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An Electron Microscopic Examination of Spermiogenesis in the Recessive Sex-Linked Male-Sterile Mutant, ms(1)10S of Drosophila melanogaster

Lizabeth A. Perkins
Utah State University

AN ELECTRON MICROSCOPIC EXAMINATION OF SPERMIOGENESIS IN THE RECESSIVE SEX-LINKED MALE-STERILE MUTANT, ms(1)10S OF *Drosophila melanogaster*

by

Lizabeth A. Perkins

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Committee Member	Dean of Graduate Studies

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Lizabeth A. Perkins

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

An Electron Microscopic Examination of Spermiogenesis in the Recessive Sex-linked Male-sterile Mutant, ms(1)10S, of Drosophila melanogaster

by

Lizabeth A. Perkins, Master of Science

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Major Professor: Dr. Hugh P. Stanley
Department: Biology

Electron microscopy of the testes of mutant ms(1)10S of Drosophila melanogaster is characterized by three major defects in the tail region of the developing spermatid. One is the apparent lack of paracrystalline body formation. The second is the loss of the primary derivative as development proceeds, and the third is the breakup and incorporation of the plasma membrane and periaxonemal sheath into membranous whorls. The mutant shows different patterns of axonemal breakup depending on the stage of differentiation in which degeneration begins. Degeneration continues until only cellular debris, scattered axonemal profiles and membranous whorls are all that are seen in the basal end of the testis. Randomly distributed, longitudinally oriented microtubules are also observed in the cyst cells surrounding spermatid bundles. The analysis of this mutant has elucidated the importance of the primary derivative to the developing spermatid and the requirement for a paracrystalline

body for normal development. It further shows that the integrity of the axonemal components increases as spermatid development proceeds.

(38 pages)

INTRODUCTION

Most of the early research on spermiogenesis in Drosophila melanogaster involved the elucidation of the normal developmental sequence. Cooper (1950) provided the early light microscopic observations on spermiogenesis and Tates (1971) and Stanley et al. (1972) were the first to propose ordered staging of spermatid development with the use of electron microscopy.

Other investigators concentrated on elucidating specific processes, functions, and structures during insect spermiogenesis. Among these were studies of nebenkern formation in the Hemipteran insect, Murgantia histrionica (Pratt, 1968), the paracrystalline body in Drosophila (Meyer, 1964), and nuclear morphology in Drosophila (Shoup, 1967). Tokuyasu (1972a,b, 1974, 1975), again in Drosophila, has elucidated the processes of individualization and coiling, and detailed relationships between the nebenkern, mitochondrial derivatives and the axoneme.

Work with male-sterile mutants of D. melanogaster has provided information concerning the roles played by various organelles within spermiogenesis (Shoup, 1967: Hess and Meyer, 1968; Kiefer, 1970; Romrell et al., 1972a,b; Wilkinson et al., 1974, 1975; Laughran et al., 1976; and Habliston, 1977). Spermiogenesis in the male-sterile, sex-linked mutant ms(1)10S analyzed here is characterized by disruption of various spermatid organelles in the tail region of the developing spermatid. The study of this mutant has provided information concerning the importance of the primary derivative and the paracrystalline

body in the normal differentiation of the spermatid and further provides insight into the integrity of the axonemal components as spermatid development proceeds.

MATERIALS AND METHODS

The mutant ms(1)10S was isolated by B. S. Shadbolt in 1971 in our laboratory from the progeny of flies treated with ethyl methanesulfonate. It is a sex-linked male-sterile which is maintained in stock balanced with FM4 (Lindsey and Grell, 1968). This mutant has been mapped to map position ca. 36. No motile sperm were observed in freshly dissected testes, which resemble the wildtype (Canton-S) in both size and sperm bundle morphology. The males were not observed to mate so that sperm transfer could not be checked.

The flies were maintained at 25 ± 1°C on standard corn meal-sucrose-yeast-agar medium with propionic acid added as a mold inhibitor.

After light etherization the testes were dissected out and fixed in 2.5% glutaraldehyde for one hour. Fixation was followed by three 20-minute rinses in 0.2 M phosphate buffer at pH 7.4. Post-fixation was in 2% osmium tetroxide for one hour, and the testes were dehydrated in a graded alcohol series for 10-15 minutes for each concentration of alcohol. The testes were then embedded in Epon 812.

Thin sections, gold to silver-grey, were cut with a Dupont diamond knife using a Sorvall Porter-Blum II-B microtome. The sections were then stained with uranyl acetate and lead citrate and examined with a Zeiss EM-9S-2 electron microscope.

The references to normal sperm development and stages of differentiation are to those of Stanley et al. (1972). Primary and secondary mitochondrial derivatives were determined by the method of Tokuyasu (1974).

RESULTS

The testes from mutant ms(1)10S are similar to Canton-S in size and gross morphology. The testicular duct, seminal vesicle, accessory gland and anterior ejaculatory duct are also morphologically normal. No motile sperm were observed in the mutant testes after squash preparations were made in fresh Drosophila Ringer's solution. Phase microscopy has revealed normal bundle elongation within the testis. Squash preparations of the salivary gland chromosomes showed a normal chromosome complement and configuration.

Electron microscopic observations have revealed normal spermatid differentiation in developmental stages 1 through 3 with occasionally normal differentiation in spermatids as late as stage 6. The onset of the abnormalities, therefore, is relatively variable. No spermatids of later development than stage 7 were observed. The abnormalities were confined to stages 4, 5, 6, and 7.

In ms(1)10S stages 1, 2, and 3 the mitochondria aggregate and form a nebenkern normal in both size and ultrastructure. The nebenkern has been observed to differentiate and divide normally to form the two mitochondrial derivates, one on each side of the axoneme.

All nuclei observed were normal through stage 6. The latter nuclei were lanceolate in shape with a normal number of longitudinally oriented microtubules lying in the nuclear concavity. Around the nuclear periphery a layer of smooth endoplasmic reticulum parallels the convex portions of the nuclear envelope (Fig. 1). The abnormalities of this

mutant, it appears, are confined to the region of the elongating axoneme, and the nuclear region can be disregarded as a site of spermatid abnormalities.

The developing axoneme was used to determine the developmental stage of a particular spermatid according to the criteria of Stanley et al. (1972). Therefore, it was necessary to distinctly see and follow the development of the arms of the A tubules, the formation of the accessory loops and the final completion of the accessory tubules. No difficulties arose in the formation of the above structures, thus no problems were encountered in staging any of the spermatids. The stage designation given to a particular sperm tail profile only applies to that spermatid at the level of that particular section. Tokuyasu (1972a, 1975) and Stanley and Bowman (1974) have shown that there is a longitudinal "wave of development" along any one sperm bundle within the testis, i.e., development is more advanced at the nuclear region of the cell, becoming less advanced as one proceeds down the tail. Therefore, to specifically stage a spermatid one should look at the nuclear region. A stage number assigned to a random section level can only be said to indicate that the spermatid has reached at least that stage.

The determination of primary and secondary derivatives follows the criteria of Tokuyasu (1974) in which the primary derivative is that derivative which has its attachment point adjacent to doublets 5 and 6. The secondary derivative is attached to the periaxonemal sheath adjacent to doublets 8 and 9.

The earliest developmental abnormalities appear in stage 4. Of the five stage 4 tail profiles observed only one was normal. Of the

other four profiles, one axoneme had only one derivative; two of the axonemes had lost connections with one and both of the derivatives, respectively; and the fourth stage 4 tail profile completely lacked the axoneme and one of the derivates.

Spermatids of ms(1)10S can be characterized by their lack of paracrystalline material in the primary derivative. Normally a paracrystalline array would be noticeable in an early stage 6 spermatid, but this was not seen in any of the spermatids observed (Fig. 2). There is also a tendency for the spermatids to fold back upon themselves within the cysts. This can be clearly seen in Figure 3; in axoneme number 1 the arms of the A tubule are pointing clockwise indicating that one is looking toward the distal end of the axoneme, whereas in axoneme number 2 the arms of the A tubule are pointing counterclockwise indicating that one is looking toward the basal end of the axoneme.

Many of the tail profiles observed lacked primary derivatives entirely (Fig. 4). Eight percent of stage 5, 60% of stage 6, and 70% of stage 7 tail profiles lacked the primary derivative. These numbers were obtained by first identifying which derivative was present using the method of Tokuyasu (1974), and then scoring only those tail profiles where no ambiguities were present, i.e., axonemes which shared derivatives due to their proximity within the cytoplasmic mass and axonemes which were not in the plane of section so that identification of derivatives was hindered were eliminated.

The number of tail profiles which lacked the primary derivative was found to be dependent on the particular stage of the bundles

sampled. The value obtained from a 2 x 3 contingency Chi-square test of independence gave a χ^2 value of 14.62 which exceeds the tabular χ^2 (df = 2, χ = .001) value of 13.815. Clearly, there is progressive loss of primary derivatives as spermatid development proceeds.

The earliest sign of primary derivative defect is an observable loss of electron density in the matrix compartment (Fig. 5) and irregularity of derivative shape. The derivative then diminishes in size (Fig. 6) and is eventually lost. In normal spermatids the primary derivative increases in interior density during stage 6.

Cell degeneration in this mutant can begin in stages 5, 6, or 7 and is characterized by gross membrane reorganization into myelinated membrane formations. The spermatid plasma membranes seem to coalesce, creating areas of cytoplasmic masses where individualization has broken down. Frequently the periaxonemal sheath is incorporated into these same whorls (Fig. 7).

The pattern of axonemal breakup depends on the stage in which degeneration begins. If degeneration begins in stage 5 or early stage 6, the periaxonemal sheath begins to assume a wavey configuration and breaks appear in it. Axonemal components in the form of doublets and joined central tubules are scattered throughout the cytoplasm (Fig. 8). If degeneration begins in late stage 6 or early stage 7, the pattern of axonemal breakup is quite different. The periaxonemal sheath is lost from around the axoneme except at the point of attachment of the secondary derivative (Fig. 9). The A tubule becomes weak and eventually disappears (Figs. 9 and 10), and the axonemal subunits

begin to disperse in the surrounding cytoplasm. Eventually the secondary derivative is lost (Fig. 10).

Degeneration continues until only cellular debris, scattered axonemal profiles and membrane whorls can be seen in the basal end of the testis (Fig. 11).

Microtubules running longitudinally in the cyst cells surrounding a bundle have been observed (Fig. 1). These microtubules are randomly distributed around the bundle and do not seem to associate with other microtubules in any particular array or configuration.

DISCUSSION

In the mutant ms(1)10S, abnormalities are confined to the region of the elongating axoneme; therefore, it is likely that the primary result of the mutation directly involves some structure or biochemical process confined to the tail region of the spermatid. Three major defects in the tail region have been observed in this mutant. One is the apparent lack of paracrystalline body formation. The second is the loss of the primary derivative as development proceeds, and the third is the breakup and incorporation of the plasma membrane and periaxonemal sheath into membranous whorls. It is impossible to tell which of the abnormalities is primary and which are secondary. All three have profound effects on the developing spermatid.

A number of hypotheses to account for the observations listed above have been considered; the following three seeming to be the most likely. The primary defect in this mutant may be the failure of the periaxonemal sheath and the primary derivative to make proper contact. A second possibility is that the primary derivative is defective in a way which leads to the abnormal configurations observed. Finally, the normal sequence of events in spermiogenesis might be altered, disrupting normal development and leading to the defects listed above. It is impossible to choose between these hypotheses based on the data obtained. Each hypothesis will be discussed below.

In mutant ms(1)10S, the periaxonemal sheath and the primary derivative may fail to make proper contact. This could explain the lack of a paracrystalline body and the eventual loss of the primary

derivative with the large masses of membranous whorls being indicative of cytoplasmic degeneration. There are two hypotheses concerning the origin and the pathway of paracrystalline proteins to the primary derivative both of which require the formation of a particular membrane association assumed by the periaxonemal sheath and the inner and outer membranes of the mitochondrial derivative. Baccetti (1975, 1976) suggests that the paracrystalline proteins are synthesized in the cytoplasm and are pumped into the primary derivative from the cisterna of the periaxonemal sheath which he considers to be an extension of the Golgi body. Stanley (personal communication) suggests the paracrystalline proteins are synthesized in the matrix of the primary derivative and are added to the growing crystalline array from the inside of the derivative (see Appendix) with the site of deposition being determined by the specific membrane configuration.

The breakdown of the periaxonemal sheath in mutant ms(1)10S can occur at various stages of development, but by late stage 6 (the time the deposition of paracrystalline protein normally begins) no intact periaxonemal sheaths have been observed. Therefore, whichever hypothesis is preferred, the defective periaxonemal sheath has failed to establish a normal membrane association with the primary derivative and paracrystalline bodies are not expected to be formed. If the paracrystalline proteins are produced in the mitochondrial derivative and if the mutation inhibited or blocked protein synthesis within the mitochondrial derivative, then one would not expect to see severe spermatid abnormalities occurring until after the time the paracrystalline body begins forming. This is not the case in ms(1)10S and

therefore the mutant can not be a paracrystalline protein mutant. Clearly, more work is needed to further define the origin and pathway of these proteins to the site of deposition within the primary derivative and further, to characterize the connection between the primary and secondary derivatives to the periaxonemal sheath.

It is possible that in mutant ms(1)10S the primary derivative is defective. The mutant shows a progressive loss of primary derivatives as spermiogenesis continues. The question arises as to why only the primary derivative is lost even in stage 5 (8% loss) where there is no obvious morphological difference between derivatives. In ms(1)10S normal nebenkern formation and division have been observed; therefore, it is reasonable to assume that the primary derivative should proceed through spermiogenesis in a normal fashion, but since it is seen to first lose electron density in the matrix compartment, assume an irregular shape, diminish in size and eventually disappear, some factor must be acting specifically on the primary derivative and not the secondary derivative. This factor must be recognizing a difference between the primary and secondary derivatives which Baccetti (1975) believes is a particular configuration assumed by the primary derivative with the periaxonemal sheath. This, then, requires the formation of two types of connections; one between each of the mitochondrial derivatives and the periaxonemal sheath. Stanley (personal communication) believes the primary derivative is determined at the "clew" and "onion" nebenkern stages, where the nebenkern may represent a way to assemble and distribute the mitochondrial DNA selectively to the primary derivative in D. melanogaster. The primary derivative, by

this hypothesis, retains all of the mitochondrial DNA and thus would be the one derivative capable of extended transcription and translation of paracrystalline proteins.

The defects seen in mutant ms(1)10S could be due to an alteration in the normal sequence of events expected during spermiogenesis, i.e., some developmental processes may be occurring ahead of their normal timing. In normal spermiogenesis at stage 9 the two unit membranes of the periaxonemal sheath are observed to have coalesced and to have broken at points on either side of the sites of attachment of the mitochondrial derivatives. Both of these "normal" events occur in ms(1)10S but at a much earlier stage. The membranes of the periaxonemal sheath are seen to coalesce and break as early as stage 5 and no stage 7 axonemes were seen that had not assumed this configuration or had degenerated beyond this point (Figs. 6, 7, 9, 10).

Evident, also in normal spermiogenesis by stage 9 or 10 is a waste bag (the product of the individualization process) consisting partly of cytoplasm, excess membranes seen as membranous whorls, ribosomes and defective spermatids (Tokuyasu, 1972a,b). This waste bag continues to break down and is eventually resorbed by the epithelium of the testis. Membranous whorls, indicative of cytoplasmic degeneration, are seen at any developmental stage in ms(1)10S (Figs. 7, 8) and are present in varying degrees in all sperm bundles. Once this membrane degeneration begins it continues until axonemal profiles are seen in large cytoplasmic masses and it is also believed to be largely responsible for producing the large regions of cytoplasmic debris seen in the basal end of the testis.

ms(1)10S, then, may be a regulatory mutant which is characterized by the precocial development of the periaxonemal sheath and the early onset of the degeneration process which in this case begins before the waste bag is formed. The early senescence of the periaxonemal sheath could explain the observation that no paracrystalline proteins are deposited within the primary derivative. This is consistent with either Baccetti or Stanley's suggestions of protein deposition as the periaxonemal sheath would fail to make proper contact with either derivative.

The lack of the paracrystalline body in the mitochondrial derivatives of this mutant could explain the observation that spermatids fold back on themselves within the developing bundle. Meyer (1964) and Phillips (1974) suggest that the mitochondrial derivatives and their associated paracrystalline body add a "stiffness" or rigidity to the flagellum which may regulate the wave lengths of flagellar movement (see Appendix). If, indeed, this rigidity is missing due to the lack of the paracrystalline body, then the growing axoneme could assume an abnormal configuration and folding back is then possible.

The pattern of breakup of the axonemal elements seems to be dependent on the stage in which axonemal degeneration begins. It has been suggested that axonemal elements in early stages of spermiogenesis are "less rigidly and exactly held in precise 40° repetition patterns about the central axis than in later stages" (Stanley et al., 1972). The pattern of axonemal degeneration in ms(1)10S clearly demonstrates this idea. When degeneration begins in spermatid stages 5 and early stage 6, axonemal components in the form of doublets, single A tubules with their arms, B tubules with their forming accessory loop, and

central tubules are scattered throughout the cytoplasm. On the other hand, when degeneration begins in late stage 6 or stage 7, the first axonemal component to be lost is the A tubule whereas the remaining axoneme maintains its general shape and integrity. The axoneme in these latter stages only loses its shape after the periaxonemal sheath is lost. The B tubule with its adjacent accessory tubule and the intermediate electron dense material remain joined as one unit. The central tubules also appear to maintain their oreintation. This finding is in conflict with the work of Kiefer (1970) in which he proposes a sequential pattern of axoneme degeneration in the Y-mutant sperm of D. melanogaster. Clearly, one pattern of axoneme degeneration is not adequate to explain the breakdown of the axonemes in ms(1)10S, therefore, it is probable that various degeneration patterns exist depending on the stage of spermatid degeneration and differences between the primary mutations.

No clear data have been obtained on the factor(s) which are responsible for the achievement and maintenance of spermatid elongation. It has been shown with the use of colcemid and vinblastine that the mitochondrial derivatives alone can not mediate spermatid elongation (Wilkinson, 1974, 1975). Habliston (1977) has shown in mutant ms(1)6S that elongation continues in the absence of an axoneme with only caudal cytoplasmic microtubules being present. Whether the caudal cytoplasmic microtubules in the absence of the axoneme can achieve normal elongation, ms(1)6S has failed to prove. The electron microscopy of ms(1)10S has revealed the presence of longitudinally oriented microtubules in

the cytoplasm of the cyst cells surrounding the spermatid bundles (Fig. 1). Tokuyasu (1972b) also reported such a finding. Since microtubules are intimately associated with spermatid shaping (Dustin, 1978) it is reasonable to propose that these longitudinally oriented microtubules are also involved in the normal elongation of spermatids of D. melanogaster. This role is at least a permissive role, facilitating the elongation of the cyst cell as the spermatid bundle elongates.

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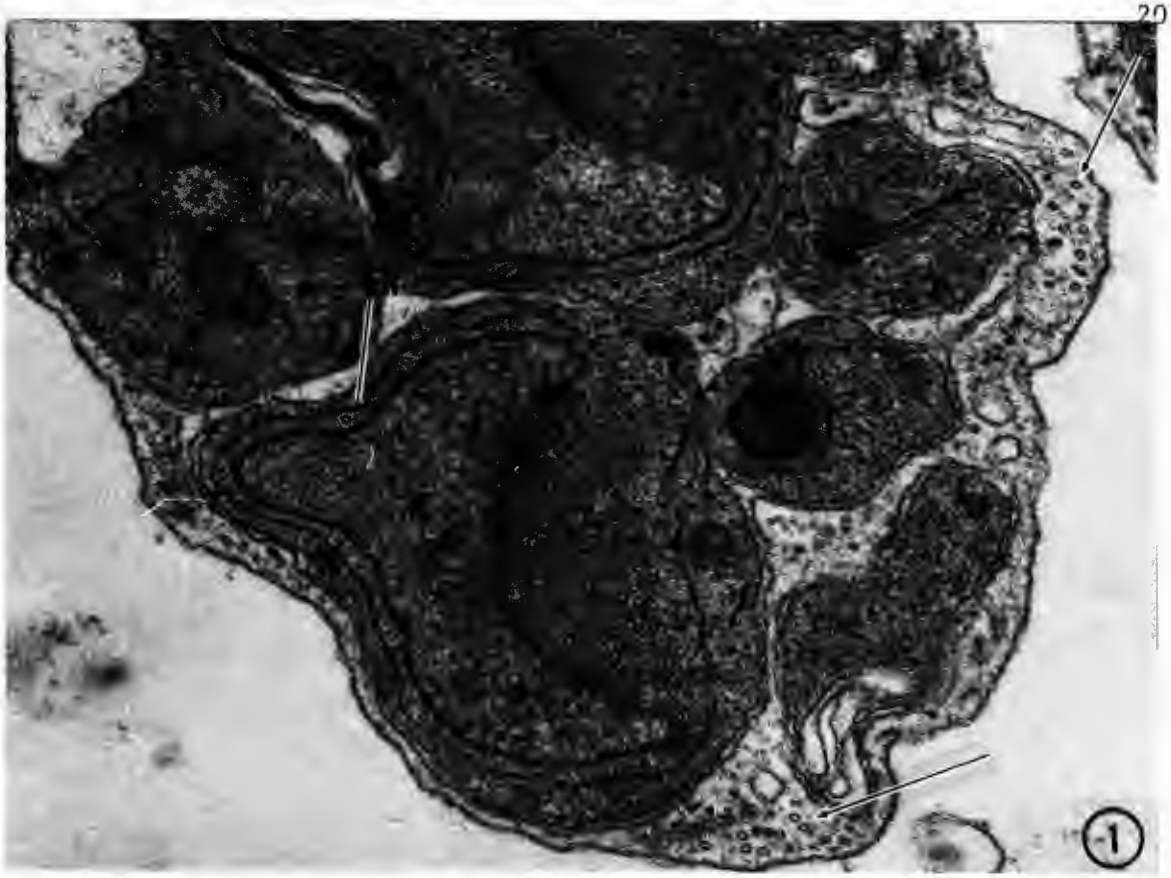
Fig. 1. A section through a stage 6 nucleus (N) showing normal nuclear development. A layer of smooth endoplasmic reticulum (ER) parallels the nuclear envelope and a normal number of longitudinally oriented microtubules are seen in the nuclear concavity. Note the microtubules (arrows) in the cytoplasm of the cyst cell. X 52,800.

Fig. 2. A stage 7 tail profile lacking a paracrystalline body in the primary derivative (MM). X 144,000.

Fig. 3. Axonemal profiles which show the spermatids have folded back upon themselves. In axoneme number 1 the arms of the A tubule are pointing clockwise indicating that one is looking toward the distal end of the axoneme, whereas in axoneme number 2 the arms of the A tubule are pointing counterclockwise indicating that one is looking toward the basal end of the axoneme. X 58,200.



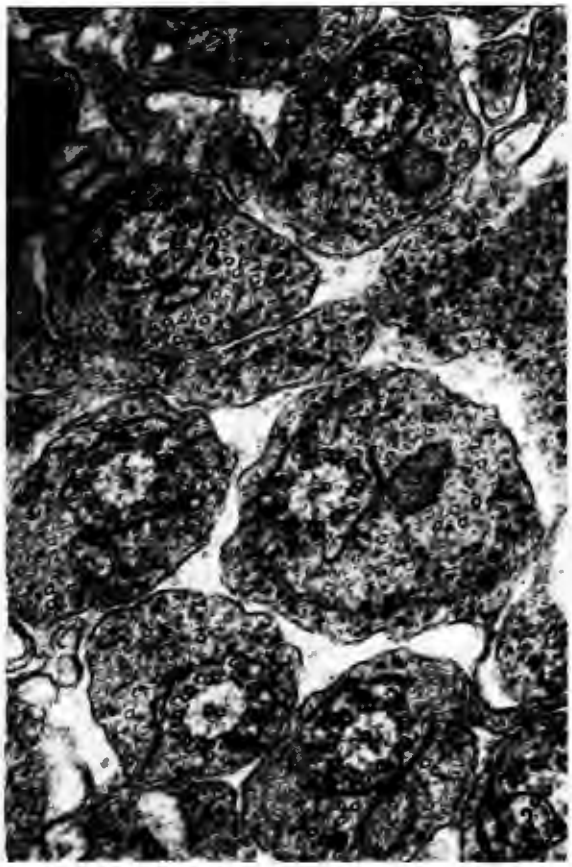


Fig. 4. Percentage of spermatid tail profiles which possess both a primary (MM) and secondary (mM) derivative, only a primary derivative, or only a secondary derivative in spermatid stages 5, 6 and 7.

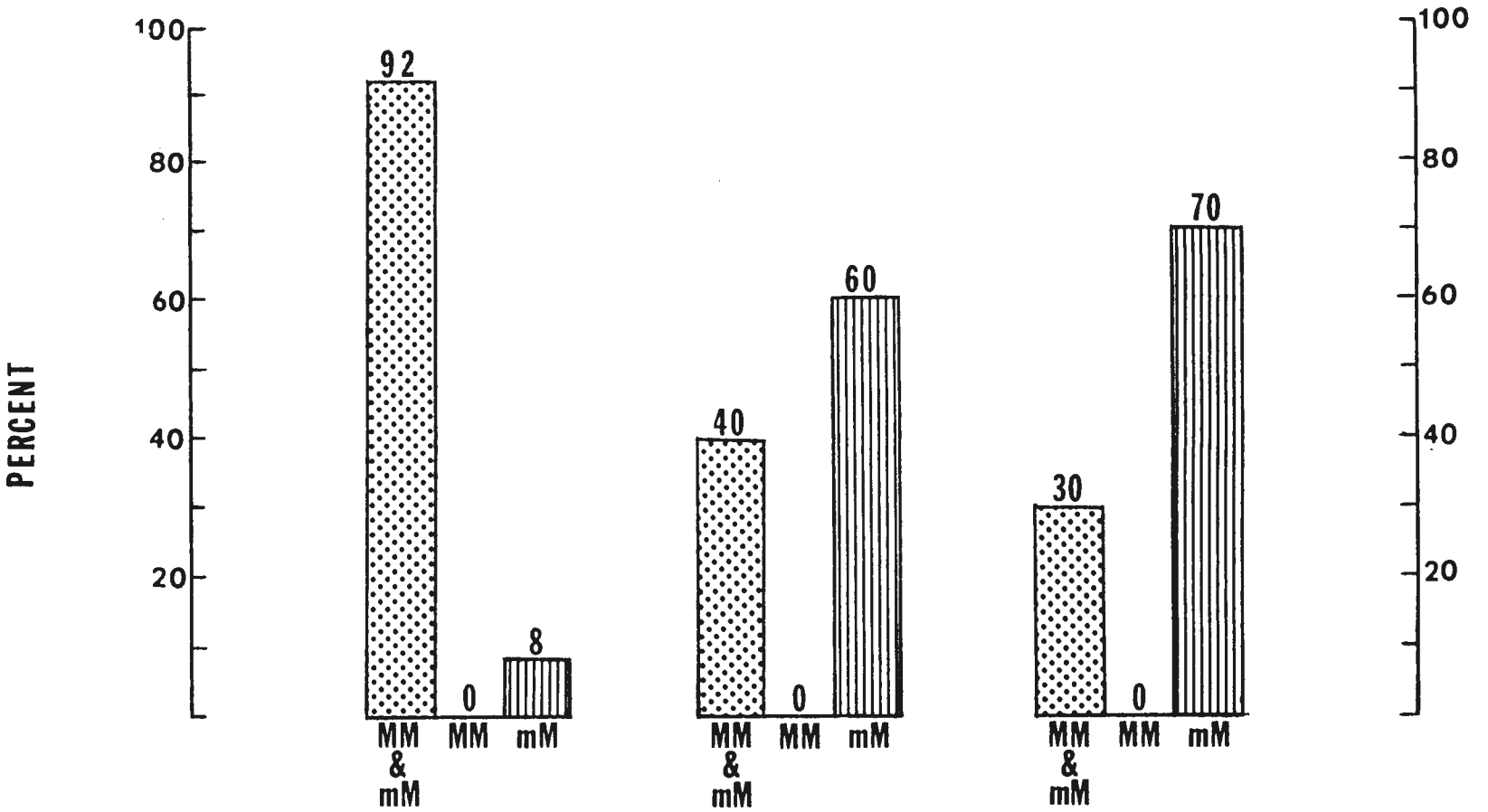

PERCENT
100
80
60
40
20
92
0
8
40
0
60
30
0
70
MM & mM
MM
mM
Stage 5
Stage 6
Stage 7

Fig. 5. A section showing an observable loss of electron density in the primary derivative (MM). X 93,400.

Fig. 6. A section demonstrating the further degeneration of the primary derivative (arrows). Notice that the primary derivatives are diminishing in size in addition to a loss of electron density. The periaxonemal sheaths (PS) are breaking up and a membranous whorl (MW) is developing. X 55,200.

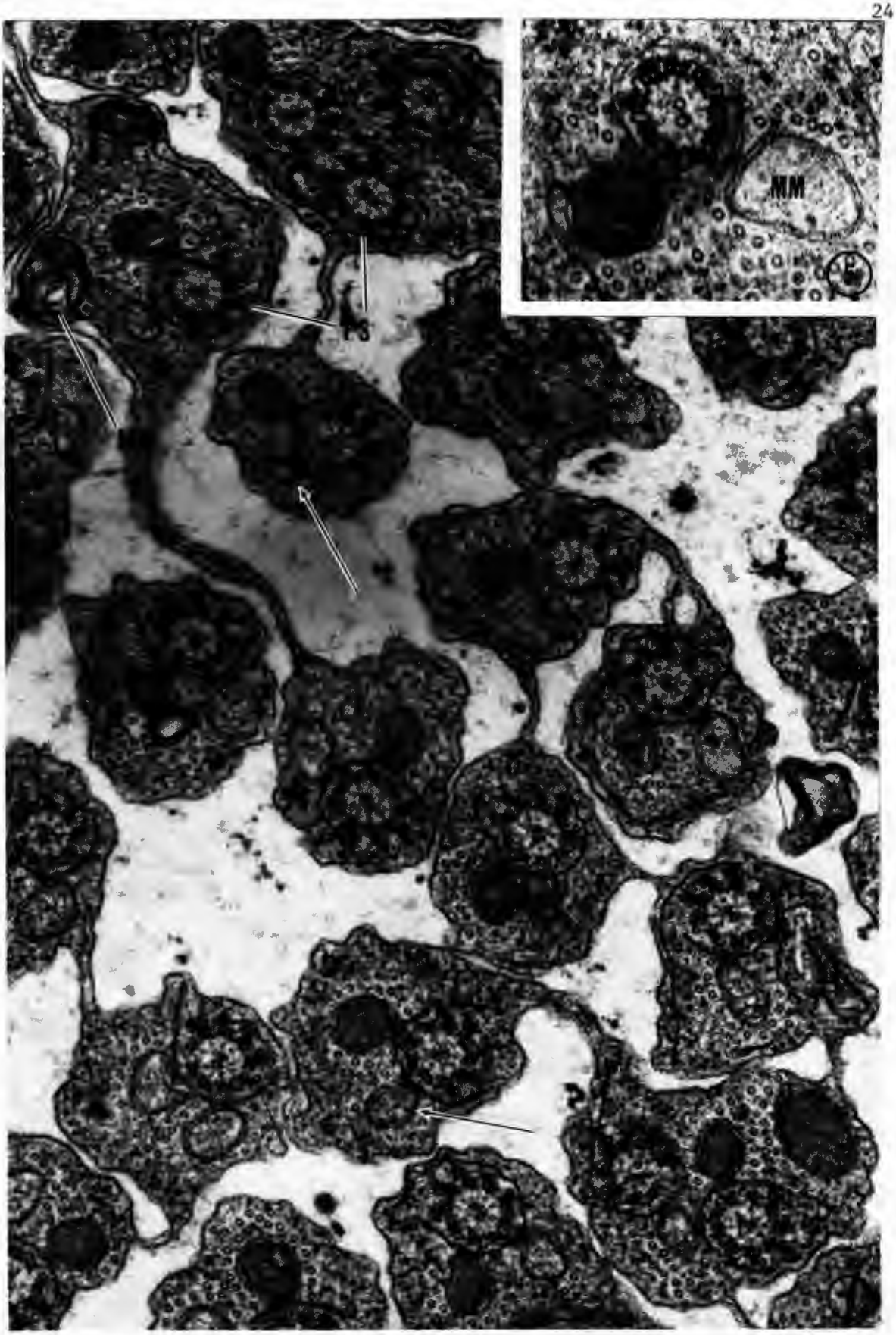
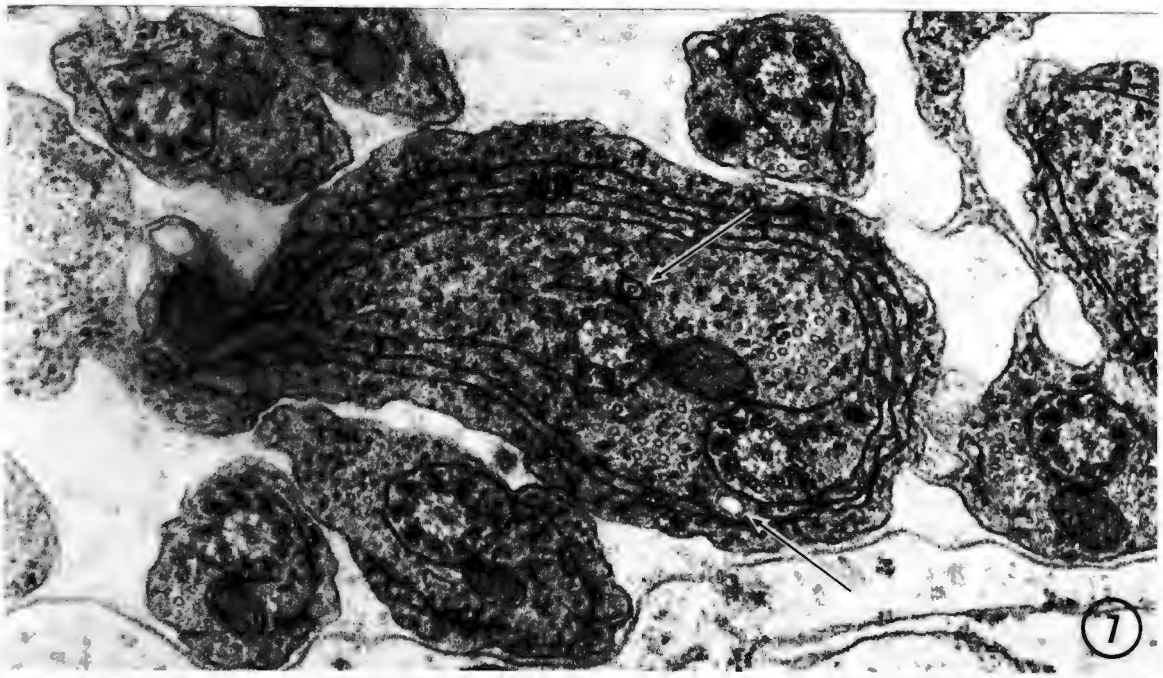

MM
PS

Fig. 7. A portion of a stage 6 spermatid bundle showing the incorporation of the periaxonemal sheaths into a developing membranous whorl (MW). Notice the size and density of the primary derivatives (arrows). X 58,800.

Fig. 8. A section which shows axonemal degeneration in stage 6. Notice the doublets and joined central tubules in the cytoplasm (arrows). Membranous whorl (MW). X 95,200.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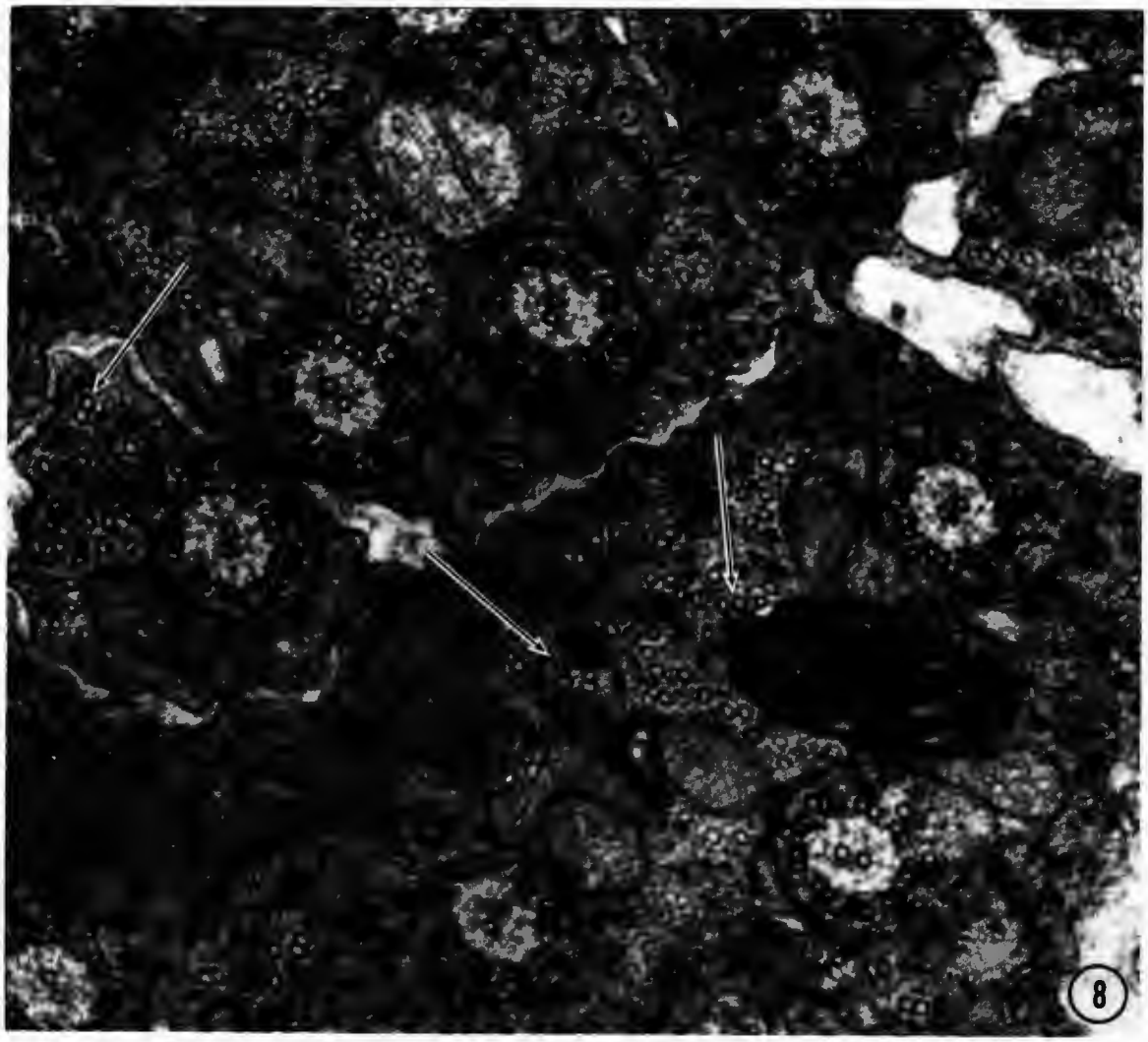
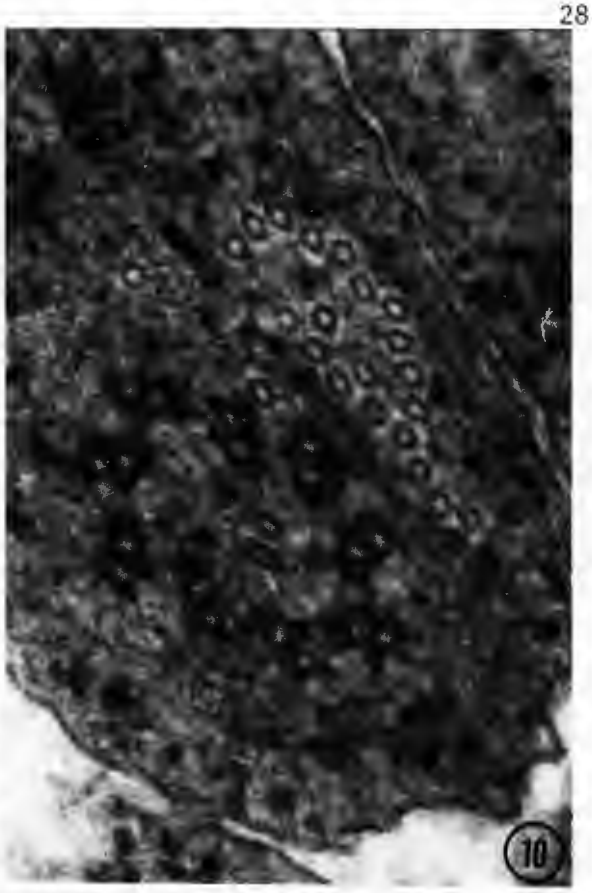
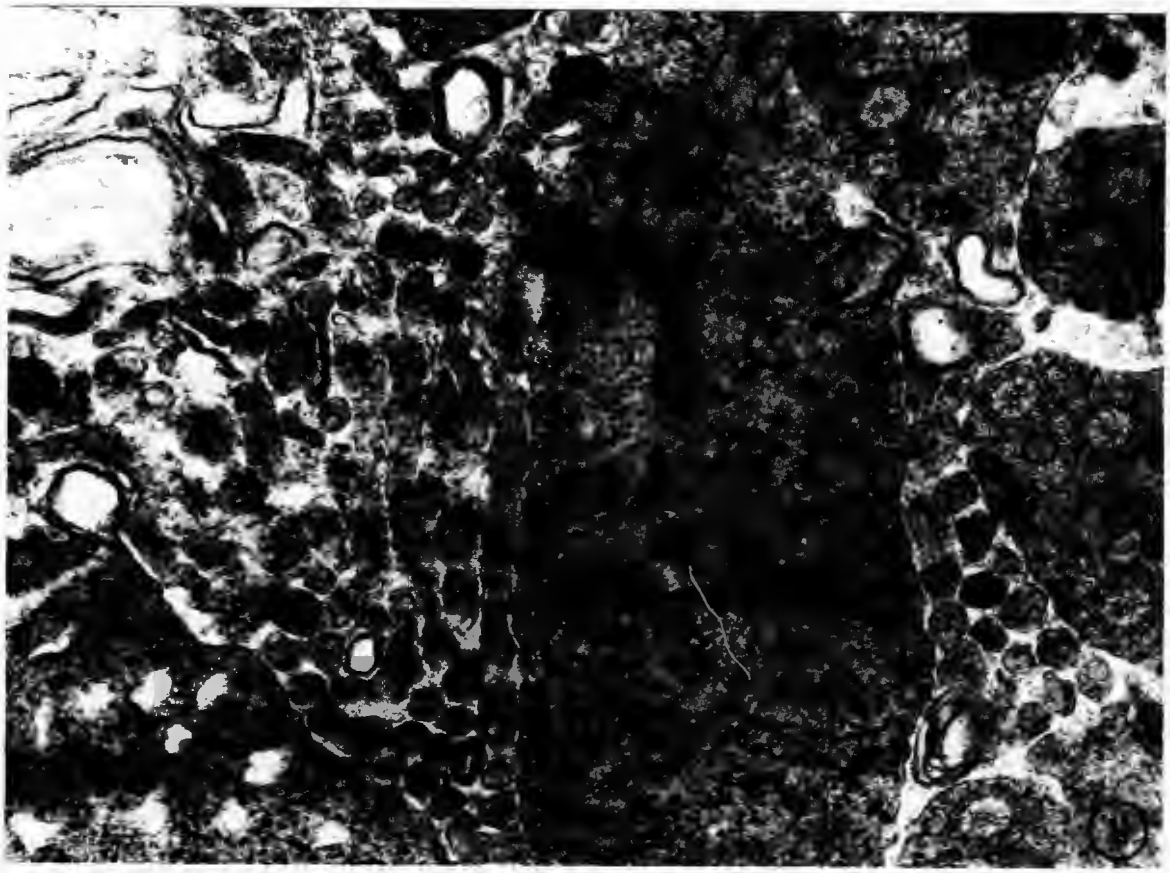

8

Fig. 9. A section through a stage 7 spermatid showing the loss of the periaxonemal sheath (PS). The A tubules are becoming weak. X 137,300.

Fig. 10. A section through a stage 7 spermatid showing the loss of the A tubule and the dispersion of the axonemal subunits into the surrounding cytoplasm. Both derivatives have degenerated. X 151,300.

Fig. 11. A section taken from the basal end of the testis showing cellular debris, scattered axonemal profiles and membranous whorls. X 50,500.





APPENDIX

POSSIBLE FUNCTIONS OF THE MITOCHONDRIAL DERIVATIVES IN *Drosophila* SPERMATOZOA

The readily observable mitochondrial derivatives of *Drosophila melanogaster* have been given considerable attention in the past, yet even the most recent literature has failed to elucidate a functional significance of these structures. As early as 1964 Meyer proposed a mechanical role for the mitochondrial derivatives. He further described the ultrastructure of the paracrystalline material in three *Drosophila* species. Since then other theories of the function of these bodies have been presented but none have been given substantial support. It is the purpose of this review to present the current thoughts on the function of mitochondrial derivatives in general, but more specifically, the function of these structures in the spermatozoon of *Drosophila melanogaster*.

The paracrystalline body in the mitochondrial derivative may represent more than 50% of the sperm volume (this is true of *Drosophila*) (Perotti, 1973) and in the Pulmonate Gastropods the body may occupy up to 95% of the sperm volume (Favard and Andre, 1970). In extreme cases the length of these mitochondria may reach several millimeters (Afzelius *et al.*, 1976). Those sperm types with large crystal filled mitochondrial derivatives, including the insects *Drosophila* and *Notonecta*, and the frog *Discoglossus* are slow moving or sluggish compared to the spermatozoa that must swim great distances to the egg. In the above mentioned species the sperm are longer than the distance

they have to travel to reach the micropyle of the egg (Baccetti and Afzelius, 1976).

In 1968, Pratt demonstrated beautifully the formation of the nebenkern in early spermatids of the insect Murgantia histrionica. Later the nebenkern divides into two halves, each half representing one mitochondrial derivative which aligns itself along the flagellum opposite the second mitochondrial derivative. These derivatives elongate along with the growing flagellum. A protein material is deposited in paracrystalline array in only one of the derivatives of Drosophila melanogaster and this defines the primary derivative while the other derivative (lacking paracrystalline material) is referred to as the secondary derivative.

Baccetti et al. (1977) have reported that the paracrystalline material found in the derivatives of insect spermatozoa contains two main polypeptides of 52,000 and 55,000 daltons molecular weight which they term "crystallomitin". These crystals, arranged as filaments, show a characteristic longitudinal periodicity of 45 nm with each period being divided into two subperiods of about 20 nm each. The two polypeptides contain a high percentage of proline and in many ways are closely related. The filaments contain a large number of disulfide cross-links which make the polypeptides insoluble in sodium dodecyl sulfate.

The manner of deposition of the crystals is unresolved at this time. Baccetti (1975) describes the periaxonemal sheath as a

"noncommittal description" of the smooth cytoplasmic membranes around the axoneme. He further states that the derivation of the periaxonemal sheath as from the Golgi complex and crystalline material begins to appear at the time the "flattened Golgi-derived cisternae [the axononemal sheath] come into contact with the mitochondrial wall". He describes the presence of an electron-opaque material within the cisterna of the periaxonemal sheath and in the space between the periaxonemal sheath and the mitochondrial membranes. This material, he believes, is the protein which makes up the paracrystalline material. Bacetti then pictures these proteins being added to the growing crystalline array from the point of contact between the periaxonemal sheath and the membranes of the mitochondrial derivative. The machinery that is responsible for the production of these proteins is present in the cytoplasm; i.e., the genes coding for these proteins are located in the nucleus, the corresponding mRNA's transcribed from these genes are transferred to the cytoplasm where cytoplasmic ribosomes and tRNA's carry out the process of protein synthesis. The proteins thus produced are transferred into the mitochondrial derivative.

Personal communication with H. P. Stanley reveals a second theory of crystalline protein deposition. Stanley pictures the proteins being added to the growing crystalline array from the inside of the mitochondrial derivative. The mRNA corresponding to these proteins is transcribed from the mitochondrial DNA and the translation of the mRNA being accomplished by the mitochondrial ribosomes and tRNA's.

The first proteins synthesized are deposited at the point of contact of the mitochondrial derivative with the periaxonemal sheath. With the use of ^{3}H-uridine incorporated into nuclear and mitochondrial RNA labeled up to the beginning of chromatin condensation and electron microscope autoradiography during spermiogenesis in Drosophila it has been shown that the nebenkern is the most radioactive cell organelle. "During chromatin condensation, nuclear RNA synthesis ceases, but mitochondrial derivatives continue to be significantly labeled up to their complete paracrystalline transformation" (Curgy and Anderson, 1972). Therefore, there must be RNA synthesis by mitochondria at the end of spermiogenesis.

The possible functions of the mitochondrial derivatives in Drosophila can be broken down into six categories. One proposed function is that the derivatives serve a mechanical role for the spermatozoon. This theory was first proposed by Meyer in 1964 and later by Phillips in 1974. Both papers state that the derivatives add a "stiffness" to the flagellum which Phillips explains as regulating the wave lengths of flagellar movement. Baccetti et al. (1977) suggest that the presence of disulfide bonds may give the paracrystalline material an elastic role similar to that of the paregrins in the mammalian and mollusk sperm.

The second proposed function is that the paracrystalline material consists of respiratory enzymes that have been reorganized for maximal respiratory efficiency. This hypothesis was proposed because of the high degree of morphological simularity between

the mitochondrial derivatives and paracrystalline material in both pulmonate gastropod and Drosophila spermatozoa. In the gastropods all of the enzymes of the respiratory chain are present and active (Anderson and Personne, 1970; Perotti, 1973). Cytochrome C oxidase activity has been shown to be completely absent from the paracrystalline material of Drosophila (Bigliardi, 1970). Only in the restricted area of the derivative where the cristae still exist is there cytochrome C oxidase and succinic dehydrogenase activity (Perotti, 1973). This indicates that the paracrystalline material is not a means of enhancing oxidative phosphorylation in Drosophila, in fact it appears that the paracrystalline body is metabolically inert (Baccetti et al., 1977). This is not true in gastropods.

The third hypothesis of paracrystalline body function is that this structure serves as a storage depot for intracellular reserves. The derivative can be disregarded as a nutritive source for the developing spermatozoon or as an energy source during sperm transfer and progression through the female reproductive tract, during storage in the female, or at the time of fertilization. Perotti (1973, 1975) has shown that the paracrystalline body remains unmodified through the active lifespan of the spermatozoon until fertilization has taken place at which time the entire spermatozoon enters the egg. After sperm entry roundish or elongated bodies, the multivesicular bodies, surround and enclose in various places the sperm tail which by this time has separated from the sperm nuclear region. Only after the sperm has been enclosed in these bodies do the first

modifications of the crystalline structure occur. The sperm membrane becomes discontinuous, the derivative separates from the axoneme, the derivative is split into fragments and the paracrystalline subunits are substituted by an amorphous or fibrous material. These fragments break down further and can be followed in the egg anterior pole until the onset of cellular blastema.

The paracrystalline material may represent a metabolically inert substance comparable to the yolk platelets in oocytes (Afzelius, 1970). This substance after copulation could provide the female with a protein-rich nutritive source both from the material which enters the egg at fertilization and the material which can be broken down and absorbed in the female's reproductive tract.

The paracrystalline array could play a role in egg activation at fertilization. This seems unlikely since the finding that the derivative is not altered until the male and female pronuclei have fused (Perotti, 1973).

The evidence that the derivative undergoes structural modifications only after fertilization and it is broken down and distributed prior to the onset of the cellular stage (Perotti, 1975) plus the fact that the derivatives contain nucleic acids (Curgy and Anderson, 1972) suggests still another possible functional role for the derivative. The derivative may be a device for the packaging, transfer and distribution of a huge amount of paternal material, namely proteins and nucleic acids which could be implicated in cytoplasmic inheritance and recombination of mitochondrial DNA, respectively.

Finally the mitochondrial derivative may serve as a mechanism to achieve reproductive isolation for a species. Phillips (1970) has said, "The mitochondrial derivatives assume precise species-specific shapes, so that in transverse section they present highly characteristic and sometimes bizarre profiles". The derivatives regulate the wave lengths of flagellar movement (Phillips, 1974) and establishes a particular swimming pattern for that species (Tokoyasu, 1974). In females sperm are stored in the ventral receptacle and in a pair of mushroom-shaped spermathecae. Sperm coming from the ventral receptacle are believed to be directly responsible for fertilization. The lumen of the proximal portion of the ventral receptacle is rather narrow which suggests that sperm with abnormal swimming patterns may fail to leave or enter the storage organs. The length and structure of the sperm tail may be species-specific which would be an important regulator in sperm compatability with the female reproductive tract. It is then possible that the evolution of highly different mitochondrial derivatives is a mechanism to achieve reproductive isolation of numerous insect species.

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