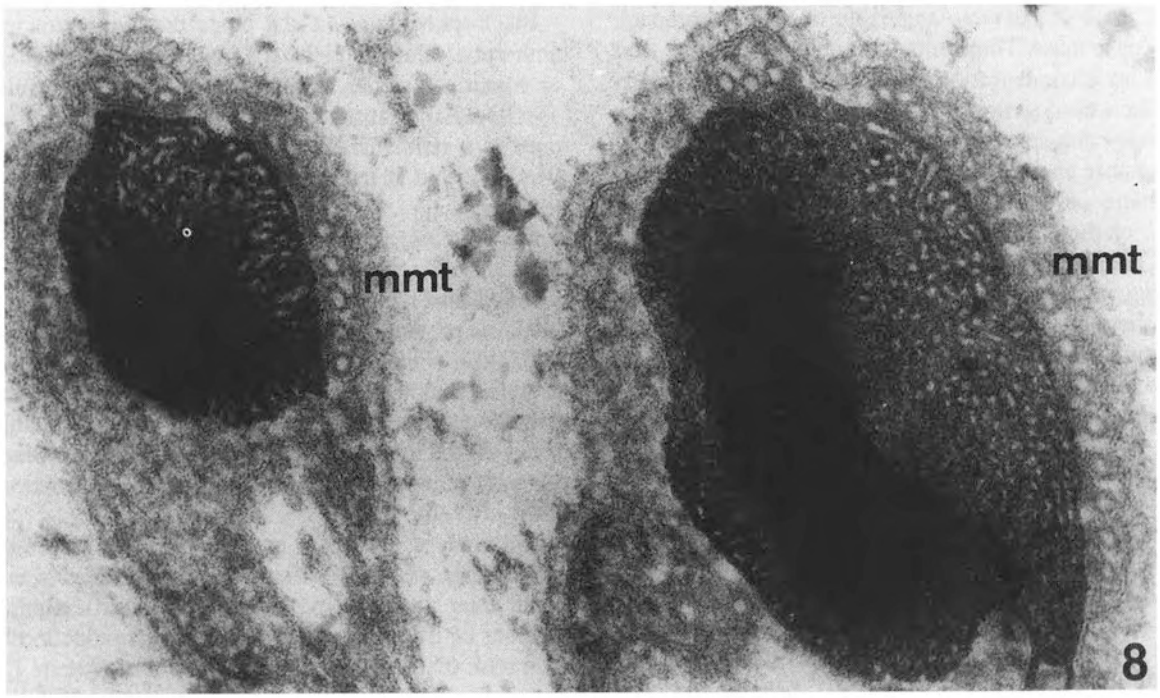
Electropheograms of the basic proteins extracted from antheridial filament cells of *Chara tomentosa* revealed, near somatic histones, protamine-like proteins (ChPlP) respectively migrating similarly to the salmon protamines used as a standard. No fraction of protamine-like proteins was observed at the early stage of spermiogenesis. At the medium stages of spermiogenesis extra two fractions of these proteins, with molecular masses about 9.1 and 9.5 kDa, were observed (Fig. 11, Table 1). The antheridial filament cells of the third population of antheridia (late) contained one protamine-like fraction with molecular mass about 8.7 kDa (Fig. 11, Table 1) Electrophoretic profiles of the proteins isolated from antheridia at a terminal stage of spermiogenesis contained three fractions of protamine-like proteins: the main one and two small protamine-like proteins with molecular masses 9.6 and 11.2, 9.1, respectively (Fig. 11, Table 1).

Although at the early, medium and late stages of spermiogenesis all the fractions of core histones were observed, their participation underwent modifications in the number of fractions and a significant in reduction of their size took place (Table 2). The amount of histone H4 fraction increased, about two fold at the late and medium stages of spermiogenesis and that of H2A only at the late stage. At the same time the proportion of H2B histone fraction became lowered while that of histone H3 decreased at the medium stage but increased at the late stage (Table 2). At the early and late stages of spermiogenesis two fractions of histone H1, with migration time and molecular masses similar to the histone H1 standard were determined (Fig. 1, Tables 1, 2) but at the late stage of spermiogenesis the fractions of histone H1 were very weak. The protein sample extracted from the spermatids during the medium period of spermiogenesis contained three fractions of histone H1 (Fig. 11, Tables 1, 2). The core and H1 histones prepared from spermatids of antheridia at the terminal stage were replaced by three fraction of protamine-like proteins (Fig. 11, Tables 1, 2).

## DISCUSSION

Ultrastructural studies of differentiating *C. tomentosa* spermatids revealed, similarly as in *C. vulgaris*, much grea-



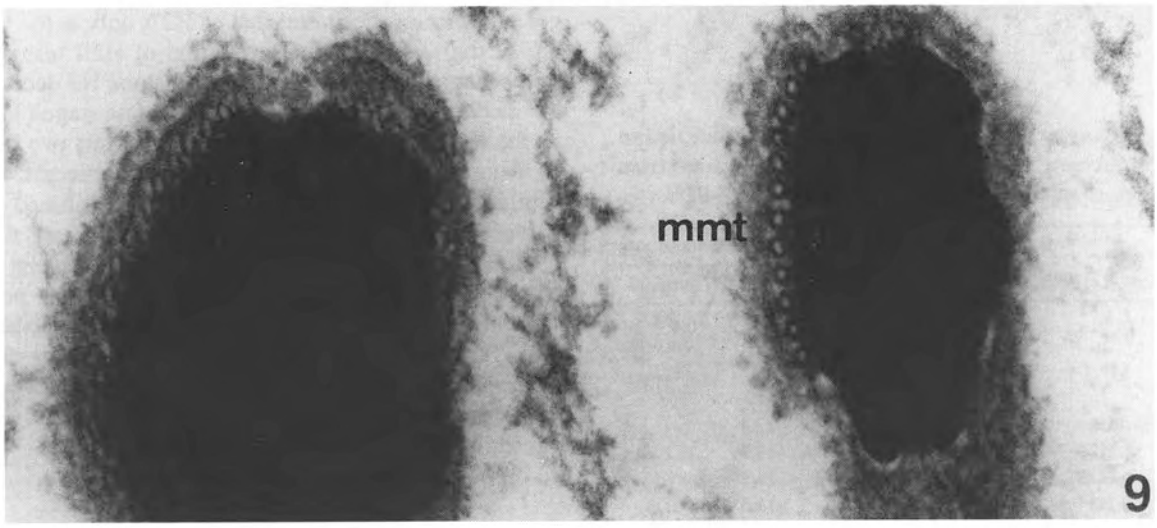




Fig. 7. Spermiogenesis phase IX. Cross section of an elongated, spiral nucleus. Chromatin forms lamelles, ~×102 000.
Fig. 8. Spermiogenesis phase IX\X. Nucleus with lamellar chromatin becoming extremely dense, ~×150 000.
Fig. 9. Spermiogenesis phase X. Cross section of a nucleus with extremely condensed chromatin, ~×134 000.

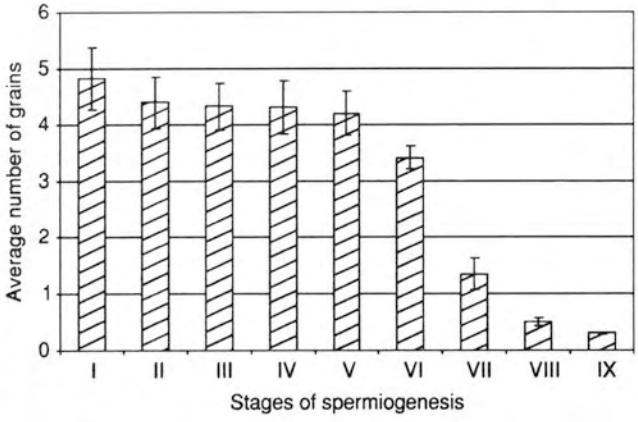



Fig. 10. $^{3}$H-arginine and $^{3}$H-lysine incorporation into spermatids at consecutive spermiogenesis phases.

ter variety of changes of the nuclear structure then those described by other authors (Pickett-Heaps 1968; Cocucci, Caceres 1976; Robert 1979). On the basis of chromatin structure, as well as of the shape and localisation of the nucleus, 10 phases of spermiogenesis were described. In electrophoretic studies on the basis of an estimated advancement of spermiogenesis 4 stages were distinguished: early, mid, late and terminal. Cytochemical analyses of basic nuclear proteins are useful in correlating biochemical and ultrastructural data. In these studies, similarly as in ultrastructural studies, 10 phases of spermiogenesis were distinguished on the basis of the same criteria (Popłońska 2002).

Cytochemical investigations have shown that during phases I-IV only histone-like proteins are found in the nucleus (Popłońska 2002). These phases correspond to the early stage in electrophoretic studies during which 4 fractions of core histones and 2 fractions of linker histones typical of somatic cells were distinguished. These data are also in agreement with nucleus ultrastructure typical of active cells apart from nucleoli which are small and lack a granular component. Autoradiographic studies show that these cells actively incorporate aminoacids: $^{3}$H-lysine and $^{3}$H-arginine. Probably, similarly as in *C. vulgaris*, demethylation of genes, which are inactive during proliferation and responsible for triggering differentiation occurs (Olszewska et al. 1997).

Mid spermiogenesis in electrophoretic studies corresponds to phases V and VI observed in cytochemical (Popłońska 2002) and ultrastructural investigations. During this period small amounts of proteins with electrophoretic mobility similar to that of salmon protamines appear. Simultaneously, linker histones undergo modification and the third fraction appears, 4 fractions of core histones are still observed. Cytochemical studies of phases V and VI also reveal, together with histones, protamine-like proteins (Po-

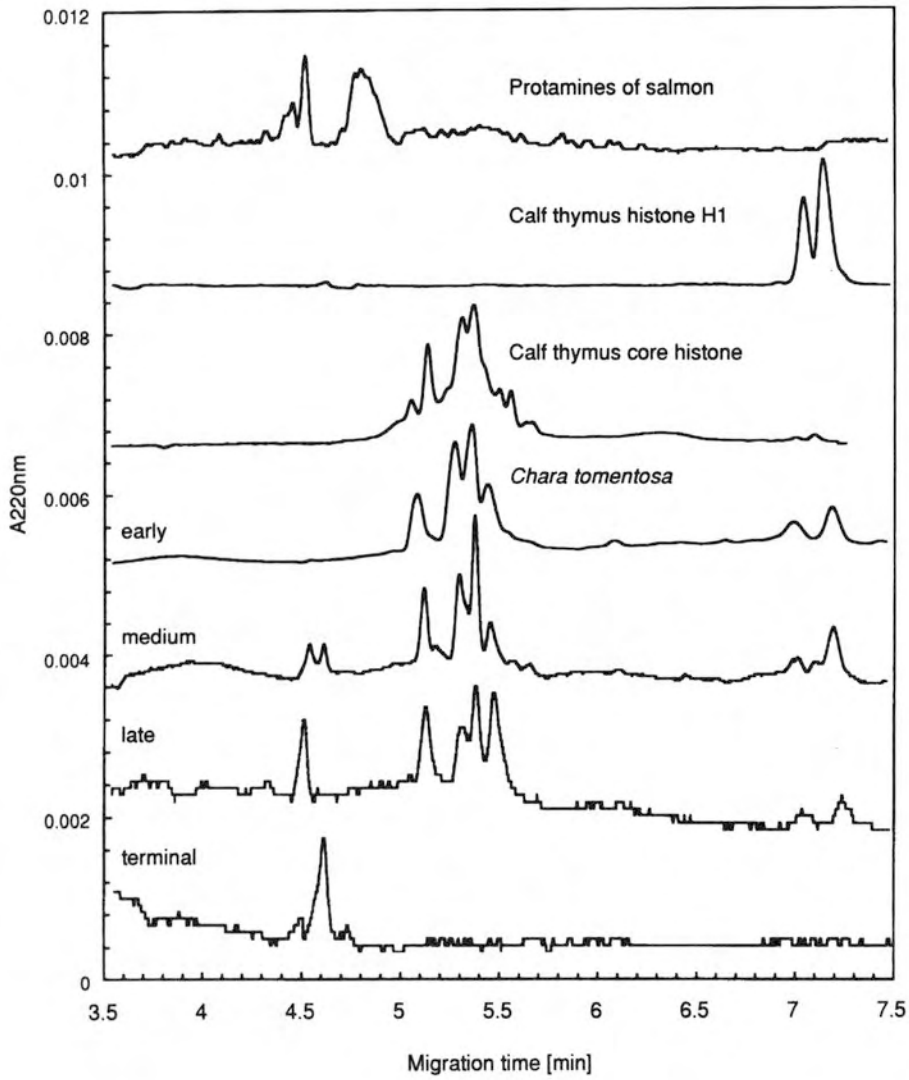



Fig. 11. Electropherograms of capillary electrophoresis in sodium dodecylsulfate (CE-SDS) of standards: salmon protamines, H1 histone, calf thymus core histones and basic proteins isolated from antheridial filaments of antheridia at: early, medium, late terminal stages of *Chara tomentosa* spermiogenesis.

płońska 2002). Ultrastructure also changes markedly during that period. While in phase V a condensed chromatin in the nucleus forms a thick layer adjacent to a nuclear envelope, in phase VI it undergoes complete reorganisation and forms a network. Similar network was observed during pre-prophase of antheridial filament cells in the course of their proliferation (Kwiatkowska, Maszewski 1978). It may be supposed that in both cases it is a preliminary state before deep ultrastructural changes of chromatin. It seems probable that these changes in nuclear ultrastructure are connected with a modification of linker histones observed in electropherograms of this stage. These observations also indicate that the appearance of a small amount of protamine-like proteins in the spermatid nucleus precedes the appearance of fibrillar structures characteristic of the generative nuclei.

During phases V and VI spermatids still actively incorporate basic aminoacids. It seems probable that it is connected with protamine-like protein synthesis in which RER may be involved (Kwiatkowska et al. in preparation).

Electrophoretic studies show that in late spermiogenesis the amount of protamine-like proteins nearly equals the maximum level reached in mature spermatozoids. Core histones are still present while the amount of linker ones diminishes. Late spermiogenesis corresponds to phases VII and VIII from cytochemical studies when strongly stained protamine-like proteins coexist with a positive but weaker staining of histone-like proteins (Popłońska 2002). In ultrastructural studies of these phases (VII and VIII) the nuclei completely lack dense chromatin. Disappearance of condensed chromatin may be correlated with the elimination of linker histones responsible for the arrangement of DNA fibrils into higher order structures (Denecke et al. 1994). Early disappearance of H1 is also characteristic of animal spermiogenesis (Gatewood et al. 1990; Gusse et al 1986).

The appearance of short randomly positioned fibrils in phase VII and long ones parallel to the spermatozoid axis in phase VIII that is in late spermiogenesis is a clear sign of chromatin reorganisation towards the generative state. During this phase spermatids incorporate basic aminoacids only very slightly.

The terminal stage distinguished during electrophoretic studies corresponds to phases IX and X in cytochemical studies (Popłońska 2002). Both studies clearly indicate that DNA is then bound only with protamine-like proteins whose 3 fractions: 9.1 kDa, 9.6 kDa, 11.2 kDa have been identified at these stages. Ultrastructural studies show that simultaneously with elimination of histones there takes place a gradual condensation of DNA throughout phase IX, when chromatin forms lamellae, to phase X, when it is extremely condensed. Sometimes very thin spirals can be seen similarly to *C. vulgaris* (Kwiatkowska, Popłońska 2002), which are probably DNA particles bound to protamines according to Ward's model (1994).

The results of *C. tomentosa* electrophoretic studies differ from those presented by Robert (1984) for *C. vulgaris* in which he showed histones together with protamines in mature spermatozoids. In *C. tomentosa* such electrophoretic results appear in late spermiogenesis. Fully matured spermatozoids derived from broken down antheridia contain only protamine-like proteins. Similar situation is observed in *Marchantia polymorpha* (Reynolds, Wolfe 1978). In mollusc *Murex brandaris* also only 3 small straight protamine molecules are present which cause an almost extreme packing of sperm DNA (Caceres et al. 1999). In man, however, ~10-15% of sperm cell proteins are histones associated with chromatin (Zalenskaya et al. 2000).

Electrophoretic analysis of *C. tomentosa* shows as well that the replacement of histone proteins with protamine-like proteins is not connected with transitory proteins which are fairly common in various animal organisms (Wouters-Tyrou et al. 1998; Steger 1999) and were also observed in *Marchantia polymorpha* (Rynolds, Wolfe 1978).

The data presented in this paper show that spermiogenesis of algae *C. tomentosa* is in many respects similar to animal spermiogenesis as far as biochemical and structural changes of nuclear chromatin are concerned. However, there are also some features specific for *C. tomentosa*: subtle remodelling of nuclear ultrastructure preceding main processes leading to the appearance of extremely condensed chromatin characteristic of male gametes of many plant and animal organisms.

## ACKNOWLEDGEMENTS

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## ULTRASTRUKTURALNE, AUTORADIOGRAFICZNE I ELEKTROFORETYCZNE BADANIA PROCESU SPERMIOGENEZY U *CHARA TOMENTOSA*

### STRESZCZENIE

Ultrastruktura jądra spermatyd zmienia się wielokrotnie podczas spermiogenezy. W stadiach I-II chromatyna skondensowana występuje w postaci nieregularnych skupień, w stadiach III-V – ciągłego pierścienia przy otoczce jądrowej, a w stadium VI – sieci wypełniającej całe jądro. W zaawansowanej spermiogenezie chromatyna zwarta zanika i w jądrze pojawiają się krótkie, nieuporządkowane fibryle (stadium VII), następnie długie fibryle ułożone równolegle (stadium VIII), które w stadium IX łączą się w lamelle. W dojrzałych spermatozoidach (stadium X) chromatyna przyjmuje postać maksymalnie skondensowaną.

Włączanie $^{3}$H-argininy i $^{3}$H-lizyny do spermatyd w ciągu 2 min inkubacji jest intensywne w stadiach I-V, zmniejsza się w stadiach VI oraz VII i ma charakter śladowy w stadiach VIII-IX.

Metodą elektroforezy kapilarnej wykazano, że w spermiogenezie *Chara tomentosa* następuje wymiana histonów na białka zasadowe o porównywalnej mobilności do protamin łososia. Na początku spermiogenezy w spermatydach występują histony rdzeniowe i łącznikowe. We wczesnej spermiogenezie pojawiają się białka typu protamin, których ilość zwiększa się w późnej spermiogenezie, gdy jeszcze obecne są histony rdzeniowe. W dojrzałych spermatozoidach występują jedynie białka typu protamin reprezentowane przez 3 frakcje: 9,1 kDa, 9,6 kDa i 11,2 kDa. Zanikanie histonów łącznikowych po ich uprzedniej modyfikacji następuje wcześniej niż zanikanie histonów rdzeniowych.

SŁOWA KLUCZOWE: *Chara tomentosa*, spermiogeneza, histony, białka typu protamin, ultrastruktura, autoradiografia, elektroforeza kapilarna.