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FERTILIZATION IN OEDOGONIUM. III. KARYOGAMY[1]

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ABSTRACT

Karyogamy is described in *Oedogonium cardiacum* from ultrastructural studies. Close proximity of the two gamete nuclei in the fusion cell is established by plasmogamy, whereas karyogamy appears to be initiated by multiple contacts formed between the outer membranes of the adjoining nuclear envelopes. Blebs of endoplasmic reticulum (ER) originate from the outer membrane of each nuclear envelope; these ER blebs presumably contact and fuse with the outer membrane of the nuclear envelope of the opposing nucleus. This is followed by the fusion of the inner membranes of the opposing nuclear envelopes, thereby resulting in a series of small connective bridges between the two gamete nuclei. It is estimated that in this manner 30–50 bridges are formed, perhaps many more. Several of these bridges enlarge relative to the others; one presumably becomes the major connection between the fusing nuclei. As it continues to enlarge, any organelles positioned between the fusing nuclei are pushed aside. There is also evidence, particularly in later stages of karyogamy, that the smaller connective bridges fuse to form larger ones. Temporary cytoplasmic channels often result at the juncture of fusion. In other instances, isolated inclusions of cytoplasm may be delimited by remnants of nuclear envelope deep within the developing zygote nucleus; these inclusions disappear with subsequent development. Throughout karyogamy the contribution of the male gamete nucleus is readily recognized by the characteristic appearance of its highly condensed chromatin. Ultimately, however, this distinction is lost and the content of the mature zygote nucleus assumes a more uniform appearance very similar to that of an egg nucleus. The complete process of fertilization in *Oedogonium* may occur within 15 min of mixing the spermatozoids with eggs.

ULTRASTRUCTURAL STUDIES of karyogamy have been made for comparatively few organisms (Austin, 1968). Early work with plants includes a study of the fern *Pteridium* by Bell (1964). Although he illustrated a spermatozoid in the egg cytoplasm, karyogamy was not observed. The earliest ultrastructural description of nuclear fusion was by Jensen (1964), who based his report on the three types of nuclear fusion that occur in the female gametophyte of cotton. "Double fertilization" in angiosperms provides three types of nuclear fusion: fusion of polar nuclei, fusion of a sperm nucleus with the fused or fusing polar nuclei, and fusion of sperm and egg nuclei. Fusion of gamete nuclei in pine was well documented by Camefort (1965). Subsequent studies by Jensen and co-workers (e.g., Jensen, 1965, 1972, 1973; Jensen and Fisher, 1967; Schulz and Jensen, 1973) have supplied the majority of the still very limited information presently known about nuclear fusion in plants.

Although a number of ultrastructural papers on algae purport to describe "fertilization," only a few include information on karyogamy. In the Chlorophyta ultrastructural documentation of nuclear fusion is available only for *Bryopsis* (Urban, 1969), *Chlamydomonas* (Brown, Johnson, and Bold, 1968), and *Hydrodictyon* (Marchant and Pickett-Heaps, 1972). Of these three accounts, the most thorough description of karyogamy is for *Bryopsis*. Early stages in the initiation of karyogamy do not appear so well documented for *Chlamydomonas* and *Hydrodictyon*.

The present paper is one of a series (Hoffman, 1973a, b) providing a detailed study of fertilization in the green filamentous alga *Oedogonium* and is the most complete description of karyogamy in any alga to date.

MATERIALS AND METHODS—*Oedogonium cardiacum* was used exclusively in this study; male and female cultures were obtained from the culture collection of algae at Indiana University (Starr, 1964). Previous reports describe details of culture maintenance (Hoffman, 1971) and the methods used to induce gametogenesis (Hoffman, 1973a). Material for electron microscopy was processed by methods previously described (Hoffman, 1970). Sections were examined with a Hitachi HU-11A at the University of Illinois or with a Siemens Elmiskop I during the author's 1969 visit to Professor Manton's laboratory in Leeds, England.

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Appreciation is expressed to Professor Irene Manton and her staff for hospitality extended during the author's 1969 visit to her laboratory in Leeds, England.

RESULTS—Plasmogamy in *O. cardiacum* always occurs with the egg nucleus positioned opposite the oogonial pore (Hoffman, 1973a). Since plasmogamy occurs in this region of the egg, it results in the close proximity of the egg and spermatozoid nuclei prior to karyogamy (Fig. 1, 2). Little, if any, nuclear migration is required in *Oedogonium* to bring the gamete nuclei together.

Karyogamy appears to be initiated as multiple contacts are formed between the outer membranes of the nuclear envelopes of the opposing gamete nuclei. This appears to be facilitated by relatively short, papilla-like blebs of ER which originate from the outer membrane of each nuclear envelope and possess relatively few associated ribosomes (Fig. 9–15). The ER blebs of one nucleus are presumably capable of contacting and fusing with the outer membrane of the nuclear envelope of the opposing nucleus. Thus, continuity is established between the outer membranes of the adjoining nuclear envelopes, thereby permitting contact and subsequent fusion at each juncture. This results in a series of small connective bridges between the two gamete nuclei (Fig. 1–15).

Figures 6–15 illustrate serial sections through two connective bridges, with the male gamete nucleus lying above and the egg nucleus below. Each bridge is in a different stage of development. The bridge on the left shows both the outer and inner membranes of the opposing nuclear envelopes fused. Continuity of the two membrane systems is apparent at the right-hand side of the bridge (Fig. 7, 8), while on the left-hand side proliferation of ER blebs somewhat obscures the connection. The connective bridge on the right is not as far along in development since only the outer membranes have fused; the inner membranes of the two nuclear envelopes lie close together but remain unfused throughout the series of sections. Figures 9–11 and 15 show near median sections through this connection. Although the inner membrane of the egg nuclear envelope is not entirely distinct in Fig. 11 and 15, that of the spermatozoid nucleus is clearly intact.

Of the many fusion cells examined, only two represented early stages in karyogamy. In both cells the opposing gamete nuclei were joined by many connective bridges—the majority of them small (Fig. 1–4, 6–11, 14, 15), but others larger (Fig. 5, 16, 17). One of these cells is illustrated in part by Fig. 1 and 6–19; the other is shown in Fig. 2–5. In the former case, a photographic record has been obtained of most sections of the series throughout the region of the fusing nuclei.

A remarkable number of connective bridges develop between opposing nuclei in the early stages of karyogamy in *Oedogonium*. In the two cells studied it is estimated that each formed 30–50 bridges, perhaps many more. Even after these connections are well established, ER blebs from the outer membranes of both nuclear envelopes can still be seen (Fig. 9–15, and elsewhere). Whether these ER blebs can continue to generate new bridges at this time is uncertain. One unusual feature, not reported in other studies of nuclear fusion, is the presence of microtubules arranged parallel to the opposing surfaces of the fusing nuclei (Fig. 6, 7, 11, 13, 15, 17). Their significance is not known.

In one of the cells the spermatozoid nucleus had formed several slender nuclear extensions. Most of these extensions occurred on the periphery of the region where connective bridges were forming with the egg nucleus (Fig. 18). They often appeared directed toward the surface of the egg nucleus and frequently bore ER blebs (Fig. 18). Although these extensions may serve another means of forming bridges between the fusing nuclei, it must be pointed out that not all of them

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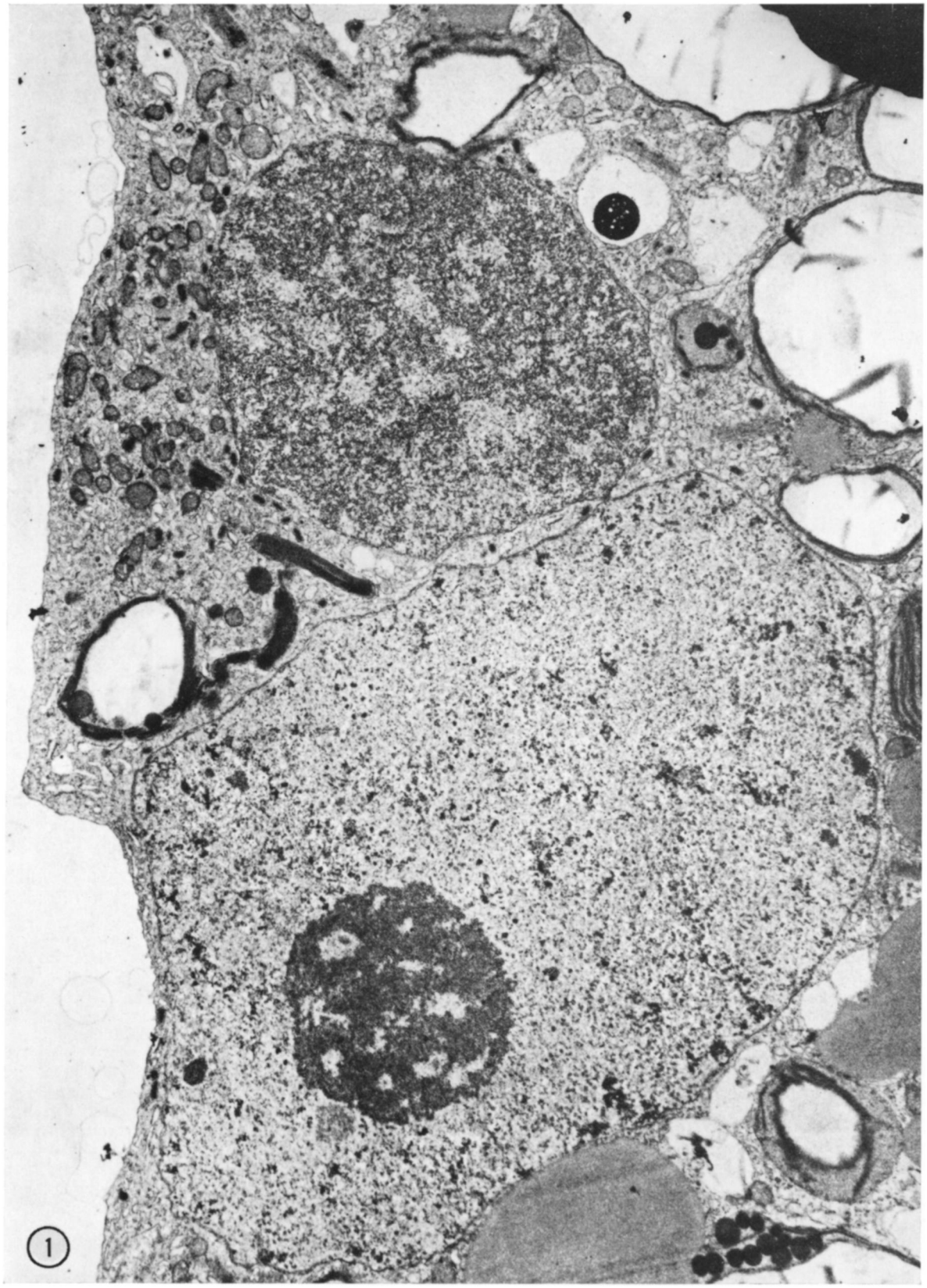
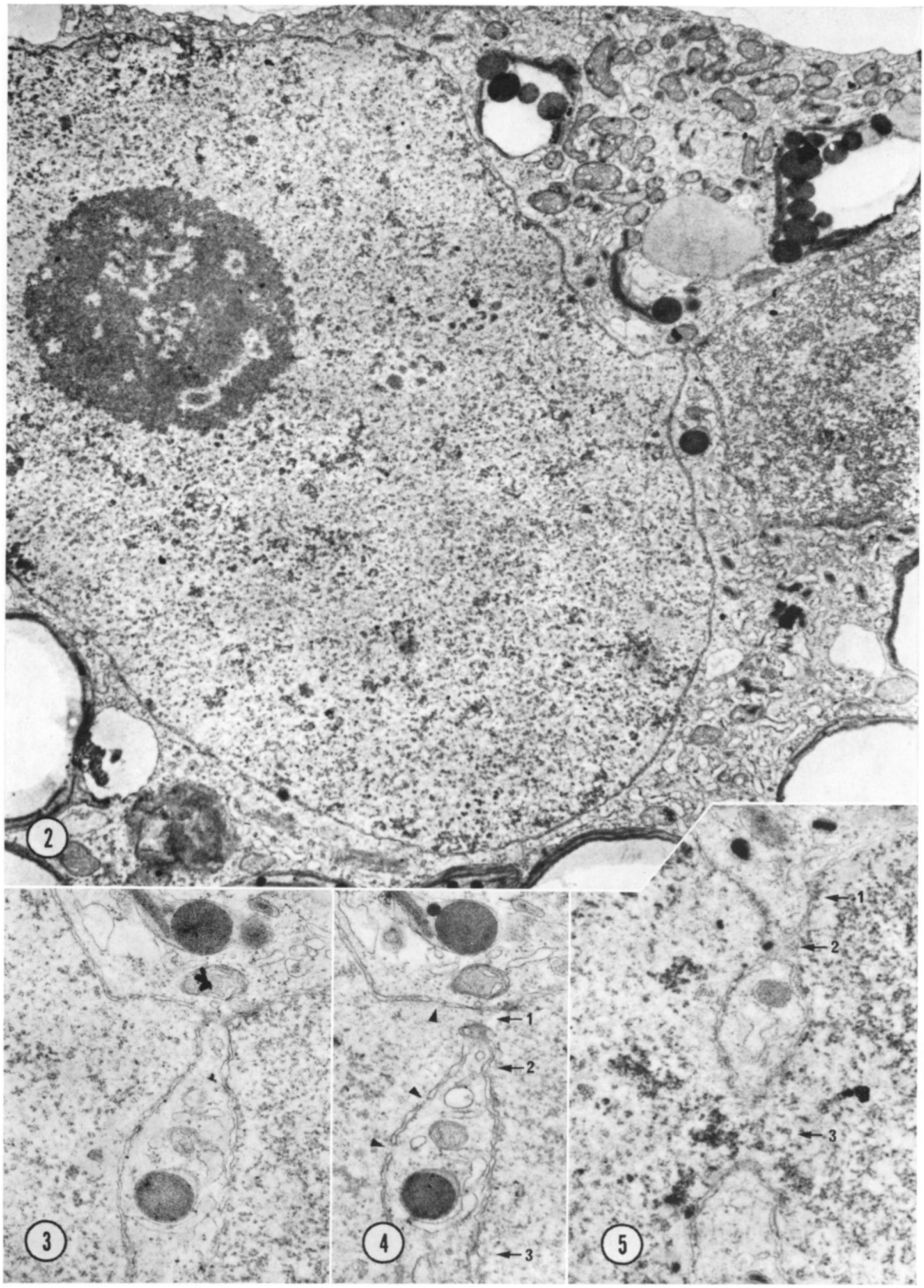
Fig. 1. Early stage in the fusion of the spermatozoid nucleus (above) and the egg nucleus (below) in *Oedogonium cardiacum*. The nucleus of the male gamete is smaller, lacks a nucleolus, and possesses more condensed chromatin. Two connective bridges are seen between the nuclei (cf. Fig. 9, 10). The surface of the fusion cell is to the left, while cytoplasmic remains of the spermatozoid, most prominently mitochondria, appear immediately to the left of the spermatozoid nucleus. Other sections of this same cell are shown in Fig. 6–19. × 11,000.

Fig. 2–5. **2.** Early stage of karyogamy in another fusion cell (cf. Fig. 3–5). The large egg nucleus (left) has a conspicuous nucleolus and has been joined by numerous connective bridges (one shown here) to the smaller spermatozoid nucleus (right). Cytoplasmic remains of the male gamete, including mitochondria and parts of the flagellar apparatus, appear above the fusing nuclei. The surface of the fusion cell is toward the top of the micrograph. × 12,000. **3.** A portion of Fig. 2 enlarged; this section represents the first of a series through a region with three connective bridges joining the nuclei of the egg (left) and spermatozoid (right). One of the bridges is shown in a grazing section, while three others are evident in adjacent sections (cf. Fig. 4, 5). Between the two nuclei are ER blebs formed from the outer membranes of the nuclear envelopes. × 25,000. **4.** An adjacent section of the series to show the same bridge seen in Fig. 3, but now in median section. Note the numerous pores in the egg nuclear envelope (arrowheads). Positions of two underlying bridges are indicated by arrows. × 25,000. **5.** Several sections further on in the series show the second and third connective bridges. The second (arrow 2) is close to the position of the first (cf. Fig. 4) but it is somewhat larger in diameter. The third bridge (arrow 3) is even larger. × 25,000.





approach the egg nucleus. In the same section illustrated in Fig. 18, one of the nuclear extensions occurred on the far side of the spermatozoid nucleus and pointed away from the egg nucleus.

Cytoplasmic intrusions or pockets occasionally occur in both the spermatozoid (Fig. 19) and egg nucleus during karyogamy. This appears to have little or nothing to do with the process of karyogamy, however, since these cytoplasmic pockets are just as frequently found in gamete nuclei prior to plasmogamy (Hoffman, 1971, Fig. 3).

The egg nucleus is generously provided with nuclear pores prior to and during karyogamy (Fig. 4, 6–11, 15, 23, 24; Hoffman, 1973a). The spermatozoid nucleus also possesses nuclear pores (Fig. 6, 8, 9, 11, 24) although they may be less abundant. This contrasts to the situation observed in *Bryopsis* (Urban, 1969) in which nuclear pores were absent in both male and female pronuclei. The possible role of nuclear pores in karyogamy was considered in this investigation of *Oedogonium*. There was no suggestion of their participation in the initiation of karyogamy.

Of the numerous connections established between fusing nuclei early in karyogamy, some enlarge more rapidly than others (Fig. 5, 16, 17).

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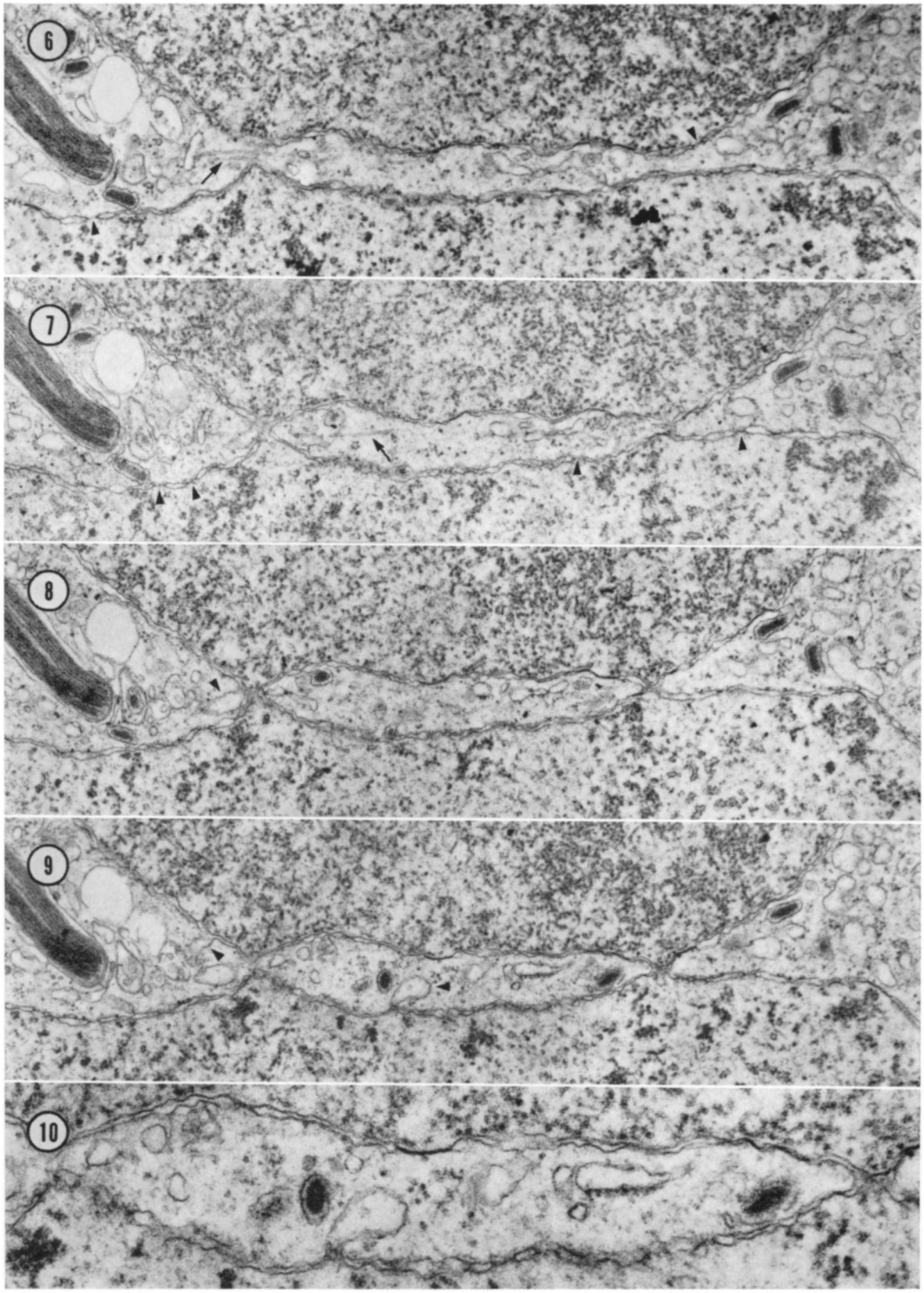
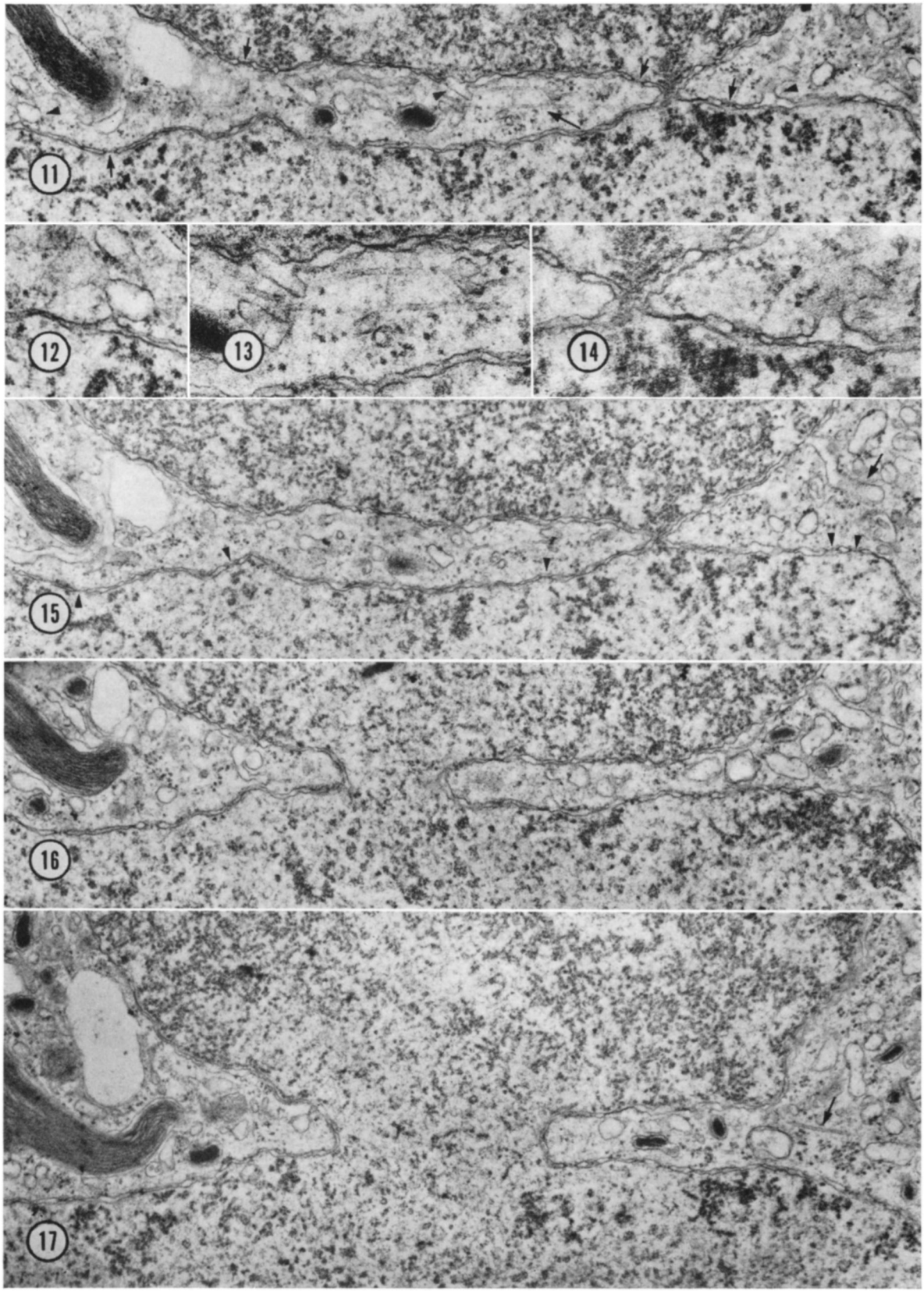
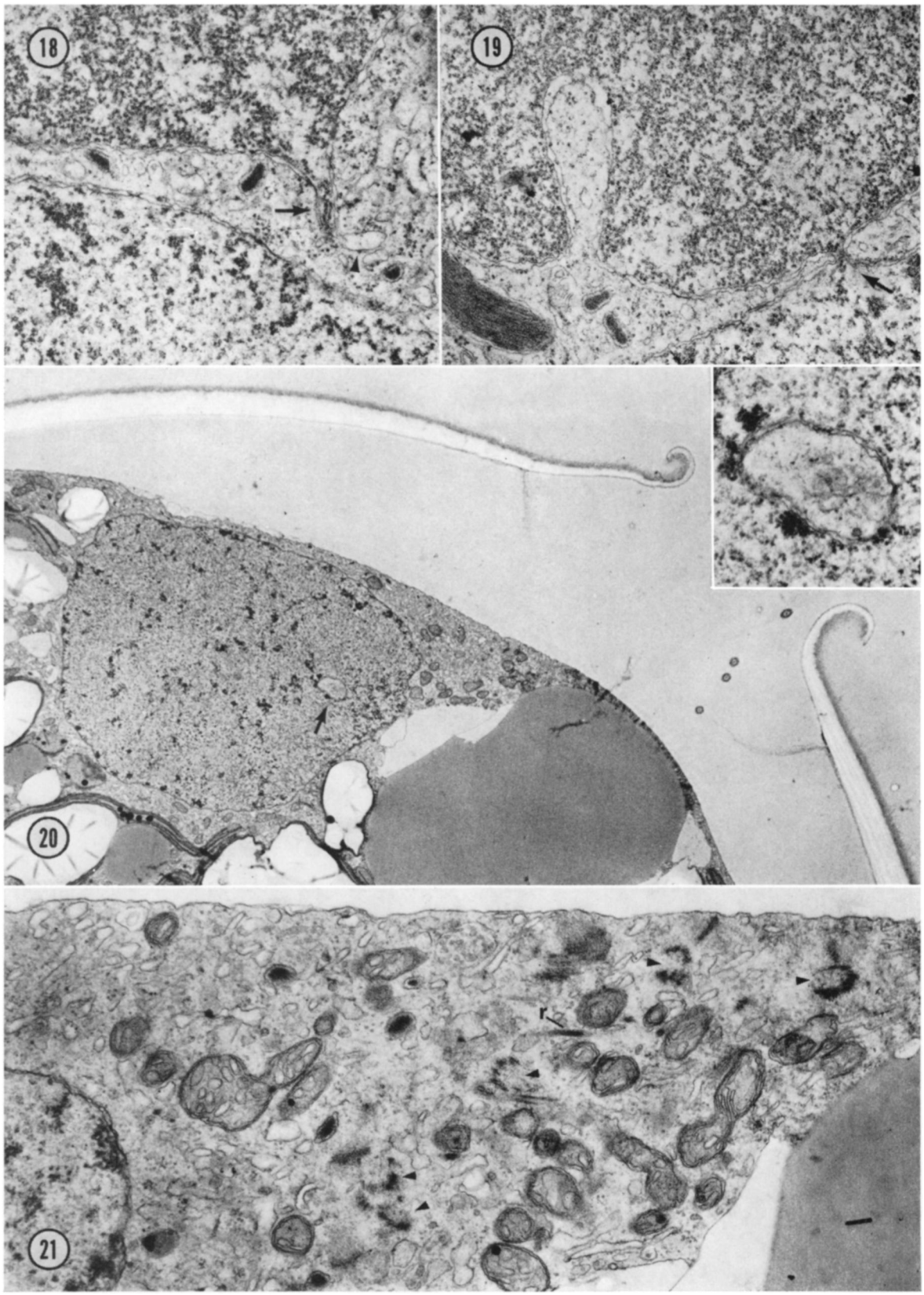
Fig. 6–10. Part of a series of sections (Fig. 6–15) through two bridges connecting the gamete nuclei early in karyogamy (spermatozoid nucleus above). The bridge on the left is complete, with fusion of both the outer and inner membranes of the nuclear envelopes; the bridge on the right is incomplete since only the outer membranes have fused. **6.** This section is at a level slightly above the two bridges, but their positions are suggested by slight evaginations of the two nuclear profiles. Note inflated ER and microtubules (arrow) between the fusing nuclei. Nuclear pores are also evident (arrowheads). × 25,000. **7.** Next section in series. The connective bridge on the left has just been grazed, whereas the bridge on the right is still not in this level of section. Note microtubule (arrow) and nuclear pores (arrowheads). × 25,000. **8.** This section passes through both bridges. The left-hand bridge is in median section and continuity of the two nuclear envelopes is evident, particularly at the right-hand side of the bridge; membrane continuity is obscurred on the left-hand side by an ER bleb (arrowhead). Note the fusion of both the outer and inner membranes. The bridge at the right has just been grazed. The outer membranes of the two nuclear envelopes have fused but not the inner membranes. × 25,000. **9.** Next section in series. The bridge on the left has passed out of the level of section. The bridge on the right shows clearly that the outer membranes of the opposed nuclear envelopes have fused, while the inner membranes are still intact (cf. Fig. 10). Note short blebs of smooth ER proliferating from the outer membrane of each nuclear envelope (arrowheads). × 25,000. **10.** Same as Fig. 9 but at higher magnification. Note the fused outer membranes of the bridge on the right. Although the inner membranes appear in contact, they remain intact; fusion appears imminent. Note inflated ER between fusing nuclei. × 50,000.

Fig. 11–17. **11.** Continuation of series shown in Fig. 6–10. The bridge on the right still has intact inner membranes. There are three good examples of ER proliferation (arrowheads); these are shown magnified in Fig. 12–14. Note nuclear pores (short arrows) and microtubules (long arrow). × 25,000. **12.** An enlargement from Fig. 11, showing the proliferation of ER from the outer membrane of the egg nuclear envelope. × 50,000. **13.** An enlargement from Fig. 11, showing microtubules and the proliferation of ER from the spermatozoid nuclear envelope. × 50,000. **14.** An enlargement from Fig. 11, showing the right-hand connective bridge and ER proliferation from the egg nuclear envelope. Although the membranes of the bridge are less distinct than in the adjacent section (cf. Fig. 10), the inner membrane of the spermatozoid nuclear envelope is intact. × 50,000. **15.** Next section in series. The right-hand bridge is just grazed at this level of section, and in the adjacent section (not illustrated) it was not present. Note long ER bleb from the envelope of the spermatozoid nucleus (arrow) and nuclear pores (arrowheads). × 25,000. **16.** This section shows the same fusing nuclei as Fig. 6–15. The bridge shown was the largest of the numerous connections established between the nuclei, and it was positioned near the center of the field of bridges. This section passes through the periphery of the bridge (cf. Fig. 17) . × 25,000. **17.** Median section through the same bridge illustrated in Fig. 16. Note microtubule (arrow). × 25,000.

Fig. 18–21. **18.** Slender extension of male gamete nucleus (arrow) approaching surface of egg nucleus (below). Note ER bleb associated with this nuclear extension (arrowhead). × 25,000. **19.** A cytoplasmic pocket extending into spermatozoid nucleus. There is also a small connective bridge between the two gamete nuclei (arrow). × 25,000. **20.** One of a series of sections through a fusion cell in which the two gamete nuclei are broadly connected through fusion although the integrity of each is still evident (cf. Fig. 22–26). This section passes primarily through chromatin of the original egg nucleus, while most of the chromatin contributed by the former spermatozoid nucleus lies in deeper levels of section to the right (cf. Fig. 25, 26). A cytoplasmic channel (arrow) delimited by nuclear envelope is evident in the juncture region with the more condensed chromatin of the male gamete nucleus evident to the immediate right. Also shown is the pore in the thick oogonial wall and transverse sections through flagella shed by the spermatozoid during plasmogamy. × 4,500. **20.** (inset). Enlargement of the cytoplasmic channel in Fig. 20 to illustrate the delimiting membranes which have been shown to be continuous with the nuclear envelope. × 25,000. **21.** Another section of the fusion cell in Fig. 20. The area shown is immediately to the right of the fusing nuclei (lower left in Fig. 21) and cytoplasmic remains of the spermatozoid are still conspicuous. These include mitochondria and parts of the flagellar apparatus, such as basal bodies (arrowheads) and flagellar roots (*r*). × 25,000.







Figures 16 and 17 show two sections through a large connective bridge in the same cell illustrated in Fig. 1 and 6–15. The section shown in Fig. 16 passes tangentially through the periphery of the bridge and is only a short distance removed from the section illustrated in Fig. 6. A median section through the same bridge is illustrated by Fig. 17. It was the largest connection joining the fusing nuclei in this cell and it was approximately centered in the field of connections between the nuclei. Although a few other relatively large connections occurred in more peripheral positions, this was clearly the most prominent bridge and is most probably the one which would continue enlargement and ultimately unite the nuclei. As has been reported in other examples of nuclear fusion (Jensen, 1972, 1973; Schulz and Jensen, 1973), progressive enlargement of this bridge would result in the gradual exclusion of any cell organelles located between the two fusing nuclei. The fate of the numerous smaller connective bridges appears to be fusion with larger bridges.

Later stages in karyogamy were more frequently encountered and examples from two such fusion cells are illustrated. In one (Fig. 20–27), the two nuclei are in an advanced stage of fusion, although there is still an obvious juncture between the portions that were formerly the egg and spermatozoid nuclei. Karyogamy in the other cell is even more advanced with little evidence remaining of a juncture (Fig. 28, 29).

These later stages of karyogamy can be readily recognized ultrastructurally since the contribution of the spermatozoid nucleus appears so different in comparison to the contribution of the egg nucleus. The spermatozoid nucleus is smaller, lacks organized nucleoli, and has more condensed chromatin (Hoffman, 1973a). As the nuclei fuse, the more condensed chromatin of the spermatozoid nucleus remains conspicuous throughout karyogamy.

With the rapid enlargement of a single connective bridge between the fusing gamete nuclei (Fig. 17) and the presumptive fusion of the smaller bridges with it as it expands, the nuclei become united by a single nuclear envelope (Fig. 20, 22, 25, 26). It is formed from the combined nuclear envelopes of the egg and spermatozoid nuclei. At this stage, the juncture between the fusing gamete nuclei may be evidenced by a constriction of the nuclear envelope as well as channels of cytoplasm delimited by invaginations of the nuclear envelope (Fig. 20, 23–25). Sometimes small pockets of cytoplasm are isolated within the nucleus near the juncture; these pockets are delimited by remnants of nuclear envelope (Fig. 22, 26, 27).

The sections shown in Fig. 20–26 are representatives from a sequence cut in a plane slightly oblique to the axis passing through the two fusing nuclei. Only sections through the juncture are illustrated. Since the plane of sectioning is somewhat oblique, the first of the sequence illustrated (Fig. 20) passes mostly through the chromatin of the former egg nucleus, with a bit of the juncture evident at the right, while Fig. 25 passes almost midway through the juncture, and Fig. 26 passes through the chromatin contributed by the former spermatozoid nucleus with the juncture on the left. A more detailed description follows.

The fusing nuclei in Fig. 20 are shown close to the surface of the fusion cell. Note the thick oogonial wall with the pore through which the spermatozoid entered. Cross sections of the spermatozoid's flagella, shed during plasmogamy (Hoffman, 1973a), may be seen between the pore and the surface of the developing zygote. Remains of the sperm cell's mitochondria and flagellar apparatus (minus the flagella) are still evident in the region to the right of the fusing nuclei (Fig. 20) and are even more obvious in another section at higher magnification (Fig. 21). Description of these components is given in an earlier report (Hoffman, 1973a).

Although other sections of this same series (not illustrated) show more of the fusing egg nucleus with its nucleolus, Fig. 20 is one of the first in the series to pass through part of the juncture between the fusing nuclei. The juncture in this and other sections can be recognized by two features: first, the sharp demarcation of less dense chromatin contributed by the egg nucleus and the more dense chromatin contributed by the

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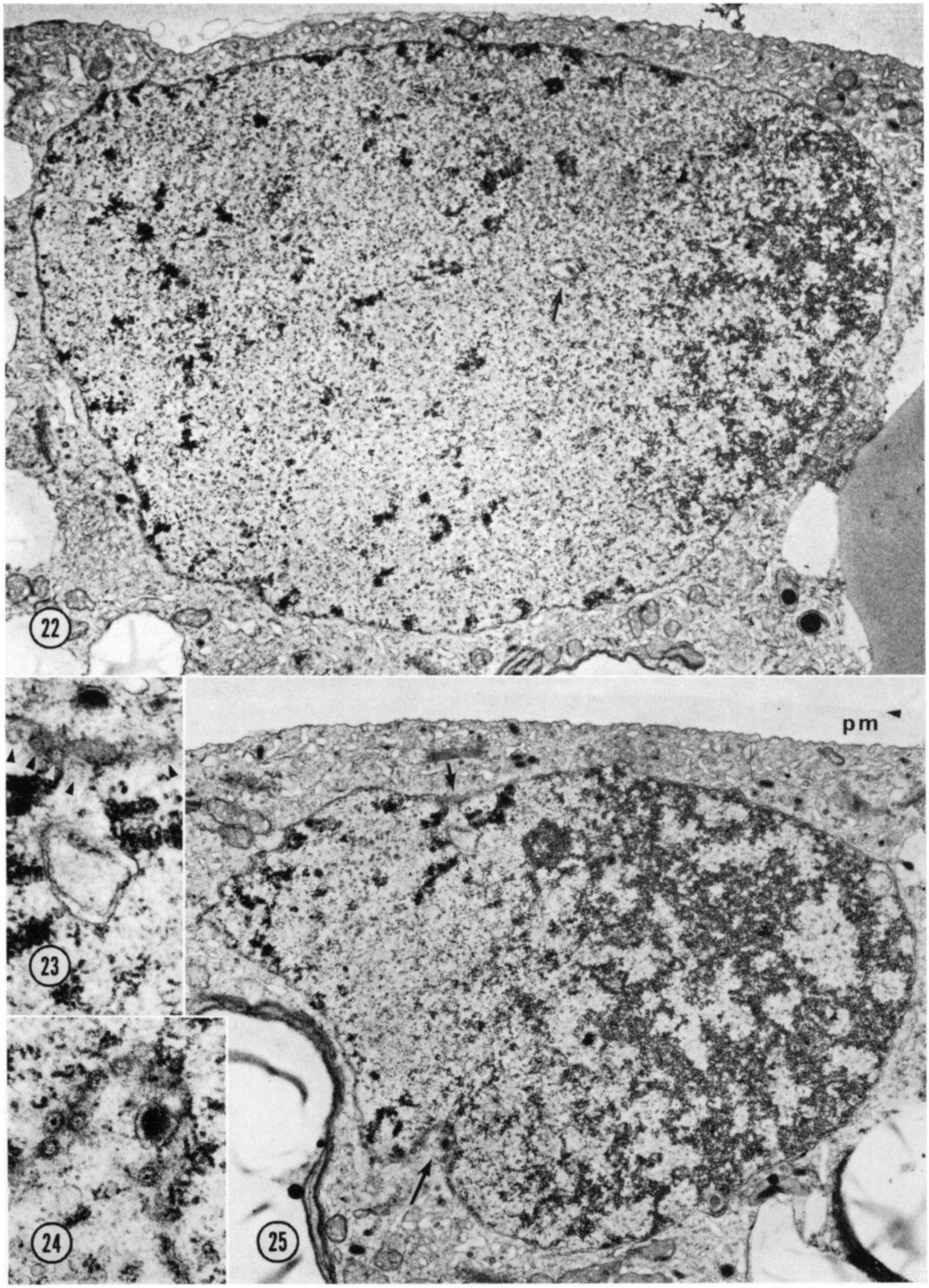
Fig. 22–25. **22.** Another section through the fusing nuclei shown in Fig. 20. In this deeper level of section the juncture between the fusing nuclei lies about ⅔ the way to the right. In this position there is an inclusion of cytoplasm delimited by a remnant of nuclear envelope (arrow); serial sections show that the delimiting membranes are unconnected to the nuclear envelope of the newly forming zygote nucleus. The denser chromatin contributed by the male gamete nucleus is evident on the right. × 11,000. **23.** An enlargement of one of the cytoplasmic channels in Fig. 25. Note pores of the nuclear envelope (arrowheads). × 35,000. **24.** Enlargement of the other cytoplasmic channel in Fig. 25. Note nuclear pores in this grazing section of the channel. × 40,000. **25.** Another, deeper level of section of the series shown in Fig. 20 and 22. This section passes obliquely through the juncture of the fusing nuclei. The juncture is evidenced by the slight constriction in the profile of the continuous nuclear envelope and by cytoplasmic channels, two of which are shown (arrows). The condensed chromatin of the spermatozoid nucleus is to the right of the juncture. External to the plasmalemma is the "primary zygote membrane" (*pm*) whose outer boundary is indicated by arrowhead. × 11,000.



nucleus of the male gamete; and second, by the presence in this region of cytoplasmic channels delimited by invaginations of the nuclear envelope and/or nuclear inclusions which are cytoplasmic pockets delimited by remnants of nuclear envelope. A section through a cytoplasmic channel is seen in Fig. 20 (arrow) and at higher magnification in the inset of Fig. 20. The paired membranes of the nuclear envelope are evident, and serial sections demonstrate that this is indeed a channel; its delimiting membranes are continuous with the nuclear envelope of the fusing nuclei. The denser chromatin of the spermatozoid nucleus lies in a small area immediately to the right of this channel.

A deeper level of section is illustrated in Fig. 22 and the condensed chromatin from the spermatozoid nucleus is evident on the right. What appears as another cytoplasmic channel lies deep within the developing zygote nucleus (arrow) at the juncture of fusion. Serial sections indicate, however, that this is not a channel since the paired membranes forming the boundary of this pocket of cytoplasm are not connected with the nuclear envelope surrounding the developing zygote nucleus. It is apparent that the paired membranes which delimit this inclusion represent remnants of nuclear envelope isolated during fusion of connective bridges in an earlier stage of karyogamy. Although the exact fate of such inclusions is not certain, they have not been observed in mature zygote nuclei.

The juncture between the fusing egg and spermatozoid nuclei is most clearly seen in the next section illustrated in the series (Fig. 25). A slight constriction is evident in the profile of the common nuclear envelope that broadly unites the fusing nuclei. A three-dimensional reconstruction would show that the general shape of each fusing nucleus is still retained. Two cytoplasmic channels appear at the juncture in Fig. 25, and these are shown at higher magnification in Fig. 23 and 24. One of the channels (Fig. 23) is sectioned obliquely so its "exit" is grazed and only vaguely apparent in the section illustrated; adjacent sections confirm that it is a channel. Nuclear pores are indicated by arrowheads. The channel illustrated by Fig. 24 is sectioned near its periphery so nuclear pores are evident in tangential view. Presumably, the more numerous pores on the left were originally associated with the nuclear envelope contributed by the egg nucleus, while those on the right were associated with that of the male gamete.

Figure 26 illustrates another section in the series which passes through the far side of the juncture in contrast to Fig. 20. Only the contribution of the spermatozoid nucleus is evident in this section and the juncture lies to the left.

Two dissimilar nuclear inclusions are shown in Fig. 27. These were observed near the juncture of the fusing nuclei in the same cell discussed above. The one on the right is delimited by a pair of membranes in close proximity to the nuclear envelope. Even with serial sections, it is not clear how these membranes are associated with those of the nuclear envelope, although there is some suggestion of membrane fusion. This inclusion is not an ordinary cytoplasmic channel, but appears to be a cytoplasmic pocket delimited by an isolated remnant of nuclear envelope. The other nuclear inclusion is delimited by a single membrane, and in adjacent sections it appears in close proximity to the first inclusion described. Although the origin and significance of this second inclusion are uncertain, it may have resulted by membrane blebbing from either the inner membrane of the nuclear envelope or from the outer membrane of the adjacent inclusion.

A late stage in karyogamy is illustrated in Fig. 29; the section passes obliquely through the juncture of fusion. Relative to the plane of section, the spermatozoid nucleus had been positioned at a deeper level and closer to the cell surface than the egg nucleus. The recently fused nuclei are broadly connected by a continuous nuclear envelope. Except for the more condensed chromatin contributed by the nucleus of the male gamete, there is little indication remaining of the juncture between the fused nuclei. There is no obvious

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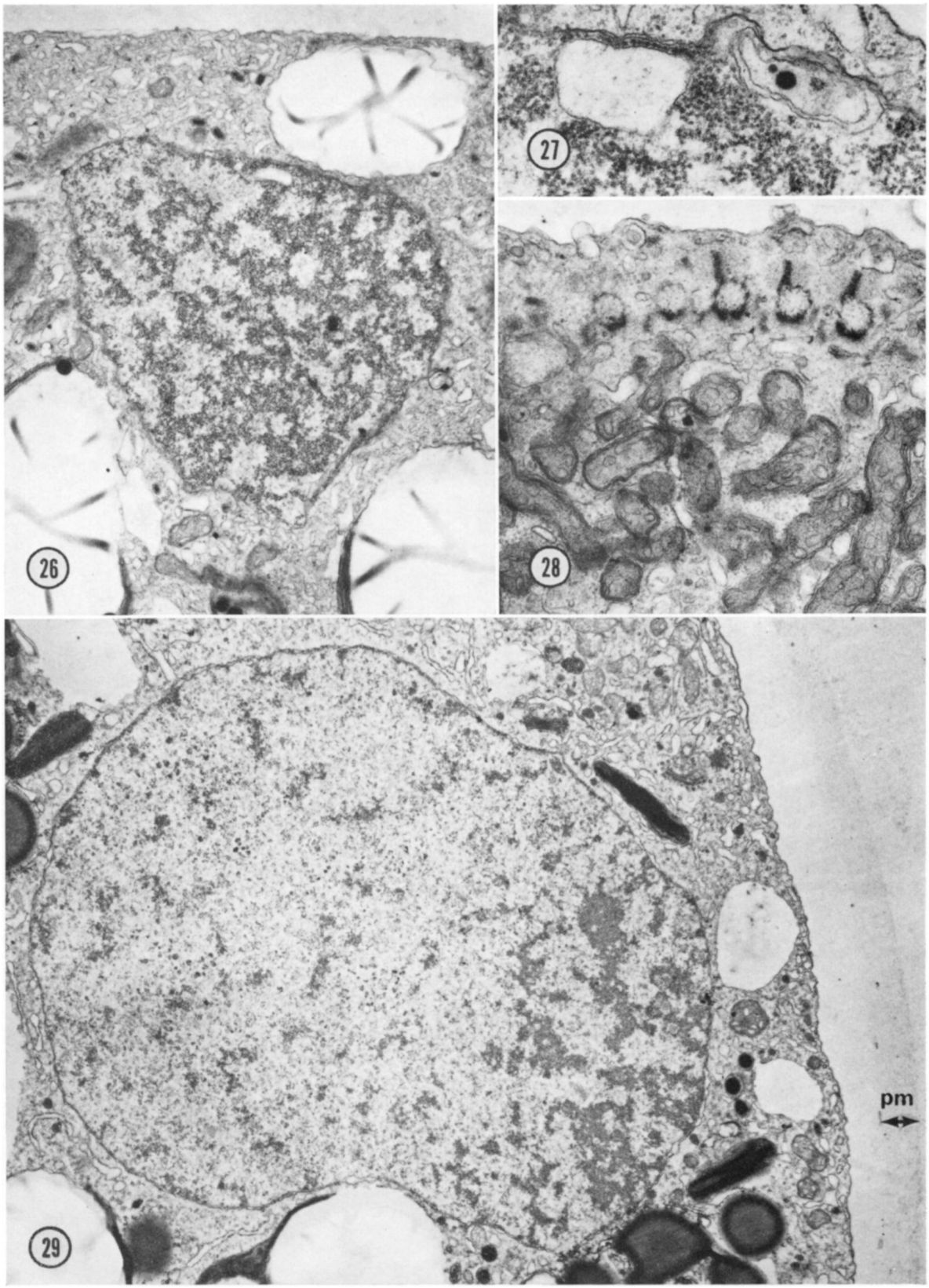
Fig. 26–29. **26.** Still deeper level of section of the series shown in Fig. 20, 22, and 25. This section passes through the former spermatozoid nucleus. The juncture between the fusing nuclei is to the left. × 12,000. **27.** Two nuclear inclusions at the juncture between fusing gamete nuclei; same cell as in Fig. 20–26. The inclusion on the right is delimited by a pair of membranes that probably represent isolated remnants of nuclear envelope, whereas the one on the left appears to be delimited by a single membrane and lies adjacent to the nuclear envelope. × 35,000. **28.** Surface of the fusion cell illustrated in Fig. 29 to show the remains of the spermatozoid cytoplasm during this late stage of karyogamy. A row of basal bodies is shown in transverse section with the remains of the connective, fibrous band. The mitochondria are also from the sprematozoid. × 25,000. **29.** A section through a fusion cell in a late stage of karyogamy. Little trace of a juncture can be seen between the fusing nuclei although the more condensed chromatin of the male gamete nucleus can be distinguished readily on the right. This section is oblique through the juncture region; before fusion, the egg nucleus was positioned above and slightly to the left of the spermatozoid nucleus. The double-headed arrow indicates the thickness of the "primary zygote membrane" (*pm*). × 10,500.



constriction in this region nor any cytoplasmic channels, although in other sections a few inclusions of cytoplasm were observed in the nucleus. In comparison to the earlier stage seen in Fig. 20–26, this young zygote nucleus has begun "rounding up."

Even at this advanced stage of karyogamy cytoplasmic components contributed by the spermatozoid can be readily recognized. Another section from the same cell (Fig. 28) illustrates the amassed mitochondria of the spermatozoid as well as the remains of a row of basal bodies from its flagellar apparatus. Portions of the fibrous connective band (Hoffman, 1973a) are still in association with the three basal bodies on the right. There appears to be even less degeneration of the components of the flagellar apparatus in this cell than in the cell illustrated in Fig. 21, which represents an earlier stage of karyogamy.

Late stages of karyogamy also show a well-developed "primary zygote membrane" (Fig. 25, 29). This is a finely fibrous layer that surrounds the young zygote. Its formation appears to be initiated by plasmogamy, as previously described (Hoffman, 1973a).

Eventually, the young zygote nucleus assumes a subspherical to ellipsoidal shape. The dense chromatin contributed by the spermatozoid nucleus gradually becomes indistinguishable from the chromatin contributed by the egg nucleus with which it presumably associates. Thus, the zygote nucelus ultimately looks much like the egg nucleus prior to plasmogamy. The zygote at this stage has been previously illustrated (Hoffman, 1971, Fig. 1).

Although it still resembles an egg to a large degree, a young zygote (i.e., before the oospore wall forms) can be distinguished by any and all of the following features: (1) remains of sperm cell cytoplasm near the zygote nucleus, particularly a concentration of mitochondria; (2) remains of the sperm cell's flagellar apparatus; (3) ruptured inner boundary of the pore substance caused by the entry of a spermatozoid into the oogonium (for explanation see Hoffman, 1973a); and (4) the presence of a "primary zygote membrane."

DISCUSSION—The literature contains numerous references to plasmogamy in *Oedogonium* (see references in Hoffman, 1973a), but only a few discuss karyogamy (Gussewa, 1931; Klebahn, 1892; Ohashi, 1930). These early cytological studies agreed that karyogamy followed soon after plasmogamy and that the two gamete nuclei differed greatly in appearance at the time of karyogamy. Klebahn (1892) and Gussewa (1931) presented the most detailed description; the illustrations of each contrast the smaller, more densely staining spermatozoid nucleus with the larger, more diffuse egg nucleus. Only in the latter was a nucleolus observed. Light microscopy revealed little else of consequence.

The present ultrastructural study describes the basic details of nuclear fusion in *O. cardiacum*. Fusion of the envelopes of opposing gamete nuclei occurs in two steps: first, the outer membranes of the two envelopes fuse in many small localized areas; then, the inner membranes fuse. It appears that the first step is often affected by short blebs of ER which are produced in abundance by the outer membrane of both nuclear envelopes. Nuclear fusion initiated by intervening elements of ER has been termed *indirect fusion* by Urban (1969), who also discusses the possible significance of the ER in initiating fusion. Indirect fusion has been observed in *Bryopsis* (Urban, 1969) and some angiosperms (Jensen, 1964, 1972, 1973). If fusion is initiated without intervening ER elements, Urban refers to it as *direct fusion*. As an example Urban cites *Chlamydomonas*, based on the published work of Brown et al. (1968).

Although the distinction between direct and indirect fusion appears clear, it may be too soon to evaluate the usefulness of these terms. Until more data are available on the fine structure of karyogamy in plants, caution must be exercised before assigning any organism into the category of either direct or indirect fusion. This is particularly true if initial study suggests the organism lacks any significant ER proliferation from the nuclear envelopes and, thus, does not appear to have ER participating in the initiation of karyogamy. Before the existence of ER blebs is ruled out, one must consider that their demonstration is somewhat dependent on the techniques employed in preparing the material. Moreover, other factors, such as those associated with the physiological state of the material, may provide for some variance in morphological expression. Organisms now suspected of demonstrating direct fusion should be critically reexamined with a variety of techniques. Indirect fusion may prove to be even more prevalent in plant karyogamy than presently thought.

The methods used in the present study of *Oedogonium* clearly demonstrate numerous ER proliferations from the envelopes of both gamete nuclei. The ER blebs lack associated ribosomes and are relatively short and inflated in comparison to the well-developed proliferations of rough ER formed around the gamete nuclei in *Bryopsis* (Urban, 1969). The proliferated ER is also more conspicuous during nuclear fusion in cotton (Jensen, 1964). Although the ER blebs are less well developed in *Oedogonium*, they do not appear to be artifacts. They are not observed when one examines nuclei of vegetative cells prepared with the same techniques.

In cotton, Jensen (1964, Fig. 2) elegantly

demonstrated that fusion occurred between proliferated ER elements from opposing nuclei. Since the proliferated ER in *Oedogonium* is so poorly developed, the chance contact between two ER elements is much less likely. Therefore, it is presumed that nuclear fusion in *Oedogonium* occurs most commonly between a single ER proliferation and the outer membrane of the opposing nuclear envelope.

The initiation of karyogamy in *Oedogonium* establishes many small connective bridges between opposing gamete nuclei. This is similar to the situation in *Bryopsis* (Urban, 1969), pine (Camefort, 1965), and the angiosperms (Jensen, 1964, 1965, 1972, 1973). As karyogamy proceeds, these many small connectives become consolidated, apparently by fusion, into a single broad connection between the fusing nuclei. In the process of consolidation there is a generation of numerous cytoplasmic channels as well as isolated nuclear inclusions consisting of small pockets of cytoplasm delimited by remnants of nuclear envelope. In angiosperms, Jensen (1973) noted that, "the two nuclei fuse completely although isolated pockets of nuclear membrane may be visible for long periods in the new zygote nucleus."

What is the fate of the channels and the isolated pockets of cytoplasm formed at the juncture of nuclear fusion in *Oedogonium*? They are not present in the mature zygote nucleus. Although conclusive evidence is not available to explain their rapid disappearance, one may speculate. The disappearance of the cytoplasmic channels would appear to be a simple matter. As the zygote nucleus matures it begins to assume a more rounded shape, during which time the channels could be readily eliminated since the delimiting membranes are continuous with the envelope of the zygote nucleus. The elimination of the isolated cytoplasmic pockets requires another explanation. It is unlikely that the zygote nucleus "absorbs" these inclusions of cytoplasm. A more logical explanation is that these isolated pockets of cytoplasm eventually migrate to the nuclear surface where their delimiting membranes fuse with the nuclear envelope to "liberate" the trapped cytoplasm. Figure 27 may provide some evidence for this mechanism. The nuclear inclusion at the right of the micrograph is delimited by a pair of membranes and lies directly beneath the nuclear envelope. If it is an isolated pocket of cytoplasm, as serial sections suggest, fusion of its membranes with those of the adjacent nuclear envelope would release the enclosed cytoplasm. Since the delimiting membranes of the inclusion are derived from remnants of nuclear envelope during fusion, they should be compatible with the nuclear envelope of the zygote.

The duration of karyogamy has not been considered in reports on other algal genera. There has only been the implication that it occurs rapidly. In *Oedogonium*, a well-formed zygote nucleus may occur within 15 min of mixing eggs and spermatozoids. Since most of that time is required for the spermatozoid to arrive at an oogonium, enter it, and establish plasmogamy, karyogamy must take place very rapidly indeed. This may be contrasted with cotton, in which fusion of the gamete nuclei appears to be quite slow, lasting a matter of hours (Jensen and Fisher, 1967; Jensen, 1972).

Cytoplasmic components contributed by the spermatozoid of *Oedogonium* are still evident in the young zygote after karyogamy. This is particularly true of the remains of the flagellar apparatus and the mitochondria (Fig. 21, 28). The components of the flagellar apparatus ultimately degenerate, a process already begun soon after plasmogamy (Hoffman, 1973a). In contrast, the sperm cell mitochondria appear structurally intact even after karyogamy. The ultimate fate of the mitochondria and other features of zygote maturation remain to be determined by future studies.

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