ULTRASTRUCTURE OF NUCLEAR DIVISION IN *PARAMECIUM AURELIA*

III.* MEIOSIS IN THE MICRONUCLEUS DURING CONJUGATION

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Abstract

Ultrastructural features of micronuclear meiosis occurring during conjugation of the ciliate *P. aurelia* are described. Following pair formation the micronuclei enter prophase. The core of condensed chromatin in the micronucleus fragments, and almost the whole nucleus becomes filled with coiled and twisted chromatin elements. After about 1·5 hr of pairing regions of condensed chromatin appear linked into strands 1–2 μm long. Somewhat later, ill-defined, synaptinemal, complex-like material lies between paired masses of chromatin. Only a few synaptinemal complex-like structures can be seen in the nucleus. A short while later, synaptinemal complex-like material may be seen free in the nucleoplasm. After about 2·5 hr of pairing, the micronucleus expands into the crescent stage. Microtubules appear in the nucleus, which expands rapidly to a length of about 20 μm, usually oriented along the long axis of the cell. Concurrently with expansion the chromatin becomes diffuse and spreads along the length of the nucleus. This stage probably corresponds to diplotene. The nucleus then shrinks, microtubules reappear, and metaphase of the first meiotic division occurs. A large number of chromosomes, with indistinct kinetochores, can be seen. At telophase the nucleus is 15–20 μm long and dumbbell-shaped. It breaks into two parts, there being no separation spindle, in contrast to micronuclear mitosis. The second meiotic division occurs immediately. Ultrastructurally it resembles the first with respect to metaphase, anaphase, and telophase. Since *P. aurelia* has two micronuclei, there are eight haploid meiotic products. The one nearest the paroral cone survives and the other seven disintegrate and are resorbed. The remaining nucleus undergoes a modified mitotic division. One of the two final products becomes a stationary ("female") pronucleus, while the other becomes a migratory ("male") pronucleus. Male pronuclei are exchanged across the fused paroral cone regions of the partners. The male pronucleus contains microtubules during its migration. After fusion to produce the synkaryon, the conjugants separate.

The macronuclei of conjugants also undergo a series of changes. At metaphase of the first micronuclear division they become irregular in outline. During the second meiotic division the nucleus becomes a twisted skein 40–50 μm long. As the third micronuclear division becomes imminent the macronucleus fragments into 30 or 40 pieces, microtubules being apparent in small numbers at this time.

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I. Introduction

The two previous papers of this series (Stevenson and Lloyd 1971*a*, 1971*b*) have described the fine structure of the division processes of the micronucleus and macronucleus of the ciliate *Paramecium aurelia*. This paper will describe the fine structure of the meiotic divisions of the micronucleus of this organism, which occur during the sexual process, conjugation.

Conjugation in *P. aurelia* occurs between animals of opposite mating type of any of the 14 known syngens. Conjugation between animals of different syngens is not usual, though it does occur in certain cases (Beale 1954). In normal conjugation, the two micronuclei of each partner undergo meiotic divisions resulting in eight haploid daughter nuclei. Seven of these nuclei disintegrate, while the eighth, protected by its special location in the cell, divides equationally, One of these nuclei migrates to the partner animal and fuses with the stationary nucleus; thus reciprocal cross-fertilization takes place. Consideration of the behaviour of gene markers establishes this sequence of events, which is also observable cytologically. During conjugation the macronucleus of each partner breaks up into 30 or more fragments. After fertilization, the cells separate, and the micronuclear synkarya divide twice mitotically. Of the four products, two remain micronuclei, and two differentiate, via structures known as anlagen, into macronuclei. The two anlagen are separated to sister cells at the first post-conjugal fission, prior to which the micronuclei divide once more. After this fission, each cell has a nuclear complement of the two micronuclei, one macronuclear anlage, and a number of fragments of the old macronucleus. The anlagen are mature by the second fission, while the fragments are resorbed over several fissions, being diluted out to daughter cells; they continue RNA synthesis during this period (Berger 1969).

Over the years, a number of authors have provided light microscopic accounts of nuclear events at conjugation in *P. aurelia* (Hertwig 1889; Maupas 1889; Sonneborn 1947, 1954; Beale 1954; Kimball and Gaither 1955; Jones 1956; Kosciusko 1965; Grell 1967). There have been some studies with the electron microscope of the nature of contact between the partners and the process of nuclear exchange (Vivier and André 1961; André and Vivier 1962; Schneider 1963; Inaba, Suganuma, and Imamoto 1966; Jurand and Selman 1969) but there are no ultrastructural accounts of meiosis. This paper, describing the nuclear events occurring during pairing of the cells, is an attempt to fill this gap. Indeed, as far as the author is aware, this is the first ultrastructural description of nuclear behaviour at meiosis in any ciliate.

II. Materials and Methods

Stock 540 of syngen 1 of *P. aurelia* was used throughout. Cells were cultured in baked lettuce extract medium, and sexual reactivity was induced as described by Sonneborn (1950). Baked lettuce extract was used because it was found extremely difficult to obtain adequate mating reactivity in the 0·1% Vegemite medium used in previous studies.

Pairs of conjugating cells were selected at known times after mixing the complementary mating types, and fixed, processed, and embedded as described earlier (Stevenson and Lloyd 1971*a*). All steps were carried out at room temperature (22°C). At this temperature the period of pairing in stock 540 is 7–7·5 hr.

All other techniques were also as described previously (Stevenson and Lloyd 1971*a*).

III. Results

In the introduction to this paper, the course of events in micronuclear meiosis was described briefly. Before describing the results of this investigation it would be advantageous to give the reader some further details of the meiotic and conjugation processes in *P. aurelia*, as shown by light microscopy. This account is based on those of Beale (1954), Kosciusko (1965), and Jurand and Selman (1969), who present some of Jones' (1956) data not previously published. It will also include some of the present author's more general observations on the processes in stock 540, at 22°C.

When reactive cells of complementary mating type are mixed there is an immediate clumping, or agglutination, of the cells into large groups. This agglutination persists for about 1–1·5 hr at 22°C. Cells then separate from the clump in pairs. During agglutination, cells are not fused as they are in conjugation, and the clump splits into individual cells if violently agitated or on the addition of fixative.

Pairs of cells are initially attached at the anterior ends (the holdfast), and then later near the mouth (the paroral cone). Once they are attached at both points, they cannot be forcibly separated. Meiotic processes do not commence until pairs are united at the anterior holdfast. The cells swim about all the time, and do not feed; later in conjugation the mouths de-differentiate, and new mouths are formed after separation.

At the start of meiosis, the micronuclei increase in size, from the interphase diameter of 3–5 μm to about 6–8 μm. A prophase-like condensation of chromatin occurs; granules are just discernible in the light microscope. Jones (1956) observed what may be heteropycnotic chromosomes. There follows a great increase in size, to a length of 20 μm (the crescent stage) with chromatin spread along the nucleus. The nucleus contracts, a brief prometaphase occurs, and then a large number of chromosomes are visible on a metaphase plate. Estimates of chromosome number vary—Dippell (1954) gives a range of haploid numbers from 33 to 51, Jones (1956) a diploid number of 88 or 90, and Kosciusko (1965) a range up to a diploid number of 126. These were in differing stocks and syngens, but it seems that chromosome number varies from stock to stock, even within a single syngen. At 22°C, the first division takes about 5 hr, prophase and the crescent stage occupying the first 3–3·5 hr of this period.

After a brief anaphase, the nuclei enter the second division. There is no interphase, second metaphase forming immediately. This is of about 1 hr duration, followed by an anaphase with somewhat more marked elongation than first anaphase. The nucleus becomes about 25 μm long at this stage, before separating. The chromosomes condense somewhat. Each cell now has eight haploid micronuclei. Seven of the nuclei disintegrate, and the eighth nucleus, always positioned in the paroral cone, undergoes an equational division, to give the two "gamete" nuclei. This division occurs after 6–6·5 hr at 22°C. Migratory ("male") nuclei are now exchanged by partners. How it is decided which nucleus is to be migratory is unknown. The exchange is not necessarily simultaneous, and takes place across the paroral cone regions of the partners, in which membrane fusion and dissolution is much more extensive than at other points of contact. Each migratory nucleus fuses with the partner cell's stationary ("female") nucleus to form the diploid synkaryon. This occurs after

about 7 hr at 22°C, and the cells then begin to separate. The synkaryon nucleus enters a brief interphase-like state at this time. In stock 540, the first division of the synkaryon does not seem to occur till after cell separation. Significant amounts of cytoplasm are not normally exchanged.

The macronucleus also undergoes changes. After 3·5–4 hr at 22°C, it develops a large number of lobes, then stretches to form a long narrow skein. In stock 540, the skein does not seem generally as long as has been described in other stocks. After about 6 hr (roughly concurrent with the end of the second micronuclear meiotic division) it fragments into 30–40 pieces, microtubules being visible (with the electron microscope) within it at this time.

A number of other changes also occur. After about 6 hr at 22°C, the gullets de-differentiate. The cells themselves become smaller as conjugation proceeds, the cytoplasm becomes full of aggregations of vesicles, and assumes a pale "washed-out" appearance in the electron microscope. Presumably this appearance and the reduction in size is associated with the utilization of many of the cell's metabolic reserves.

After separation, the nuclei divide and differentiate into macro- and micronuclei, a new gullet is formed, and the cell feeds again, growing to a larger size than normal. Normal size is restored after the first postconjugal fusion. The events of this period will be dealt with in a succeeding paper.

(*a*) *Micronuclear Events in Conjugation*

(i) *Agglutination*

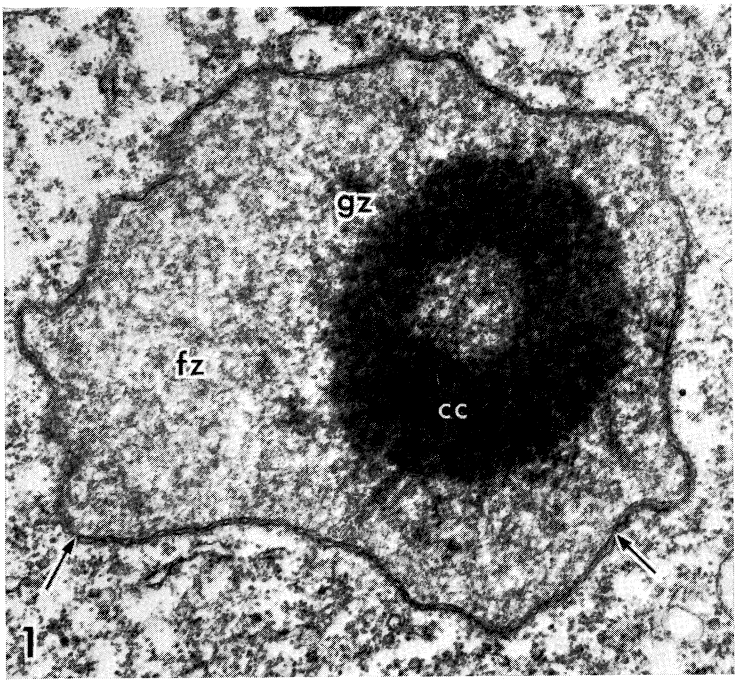
Sexually reactive cells have nuclei with the ultrastructure typical of interphase: a diameter of about 4 μm, an electron-dense core of condensed chromatin, a granular zone, and an outer fibrous zone (Fig. 1). The core may show regions of variation in electron density, or may be ring-shaped. There are no significant changes during the period of agglutination; at the end of this time the micronuclei still have an interphase ultrastructure.

(ii) *Prophase I*

As in other organisms, prophase is the most distinctive stage of meiosis in *P. aurelia*. It cannot be divided into the classical substages, however, as in a number of respects the events are atypical.

Meiosis begins as the organisms become joined in pairs. The micronuclei increase somewhat in size (to 5–6 μm in diameter), and the core of condensed chromatin fragments into a large number of tightly coiled and twisted elements (Fig. 2). It is difficult to infer whether there are any relationships between these elements. The chromatin elements resemble chromosomes, but it cannot be ascertained whether they are single or double. Thus it seems that leptotene and zygotene stages do not occur as such, chromosomal elements remaining coiled and condensed throughout.

Fig. 2.—Earliest stage of prophase, about 45 min after start of cell pairing. The core has fragmented into a large number of chromatin elements (*chr*). Much of the chromatin is apparently linked up, but the whole mass is coiled and twisted. ×15,200.



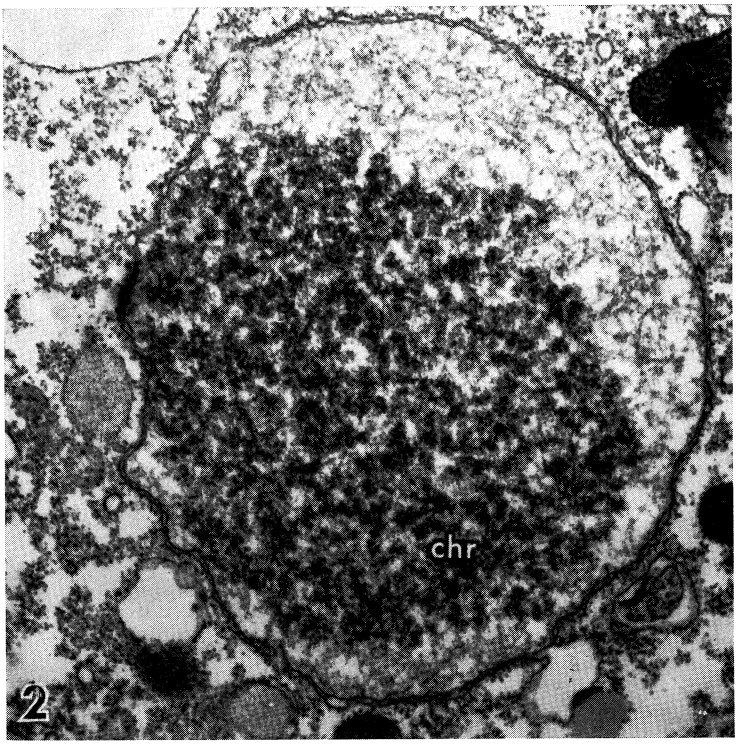


Fig. 1.—Micronucleus of cell after 1 hr of agglutination. The ultrastructure is that typical of interphase, showing a dense core of condensed chromatin (*cc*), in this case doughnut-shaped in section, a granular zone (*gz*), and a fibrous zone (*fz*). Pores are evident in the nuclear membrane (arrows). ×15,200. (Figures 1–11, 13–15, 17–20, and 22–28 are electron micrographs. Figures 12, 16, and 21 are light micrographs of toluidine blue-stained sections.)

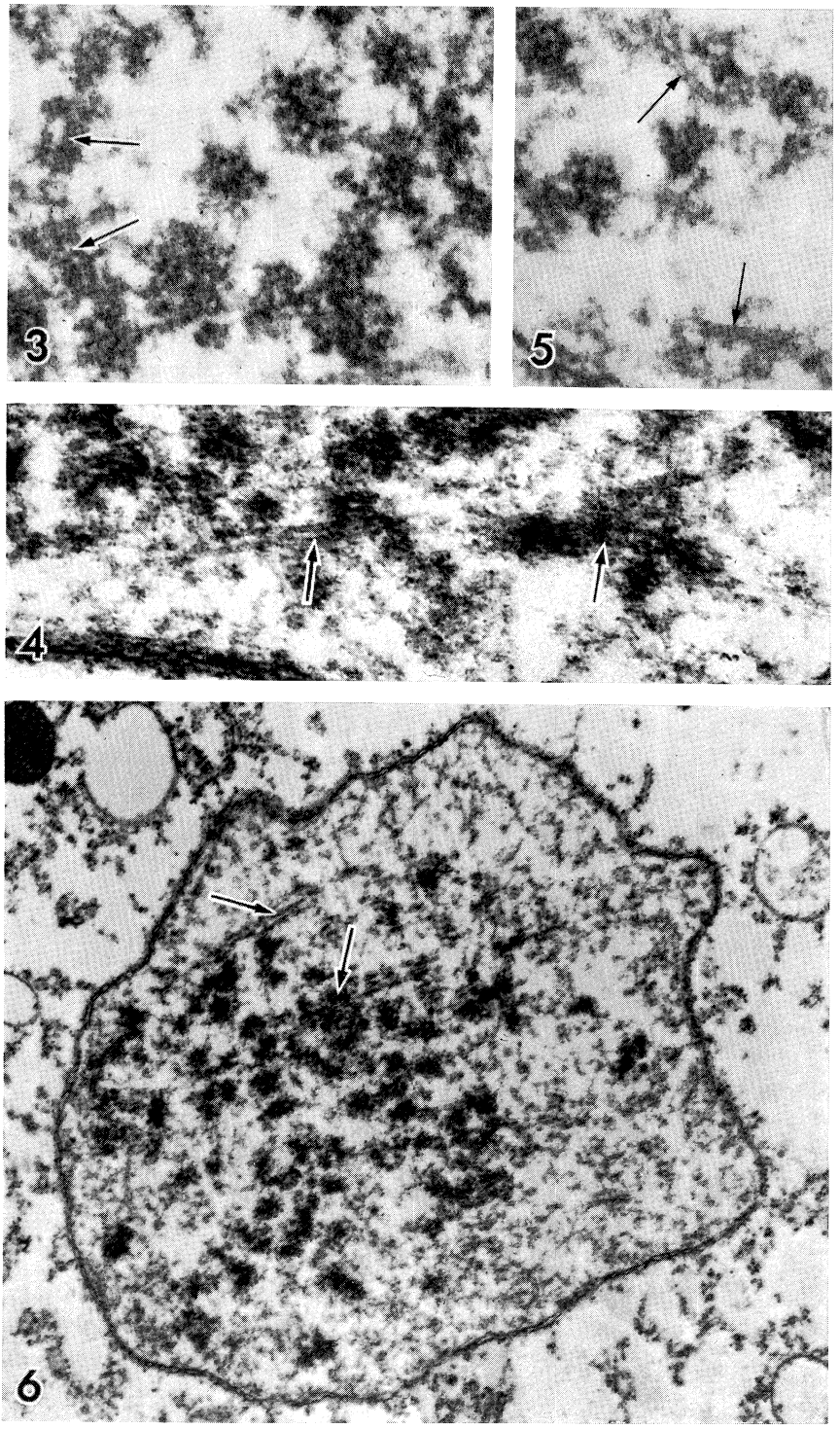


Fig. 3.—After about $1\frac{3}{4}$ hr of pairing a series of regions of condensed chromatin is apparently joined by an ill-defined continuous structure (arrows), the total length of the joined regions being about $1 \cdot 5$ μm. $\times 65{,}000$.

This occurs in some other organisms also, for example some Ascomycetes (Westergaard and von Wettstein 1970).

After about 1–1·5 hr of conjugation, structures somewhat resembling the axial cores described by Moens (1968) can be seen, distinguished by continuity rather than any marked feature (Fig. 3). Frequently the core-like material appears to bridge regions of chromatin; it has a diameter of 50–60 nm. It frequently runs for lengths of 1–2 μm, a greater length than that of any of the chromosomes in *P. aurelia*.

After about 2 hr of conjugation what may be a stage of chromosome pairing occurs. Chromatin elements occasionally appear paired, and have a structure resembling the synaptinemal complex, described in other organisms, lying between them (Fig. 4). The complex-like structure, clearly evident at lower magnification, but rather elusive at high power, consists of two tubular elements lying parallel to one another, with a space between them. Each lateral component is about 40 nm across, and the space between them is 60–70 nm across. No structure is apparent in the central space. Chromatin surrounds this complex in a rather amorphous clump. The whole complex-like structure is very poorly defined; despite repeated efforts and some variation of the method of preparation, better results could not be obtained. Although there are large amounts of condensed chromatin, only a few of these complex-like structures are evident in any one nucleus. If these structures correspond to synaptinemal complexes, then it may be that not all the chromosomes pair and form chiasmata. In nuclei showing these synaptinemal complex-like structures, similar structures which are not associated with chromatin may be seen (Fig. 5). These could correspond to "stripped complexes" (Moens 1968), discarded after synapsis and chiasma formation has taken place. On occasions, several of these may be evident (Fig. 6).

The nucleus begins to enter the crescent stage after about 2·5 hr conjugation. This is the most distinctive stage of meiosis in *P. aurelia*. The nucleus, which up till now has been irregularly round in section, becomes rod-shaped with a length of 20 μm or more, and a diameter of 2–3 μm. Expansion takes place rapidly, often but not always, along the long axis of the cell. Longitudinally oriented microtubules are found, particularly near and just below the membrane (Fig. 7). As the nucleus expands, projections which have microtubules running into them (Fig. 7) are found, reminiscent of similar projections which occur during the elongation phase of macronuclear amitosis in *P. aurelia* (Stevenson and Lloyd 1971*b*). Possibly such projections are equivalent to the extrusion strands observed by Jones (1956) at this stage. The membrane of the micronucleus shows a large number of pores (Fig. 7): these appear similar to the pores of the macronuclear membrane in fine structure

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Fig. 4.—2 hr after commencement of pairing. Poorly defined synaptinemal complex-like structures can be seen (arrows), as two parallel tubular elements separated by a space. Chromatin surrounds the structures, and may obscure them. × 45,600.

Fig. 5.—Regions of synaptinemal complex-like material not associated with condensed chromatin (arrows). These could correspond to "stripped" complexes. Material of this type is often found concurrently with complex-like structures. × 65,000.

Fig. 6.—A micronucleus about 2 hr 15 min after the start of pairing, showing a number of both free ("stripped") and chromatin-associated, synaptinemal-complex-like structures (arrows). × 15,200.

(Stevenson and Lloyd 1971*b*). Initially in the crescent stage the chromatin elements remain condensed (Fig. 8) but they became scattered throughout the nucleus, becoming more diffuse, particularly towards the end of nuclear expansion. When the nucleus has attained full size, the chromatin is completely diffuse (Fig. 9). Microtubules are also no longer well-defined at full expansion (Fig. 9). This stage, with the nucleus some 20 μm long, sometimes irregularly twisted, and containing diffuse, fibrous chromatin, persists for 0·5–1 hr.

(iii) *Metaphase I*

After the crescent stage, 3·5–4 hr after the commencement of conjugation, the micronuclei shrink in size. Condensed chromatin reappears, and microtubules also reappear. These events occur as the nucleus is shrinking. A large number of chromosomes can now been seen (Fig. 10). Over 60 have been observed in a single section, though 20–30 is more usual. Each chromosome is small (up to 1 μm long, usually less), and many appear round, attached to a bundle of microtubules at an indistinct site (Fig. 10). Microtubules are not well organized in the regions of the nucleus away from the metaphase plate, and no distinctive poles are evident on the nucleus. The membrane stays intact.

(iv) *Anaphase and Telophase I*

The nucleus lengthens again at anaphase (Fig. 11), and microtubules run from the poles as well as the centre of the nucleus. The nucleus expands again to a length of 15–20 μm, becoming dumbbell-shaped, and lying at any angle in the cell (Fig. 12). The separation spindle is not pinched off close to the terminal knob, and does not leave a remnant in the cytoplasm, in contrast to mitosis (Stevenson and Lloyd 1971*a*). Instead the spindle breaks approximately in the centre, giving pear-shaped daughter nuclei about 10 μm long (Fig. 13) and a good deal of microtubule depolymerization takes place. Anaphase and telophase occur rapidly (in less than 0·5 hr) about 4·5 hr after the start of conjugation.

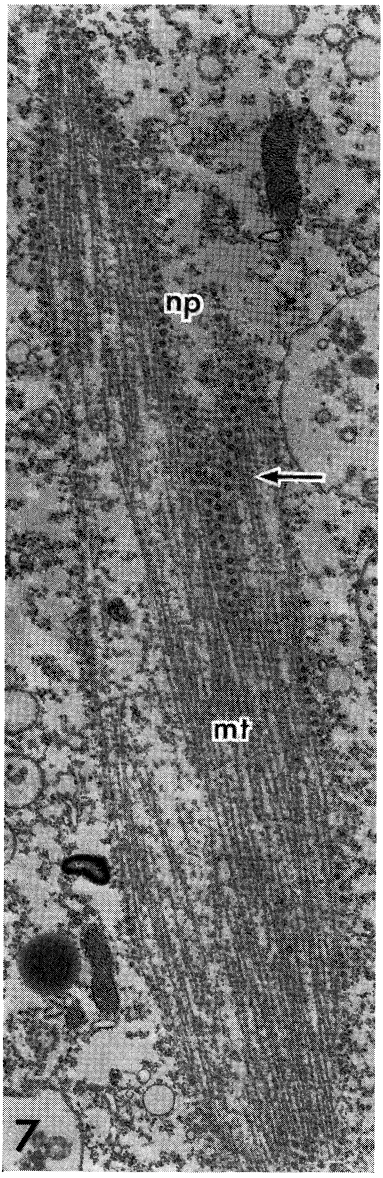
(v) *Metaphase II*

The second nuclear division occurs more rapidly than the first, and there is neither an interphase nor a clear prophase between divisions. The second division takes about 1 hr instead of 4·5 hr. After the daughter nuclei have separated at telophase I a metaphase quickly forms, with the chromosomes arranged on a metaphase plate which usually appears more compact than at the first division (Fig. 14). Again kinetochores are indistinct, and microtubules not well organized in the polar regions. The metaphase configuration appears to last for about 45 min, and then elongation of the nucleus commences again.

(vi) *Anaphase and Telophase II*

Extension takes place much as before, the nucleus eventually reaching a length of 25 μm. The nucleus usually does not have such sharply pointed poles at this

Fig. 8.—An expanding micronucleus, after about 2 hr 45 min of pairing. Chromatin, still condensed, is spread throughout the nucleus, and longitudinal microtubules (*mt*) are apparent. The cytoplasm now contains some of the vesicles (*v*) which later become very numerous. ×14,000.



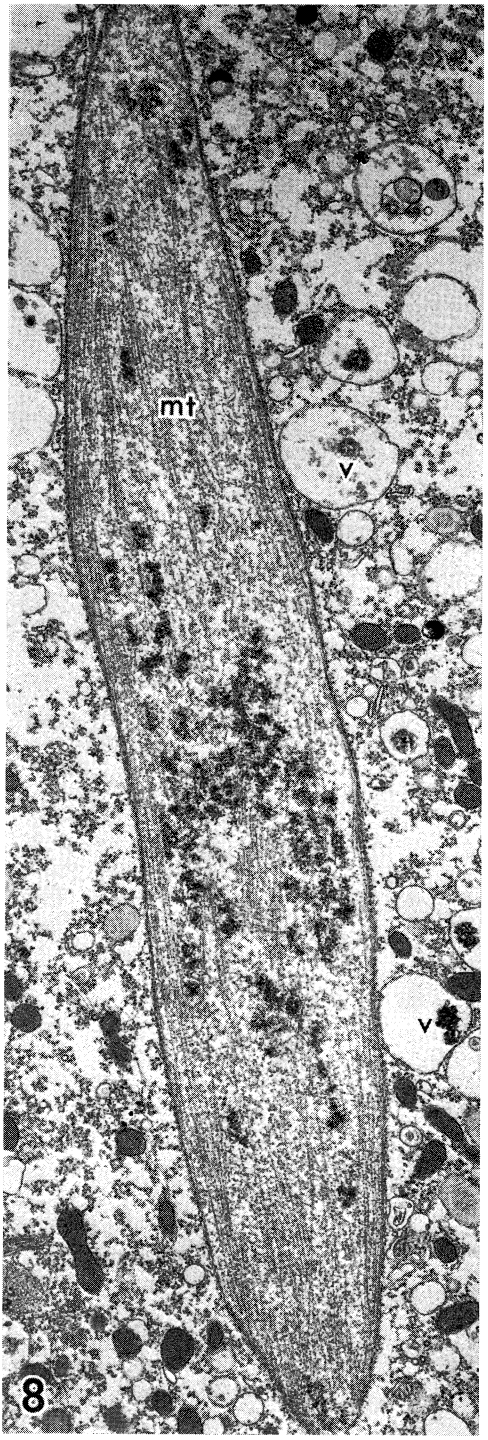
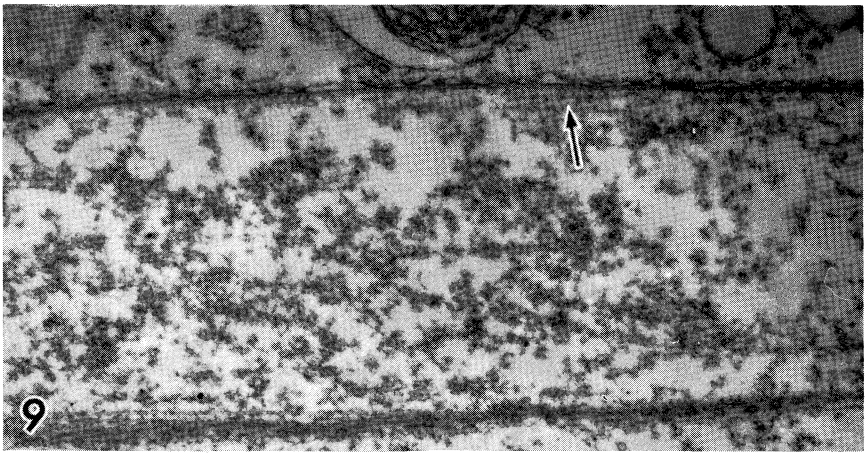


Fig. 7.—Micronucleus about 2·5 hr after the commencement of pairing. This is a glancing section of a nucleus undergoing extension at the beginning of the crescent stage. Large numbers of longitudinally oriented microtubules (*mt*) are apparent. The nucleus shows a projection with microtubules running into it (arrow). Many nuclear pores (*np*) are evident in the membrane, which is seen in face view. ×11,200.



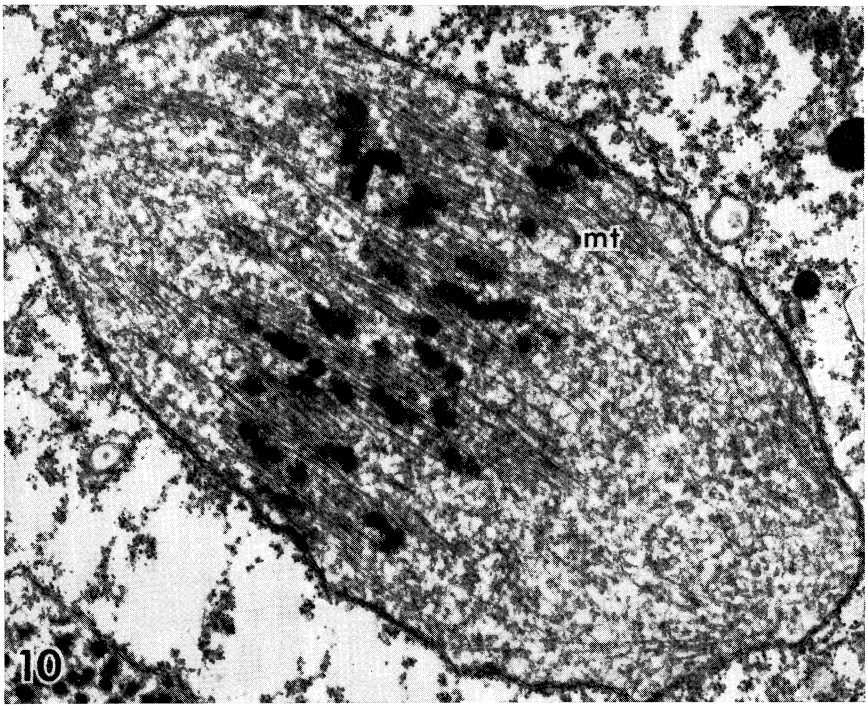


Fig. 9.—Detail of part of a fully expanded crescent-stage micronucleus. The chromatin is diffuse. What may be the remnants of microtubules are evident (arrow). × 19,600.

Fig. 10.—The chromosomes congregate to form a typical metaphase. A variety of shapes and sizes are evident. Microtubules (*mt*) are not so well organized away from the metaphase plate. There are no well-defined poles at this stage, and no centrioles are associated with the nucleus. × 15,200.

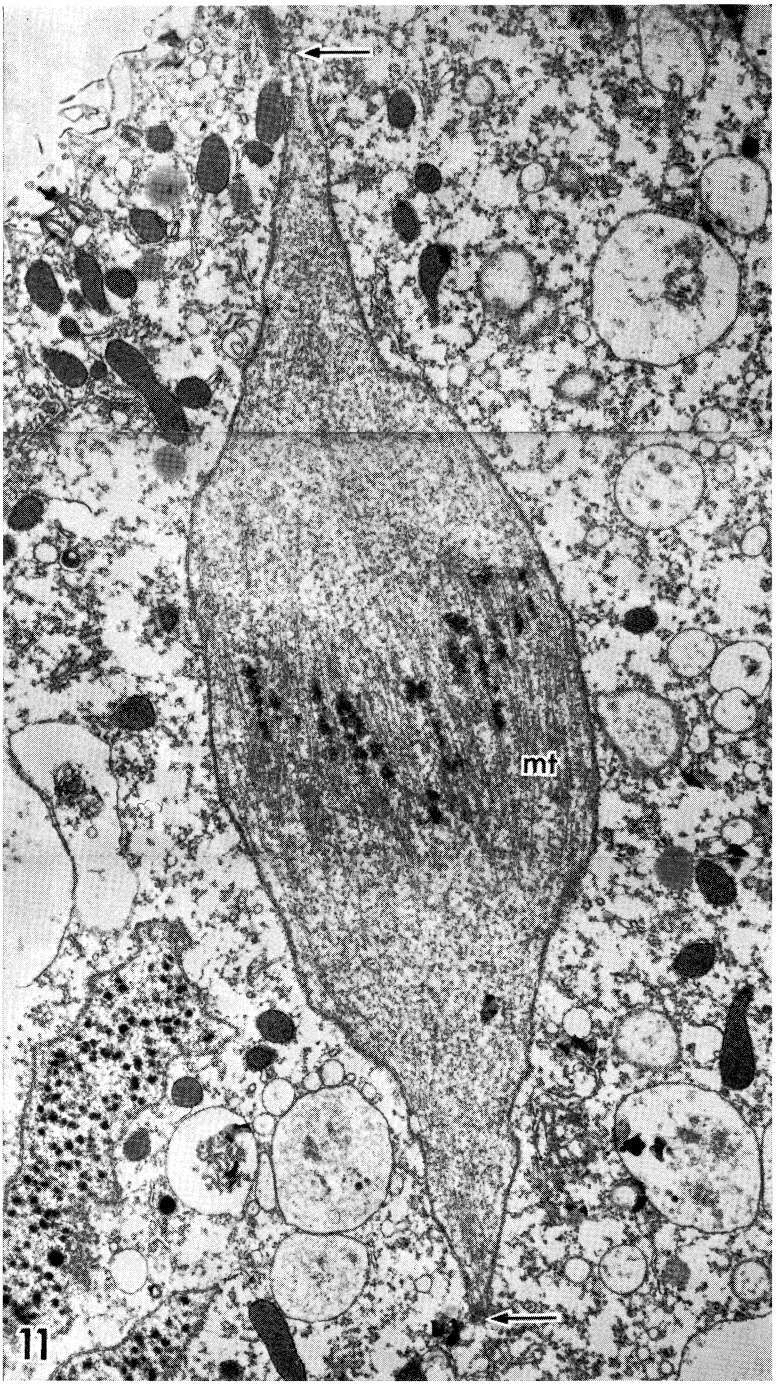


Fig. 11.—A montage of anaphase of the first meiotic division. The nucleus has lengthened and microtubules (*mt*) may been seen running up to the poles (arrows) which are sharply pointed. ×8400.

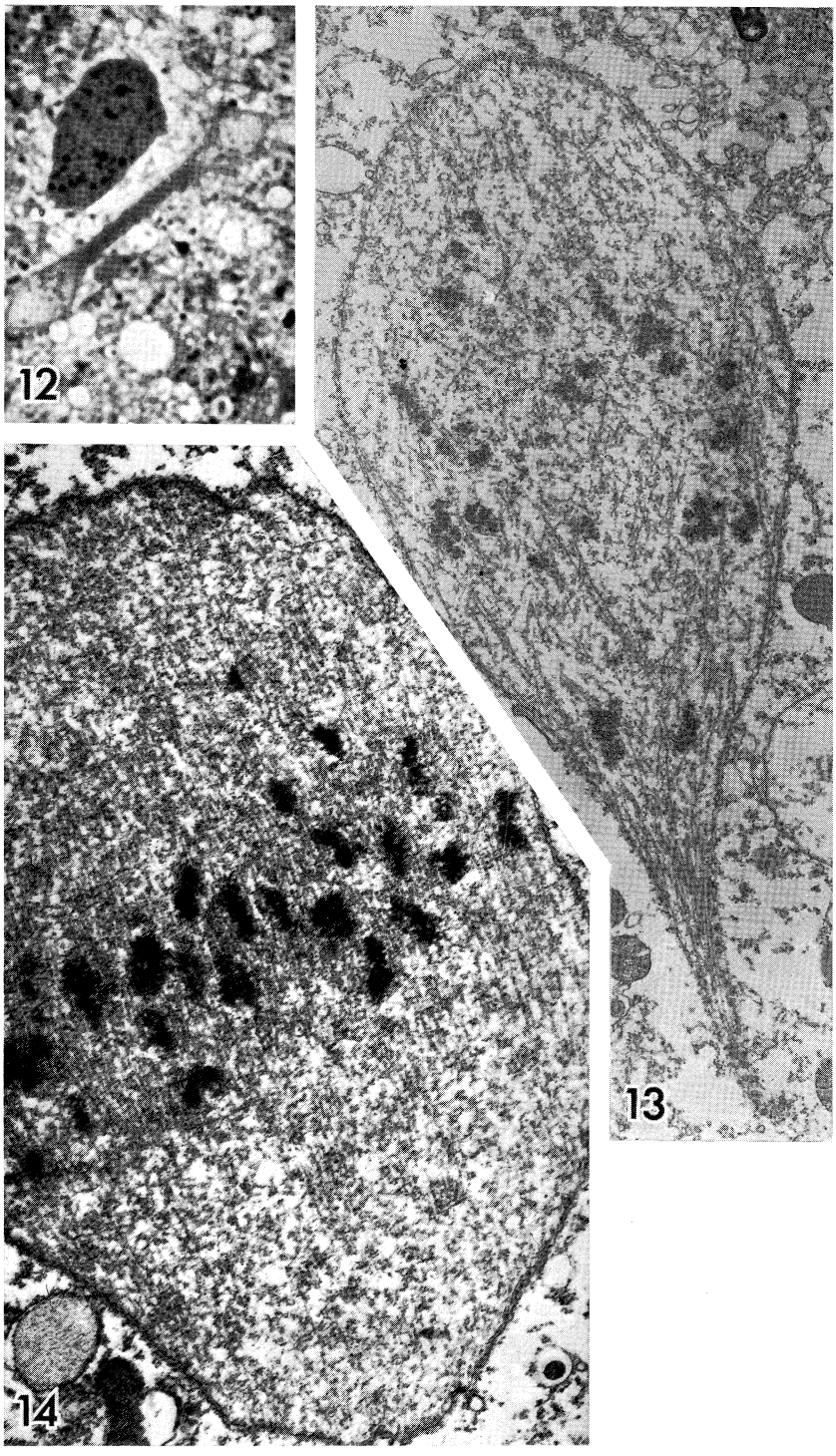


Fig. 12.—Light micrograph of toluidine blue-stained section, showing a micronucleus at telophase of the first meiotic division. The orientation of the nucleus is very variable; here the long axis of the cell is vertical on the micrograph. $\times 1200$.

anaphase, but may on occasion bear a distinct scar of the previous division, in this case at right angles to the plane of the second division (Fig. 15). The nucleus also seems to be more oriented to the long axis of the cell at this division (Fig. 16). Microtubules attain considerable lengths (10 μm or more), and the nucleus reaches telophase with the individual chromosomes still condensed (Fig. 17); they seem to separate at a more uniform rate in this division than during the first, as can be seen by comparison of Figures 13 and 17. The separated nuclei after the second division look similar to those after the first.

(vii) *Nuclear Degeneration and the Third Division*

Since there are two micronuclei in *P. aurelia*, the meiotic divisions have produced eight haploid products. These eight products usually lie in the central to rear area of the cell, in the region of the gullet and the paroral cone. Only one of these meiotic products is to survive, the one nearest the paroral cone (Sonneborn 1954). The other seven nuclei degenerate. This seems to be a rapid process in stock 540, taking less than 30 min. During degeneration the nuclear membrane is recognizable, though it is usually broken, while the chromosomes become a compact mass of dense material; the microtubules become indistinct (Fig. 18). Only this stage has been clearly recognized in the electron microscope.

The third micronuclear division, although mitotic in nature, differs somewhat from normal mitosis. It always occurs close to the paroral cone and does not seem to have a very well-defined metaphase or anaphase (Fig. 19). At anaphase, it increases in length to about 10 μm, but seldom much longer. A dumbbell-shaped telophase has not been seen; instead the nucleus appears to split into halves which appear identical (Fig. 20) but which are different in function. As at other divisions, the kinetochores are indistinct.

(viii) *Nuclear Exchange and the Synkaryon*

The two haploid nuclei produced at the third division are different in function; one is the migratory, or male, pronucleus, and the other the stationary, or female, pronucleus. Exchange is effected across the paroral cone, in which much more extensive membrane fusion and dissolution takes place than at other regions of the cell pellicle. The region of fusion is several microns across (Fig. 21). This finding is in agreement with that of Schneider (1963), who termed the extensive fusion in the paroral cone region the "grosse Auflösungszone", as opposed to the "kleine Auflösungszone", the fusion of the ectoplasmic ridges (Fig. 20). Inaba, Imamoto, and Suganuma (1966), however, found that in *P. multimicronucleatum*, membrane fusion was less extensive in the paroral cone region, being often only 1 μm across. As this is the case in *P. caudatum* also (André and Vivier 1962) this more extensive fusion seems confined to *P. aurelia*.

Fig. 13.—Telophase of the first meiotic division, occurring about 4·5 hr after the start of pairing. The nucleus shows a "tail" where it has separated and the microtubules are somewhat depolymerized. Chromosomes are scattered throughout the nucleus. ×11,200.

Fig. 14.—The second metaphase of meiosis, occurring about 5 hr after the commencement of pairing. This appears rather similar to metaphase of the first division. ×15,200.

Male and female pronuclei can be distinguished. The female pronucleus resembles superficially an early prophase nucleus (Fig. 22), becoming round in section with condensed chromatin in its centre, surrounded by fibrous material. The male pronucleus is more striking. It swells somewhat, becomes rather elongated, with a pointed anterior end. The shape is rather slug-like (Fig. 23). Chromatin is usually spread between the centre and the anterior end, the nucleus in Figure 23, for example, having chromatin in a condensed state at the anterior end in serial sections. Microtubules ensheath the chromatin and run from the anterior end. No structures occur in the cytoplasm surrounding the nucleus, and how it moves the several microns distance to make contact with the female pronucleus is obscure. The reciprocal exchange is rapid, occurring in the last half hour of conjugation.

The synkaryon (Fig. 24) does not appear very different to the female pronucleus; indeed fertilization can only be inferred to have taken place by the absence of male pronuclei. The chromatin remains fragmented as in Figure 24 until the cells separate. Pictures of fusion itself were not obtained, despite a number of efforts. With the formation of the synkaryon, the cells separate. The synkaryon then undergoes divisions and some of the products differentiate into macronuclei; these processes will be described in a later paper.

(*b*) *Macronuclear Changes during Conjugation*

The macronuclei of conjugants undergo a series of changes. At the beginning of conjugation the macronucleus has a typical interphase shape and ultrastructure. It stays like this until the micronuclei are in the first metaphase of meiosis and then develops a more irregular outline with a number of lobes. As the second meiotic division occurs the macronucleus becomes a long twisted skein (Fig. 25), 40 or 50 μm long. It quite frequently loops around one or both micronuclei, as may be seen in Figure 16. No ultrastructural changes in the macronuclear contents are evident during these stages. As the third micronuclear division approaches the macronucleus begins to fragment. It usually breaks into 30–40 fragments. During fragmentation, microtubules are apparent in the nucleus, often at the points of breakage (Fig. 26). They are usually oriented in the direction of the long axis of the nucleus at the skein stage. By the time of cell separation, fragmentation is complete. Fragments have normal macronuclear fine structure, but microtubule-like elements sometimes persist (Fig. 27) and this is retained for the period of survival of the fragment.

(*c*) *Cytoplasmic Changes during Conjugation*

The cytoplasmic changes during conjugation are quite well marked. With the fusion of the cells at the paroral cone region, they cease to feed, and food vacuoles

Fig. 16.—Light micrograph of toluidine blue-stained section, showing telophase of the second meiotic division. This occurs more regularly along the axis of the cell. The macronucleus (*ma*) is at the skein stage. $\times 1200$.

Fig. 17.—Telophase of the second meiotic division. The chromosomes tend to form a more compact group at this division, while microtubules are not apparent in the region between the chromosomes and the poles. A trichocyst (*tr*) which has discharged into the cytoplasm, probably as a result of the action of the fixative, lies beside the nucleus, while a number of vesicles (*v*) can be seen also. $\times 7500$.

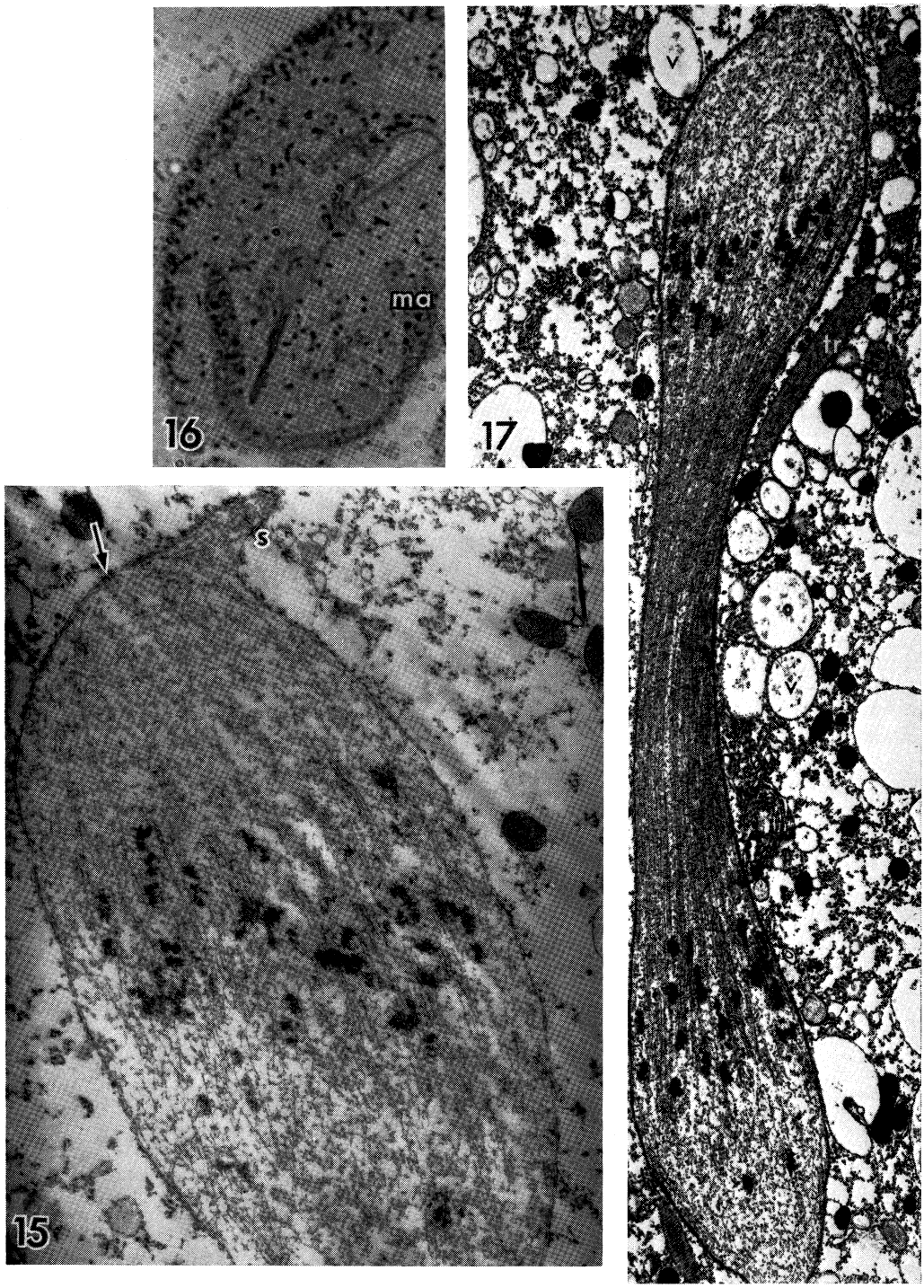
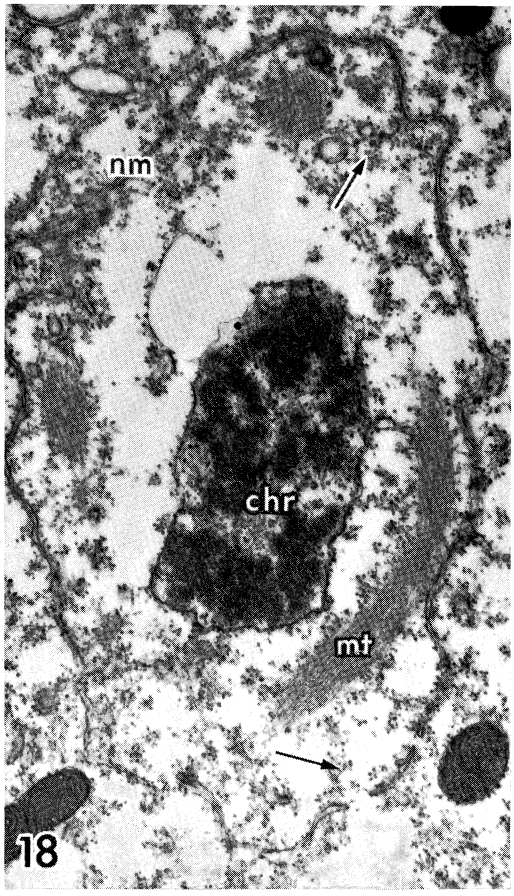


Fig. 15.—Anaphase of the second meiotic division. The nucleus shows a scar (*s*), indicating that the plane of the first division was at right angles to the plane of this division. The pole of the second division would seem to be at the point indicated by the arrow. ×15,200.



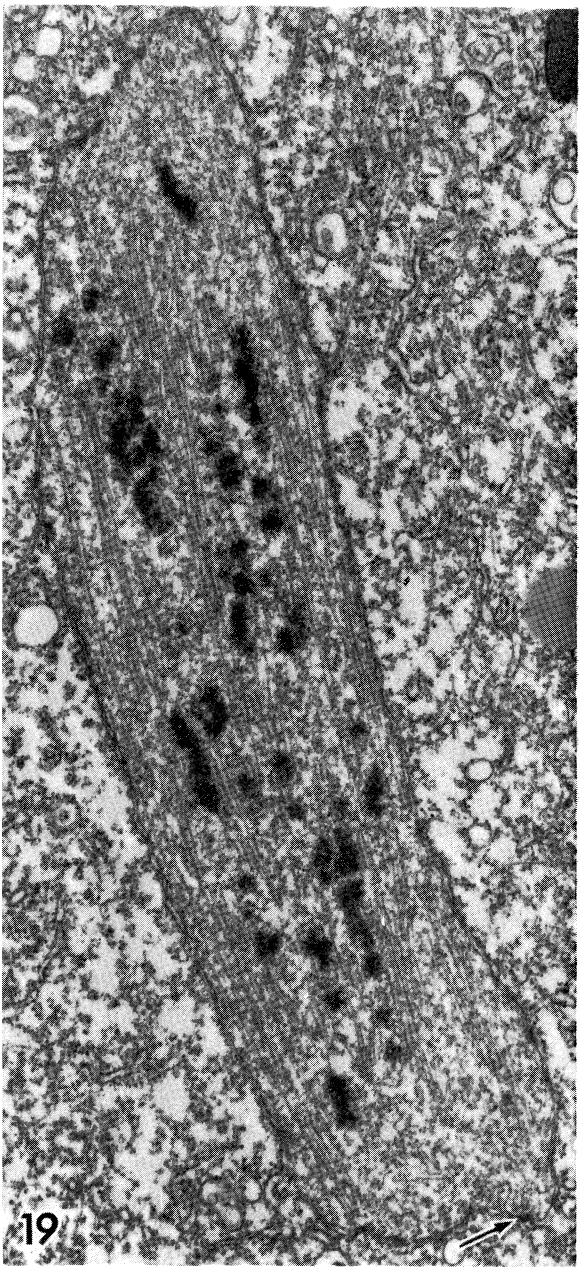


Fig. 18.—Nuclear degeneration, occurring about 6 hr after the start of pairing. The major nuclear components are still recognizable—nuclear membrane (*nm*), indistinct microtubules (*mt*), and condensed chromatin (*chr*) which is apparently partially delimited from the remainder of the nucleus. Ribosome-like granules (arrows) also occur inside the nuclear membrane. × 19,000.

Fig. 19.—Anaphase of the third division. There are no sharp poles in this division, though the nipple-like protuberance (arrow) of the nucleus is fairly common. × 19,000.

are not formed. After about 3 hr of conjugation, food vacuoles are no longer apparent in the cells. The cytoplasm becomes much less dense in appearance, and membrane-limited vesicles become numerous in the cytoplasm; these are of varying sizes, and are often found in aggregates (Fig. 28). They increase in number throughout conjugation. These changes are presumably associated with the utilization of reserve food materials. The cells shrink, often becoming half their normal size by the end of pairing. Swimming activity is, however, undiminished and the contractile vacuoles continue to function.

As mentioned above, cell union shows two distinct patterns; small areas of membrane dissolution at the peaks of the ectoplasmic ridges (see Fig. 20) and much greater areas of dissolution at the paroral cone (Figs. 21 and 23). Significant amounts of cytoplasm are not normally exchanged, as was shown by Sonneborn (1947). The regions of fusion are very quickly healed with new membrane at cell separation. No other cytoplasmic processes have been studied.

IV. Discussion

Meiosis in *P. aurelia* undoubtedly leads to the same result as in other organisms, namely gene reassortment. This is well established from the behaviour of gene markers (Sonneborn 1947; Beale 1954). Cytologically, many of the difficulties of interpretation undoubtedly spring from the unsuitability of ciliate Protozoa for these studies. Meiotic prophase of *P. aurelia* is the most difficult stage to understand.

The normal sequence of events in meiotic prophase may be summarized as follows: chromosomes appear as single threads, often having numbers of chromomeres, homologues pair, a doubling process occurs (except at the kinetochores), chiasmata form, and then separation occurs. Observations with the electron microscope have shown that axial cores (Moens 1968) and synaptinemal complexes (review—Moses 1968) are associated with synapsis and chiasma formation. In some organisms these processes and structures are very clearly defined, but unfortunately this is not the case in *P. aurelia*. Nevertheless, some interpretation of the events of prophase is possible.

When chromatin becomes evident in the *P. aurelia* micronucleus it appears condensed, as was seen in the early light microscope studies. The granular condensed chromatin can barely be resolved in the light microscope, and in the electron microscope masses of chromatin often appear linked (see Fig. 2). Perhaps these should be regarded as chromomeres, and the high number of chromatin masses would support this. Some condensation seems to have taken place when synapsis commences. Synapsis, although normally the preliminary to chiasma formation, does not necessarily lead to it; in a number of organisms, perhaps the best known being male *Drosophila*, chiasmata are not formed, although pairing of homologues occurs. The synaptinemal complex is associated with the formation of chiasmata (Moses 1968). In *P. aurelia*, synaptinemal complex-like structures occur, but only a few (less than 10) seems to occur in any one nucleus. On this basis, it seems safe to say that only some of the chromosomes (groups of chromomers) are associated with them. This could simply mean that only a minority of the chromosomes form chiasmata. The chromosomes, as observed at metaphase, are very small, so the lack of chiasmata

could be because many of the chromosomes are physically too small for crossing over to be an event of high frequency.

The crescent stage of prophase probably corresponds to diplotene. While many descriptions of meiosis refer to chromosomes becoming "ill-defined", at this time, in *P. aurelia* they vanish as condensed entities, and all the chromatin is diffuse and has no electron density for a while. Presumably this may somehow be associated with crossing over and separation of chromatids. In *Tetrahymena pyriformis*, which has a similar sequence of events in meiotic prophase to *P. aurelia*, but which has a haploid chromosome number of only 5, as opposed to 40–50 for *P. aurelia*, chromatin threads can be seen in the light microscope to be paired at the crescent stage, and the chromosomes are described as late pachytene–early diplotene (Ray 1956). No ultrastructural studies have been published, however. In *T. pyriformis*, bivalents are clearly evident, but in *P. aurelia*, bivalent-like structures are only occasionally seen. The chromosomes then contract very rapidly, becoming very tight and compact chromatin. Diakinesis and prometaphase thus appear to be short, a conclusion found in light microscope studies also.

Prophase seems thus only to be unusual in its cytology, not in its functional significance. Crossing-over may be infrequent, but if the haploid chromosome number is 40–50, then simple assortment of chromosomes results in a very large number of gametic possibilities. However, all linked genes then are assorted together. But in connexion with this it should be noted that *P. aurelia* is frequently completely homozygous as a result of autogamy (meiosis followed by internal self-fertilization); in this case crossing-over cannot of course result in any recombination.

The remainder of the meiotic division process is not strikingly different ultrastructurally to mitotic division of the micronucleus in *P. aurelia*. The persistence of the nuclear membrane is usual in ciliates, as is the non-involvement of centrioles. Microtubules are associated with meiotic division in a role that seems particularly concerned with extension in length, particularly at the crescent stage and the anaphase stages, and seem also to be involved with the movement of the male pronucleus. As in mitotic divisions, microtubules must be polymerized inside the nucleus. In meiosis they are polymerized and depolymerized several times as the nucleus passes through its cycles of division. Presumably there is a pool of subunits in the nucleus or in its vicinity for this purpose. Kinetochores in *P. aurelia* are indistinct, and often cannot be unequivocally distinguished from the rest of the chromosome. Ill-defined kinetochores seem usual in ciliates (Roth and Shigenaka 1964; Jenkins 1967; Tucker 1967; Stevenson and Lloyd 1971*a*).

One persistent difference between meiosis and mitosis in *P. aurelia* is the structure of the nuclei at telophase of each division. At telophase of mitosis in *P. aurelia*, the dumbbell-shaped 35 μm long nucleus splits into three parts—the two daughter nuclei and the separation spindle, a bundle of continuous microtubules

Fig. 21.—Light micrograph of toluidine blue-stained section, showing region of fusion at paroral cone (arrow). A male pronucleus (large arrow) is entering the paroral cone. This is a serial section of the nucleus shown in Figure 23. $\times$1200.

Fig. 22.—A female pronucleus, before fertilization. The nucleus has rounded up, microtubules are no longer evident, and the chromatin is compacted. The "washed-out" appearance of the cytoplasm of late conjugants is clearly evident. $\times$15,200.

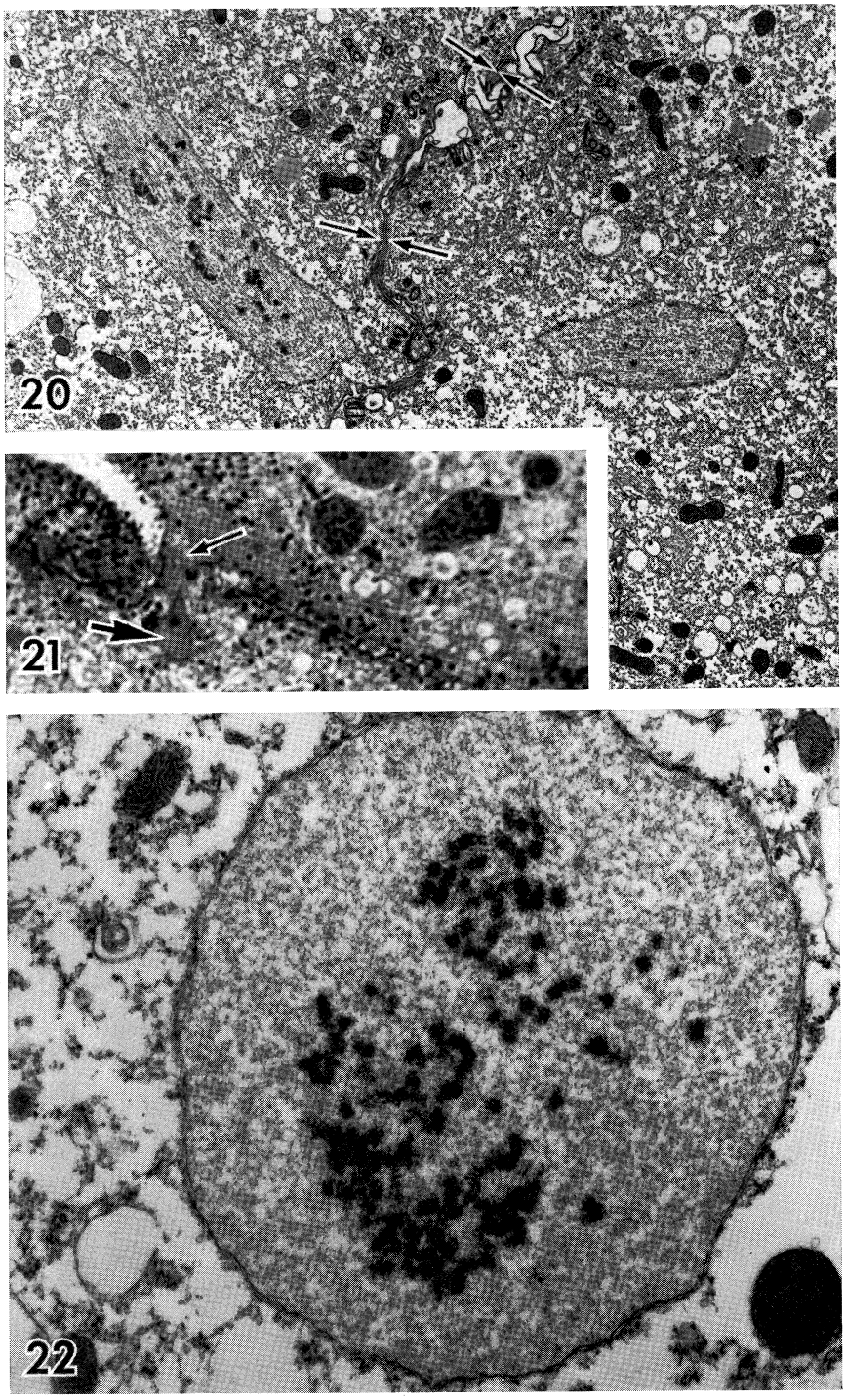


Fig. 20.—A survey of the paroral cone region, showing in the left-hand conjugant the anaphase nucleus of Figure 28, and in the right-hand conjugant, a telophase nucleus, which has not been sectioned at a plane showing any chromosomes. The different modes of fusion at the paroral cone with extensive deciliation and the ectoplasmic ridges (arrows) are shown also. ×5500.

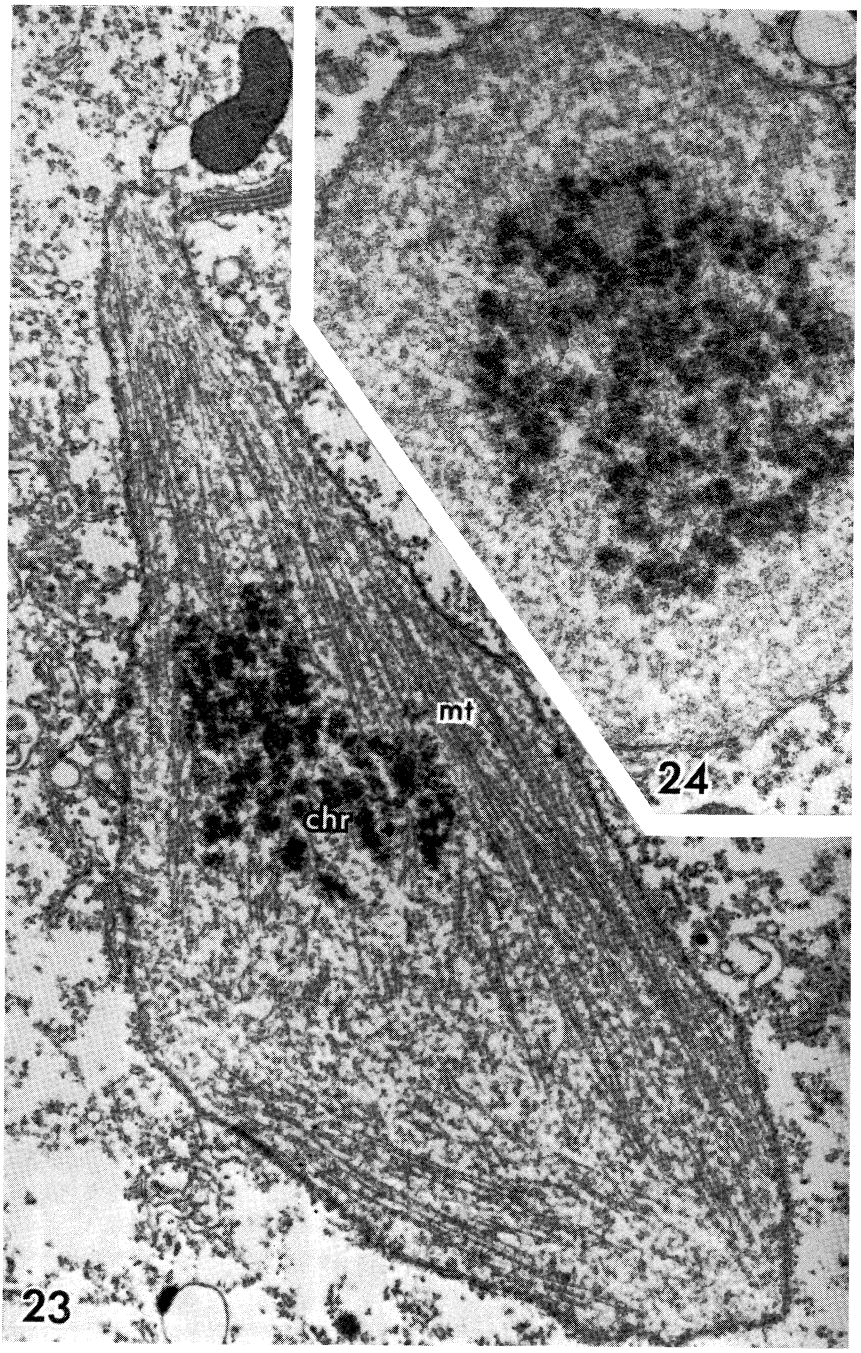
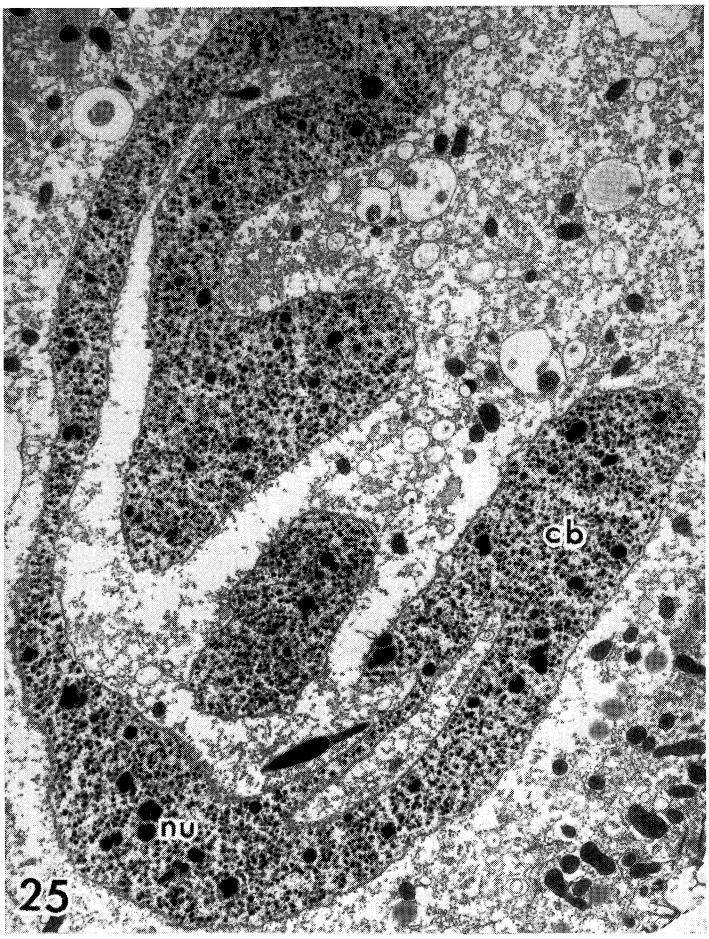


Fig. 23.—A male pronucleus during migration, passing across the paroral cone. A region of complete fusion is evident to the left of the nucleus, and closely appressed cell membranes in the paroral cone to the right. In the nucleus itself the chromatin (*chr*) is ensheathed by microtubules (*mt*) which run longitudinally. No elements are evident in the cytoplasm which might be connected with nuclear movement. × 14,000.

Fig. 24.—Synkaryon. The size is somewhat larger than that of the female pronucleus, but the general appearance is very similar. × 15,200.



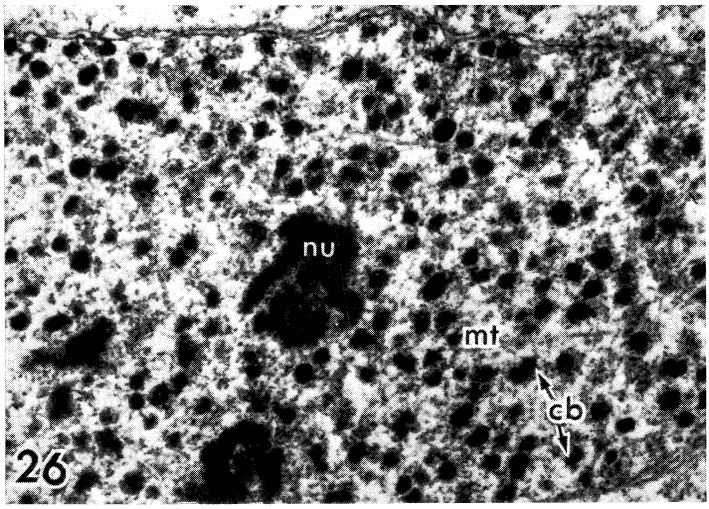


Fig. 25.—Macronucleus at time of change from lobed to skein stage, about 5 hr after commencement of conjugation. Appearance of macronuclear contents is unaltered, showing nucleoli (*nu*) and chromatin bodies (*cb*). ×5500.

Fig. 26.—Macronucleus at time of fragmentation, showing region between two fragments. Microtubules (*mt*) are evident within it at this time, oriented along what was the long axis at the skein stage. Nucleoli (*nu*) and chromatin bodies (*cb*) still appear normal. ×19,600.

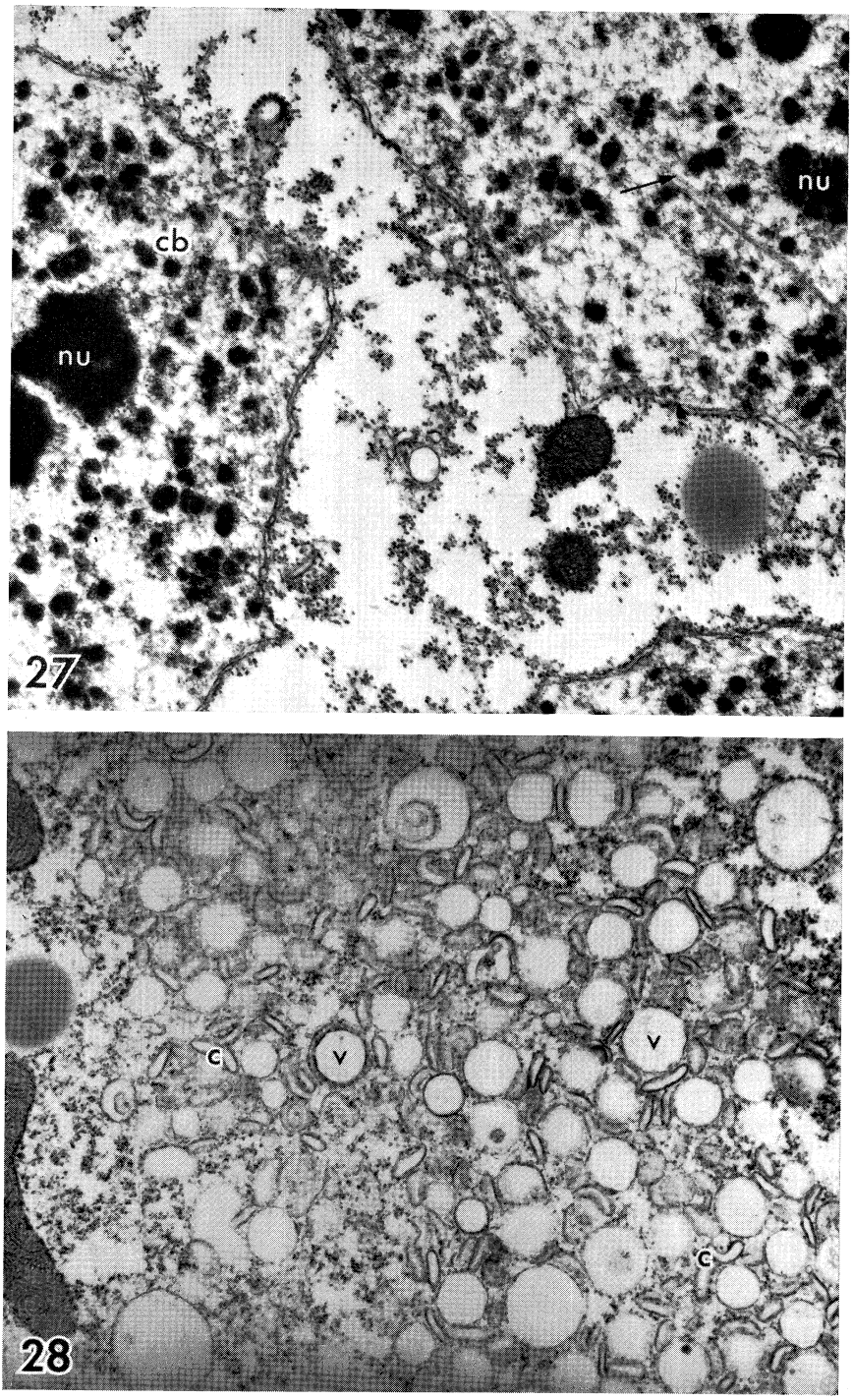


Fig. 27.—Parts of three macronuclear fragments. The ultrastructure is that typical of the macronucleus, with chromatin bodies (*cb*) and irregular fibrogranular nucleoli (*nu*). Microtubule-like elements occasionally persist (arrow). ×19,600.

enclosed by a sleeve of nuclear membrane (Stevenson and Lloyd 1971*a*). A separation spindle does not seem to occur in meiotic divisions. Instead the nucleus breaks into pear or teardrop-shaped halves each time. This may be to conserve microtubule subunits for later use in the nucleus, as the repeated polymerization and depolymerization must use up much raw material and energy.

The meiotic product that survives is always found in the paroral cone region of the cell. Clearly this area exerts some protective function on the nucleus. If all eight haploid products lie outside the paroral cone, then they all disintegrate (Sonneborn 1954). The nuclear degeneration is a very rapid process; within half an hour or less no nuclei are evident. Possibly because of the large physiological changes occurring in the cells all possible sources of raw material are rapidly utilized. The third mitosis-like division of the protected nucleus always occurs in the paroral cone region. This division does not resemble normal mitosis of the micronucleus principally because a large anaphase spindle is not formed. This is probably connected to the necessity to keep both daughter nuclei in the paroral cone region, one to become the male pronucleus and the other the female. Also it might be that if the division products left this area they would not be protected against dissolution.

The movement of the male pronucleus is a puzzling process. No cytoplasmic elements are associated with it, and it seems that the microtubules contained within it have the major part to play. Possibly they may be associated with alternate contractions and expansion so that the nucleus may "wriggle" along. In *P. caudatum* (Inaba, Imamoto, and Suganuma 1966) the micronucleus at this time looks very similar to a *P. aurelia* micronucleus, although it has to get through a smaller gap than the *P. aurelia* nucleus, as the paroral cone fusion is not so extensive in this species.

Cell fusion at the paroral cone covers a zone several micrometres long in *P. aurelia*, as Schneider (1963) also found. In *P. multimicronucleatum* and *P. caudatum* it is not so extensive (André and Vivier 1962; Inaba, Imamoto, and Suganuma 1966; Hiwatashi 1969). In view of this amount of cell fusion, it is surprising that cytoplasmic exchange is not usual during conjugation in *P. aurelia*. However, it is not, for it has repeatedly been observed that cytoplasmic endosymbionts are not normally transferred from bearer to non-bearer cells during conjugation, even though the non-bearer cells possess a suitable genotype (see for example Sonneborn 1944; Beale 1954). Cytoplasmic exchange occasionally occurs spontaneously or may be induced by antiserum treatment (Sonneborn 1950) but normally only male pronuclei are exchanged, which further suggests that cytoplasmic streaming or other processes are not involved in the fertilization process.

The behaviour of the macronucleus during conjugation is clearly a prelude to its replacement, by a division product of the synkaryon. The fragmentation process is probably not entirely random, judging from the preliminary occurrence of lobing and the skein stage, and then the involvement of microtubules in fragmentation. The role of the microtubules is, however, obscure. The occurrence of macronuclear regeneration, in which one fragment can reconstitute an entire macronucleus (Sonne-

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Fig. 28.—An accumulation of the membrane-limited vesicles (*v*) and cisternae (*c*) which become numerous in the cytoplasm during conjugation. Aggregates such as this are very common. × 15,200.

born 1947) shows also that each fragment has sufficient genetic information to control cell functions, but this might be expected since if there are 30 fragments, each would have 25 times the haploid complement of DNA, since the mature macronucleus has about 860 times the haploid complement of the micronucleus (Woodard, Gelber, and Swift 1961).

Meiosis in *P. aurelia*, although unusual in cytology and procedure, has the same function as meiosis in other organisms, the production of haploid gametes bearing reassorted genes. It is unusual in producing only two gamate nuclei, identical in genotype, but differentiated in function, and in the fact that vegetative cells participate directly in the sexual process, but despite the atypical means the end result of sexual reproduction is achieved.

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