Reprinted from *Br. phycol. J.* **12**: 23–44 1 March 1976

ULTRASTRUCTURE OF MEIOSIS IN *CHLAMYDOMONAS REINHARDTII*

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Ultrastructural details of meiosis in *Chlamydomonas reinhardtii* are presented. Zygotes undergo a resting stage prior to meiosis, during which a multi-layered zygote wall is deposited. Basal bodies reappear at interphase. Heterochromatin is not visible in the interphase nucleus. Leptotene is characterized by the appearance of axial cores and the condensation of chromonemata. By early zygotene, axial elements associated with the condensing chromatin associate with the nuclear envelope. Tubular elements appear in the perinuclear space adjacent to the associated chromosomes. Bivalents begin to associate with the nucleolus. By pachytene, typical synaptinemal complexes have formed. The synaptinemal complexes possess two lateral elements associated with the chromosomes and separated by a central space of about 130 nm. A central element may form midway between the lateral elements and be joined to them via transverse elements. Separated synaptinemal complexes appear in the cytoplasm by diplotene–diakinesis. At this stage the chromosomes become most condensed and chiasmata occur. The nucleolus disappears. The later stages of meiosis are described. By metaphase I the nuclear envelope is fenestrated and a spindle develops. Daughter nuclei reform at telophase. Basal bodies are associated with a ribosome-free zone adjacent to the nuclear membrane. The interzonal spindle degenerates. Cleavage occurs via a phycoplast. At the initiation of the second division, basal bodies are present at the incipient cleavage furrow. Basal body replication appears to occur at pre-prophase. By metaphase II, the basal bodies migrate to the poles of the fenestrated spindle. Completion of the second meiotic division results in the formation of haploid zoospores.

Cytological aspects of meiosis in *Chlamydomonas* have been incompletely understood mostly because of technical difficulties in germinating and adequately fixing the zygote. Once karyogamy occurs, the zygote enters a resting stage. In nature, this serves as an over-wintering state. Zygotes have been induced to germinate in the laboratory (Levine & Folsome, 1959). In 1956, Schaechter & DeLamater studied meiosis in *Chlamydomonas* at the light microscopic level. Zygote germination in their cultures was not synchronous, and this difficulty complicated the procedure of accurately documenting the sequence of events in meiosis. In 1959, Levine and Folsome re-examined meiosis using synchronous cultures and followed the meiotic process using Azure B staining and the Feulgen reaction to visualize the chromosomes. Levine & Folsome (1959) noted that meiosis is initiated about 10 h after the zygotes are placed in the light. Cleavage results in the four-cell stage by the twelfth hour. These investigators reported a haploid chromosome number of eight confirming the earlier values reported by Buffaloe (1958) and Kater (1929).

Owing to extreme impermeability of the zygote wall to fixatives, it has not been feasible to demonstrate meiosis at the ultrastructural level. Using a fortuituous pre-treatment of zygotes with trypsin, we were able to preserve

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successfully ultrastructural details of meiosis in *C. reinhardtii*. This study will document these stages of meiosis in *Chlamydomonas* and compare them with meiosis of other algae, plants and animals.

MATERIALS AND METHODS

The cells of *Chlamydomonas reinhardtii* Dangeard were derived from isolates (137+ and 137−) made by Dr Gilbert Smith (Amherst, Mass. collection) and provided by Dr N. Gillham at Duke University. The culture media used to maintain the cells are similar to those used by Sueoka (1960) (Table I). Cells were grown on agar at 20°C under a 16/8 h light/dark cycle at 1500–2000 lx provided by 40 W cool fluorescent bulbs.

Gametes of *C. reinhardtii* were obtained by washing 12–20 day old vegetative cells from agar plates with sterile induction medium (Table I). The cells were then placed in sterile Petri dishes filled with about 35 ml of induction medium, and placed in the light (2500 lx) for 10 h.

Active gametes of opposite mating types were then mixed in approximately equal numbers. Mating occurs within minutes after mixing. To obtain zygotes, the gametes were allowed to mate for 30 min. The planozygotes formed by this time were then transferred to agar plates containing zygote maturation medium (Table I). These plates remained in the light for an additional 18 h and were then placed in the dark for 6 days at room temperature (23°C). After this, they were transferred to fresh agar plates containing growth medium and placed in the light (2500 lx) at room temperature (23°C). Zygotes began to germinate approximately 10–12 h after being placed in the light.

Because of the impermeability of the zygote wall to fixatives, the zygotes were treated with 0·3% trypsin (Sigma, 3X crystallized from bovine pancreas; no. T-6882) made up in *C. reinhardtii* induction medium (Table I) adjusted to pH 7·8 with 1 N NaOH. The treatment time was determined by checking the effect of the trypsin on the penetration of 0·1% acridine orange (Chroma-gesellschaft, Schmidt Co.; Stuttgart–Untertürkheim, no. 10010), which does not penetrate untreated zygotes. The zygotes were placed in the trypsin solution at room temperature and samples were removed periodically at 30 s intervals. After 1 min of trypsin treatment, slight fluorescence could be seen in the cytoplasm adjacent to the zygote wall. A Zeiss Photomicroscope II equipped with accessories for fluorescence microscopy was used in this procedure. Only zygotes treated by this procedure were fixed adequately enough to allow ultrastructural observations. Following trypsin treatment, zygotes were washed three times in ice cold induction medium (Table I) to which a few drops of 2% glutaraldehyde (Serva) had been added.

Zygotes were fixed at 30 min intervals for 5 h beginning 9 h after they were introduced to the light. Fixation was in 2% glutaraldehyde (Serva) in *C. reinhardtii* induction medium (Table I) for 2 h at 5°C in the dark. This was followed by three successive washes in cold induction medium (Table I) at 15 min each. Cells were post-osmicated in 2% OsO_4 made up in induction medium (Table I) for 2 h at 5°C in the dark. A 5 min wash in distilled H_2O followed osmication and the cells were then embedded in 2% agar. Dehydration and infiltration were carried out as described for *C. moewusii* Gerloff (Triemer & Brown, 1974).

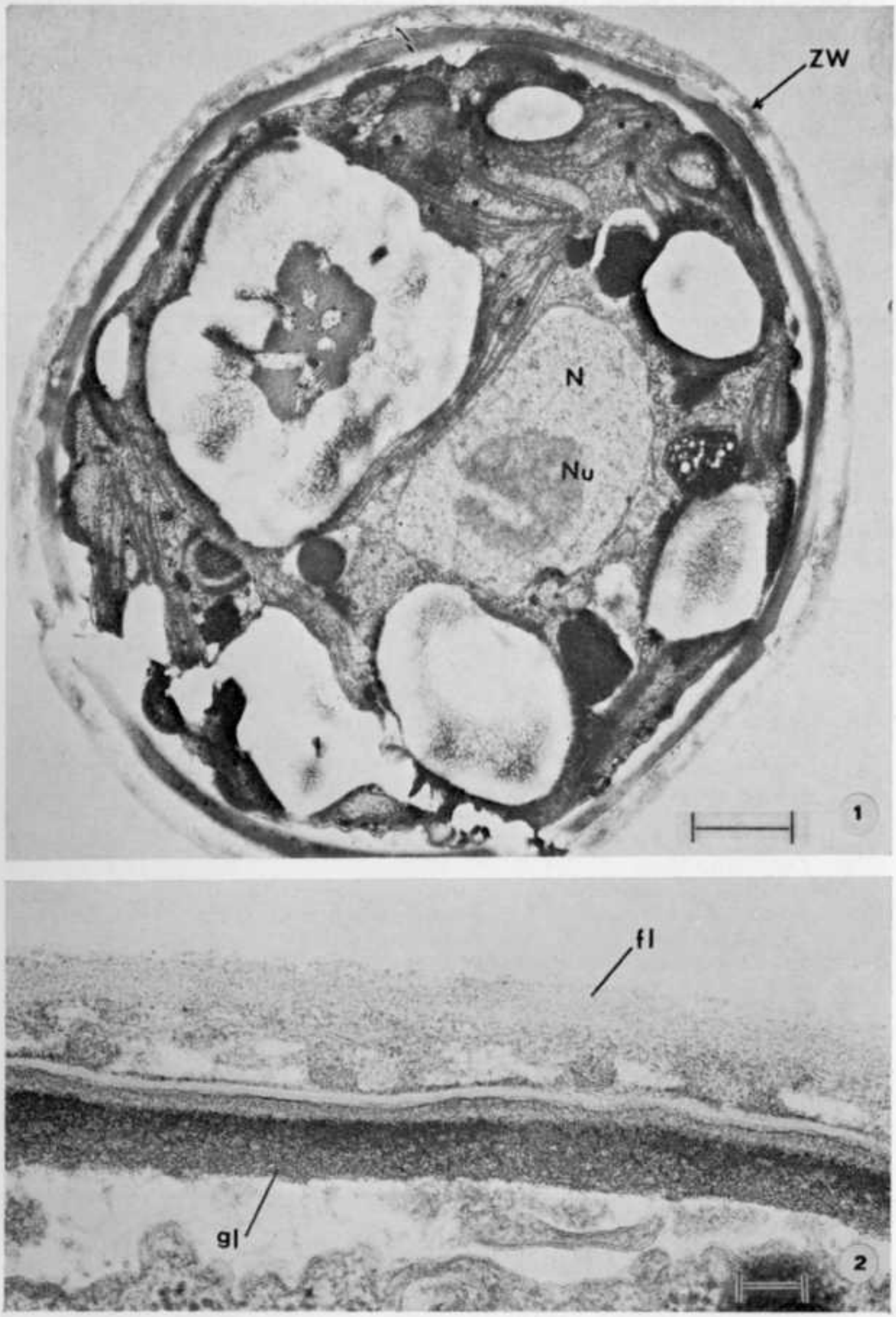
Silver sections were cut for electron microscopy on a Reichert OM U2 ultramicrotome with a Dupont diamond knife, and mounted on Formvar-carbon coated grids. The sections were post-stained with 2% aqueous uranyl acetate for 15–30 min at room temperature, followed by staining for 15 min in Reynold's lead citrate, also at room temperature. Material was examined on a Hitachi HU 11 E electron microscope at 75 kV.

OBSERVATIONS

Eighteen hours after mating, zygotes were placed in darkness on minimal media (Table I). The cells were then returned to the light and placed on fresh minimal media. Meiosis was initiated 10–14 h later.

During the dark period, a thick secondary zygote wall develops (Fig. 1). The thickness and stratification of the wall varies among zygotes (Fig. 2). The innermost layer is extremely dense and granular, while the outermost layer appears less dense and fibrillar (Fig. 2). Intermediate layers vary in density and thickness (Fig. 2).

Successful fixation depends upon rapid and complete penetration of the fixative. The trypsin treatment apparently degrades the zygote wall to a degree



FIGS 1, 2. Interphase zygote of *C. reinhardtii*. Fig. 1. Six-day-old premeiotic zygote. Note absence of heterochromatin, prominent pyrenoid and abundant starch in the chloroplast. N, nucleus; Nn, nucleolus; ZW, zygote wall. Scale = 1 μm. Fig. 2. Zygote cell wall. Note dense granular inner layer (gl) adjacent to cytoplasm, outer fibrillar layer (fl) and intervening layers of variable density and thickness. Scale = 0·1 μm.

sufficient to allow penetration of the fixative. The outermost wall layer appears most affected by the trypsin treatment (Fig. 1). Other layers may be degraded, but they are not significantly different at the ultrastructural level.

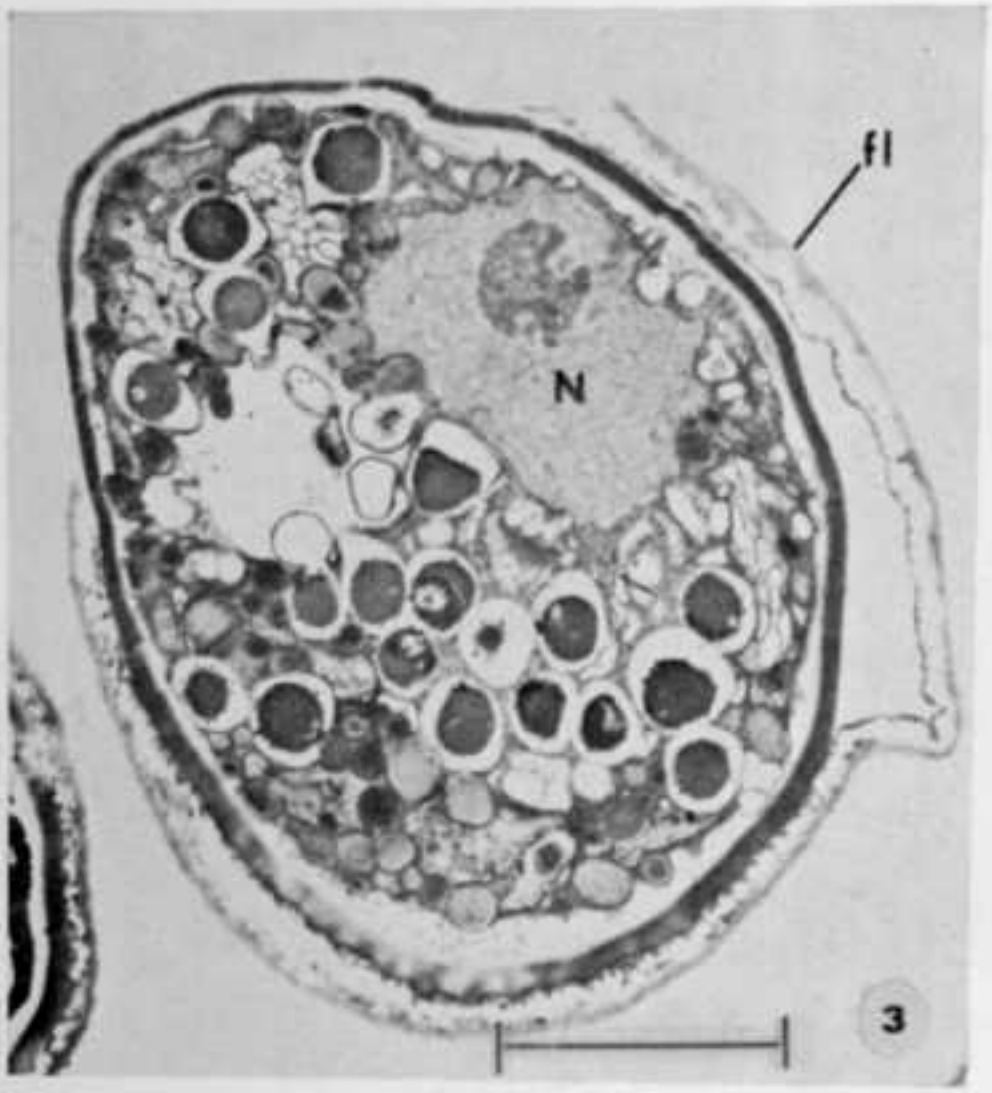
Meiosis will be described in terms of the classical stages. It is important to understand that only a relative approximation to a stage is possible since the chromosomes are too small to analyse at the light microscopic level and because of batch differences in zygote synchronization.

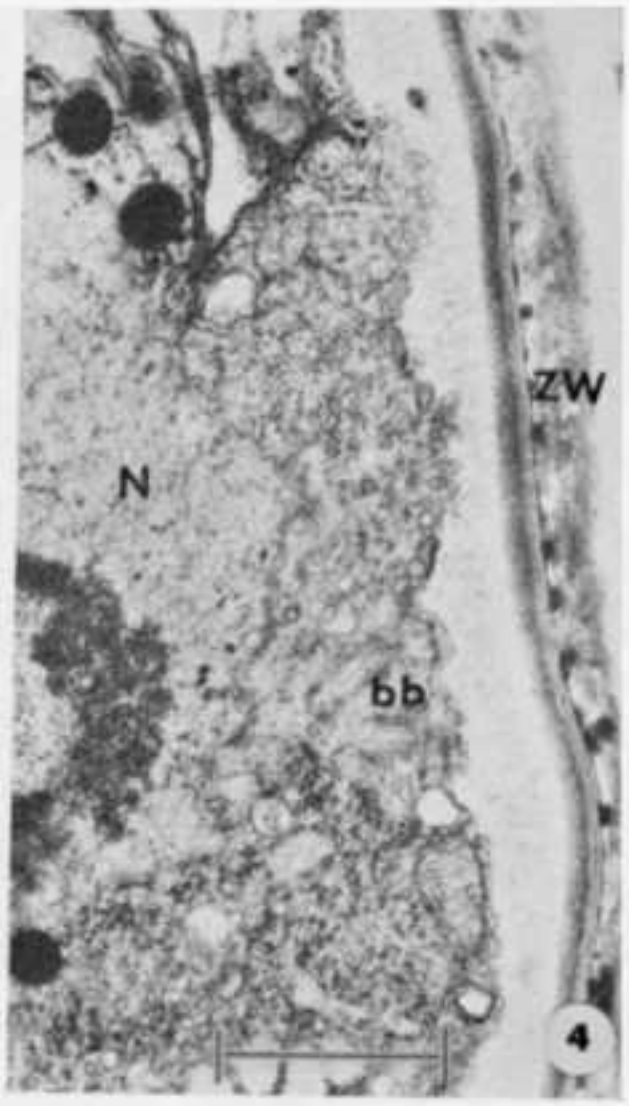
TABLE I. *Chlamydomonas reinhardtii*: Stock Reagents
(Sueoka, 1960; Gillham, Boynton & Burkholder, 1970)

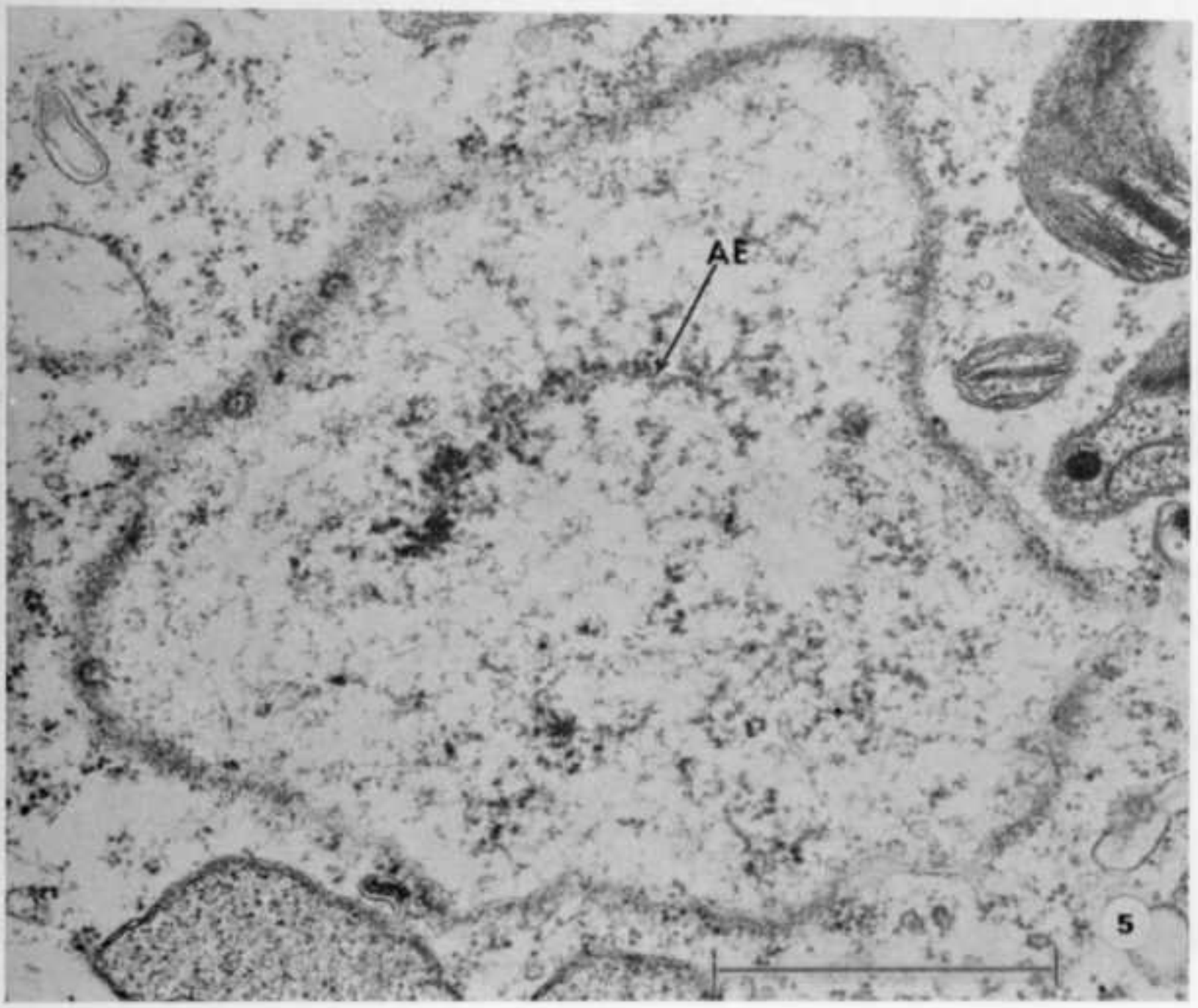
Beijerinck's solution no. 1	
NH_4NO_3	1·00 g
K_2HPO_4	0·04 g
$MgSO_4 \cdot 7\,H_2O$	0·04 g
$CaCl_2 \cdot 2\,H_2O$	0·02 g
Deionized H_2O	1000·00 ml
Beijerinck's solution no. 2	
NH_4Cl	10·00 g
$MgSO_4 \cdot 7\,H_2O$	0·40 g
$CaCl_2 \cdot 2\,H_2O$	0·20 g
Deionized H_2O	1000·00 ml
Phosphate buffer (pH 6·8, refrigerate)	
K_2HPO_4	14·34 g
KH_2PO_4	7·26 g
Deionized H_2O	1000·00 ml
C. reinhardtii growth medium (to prepare 1 litre)	
Beijerinck's solution no. 2	50 ml
Phosphate buffer	50 ml
Hutner's trace elements (Hutner et al., 1950)	1·0 ml
Na acetate	2·0 g
Deionized H_2O	900 ml
Difco Bactoagar	15 g
C. reinhardtii induction medium (to prepare 1 litre)	
Beijerinck's solution no. 2 (without NH_4Cl)	50 ml
Phosphate buffer	50 ml
Hutner's trace elements (Hutner et al., 1950)	1·0 ml
Na acetate	2·0 g
Deionized H_2O	900 ml
C. reinhardtii zygote maturation medium (to prepare 1 litre)	
Beijerinck's solution no. 1	50 ml
Phosphate buffer	50 ml
Hutner's trace elements (Hutner et al., 1950)	1·0 ml
Deionized H_2O	900 ml
Difco Bactoagar	40 g

PREMEIOTIC INTERPHASE

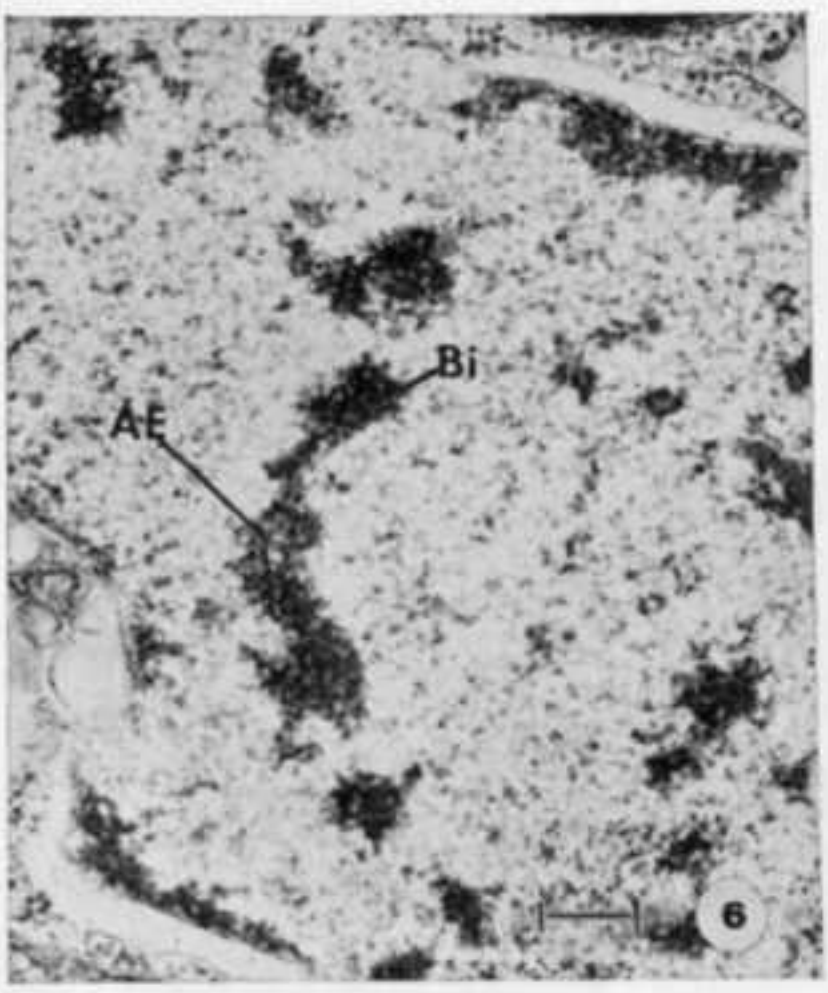
The interphase or resting zygote is densely granular and starch is abundant in the dormant chloroplast (Fig. 1). Heterochromatin is not visible in the premeiotic nucleus; however, a prominent nucleolus is present. Few mitochondria, ER membranes and dictyosomes are evident. Frequently, the outermost zygote wall layer is shed at the onset of meiosis, leaving only the dense inner wall layer surrounding the zygotes (Fig. 3). Basal bodies reappear in the zygote at interphase or early leptotene (Fig. 4).

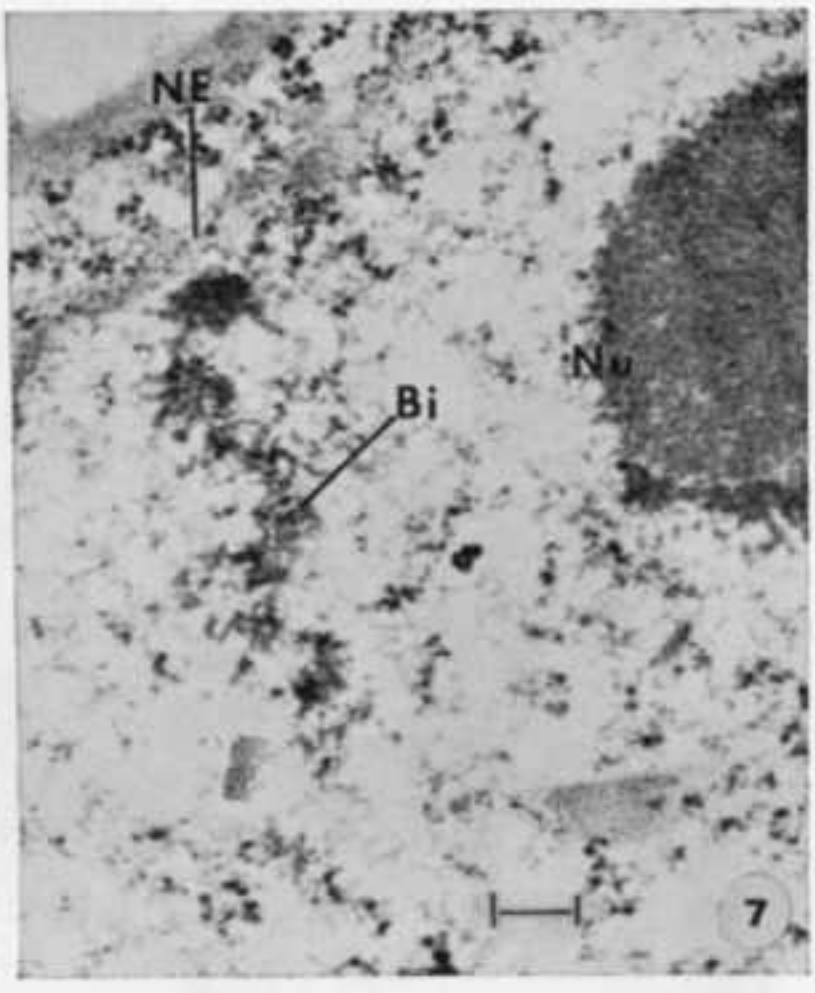


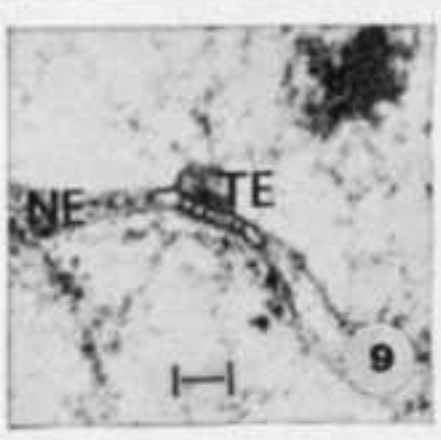




FIGS 3–5. Interphase–Early leptotene. Fig. 3. Note abscission of outer fibrillar layer (fl) of the zygote wall which occurs most frequently prior to leptotene. N, nucleus. Scale = 1 μm. Fig. 4. Early formation of basal bodies (bb) prior to meiosis. N, nucleus; ZW, zygote wall. Scale = 1 μm. Fig. 5. Grazing section of the nucleus at leptotene showing condensing chromatin and appearance of the axial element (AE). Scale = 1 μm.







FIGS 6–9. Leptotene–Zygotene. Fig. 6. Note dense axial element (AE) and continued condensation of chromatin. Bi, bivalent. Scale = 0·1 μm. Fig. 7. Early zygotene stage in which bivalents (Bi) associate with the nuclear envelope (NE). Nu, nucleus. Scale = 0·1 μm. Fig. 8. Midzygotene, illustrating further condensation of bivalents (Bi). Note tubular elements (TE) present in the perinuclear space associating with the chromosomes at the nuclear envelope. Scale = 0·1 μm. Fig. 9. Cross-section of tubular elements (TE) bridging perinuclear space and associating with the chromatin. NE, nuclear envelope. Scale = 0·1 μm.

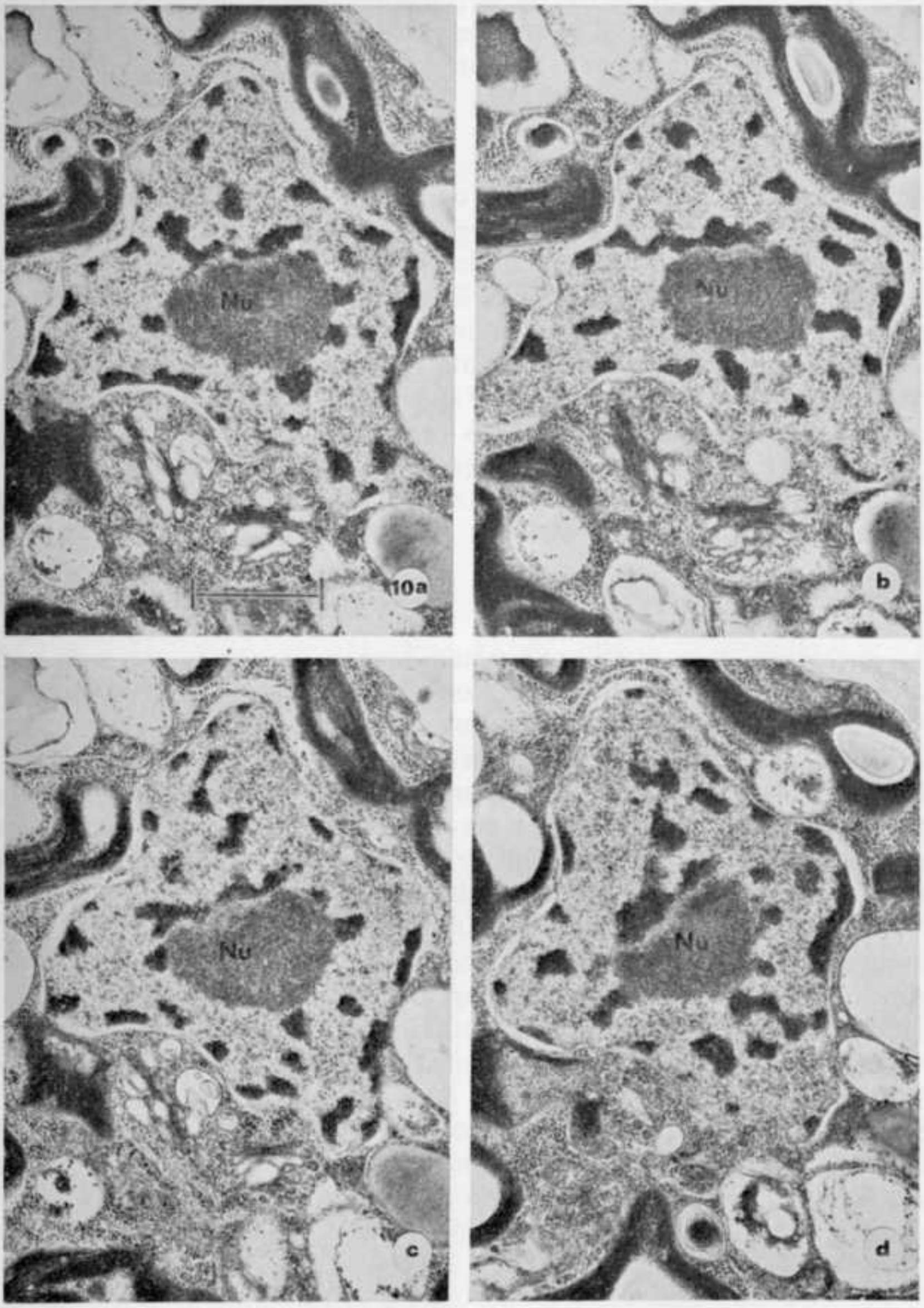


FIG. 10(a)–(d). Serial sections through zygotene nucleus. Note continuity of chromosomes between nuclear envelope and nucleolus (Nu). Scale = 1 μm.

LEPTOTENE

Fine axial cores appear in the leptotene chromosomes as they begin to condense in the nucleus (Fig. 5). By late leptotene or early zygotene distinct cores are present in the homologues (Fig. 6).

ZYGOTENE

Axial elements of the condensing chromatin can be observed to associate with the nuclear envelope by early zygotene (Fig. 7). Tubular elements (25 nm in diameter) are present in the perinuclear space in the region of chromosome contact at the nuclear envelope (Fig. 8). In cross-section the tubular structures appear as short rods bridging the perinuclear space (Fig. 9).

During zygotene the bivalents remain attached to the nuclear envelope and seem to associate with the nucleolus [Fig. 10(a)–(d)]. Frequently a dense fibrillar material (Fig. 11) or a dense layer is present between the nucleolus and the chromosome (Fig. 12). The narrow dense layer (Fig. 12) is approximately one-fifth (6·5 nm) the diameter of the central element of the synaptinemal complex visualized during pachytene.

PACHYTENE

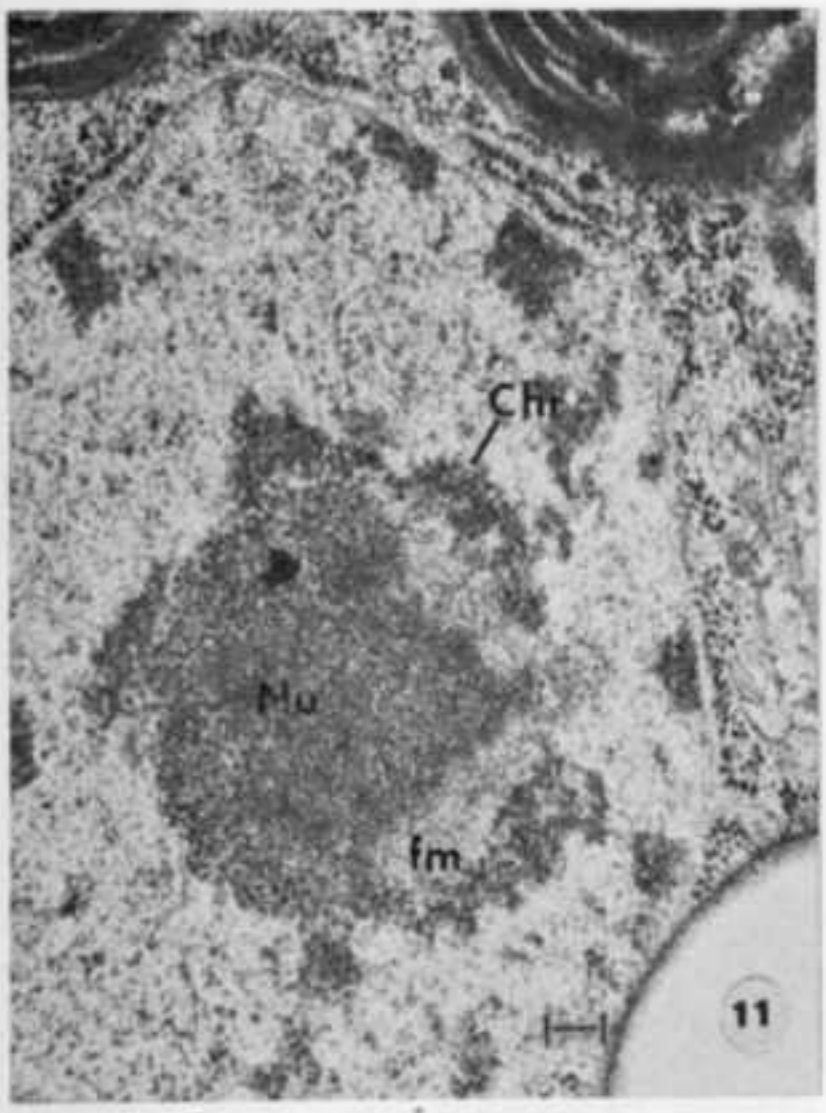
By pachytene, fully developed synaptinemal complexes are seen and the bivalent chromatin becomes further condensed. In this stage the chromosomes still remain attached to the nuclear envelope, though tubular elements are no longer present in the perinuclear space.

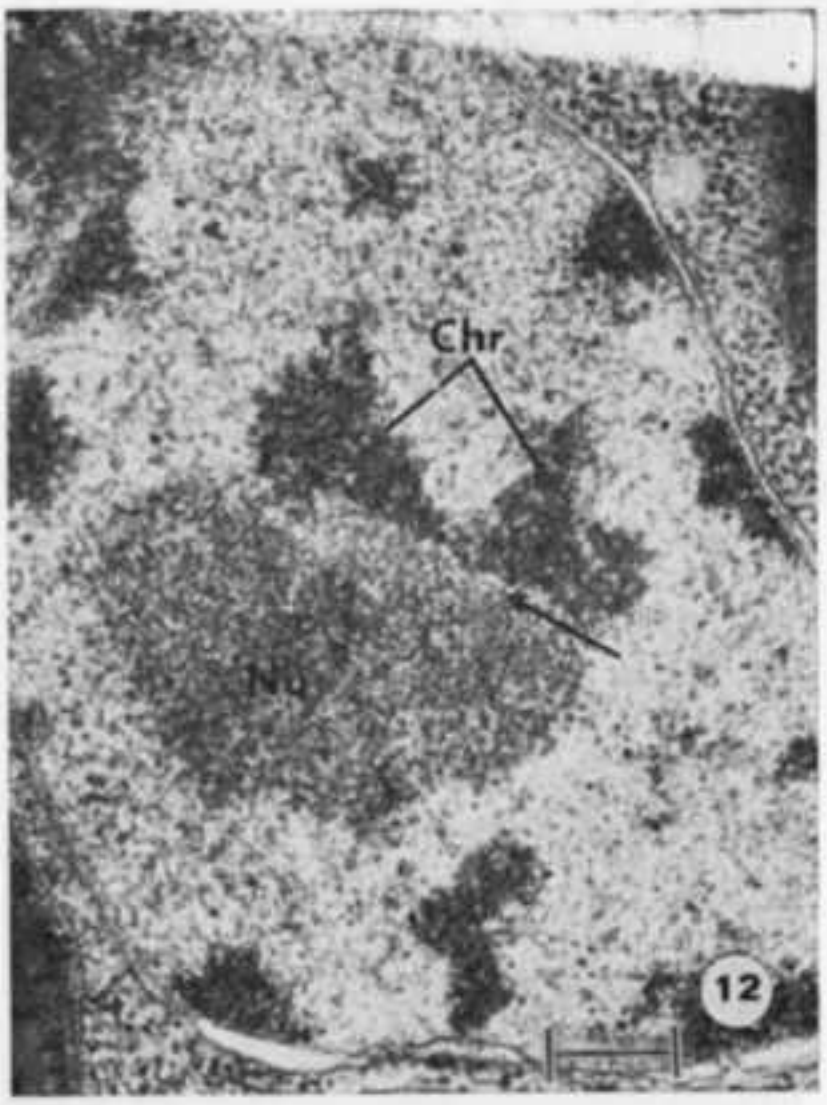
The synaptinemal complex in longitudinal section is shown in Figs 15 and 16. It is constructed of two lateral elements separated from one another by a space of approximately 125–135 nm (Fig. 19). A 30–40 nm central element, possibly composed of a series of stacked disc-like components, lies midway between the lateral elements [Figs 14, 17, 18(b)]. Transverse elements (approximately 7 nm) extend between the lateral elements and the central element (Fig. 14). In cross-section, the synaptinemal complex appears as a dense ring of chromatin surrounding a dense central element with the lateral elements adjacent to the chromatin (Fig. 17). Synaptinemal complexes in both tangential and longitudinal section are shown in Fig. 16.

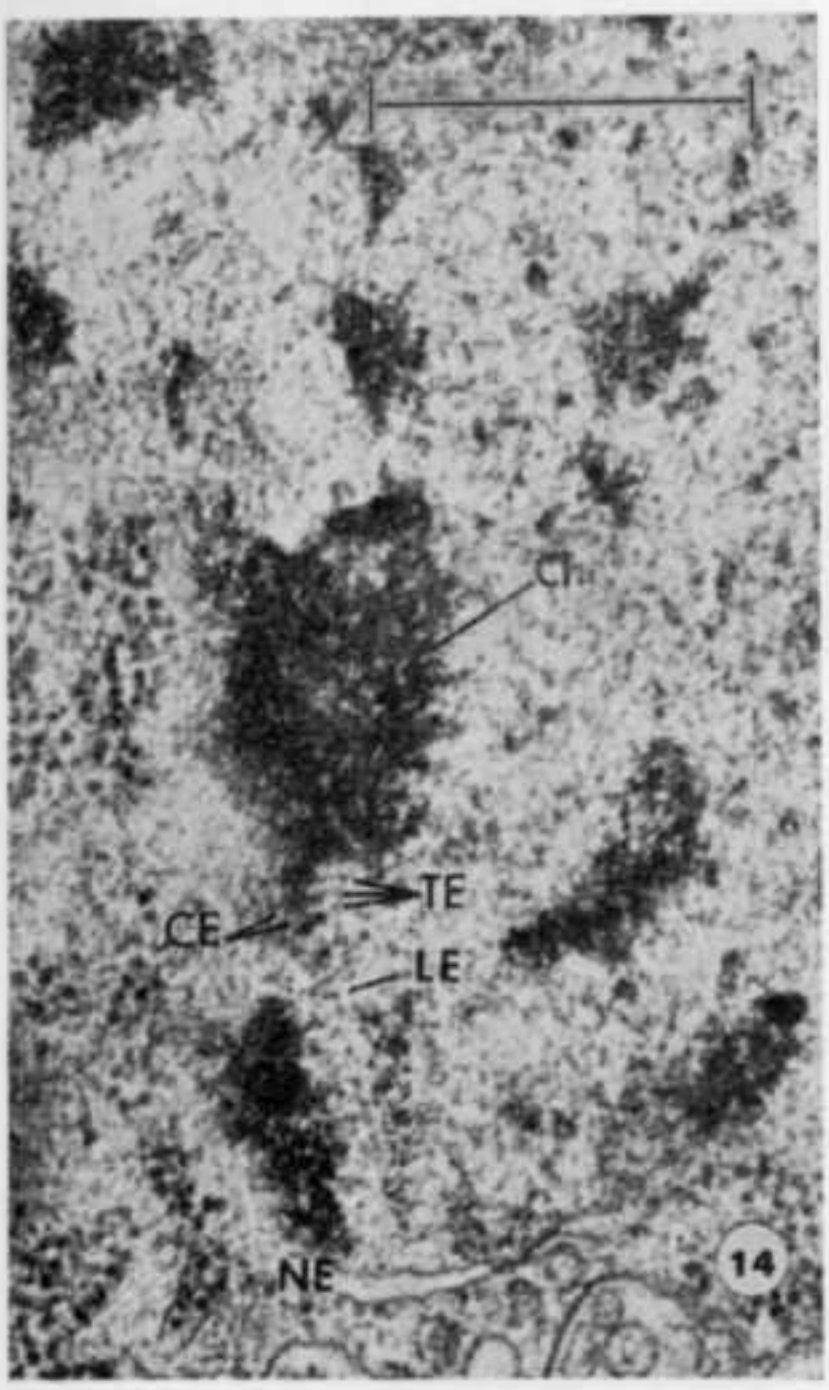
Serial sections demonstrate that most, if not all, of the chromosomes are attached to the nuclear envelope during pachytene. At the attachment site the synaptinemal complex is embedded in a dense matrix [Fig. 18(a), (b)]. A diagrammatic interpretation of the synaptinemal complex in *Chlamydomonas* is presented in Fig. 19.

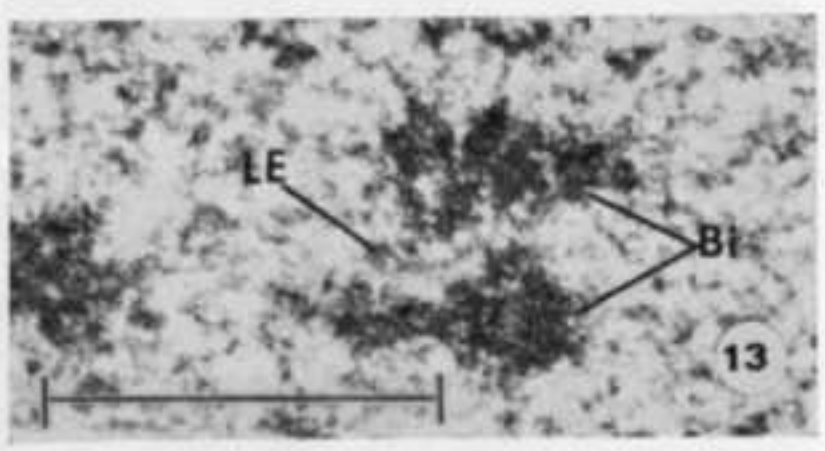
DIPLOTENE–DIAKINESIS

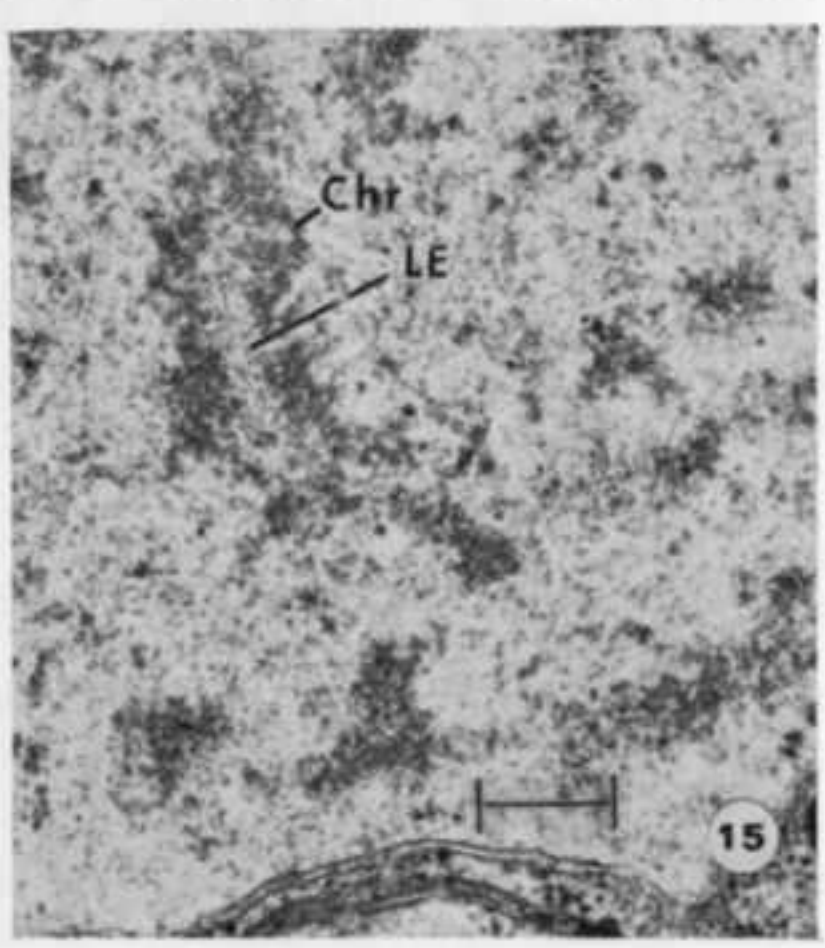
By diplotene, remnants of the synaptinemal complex are observed, suggesting that they either degenerate or separate from the chromosomes (Fig. 21). At this stage the chromosomes are strongly contracted and appear fuzzy [Figs 20(a), (b), 21]. The nucleolus is no longer present. A possible chiasma is shown in serial section in Fig. 20.



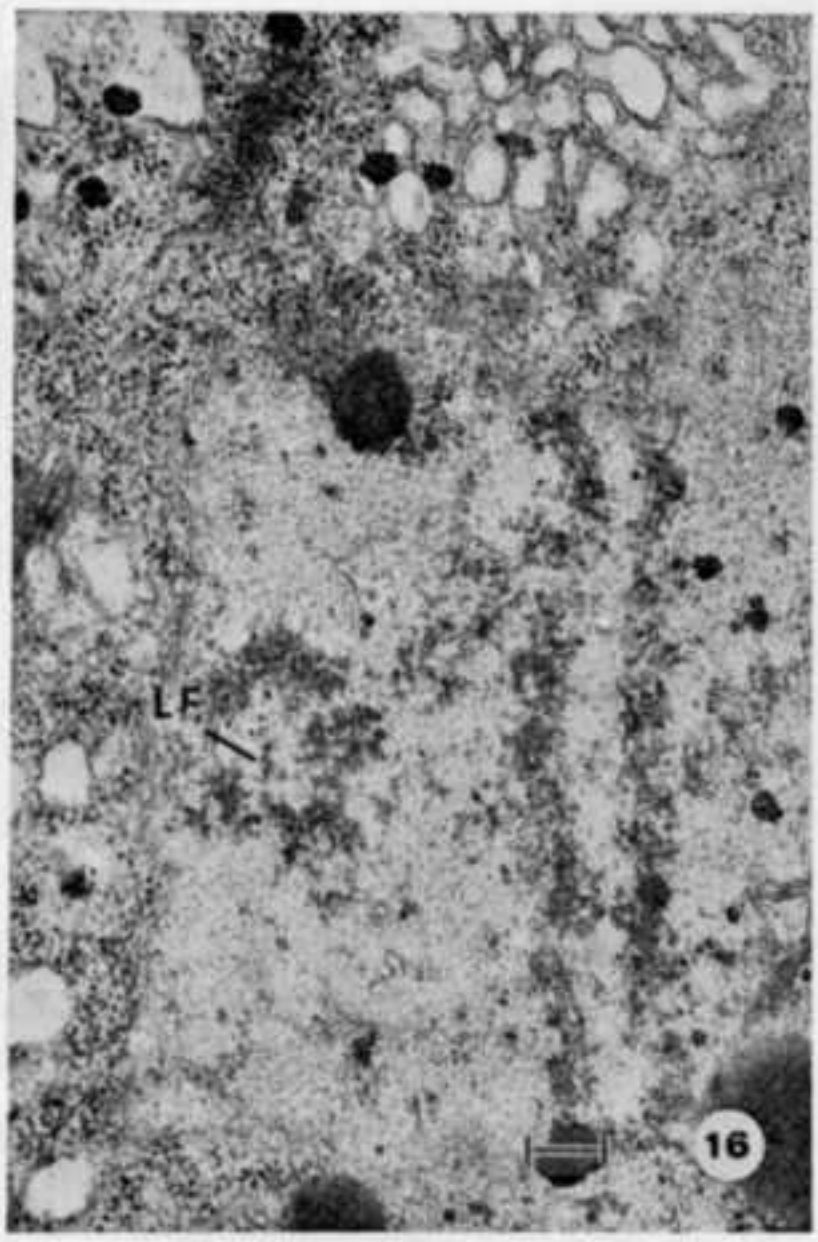


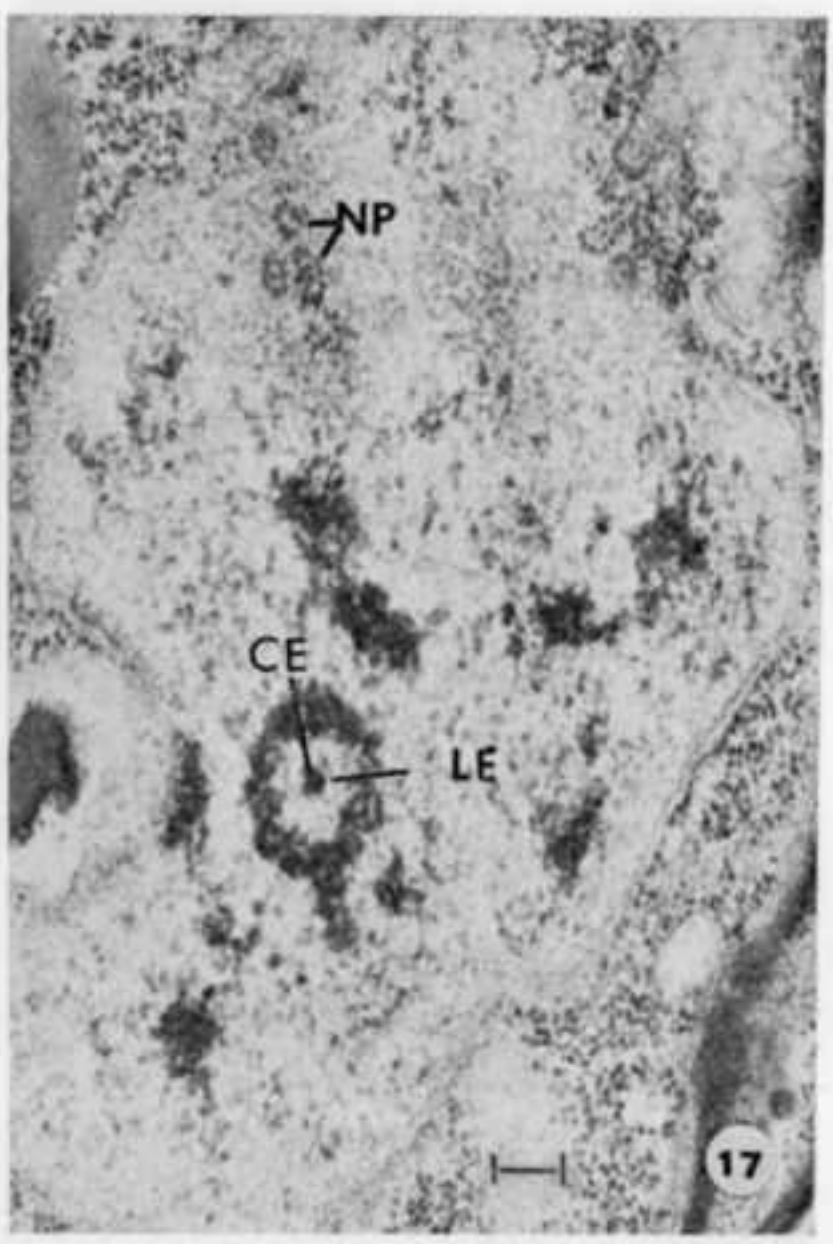


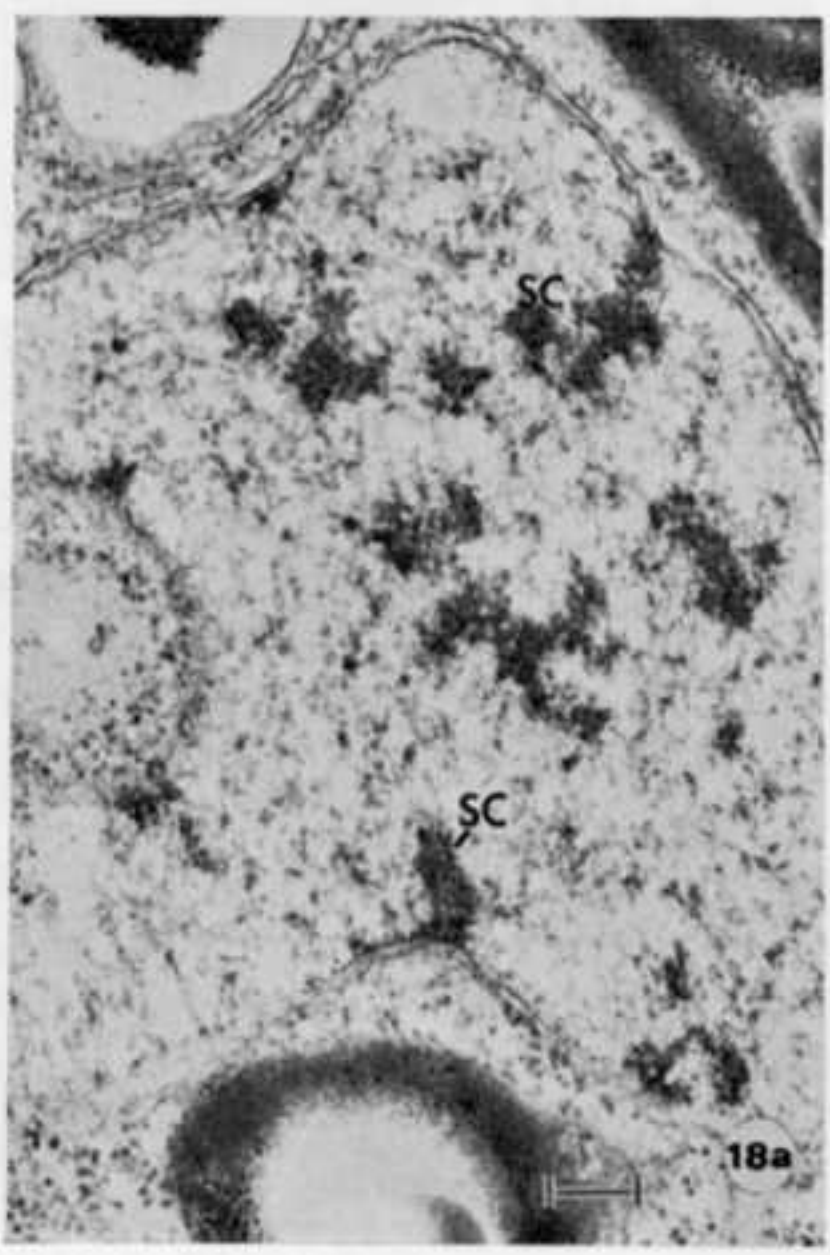


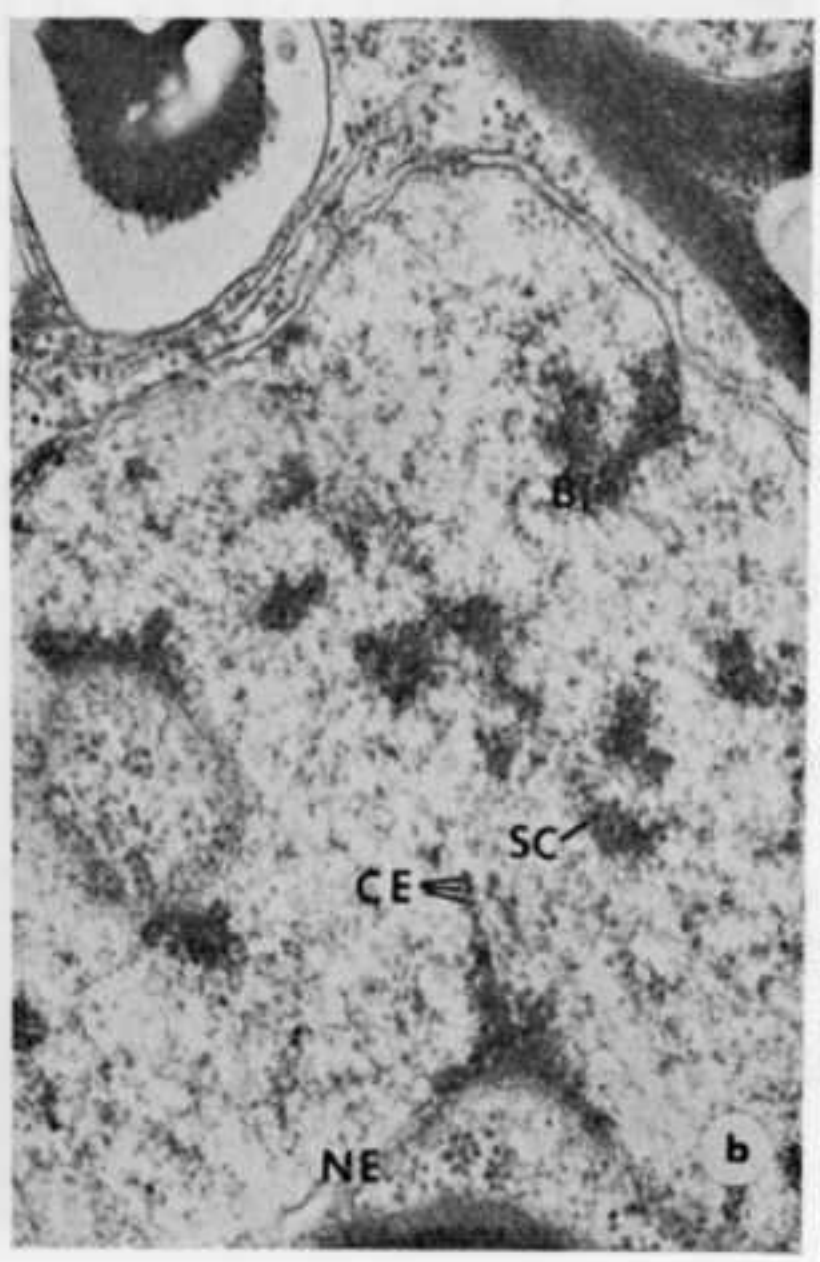


FIGS 11–15. Zygotene–Pachytene. Fig. 11. Zygotene. Note fibrillar material (fm) between nucleolus (Nu) and chromosome (Chr). Scale = 0·1 μm. Fig. 12. Zygotene. Note dense band (developing central element ?—arrow) between chromosome (Chr) and nucleolus (Nu). Scale = 0·1 μm. Fig. 13. Pachytene. Oblique section through bivalent (Bi) showing possible lateral element (LE). Scale = 0·1 μm. Fig. 14. Pachytene. Longitudinal section through synaptinemal complex. Note lateral element (LE), transverse elements (TE) and possible disc-like components of the central element (CE). Chr, chromosome; NE, nuclear envelope. Scale = 0·1 μm. Fig. 15. Pachytene. Oblique section through chromosome (Chr) revealing portion of possible lateral element (LE). Scale = 0·1 μm.









FIGS 16–18. Fully developed synaptinemal complexes at Pachytene. Fig. 16. Transverse and longitudinal sections through bivalents. Note portion of lateral element (LE). Scale = 0·1 μm. Fig. 17. Tangential section through nucleus. Note nuclear pores (NP) and cross-section through central (CE) and lateral (LE) elements of the synaptinemal complex. Scale = 0·1 μm. Fig. 18(a)–(b). Serial sections illustrating synaptinemal complex (SC) and association with nuclear envelope (NE). Possible disc-like components of the central element (CE) are noted in Fig. 18. Bi, bivalent. Scale = 0·1 μm.

During diakinesis the chromosomes achieve their most condensed configuration and become evenly distributed in the nucleus [Fig. 22(a)–(d)]. Many do not seem to be associated with the nuclear envelope. This stage merges into metaphase.

METAPHASE I–CLEAVAGE I

Apparently metaphase is a relatively short stage, and it has been particularly difficult to obtain a representative set of electron micrographs to fully document this phase.

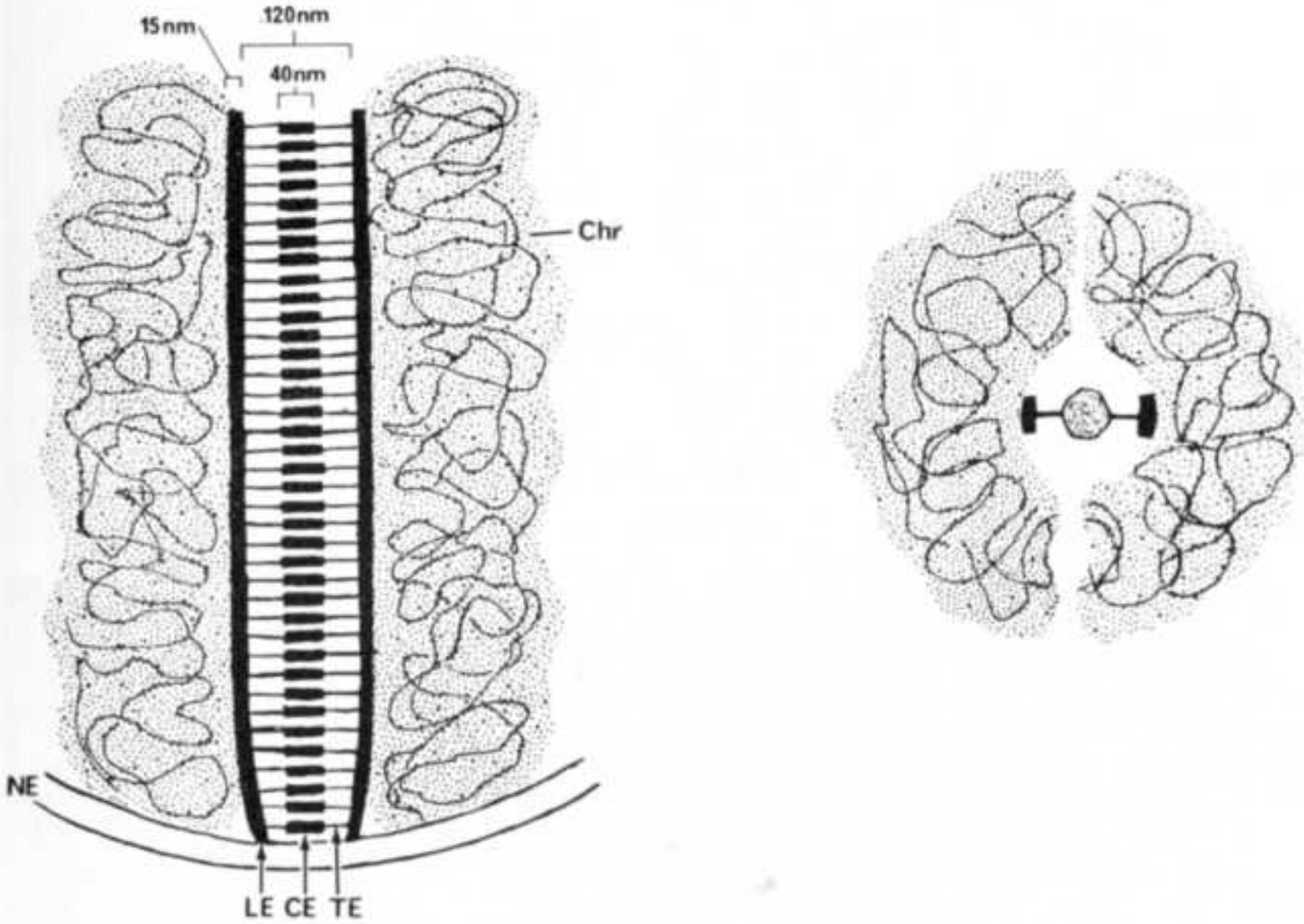
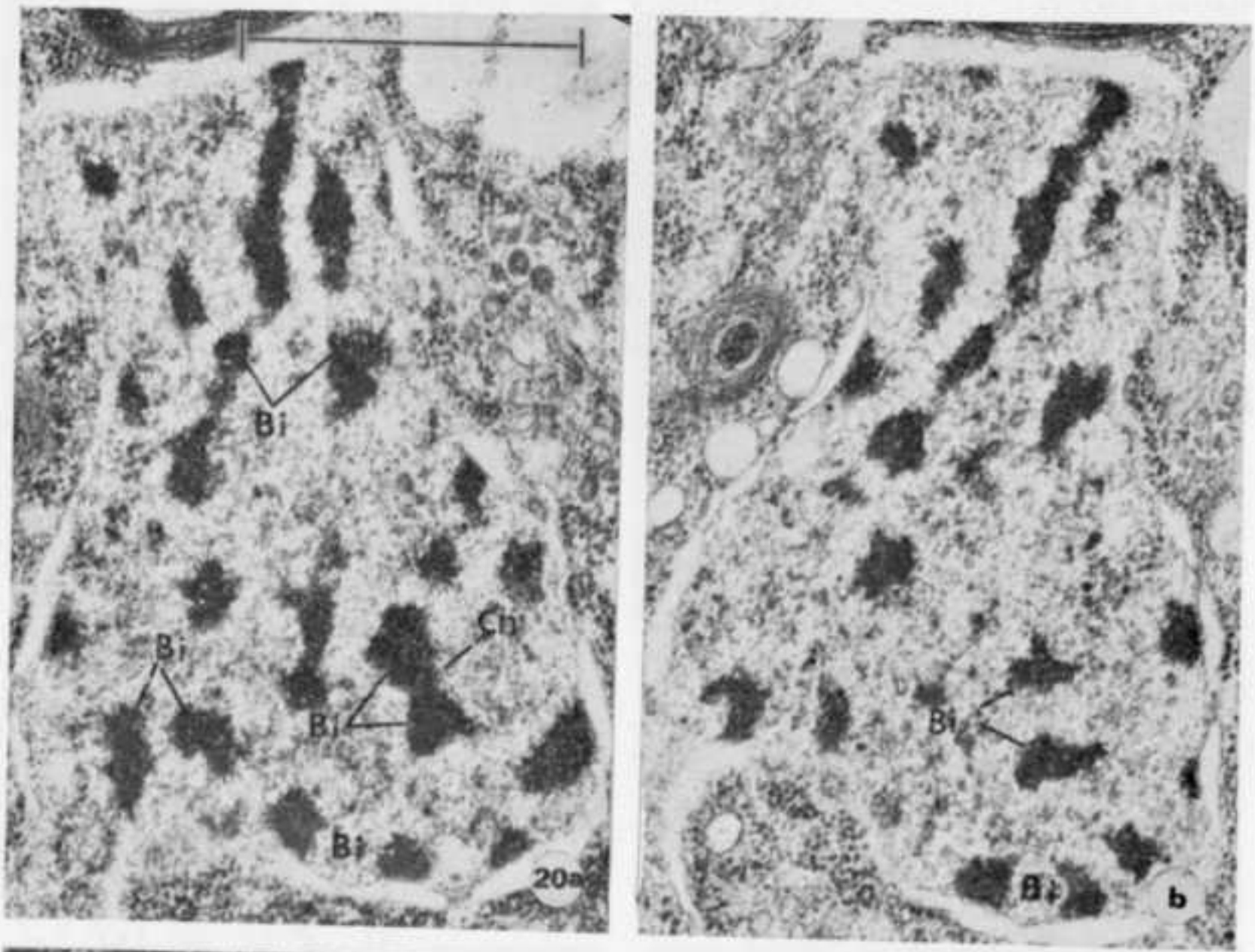


FIG. 19. Diagrammatic representation of the possible structure of the synaptinemal complex in *Chlamydomonas reinhardtii* (in transverse and longitudinal section). Two lateral elements (LE, 18–24 nm) associate with the bivalent chromosomes (Chr) which are separated by a central space of 120–130 nm. A central element (CE, 30–40 nm) is present along specialized regions of the synapsed bivalents. Transverse elements (TE, approx. 7 nm) extend from the lateral elements to the central element in regions where the complex is formed. Note the association of the complex with the nuclear envelope (NE).

By metaphase, the nuclear envelope has become fenestrated and spindle microtubules have formed and attached to the chromosomes (Fig. 23). A possible anaphase is shown in Fig. 24. Fenestrae seem to persist at the poles.

Daughter nuclei form late in telophase (Fig. 25). Basal bodies are associated with the nuclear envelope in a ribosome-free zone, and remnants of the interzonal spindle are present in the cytoplasm by late telophase (Fig. 25). Microtubules associated with the incipient cleavage furrow are seen in cross-section (Fig. 25).

Cleavage occurs utilizing a phycoplast as in vegetative mitosis (Fig. 26). Two daughter cells enter a brief interphase condition before proceeding with the second meiotic division (Fig. 27).



FIGS 20, 21. Diplotene. Fig. 20(a)–(b). Serial sections through diplotene chromosomes. Note possible chiasma (Ch) and lack of structure between bivalents (Bi) typical of this stage. Scale = 1 μm. Fig. 21. Note possible remnants of synaptinemal complexes ("stripped cores", SCR) free in the nucleoplasm. Nucleolus is no longer present. Bi, bivalents. Scale = 1 μm.

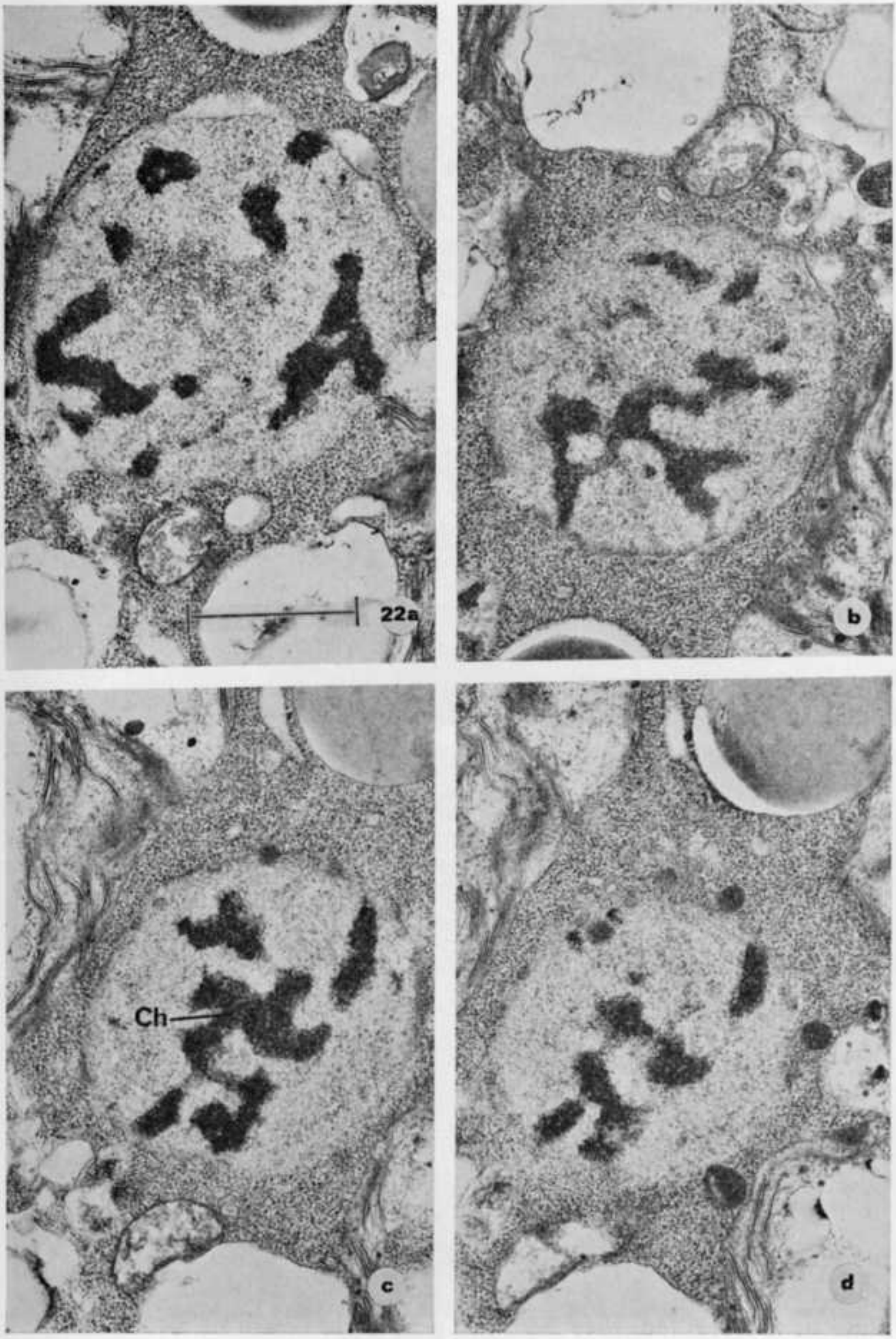
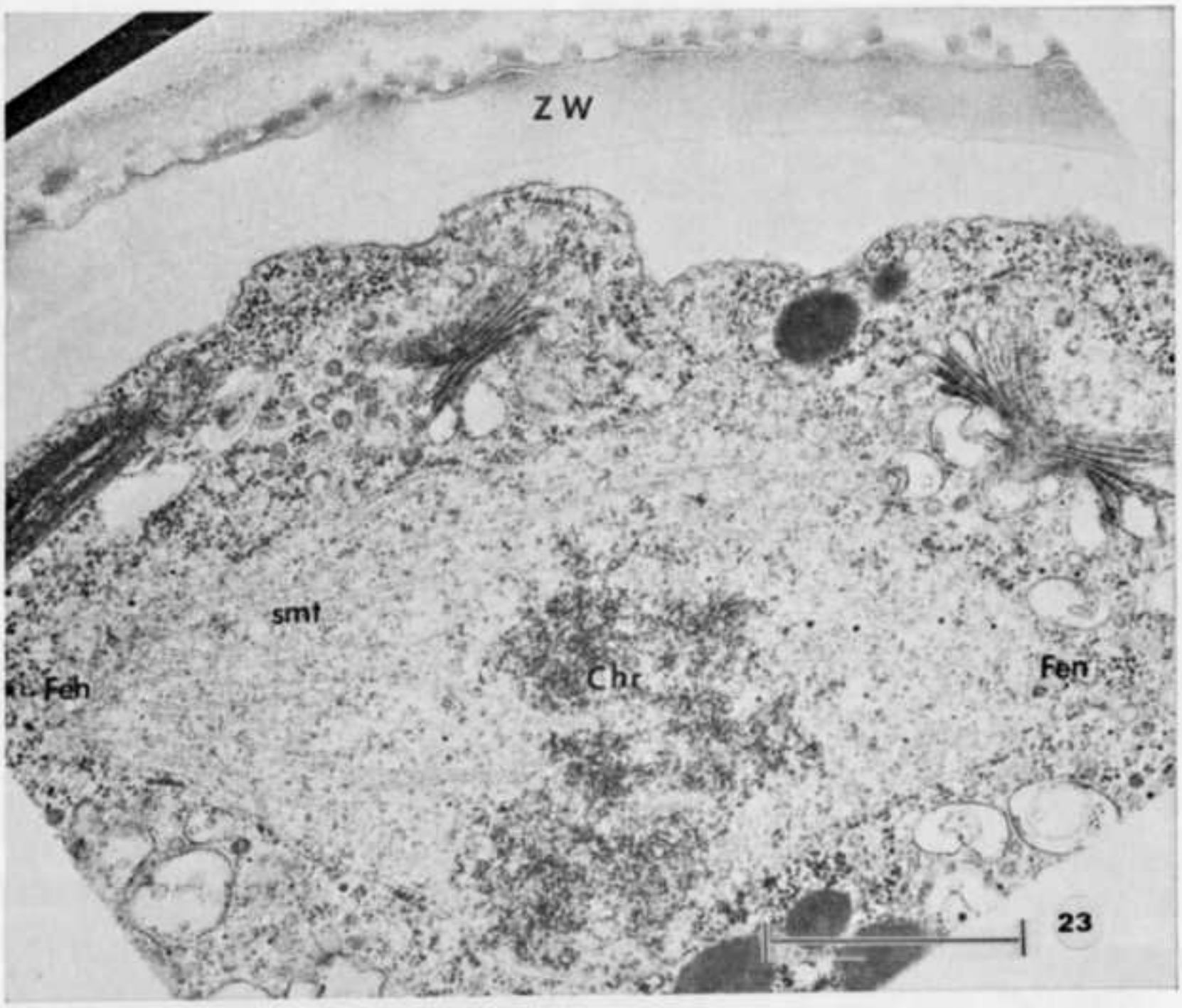
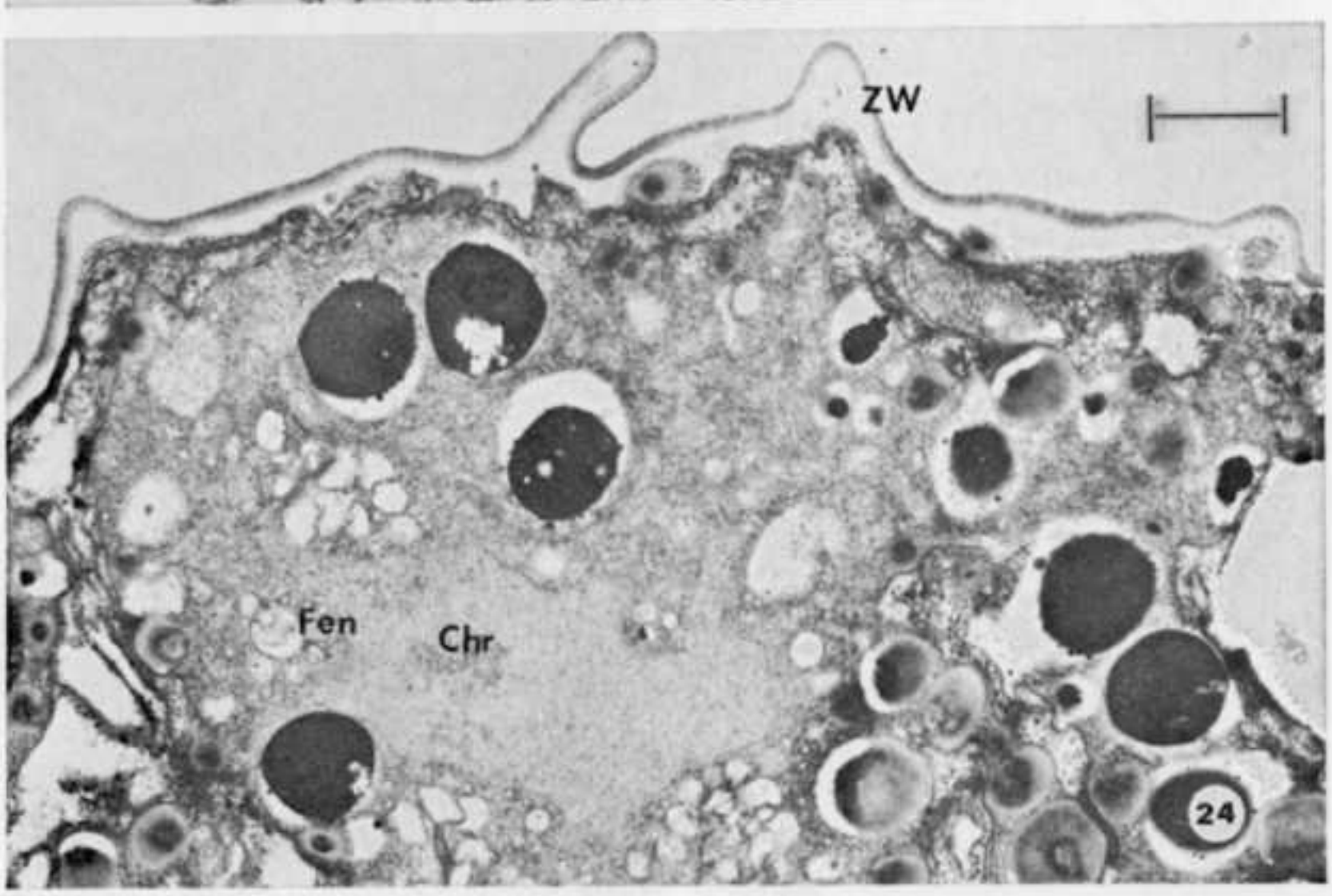
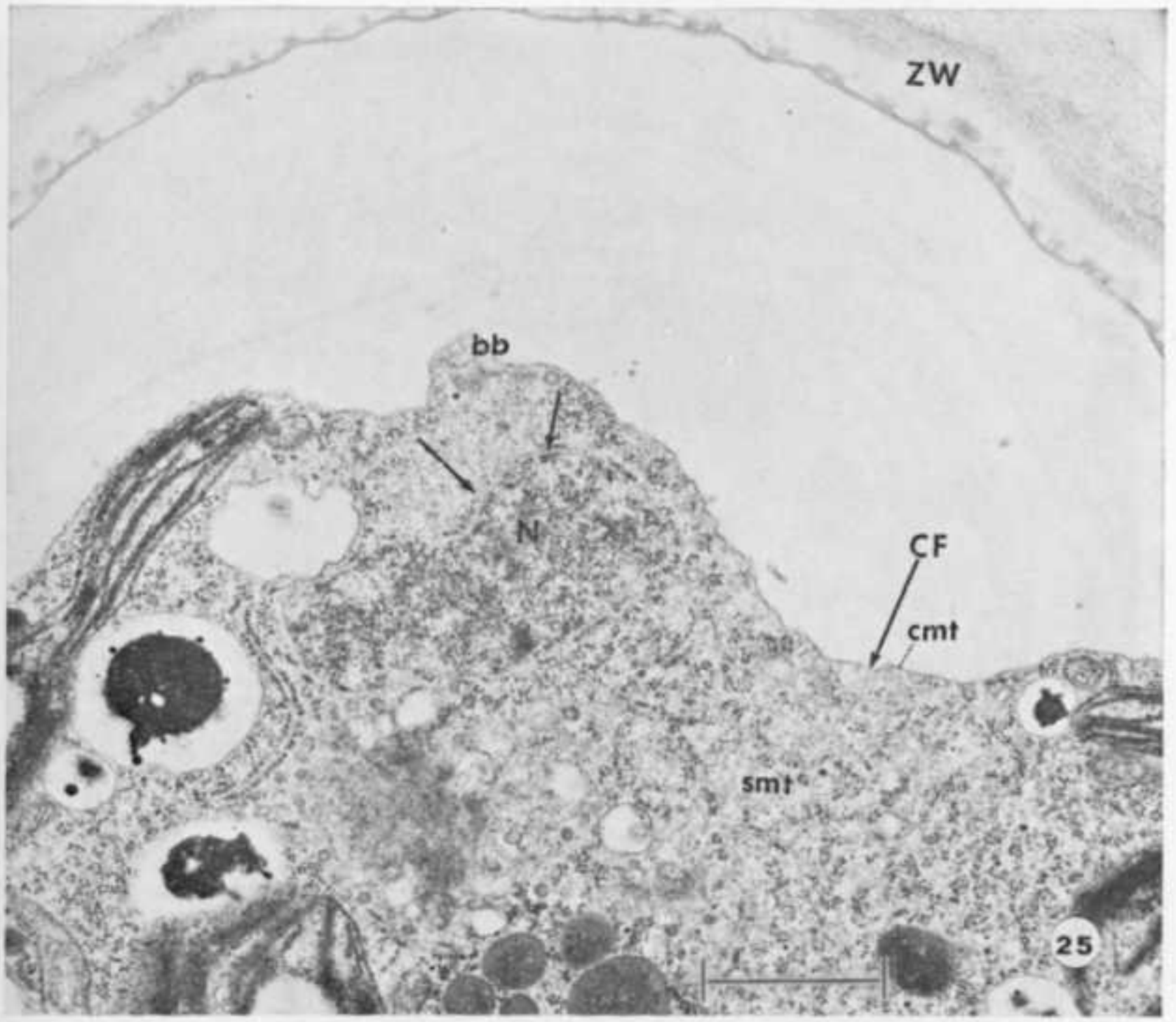


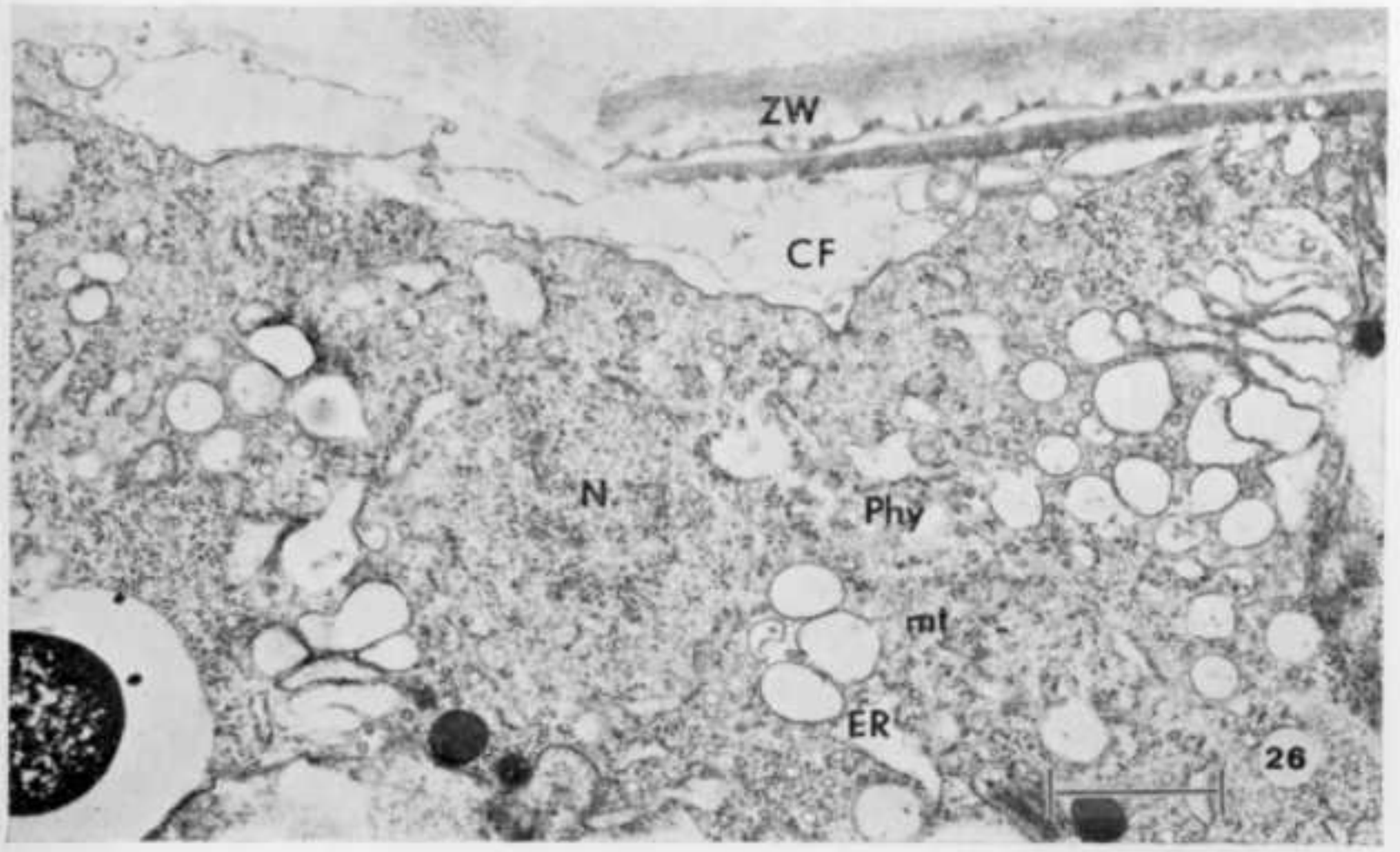
FIG. 22(a)–(d). Serial sections of nucleus at diakinesis. Chromosomes reach their most condensed state, nucleoplasm appears dense and granular. Possible chiasma (Ch) is shown in Fig. 22(c). Scale = 1 μm.



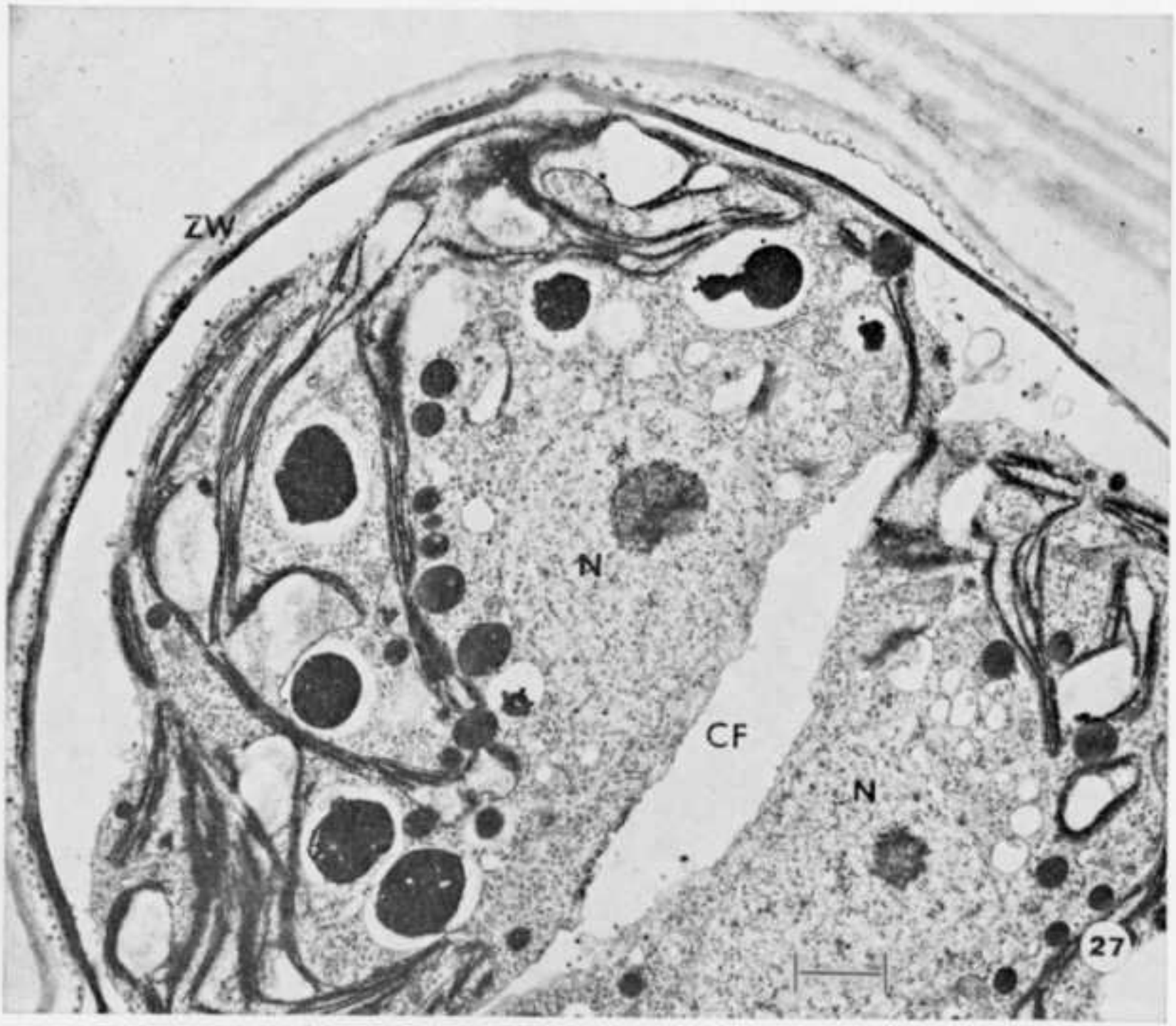


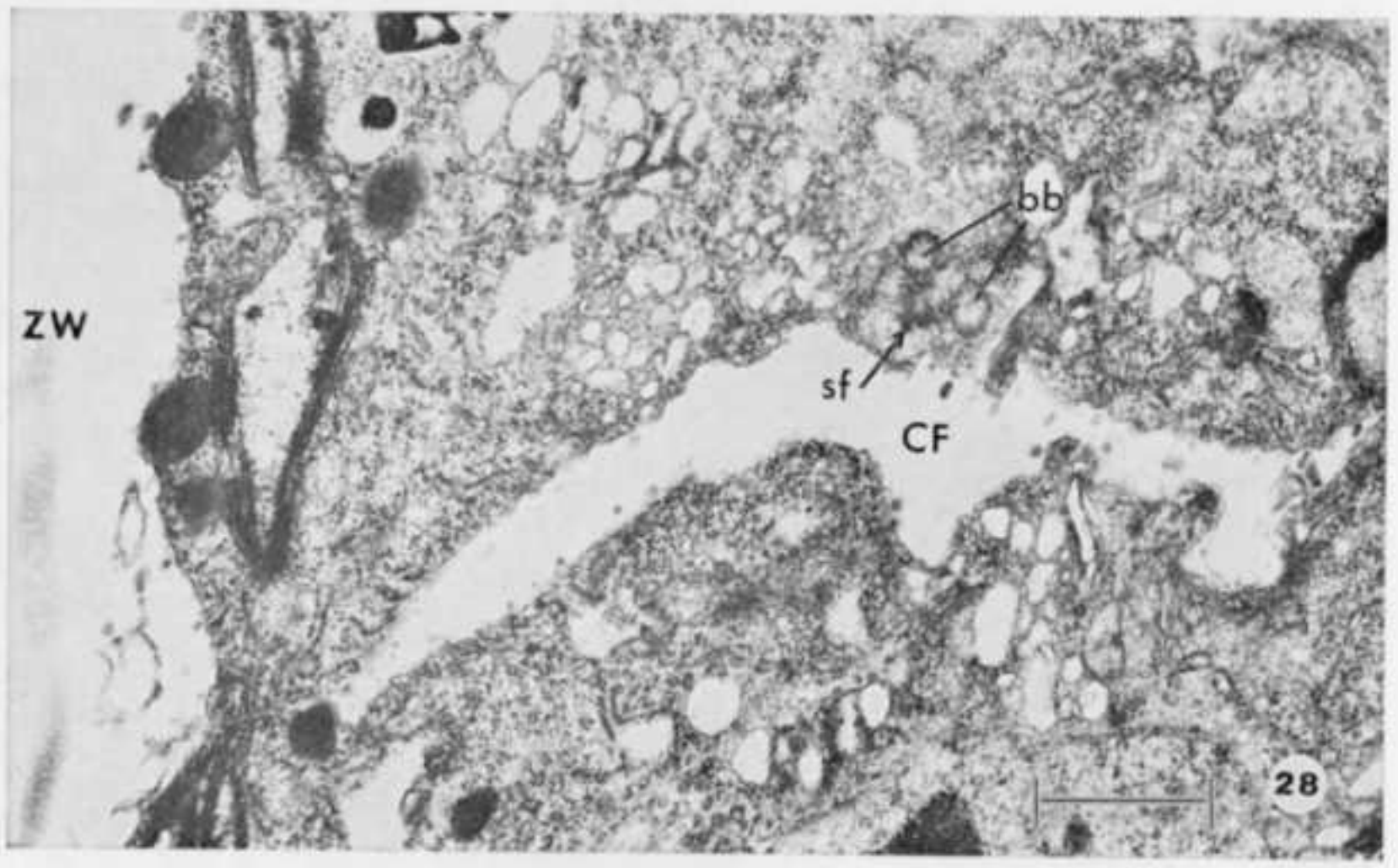
FIGS 23, 24. Later stages of meiosis. Fig. 23. Metaphase plate formed, spindle microtubules (smt) associated with the bivalents. Note polar fenestrae (Fen). Chr, chromosomes; ZW, zygote wall. Scale = 1 μm. Fig. 24. Possible anaphase (one pole out of the plane of section). Nuclear pole appears to be fenestrated (Fen). Chr, chromosome; ZW, zygote wall. Scale = 1 μm.



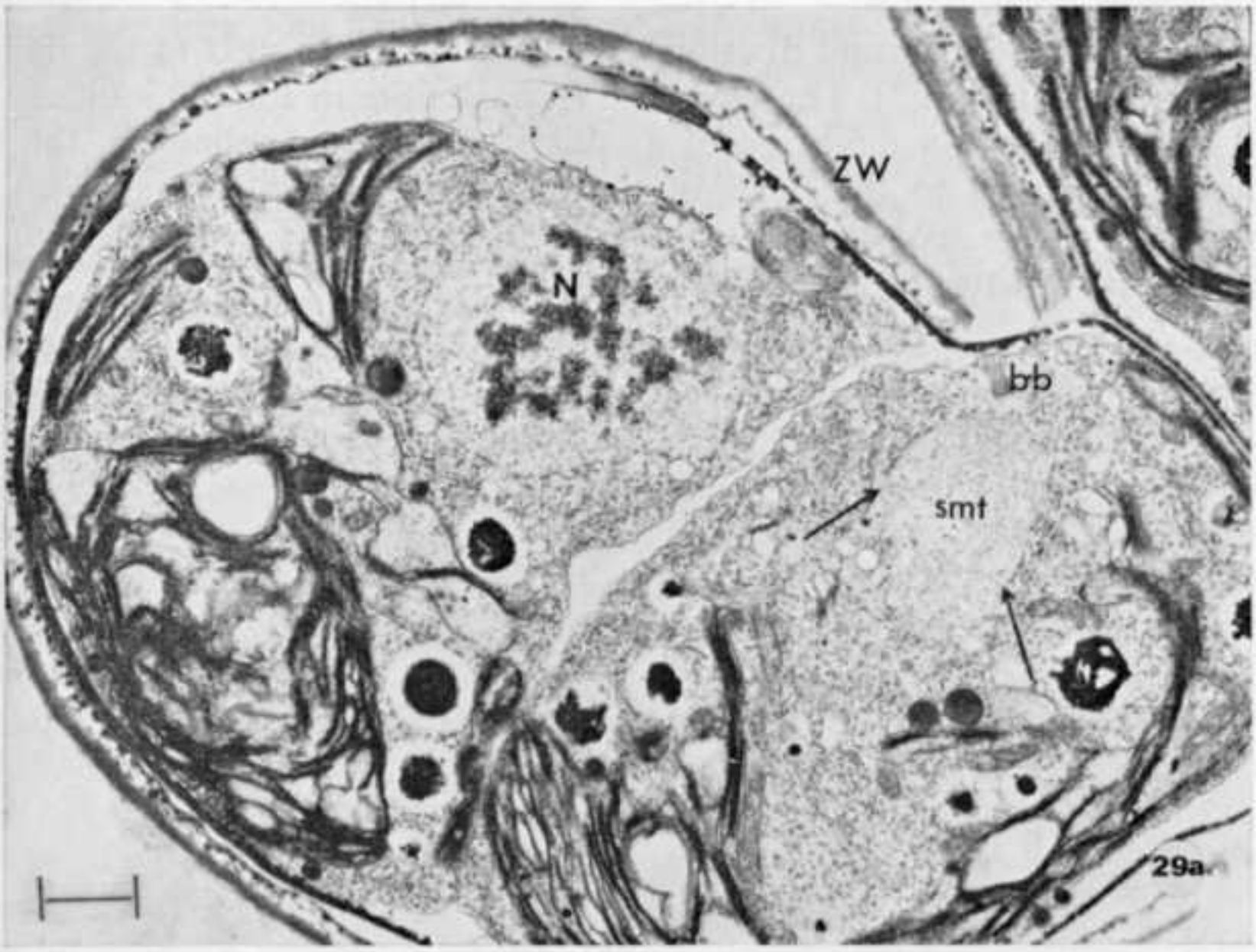


FIGS 25, 26. Late telophase-cleavage. Fig. 25. Basal body (bb) is associated with the newly formed daughter nucleus (N) in a ribosome-free zone (arrows). Note degenerating spindle microtubules (smt) in longitudinal section and cleavage microtubules (cmt) in cross-section near incipient cleavage furrow (CF). ZW, zygote wall. Scale = 1 μm. Fig. 26. Early cleavage. Note endoplasmic reticulum (ER) and phycoplast microtubules Phy, mt). CF, cleavage furrow; ZW, zygote wall. Scale = 1 μm.





FIGS 27, 28. Interkinesis. Fig. 27. Cleavage furrow (CF) is completed. Note fully reformed daughter nuclei (N) with nucleoli. ZW, zygote wall. Scale = 1 μm. Fig. 28. Possible replication of basal bodies (bb) at interkinesis. Note separated striated fibre (sf). CF, cleavage furrow; ZW, zygote wall. Scale = 1 μm.



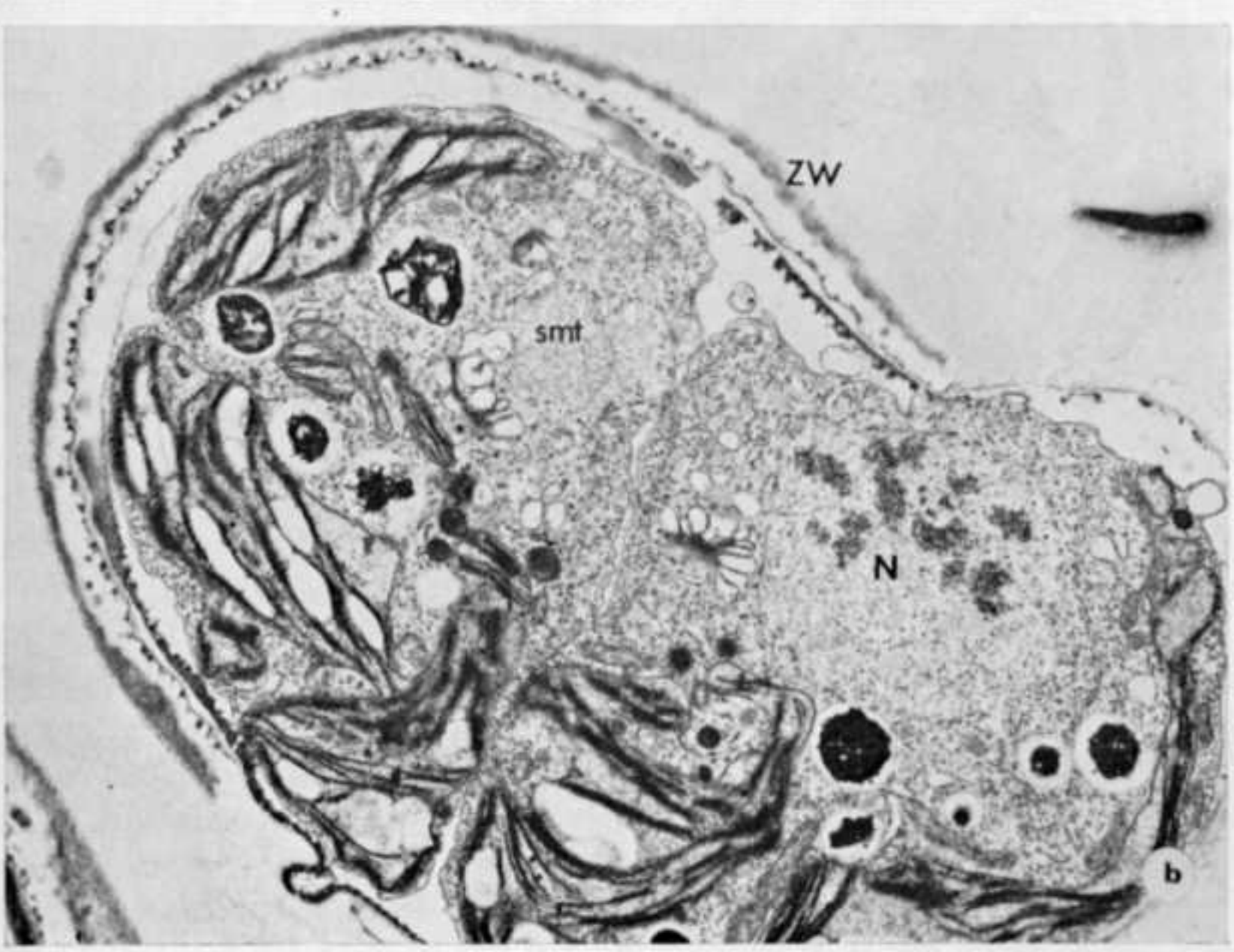


FIG. 29(a)–(b). Metaphase II. Serial sections demonstrating the polar fenestrae (arrows) and metaphase plates of the daughter nuclei (N). Note basal body (bb) present in ribosome-free region of nuclear pole in Fig. 29(a). smt, spindle microtubules; ZW, zygote wall. Scale = 1 μm.

METAPHASE II–CLEAVAGE II

At the start of the second division, the basal bodies lie next to the incipient cleavage furrow (Fig. 28). The separated striated fibre in Fig. 28 indicates that basal body replication might occur at this stage. By metaphase II, the basal bodies have moved to the poles of the fenestrated spindle [Fig. 29(a), (b)]. Completion of the second meiotic division results in the production of four daughter cells within the zygote wall (Fig. 30).

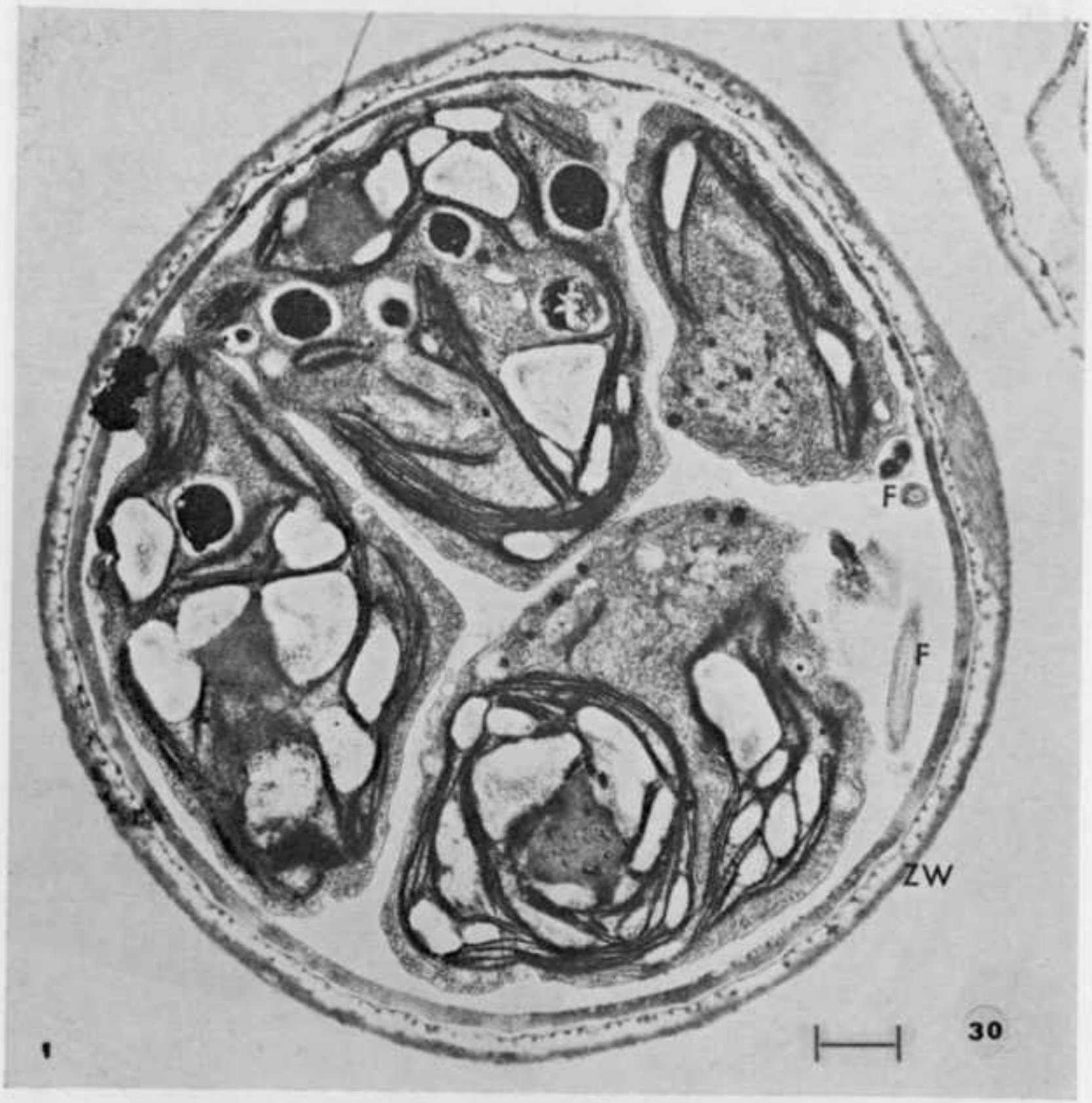


FIG. 30. Late cytokinesis–zoospore maturation. Note developing flagella (F) in zoospores prior to liberation from the zygote wall (ZW). Scale = 1 μm.

DISCUSSION

Meiosis has been investigated at the ultrastructural level in more than 40 genera of plants (Westergaard & von Wettstein, 1972). Of these studies at least 16 have dealt with fungi, 20 have dealt with higher plants and four have dealt with the algae. Probable reasons for the infrequent reports in the algae are culturing and fixation problems. Sexual stages of many algae do not occur in culture or, at least, are very difficult to obtain. Frequently, extracellular coatings

or cell walls form permeability barriers for fixatives, allowing only slow penetration and therefore poor fixation. In those algae investigated (*Ulva*, Bråten, 1973; *Chorda* and *Pyaliella*, Toth & Markey, 1973; *Lithodesmium*, Manton, Kowallik & von Stosch, 1969, 1970a, 1970b; *Janczewskia*, *Levringiella*, *Gonimophyllum* and *Polycoryne*, Kugrens & West, 1972) only the studies by Bråten, and Manton, Kowallik & von Stosch have dealt with various stages of the meiotic process; others have focused only on pachytene, the stage at which synaptinemal complexes are most evident.

The synaptinemal complex is a term introduced by Moses (1956) to describe the tripartite structure associated with meiotic chromosomes during pachytene. Further investigations have shown these structures to be characteristic features of meiotic synapsis in animals and plants (see Westergaard & von Wettstein, 1972). For instance, complexes have been reported in haploid tomato (Menzel & Price, 1966) and maize (Ting, 1971), possibly formed during non-homologous or fold-back pairing. In haploid *Petunia* (Sen, 1970) pairing segments between non-homologous chromosomes have been shown at pachytene with light microscopy. At the ultrastructural level, short segments of synaptinemal complex are found at pachytene, and by diakinesis abundant univalents were observed. From these observations, Sen (1970) suggested that axial elements form irrespective of chromosomal homology, and may therefore form nonspecific synaptinemal complexes.

The dimensions and architecture of the synaptinemal complexes are relatively constant in the wide range of organisms so far studied (central space = 90–120 nm, central element = 20–30 nm). The dimensions of the synaptinemal complex in *Chlamydomonas* fall within this range.

Certain aspects of the mode of development of the synaptinemal complex are open to speculation. In serial section reconstructions of *Locusta* testis (Moens, 1969), axial elements are shown first to appear in the nucleus at leptotene as the chromosomes begin condensation. By zygotene the axial elements and terminal points of the pairing chromosomes associate with the nuclear envelope. This same sequence has been observed in *Chlamydomonas* and seems to be quite common. It has been reported, for example, in rat spermatocytes (Esponda & Giménez-Martín, 1972), *Labyrinthula* (Moens & Perkins, 1969), *Solanum* (Menzel & Price, 1966), mosquito oocytes (Roth, 1966), *Neottiella* (Westergaard & von Wettstein, 1970), *Neurospora* (Gillies, 1972), *Zea* (Underbrink, Ting & Sparrow, 1967) and *Coprinus* (Lu, 1967).

The synaptinemal complex is frequently observed terminating against the nuclear envelope. This attachment has been observed, for example, in *Physarum* (Aldrich, 1967; Aldrich & Mims, 1970) and *Lilium* (Moens, 1968).

In *Chlamydomonas*, short tubular elements span the perinuclear space in the regions of the chromosomal attachment. Esponda & Giménez-Martín (1972) also noted modifications at the attachment sites in rat spermatocytes. They described fibrillar material in the perinuclear space at the locus of the "attachment plates" and in the adjacent cytoplasm. Since synapsis begins in rat spermatocytes at the nuclear envelope, these authors have suggested that the nuclear envelope attachment may play an important part in initiating synapsis, especially since attachment sites in rat spermatocytes do not appear until leptotene at which time synapsis begins in this organism (Esponda & Giménez-Martín, 1972).

Regardless of the mechanism, the attachment of homologous chromosomes to the nuclear envelope is certain and may be necessary in providing a spatial relationship for a functioning synaptinemal complex.

The central element of the synaptinemal complex appears between zygotene and pachytene. Two biogenic pathways of the central element have been suggested. Moens (1968) believes that the central element is derived from a fusion of transverse elements, particularly in the central region between the chromosomes at the interface of the transverse elements. On the other hand, Westergaard & von Wettstein (1970) propose that fibrillar regions arising in the nucleolus at zygotene are involved in the development of the central element and may actually be components of the central elements. In *Chlamydomonas*, as in *Neottiella* (Westergaard & von Wettstein, 1970), *Echinostelium* (Haskins, Hinchee & Cloney, 1971) and *Saccharomyces* (Moens & Rapport, 1971), dense fibrillar aggregates are present in the nucleolus during zygotene. Frequently these aggregates form dense bands resembling central elements between the nucleolus and the associated chromosomes. In *Neottiella* (Westergaard & von Wettstein, 1970), the nucleolus is attached to the nuclear envelope during zygotene and fibrillar aggregates form a band between the two structures at this stage. If one accepts the hypothesis of Westergaard & von Wettstein for development of the central element, some mechanism functioning in moving the central element from the nucleolus to the chromosomes must be involved. Westergaard & von Wettstein suggest that the nucleolus is transported to the bivalents but do not suggest the mechanism by which this may occur. In *Chlamydomonas*, however, the fibrillar element is formed between the chromosomes and the nucleolus and may be synthesized in association with the chromosomal–nucleolar complex, making an elaborate transport mechanism unnecessary.

In most organisms (Westergaard & von Wettstein, 1972), synaptinemal complexes disappear by diplotene–diakinesis and this has been confirmed for *Chlamydomonas*. In *Neottiella*, however, Westergaard & von Wettstein (1970) reported short pieces of synaptinemal complex in the loci of what were believed to be chiasmata, and suggested that they function in crossing over. If the role of the synaptinemal complex in crossing over is reflected in its involvement with chiasmata at diplotene–diakinesis, it would seem that it should have been observed more frequently at this stage. Perhaps synaptinemal complexes are instrumental in establishing a spatial relationship for crossing over to occur but do not participate in the actual process.

Among the algae, later stages of meiosis (metaphase-cleavage) have been reported only for *Ulva* (Bråten, 1973) and *Lithodesmium* (Manton, Kowallik & von Stosch, 1969, 1970a, 1970b). At these stages, meiosis closely resembles mitosis with the exception of prophase I. In *Ulva* and *Chlamydomonas*, basal bodies are present prior to the onset of meiosis, while in *Lithodesmium* (which does not possess centrioles during the vegetative state) they reappear at interkinesis between the first and second meiotic divisions. At metaphase I, centric spindles with polar fenestrae are formed in *Ulva* and *Chlamydomonas*. The nuclear envelope breaks down at meiosis in *Lithodesmium* as it does during mitosis. In contrast, the nuclear envelopes of *Chlamydomonas* (Triemer & Brown, 1974) and *Ulva* remain intact throughout mitosis and meiosis except at the nuclear poles. Following cleavage the basal bodies associate with the nucleus

in a ribosome-free zone in *Ulva* (Bråten, 1973) and *Chlamydomonas*, although this zone was not reported by Bråten (1973).

The first cytokinesis usually is followed by one or more mitotic divisions, the number of which is characteristic of the organism. In *Ulva* and *Chlamydomonas*, haploid vegetative zoospores are released; in *Lithodesmium* gametes are formed.

In summary, a brief comparison of mitosis with meiosis in this organism (Triemer & Brown, 1974) is useful. Basal bodies are present at the onset of both divisions. Polar fenestrae are characteristic of both divisions. A ribosome-free zone exists at the poles in mitosis as in meiosis. At telophase of mitosis and meiosis, the interzonal spindle is abscised and daughter nuclei reform. Cleavage in meiosis and mitosis occurs via a phycoplast. A brief period of interkinesis ensues following the first division in both cases. Both mitosis and meiosis yield haploid zoospores, but the meiotic zoospore has the advantage of gene recombination during its formation.

This investigation has demonstrated that the post-prophase stages of meiosis appear similar to mitosis. However, the differences (association of the chromosomes with the nuclear envelope, formation of the synaptinemal complex, crossing over and recombination) occurring in meiotic prophase provide the basis for sexual reproduction and genetic evolution in *Chlamydomonas*.

ACKNOWLEDGEMENTS

We wish to express our appreciation to Dr H. Stanley Bennett, Laboratories for Reproductive Biology, for his generous financial support of these studies (Rockefeller Foundation); and Mr Richard Santos, Dr Montrose Moses and Dr Jeremy Pickett-Heaps for their valuable discussions and helpful criticisms.

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