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A Fine-Structural Study of Aberrant Meiotic Cytokinesis in an Autosomal Male Sterile Mutant (ms(2)3R) of *Drosophila melonogaster*

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A FINE-STRUCTURAL STUDY OF ABERRANT MEIOTIC CYTOKINESIS IN AN AUTOSOMAL MALE STERILE MUTANT (*ms*(2)3R) OF *DROSOPHILA MELANOGASTER*

by

Laura J. Laughran

A thesis submitted in partial fulfillment
of the requirements for the degree

of

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in

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Major Professor

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ABSTRACT

A Fine-Structural Study of Aberrant Meiotic Cytokinesis in an Autosomal Male Sterile Mutant (ms(2)3R) of Drosophila melanogaster

by

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The ultrastructure of abnormal meiotic cytokinesis in an autosomal male sterile mutant of Drosophila melanogaster is described. In the mutant ms(2)3R mitotic cytokinesis proceeds normally, but abnormal meiotic divisions give rise to four spermatids developing in one cytoplasmic mass. The contractile ring which is responsible for cell constriction during cytokinesis appears to form and function normally during meiosis. Ring canals which form intercellular bridges between synchronously developing spermatids are also apparently normal. However, there is an apparent adhesion and subsequent disintegration of the furrow membranes after division is completed. In addition to double membrane fragments in areas of open communication between germ cells, occasional unattached ring canals within the spermatid cytoplasm were found. These findings suggest that cell fusion is the anomaly in meiotic cytokinesis of ms(2)3R. Some observations on abnormal nebenkern formation are also reported.

(32 pages)

INTRODUCTION

In 1972, Romrell *et al.* (19) published an ultrastructural study on spermatogenic anomalies in an autosomal male sterile mutant (*ms*(2)3R) of *Drosophila melanogaster*. It seemed apparent that meiotic cytokinesis had failed, resulting in four spermatids developing in a common cytoplasm. Evidence for this assumption came from observations of "giant" nebenkernen four times the normal volume inside abnormal spermatids, up to four nuclei inside a single cytoplasmic unit, and cross sections through tails showing four axonemes. It was observed that each "giant" nebenkern later divided to form as many as eight mitochondrial derivatives.

Two possibilities were suggested to account for the alleged failure of meiotic cytokinesis. The first suggestion was that the microfilaments forming the contractile ring, an organelle that constricts in the division plane resulting in cell cleavage (20-23), do not form, and cytokinesis does not occur. The second possibility was that cleavage furrows and intercellular bridges were formed but that they were unstable, allowing the furrows to recede and the partially separate cytoplasmic masses to become broadly confluent.

In this study these two possibilities were investigated by means of ultrastructural comparison of meiotic stages and early spermatids in *ms*(2)3R and wild-type testes. The results indicated that neither of the above postulates was correct, but rather that breakdown of furrow membranes accounts for aberrant meiotic cytokinesis in the mutant.

In addition, new information concerning the mutant nebenkernen examined in the Romrell study is reported.

MATERIALS AND METHODS

The recessive autosomal male sterile mutant ms(2)3R of Drosophila melanogaster is located on the second chromosome at map position 51. It is characterized by an anomaly of cytokinesis during meiotic division. Although nuclear division occurs in the primary and secondary spermatocytes, the cytoplasm fails to remain separated, thus giving rise to four nuclei within one cytoplasmic mass.

Isolation of mutant pupae

The mutant is maintained in stock using a balancer chromosome (SM5). Only heterozygotes and male sterile homozygotes survive. According to Cooper (4), the number of cells undergoing meiosis in the male reaches a maximum during the midpupal stage of development, i.e., at approximately 150 hours after the egg is laid. Since this study involved the investigation of abnormal cytokinesis during meiotic division in males, it was necessary to find a means of distinguishing heterozygotes from male sterile homozygotes at the midpupal stage.

To accomplish this, the heterozygote $\frac{ms(2)3R}{SM5}$ was mated to another heterozygote $\frac{M(2)H^{S5}}{SM5}$. This second fly has a "Minute" mutation on the second chromosome at map position 53.5. It is a dominant trait, lethal when homozygous, having short (minute) body bristles and increased development when heterozygous. SM5, the same balancer chromosome used with ms(2)3R, gives heterozygotes the distinctive trait of curly wings. From the cross

$$\frac{ms(2)3R}{SM5} \times \frac{M(2)H^{S5}}{SM5}$$

only those offspring having straight wings, $\frac{M(2)H^{S5}}{ms(2)3R}$, were taken. The map locations of the genes specifying these two traits are only about 2.5 units apart. Matings between these heterozygotes,

$$\frac{M(2)H^{S5}}{ms(2)3R} \times \frac{M(2)H^{S5}}{ms(2)3R},$$

produce offspring of the following genotypes:

$$\frac{M(2)H^{S5}}{ms(2)3R} \text{ and } \frac{ms(2)3R}{ms(2)3R}.$$

The Minute heterozygotes have the longer development time. Therefore, the early pupating males were collected and allowed to develop to midpupal stage.

To insure that only ms(2)3R flies were being studied, several pupae from the collected group were allowed to mature. After hatching they were mated with virgin females and no offspring resulted. In addition, upon ultrastructural observation only those testes which contained spermatids showing distinctly mutant characteristics were examined.

EM preparation

Two different fixation methods were used. In all cases, as a control, midpupal wild-type Canton-S males were processed in a manner identical to the mutants.

a) Midpupal testes were dissected in 3% glutaraldehyde buffered with 0.05M phosphate buffer at pH 7.3. Samples were transferred to a fresh vial of the same fixative and fixed for one hour at room temperature followed by three washes of 0.05M phosphate buffer. Postfixation was in

2% osmium tetroxide for one hour at 4 C.. This was followed by three more buffer washes. (Figs. 1, 2, 6, 8, 9, 10, 14, 15.)

b) Midpupal testes were dissected in Drosophila Ringer's solution. The testes were then transferred into "Luft's cocktail" (24, 27) which consisted of one part of 5% glutaraldehyde, one part of 5% OsO_4, and two parts of 0.05M phosphate buffer at pH 7.3 mixed immediately before use. This mixture was set in an ice bath, and here the tissue was fixed for one hour.. Three washes of cold 0.05M phosphate buffer followed. (Figs. 3, 4, 5, 7, 11, 12, 13, 16, 17.)

Following either of the above protocols, testes were dehydrated through a graded alcohol series and embedded in Dow epoxy resin (12). One micrometer sections were cut and observed under the light microscope for purposes of orientation and localization of promising areas for thin sectioning. Thin sections, silver to gold, were cut with glass knives, double stained with uranyl acetate and lead citrate (18), and examined with a Zeiss EM9-S2 electron microscope.

RESULTS

Light microscopic observations of midpupal testes of Drosophila melanogaster (approximately 60 hours post pupation) revealed that the apical tip of each testis contained spermatogonia; primary spermatocytes occupied most of the middle region. Secondary spermatocytes were rarely observed, and the few that were seen were difficult to distinguish from the primary spermatocytes. These cells were located nearer the basal end of the testis while spermatids, if present, were always found at the extreme basal end. Division figures were identified by the large numbers of elongate mitochondria stretching along the spindle apparatus.

At the ultrastructural level a few spermatocytes were observed in first meiotic division. Interphase of the secondary spermatocyte is very brief; for this reason few were observed, and no abnormality was found in mutant cells. Most observations were made on cells undergoing the second meiotic division and on early spermatids. No mature spermatozoa were found in the midpupal testes of either the mutant or the wild-type specimens.

Contractile rings

The contractile ring is a collar-like zone of microfilaments encircling the area of cell constriction in cells that are in the process of cleaving (21). In both wild-type and mutant Drosophila the contractile ring begins to form at the end of anaphase, causing a slight constriction in the division plane. It is not formed simultaneously all the way

around the cleavage furrow. A dense band of filaments may be found directly beneath the plasma membrane on one side of the furrow with little or no evidence of a contractile ring on the opposite side (Fig. 1). No difference was discernable between wild-type and mutant germ cells.

Measurements of the thickness of contractile ring material were recorded throughout the process of cleavage furrow formation in both wild-type and mutant germ cells (Table 1). Although an analysis of variance showed the means to be homogeneous, in both ms(2)3R and Canton-S cells there was an apparent increase in the thickness of the ring material from 0.07 microns in early telophase to 0.20 microns in late telophase. No abnormality was noted in the formation of contractile rings and cleavage furrows in the mutant cells Figs. 2 and 3). The decrease in thickness recorded in the wild-type contractile ring at mid-telophase was probably an artifact due to variable planes of section and smallness of the sample size.

Table 1. Thickness of contractile ring material in Canton-S and ms(2)3R germ cells measured in micrometers.

		Early Telophase	Mid-Telophase	Late Telophase
Canton-S	Number measured	3	3	8
	Mean thickness	0.11	0.09	0.13
	Standard error	0.02	0.01	0.02
ms(2)3R	Number measured	8	10	7
	Mean thickness	0.12	0.14	0.15
	Standard error	0.01	0.01	0.01

Ring canals

In the Drosophila male the secondary spermatogonium undergoes four mitotic divisions to form a cluster of 16 primary spermatocytes. These 16 cells remain connected by intercellular bridges stabilized as ring canals (17). Koch et al. (10) state that the ring canal is formed when the advancing cleavage furrow contacts the spindle fibers and the plasma membrane flows around the spindle forming a ring. Even after the spindle dissolves, a stable ring of plasma membrane together with contractile ring filaments remains.

There is no evidence that once a ring canal is formed cell constriction can be reactivated to separate the connected cells (7). Each primary spermatocyte undergoes meiosis to produce four spermatids, again connected by ring canals. Thus each secondary spermatogonium gives rise to 64 interconnected spermatids.

If the four-nucleate spermatids described by Romrell et al. (19) result from the breakdown of ring canals, only ring canals formed by the last two divisions would be involved. Therefore both normal ring canals resulting from the mitotic divisions and defective ring structures of meiotic origin would be expected. Ring canal breakdown might be evident as broader than normal cytoplasmic bridges and/or as displaced or disappearing ring structures. Comparing dimensions of mutant and wild-type ring canals (Figs. 4 and 5) at the same stage of spermiogenesis, it was found that the mean ring canal width in the mutant was 1.24 micrometers and

in the wild-type, 1.06 micrometers (Table 2). These means are not significantly different. Calculations of thickness of the ring material (Table 3) resulted in a mean of 0.08 micrometers for both mutant and control spermatids. Therefore breakdown of ring canals was not evident.

Plasma membranes

In the mutant but not in normal spermatids, adjacent cells were partially confluent due to a large portion of their plasma membranes being absent. This was made evident by following the plasmalemma around each cell until the two meet to extend between the neighboring cells. Here they ended abruptly in a common cytoplasm (Fig. 6). Measurements showed that these membranes were lying much closer together than adjacent spermatid membranes in the wild-type testes. The distance across the two abruptly ending membranes plus the intervening space in mutant spermatids was 19.5 $\pm$ 1.1 nm. Similar measurements across membranes between wild-type spermatids gave a distance of 27.5 $\pm$ 1.6 nm. This is significant at the 0.0005 level. Across these closely apposed plasmalemmas of the mutant, evidence of cross striations was noted (Fig. 7). The striations were absent in the normal specimens (Fig. 8).

Fragments of similar closely apposed unit membranes were frequently found within multi-nucleate spermatids of ms(2)3R. These were identified as plasma membranes on the basis of occasionally finding one lying along the path of abruptly ending membranes that could be followed until they separated to enclose their specific spermatids as do the

Table 2. Diameter of spermatid ring canals measured in micrometers.[a]

		Stage 2	Stage 3	Stages 4-6
Canton-S	Number measured	9	5	1
	Mean diameter	1.0	1.1	1.3
	Standard error	0.14	0.02	—
ms(2)3R	Number measured	17	8	7
	Mean diameter	1.2	1.5	1.2
	Standard error	0.04	0.16	0.18

Table 3. Thickness of spermatid ring canal rim material measured in micrometers.[a]

		Stage 2	Stage 3	Stages 4-6
Canton-S	Number measured	9	5	1
	Mean thickness	0.09	0.07	0.06
	Standard error	0.01	0.01	—
ms(2)3R	Number measured	17	9	6
	Mean thickness	0.09	0.06	0.06
	Standard error	0.01	0.00	0.00

[a]For criteria used in staging spermatids refer to Stanley et al. (25).

ones seen in figure 6. Also, the density of these fragments was similar to that of the plasmalemmas and dissimilar to that of nearby endoplasmic reticulum. Endoplasmic reticulum, ribosomes, and occasional mitochondria occupied the area between such fragments. Figure 9 shows an apparent fragment of apposed plasma membranes separated by a distance of 1.4 micrometers from plasmalemmas that end abruptly between two spermatids, a portion of which is shown at the left. In another instance more than 5 micrometers separated two plasma membrane fragments. An isolated protion of membrane, 2 micrometers long, was found within another multi-nucleate spermatid (Fig. 10). No such configurations were seen in wild-type germ cells.

Further investigation of mutant germ cells revealed cases where intact plasma membranes were absent in a short space between neighboring cells but membrane remnants were evident (Fig. 11). The earliest stage at which this was noted was metaphase of the second meiotic division. A similar case was seen at a slightly later stage, anaphase II. Figure 12 shows another instance; almost no remnants are present and an unobstructed connection exists between the two cells.

On a few occasions in mutant spermatid units, ring canals were found which appeared to be unattached from surface membrane. Figure 13 represents a transverse section through an apparently free-floating ring canal. It has bits of membrane still attached to its ends but instead of

being connected with a plane of plasma membrane, the free ends have fastened to the middle of the outer surface of the ring canal.

The phenomenon of occasional unattached ring canals, in addition to the finding of partially joined spermatids and apparent membrane fragments within four-nucleate spermatids, strongly suggests that fusion of meiotic division products followed by dissipation of the fused membranes is responsible for the cytokinetic anomaly in ms(2)3R male germ cells. The proposed fusion process proceeds in the following manner. 1) Plasma membranes along the meiotic division plane come to lie very close together and cross linkages are formed. Poste and Allison (16) suggest that stable intermembrane linkages must be established before fusion can proceed. The cross striations seen in figure 7 may therefore represent the macromolecules forming intermembrane linkages. 2) Portions of the membranes become closely adherent and rapidly break up leaving remnants of the two membranes lying in the fusion area. 3) These remnants also disperse resulting in an unobstructed connection between the two cells. A diagram of the proposed sequence of events in cell fusion is presented in figure 18.

Supportive cells (nutritive cells (4); cyst cells (26)) surround each group of 64 spermatids. Cytoplasmic processes of these cells interdigitate with, but do not completely surround, the developing germ cells. These supportive cells are thought to be analogous to the Sertoli cells of mammals (26). Fusion was never observed between a supportive

cell and a germ cell. Neither was there any evidence of supportive cell organelles intermingling with germ cell organelles. Instead, the evidence indicates that the fusion process stops when it reaches an intervening supportive cell process (Figs. 11, 12, 14). As the unfused membrane remaining between the united cells is pushed outward by the fluid cytoplasm, the cells become completely confluent (Figs. 14-17).

Nebenkernen

The previous study of ms(2)3R testes by Romrell et al. (19) demonstrated that the mitochondria of the multi-nucleate spermatid could fuse to form a single "giant" nebenkern four times the normal volume. This "giant" nebenkern then underwent a number of divisions to form as many as eight mitochondrial derivatives.

"Giant" nebenkernen were occasionally observed in this study also; however, another variation was noted. Occasionally, two nebenkernen were found inside the same cytoplasmic mass (Fig. 17). Sometimes remnants of plasma membranes were found between them. The presence of the membrane fragments between two nebenkernen suggests that they were never part of a nebenkern with four times the normal volume.

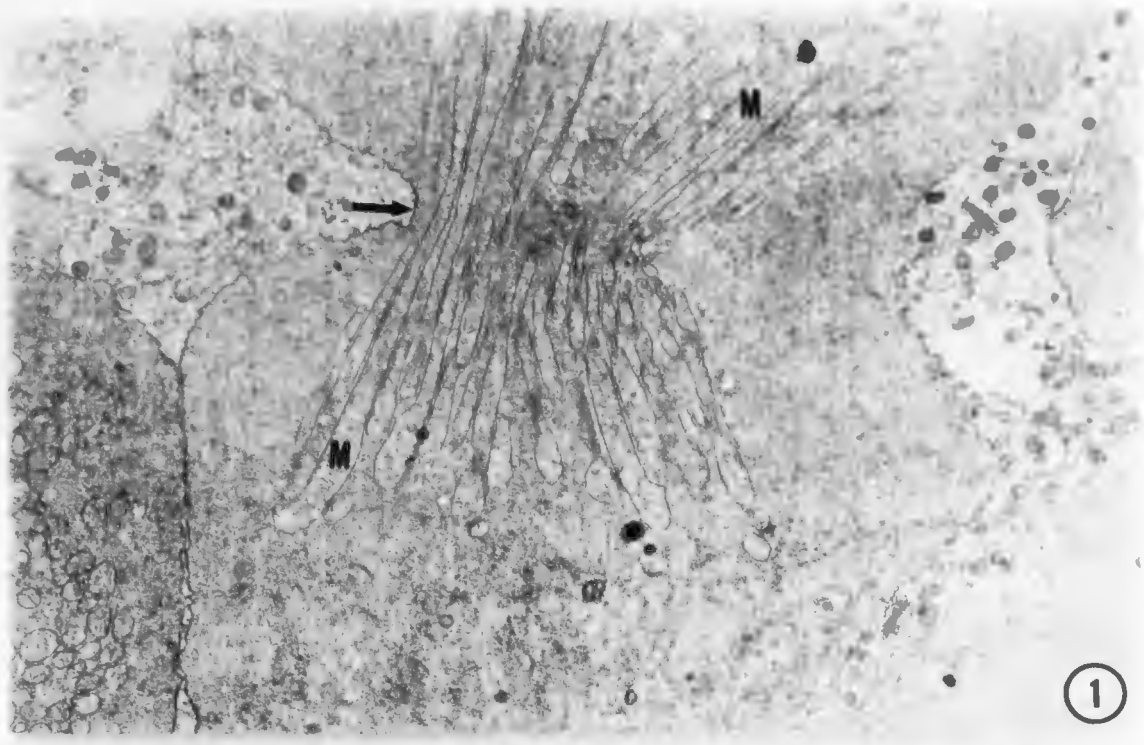
Finding variable sizes and numbers of nebenkernen leads to the conclusion that fusion of the plasma membranes does not occur at any one specific time after cleavage. Extent of supportive cell processes and distance between daughter cell membranes along the cleavage plane may vary between cells and result in variable rates of membrane fusion.

Normal, twice normal, or four times normal size nebenkernen could be formed depending upon when fusion began and how fast it proceeded.

Fig. 1. Wild-type secondary spermatocyte in early telophase. Contractile ring material (arrow) is evident on only one side of the cleavage furrow. M, mitochondria. x6700.

Fig. 2. Late telophase in wild-type spermatocyte. Transverse section through a contractile ring (CR) composed of microfilaments under the plasma membrane. x17,500.

Fig. 3. Late telophase in mutant spermatocyte showing a normal contractile ring (CR). x18,000.



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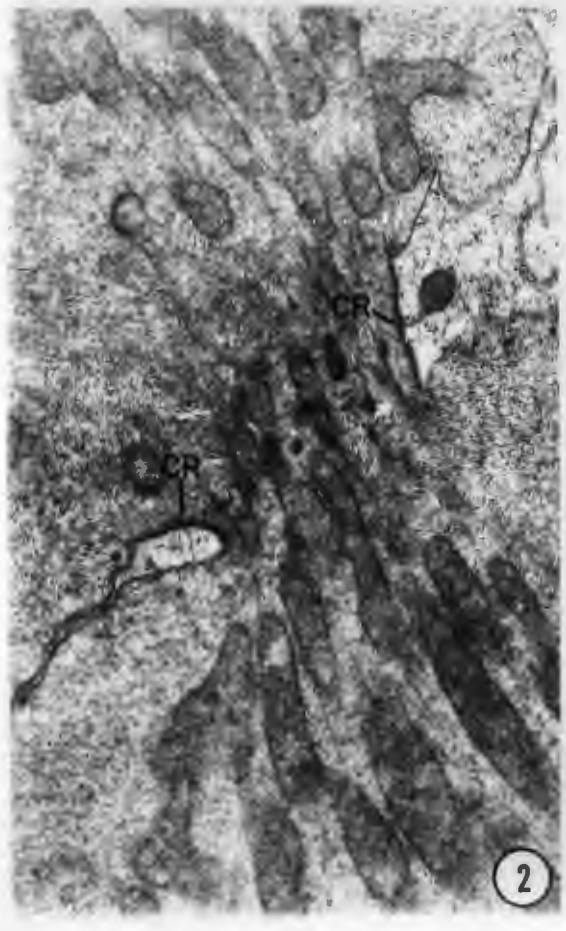

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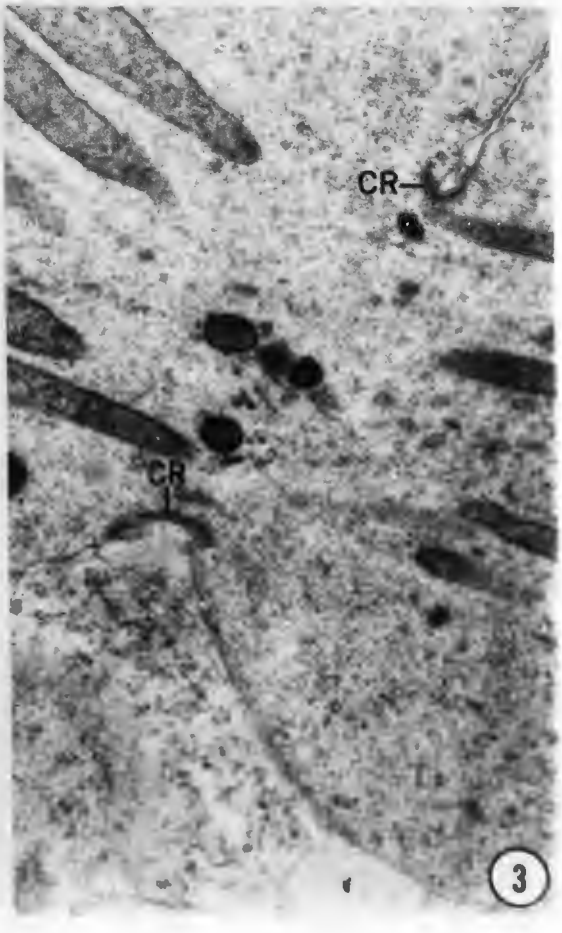

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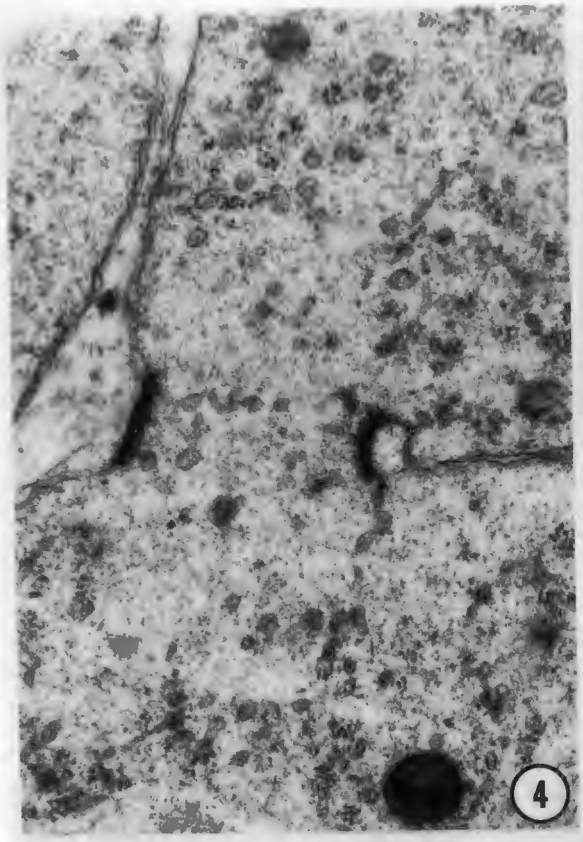
Fig. 4. Section through ring canal connecting spermatids in a wild-type testis. It is composed of microfilament remnants of the contractile ring and an electron dense material in addition to plasmalemma. x27,500.

Fig. 5. Section through ring canal between two spermatids in ms(2)3R. Filaments and electron dense material are abundant. x27,500.

Fig. 6. Membranes between two mutant spermatids. The plasma membranes of each individual cell can be followed (arrows). They meet and continue inward, ending abruptly within the cytoplasm. x7300.

Fig. 7. Plasma membranes of mutant spermatids during an early stage of fusion. The space separating the membranes is very narrow. The arrow indicates an area where cross striations are evident. x150,000.

Fig. 8. Plasma membrane between two wild-type spermatids. The space between the membranes is at least as wide as each membrane. In the lower portion of the micrograph the cells are separated by an interdigitating supportive cell process (S). x150,000.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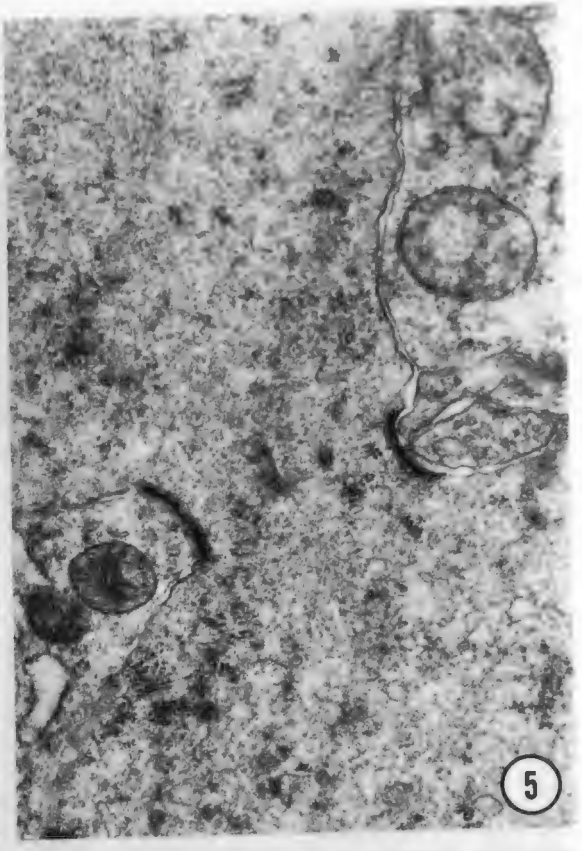
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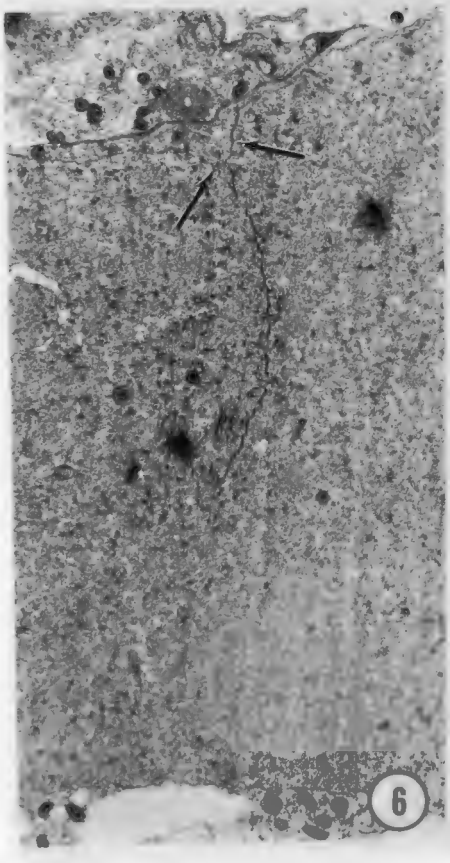
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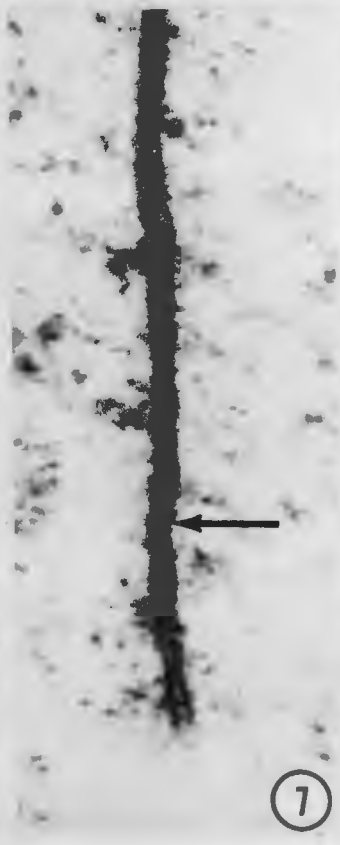
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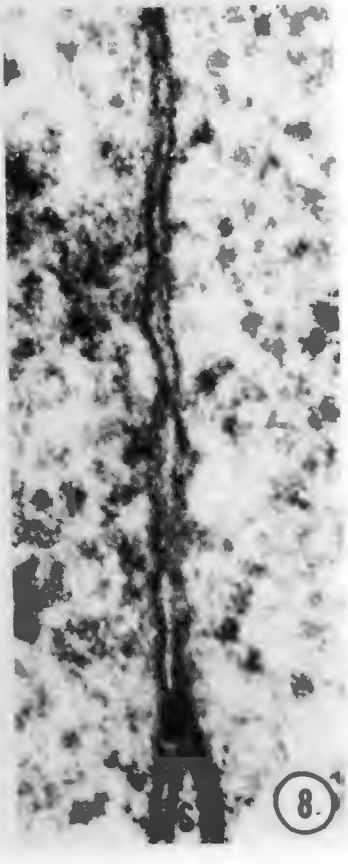
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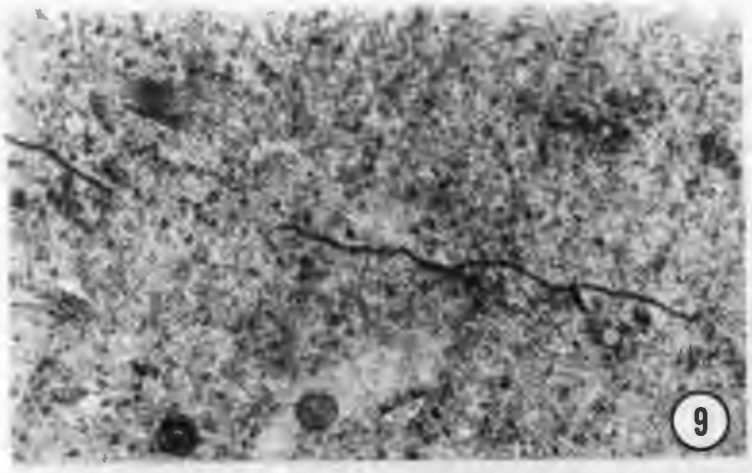
Fig. 9. Portion of a plasma membrane fragment inside conjoined mutant spermatids. Such fragments are often located near another portion of membrane which is continuous around the cell. x15,000.

Fig. 10. An isolated bit of plasma membranes within a multinucleate spermatid of ms(2)3R. Cross linkages (arrows) are discernable in several locations. x31,500.

Fig. 11. A more advanced stage of membrane fusion in mutant spermatids. The cytoplasm of the two cells is confluent. Remnants of plasmalemma remain in the fusion zone. The arrow indicates an area where bits of membrane from each cell appear to rest side by side. No fusion has occurred where supportive cell processes (S) intervene. x31,500.

Fig. 12. Fusion nearly completed along a portion of membrane between mutant secondary spermatocytes. Possibly one small membrane remnant (arrow) is still present. x31,500.

Fig. 13. Transverse section through an unattached ring canal inside a mutant spermatid. A bit of membrane (Mb) is attached to one side. x31,500.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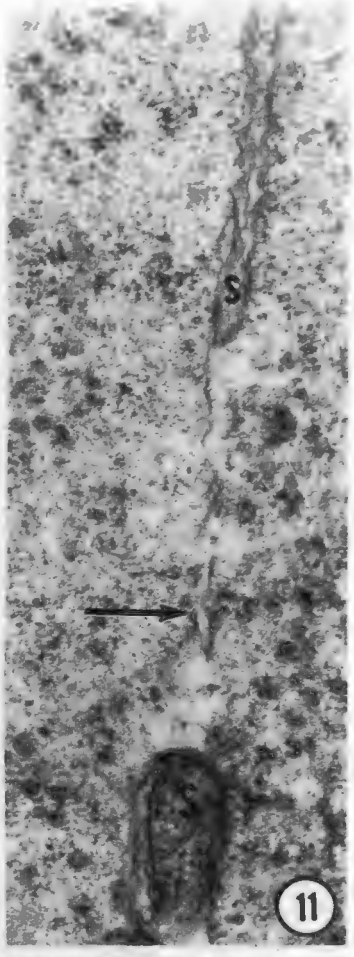
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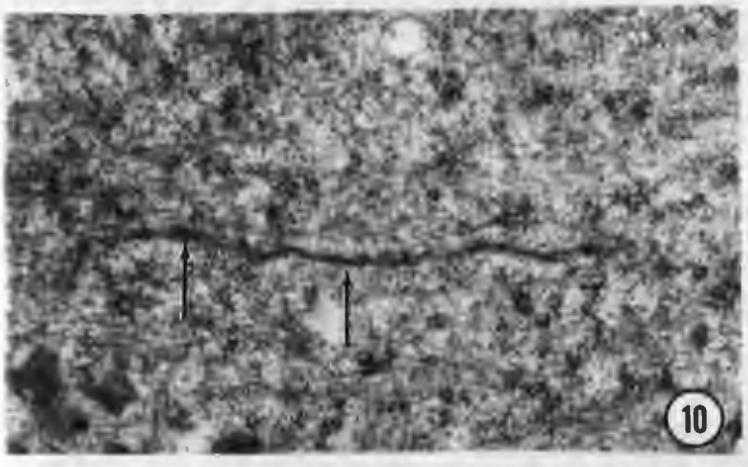
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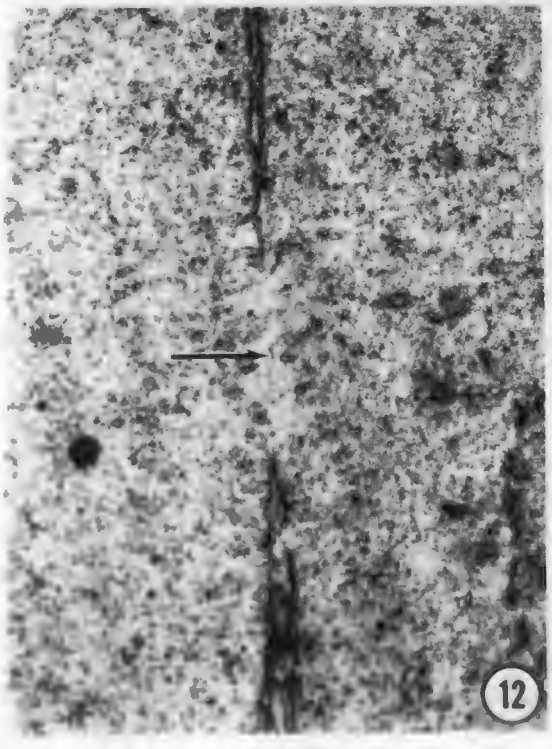
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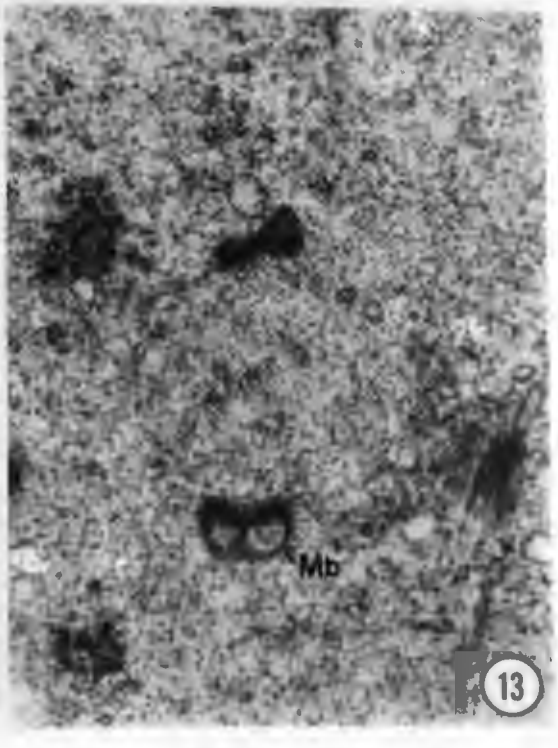
Mb
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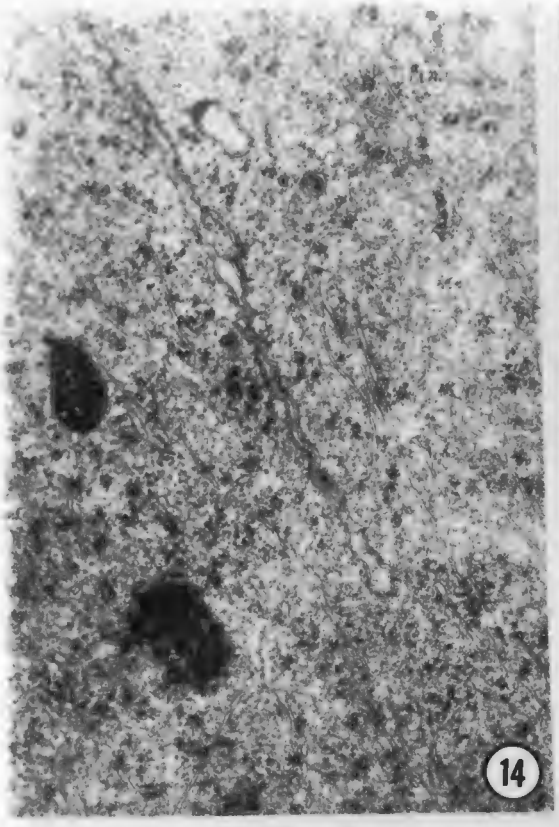
Fig. 14. Section through untied mutant spermatids. A supportive cell process extends for some distance between the cells. No evidence of fusion was seen where such processes intervened. Remnants of the fused membranes are no longer apparent. x21,500.

Fig. 15. Fused mutant spermatids. The closely adjacent nuclei (Nu) at the right suggest that fusion between two cells occurred earlier. Communication with a third cell has become quite extensive. Two axonemes (A) can be distinguished. x6400.

Fig. 16. Fused spermatids of ms(2)3R. The area between the two cells is completely free of membrane. After fusion is completed the newly joined cells appear to open so that individual cell boundaries cannot be distinguished. A ring canal (RC) has been sectioned transversely in the lower right.. An oblique section through another ring canal (arrow) is also evident. x5500.

Fig. 17. Fused mutant spermatids.. Two nebenkernen (N) are present. The lower one has a diameter which corresponds to twice the normal volume. The upper one has a normal diameter, possibly due to either the plane of section or late membrane fusion allowing a normal nebenkern to form. A slight indentation of the cell surface suggests that plasma membranes once separated the nebenkernen at this point. x7300.



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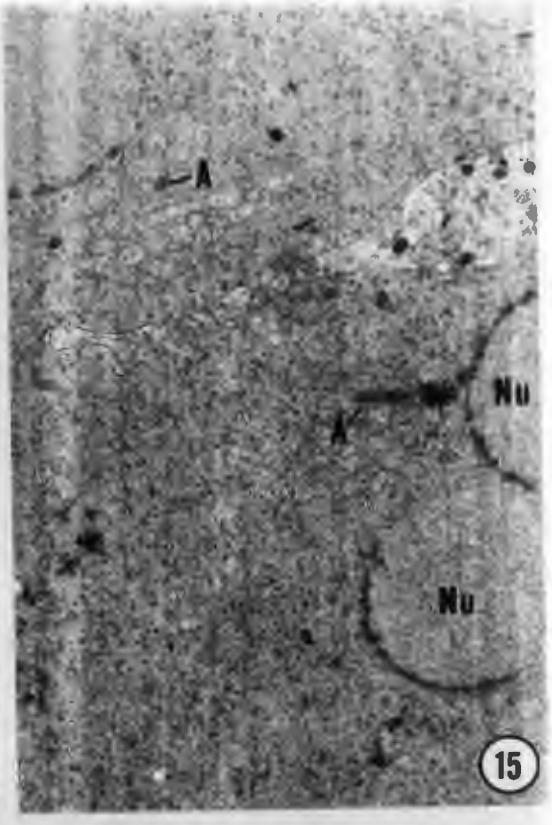

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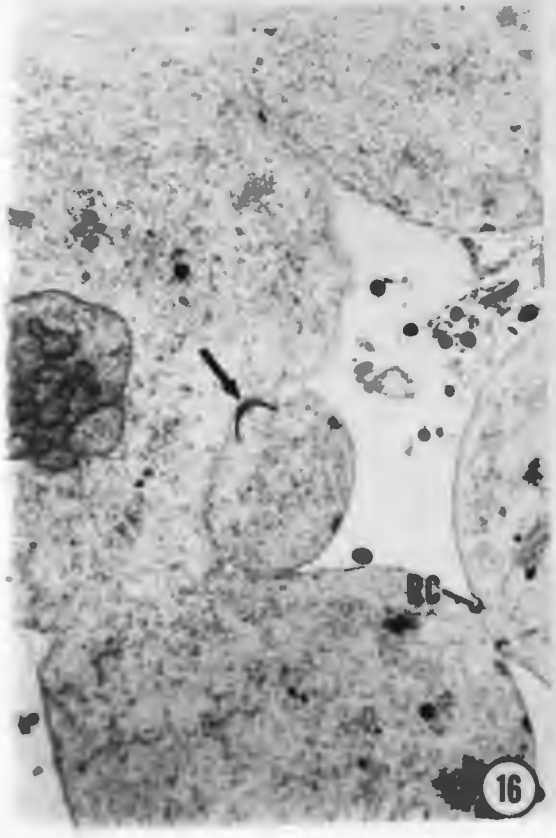

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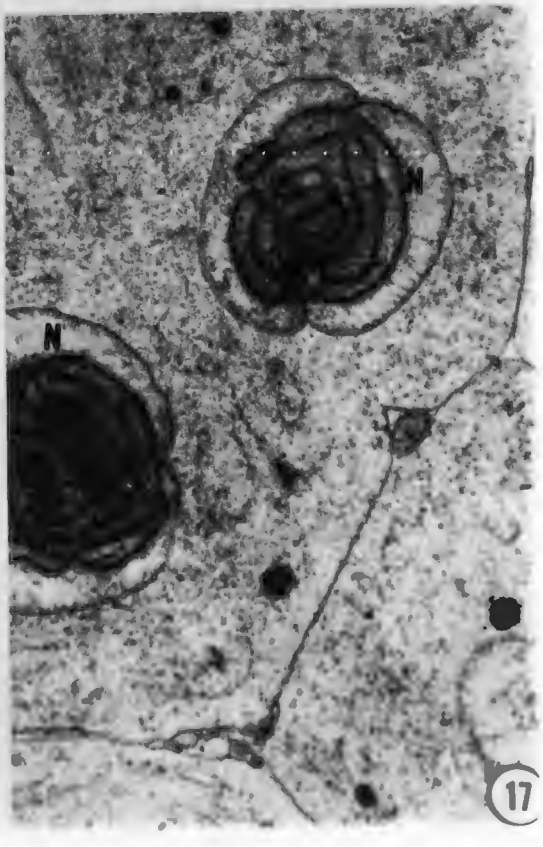

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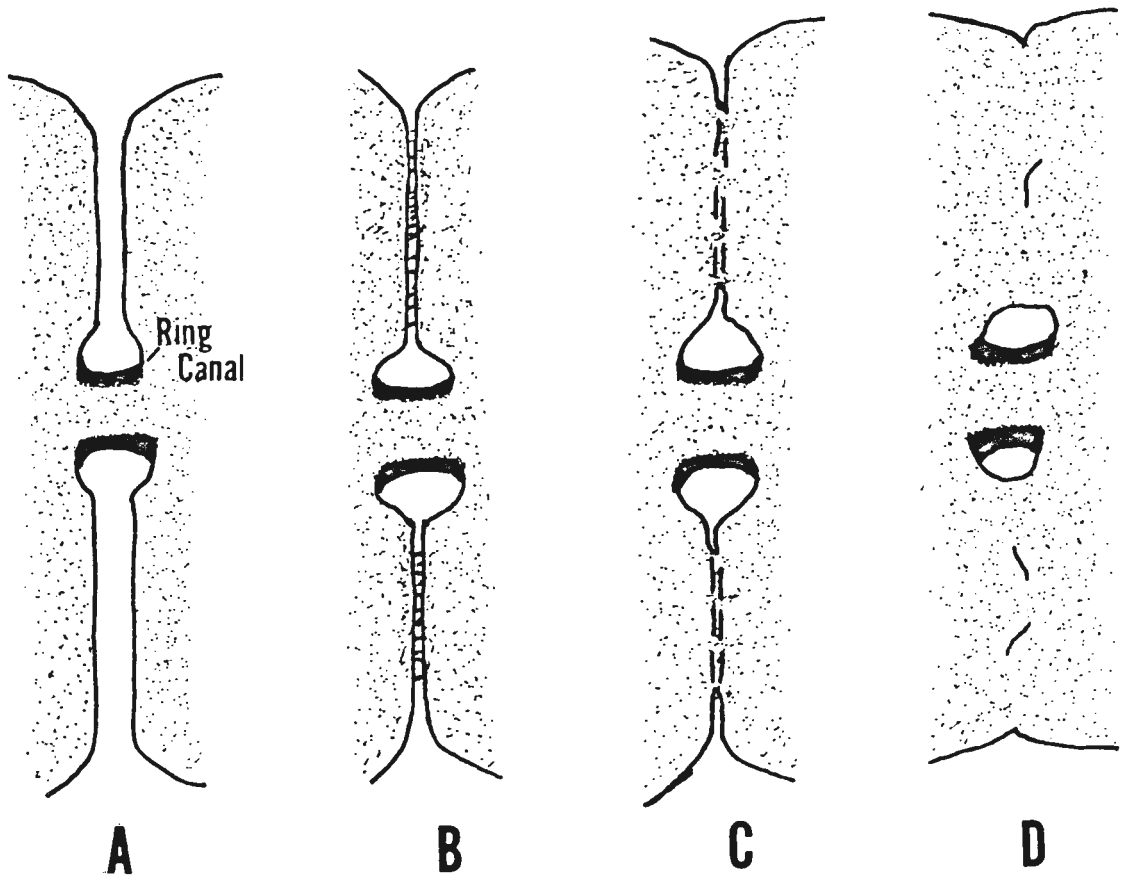

Fig. 18. Diagram of the proposed sequence of events of cell fusion. A. A meiotic division has been completed. Contractile ring elements remain in the form of a ring canal stabilizing an intercellular bridge. B. Membranes of sister cells have become closely apposed and cross linkages are evident between the membranes. C. Membranes have partially broken down allowing the sister cells to become confluent. D. Membrane elements have dispersed. An unattached ring canal remains in the cytoplasm of the fused cells.

DISCUSSION

In the previous study of mutant ms(2)3R of Drosophila melanogaster, Romrell et al. (19) reported that in spermatogenesis the meiotic cell divisions are abnormal. Mitotic divisions of each spermatogonium to form 16 primary spermatocytes appeared to proceed normally as did all somatic development. Romrell described the effects of the failure of meiotic cytokinesis on spermatid differentiation. The present study was undertaken to elucidate the morphological basis of the abnormal meiotic cytokineses by untrastructural examination of mutant testes..

The logical place to begin such a study was by investigations of the organelle whose function is uniquely essential to cytokinesis, the contractile ring.. There is general agreement that the contractile ring is the agent responsible for the mechanical act of cytokinesis in animal cells (20, 21, 23).. Recent studies (15, 22) have presented strong evidence indicating that the microfilaments of the contractile ring are composed of actin or an actin-like molecule. Cell constriction, therefore, may be achieved by mutual sliding between neighboring contractile ring filaments in a manner similar to the sliding filament model of muscle contraction.

An abnormal or absent contractile ring would result in abnormal cytokinesis or none at all. Although the nuclear elements might proceed through a regular sequence of division events, they would be enclosed within a single cytoplasmic mass.

The present investigations revealed that this is not the case. Cells in various stages of meiotic division were observed, and normal contractile rings were evident throughout telophase.. A critical comparison of contractile rings in wild-type and mutant germ cells showed no differences in structure..

Another way in which the four-nucleate spermatids might form is by fusion of cells following the failure of some mechanism after initial separation occurs.. The primary suspects were the ring canals which form intercellular bridges connecting all of the germ cells formed from a single spermatogonium. Being formed by stabilization of contractile ring elements at the end of telophase (3, 10), they are composed of an electron dense material, possibly a sort of cellular cement, in addition to the microfilaments of the contractile ring. If the ring canals are not stabilized after meiotic divisions, they might gradually open, producing multi-nucleate spermatids. This hypothesis was also proven to be incorrect when measurements revealed no significant differences between ring canal dimensions of wild-type and mutant spermatids.

Discovery of membrane fragments within spermatids, membranes extending from the outer surface and ending abruptly within spermatids, and occasional unattached ring canals inside the cytoplasm of mutant spermatids suggested a third alternative, membrane fusion and breakdown. Areas were also found where membranes were evidenced only by a few small remnants along the former path of the cleavage furrow.

Finding membrane fragments separated from each other by short distances in the cytoplasm strengthened the argument that the multi-nucleate condition did not arise from a ring canal anomaly. Instead of a single area between spermatids being devoid of membrane, as would be expected if a ring canal were opening, several locations between two cells lacked plasma membrane. This suggested that fusion was occurring in a number of places between plasma membranes along the cleavage furrows formed during meiosis.

Two factors support the postulate that fusion occurs along the plane of the meiotic cleavage furrow in ms(2)3R. First, Koch et al. (10) postulated that cleavage furrows always develop at right angles to the plane of the previous furrows. This accounted for their observation of branching chains of cystocytes formed during Drosophila oogenesis. In the present instance, where fusion was noted between mutant dividing secondary spermatocytes, the degenerating membrane was located in an area roughly normal to the plane of the oncoming cleavage furrow. Therefore, the fusion areas were situated in a plane which corresponded to that in which the previous cleavage furrow was presumably located.

Second, and more importantly, mutant spermatids never contained elements from more than four single cells. If membrane fusion were random, large cells containing elements of many, perhaps all 64, interconnected spermatids would be expected. This was not the case. Therefore, only products of the last two divisions, the meiotic divisions, fused.

Assuming that this reasoning is valid, it follows that there must be something unique about plasma membrane along meiotic cleavage furrows. To adequately cover the additional surface area resulting from the division of one cell into two, approximately 25% additional surface material is required, even if no growth occurs as is the case in dividing germ cells. The most widely accepted hypothesis for surface growth during division states that some of the new surface components are inserted exclusively into the walls of the cleavage furrow (1, 5, 6). This does not, however, exclude the concept that much of the surface area is increased by the smoothing out of pre-existing rough surface, e.g., during lengthening of cells in metaphase and anaphase. Thus, the cell surface along the cleavage furrow contains some newly inserted components not found elsewhere along the surface. An abnormality in one of these components would be evident solely along the cleavage furrow membrane.

It is generally accepted that developmental processes, including cytodifferentiation, require differential utilization of genetic information. Although it is not yet clearly evident how this is accomplished, Britten and Davidson (2) have proposed a model that includes the necessary elements to account for the observed phenomena. This model allows for the differential activation of genetic information by a number of different processes. For example, the same information can be evoked by different signals, or different genes can be activated by the same signals.

It is fairly clear from a number of investigations that during spermatogenesis, transcription of information occurs at a very high level during meiotic prophase and ceases prior to metaphase I. In Drosophila and Urechis, transcription is not resumed until after fertilization (11, 14), whereas in some other animals, low level transcription may be reinitiated during prophase II (locusts and grasshoppers (25)) or in developing spermatids (mouse (13)). In addition, the work of Hess and Meyer (9) on Drosophila hydei and Williamson (28) on D. melanogaster suggest the unique participation in spermatogenesis of Y-chromosome information transcribed in the primary spermatocyte. Therefore, the primary spermatocyte contains the large number of autonomously synthesized macromolecules that are necessary for cellular activity throughout meiosis, spermiogenesis, and fertilization. These macromolecules presumably include surface components i.e., membrane and cell coat components, which will be inserted during cytokinesis and spermatid elongation, or the appropriate messages for their synthesis.

In the case at hand a process common to both meiosis and mitosis, namely cytokinesis, is abnormal only in meiotic divisions. Since the cytokinetic process is presumably essentially the same in both meiosis and mitosis, the elementary assumption may be made that the same genetic information is utilized in both events. Although this study is not adequate to test the manner in which ms(2)3R must regulate the production of cell surface components, it is possible that in this mutant an inadequate level of a component is produced,

resulting in a variable instability of the membrane of the cleavage furrow due to large concentrations of new surface material. As noted by Romrell *et al.* (19), these mutant spermatids might reach a fair degree of elongation, since during elongation, as in cell growth, surface components are presumably inserted into the membrane at numerous locations. The effects of missing surface components in the mutant, therefore, would be masked in the case of elongation as the new material is not concentrated in a specific area of the membrane.

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