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ELECTRON-MICROSCOPIC OBSERVATIONS ON THE MACRONUCLEAR DEVELOPMENT OF *STYLONYCHIA MYTILUS* AND *TETRAHYMENA PYRIFORMIS* (CILIOPHORA-PROTOZOA)

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SUMMARY

This report describes an ultrastructural investigation of macronuclear development following conjugation in *Stylonychia mytilus* (a spirotrichous ciliate) and *Tetrahymena pyriformis* (a holotrichous ciliate).

In *S. mytilus*, polytene chromosomes are formed in the young macronucleus (macronuclear *Anlage*). They are subsequently broken between the bands by 'membranous' partitions; the assembly of the membranes appears to be concomitant with the formation of the polytene chromosomes. The membranes in the Anlage appear to originate from fibrous material seen in the early Anlage. This fibrous material in the earlier stages is seen concentrated at several points along the border of the inner nuclear membrane. In the later stages it is seen in the interior of the Anlage, outlining the developing polytene chromosomes. As the chromosomes reach the maximum degree of polyteny, the fibrous material condenses to acquire a membranous appearance and extends into the interband regions. The Anlage throughout this period shows a progressive increase in size.

Subsequently, the membranes enclose individually each band plus portions of the 2 adjacent interband regions of the polytene chromosomes to form a large number of vesicles. After vesicle formation the Anlage shrinks, and the chromatin inside the vesicles shows degradative changes. Finally, the vesicles disappear, the membrane degradation products appear at the nuclear membrane, and the Anlage now contains nucleoli. The Anlage increases its DNA content by multiple rounds of replication to become a mature macronucleus. The ultrastructural changes observed in the Anlage support the idea of genetic diminution (i.e. extensive DNA synthesis, elimination of many DNA nucleotide sequences, and amplification of the remaining DNA nucleotide sequences in a second period of DNA synthesis) proposed earlier on the basis of cytochemical, biochemical, and limited electron-microscope studies.

In *T. pyriformis*, the macronuclear development differs substantially from that of *Stylonychia*. Features such as the formation and degradation of polytene chromosomes are absent in the macronuclear development of *Tetrahymena*; the young macronucleus in this cell becomes a mature macronucleus by progressive increment in size and chromatin content with no apparent genetic diminution. These observations agree with cytochemical studies on the macronuclear development of *Tetrahymena*.

INTRODUCTION

The process of macronuclear development following conjugation in hypotrichous ciliates (e.g. *Stylonychia* and *Euplotes*) has been the subject of several investigations (Ammermann, 1964, 1965, 1968, 1969, 1971; Gil, Alonso & Perez-Silva, 1972; Kloetzel, 1970; Rao, 1968; Rao & Ammermann, 1970; Sapra & Dass, 1970). After conjugation in these ciliates, the young macronucleus (macronuclear Anlage), which in

the initial stage is indistinguishable from a diploid, mitotically dividing micronucleus, appears to undergo qualitative and quantitative changes in its genetic apparatus before maturing into a 'polyploid', somatic, amitotically dividing macronucleus.

In *Stylonychia mytilus*, Ammermann (1971) has divided macronuclear development into 3 stages. In the first, the DNA content of the macronuclear Anlage increases 15 times over the amount contained in the diploid micronucleus. As a consequence of this DNA synthesis, polytene chromosomes are formed (Ammermann, 1964; Gil *et al.* 1972; Kloetzel, 1970; Sapra & Dass, 1970). The polytene chromosomes are next broken up by 'membranous' partitions that transect the polytene chromosomes in between the bands (Kloetzel, 1970). Subsequently, every band of the polytene chromosomes becomes completely enclosed by the membranes and the Anlage at this time is filled with a large number of physically independent 'vesicles', each enclosing a band of the polytene chromosome. The formation of these vesicles marks the beginning of the degradation of DNA in the Anlage. More than 90% of the DNA is broken down into acid-soluble products that are excreted into the medium (Ammermann, 1969). This destruction of DNA results in the so-called 'DNA-poor' stage. In the subsequent, third stage, the vesicles disappear with dissolution of intranuclear membranes, nucleoli appear, and the Anlage synthesizes DNA by means of replication bands (Ammermann, 1971). During this second period of DNA synthesis, the DNA content of the Anlage increases to the level of the mature macronucleus (29-fold over that of DNA-poor stage: Ammermann, 1971). It has been suggested (Rao & Ammermann, 1970; Kloetzel, 1970; Ammermann, 1971) that the above series of events may produce a mature macronucleus that contains only a part of the gene complement of the micronucleus amplified many times.

This hypothesis of selective genetic diminution in the macronuclear Anlage of *S. mytilus* is borne out by comparisons of the buoyant density and melting patterns of DNA obtained from the mature micro- and macronuclei of the vegetative cells of *S. mytilus* (Bostock & Prescott, 1972). It is clear that many of the DNA components present in the micronucleus are absent from the macronucleus. In keeping with the events in the macronuclear Anlage the purified macronuclear DNA of *S. mytilus* exists as short pieces ranging in length from 0·2 to 2·2 μm (Prescott *et al.* 1971). The small size of the macronuclear DNA is believed to be due to the scissions ('membranous' partitions described by Kloetzel) introduced in the interband regions of the polytene chromosomes and the subsequent degradation of DNA in the vesicles (Prescott, Murti & Bostock, 1973).

By comparison, the limited available evidence suggests that the holotrichous ciliates (e.g. *Paramecium* and *Tetrahymena*) have a much simpler pattern of macronuclear development. In *Paramecium aurelia* (Woodard, Woodard, Gelber & Swift, 1966) and *Tetrahymena* (Johansson and Zech, unpublished observations quoted by Ammermann, 1971), microspectrophotometric measurements have shown a steady increase in the DNA content of the macronuclear Anlage until it becomes a mature macronucleus. According to light-microscopic observations polytene chromosomes are never present in the macronuclear Anlage of *Tetrahymena* (Ammermann, 1971). The only published electron-microscopic investigation on the macronuclear develop-

ment in holotrichs was made on *P. aurelia* (Jurand, Beale & Young, 1964), and no notable changes were detected in the structure of the chromatin of the developing macronuclear Anlage.

In this report an ultrastructural investigation of the sequence of macronuclear development in *S. mytilus* and *T. pyriformis* is presented. The observations demonstrate that macronuclear development is fundamentally different in these 2 types of ciliates.

MATERIALS AND METHODS

Stylonychia mytilus was cultivated under sterile conditions at room temperature in a medium containing 0·2 % cerophyll (Cerophyll Laboratories Inc., Kansas City), 0·001 % KCl, 0·001 % $MgSO_4$ and 0·002 % $CaCl_2$ (pH 6·8). Living *Tetrahymena* grown asceptically in synthetic medium was used as the food organism. Extensive intraclonal conjugation was induced by starving the cells for 24–48 h. After 80–90 % of the cells had formed conjugal pairs (which occurred over a period of 3–4 h during starvation), cells that had not initiated conjugation were removed from the culture dish. After cells had completed conjugation, groups of exconjugants were collected with a braking pipette (Stone & Cameron, 1964) over a 5-h period at different times (5–10 h, 20–25 h, 35–40 h, 50–55 h, 65–70 h, and 85–90 h) and prepared for electron microscopy. In *Stylonychia* the maturation of the macronucleus begins in the newly separated exconjugants and is completed in about 100 h.

The exconjugants were fixed in a 3 % solution of glutaraldehyde, buffered at pH 7·25 with 0·1 M cacodylate, at room temperature for 20–30 min (Allen, 1969). They were washed with 0·1 M cacodylate (containing 10 % sucrose), embedded in agar (Flickinger, 1969), and post-fixed for 45 min at room temperature in 1 % OsO_4 in 0·1 M cacodylate buffer. The agar blocks were rinsed again in the buffer containing sucrose, dehydrated in a graded series of ethanols, immersed in propylene oxide, and embedded in a mixture of Epon and Araldite (Mollenhauer, 1964). Sections showing pale gold interference colour were cut with a diamond knife on a Sorvall MT-1 or MT-2 ultramicrotome and mounted on 200-mesh copper grids. The grids with sections were stained for 1 min with a 1 % aqueous solution of uranyl acetate and for 2–4 min with lead citrate (Reynolds, 1963) and examined in a Philips EM 200 electron microscope.

Tetrahymena pyriformis syngen I mating types 1 and 2 were maintained axenically at room temperature in 1·5 % proteose peptone medium. To obtain conjugants, the 2 strains were washed several times with a dilute salt solution (Prescott & Carrier, 1964) and mixed. Usually by 6–7 h after mixing, about 80–90 % of the cells had formed conjugal pairs. Unlike *Stylonychia*, synchronous conjugation was not obtained in *Tetrahymena*; the samples collected at a particular time often contained conjugating cells at different stages of nuclear organization. Therefore, 5 batches of cells were induced to conjugate 5 h apart, all of them were combined at the end of a 24-h period, fixed, and processed for electron microscopy. Conjugating and exconjugant *Tetrahymena* (in *Tetrahymena* part of the macronuclear development occurs while the cells are still paired) were prepared for electron microscopy following the same procedures as for *Stylonychia*.

OBSERVATIONS

Macronuclear development in Stylonychia

Ammermann (1965) has described the process of conjugation in *S. mytilus*. Briefly, the events of conjugation are as follows. After the cells have formed conjugal pairs, the micronuclei undergo 3 successive prezygotic (2 meiotic followed by 1 mitotic) divisions in each cell. During this time the macronucleus begins to disintegrate by breaking into 4 pieces. Of the 8 haploid micronuclear division products in each cell,

all disintegrate except for 2, the pronuclei. These are designated as the stationary pronucleus and the migratory pronucleus. The migratory pronucleus of one cell migrates across the cytoplasmic bridge that links the 2 cells and fuses with the stationary pronucleus of the other cell to form the zygote nucleus or synkaryon. After the formation of synkaryon, the conjugants separate. The synkaryon undergoes 2 successive post-zygotic divisions to produce 4 nuclei. Of these nuclei 2 become the new micronuclei, a third becomes the presumptive macronucleus (or macronuclear Anlage), and the fourth degenerates.

According to Ammermann (1971) there are 3 stages in the maturation of the macronuclear Anlage. (1) An initial period of DNA synthesis during which the amount of DNA in the Anlage is increased 15-fold over the amount in the diploid micronucleus. This DNA synthesis is initiated in the macronuclear Anlage of a newly separated exconjugant and is completed in about 40 h. (2) A period in which over 90% of the DNA is lost from the Anlage (resulting in the DNA-poor stage). This period extends between 40 and 70 h. (3) A second period of DNA synthesis in which the remaining DNA is replicated many times to produce the amount (29-fold over that of the previous stage) in the mature macronucleus. This second period of DNA synthesis begins at about 75 h post conjugation and is completed by 100 h.

From an ultrastructural viewpoint, the above 3 stages roughly correspond to: (1) formation of polytene chromosomes, (2) degradation of polytene chromosomes, and (3) final construction of the macronucleus by means of replication bands. These stages are now described.

Formation of polytene chromosomes. The following sequence in the formation of polytene chromosomes is constructed from observations on a number of sections of macronuclear Anlagen derived from samples of exconjugants collected at 5–10, 20–25, and 40–45 h after conjugation. Figs. 1–4 show sections of macronuclear Anlagen of 5- to 10-h-old exconjugants. Fig. 1 represents the earliest Anlage in this sample. The Anlage is spherical, measures 10 μm, and contains diffusely distributed chromatin. In Fig. 2, a somewhat older Anlage appears swollen and contains condensed chromatin. In some sections fibrous material is seen inside the Anlage concentrated at various points along the inner border of the nuclear membrane (Fig. 3). These deposits are thought to contain the precursors of the membranes which in later stages transect the polytene chromosomes. In Fig. 4, the chromatin appears to be differentiated into dense and diffuse components and is organized in the form of a reticulum. In between the chromatin network are seen masses of fibrous material (Fig. 4). In samples fixed between 20 and 25 h, the chromatin and the fibrous material show progressive organization. The chromatin is seen as distinct compact bodies; the fibrous material has now become somewhat more condensed and outlines the chromatin bodies (Fig. 5). In a later stage, elongated chromosomes, each outlined by the fibrous material, are visible (Figs. 6, 7). The chromosomes in these sections show a faint banding pattern. In samples fixed between 40 and 45 h, the fully developed polytene chromosomes are already in the process of breakdown (Fig. 8). The fibrous material has become condensed to give the appearance of 'membranes'; the membranes outline the polytene chromosomes and are also seen in the interband

regions (Fig. 8). Thus, the formation of polytene chromosomes appears to be concomitant with the assembly of the membranous material that eventually encloses the individual bands and portions of the adjacent interbands of the polytene chromosomes. The Anlage throughout this period progressively increases in size.

Degradation of polytene chromosomes. In samples of exconjugants fixed between 55 and 60 h the polytene chromosomes are no longer visible. The Anlage contains instead a large number of membrane-bound vesicles (1–2 μm in diameter) each enclosing a single band and portions of the 2 adjacent interbands of the polytene chromosomes (Fig. 9). In some sections, vesicles derived from a single polytene chromosome can be identified by their linear arrangement (Fig. 9). The vesicles are packed with chromatin, and the interstitial space (between the vesicles) contains material of lesser electron density. In a single section of the Anlage the number of the vesicles may vary between 200 and 300, and their total number in the Anlage probably approaches several thousand. In some sections of the sample the Anlage appears shrunken, and the vesicles are seen more compactly arranged with little or no interstitial space (Fig. 10).

The material inside the vesicles undergoes progressive changes. In some the chromatin appears to be concentrated in clumps; in others, the amount of chromatin is diminished and is diffusely distributed within the vesicles. In some Anlagen the vesicles appear more tightly packed, and the number of vesicles that contain chromatin clumps is considerably diminished (Fig. 11), in some cases to less than one third. Many of the vesicles contain particles and aggregates of particles with the individual particles measuring 30–50 nm (arrow, Fig. 11). The nature of the particles has not been determined. On one occasion a structure with a central amorphous region and several peripheral clumps of chromatin was seen inside a vesicle (Fig. 11, inset). The significance of this structure is not known, but it may be a nucleolus. The progressive shrinkage of the macronucleus and the changes in the vesicles inside the Anlage are undoubtedly related to the loss of DNA in the macronucleus.

The membranes of the vesicles do not show the trilaminar structure typical of lipoprotein membranes. They are composed of a single layer measuring about 10 nm in thickness.

Final construction of the macronucleus. Stages of the final construction of the macronucleus are seen in the samples of exconjugants fixed between 65 and 70 h, and 85 and 90 h. At 65–70 h, dissolution of the vesicles is apparent. Many fuse together to form a few large compartments, and channels develop in between these compartments (Figs. 12, 13). These channels contain fibrous material and traverse the interior of the nucleus to the nuclear membrane. The electron density of this fibrous material is similar to that of the fibrous material seen in the early Anlage before the formation of the vesicles (see Fig. 3). The large compartments contain a few chromatin aggregates and loosely distributed chromatin. In some sections two or three nucleoli are apparent (Fig. 13). The internal structure of the Anlage remains the same after the total dissolution of the compartments (Fig. 14). The Anlage in the samples fixed between 85 and 90 h resembles the mature macronucleus (Figs. 15, 16) and contains a large number of dense chromatin granules and a few nucleoli embedded in a finely

granular nucleoplasm. That the macronucleus is in the second period of DNA synthesis is evidenced by the presence of 'replication bands' (structural manifestation of DNA synthesis in the macronucleus of hypotrichous ciliates, see Figs. 15, 16).

Macronuclear development in Tetrahymena pyriformis

Several light-microscopic studies on the conjugation of *Tetrahymena* have been described (Elliot & Hayes, 1953; Nanney, 1953; Ray, 1956). The sequence of macronuclear development presented in this study has been constructed from electron-microscopic observation on a sample of cells that contained conjugating and exconjugant cells at various stages of development.

After the meiotic divisions of the micronucleus and the exchange of the haploid pronuclei, a synkaryon is formed. The synkaryon in the early stage measures 5 μm and contains dense clumps of chromatin. Microtubules are present in the nucleoplasm and around the inner border of the inner membrane of the nuclear envelope (Fig. 17). Fig. 18 illustrates the synkaryon in mitosis and the old macronucleus. The synkaryon in each conjugant divides twice. Of the 4 division products, 2 situated at one pole become the presumptive micronuclei while the other 2 at the opposite pole enlarge and form the macronuclear Anlagen (Fig. 19). The Anlagen are equal in size and contain diffuse chromatin and a few chromatin clumps restricted to the central region. The peripheral region of the Anlagen is vacuolated and contains no discernible structures. Occasionally, the Anlagen are seen to pinch-off a small amount of chromatin in the form of an extrusion body (Fig. 20). These extrusion bodies are never observed in later stages of development and are probably degraded in the cytoplasm.

After formation of the Anlagen the conjugants separate. In the exconjugants, the presumptive micronuclei (situated at one end of the cell in the conjugants) migrate to a new position in between the macronuclear Anlagen (Fig. 21). Subsequently, 1 of the presumptive micronuclei degenerates, and the cell now contains 2 Anlagen and a single micronucleus (Fig. 22). The micronucleus undergoes a mitotic division, and this is accompanied by the cytoplasmic division of the cell such that each daughter cell contains a micronucleus and a macronuclear Anlage. Throughout this period the Anlagen enlarge progressively and display chromatin clumps of varied dimensions that are distributed over a less-dense background. The peripheral region of the Anlagen still shows no discrete structures.

In the daughter cells, the Anlagen enlarge considerably, the chromatin clumps appear more discrete and increase in number, and some of the larger chromatin clumps are seen at the peripheral region of the Anlage (Figs. 23, 24). Finally, the mature macronucleus contains many evenly distributed clumps of chromatin and a large number of crescent-shaped nucleoli situated at the periphery (Fig. 25).

DISCUSSION

Macronuclear development in Stylonychia mytilus

The ultrastructural changes of the macronuclear Anlage observed in the present study are in agreement with the light-microscopic and radioautographic studies of Ammermann (1971) and electron-microscopic observations of Kloetzel (1970). In addition, the present study provides detailed information concerning the sequential structural changes in macronuclear development. In this section some of the important features in the macronuclear development are considered.

Formation of membranes in the Anlage. The membranes in the Anlage appear to originate from fibrous material seen in the early Anlage. This fibrous material in the earlier stages is seen concentrated at several points along the border of the inner nuclear membrane. In the later stages it is seen in the interior of the Anlage outlining the developing polytene chromosomes. As the chromosomes reach the maximum degree of polyteny, the fibrous material condenses to acquire a membrane appearance and is seen in the interband regions. Finally, each of the bands of the polytene chromosomes is completely enclosed by the membranes to form a large number of vesicles. After the DNA degradation stage (to be discussed below), the vesicles fuse to form larger compartments, and the membrane degradation products are conveyed to the nuclear membrane.

Although it appears from the present ultrastructural study that the membrane assembly is concomitant with the formation of polytene chromosomes, Gil *et al.* (1972) have failed to detect either the formative stages of the membranes (in 16 to 32-h-old Anlagen) or their presence at the polytene chromosome stage (in 40-h-old Anlagen). However, these workers have noticed bundles of twisted coils whose longitudinal axis appears to run parallel to the long axis of the polytene chromosomes. In several exconjugants of the polytene chromosome stage, we have consistently observed the membranes around the polytene chromosomes as well as in the interband regions (also seen by Kloetzel, 1970). The discrepancy between our observations and those of Gil *et al.* may be due to the different procedures used in the preparation of the exconjugants for electron microscopy.

Polytene chromosomes in the Anlage. The function of the polytene chromosomes in the Anlage of *S. mytilus* remains unclear. They do not seem to contain structural manifestations of transcriptional activity (e.g. puffs, Balbiani rings). Ammermann (1965) has failed to detect RNA synthesis in the polytene chromosomes by light-microscope radioautography.

Gil *et al.* (1972) have reported many nucleolus-like bodies in the 32-h-old Anlage of *S. mytilus*. These bodies were seen at the periphery of the Anlage. At this time the chromosomes showed no clear banding pattern. Gil *et al.* have suggested that these nucleolus-like bodies represent synthetic activity of the developing polytene chromosomes. We have not fixed exconjugants between 25 and 40 h and it is possible that we have missed such a stage. However, in our earlier samples in which the Anlage contained chromosomes at the beginning of polytenization (Fig. 4), we have not observed such nucleolus-like bodies. The nucleoli were only discernible in the

Anlage at a much later time, i.e. in the exconjugants fixed between 65 and 70 h. Ammermann (1965, 1971) and Kloetzel (1970) have also observed the appearance of nucleoli at the final stages of macronuclear development, i.e. after the vesicle stage. Similarly, Sapra & Dass (1970) have noticed the formation of nucleoli at the end of macronuclear development in *S. notophora*.

Degradation of DNA in the Anlage. Ammermann (1969, 1971) has found that after the formation of vesicles the Anlage shrinks, and over 90% of the DNA in the Anlage is degraded into acid-soluble products that are released into the culture medium. Bostock & Prescott (1972) have suggested that the degradation of DNA in the Anlage at this stage involves selective elimination of certain DNA nucleotide sequences from the Anlage. In this study, macronuclear DNA was found to consist of a single buoyant density component and melted as if it were a single component. Micronuclear DNA, on the other hand, consists of 4 or more buoyant density components and melted as if it were a mixture of several DNAs of different base composition. A mathematical analysis of the micronuclear profile has indicated that more than 90% of the DNA sequences in the micronucleus are not present in the macronucleus.

The degradation of DNA probably occurs in 2 steps. First, the continuity of the polytene chromosome is interrupted as the membranes extend into the interband regions. Secondly, the DNA is degraded within the vesicles. According to the 2 simplest hypotheses concerning the reduction of DNA in the vesicles, either all of the DNA in most of the vesicles is destroyed or over 90% of the DNA in each vesicle is destroyed.

If the physical continuity of the *Stylonychia* polytene chromosomes is maintained by DNA molecules (see Prescott, 1970, for a review), the present electron-microscopic observations, as well as Ammermann's (1971) light-microscopic observations, mean that these DNA molecules are broken at the interband regions. In the present observations membranes are seen clearly separating portions of polytene chromosomes at the interband regions. Although the vesicles derived from the polytene chromosomes appear to be closely associated in the Anlage (Figs. 9–11), there is no evidence of any continuity of the material inside one vesicle with the other. Ammermann (1971) has shown that the vesicles are physically independent by disrupting the Anlage of this stage in a drop of distilled water. He observed release from the Anlage of a large number of granules (presumably the vesicles observed in the electron microscope).

The process of degradation of DNA in the vesicles, however, cannot be inferred from the present electron-microscopic observations. At an advanced stage of DNA degradation (a stage at which the vesicles are still present but the Anlage is much shrunken), 2 types of vesicles can be identified. One contains very little chromatin, which is diffusely distributed, and the second has much more chromatin, which is concentrated in the form of a single clump in each of the vesicles (Fig. 11). The former type of vesicles could be interpreted as those in which the DNA is totally degraded and the latter as those in which the DNA is 'protected' and therefore eventually retained. This hypothesis is considered because of the resemblance of the chromatin clumps in the second type of vesicles to the chromatin granules in the mature macronucleus. These vesicles, however, may be derived from the larger

bands of the polytene chromosomes or represent stages of incomplete DNA degradation.

Experiments are in progress to determine the process of degradation of DNA in the Anlagen. These involve isotopic labelling of DNA in the Anlagen and subsequent localization of the label by electron-microscope radioautography.

Macronuclear development in Tetrahymena pyriformis

The ultrastructural observations reported here show that macronuclear development in *Tetrahymena* differs considerably from that of *Stylonychia*. Features such as the formation and degradation of the polytene chromosomes, the vesicle stage in the Anlage, and shrinkage of the Anlage observed in *Stylonychia* are absent in *Tetrahymena*. Although there is evidence of the loss of a small amount of DNA (chromatin extrusion) from the Anlagen of *Tetrahymena*, the loss (at least quantitatively) is not comparable to that in *Stylonychia* Anlagen. This DNA loss apparently was not detectable in the microspectrophotometric measurements showing a progressive increase in the DNA content of the developing Anlage of *Tetrahymena* (Johansson & Zech, unpublished observations – quoted by Ammermann, 1971). Electron-microscopic observations on purified DNA have also revealed that the macronuclear DNA of *Tetrahymena* is present in much larger pieces (greater than 35 μm in length) than is macronuclear DNA of *Stylonychia* (Murti, 1972).

The above observations are in agreement with the pattern of macronuclear development observed in *Paramecium* in which the available electron-microscopic observations (Jurand *et al.* 1964) and microspectrophotometric measurements of the DNA content in the developing macronuclear Anlage (Woodard *et al.* 1966) suggest a pattern of macronuclear development similar to *Tetrahymena*.

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Fig. 1. A section through a *Stylonychia* exconjugant showing the early Anlage. × 4200.

Fig. 2. Early Anlage in the process of elongation. Note the condensation of chromatin. × 3200.

Fig. 3. Early Anlage showing deposits of diffuse material (arrows) around the inner nuclear membrane. × 3200.

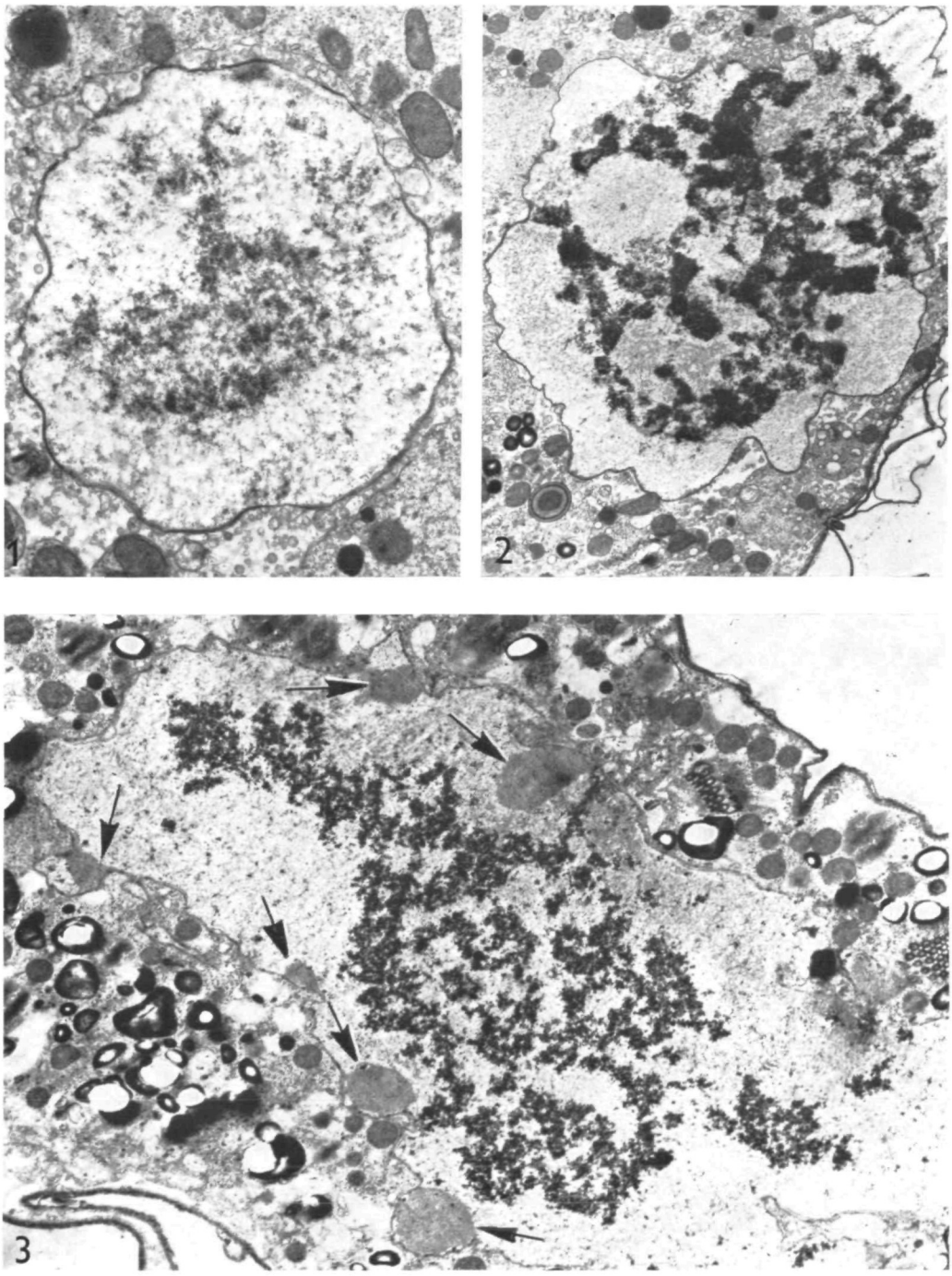
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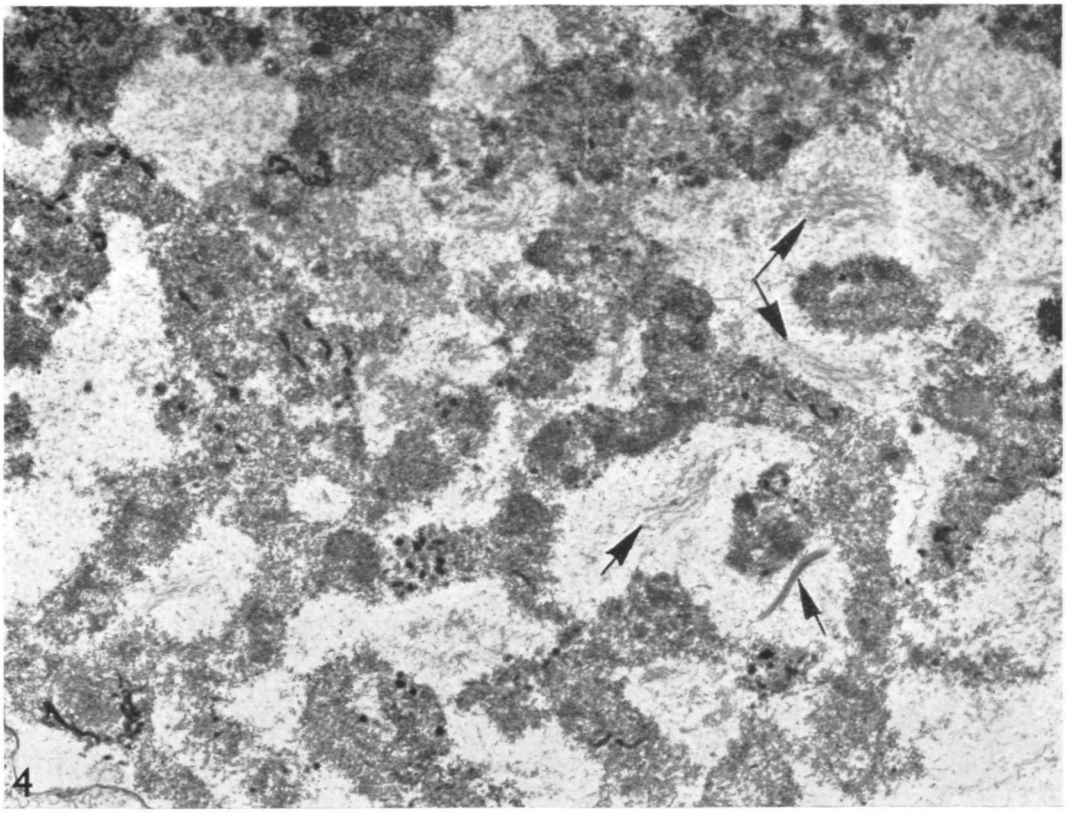
Fig. 4. A portion of the Anlage showing reticular organization of chromatin. The chromatin consists of dense and diffuse components and at places is highly condensed. Stacks of fibrous material are seen (arrows) inside the chromatin reticulum. × 11 550.

Fig. 5. The chromatin (*ch*) in the Anlage is organized into distinct aggregates. Thin layers of fibrous material (arrows) are seen surrounding the chromatin aggregates. × 7700.



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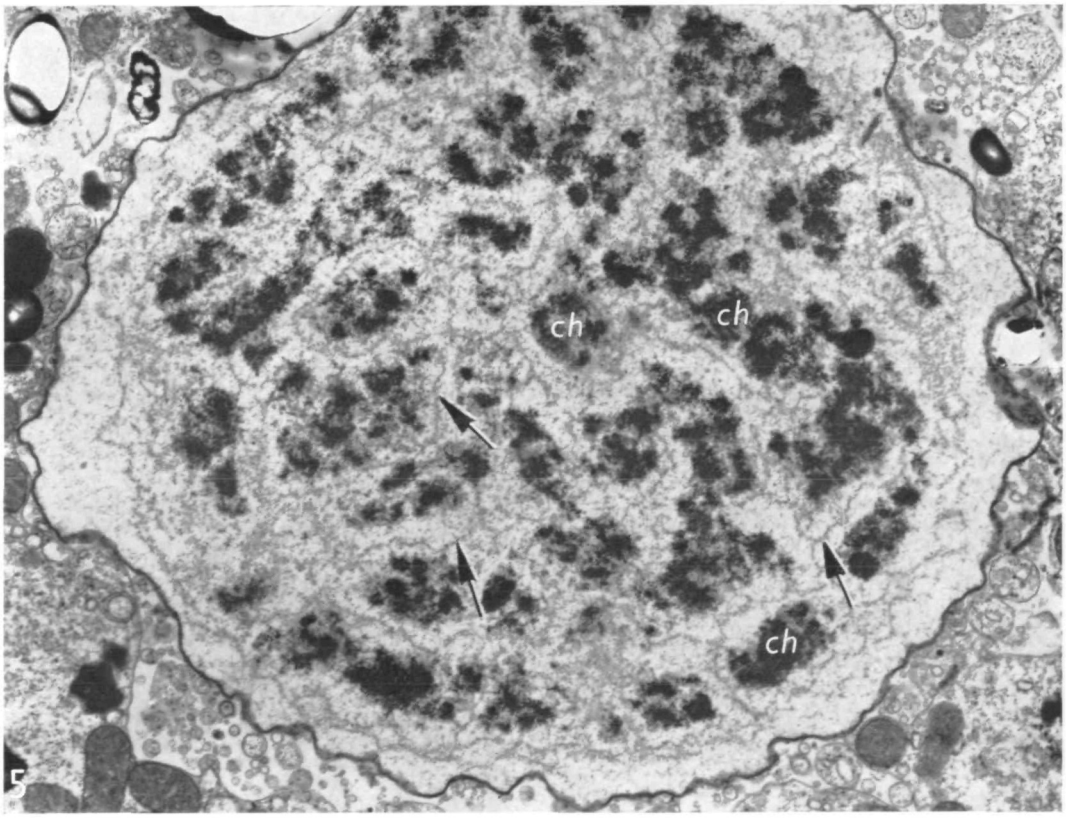

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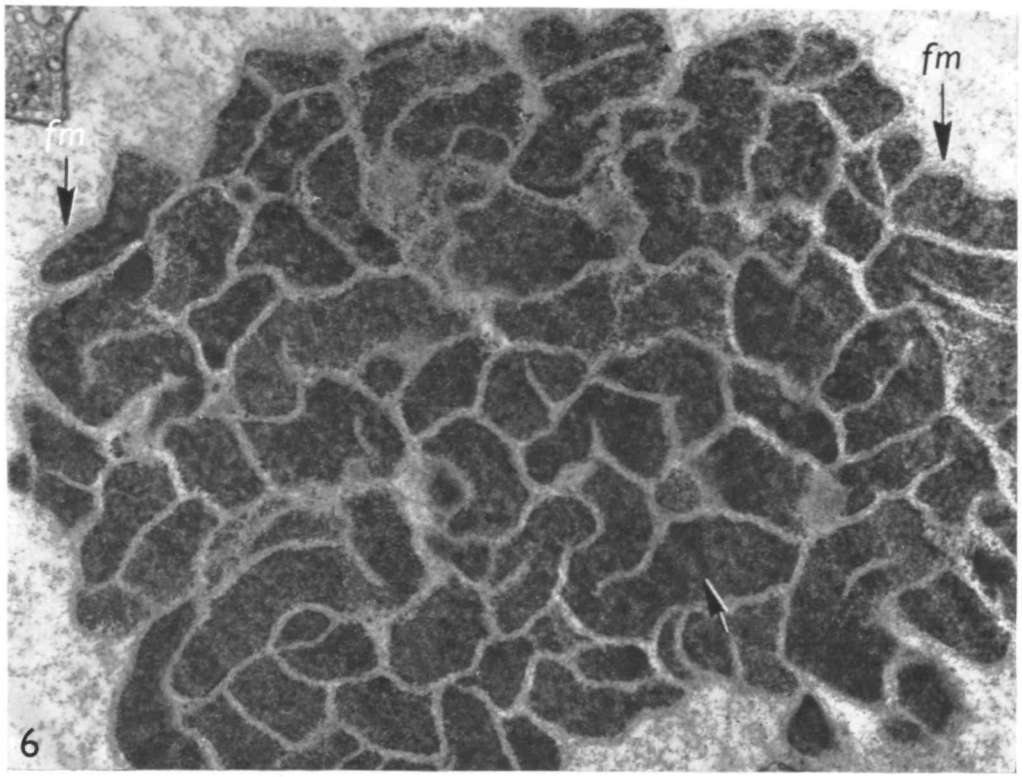
Fig. 6. A portion of the Anlage at the beginning of the polytene chromosome formation. The chromosomes are surrounded by fibrous material (*fm*) and show faint banding pattern (arrow). ×7350.

Fig. 7. A portion of the Anlage showing the early polytene chromosomes. The chromosomes are more elongated than in the previous figure, show evidence of banding (arrows), and are outlined by fibrous material (*fm*). ×9800.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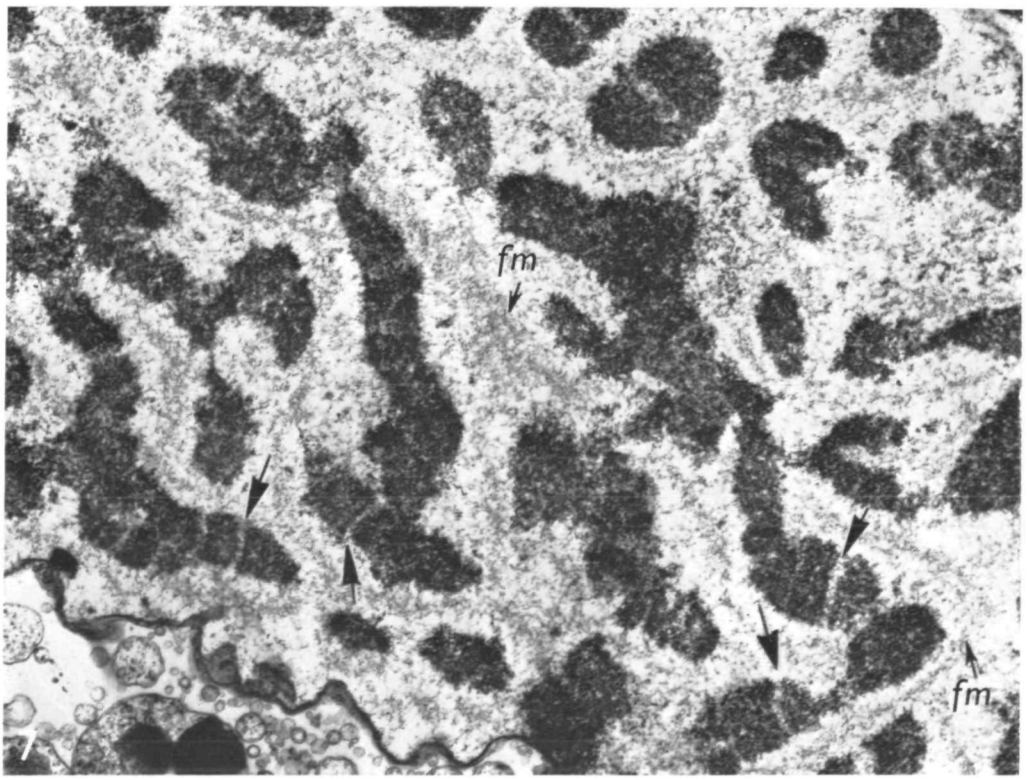

fm
fm
7

Fig. 8. A portion of the Anlage showing chromosomes at the peak of polyteny and at the beginning of degradation. The fibrous material seen in the previous section appears to have condensed into membranes and the membranes are seen around the polytene chromosomes and in the interband regions (arrows). × 13250.

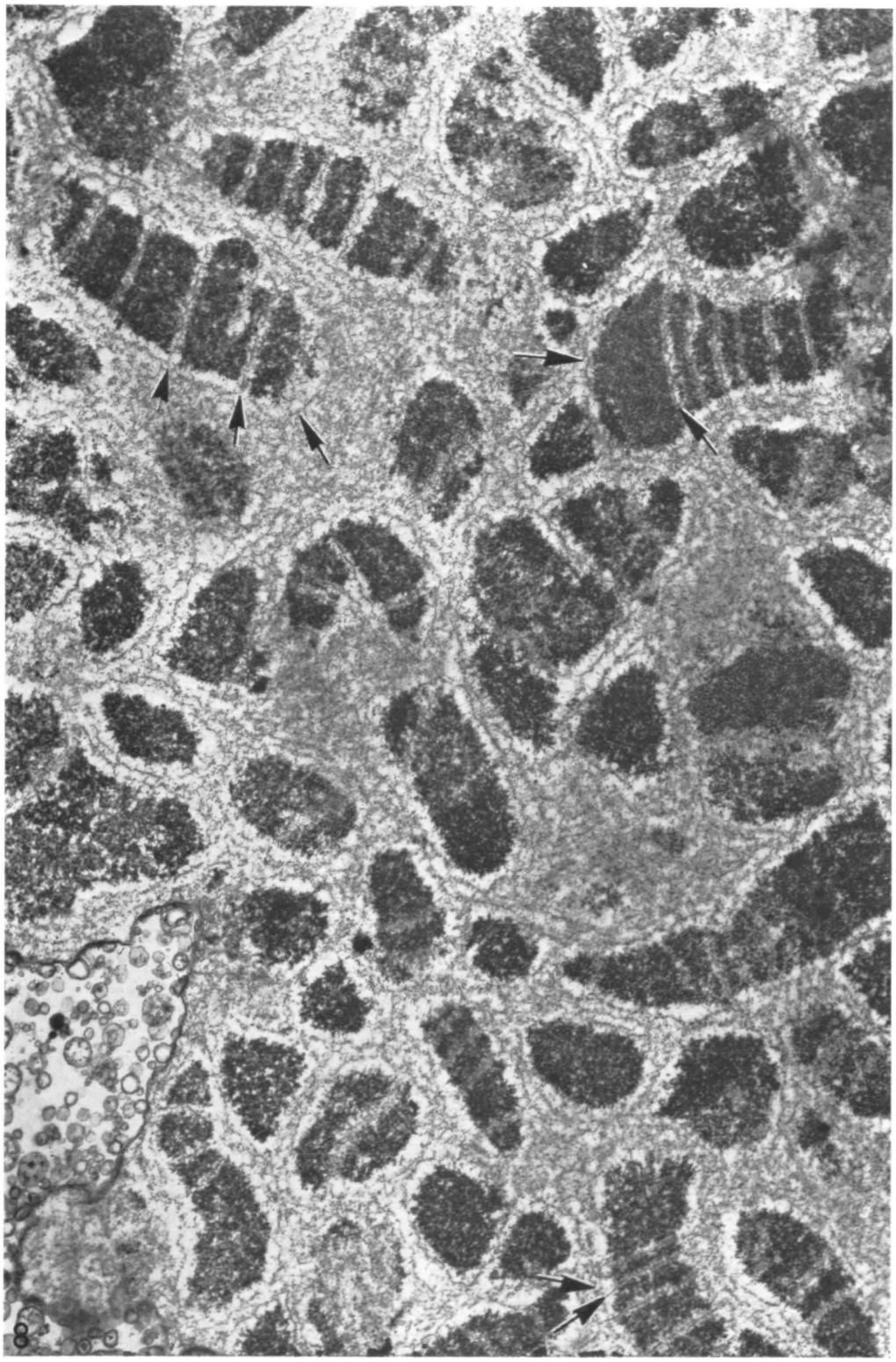
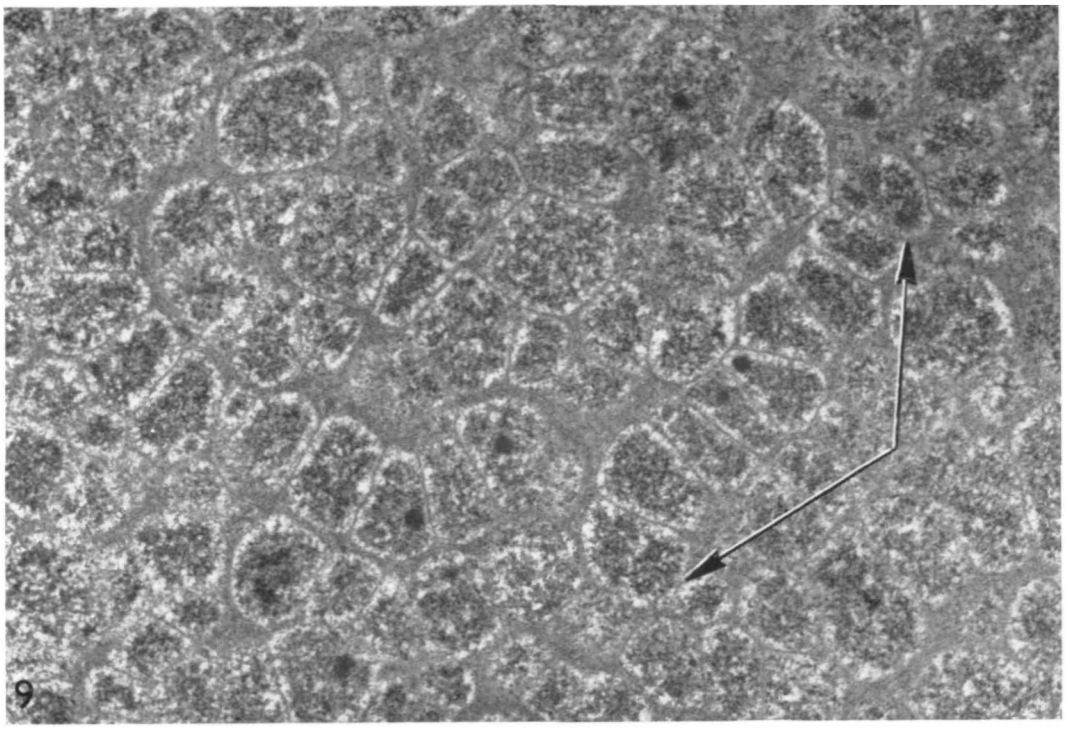


Fig. 9. A portion of the Anlage showing degraded polytene chromosomes. The Anlage is filled with a large number of vesicles which have presumably enclosed chromatin derived from a band and portions of the 2 adjacent interbands of the polytene chromosomes. Some of the vesicles that belong to a single polytene chromosome are seen in linear association (arrows). × 18650.

Fig. 10. A later stage in the degradation of polytene chromosomes. The Anlage contains tightly packed vesicles. Some of the vesicles contain dense clumps of chromatin while others enclose diffuse chromatin. × 14600.



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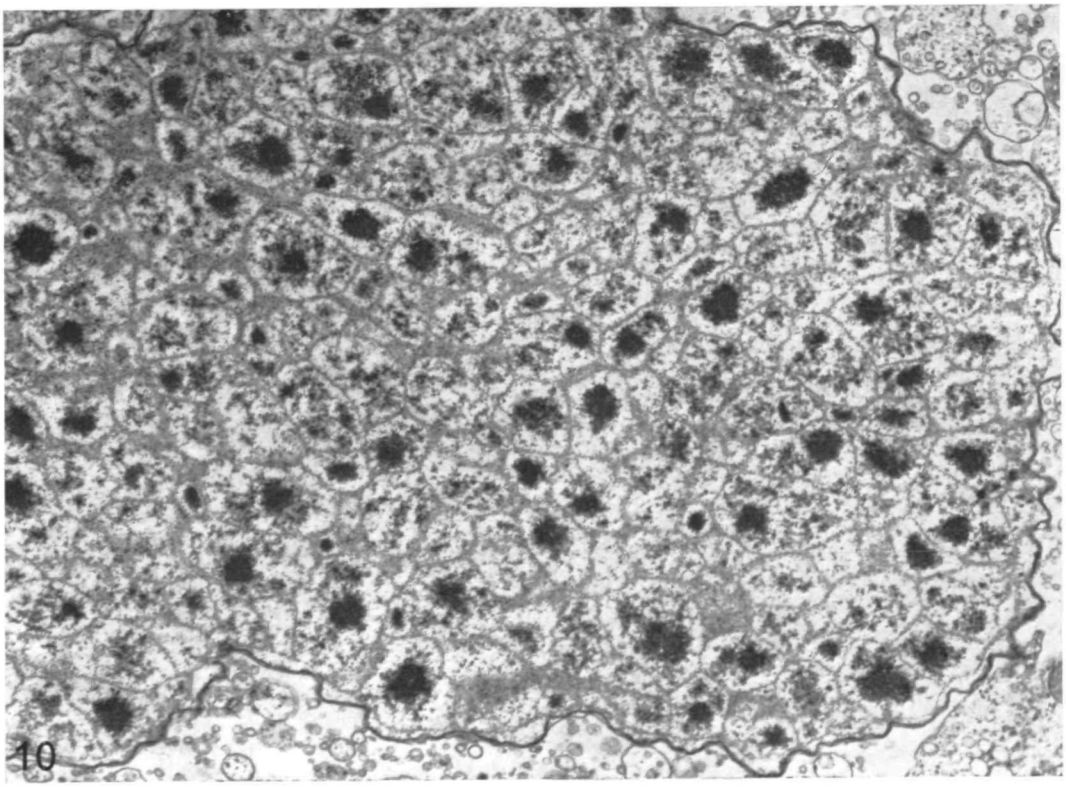

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Fig. 11. A portion of the Anlage illustrating an advanced stage in DNA degradation. The Anlage is much shrunken and the vesicles are tightly packed with no interstitial space. In most of the vesicles 30–50 nm particles are seen (arrow). × 10000. Inset: a nucleolus-like structure (*nu*) in one of the vesicles. × 21000.

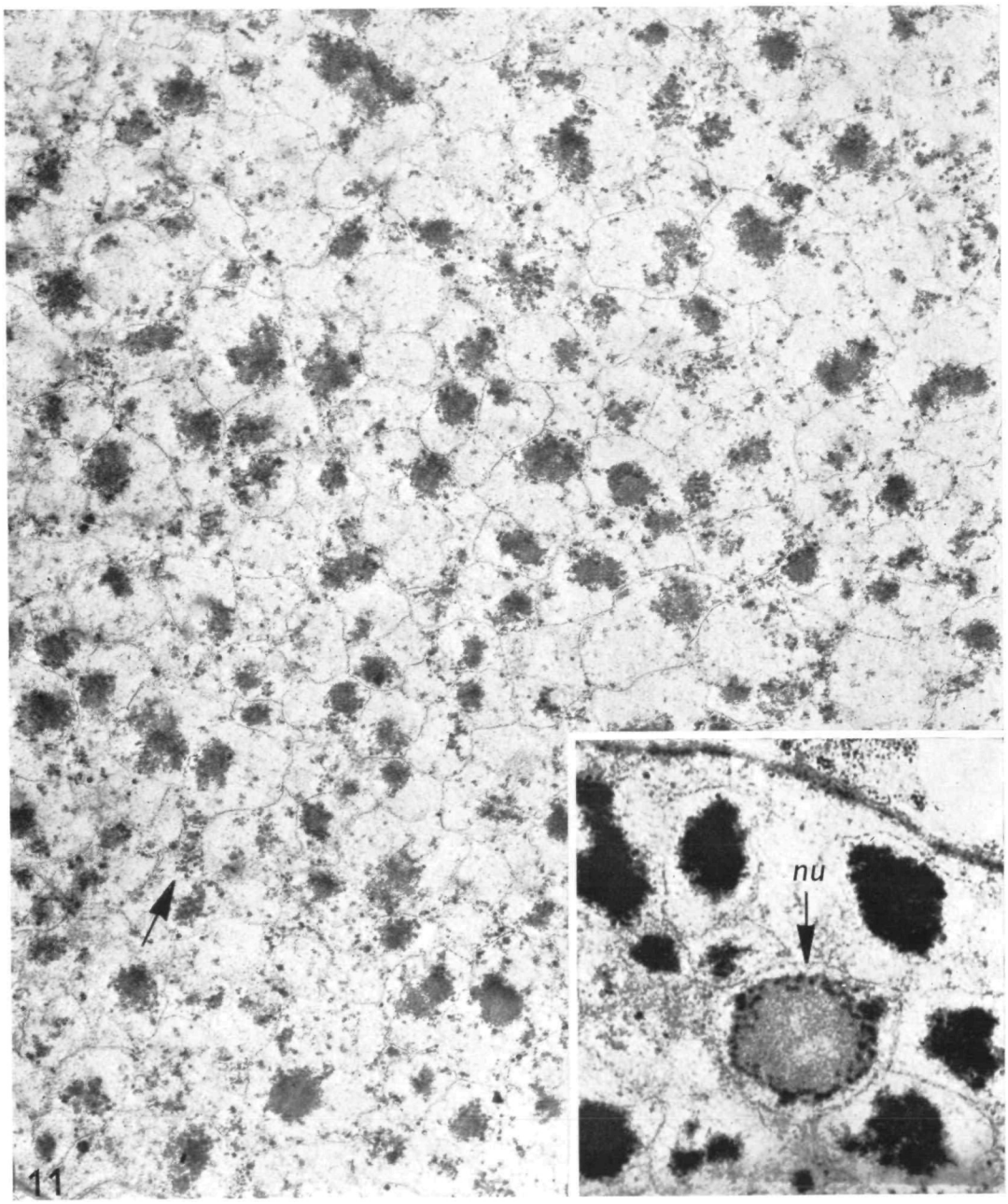
nu
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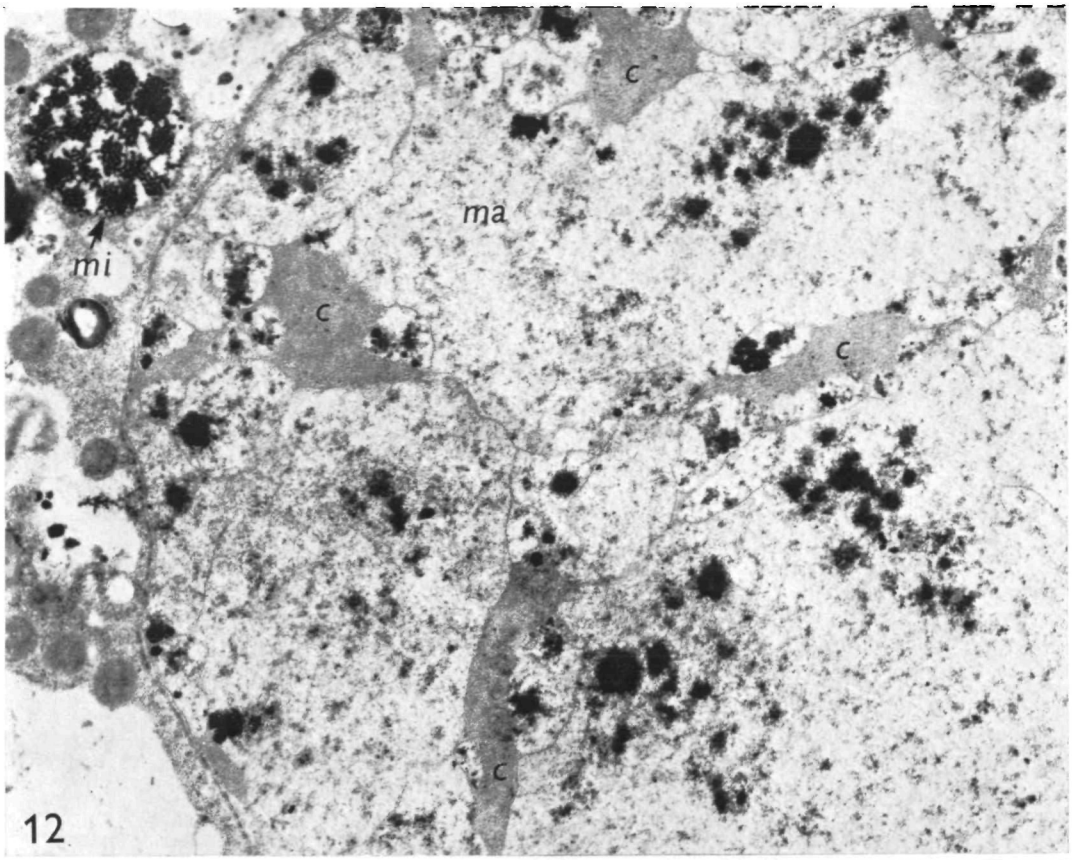
Fig. 12. A section showing the micronucleus (*mi*) and a portion of the Anlage (*ma*). The vesicles in the Anlage are in the process of dissolution. Note the channels (*c*) containing the diffuse material. × 7200.

Fig. 13. A magnified view of a portion of the Anlage showing the same structural features as in the previous figure but illustrating the nucleoli (*nu*). × 13 100.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mi
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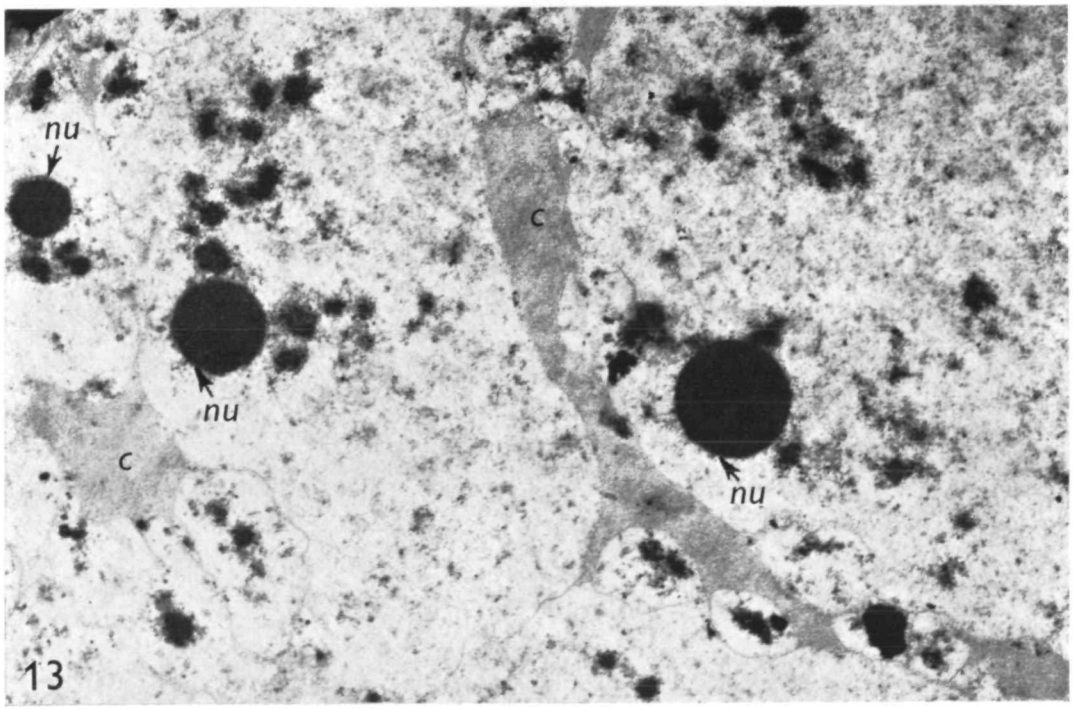
c
12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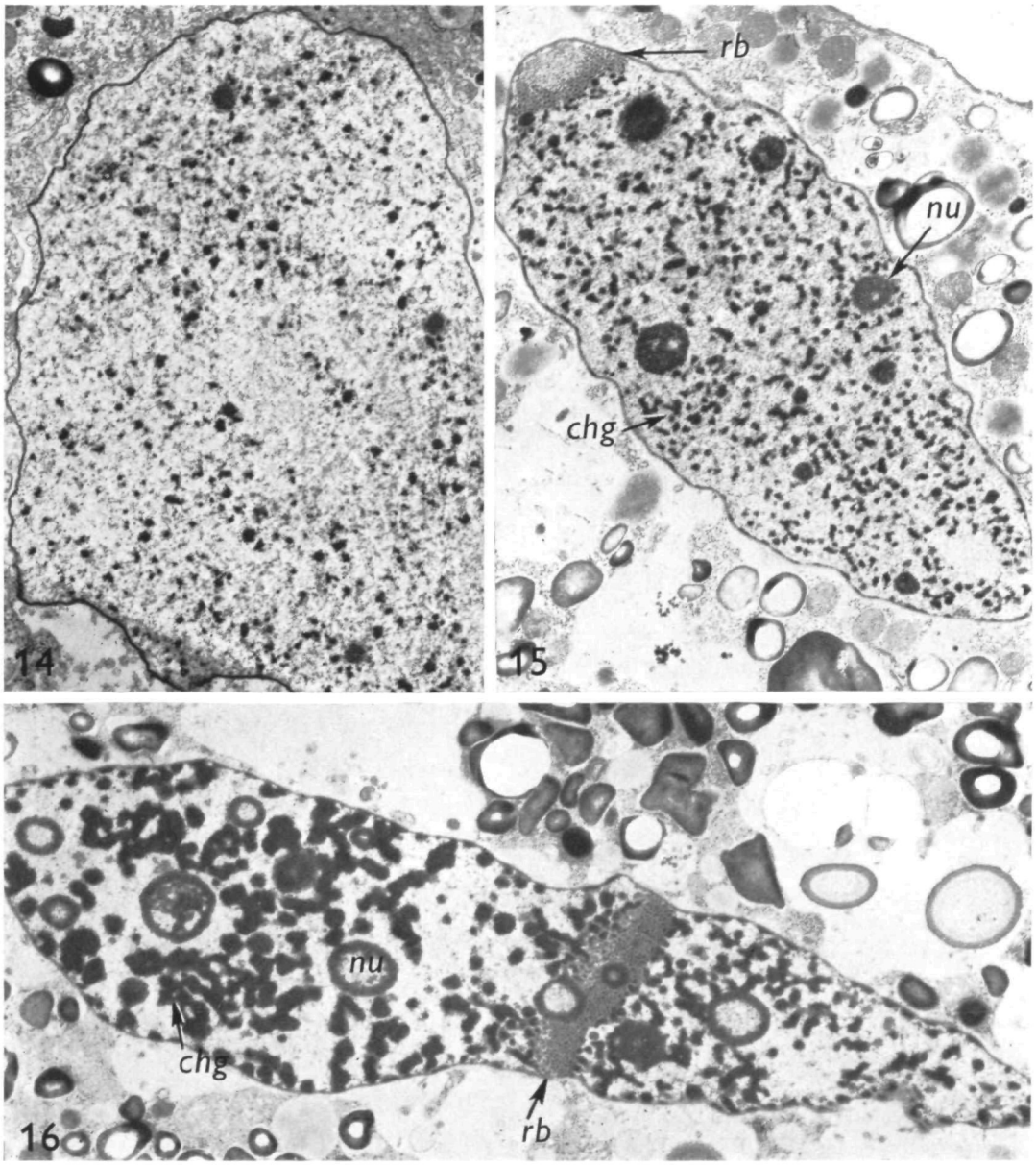


Fig. 14. An Anlage after the dissolution of the vesicles. × 6500.

Fig. 15. An Anlage in the final stages of reconstruction. It contains chromatin granules (*chg*), nucleoli (*nu*), and a replication band (*rb*). × 8200.

Fig. 16. A mature macronucleus in the process of replication. *chg*, chromatin granules; *nu*, nucleoli; *rb*, replication band. × 7200.

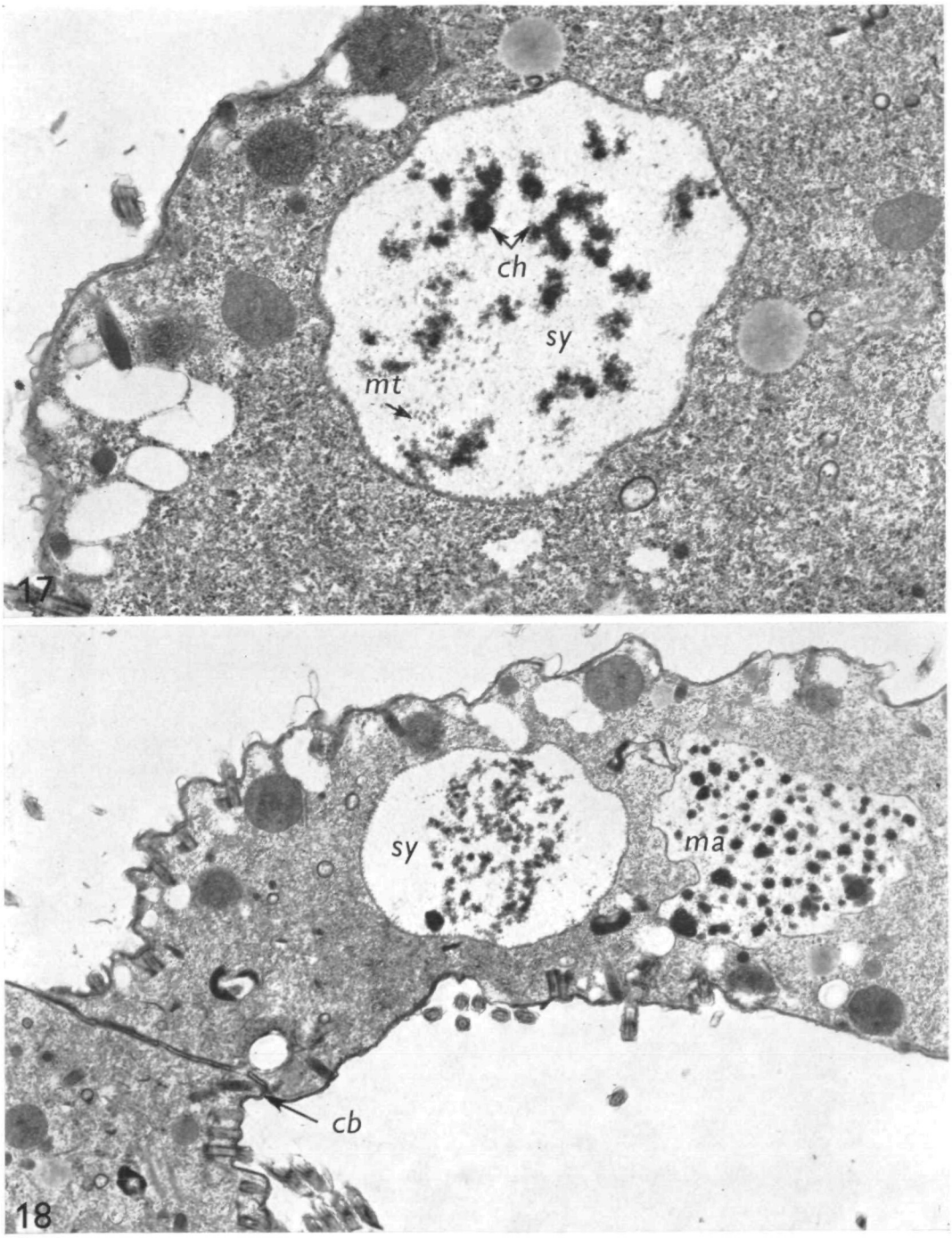
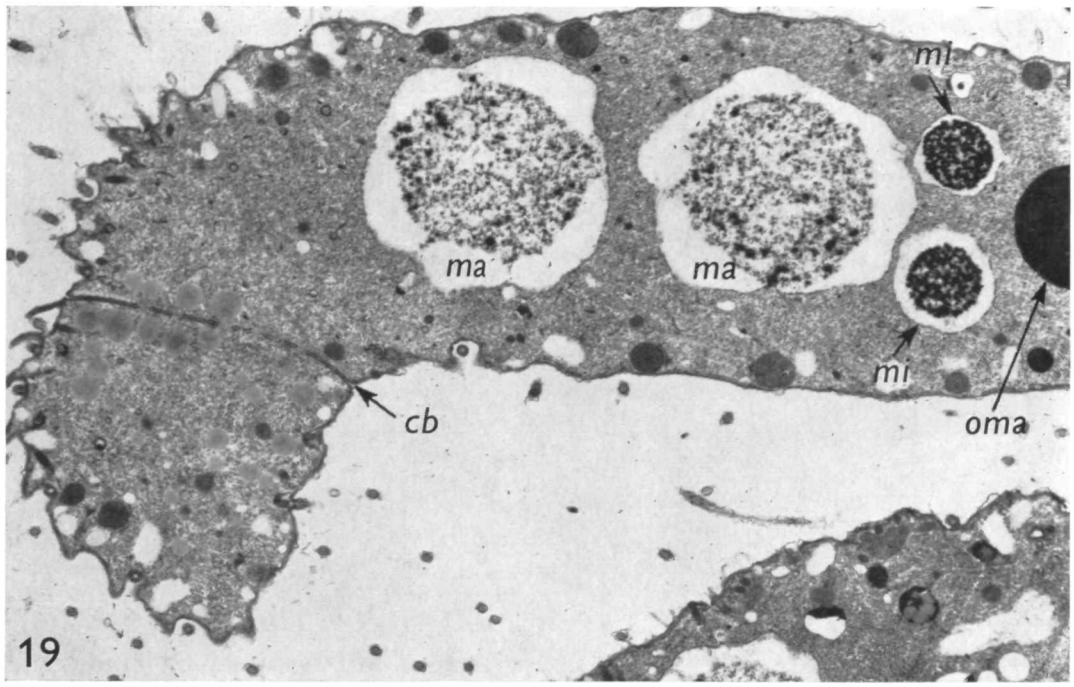


Fig. 17. A section through a conjugating *Tetrahymena* cell illustrating the synkaryon (*sy*) which contains chromatin clumps (*ch*) and microtubules (*mt*). × 11700.

Fig. 18. A section through a conjugating cell showing the synkaryon, the macronucleus (*ma*), and the conjugation bridge (*cb*) that links both cells. × 6000.



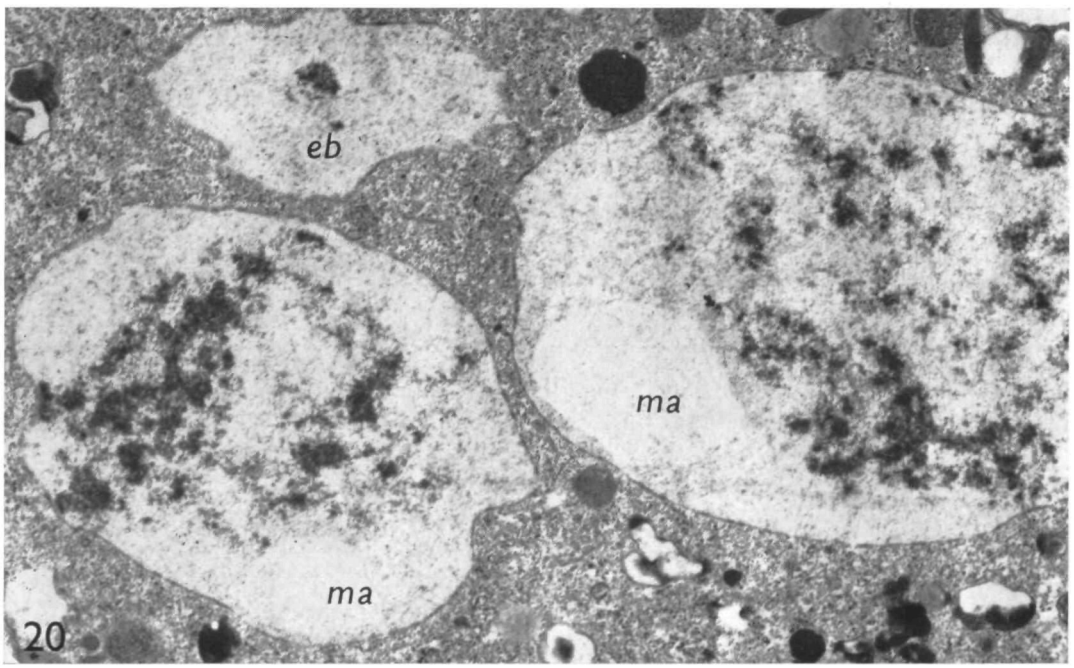
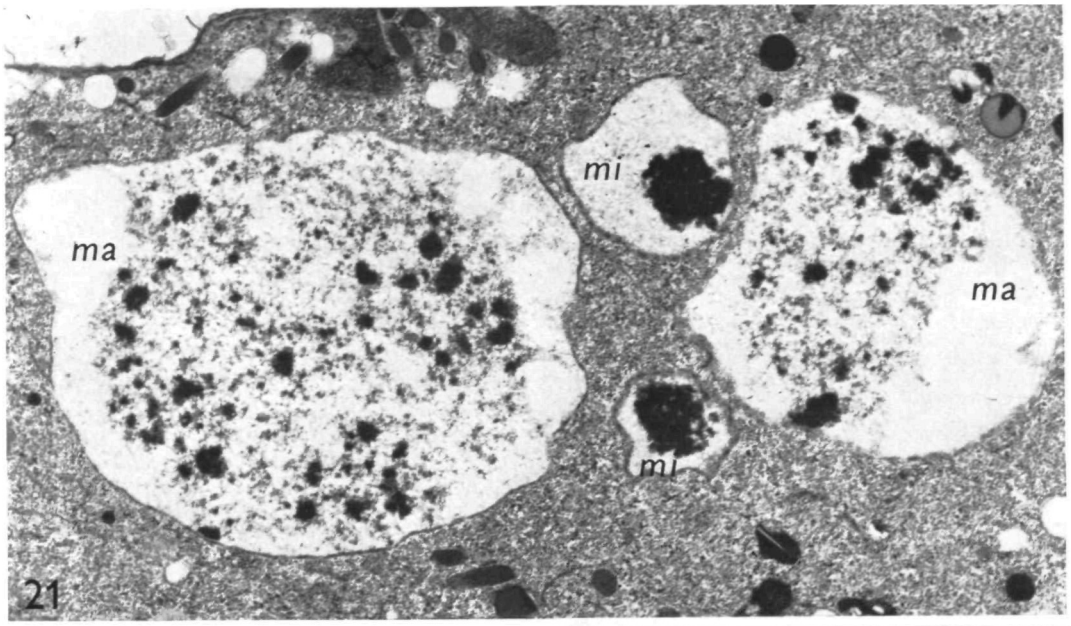


Fig. 19. A section showing the 4 division products of synkaryon. The 2 macronuclear Anlagen (*ma*) are situated at the anterior end and the presumptive micronuclei (*mi*) at the posterior end. Also included in the figure are the old macronucleus (*oma*) and conjugation bridge (*cb*). × 3700.

Fig. 20. The Anlagen showing extrusion of chromatin in the form of an extrusion body (*eb*). *ma*, macronuclear Anlage. × 6500.



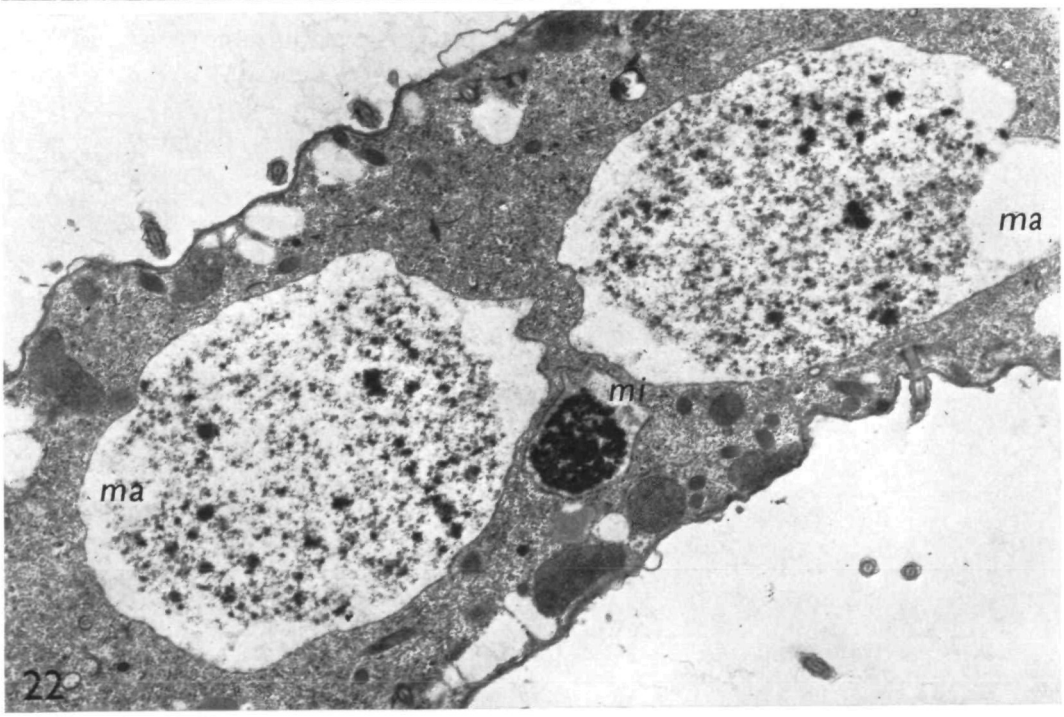


Fig. 21. A section through an exconjugant cell of *Tetrahymena* showing the presumptive micronuclei (*mi*) situated in between the Anlagen (*ma*). × 5900.

Fig. 22. Degeneration of one of the presumptive micronuclei seen in the previous section. *ma*, macronuclear Anlage; *mi*, micronucleus. × 6000.

Fig. 23. Macronuclear Anlagen (*ma*) in the cells that had been through the third postzygotic division. In the daughter cells the Anlagen contain dense chromatin clumps (*ch*), one of which is situated at the inner nuclear membrane in one of the Anlagen (arrow). ×4000.

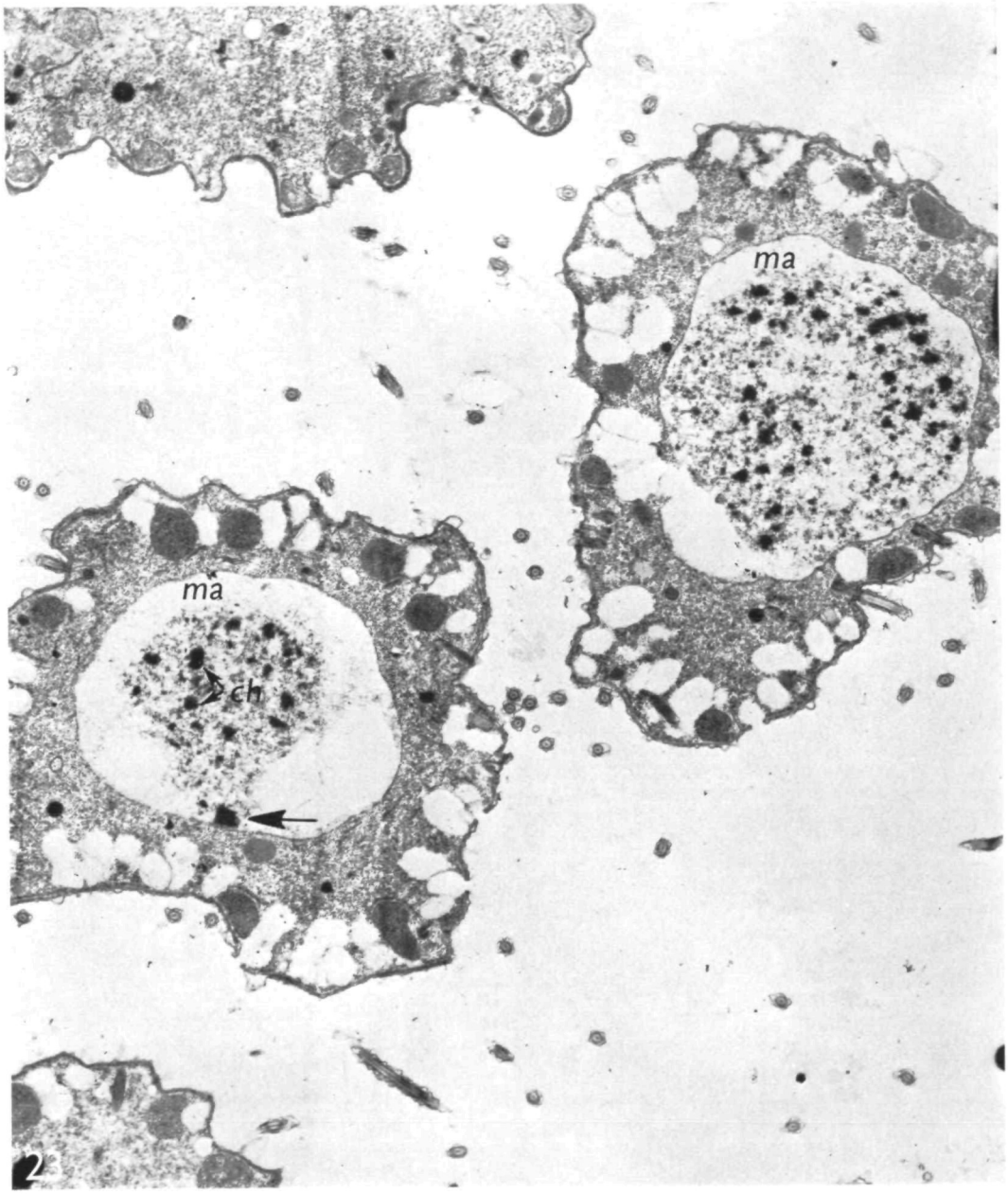
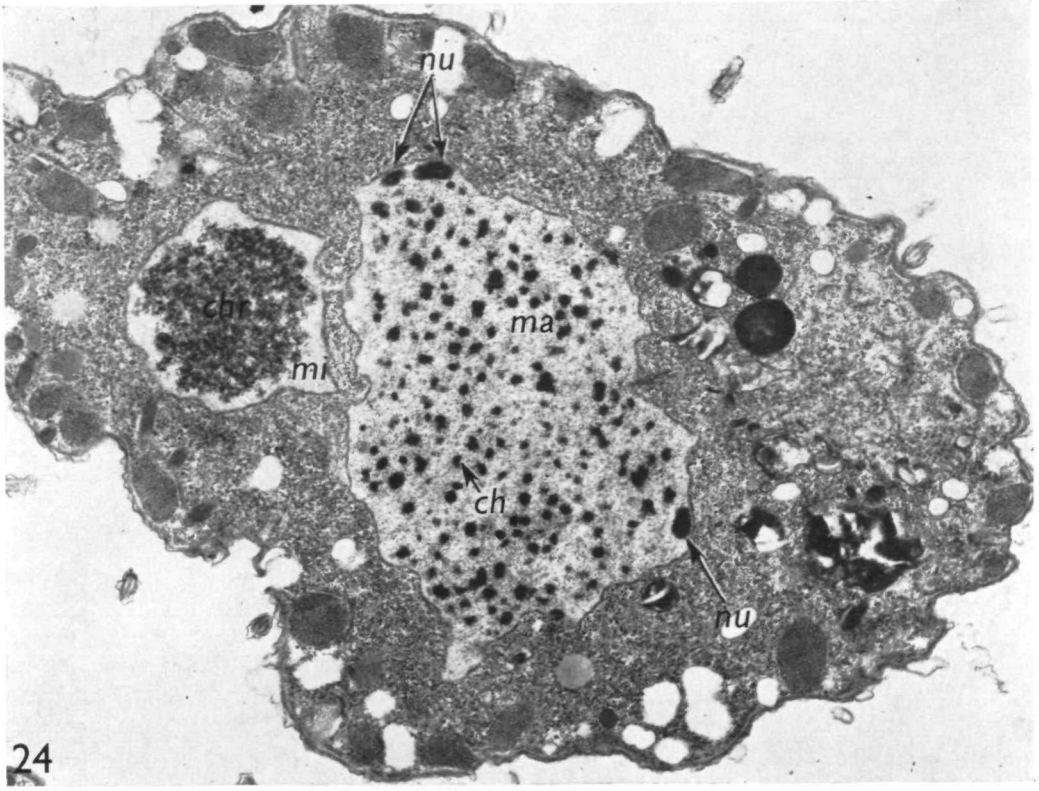

ma
ma
ch

Fig. 24. A maturing *Tetrahymena* illustrating the micronucleus (*mi*) and the macronucleus (*ma*). The micronucleus contains condensed chromatin (*chr*) and the macronucleus contains chromatin clumps (*ch*) and few nucleoli (*nu*). × 5900.

Fig. 25. Macronucleus (*ma*) in a mature cell; it contains many crescent-shaped nucleoli (*nu*) at the periphery and chromatin clumps (*ch*) in the interior. × 4000.



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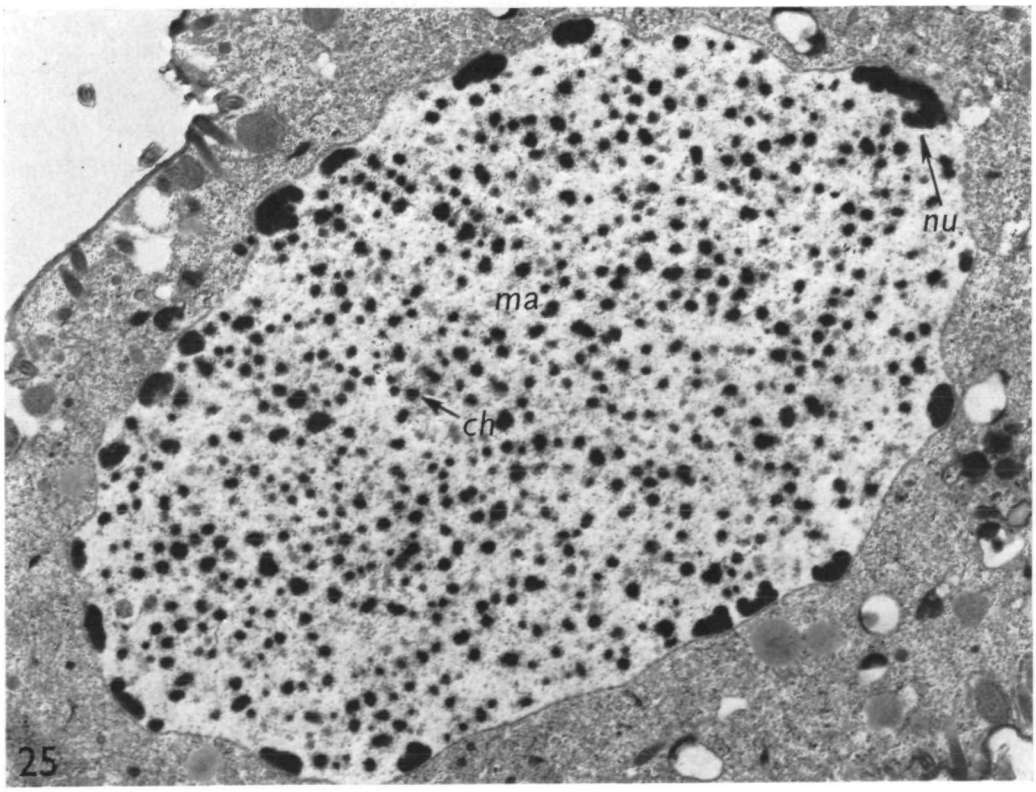

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