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An ultrastructural study of spermiogenesis in the normal (Rock) and male producing strains of the mosquito *Aedes aegypti*.

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AN ULTRASTRUCTURAL STUDY OF SPERMIOGENESIS
IN THE NORMAL (ROCK) AND MALE PRODUCING
STRAINS OF THE MOSQUITO AEDES AEGYPTI.

A Thesis

Presented to the School of Graduate Studies
of

The University of Windsor

through the Department of Biology

by

Keith D. Hutcheson

In partial fulfillment of requirements for the
degree of Master of Science

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ABSTRACT

The ultrastructure of spermiogenesis is described in the normal (Rock) strain and the male producing strain of the mosquito Aedes aegypti. The changes in the differentiation of the germ cells into the spermatozoon are discussed. Formation of the axonemal complex, nebenkern and mitochondrial derivatives are described, together with the accompanying changes of the nucleus. A unique mechanism is observed for the elimination of superfluous cytoplasm through the formation of membranes which package it into concentric rings which are later sloughed from the spermatid. A possible mechanism for the formation of the centriole adjunct from the Golgi complex is suggested. The male producing strain is characterized by the occurrence of a "jumbled" area at the lower end of the testes in which normal sperm lie together with abnormal bundles of sperm tails which fail to separate. Pieces of free axonemal complexes, cellular debris, together with large cytoplasmic bodies containing portions of sperm tails indicate phagocytic activity. It is postulated that these abnormal bundles are composed mainly of the female determining sperm which result in the distorted sex ratio characteristic of the male producing strain.

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INTRODUCTION

The sequence of events taking place in the formation and maturation of the spermatozoa within insect testes presents a fascinating problem in cellular differentiation; a problem that has attracted much attention in the past few years by workers using the methods of classical cytology, light microscopy and electron microscopy.

A typical pattern of spermatozoa development is presented as follows: the germ cells within the testes are generally arranged in a sequence that reflects the temporal changes involved in spermatogenesis. Thus, at the apex of the testes lie the undifferentiated germ cells or spermatogonia, followed by a zone in which the products of spermatogonial mitotic division, the primary spermatocytes, become grouped into cysts. Each cyst contains the daughter cells derived from a single primordial germ, enclosed in a follicle or sheath of somatic cells. Posterior to these, arising by the first and second meiotic divisions are the secondary spermatocytes and the spermatids. The last phase of spermatogenesis is the development of the spermatid into the functional spermatozoa or sperm through the process of spermiogenesis or spermateleosis. During spermiogenesis, the process which occurs in the lower end of the testes, the spermatid develops a tail, the chromatin or nuclear

material becomes compressed, the nucleus elongates, parts of the mature spermatozoa develop from organelles of the spermatid and excess cytoplasm is discarded.

Studies with the electron microscope have contributed much to our understanding of spermiogenesis. For example, the Golgi complex and its role in egg penetration at fertilization (Kay, 1962); the formation of the motile apparatus in association with a centriole (Gall, 1961); and the role mitochondria play in formation of the nebenkern and its subsequent division into twin organelles which associate with the flagellum (Pratt, 1968); and the complex nuclear changes which occur as the gamete differentiates and matures into the mature sperm (Kaye and Kaye, 1966). At the same time however, the greatly enhanced resolution and magnification has revealed that not only are the cellular changes leading to maturation even more complex than was earlier suspected, but the interspecific variation in detail and plan even between closely related species is wide and not completely understood.

While Nath (1965) set up certain criteria for development of sperm in general, by no means do all sperm follow this typical pattern. Insect spermatozoa particularly show considerable variation. Selective studies of insect spermiogenesis in a number of insect orders have been carried out by Kessel (1960), Breland (1970), Lindsey (1970) and Phillips (1970, 1971). The events of sperm

development in mosquitoes have received little attention. Mescher and Rai, (1966) completed a study at the light microscopic level of events involved in normal meiosis during spermatogenesis in Aedes aegypti Rock strain. Ultrastructural studies have been even more limited. Nunez (1963), and Breland (1966, 1968) did preliminary electron microscopic studies of mosquito sperm. Breland's work, however, was a general study of insect spermiogenesis including several orders of insects as well as several species of mosquitoes.

Two objectives are proposed in this study. The first is to study or describe the events of spermiogenesis at the ultrastructural level in the Rock (normal) strain of the mosquito Aedes aegypti, a laboratory reared strain which has been maintained for over 20 years at the Rockefeller Virus Foundation and since 1959 at Notre Dame University, Southbend, Indiana (Hickey and Craig, 1966). This strain consistently produces progeny in a ratio of fifty per cent male to fifty per cent female.

The second objective is to compare and contrast the events of spermiogenesis as they occur in the normal strain to the events occurring in the male producing strain of the mosquito, Aedes aegypti. The male producing strain was synthesized from matings of the Red and Wart strains of Aedes aegypti. These strains have been maintained as mass breeding colonies for several years at Notre Dame University, Southbend, Indiana. Hickey and Craig (1966) consistently

found in a number of crosses that only 12% of the offspring were female. In 1960, they reported a hereditary factor, transmitted by the male, was responsible for the high male ratios. This factor was designated as male producing or MP or distorter, and was passed to subsequent generations irregardless of the type of female to which the MP males were crossed. The mechanism of this distorted ratio has been investigated but not satisfactorily answered. It was shown that relatively few female-determining gametes from MP males succeed in fertilizing eggs. Reduced fecundity of MP males has been noted, as well as the observation that MP males deplete their sperm supply earlier than normal mates (Craig and Hickey, 1966). These observations led to the hypothesis that MP males produce a full complement of male sperm, but relatively few sperm that are female determining. Perhaps some abnormal meiotic event incapacitates female carrying chromosomes or if they are formed, they are non-functional. The mechanism may be similar to the meiotic drive system demonstrated in Drosophila (Sandler et al., 1959).

In a cytological study of spermatogenesis in Drosophila, Erickson (1965), found fragmentation of the Y chromosome frequently appears during the second meiotic division. He assumed that the fragmented Y had a lethal effect resulting in reduced recovery of the Y bearing sperm. Sperm bundle counts showed that sperm production was reduced in proportion to the deficiency in Y chromosomes.

This mechanism explained the predominance of females in progeny of the males. Novitsky, Peacock and Engel (1965) found the Y chromosome degenerated and was not included in a spermatid. Further investigations are needed to determine if any of the Drosophila mechanisms explain the distortion in Aedes aegypti. Studies have been undertaken at all steps of spermiogenesis to determine the precise stage where the female determining chromosomes are lost or to determine if unknown mechanisms are involved.

The mosquito, Aedes aegypti, a blood sucking insect, is the vector of yellow fever, dengue fever, haemorrhagic fever, encephalitides and many other diseases throughout much of the world. Aedes aegypti may thus be of greater importance to more people in more places than most other insect species. Aedes aegypti now demonstrates resistance to all the major insecticides, rendering the development of new control methods imperative. The success of the sterile-male technique for control of insect population, notably that of the screw worm fly, Callitroga hominivorax, (Knipling, 1959, Proverbs, 1969), has aroused much interest in insect reproduction systems. The principal advantage of the male producing method of control is that the sterility factor is passed to subsequent generations until the population is theoretically extinct. Perhaps knowledge of normal and abnormal spermiogenesis and the genetics which govern it will contribute to a mosquito eradication program as well as furthering our understanding of the events of spermiogenesis.

MATERIALS AND METHODS

Mosquitoes of the Rock strain and male producing strain were reared in a growth chamber at 26°C.-29°C. with a relative humidity ranging from 70-80%. Rearing was conducted in accordance with the methods of Craig and VandeHey (1962). Eggs were hatched in deoxygenated water, then the growing larvae were allowed to pupate in white enamel pans in 2500 ml. of tap water containing approximately 100 larvae per pan. At daily intervals larvae were fed a suspension of liver powder at a concentration of 10gms. powder per 1000 ml. of tap water. After pupation the pupa were sexed by size and transferred to pint ice cream containers covered with nylon gauze.

With proper feeding and environment control of the larvae, pupation begins on the fifth day after hatching and almost all pupation was completed by the seventh day following hatching. Male emergence began on the seventh day. Males were fed a moistened sugar cube placed on top of the mesh covering of the cage.

In this study early pupa (1st day of pupation) late pupa (3rd day following pupation) and adult insects from both the rock strain and male producing strain of Aedes aegypti were used. The gross anatomy of pupae and adult testes was examined using phase contrast microscopy. For light microscopic studies, mosquitoes of both strains,

from each of the above stages were decapitated to allow rapid penetration of the fixing solution. Mosquitoes were fixed in Carnoy solution for one hour or Carnoy le Brun for one hour and a half, or in Bouins overnight. Tissues were then dehydrated, cleared and embedded in Paraplast Plus Parafin. Sections were cut at 5 μ and stained with Heidenhain's iron hematoxylin and eosin for general study. For histochemical studies, sections were stained by the following procedures. 1. Toluidine Blue O for RNA and DNA 2. Mercury Bromphenol blue for proteins 3. Methyl green-pyronin Y for RNA and DNA.

For electron microscopic studies early pupa, late pupa and adults from both strains were randomly removed from their respective cultures, decapitated in cold glutaraldehyde (4°C), buffered to pH 7.2 with .1M Sorensens phosphate buffer (Sabatini, Bensch and Barnett, 1963). The testes were then dissected free from the terminalium using Dumont #5 microforceps and finely sharpened needles into the cold glutaraldehyde buffered solution. Testes were then transferred to fresh buffered glutaraldehyde and fixed for one hour at 4°C. Adult insects were rendered immobile with ether, then dissected in the same manner as described above. Glutaraldehyde fixation was followed by a two hour wash in 3 successive changes of phosphate buffer. Tissues were post-fixed for one hour in 1% osmium tetroxide buffered at pH 7.2 with phosphate buffer. Tissues were dehydrated through a graded series of ethanols

at 4°C, then slowly brought to room temperature in the 100% ethanol. All vials were continuously agitated to ensure rapid penetration of the tissues by the various chemicals. Following dyhydration with propylene oxide, tissues were infiltrated and embedded in an epon-araldite mixture. The capsules containing the tissues were exposed to a mild vacuum for one hour to remove trapped air from the plastic and tissue. The plastic was polymerized for 2 days at 70°C. Thin sections were cut with a Dupont diamond knife on a Reichert Om U2 ultramicrotome. Sections were stained in a saturated solution of uranyl acetate in 70% alcohol, followed by a lead citrate stain (Reynolds, 1963) then examined in an RCA EMU 3H electron microscope. Thick sections (1 u) for light microscopic examination were cut with a glass knife and stained with Toluidine Blue O (Kay, 1965) to correlate the findings from the electron microscope.

RESULTS

Gross and Light Microscopic Features

The basic plan of the male reproductive system of the mosquito Aedes aegypti is shown in Fig. 1. The testes are pear shaped or elongate bodies situated dorsolaterally in the fifth or sixth abdominal segments. They lie internal to the peripheral fat body and in all life stages are surrounded by a light brownish layer of fat. The two organs are not always of the same size, nor are they necessarily in the same physiological stage of development. The average size of each testis is 0.4mm in length and 0.1mm wide in the emerging adult and is covered by a connective tissue capsule enclosing the testicular tubules or follicles (Fig. 2). The wall of the testis is a simple epithelial sheath and the testis itself can be divided into a number of zones.

Apically there is a portion consisting of undifferentiated sexual cells, the spermatogonia. Several spermatogonia are set free and become enclosed in a capsule of somatic cells to form cysts. The cells in each cyst are approximately in the same developmental stage at all times. Cysts with actively differentiating cells tend to be larger than the ones more anterior to them. There is continuous differentiation throughout the linear series of cysts, so

that spermatocytes, spermatids and mature spermatozoa may be found in a testis at any one time. The metamorphosis from spermatid to spermatozoa occurs towards the proximal end of the testes. At emergence the passage of the spermatozoa into the vas deferens is blocked by a mass of secretion at the distal end of the testes. Above this the spermatozoa tend to be arranged circularly with the heads towards the surface. In older males, the greater part of the testes contain differentiated spermatozoa and the lower portion of the testes may be much swollen due to accumulations of these forms. Differentiated spermatozoa measure approximately 310 u in length - the head 40 u and the tail 270 u. Ultimately the vas deferens leads to the seminal vesicles and thence into the ejaculatory duct and aedaegus or intromittent organ. Associated with the vas deferens are two pear shaped accessory glands (Fig. 1). The walls of the seminal vesicles, accessory glands and ejaculatory ducts all contain muscle fibres. Mescher and Rai (1966) completed a study of the chromosomes during spermiogenesis in the mosquito, thus this aspect is not considered in this study. The upper germinal cells exhibit a diffuse chromatin material while in the primary spermatocytes the chromatin is condensed in the nucleus. Following the primary spermatocyte stage, the chromatin material in each spermtocyte no longer stains deeply and appears diffuse and remains so until it appears in a condensed state in the spermatid nuclei. Basophilic

staining is most intense in the primary spermatocyte, then less intense in the secondary spermatocyte and early meiotic stages, but becomes slightly more intense in the spermatid again. In the lower region of the testes the spermatids are orientated with their heads near or attached to the testicular walls and their tails projecting in to the centre of the testes. In adult males the spermatids are in tightly packed groups in the lower testes. At the extreme lower end of the testes, in the normal strain, there may be a "jumbled" area which exhibits various dense staining cytoplasmic bodies and tail filaments; however, in the male producing strain this "jumbled" area occupies from one third to one half or more of the entire length of the testes (Fig. 3). Examination of the vas deferens, in adult normal males, leading from the lower testes shows large numbers of sperm contained within, while the male producing adult males show a smaller vas deferens and fewer sperm.

Ultrastructural Characteristics of the Normal (Rock) System

Each testis is sheathed in a connective tissue capsule beneath which lies the testicular wall. Squamous like cells form both the testicular wall and the cyst wall (Fig. 4). Groups of spermatocytes are partitioned off into cysts by thin cytoplasmic extensions of the outer testicular walls. The cross walls from two adjacent cysts lie closely against one another, appearing as a double layer of cytoplasm with a distinct intracellular space between. Within the intracellular space cytoplasmic debris is visible (Fig. 5).

In the wall cells, the nucleus is characterized by a granular nucleoplasm with small masses of chromatin collected along the inner nuclear membrane. Large numbers of round and threadlike mitochondria are present in the cytoplasm of both the cross wall and testicular wall cells. Cisternae of rough endoplasmic reticular, free ribosomes, Golgi complexes and microtubules are also present.

As the cells in a cyst develop, the cyst wall appears to disintegrate. The lack of a cell membrane in the surface adjacent to the spermatocyte of the cyst wall cells is perhaps the first step in the disintegration process. In several cysts, there is a close association of the spermatocyte with the cyst wall (Fig. 5). Mature sperm are also found closely associated with the wall or in

projections from the wall (Fig. 6), which sometimes are joined together by desmosomes.

The compartmentalization of the germ cells by the cyst walls into cellular groups is evident in Fig. 7. The most obvious component of the germ cells at all developmental stages is the nucleus. The primordial germ cell at the apical end of the testis is characterized by a small cytoplasmic area compared to the nuclear area. Cytoplasmic bridges are evident between a number of cells and are indicative of mitotic activity (Fig. 7).

The strands of chromatin material are well defined in the homogenous nuclear matrix, a nucleolus is accentrally placed in the nucleoplasm. The nucleolus proper is made up of threadlike elements, usually arranged as a network which connect or intercommunicate giving an irregular structure. This nucleolonema is organized around a number of spherical areas of lower electron density than the surrounding nucleolus, referred to as the nucleolar vacuoles. The nuclear membrane is often poorly defined at this stage. Some Golgi bodies and few mitochondria are noted in the cytoplasm. Tiny cytoplasmic extensions project from each cell. Between the cells in a cyst evident from the early germ stage on are varying amounts of cellular debris, whorls of membranes and odd fragments of cytoplasm (Fig. 7).

As development proceeds the number of spermatocytes in

a cyst increases. A typical spermatocyte is characterized by increase cytoplasmic volume (Fig. 8). Several mitochondria, short agranular endoplasmic reticulum membranes and Golgi complexes are randomly dispersed in the cytoplasm. The agranular endoplasmic reticulum can be observed in close association with both the Golgi complex and the nuclear membrane (Figs. 8, 9). Free ribosomes and polysomes are abundant in the cytoplasm (Fig. 9). Mitochondria of the round type are in abundance though some of the spermatocytes show varying numbers of filamentous mitochondria. The cristae of several mitochondria are in parallel folds resembling a lamellar structure (Fig. 9). Some endoplasmic reticulum is of the rough form, but the majority is of the agranular cisternae type.

Masses of slightly electron dense material often appear in the cytoplasm adjacent to the nuclear membrane and this is probably RNA plus associated protein originating from the nucleus (Figs. 8, 9). Such areas resemble the material situated next to the inner nuclear membrane. Usually the nucleolus is accentually placed in the nucleus and is composed of irregular masses of an electron dense material. At times, sphere-like concentrations of material are noticeable either in proximity to the nucleolus or the peripheral nucleoplasm which suggest a movement of the spheres. Numerous nuclear pores are noted in many

spermatocytes (Fig. 10) and some micrographs show material passing through the nuclear pores (Fig. 11).

Many of the spermatocytes in the last mitotic phase are still connected to one another by cytoplasmic canals (Fig. 12). In Fig. 12, three canals, one between each of four spermatocytes are large enough to allow passage of organelles such as mitochondria and endoplasmic reticulum (Fig. 13). Microtubular elements arising from one sister spermatocyte terminate and interdigitate with microtubular elements coming from the other sister spermatocyte near the mid point of the canal (Fig. 12). The beginning of a midbody as defined by King (1971) is indicated by a darkening of the cytoplasm immediately surrounding the microtubules (Fig. 12). The canals result once the midbody and its associated microtubules dissolve. In the mitotic region of the testes such canals are abundant and in some sections three such canals have been found (Fig. 12). As the intercellular bridges decrease in size a ring of thickened cortical material 30-40 μ thick is noted along the inner surface of the cell membrane. The limits of this material is indicated by arrows in Fig. 12. The canals are semi-permanent in that they persist between the spermatocyte until the meiotic stage is reached.

The onset of the meiotic phase is characterized by

the development of the synaptonemal complex within the spermatocyte nucleus (Fig. 14). The association of the chromatin material within the structure constitutes a bivalent; the nuclear material is easily discernible, gathered along the outer edge of the complex (Fig. 15). When sectioned parallel to its long axis the synaptonemal complex appears as a twisted ribbon (Fig. 16). It is composed of lateral elements each about .05 μ wide and separated by a space about .1 μ wide (Fig. 15). The centre of the space is occupied by a medial structure which is made up of filaments or zygosomes (King, 1971) that are orientated perpendicularly to the inner surfaces of the lateral elements.

As further development of the spermatocyte proceeds, an increase in the agranular endoplasmic reticulum is observed in the cytoplasm. Concentric layers of membrane form parallel to the nuclear membrane in concentric rings which partition off the cytoplasm and associated organelles (Fig. 17). Many mitochondria remain in the central cytoplasm partition around the nucleus, but several are trapped between partitions further from the nucleus. The mitochondria are of either the round or filamentous types, but in almost all cases are characterized by parallel cristae giving a lamellar appearance. Fig. 17 shows both type of mitochondria while Fig. 18 shows only the round type.

Following the synaptic stage of meiosis the second stage of meiosis is reached and two spermatids result from this division. Each spermatid is characterized by the possession of a nebenkern body, an oblong type nucleus, and a large cytoplasmic area. One or more nucleoli are evident. The nuclear membrane retains its double unit structure. Cytoplasmic membranes and golgi apparatus are prominent features of the cytoplasm (Fig. 19). The peripheral cytoplasm and organelles becomes completely partitioned off in concentric rings by the fusion of double cytoplasmic membranes, leaving the central mass containing the nucleus, the majority of the mitochondria and Golgi complexes (Fig.10). These superfluous rings of cytoplasm are shed by the developing spermatids as the space between the double membrane expands (Figs. 19, 20, 21). Progressive shrinkage of the membranes and contained cytoplasm takes place, giving the membranes a beaded structure. Tiny beaded remains of the cast off or discarded rings are found in the lower ends of the testes in varying states of disintegration (Fig. 22).

Nuclear Changes From the End of Meiosis to Maturation

A major event in mosquito spermiogenesis is the shaping of the nucleus and the condensation of the nuclear material. Following the last meiotic division the nucleus

takes on an oblong shape, but the nuclear matrix appears homogenous. The nuclear membrane retains its double unit structure, however, a portion of the membrane is indented to form a pocket in which the centriole lies. Rarely does the envelope extend towards the nebenkern as redundant nuclear envelope.

At various points in the membrane, nuclear pores are observed (Fig. 23). Across each pore is a dense line which resembles the pore structures termed karyo-occludentes by Anderson (1965).

The nucleolar vacuoles are no longer visible in the nucleolus leaving it as an electron dense body (Fig. 23). As maturation of the spermatid proceeds the nuclear matrix condenses, becoming more granular and the nucleus itself becomes encased in a sheath of microtubules which extend above and below it, similar to the manchette of mammalian sperm (Figs. 24, 25). The nucleus also begins to elongate while the microtubular elements remain orientated parallel to the long axis of the cell (Fig. 25). As the nucleus elongates the nuclear diameter decreases and the chromatin material continues to condense forming scattered filaments of variable length (Figs. 26, 27).

After the microtubule sheath becomes visible around the nucleus, the nuclear envelope appears single layered, with a discontinuous row of dots of chromatin material on the nucleoplasm adjacent to the inner envelope

(Fig. 25). In the differentiated state the nucleus has elongated and decreased its diameter until it is approximately the same diameter as the tail (Fig. 28).

Nebenkern Formation

During teleophase II of meiosis the mitochondria become spherical, cluster together in one area of the cell and begin a complex series of rearrangements and fusions to form the nebenkern body (Fig. 29). The early cluster nebenkern forms as an aggregate of mitochondria and becomes more compact through additional fusions of the mitochondria with themselves, and take on a filamentous appearance (Figs. 29, 30). Additional points of fusion form between mitochondria with mitochondria laying adjacent to and immediately above and below one another to form a network. By a late stage of nebenkern formation, interconnections have formed between nearly all mitochondria present to give a sheet of mitochondria. Throughout development of the nebenkern, it remains in close association with both the nucleus and the centriole filament (Figs. 19, 31). The sheet of mitochondria which has formed then divides in two and the halves take up positions on opposite sides of the developing axial filament. At this stage, each mitochondrial half begins to elongate along the axial filament (Fig. 32).

Elongation of the mitochondria halves continues until they lie adjacent to the axial filament for most of its length, at which time the halves begin a process of internal reorganization and differentiation that eventually endow the spermatid with two highly ordered mitochondrial derivatives (Fig. 33). The beginning of this reorganization is marked by a disappearance of the cristae and by the appearance, at the point where the axial filament contacts the nebenkern derivatives, of a paracrystalline structure (Figs. 32, 39). The paracrystalline material will continue to form until the entire mitochondrial derivative exhibits a paracrystalline structure (Fig. 34). In mature sperm of Aedes aegypti two mitochondrial derivatives of approximately equal size are typical although some spermatozoa may show three derivatives (Fig. 34). The mitochondrial derivatives are separated from the axial filament by the dense membrane extension which also extends between the two mitochondrial derivatives in several of the spermatozoa shown in Fig. 34.

Axial Filament Development

The flagellum begins its development from the centriole which lies at the posterior end of the nucleus. As development proceeds the nucleus and the centriole become closely associated (Figs. 35, 36). In a later stage of

spermatid development the centriole lies in a nuclear indentation (Fig. 25). As the flagellum first begins to elongate very little cytoplasm is carried with it (Fig. 36). The nebenkern then becomes closely associated with the nucleus and centriole and elongates along the axial filament while at the same time the cytoplasmic area of the flagellum increases (Fig. 32).

The parts of the flagellum are designated according to conventions of Gibbons and Grimstone (1966). The nine outer doublet fibres are made up of two subfibres, the subfibre having a transverse arm is called A and the other B. The nine single fibres peripheral to these doublet fibres are called accessory fibres (Fig. 34). The typical pattern of flagellar development in the mosquito Aedes aegypti is the 9+9+1 pattern. Subfibre A of each doublet appears solid, as does the central fibre while subfibre B has a clear lumen (Fig. 34).

In an early state of axial filament development the nine accessory fibres and the central fibres are lacking (Figs. 37, 38) but as development proceeds, the nine accessory fibres and the central fibre appears (Fig. 39). During the early state of development when only the 9 doublet fibres are present, all doublet fibres appear hollow, but a transverse arm appears on the outer side of doublet subfibre A. Radial linkages between the doublets and the future central fibre are established. A circular membrane

surrounds the entire developing flagellum and in most cases this membrane contacts the nebenkern derivative on its outer edge (Fig. 39). In the differentiated state of the spermatid this membrane no longer surrounds the tubules but appears as a dark partial membrane separating the flagellum from the mitochondrial derivative. Fig. 34 show the tightly packed nature of the spermatids as they enter the seminal vesicles and their association with the muscular wall which encloses them in convolutions. Each spermatid is separated from the wall and its neighbour by a double membrane structure.

Centriole Adjunct Development

One of the most obvious structures associated with the flagellum and the nucleus of the spermatozoa is the centriole adjunct (Figs. 40, 41). This structure lies at the posterior end of the nucleus, separated from it by a distinct area of nuclear membrane. The adjunct forms a cylindrical dense sheath around the base of the flagellum where the flagellum emerges from the centriole in its depression within the nucleus. Further posteriorly, along the flagellum it forms only a partial sheath extending between the two mitochondrial derivatives. Two membranes, one on either side of the adjunct, extend the length of it or slightly beyond into the cytoplasm

of the tail. Either a third membrane may occur beneath the adjunct or the two membranes along the side may simply bend beneath the adjunct to form a base.

The sheath of microtubules surrounding the nucleus also encases the centriole adjunct as it extends into the cytoplasm of the tail (Figs. 40, 41).

In a number of micrographs examined, no evidence of a adjunct formation has been seen prior to elongation of the nebenkern, yet a distinct adjunct is present by the time the nucleoplasm has entered the condensation stage suggesting the adjunct undergoes a rapid development.

The Golgi complex appears to play a role in the formation of the centriole adjunct. Prior to the appearance of the structure, osmophilic dense bodies are noted in association with the Golgi complex (Figs. 42, 43). The Golgi apparatus also becomes more dense and several bodies have increased much in size (Figs. 43, 44). The dense bodies exhibit a vesicular substructure and this same peculiar substructure has been noted in some completely formed centriole adjuncts. Many of the bodies lie in close proximity to both the axial filament, nucleus and nebenkern derivatives (Figs. 42, 44). Fusion or rupturing of the bodies to form a larger structure is suggested in Fig. 45, and an accumulation of material is seen in Fig. 46, while a more mature adjunct shows a

vesicular substructure as well as the fusion of a body at the upper left. Occasionally the centriole adjunct shows less dense vacuolated areas (Fig. 47). The Golgi complex either becomes obscured by the developing centriole adjunct or disappears from the spermatocyte soon after the formation of the dense bodies as they are no longer visible once the adjunct is recognizable.

Ultrastructural Characteristics of the Male Producing System

The spermatocyte development of the male producing strain of the mosquito Aedes aegypti follows exactly the pattern of spermatocyte development as in the normal strain of the mosquito. The spermatocytes arise from germ cells which appear identical to the normal spermatocytes. The typical male producing strain spermatocyte is characterized by short cisternae of agranular endoplasmic reticulum, some vesicular profiles of endoplasmic reticulum, mitochondria of both filamentous and circular types and agranular cytoplasm with free ribosomes (Fig. 48). Compare Fig. 48 with Fig 8 of the normal. Small spheres of electron dense material also appear in the cytoplasm adjacent to the nuclear envelope. Some spermatocytes have fewer of these spheres in the cytoplasm, but similar chromatin material is visible, associated with the inner nuclear envelope (Figs. 48, 49). This material can sometimes be seen in areas where nuclear pores are abundant. The nucleoplasm is limited by the double unit nuclear membrane (Figs. 48, 49). A nucleolus lies eccentrically placed in the nucleoplasm as in the normal strain spermatocyte (Fig. 49), and has the same structure as described for the normal.

Cytoplasmic bridges are evident between a number of spermatocytes and are identical to the bridges described in the normal.

In Fig. 50, a portion of the spindle apparatus can be seen developing from the centriole. Also in this figure the nuclear membrane is perforated by many nuclear pores.

Among the spermatocytes in the male producing strain however, there are occasional cells which do not appear typical. These cells are characterized by a much denser nuclear matrix which is filled with spheres of darker material and patches of lighter material (Figs. 51, 52). The cytoplasm of these cells contain many apparently abnormal mitochondria in which few cristae are evident and vacuolated spaces also appear. Fig. 51 also shows a bundle of fibrous material. Cells of this type are not seen in the normal strain nor are such large numbers of mitochondria noted in the adjoining spermatocyte cells (Figs. 51, 52). The cell itself is smaller than those immediately surrounding it.

Spermatocytes reaching the synaptic stage of meiosis begin to show formation of the synaptonemal complex as do those of the normal strain. Compare Figs. 53, 54 to Figs. 14, 15 of the normal. Many common features between the spermatocytes of the male producing and normal (Rock) strain are noted. The nuclear membrane is double with several pores perforating the envelope, while the nucleoplasm is homogenous and the chromatin is beginning to clump about the synaptonemal complex to form the bivalent (Fig 54) as do the normal. The remains of a spindle apparatus or canal microtubular system is seen in the peripheral cytoplasm of Fig. 54.

Excess cytoplasm of the spermatocyte is lost in the same manner as in the normal strain with partitioning of the cytoplasm by extra membranes. In the lower end of the testes the tightly packed beaded remains of the superfluous cytoplasm are found (Fig. 55). Pieces of tail filament and several small membrane enclosed bodies are also present.

Nuclear Changes

Nuclear condensation and elongation for the most part is identical to that described for the normal, with formation of the microtubular sheath about the nucleus and the condensation of the chromatin into short fibres. Compare Fig. 56 to Figs. 26, 27 of the normal. The near mature state of the majority of the male producing spermatozoa is identical to those represented in the normal strain, however, in the area of the vas deferens where the mature spermatozoa becomes packed into parallel rows, the head region of certain spermatozoa appears devoid of nucleoplasm (Fig. 57).

Nebenkern Formation

Nebenkern development follows the same pattern as does the normal, with grouping of the mitochondria into one area of the cell, fusion of the layers and splitting of the nebenkern into two halves which elongate alongside the axial filament. A comparison of the nebenkern of the distorter strain to the normal indicates no size or appearance difference or in the transformation of the mitochondrial derivative into the paracrystalline structure in the mature sperm. Compare Fig. 58 to Fig. 29 of the normal.

Centriole Adjunct Formation

Centriole adjunct development is identical to the normal process in the sections examined. The same association of the Golgi complex with the nucleus, nebenkern and axial filament is noted. A similar vesicular substructure is seen in the maturing centriole adjunct. Fig. 57 shows the appearance of centriole adjunct in longitudinal section.

Axial Filament Formation

One of the earliest differences in the male producing strain is the development of a number of axial filament within a common cytoplasm. In Fig. 59, two developing axial filaments are seen extending into a vacuolar area. The regular occurrence of a number of axial filaments and associated mitochondrial derivatives grouped together within a membrane is characteristic of the male producing distorter system (Figs. 60, 61). Fig. 62 shows one of these units in longitudinal section. Normally, only a single filament and two mitochondrial derivatives, occasionally three, are enclosed in a membrane. Units which contain two filaments are not uncommon in either the male producing system or the normal system (Fig. 63) however, the characteristic membrane bound units of the male producing system can contain from four to ten or more of these structures and each of them may or may not have the associated mitochondrial derivatives. The development of the individual components of the axial filament is relatively normal with paracrystalline bodies developing from the nebenkern derivatives. Radial linkages and accessory fibres in most cases appear to develop normally (Figs. 60, 61). At all stages after the establishment of the axial filament sections of male producing spermatid tails can show both normal and

disrupted axonemes (Fig. 60). The presence of normal complexes at one level does not establish that they are normal throughout their length. In the disrupted axonemes, the scattering of the doublets is random, however, one half of the nine doublet arrangement is usually evident and the central filament can be identified. In Fig 60 a section from the lumen of the lower end of the testes, a large number of axial filaments are seen apparently free from any association with other structures. These "free" filament have no surrounding membrane, but do exhibit the 9 doublet pattern in a circular formation in many cases. Several of the doublets are open giving a "C" shaped profile or there can be a random distribution of the doublet members. Radial linkages are present in these "free" filament, but there is no definite central filament. Some of the "free" filaments are in association with two mitochondrial derivatives, but the membrane which usually surrounds the set is lacking. Several spermatid tails in longitudinal section show their fibre elements in a sprayed out fashion (Fig. 64).

In the lower region of the testes, as would be expected, there is much evidence of degenerating material. Large cytoplasmic bodies are evident and numbers of myelin figures and vacuoles are found situated in them, a sign of degeneration (Figs. 65, 66). Many axial filaments in cross and longitudinal section and mitochondrial derivatives

are also abundant in several membrane bound units (Fig. 67). The cytoplasm of these units appear to vary from granular to slightly fibrous. Many of these bodies contain a fibrous area of unknown composition or origin (Figs. 66, 68). Several of these bodies and isolated myelin figures when near the wall appear to become attached or embedded in the wall or in extensions of the wall (Figs. 68, 69). The numerous pseudopoda of some bodies suggests a phagocytic function (Fig. 70). These bodies are similar to bodies described by Hagopian (1971) as hemocytes. In Fig. 70, a number of membrane enclosed bodies are evident at one end of the cell, while at the other end axial filaments and mitochondrial derivatives are present. These large cells are clearly visible with the light microscope in the smaller abnormal lower regions of male producing testes. In the normal adult testes a few similar appearing bodies are noted, (Fig. 71) but they are not present in such concentration as in the male producing strain.

DISCUSSION

A necessary prerequisite to the study of the events of spermiogenesis in the male producing strain of Aedes aegypti was a thorough knowledge of the events at all developmental levels as they occurred in the normal (Rock) strain of the mosquito Aedes aegypti. Very few studies dealt with the mosquito specifically and the studies that did were concerned only with certain specific developmental aspects i.e. centriole adjunct or axial filament development (Breland, 1966). The whole process from formation of the primary spermatocyte to the mature sperm was examined in this study, since it had not been reported before in the mosquito.

Nuclear Events

The condensation and elongation of the nucleus in mosquito spermiogenesis is a major event. The end result of the shaping of the nucleus is the highly elongate narrow head of the mature spermatozoa. The presence of the microtubular sheath surrounding the elongating nuclear head suggested that the microtubules played a role in the elongation process. Kessel (1966, 1967) in studies of the microtubular system of dragonfly spermatids suggested that the microtubules first assumed a helical structure about the nucleus and then straightened out as

the nucleus elongated. Unlike the microtubular system of Aedes aegypti, which formed a complete sheath about the nucleus, the microtubular system of the dragonfly was located in indentations of the nucleus and did not form a complete sheath around it. In micrographs of tranverse sections of the elongating spermatid nucleus of Aedes aegypti there was no evidence that the microtubules assumed a helical structure about the nucleus. Yasuzumi (1971) in studies of the microtubular system in grasshopper spermatids suggested that the microtubules played a role in the paracrystalline polymerization of the chromatin material. He found that as the microtubules developed the nuclear envelope became single and the randomly dispersed chromatin fibres became organized into a paracrystalline structure. In the mature state of the spermatid the microtubules were lost simultaneously with the loss of the paracrystalline nature of the chromatin, establishing the relationship of the microtubules with this process. In the mosquito, Aedes aegypti, a similar association of the microtubules with the nucleus was noted as the chromatin material began to condense. However, the condensation of the nuclear material occurred, simultaneously with the most extreme elongation of the nucleus, suggesting a dual role of the microtubule system. It may draw the nucleus along as the tubules elongate and the association of the tubules with the nucleus may be the necessary stimulus to initiate the condensation of

the chromatin material. That the nucleus is actually carried along as the microtubules lengthen is suggested by the absence of a helix and uncoiling process as seen in the dragonfly. The microtubules were evident in sperm nearing maturity and they also persist into the tail piece, giving credence to the functional role they play in support, stabilization, conduction or transportation (Porter, 1965). On the basis of this study the supportive role of the microtubule in the elongating nucleus is favored. It appears that the polymerization of the nuclear material occurs concurrently with microtubular formation rather than a result of it.

The origin of the cytoplasmic microtubules seems to be different in different animal spermatids; for example, in the earthworm, they developed in association with the centriole, (Anderson, 1969) in the grasshopper in association with the nuclear envelope, (Kessel, 1967, Yasuzumi, 1969, 1970) and in the lepidoptera in association with the plasma membrane (Yasuzumi, 1971). In Aedes aegypti, the microtubules appeared to originate in association with the nuclear envelope.

In this study the nuclear membrane did not form a redundant nuclear envelope as in mammalian spermiogenesis; (Rattner et al., 1971) however, the agranular endoplasmic reticular did form a boundary about the centriole adjunct much in the same manner in which the redundant nuclear envelope bounds the midpiece of mammalian sperm.

The organization of the diffuse chromatin material into the paracrystalline or fibrous structure of the elongating spermatid is brought about by an unknown mechanism. In studies of elongating grasshopper spermatid nuclei, Block and Brack, (1963) found that the nuclear histone become increasingly resistant to the effects of deamination which indicate the formation of a histone with a higher arginine to lysine ratio. Autoradiographic studies also showed a rapid incorporation of labelled arginine into Drosophila and grasshopper spermatid nuclei (Das et al., 1964, 1965). Bloch et al., Das et al., suggest that it is this shift in the histone base ratio which results in the condensation of the chromatid fibres. Similar autoradiographic studies should be conducted with Aedes aegypti to establish conclusively if such a mechanism is functioning in the condensation of the chromatid fibres.

An obvious organelle associated with the meiotic stage nucleus is the synaptonemal complex. Moses (1965) first discovered the organelle in crayfish spermatocytes. Moses (1968) in a review and Comings (1970) in studies of whole mount preparations of meiotic chromosomes summarized some of the conclusions concerning the complex which are as follows. 1. The synaptonemal complex (lateral and central elements) is composed of protein. The lateral elements constitute the central axis of homologous chromosomes. 2. Crossing over requires the presence of

the synaptonemal complex, but the synaptonemal complex does not ensure that crossing over will occur. 3. The complex may serve to provide highly specific pairing of chromatids and a structural framework for recombination. 4. Short segments of the chromatin fibres attach to the lateral elements. The attachment sites may occur in clusters to produce the chromosomes visible by light microscopy. Enzyme digestions by Comings (1970) demonstrated that the synaptonemal complex is made up of a basic protein and contains no DNA or RNA.

King (1971) in studies of the synaptonemal complexes in Bombyx mori found attachment of the complex to the nuclear membrane at each end of the bivalent. Although attachment of the synaptonemal complex to the nuclear envelope was not observed in the micrographs of spermatocytes of Aedes aegypti examined, serial sections would establish whether such an attachment exists or not. Since the haploid number of chromosomes for Aedes aegypti is three, then six attachment points of the synaptonemal complex should exist. Moens (1969) postulates that the attachment points of the homologous chromosomes move closer during synaptic pairing of the homologous and that pairing is initiated near the nuclear membrane. Pairing involved a two step process (Comings, 1971) with chromosomal pairing during which the homologous are brought in close approximation with one another by the synaptonemal complex and molecular pairing involving the

intimate pairing of homologous DNA bases which leads to recombination and production of chiasmata. The synaptonemal complex found in Aedes aegypti does not differ appreciably from those observed in either the silkworm or the grasshopper thus its function is probably identical.

Cytoplasmic Bridges

Cytoplasmic bridges are definite structures in spermatocytes of Aedes aegypti which persist until the meiotic phase of development. Midbodies similar to those described in other species (King, 1971, Buck and Tesdale 1962) are found at first in the cytoplasmic bridges in partially cleaved Aedes aegypti. Cells, when they have dissolved the canals result. The persistence of these cytoplasmic bridges as canals between sister spermatocytes until meiosis is reached may be the cue which informs the spermatocyte when to begin meiotic division. In Bombyx mori King(1971) suggests that the attainment of a certain number of canals is the mechanism by which mitosis is stopped and meiosis begin.

Immediately beneath the plasma membrane of the cleavage furrow a thickened area of cortical material was indicated. This ring is similar in morphology to the contractile ring described by Schroeder, (1970) in dividing HeLa cells and in spermatocytes of Bombyx mori

by King and Akai (1972). The contractile ring was found to play a direct role in cytokineses by Schroeder (1970) who showed that the ring is composed of cytoplasmic filaments that are aligned circumferentially along the equator of the cell. Koch and King (1969, 1970) have suggested that in Drosophila and Bombyx mori the ring becomes coated with a cortical material and refer to them as canal rims. In spermatocytes of Aedes aegypti, the rings also are coated, perhaps as a supportive function to maintain the canal until meiosis.

Nebenkern Formation

The formation of the nebenkern body in Aedes aegypti does not differ to any appreciable extent from that described by Pratt (1968), in the Hemipteran Murgantia. Early cluster nebenkern formation was identical and formation of the two networks of nebenkern followed in the late cluster stage with elongation of the halves along the axial filament. The two halves of the nebenkern, though more complex in form, appear to be structurally similar to other mitochondria (Chance and Thorell, 1959). The final realignment of the nebenkern derivatives is related to the growth of the flagellum and is parallel to the flagellum and does not coil about it as in many insect species (Nath, 1956, Wilkes and Lee, 1965).

At the point where the mitochondrial derivatives

contact the axial flagellum, a paracrystalline material appears in the mitochondrial matrix suggesting a contact relationship. Warner, (1971) noted a similar association and development of the paracrystalline rod in the blowfly. This association supports Andre's (1962) suggestion that the paracrystalline material represents a particular state of the respiratory protein derived from the cristae. Meyer (1964), suggested the rod may provide support for the sperm. Foor (1970), suggested it may be involved in reducing the number of functional mitochondria in the cytoplasm. The presence of the paracrystalline material is by no means unique to spermatids of mosquitoes. It was reported in the spermatozoa of lepidoptera of Andre (1962, 1963), Drosophila by Meyer (1964), in Sciara by Makielsky (1966), in nematodes by Foor (1970).

Axial Filament

It is well known from electron microscopic and other studies that the basic structure of the flagellum or axial filament of spermatozoa is similar in many groups of organisms. This basic structure consists of nine outer doublet fibril groups and an inner centrally located pair, the plan being referred to as the 9+2 pattern. Variations in this plan have been noted in the Diptera by Nunez (1963) who noted an additional row of accessory fibres outside the 9 doublets. These accessory fibres were noted in this

study of Aedes aegypti and also in Aedes aegypti and other species of mosquito examined by Breland et al., (1966, 1968), Phillips (1969a,b). Another deviation from the general 9+2 pattern in Aedes aegypti was the presence of only a single central element rather than a pair of elements. The resulting fibre formula is expressed as the 9+9+1 pattern. In Aedes aegypti as in many insects (Friedlander, 1966, 1971) spermatids retain only one centriole after the spermatocyte division which becomes the flagellar based body. The axial filament assembled in a sequence identical to that described by Warner (1971) in the blowfly with the doublet fibre A forming first followed by the radial linkages and the B subfibre, and last the accessory fibres in a rapid sequence.

Cytoplasmic Membranes

During the elongation of both the spermatid and its developing flagellum large amounts of cytoplasm are associated with the spermatid. Mechanisms for the elimination of excess cytoplasm are varied in the insects.

Widely differing membranous structures called whorls have been described to play a role in elimination of superfluous cytoplasm. Reger (1968), showed in foldings of the surface membranes to form multivesicular, multilemiar and granular bodies in millipedes which are later sloughed. Phillips (1970) showed the formation

of large membrane whorls in spermatids of the grasshopper Melanoplus. Warner (1971) showed the formation of membranes of nuclear origin which enclosed a small amount of cytoplasm and are sloughed from the maturing sperm. In Aedes aegypti, the mechanism of cytoplasmic sloughing is unique. The membranous partitions formed early in spermiogenesis package the superfluous cytoplasm into concentric rings which are lost as the spermatid elongates. Progressive shrinkage and breakage of the rings occurred in the lower end of the testes and the tiny beaded remains may be phagocytized by the testicular wall or by hemocytes.

The origin of these smooth membranes which partition the cytoplasm is questionable. Many micrographs examined showed a close association of the agranular membrane with the Golgi complex while other micrographs suggested an association with the nuclear membrane. From these studies it is difficult to form any definite conclusions as to the origin of these membranes.

Centriole Adjunct

Species of mosquitoes examined by Breland et al., (1966) in which a centriole adjunct was found were Aedes aegypti, Culiseta inornata and Culex molestus. The origin of the centriole adjunct is still a matter of discussion. Gatenby and Tahsmisian (1959) stated that in the ground cricket Nenobius, the centriole adjunct

was not secreted by the proximal centriole, but that it arose as spheres or granules that fused and formed around the centriole. Gatenby (1961) also stated there was some possibility that the structure might have originated from the centriolar matrix. Yasuzumi (1970), in studies of the centriole adjunct formation in the grasshopper Acrida lata showed that chromatid bodies of nuclear origin were related to the formation of the centriole adjunct and were at times seen to fuse with it. He succeeded in reducing the granular component of the centriole adjunct from 360°A to 120°A by extraction with perchloric acid (PCA) indicating that a part of the centriole adjunct was composed of RNA. The fact that their chromatid bodies have a similar chemical composition to the centriole adjunct, appear to join with the adjunct, and that final development of the centriole adjunct is marked by the disappearance of these bodies from the cytoplasm suggest a plausible explanation of its origin. Lindsey and Biesele, (1970) in studies of centriole adjunct formation in the cockroach Eublabeus posticus have divided the formation into four stages - the presumptive granular stage, endoplasmic reticulum associated stage, the granular and vacuolate stage and the osmophilic dense stage. The first step occurs in the early spermatocyte where extremely fine granules coalesce on the nuclear envelope. The endoplasmic reticulum then fragments in association with the granules. The granules increase in size, fuse and by the final stage

there is no evidence of granules, rather a homogenously dark staining structure results. Barker and Biesele (1967) hold the impression that in the spermatid of the salamander, Amphiuma tridactylum, distinct nuclear pores allow material to pass from the nucleus into the neck piece and that additional material may originate in the region of the Golgi complex. The neckpiece of mammalian sperm has been homologized with the centriole adjunct in insects although any homologies of insect sperm with mammalian sperm are confusing (Breland et al., 1966).

Examination of micrographs of early spermatocytes of Aedes aegypti revealed no granular associations with the nuclear envelope such as those described by Lindsey (1970). Chromatid bodies similar to those described by Yasuzumi (1970) were seen in the cytoplasm of the spermatocytes but these bodies were not found to merge into the adjunct area.

The presence of the pore complex in the spermatids of Aedes aegypti would seem to indicate, at least in part, that some nuclear RNA material may be released to form a portion of the centriole adjunct, however, there is no evidence of material accumulating between the centriole adjunct and the nucleus.

Prior to the elongation of the nebenkern, no sign of adjunct formation was seen, indicating that its formation is rapid. Once nebenkern elongation was completed, the centriole adjunct appeared completely formed.

The association of electron dense material with the

Golgi complex which lies in close relation with the nucleus and the centriole seems to suggest a possible role of the Golgi complex in the formation of the centriole adjunct. A series of micrographs indicated the enlargement of a number of Golgi vesicles with a substructure appearing similar to the later substructure of the centriole adjunct, indicating that the Golgi apparatus may contribute in a visible way to centriole adjunct formation. However, before definite conclusions could be made as to the role of the Golgi complex in formation of the centriole adjunct, then labelling experiment should be done to trace the passage of material to the adjunct.

Although the Golgi complex commonly gives rise to the acrosome in most insect species (Breland et al., 1968, Phillips, 1970) no acrosomic system is present in Aedes aegypti. The acrosome is generally thought to be the means by which the spermatid penetrates the egg and the lack of an acrosome is particularly confusing to investigators. However, the lack of an acrosome is not entirely uncommon to invertebrates since mosquitoes (Breland et al., 1968), some caddis flies (Phillips, 1970) nematodes (Foor, 1970) and hydra (Schincariol and Habowsky, 1972) do not exhibit the structure. Studies of the spermatozoa in the female system after mating would perhaps determine the mechanism of egg penetration by the spermatozoa.

It should be remembered that the event of spermatogenesis

while discussed here as a series of steps are parts of a continuous dynamic process. The end result of these events is a mature sperm consisting of an anterior head, centriole adjunct, and a tail. The appearance of normal spermatid tail in cross section is a central axial filament with two mitochondrial derivatives enclosed in a membrane which surrounds the three structures. Occasionally, however, spermatids in the normal system exhibit two axial filaments. These variant spermatids probably were the result of failure of the second meiotic division (Friedlander and Wahrman, 1965). Presence of a few cells showing variations among cells in the normal tissues is a common occurrence and should not be emphasized as abnormalities. This was noticed in mosquitoes and pointed out previously by Breland et al., (1968) and was also particularly evident in this study where the truly abnormal was examined as described in the following sections.

The Male Producing System

Only changes in events of spermiogenesis of the male producing strain from those in the Rock strain are discussed. Events which have not changed are considered normal and thus are discussed under the normal (Rock) system. The most distinctive change in the male producing system was the occurrence of the "free" axial filament elements. In the early spermatocytes some abnormal cells were described, in which the vacuolated mitochondria suggested a degenerative change or a change that would result in abnormal mitochondrial derivatives. The fact that in the final stage of differentiation all mitochondrial derivatives appeared as those seen in the normal with regards to size and paracrystalline rod formation supports the contention that these cells degenerate and do not differentiate further. A further abnormal change seen in some micrographs was a clear head area apparently devoid of nuclear material, which suggested a cytoplasmic division without a normal nuclear division had occurred. The majority of the sperm in the male producing system, however, showed no deviation from the normal pattern. Since the male producing system gives a sex ratio of approximately 75% males to 25% females, one would expect only 25% of the sperm to be distorted or abnormal. In spite of the appearance of abnormalities, all organelles which appear in the normal were also present in the distorted sperm. The free axial filament elements

suggest that a membrane was never formed around them, or if it was, it was relatively unstable and was easily lost. Some of the axial filaments in the bundles and those that were free were distorted. Romrell (1971), in studies of spermatocytes of Drosophila, found that while axial filaments may appear normal in sections taken from near the centriole, those taken distal to the centriole may appear distorted though complete in their development. Many of the "free" disrupted filaments in Aedes aegypti did not show a central element suggesting incomplete development or a degenerative change of the filament.

In Drosophila the sperm occur in bundles and then through a process of individualization, the sperm are separated from one another (Tokuyasu, 1972a). This process produces a bag of discarded organelles referred to as a "waste bag" by Tokuyasu et al., (1972) and a free spermatozoa. A coiling process has also been observed by Tokuyasu (1972b) in Drosophila and this process acts as a mechanism to separate abnormal sperm from normal sperm which results in the formation of a bundle of abnormal sperm. "Waste bags" of organelles and bundles of sperm similar to those of Drosophila are found together in the lumen at the lower end of the testes in the male producing strain. The "waste bag" in the male producing males contained membrane whorls, mitochondria, bits and pieces of cytoplasm and portions of sperm bundles which suggested that the "waste bag" maintains a link with the abnormal tails or

is not completely sloughed off. A similar connection of sperm bundles and "waste bag" was noted in Drosophila (Tokuyasu, 1972). Romrell (1972), in investigations of an autosomal mutant in Drosophila showed a different mechanism which resulted in the formation of sperm bundles. He found a failure of meiotic cytokineses in which the primary spermatocyte underwent nuclear division, but a subsequent failure of cytoplasm division left four spermatids to develop in a common cytoplasm. The mechanism operating in Aedes aegypti may be a failure of meiotic cytokineses, but this would result in a set number of axial filaments within a bundle as in Drosophila which shows 4, whereas in Aedes aegypti, the number varies from 4 to 10 or more axial filaments per bundle.

Among the sperm bundles and "waste bags" other cellular bodies with phagocytic projections were noted and are thought to be hemocytes. Hemocytes have been reported to move among cells in differentiating tissues in order to lyse degenerating cells and debris (Jones, 1962). Hemocytes are reported to be of many different types (Hagopian, 1971) and may also have a taxonomic significance (Arnold, 1972) and thus in Aedes aegypti the hemocytes appear similar to those of other species of insect. Testicular wall cells are also known to perform a phagocytic function (Tokuyasu, 1972) and in Aedes aegypti membrane whorls and large cytoplasmic bodies were often found partially or totally contained in the wall. In the male producing strain of

Aedes aegypti such bodies accumulated in the lower lumen of the testes suggesting they are phagocytized slowly. In studies of older male producing males, Craig (1966) has shown that they depleted their sperm supply more rapidly than did normal males. This not only suggested they had a lower number of functional sperm, but also as the "waste bodies" and hemocytes collected in the lower testes then normal sperm may be impeded from reaching the vas deferens. The vas deferens in the male producing mosquitoes showed fewer spermatozoa in the lumen than did the vas deferens in the normal mosquitoes. Light microscopic studies have shown that in older males up to or over one half of the testes were filled with accumulation of abnormal appearing material in a "jumbled" area at the lower end of the testes.

It appears that the main action of the male producing factor is the production of bundles of incompletely separated tail elements which render the sperm in these bundles useless in fertilization. The free axial filaments are perhaps, a further degenerative form of portions of bundles that have been incompletely phagocytized. The empty nuclear head was not a frequent observation and would thus account for only a small proportion of the abnormal sperm.

In Drosophila the sperm were found in bundles of 64, and the abnormal sperm from a bundle became localized into one particular area of the lumen of the testes, thus

relative percentages of abnormality were easily calculated (Tokuyasu, 1972). In the Hymenopteran Dalhlbominus dextral and sinistral coiling of the head was observed (Lee and Wilkes, 1965) and evidence was presented which showed that the sex ratio was related to the proportion of the two types of spermatozoa. The dextral coiled heads always occurred in a lower proportion in the sex ratio strain which had few females while the wild stock had many females in their progeny. Again this represented a structure change which is relatively easy to identify and to relate to abnormal percentages. The change demonstrated in Dalhlbominus was not drastic and the sperm remained functional. Aedes aegypti, on the other hand did not demonstrate any particular defect which would allow one to identify individual sperm that are abnormal, but rather a drastic change which results in the bundles of abnormal sperm. From the altered sex ratio one can assume that the female sperm constitute the majority of these abnormal forms. Further studies should be done to explain their susceptibility to non separation.

SUMMARY

1. The changes involved in spermiogenesis of the normal (Rock) strain and the male producing strain of the mosquito Aedes aegypti were described.

2. Beginning with the primordial germ cell the formation of the nebenkern, mitochondrial derivatives, axonemal complex was discussed together with the accompanying nuclear changes.

3. Formation of the centriole adjunct was described and a possible mechanism for its formation from the Golgi complex was suggested. A unique mechanism was observed for the elimination of superfluous cytoplasm by the formation of membranes which package it into concentric rings which were later sloughed by the spermatid.

4. The male producing strain was found to show little abnormality in the earlier stage of spermiogenesis, however, late differentiation stages showed the formation of abnormal bundles of tails, free axonemal complexes, cellular debris and large cytoplasmic bodies which accumulated in a jumbled area at the lower end of the testes.

5. The large cytoplasmic bodies which contained portions of sperm were thought to be "waste bags" of sloughed cellular material which may be phagocytosed by

hemocytes and testicular wall cells.

6. It was postulated that the abnormal bundles were composed mainly of the female determining sperm which resulted in the distorted sex ratio characteristic of the male producing strain.

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VITA AUCTORIS

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Secondary Education:

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Post-Secondary Education:

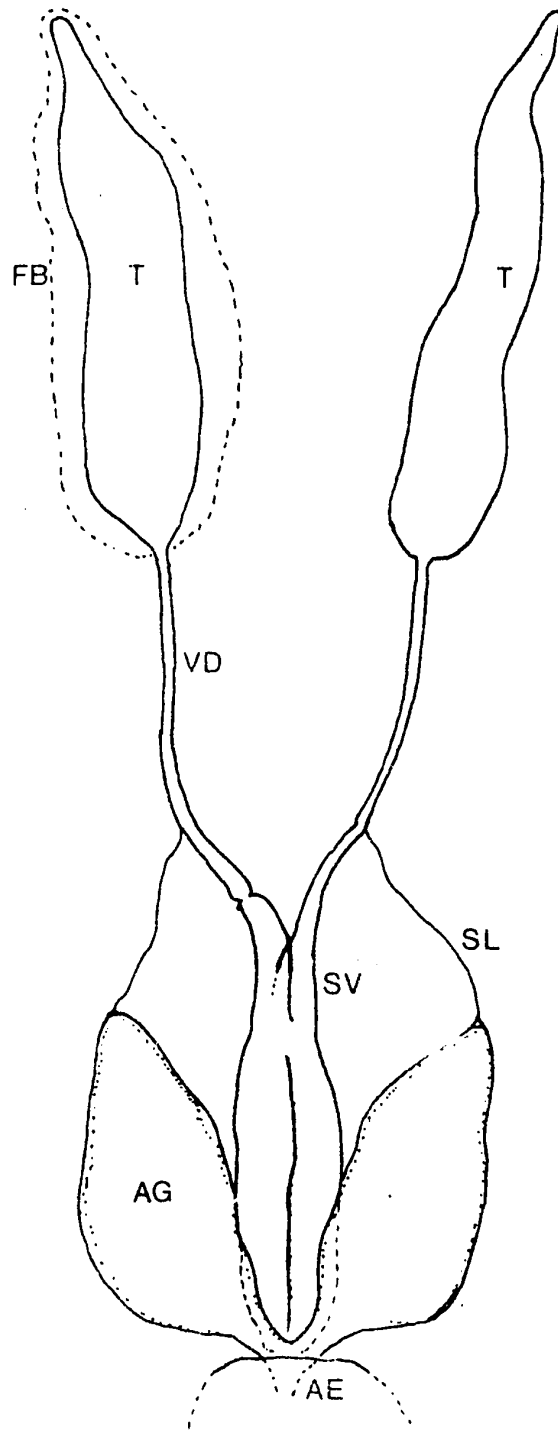
Ottawa Teachers College, Ottawa, Ontario.

1966-1967

University of Windsor, Windsor, Ontario.

1967-1970 (B.Sc.).

FIGURE 1. The basic plan of the male reproductive system is shown. Note testes (T), vas deferens (VD), seminal vesicles (SV), intromittant organ or aedaegus (AE) and the accessory glands (AG). The accessory glands are attached by suspensory ligaments (SL) to the vas deverens. The testes are surrounded by a sheath of fat body (FB).



①

FIGURE 2. A phase contrast photograph of a testis showing many spermatocytes contained in cysts. The cyst walls are noted as dark partition across the testes.

x 200

FIGURE 3. The lower end of the testes in the male producing strain showing the "jumbled" area of cytoplasmic debris and bit and pieces of spermatids.

x 600



FIGURE 4. A portion of the testicular wall showing a nucleus (N), rough endoplasmic reticulum (RER), mitochondria (M), Golgi complex (GC) and spermatocytes (S).

x 11,500

FIGURE 5. A portion of the cyst wall (CW) which is confluent with the testicular wall. Note a distinct intracellular space (IS) between the cyst cells, rough endoplasmic reticulum (RER), microtubules (MT) and spermatocytes (S). The outer cyst wall membrane appears to be disintegrating (arrow).

x 10,700

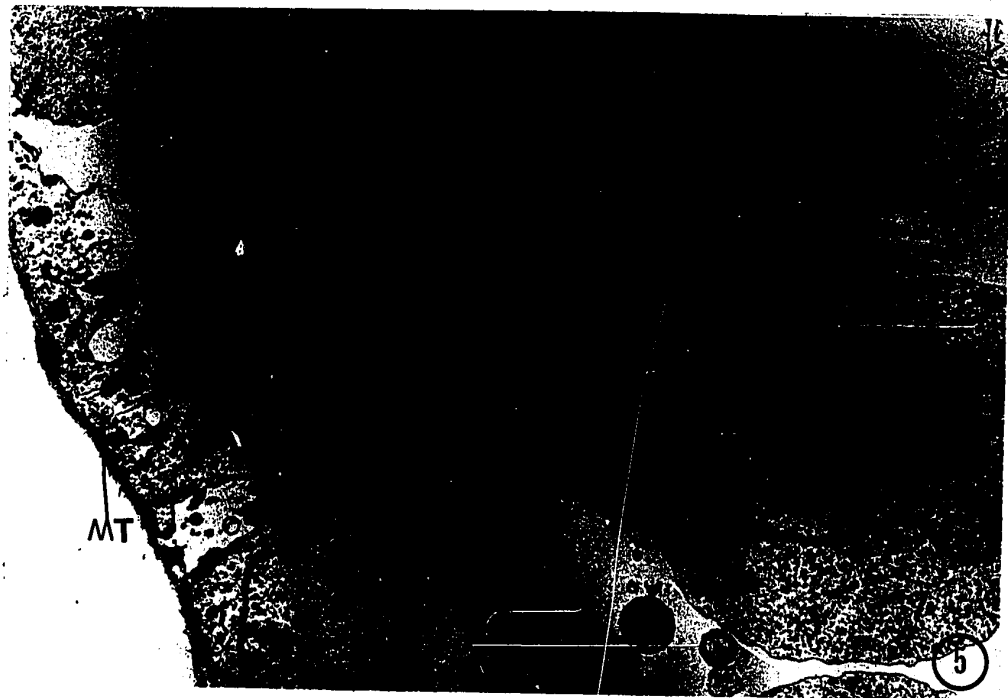
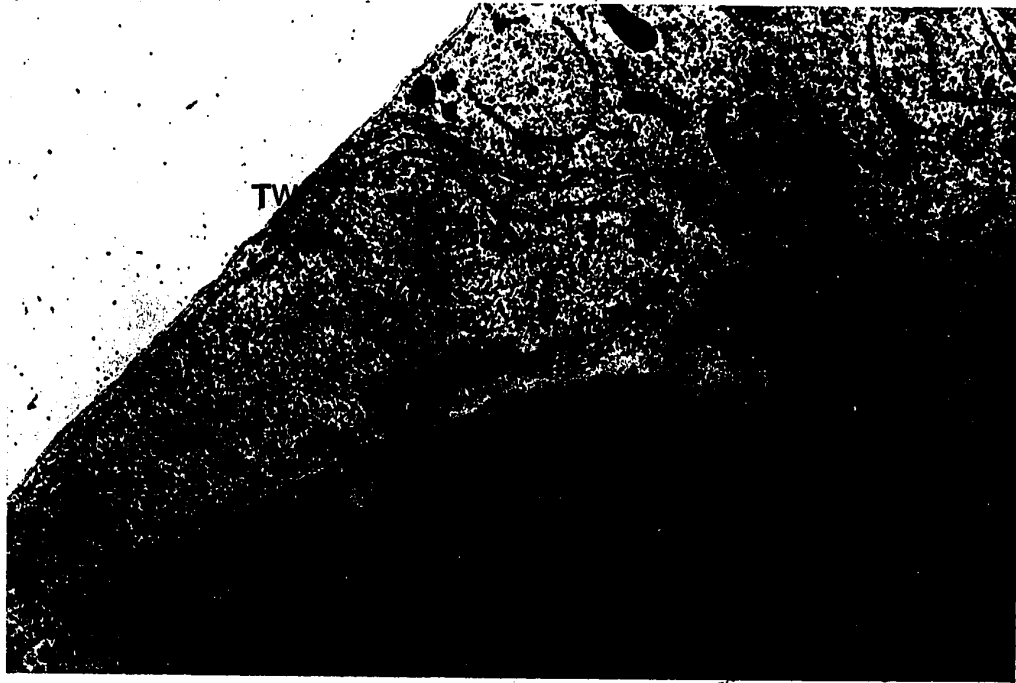


FIGURE 6. Spermatids (SP) are found in close association with the testicular wall (TW) or in projections of the wall. Note desmosomal areas (D) in the projections.

x 17,600



FIGURE 7. The primordial cells in the upper end of the testes are characterized by a large nuclear volume (N) and a small cytoplasmic area (C). Note nucleoli (NL), nucleus (N) and cytoplasmic debris between the spermatocytes. Cyst walls (CW) package groups of spermatocytes into cysts.

x 11,600



FIGURE 8. Typical spermatocytes showing increased cytoplasmic volume. Note mitochondria (M) and the association of the endoplasmic reticulum (ER) with the nuclear envelope (NE) and the Golgi complex (GC) indicated by arrows. A granular material is associated with the nucleolus (NL) and peripheral nucleoplasm (indicated by circles). Slightly electron dense spheres (ES) of material are associated with the nuclear membrane.

x 9,400

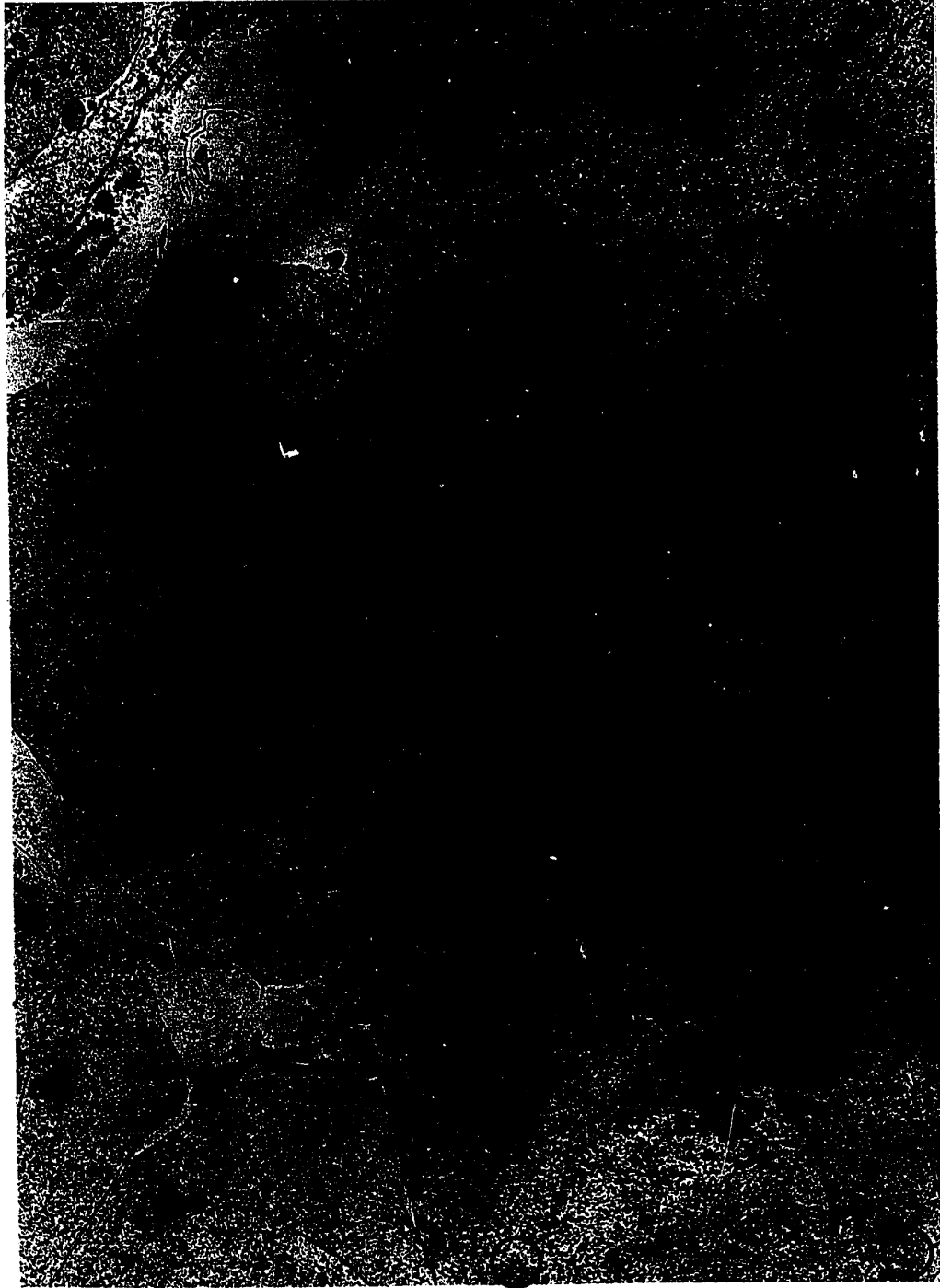


FIGURE 9. A slightly more mature spermatocyte showing increased amounts of endoplasmic reticulum (ER) and mitochondria (M). Free ribosomes (R) are evident. The Golgi complex and endoplasmic reticulum are closely associated. Nucleus (N), nucleolus (NL). The electron dense spheres (ES) cristae of the mitochondria are parallel giving a lamellar appearance.

x 17,000

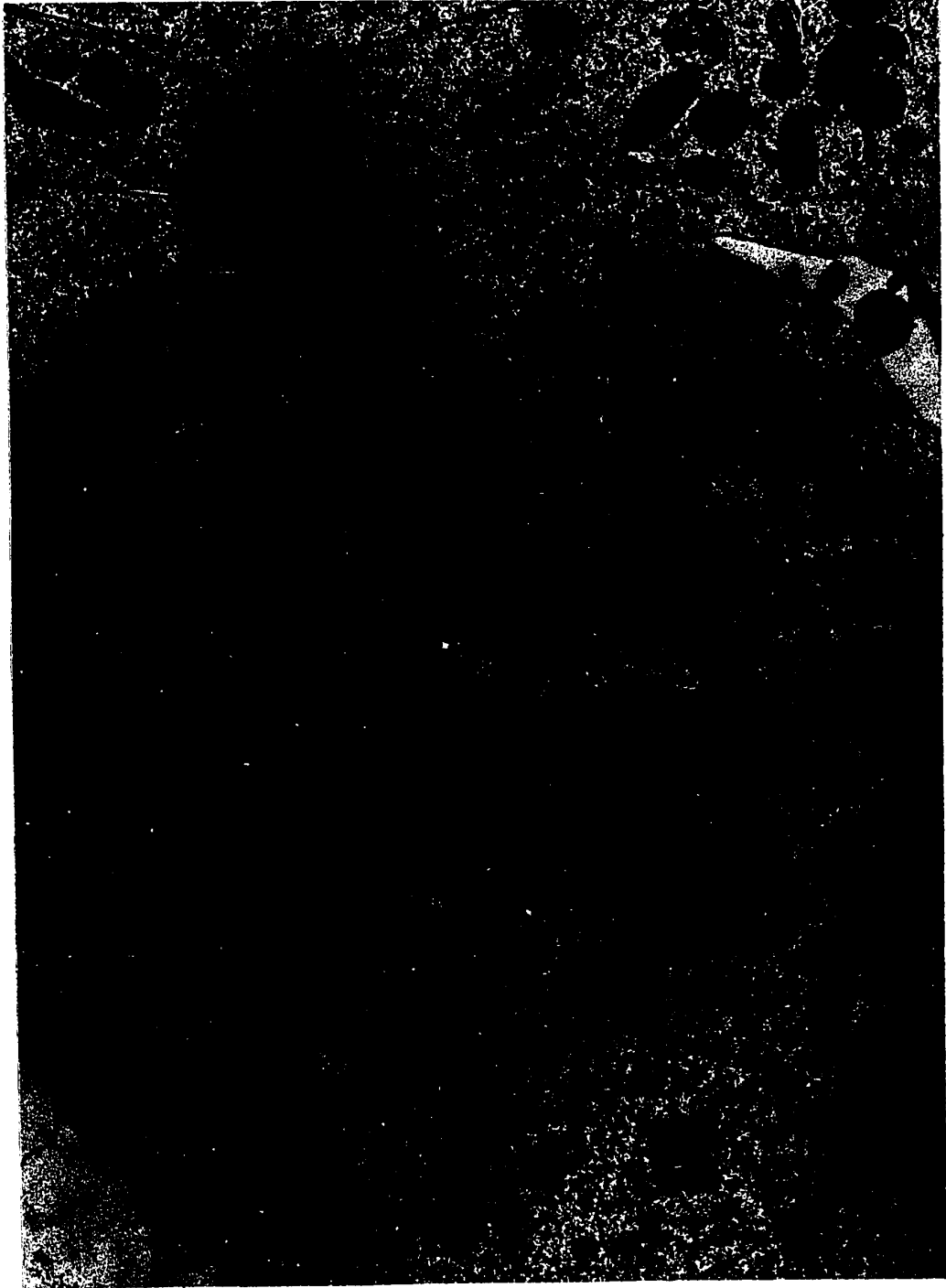


FIGURE 10. Numerous nuclear pores (NP) are evident
in many spermatocytes.

x 21,000

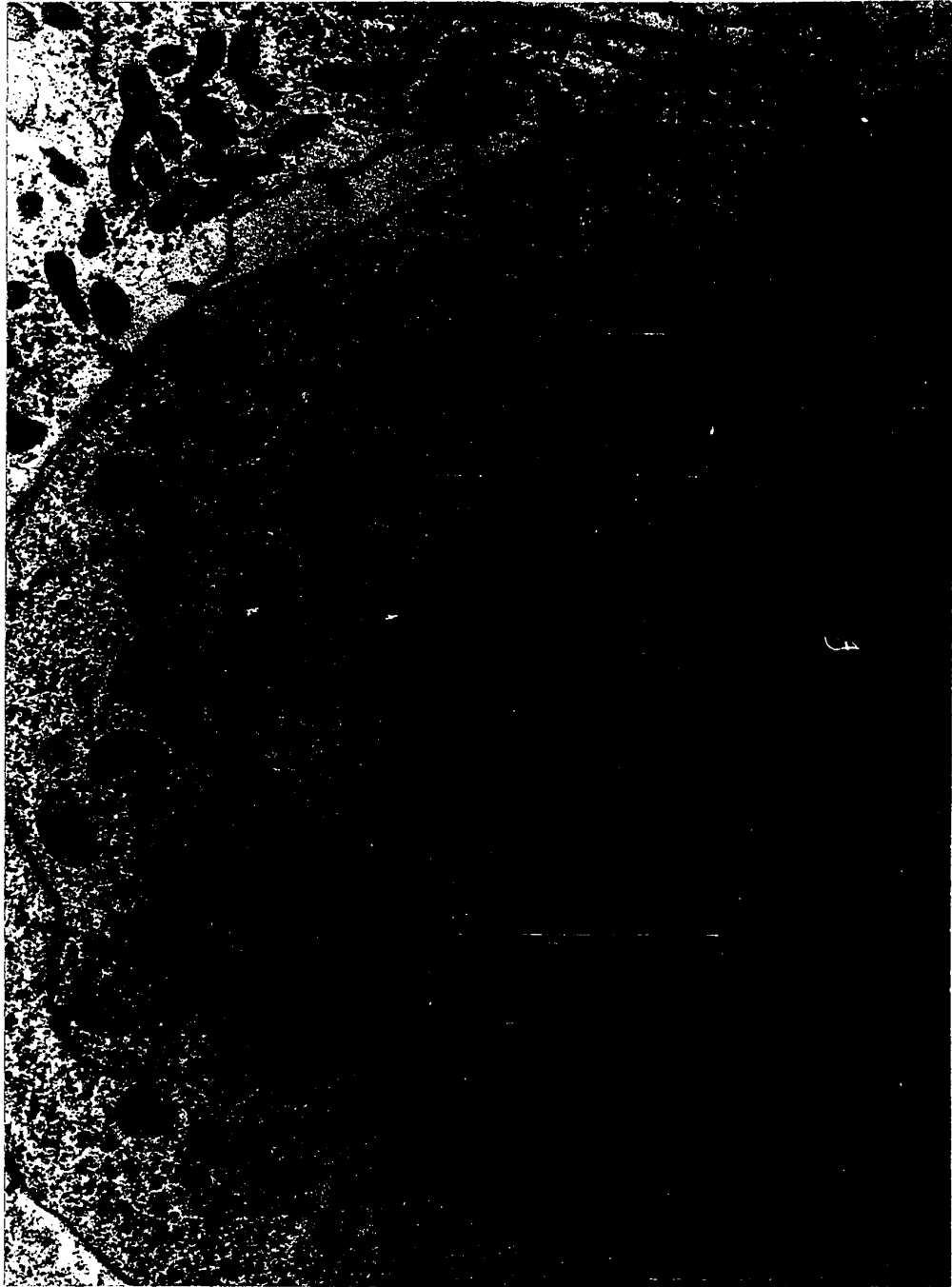


FIGURE 11. A spermatocyte showing slightly electron
dense material (ES) passing through a nuclear
pore.

x 29,000

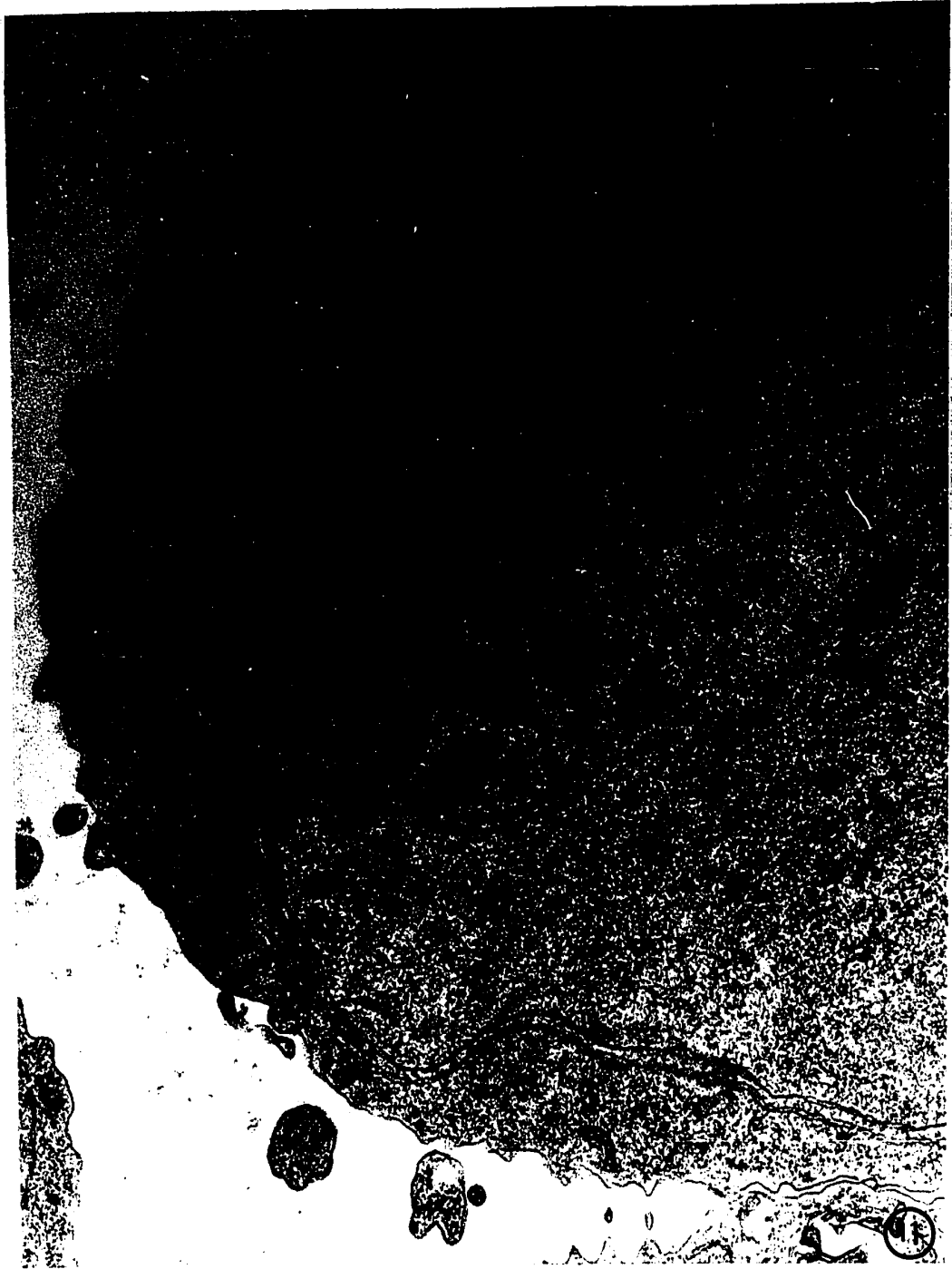


FIGURE 12. Spermatocytes remain connected to one another by cytoplasmic canals (CC). A early midbody (MB) is seen arising around the microtubules (MT) in one canal. The canal rims become thickened indicated by arrows.

x 16,000

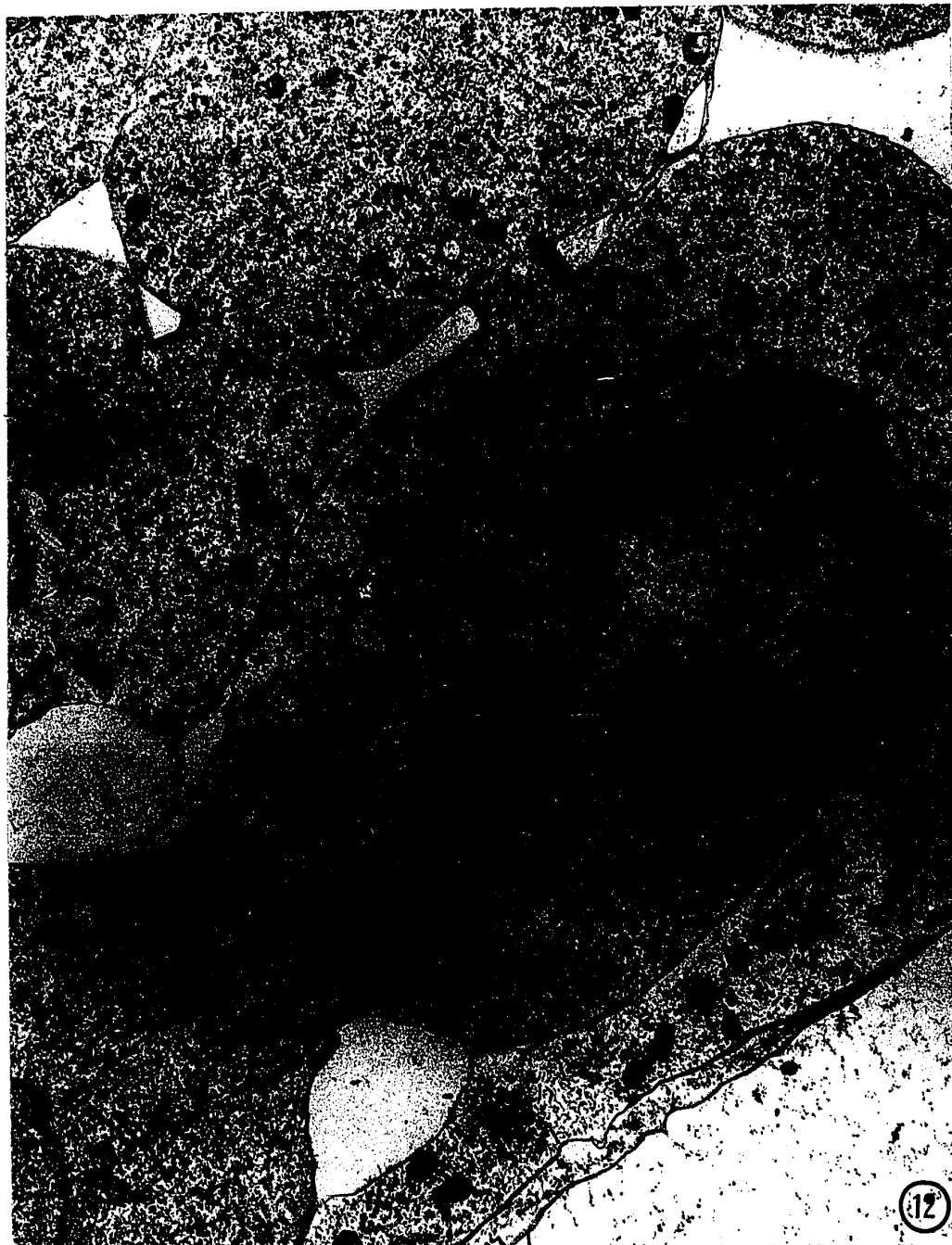


FIGURE 13. A Cytoplasmic canal (CC) which is large enough to allow passage of organelles. Note mitochondria (M) nucleus (N).

x 17,000

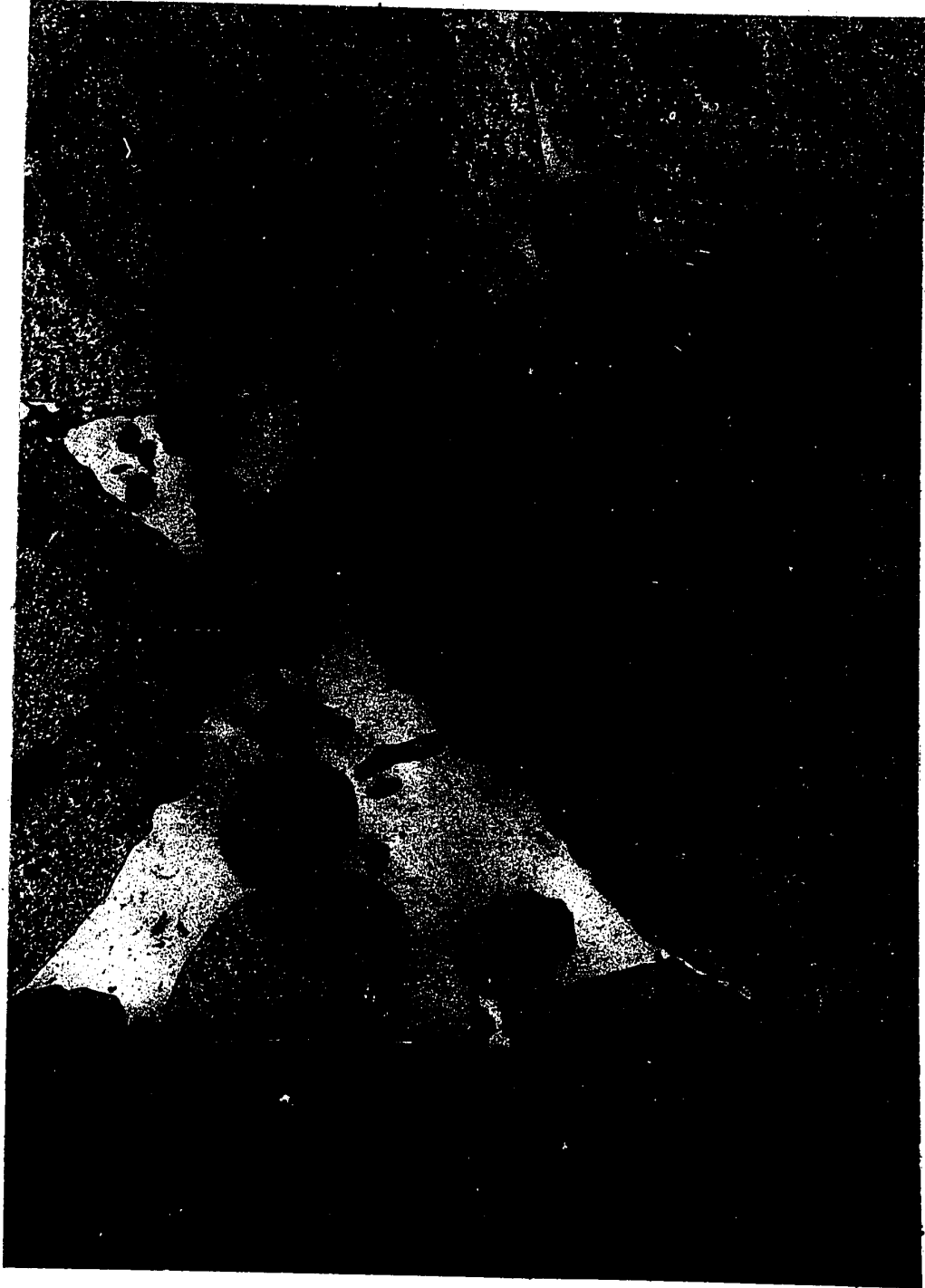
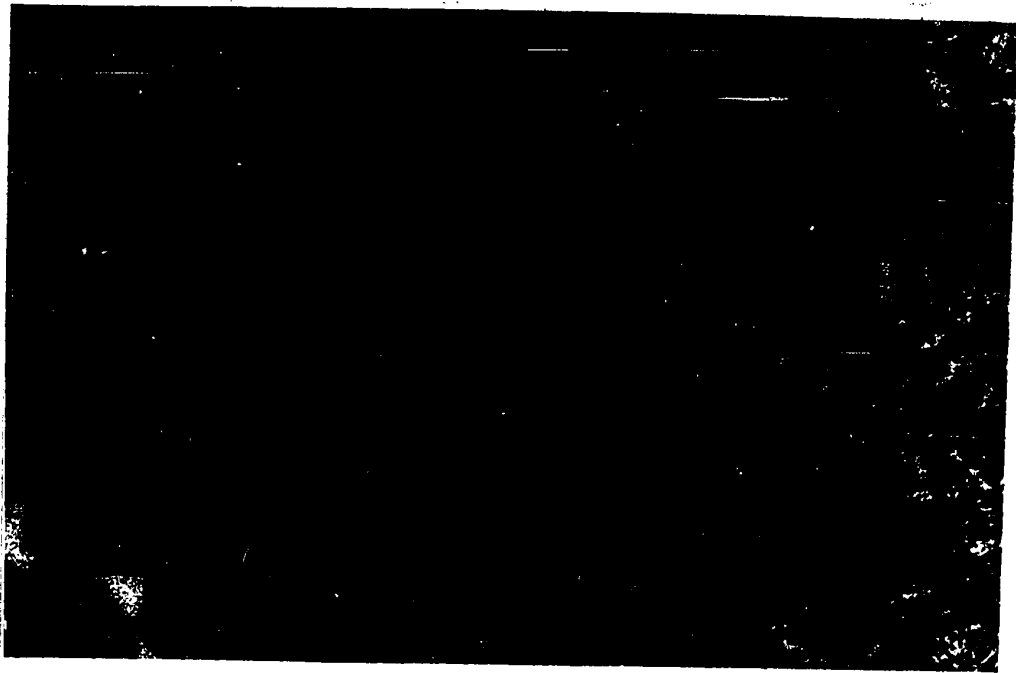
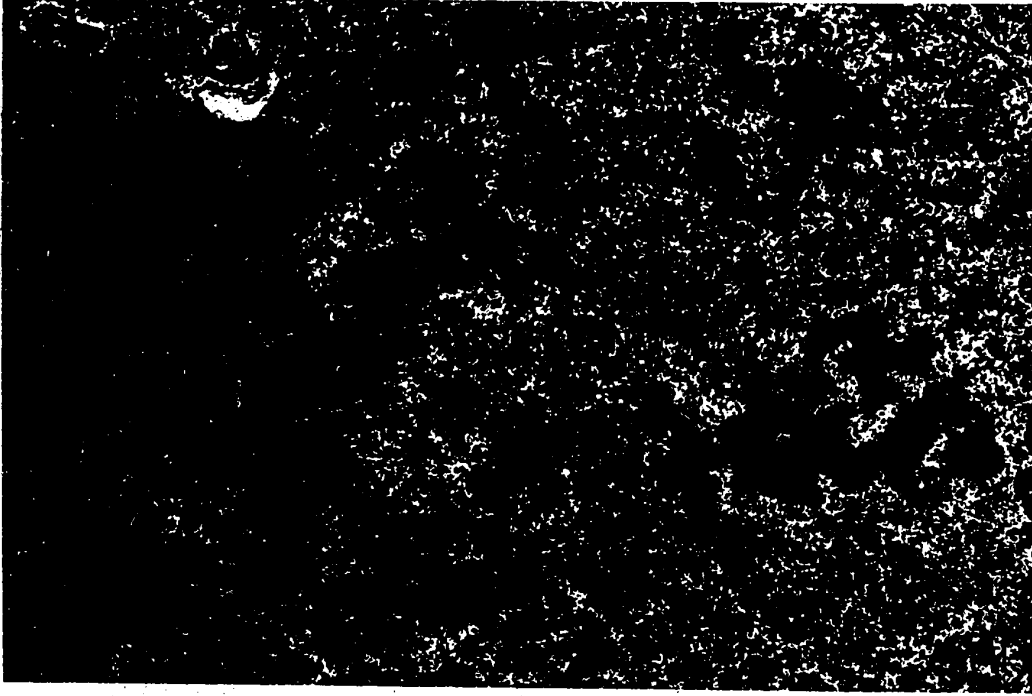


FIGURE 14. A synaptonemal complex (SC) is shown forming in the nucleus (N).

x 20,000

FIGURE 15. The association of chromatin material with the synaptonemal complex forms a bivalent (BV). The centre of the complex is composed of zygosomes (Z). Note the centriole (CT) in the lower left.

x 42,000



7

FIGURE 16. The synaptonemal complex (SC) appears as
a twisted ribbon when sectioned parallel to its long
axis.

x 17,000

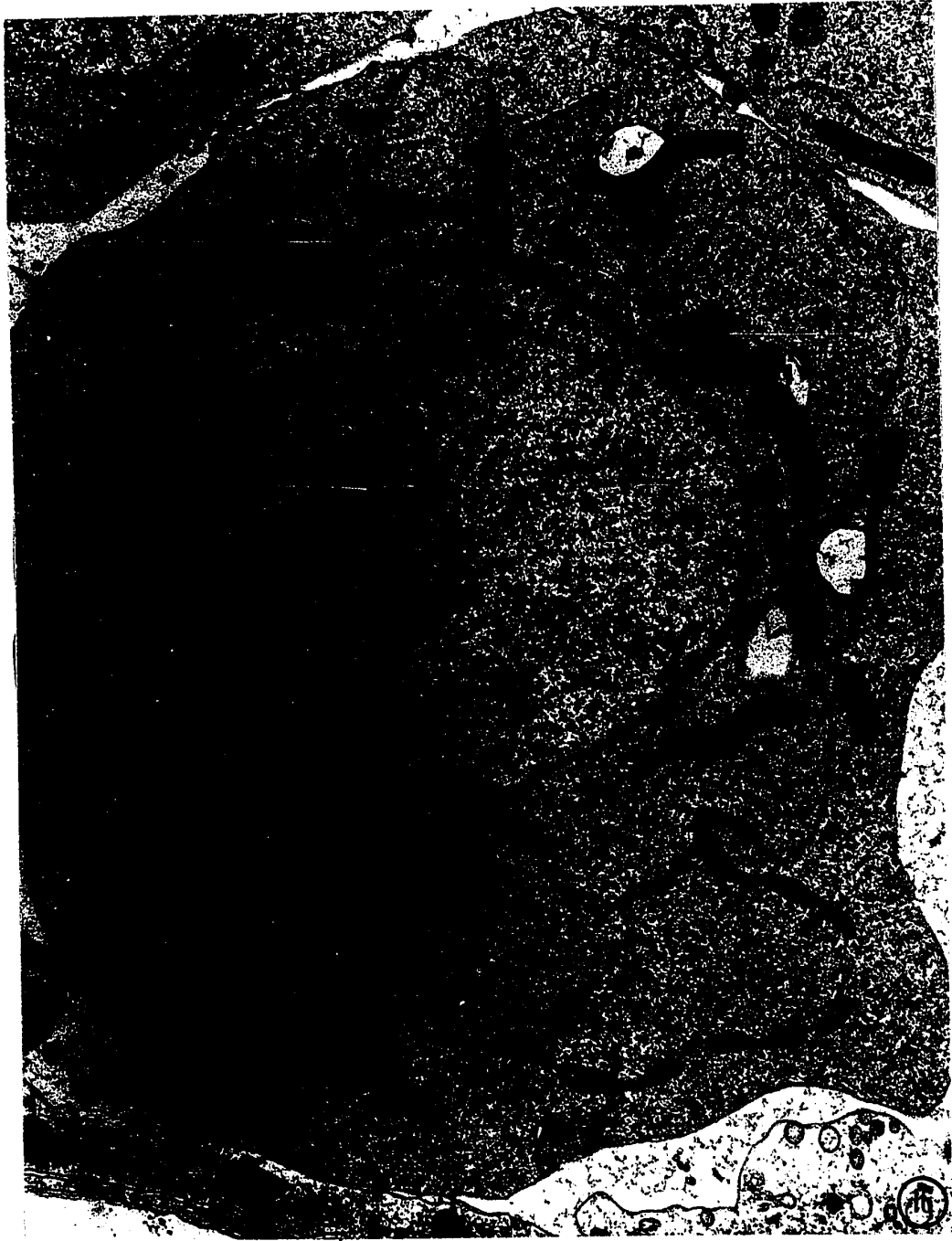


FIGURE 17. Concentric layers of endoplasmic reticulum (ER) are shown forming around the nucleus. Note the partitioning of the mitochondria (M) by the membranes. Mitochondria are of the round and filamentous type.

x 14,600

FIGURE 18. Layers of membranes are also present. Mitochondria (M) are of the round type.

x 20,000

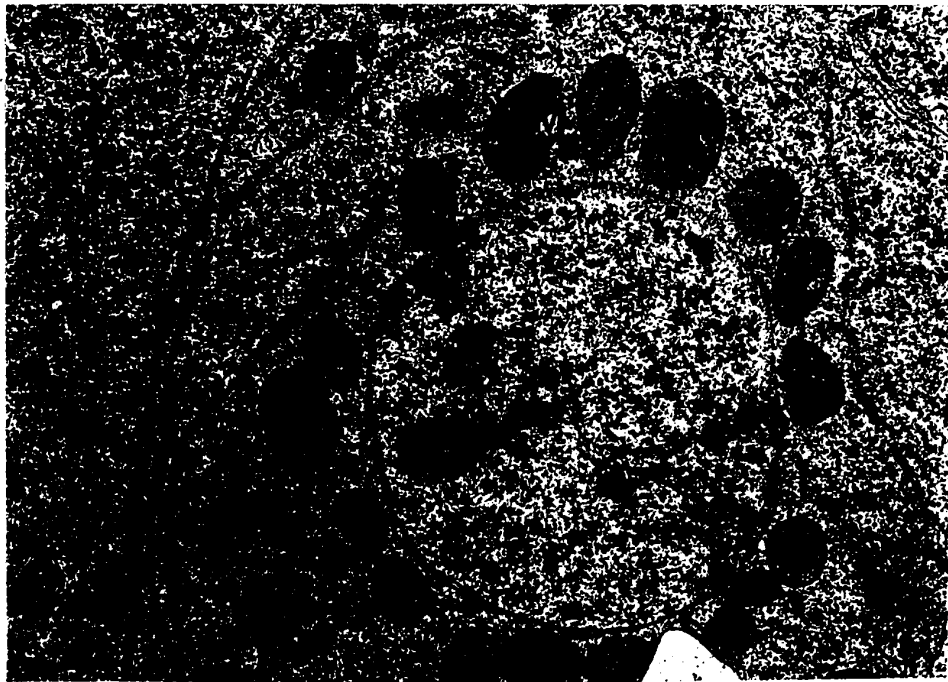
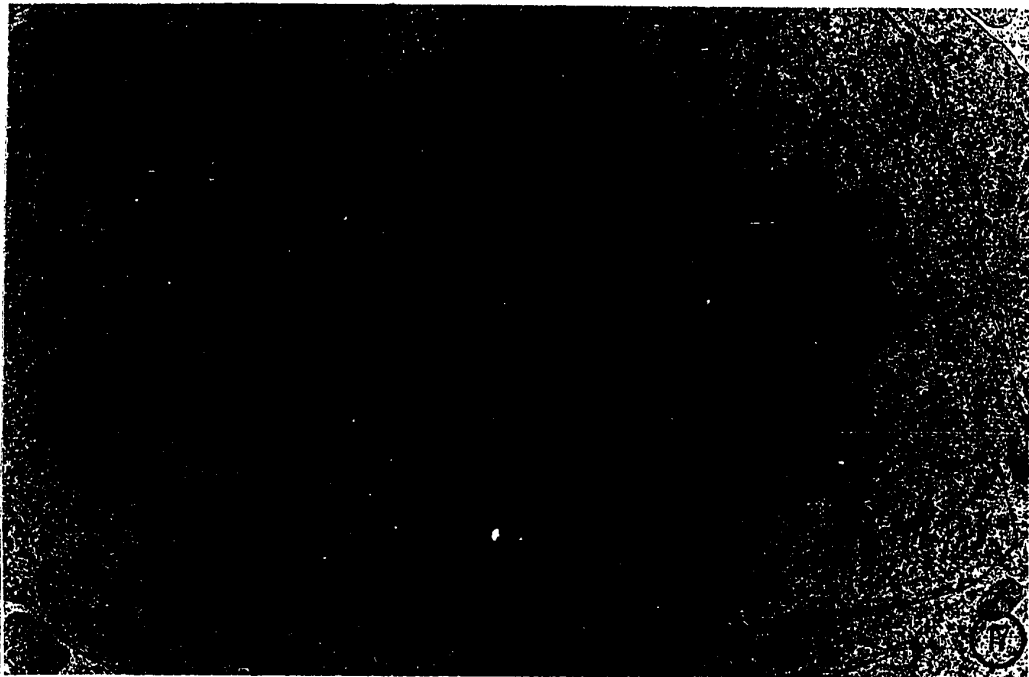


FIGURE 19. Two spermatids are shown after the second meiotic division. Each is characterized by the possession of a nebenkern body (NK), an oblong nucleus (N), Golgi complex (GC) and much endoplasmic reticulum (ER). Note the axial filament (AF).

x 19,000

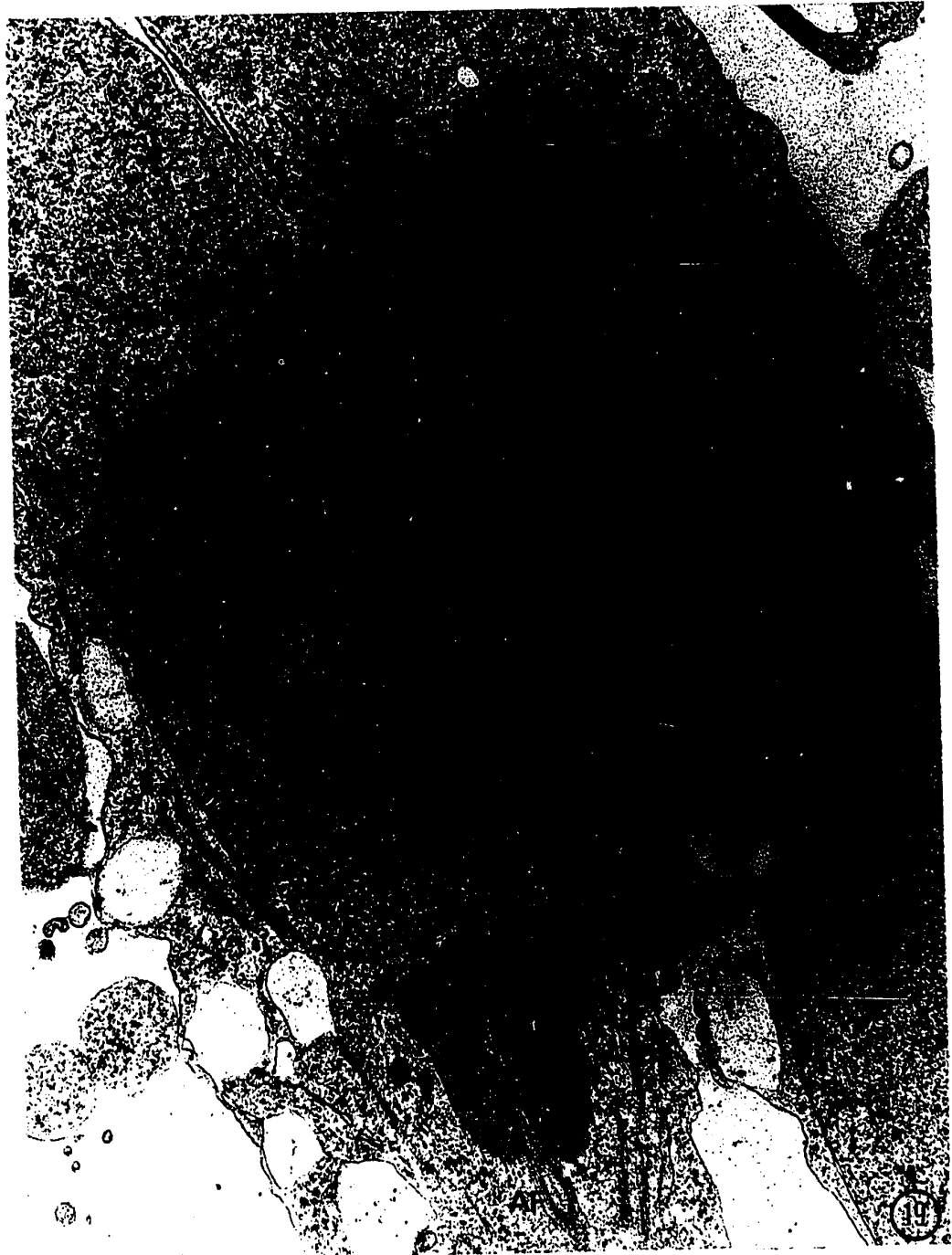


FIGURE 20. The membranes (ER) partition off the
cytoplasm leaving the central nucleus.

x 11,800

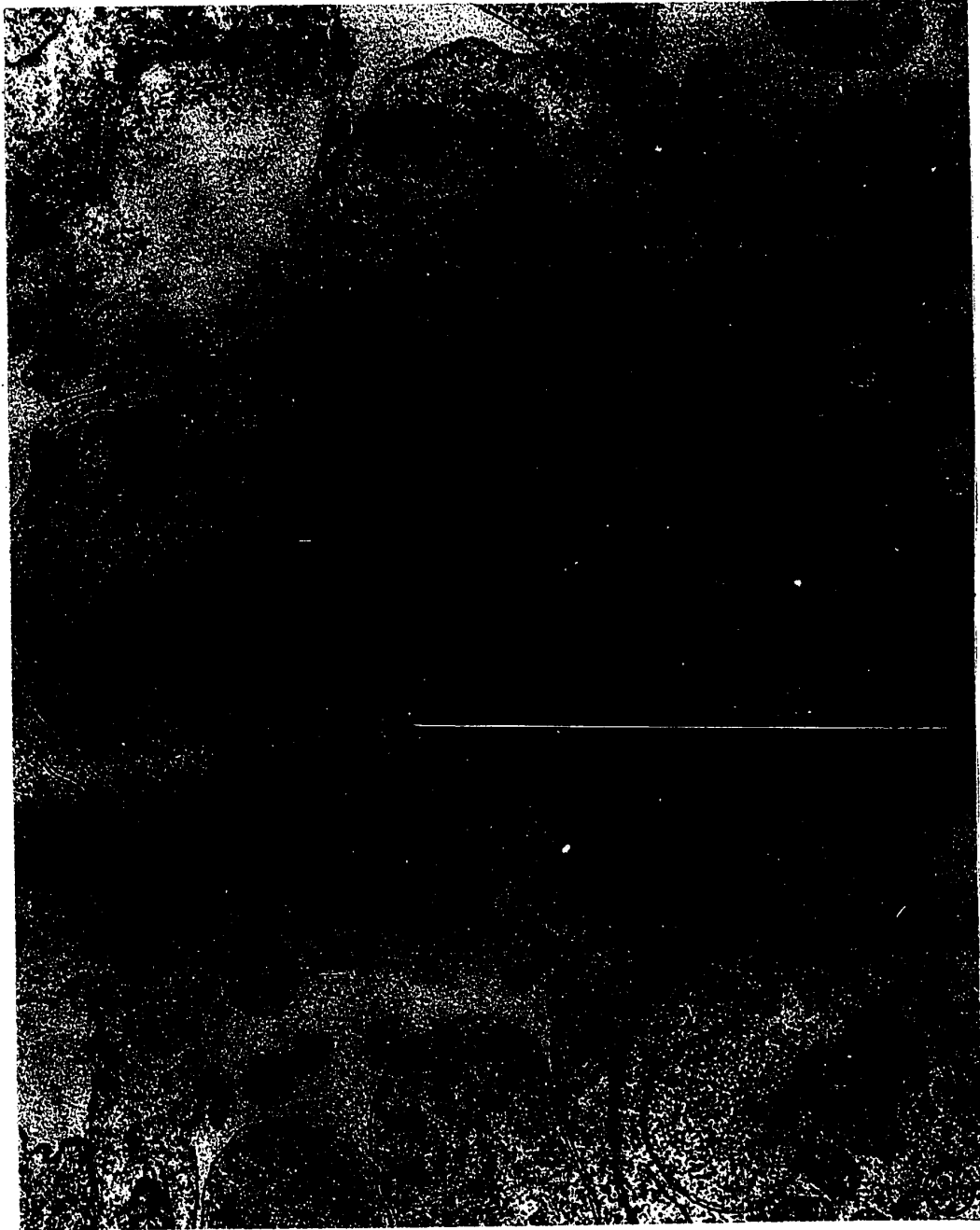


FIGURE 21. The concentric rings are shed as the space between the membranes enlarge. Note cytoplasmic debris (CD) in the rings.

x 18,000



FIGURE 22. The membranes degenerate and tiny beaded
remains occur in the lower end of the testes. Note
portions of spermatids (SP).

x 24,000



FIGURE 23. After the last stage of meiosis the nucleus(N) becomes oblong and the nucleolus (NL) becomes dense. Note numerous nuclear pores (NP) appearing as a dark line.

x 29,000

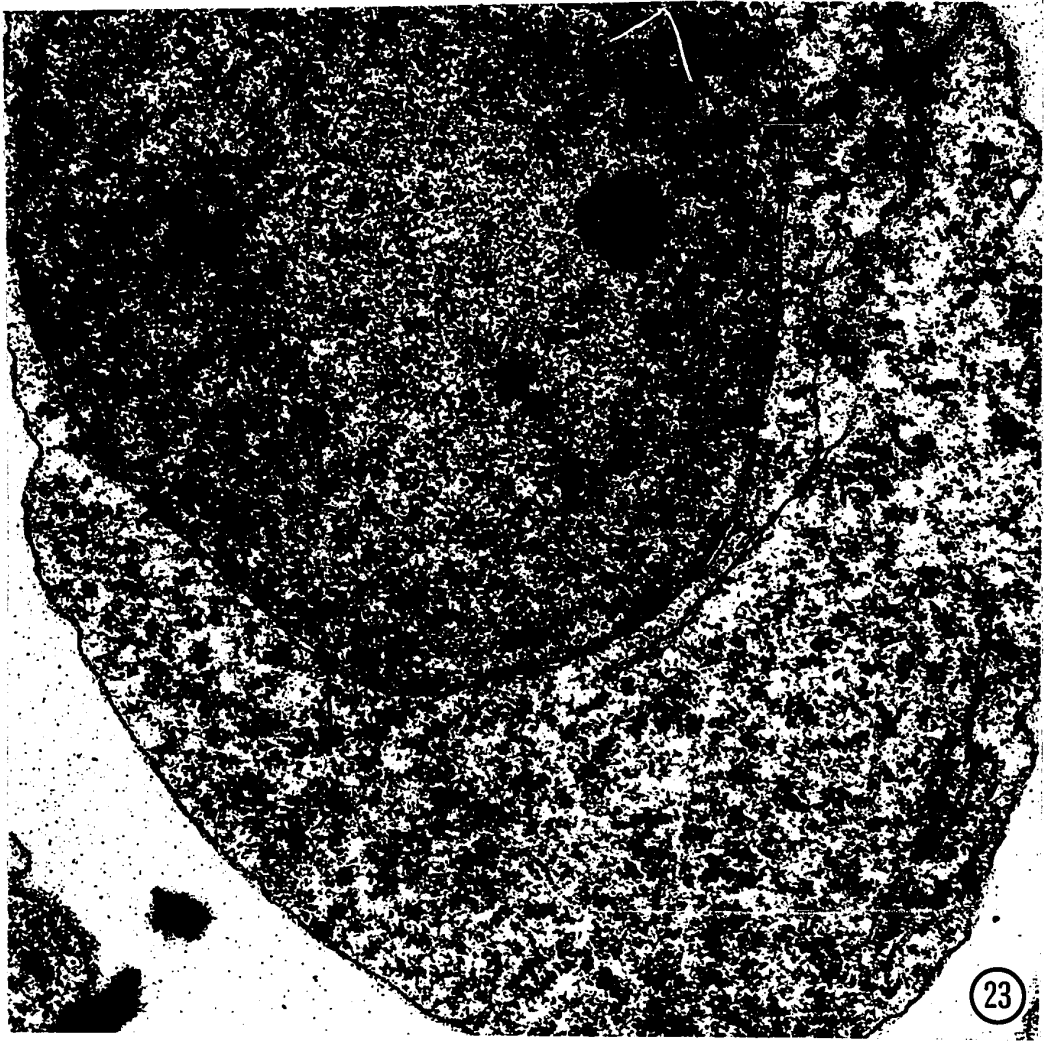
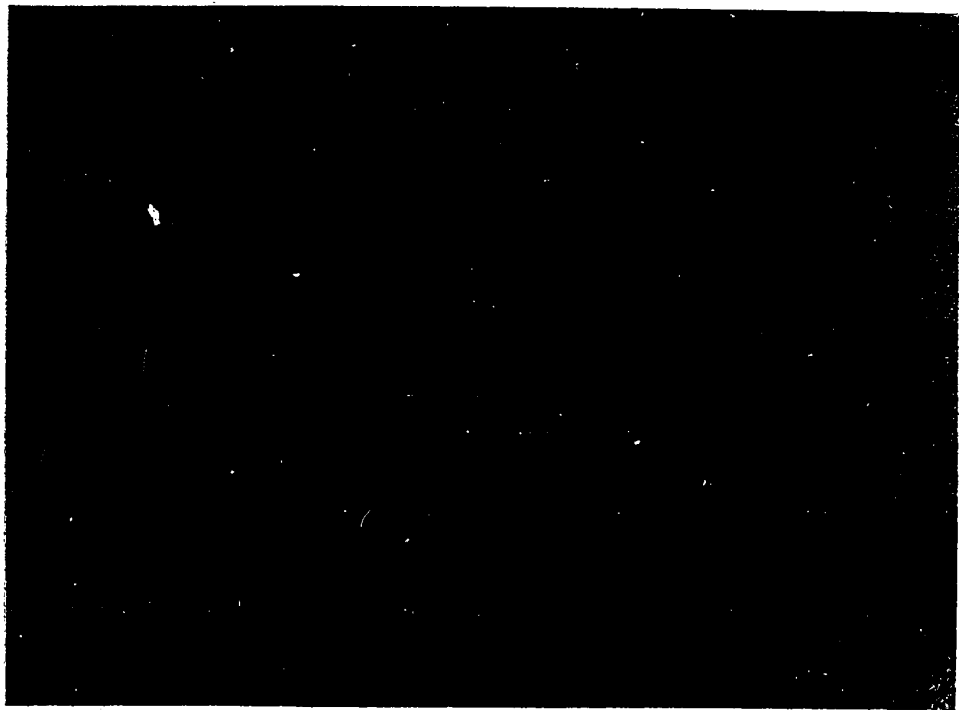
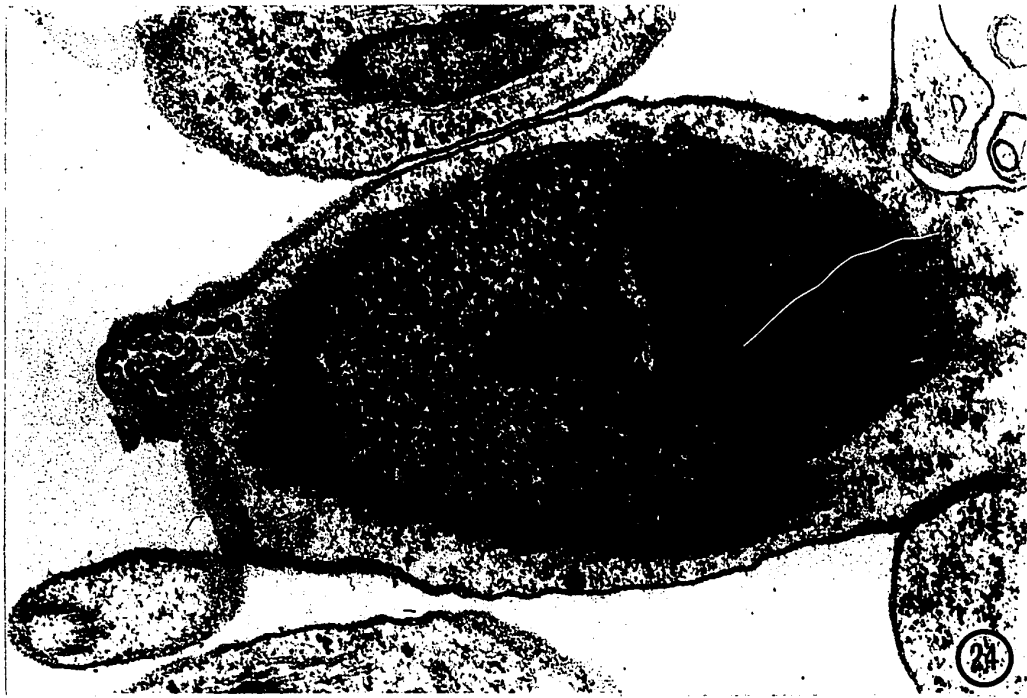


FIGURE 24. The nucleus (N) is surrounded by a sheath of microtubules (MT). The centriole adjunct (CA) exhibits a vesicular substructure.

x 33,000

FIGURE 25. The nucleus begin to elongate and the nuclear envelope appears single. Note the indentation for the centriole (CT) and the microtubules surrounding the nucleus (N) and centriole adjunct (CA).

x 33,000



FIGURES 26 and 27. The condensation of the chromatin material forms scattered filament of varying length.

Fig. 26 x 16,500

Fig. 27 x 33,000



FIGURE 28. The near mature spermatid nucleus (N) has a diameter approximately the same diameter as the tail piece (TP).

x 25,000

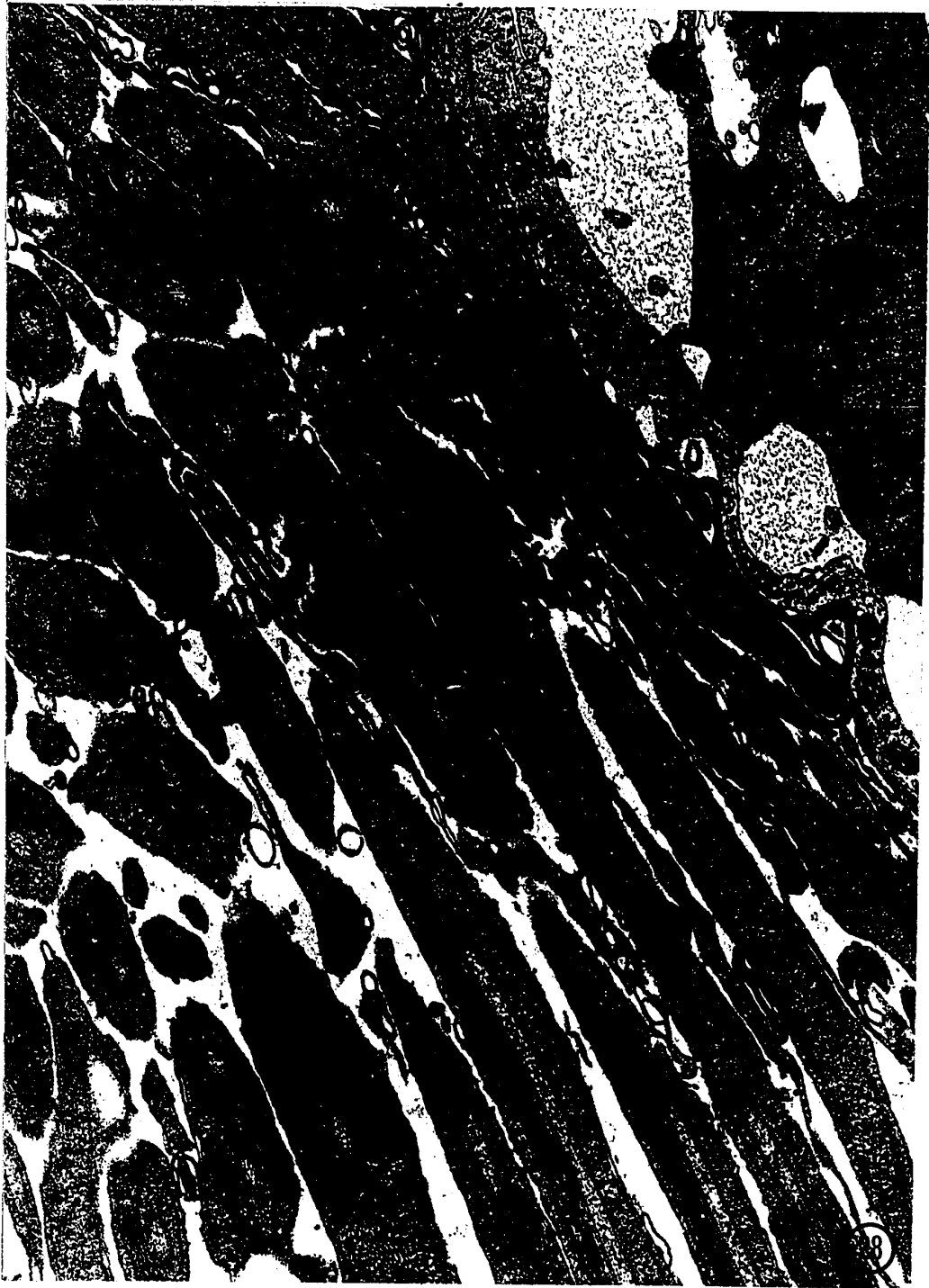


FIGURE 29. Nebenkern body formation (NK) is observed
as a clustering of mitochondria in one area of the
spermatocyte.

x 22,000

FIGURE 30. Fusion of the mitochondria (M) in the
nebenkern give them a filamentous appearance.

x 15,000

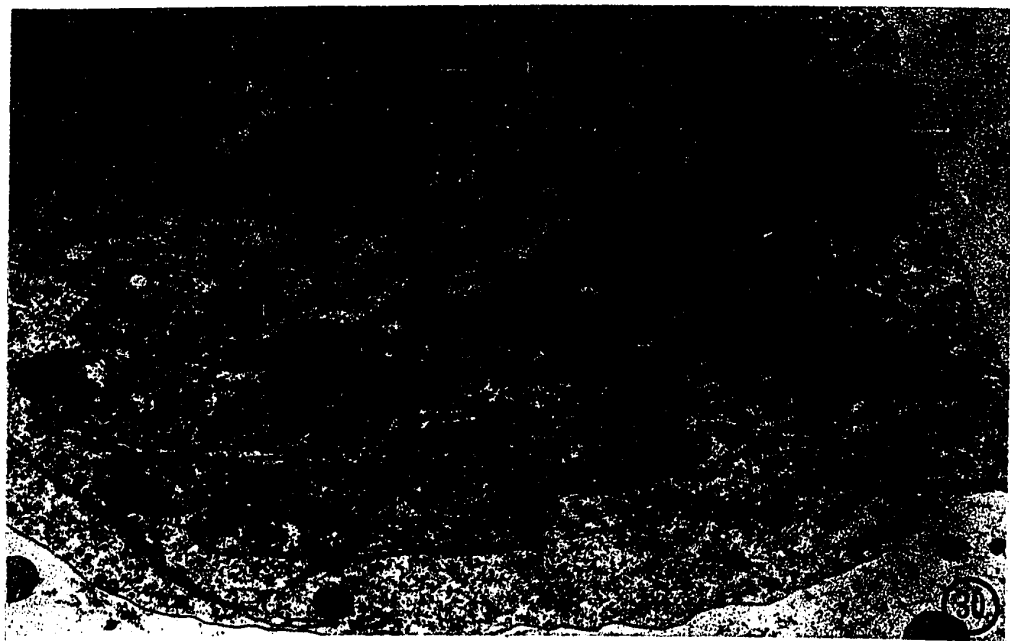
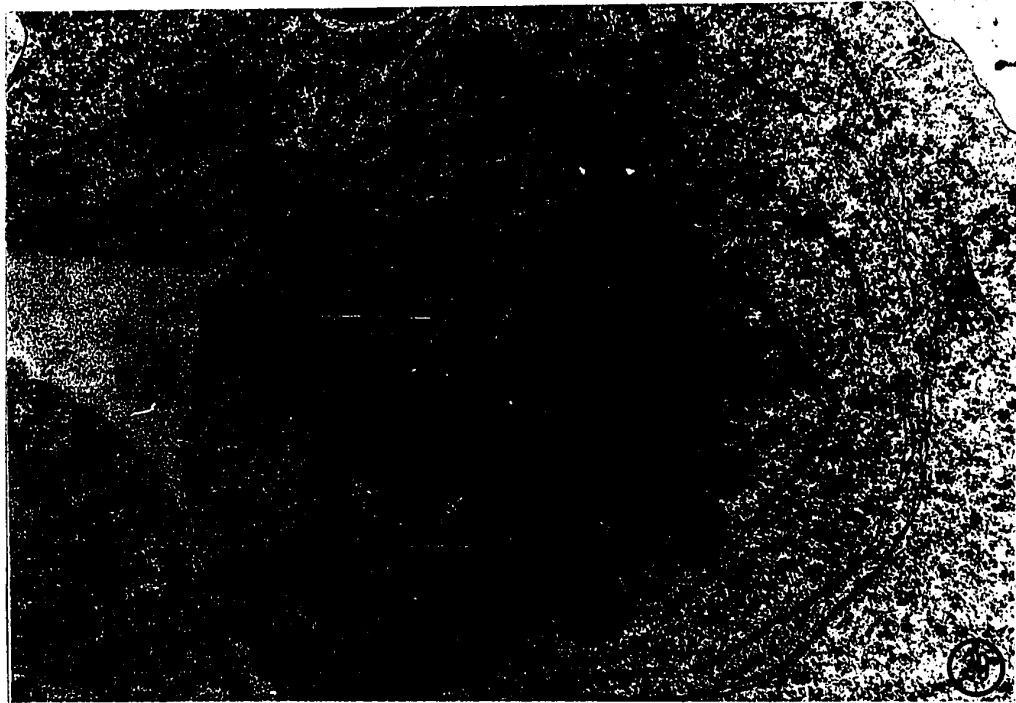


FIGURE 31. The nebenkern body (NK) remains closely associated with the nucleus (N) axial filament (AF) and the centriole (CT).

x 16,000

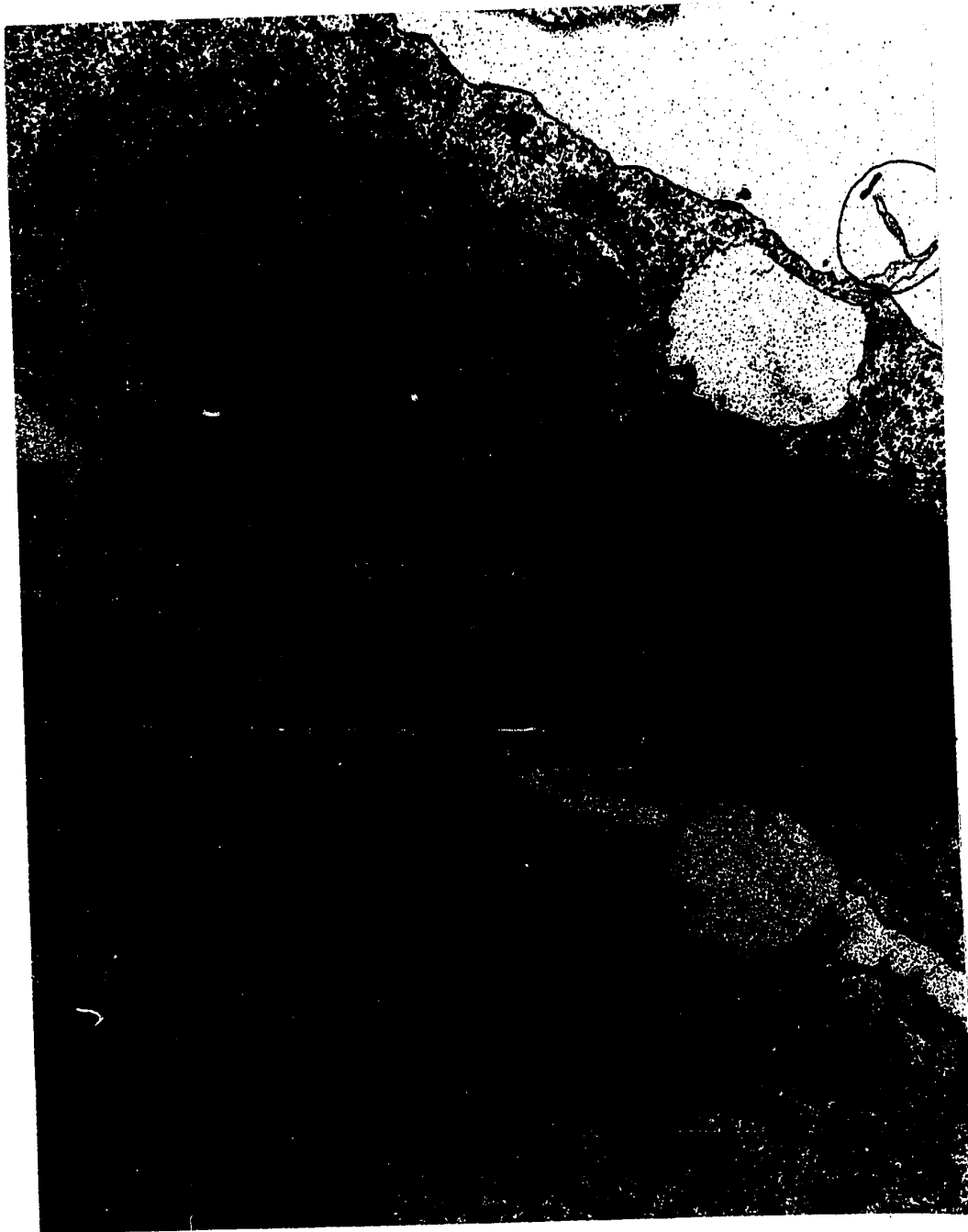


FIGURE 32. The nebenkern halves (NK) begin to elongate along the axial filament (AF).

x 22,000

FIGURE 33. The nebenkern halves begin an internal reorganization to form the mitochondrial derivatives (MD) which extend along the axial filament (AF).

x 38,000

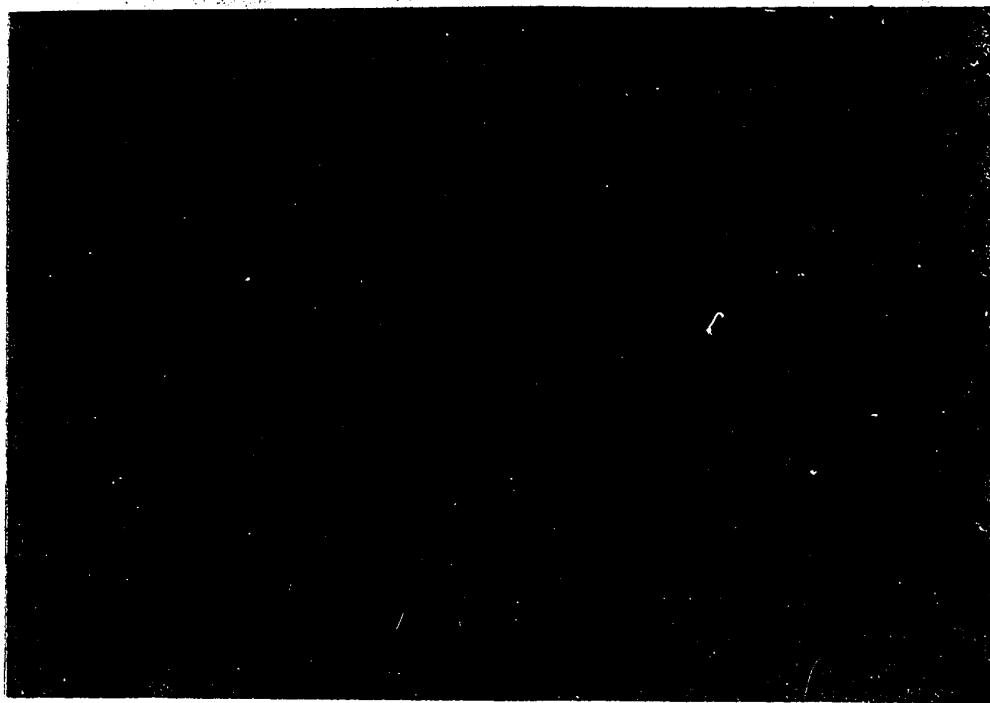
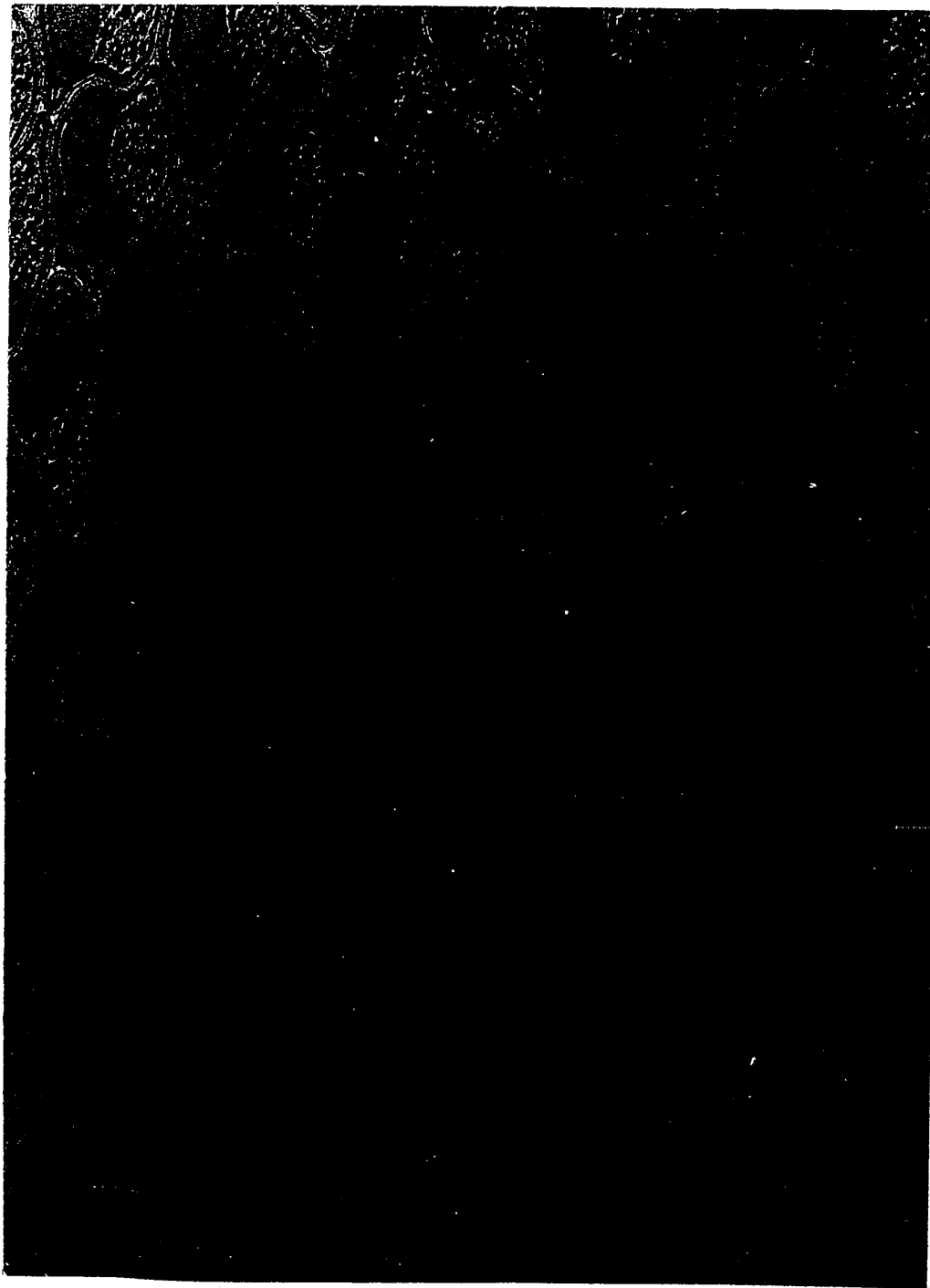


FIGURE 34. The axial filament of the spermatid are shown in cross section. Note central fibres (f), outer doublets (a and b) and the accessory fibres (ac), mitochondrial derivatives (MD), the muscular wall (MW) of the duct and the dark membrane between the mitochondrial derivatives and axial filament.

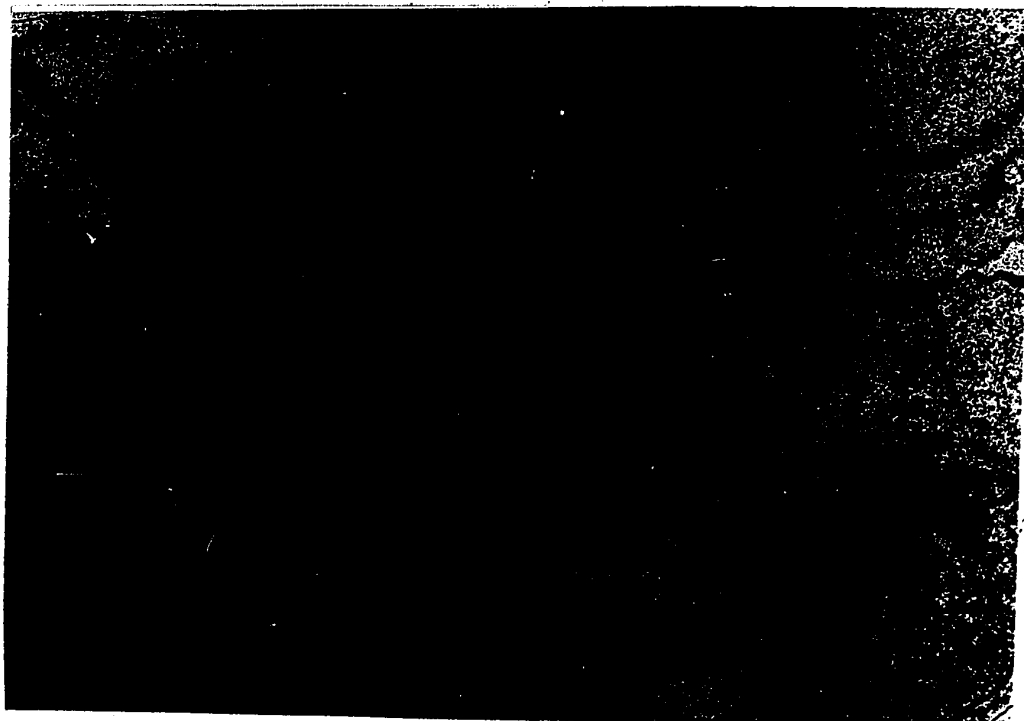
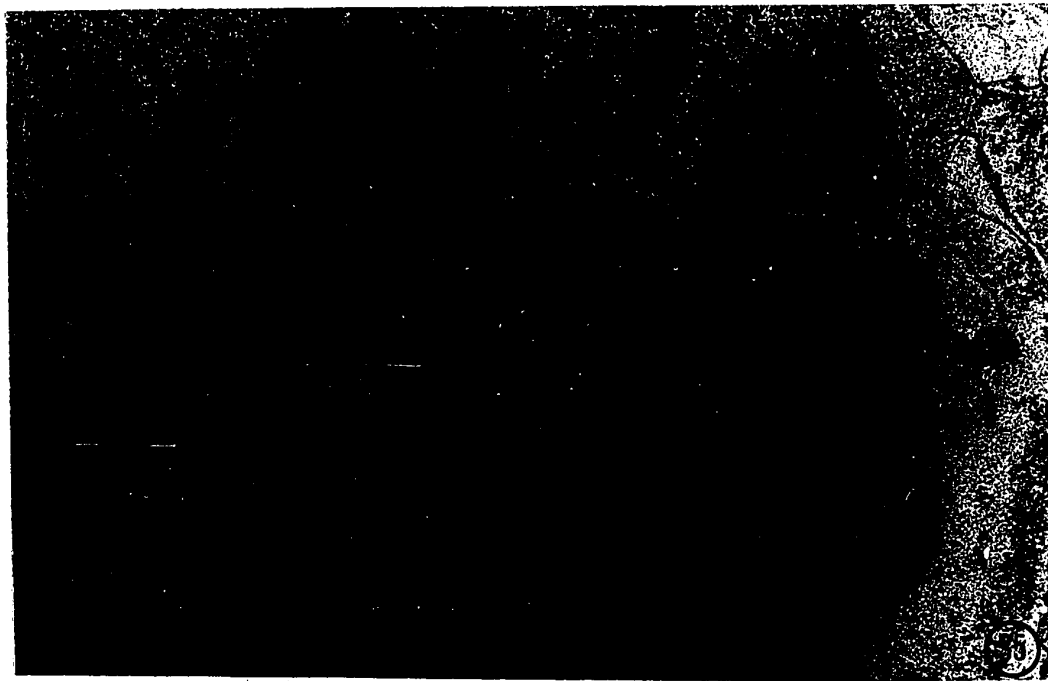
x 23,000



FIGURES 35 and 36. The nucleus (N) becomes orientated towards the centriole (CT). The axial filament (AF) begin to elongate. Note the association of the nebenkern (NK) with nucleus and the developing axial filament.

Fig. 35 x 19,000

Fig. 36 x 11,000



FIGURES 37 and 38. Note the lack of the accessory and central fibres in the developing axial filament. All fibres appear hollow.

x 25,000

FIGURE 39. Note the addition of all radial linkages (r) and the association of the mitochondrial derivative (MD) with the membrane of the axial filament.

x 34,000

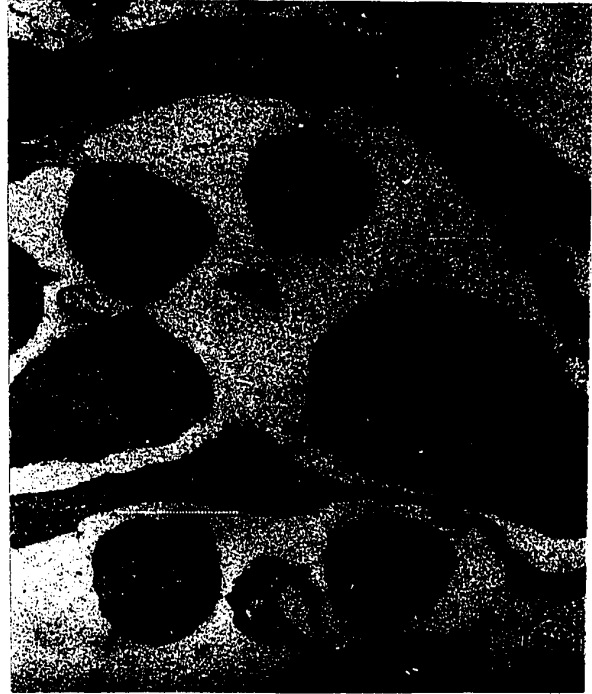
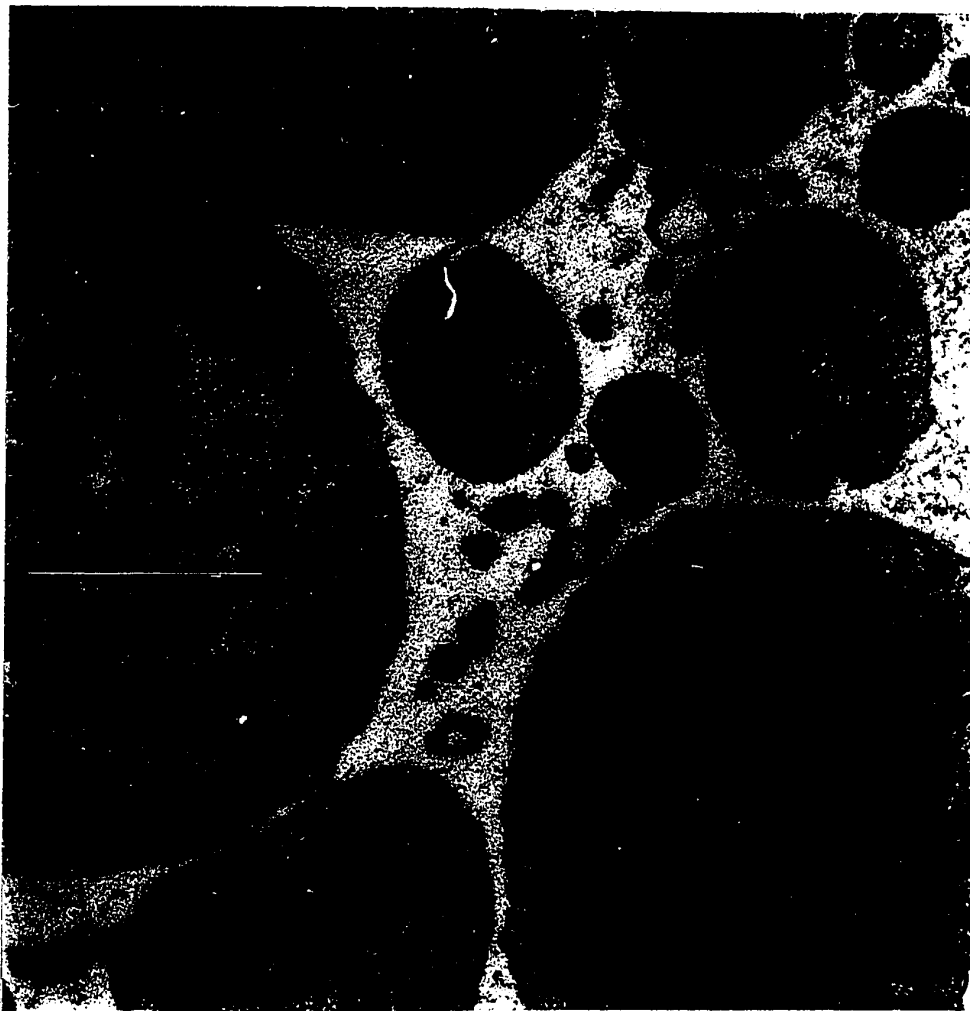


FIGURE 40. The centriole adjunct (CA) surrounds the base of the flagellum (F). The sheath of microtubules (MT) extending from the nucleus surround it.

x 34,000



7

FIGURE 41. A longitudinal section of a spermatid showing centriole adjunct (CA), nucleus, centriole (CT) and microtubules (MT). Note the distinct membrane extending along and under the adjunct (arrows).

x 51,000



FIGURE 42. Note the association of slightly osmophilic
dense masses (DB) with the Golgi complex (GC) and
the proximity of the Golgi complex with the
nucleus (N).

x 36,000



FIGURE 43. The Golgi complex (GC) has become more dense
and several bodies (DB) have increased in size.

x 24,000



7

FIGURE 44. Note the association of large bodies (DB) with the Golgi complex (GC) and the developing flagellum (F). The bodies exhibit a vesicular substructure.

x 29,000

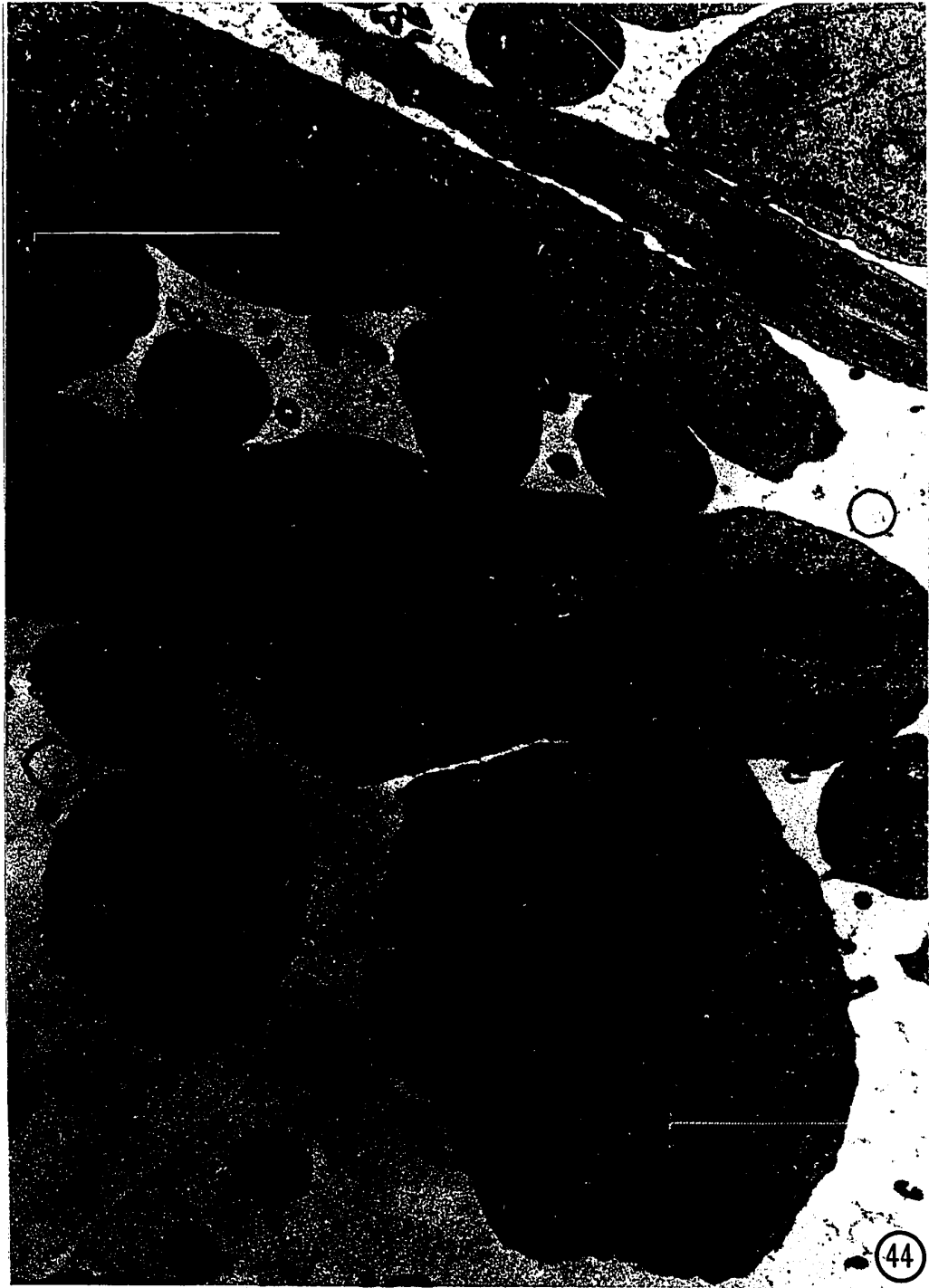


FIGURE 45. Fusion or rupturing of the dense bodies (DB)
is suggested.

x 28,000



FIGURE 46. Note the vesicular substructure of the centriole adjunct (CA) and the fusion of a body at the upper left of the adjunct.

x 36,000



FIGURE 47. The centriole adjunct may show vacuolated areas. Note also the large nuclear pores (NP) in the nuclear envelope.

x 31,000



FIGURE 48. Typical male producing spermatocytes showing endoplasmic reticulum (ER), mitochondria (M), Golgi complex.

x 9,000

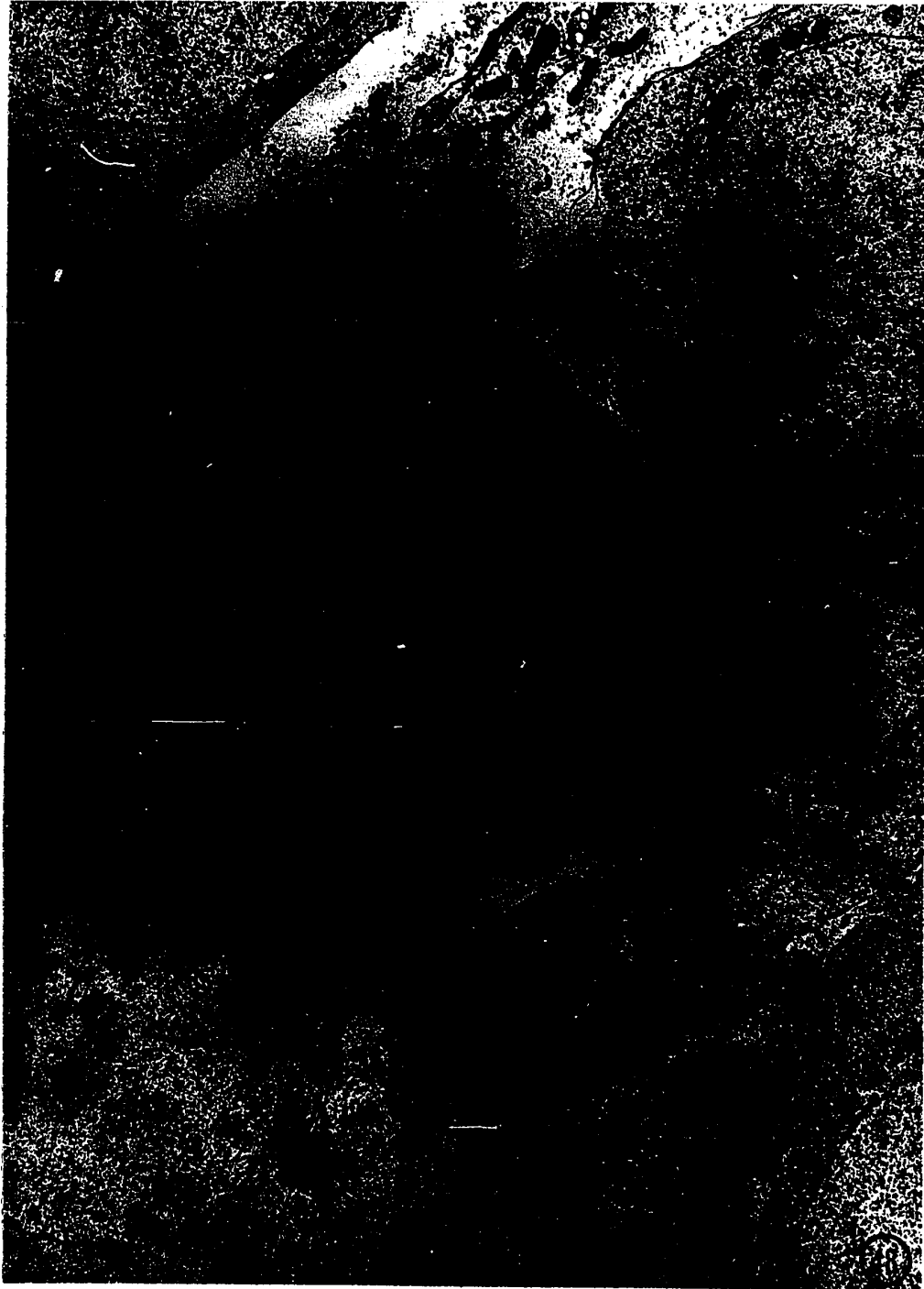
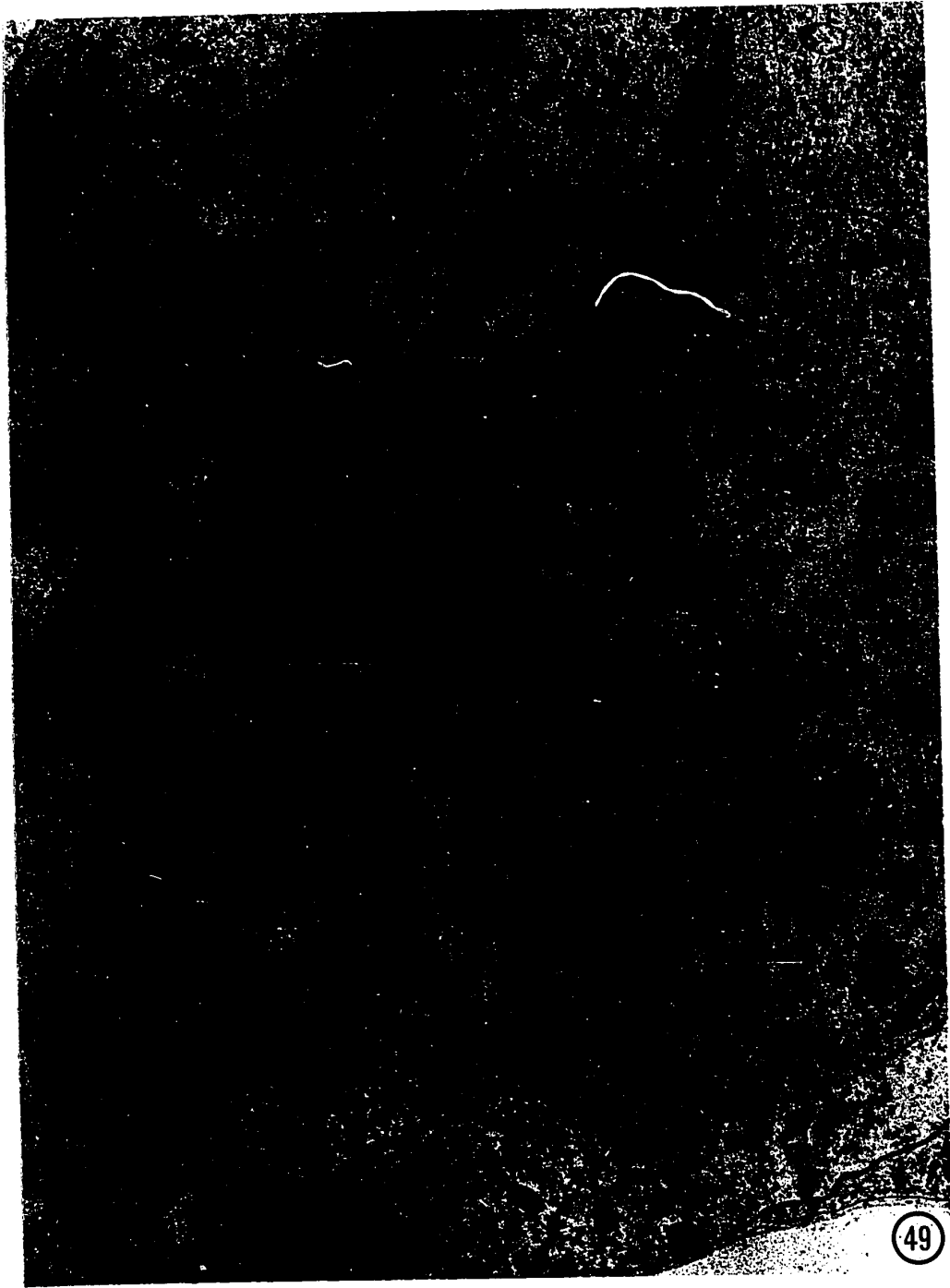


FIGURE 49. Note the association of electron dense material (ES) along the nuclear envelope, the double unit nuclear envelope (NE) and the eccentrically placed nucleolus (NL).

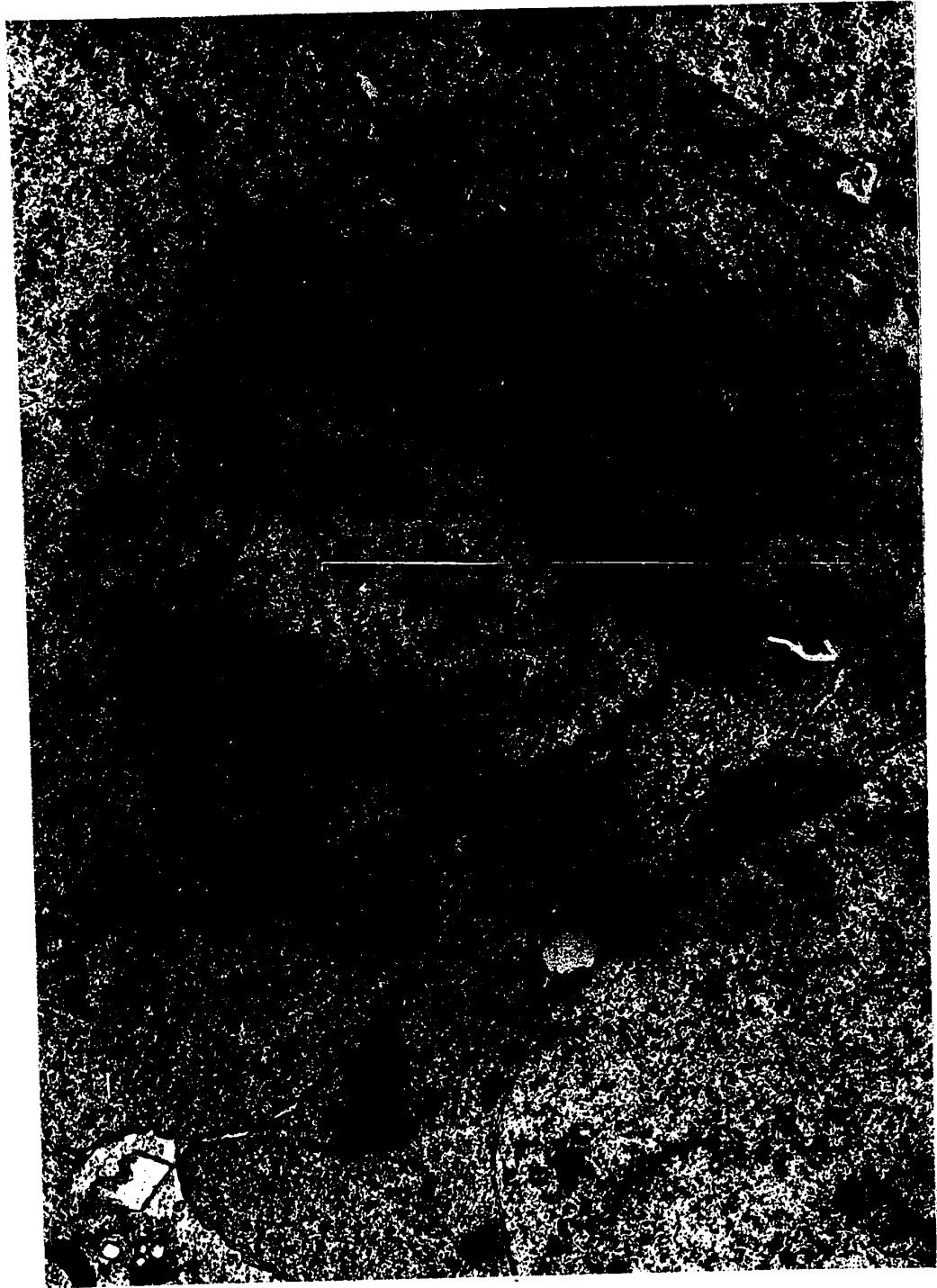
x 16,000



49

FIGURE 50. Note the numerous nuclear pores (NP) in the nuclear envelope, the remnants of the spindle fibres (SF) from the centriole (CT) and the Golgi complex (GC).

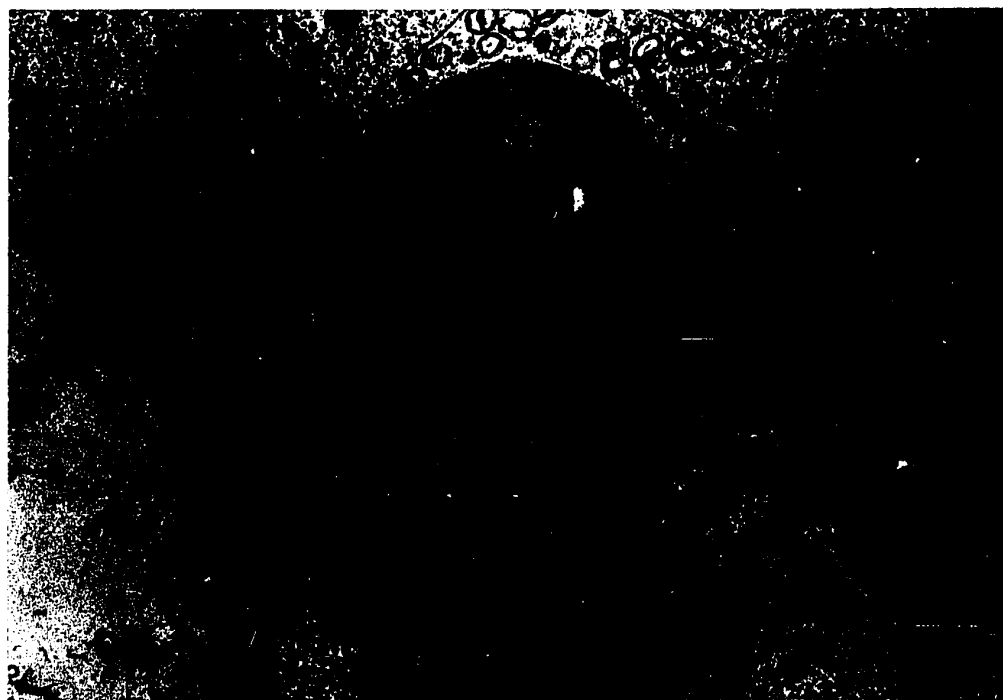
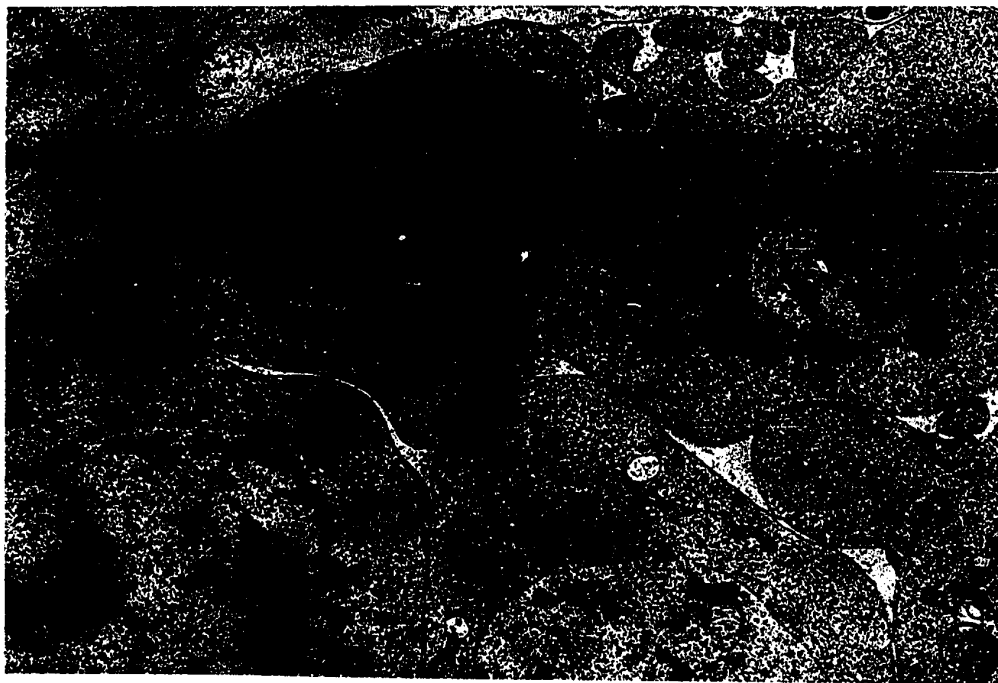
x 30,000



FIGURES 51 and 52. Note **one** spermatocyte (S) in each figure which appears abnormal. The nuclear matrix is more dense and the mitochondria (M) appear abnormal. Normal spermatocytes are found adjacent to these cells. A fibrous bundle (FB) appears in Fig. 51.

Fig. 51 x 11,000

Fig. 52 x 15,000



FIGURES 53 and 54. Note the formation of the synaptonemal complex (SC) and the association of the chromatin material with the complex to form the bivalent (BV). Remains of a spindle fibre (SF) is seen in Fig. 53.

Fig.53 x 24,000

Fig. 54 x 35,000

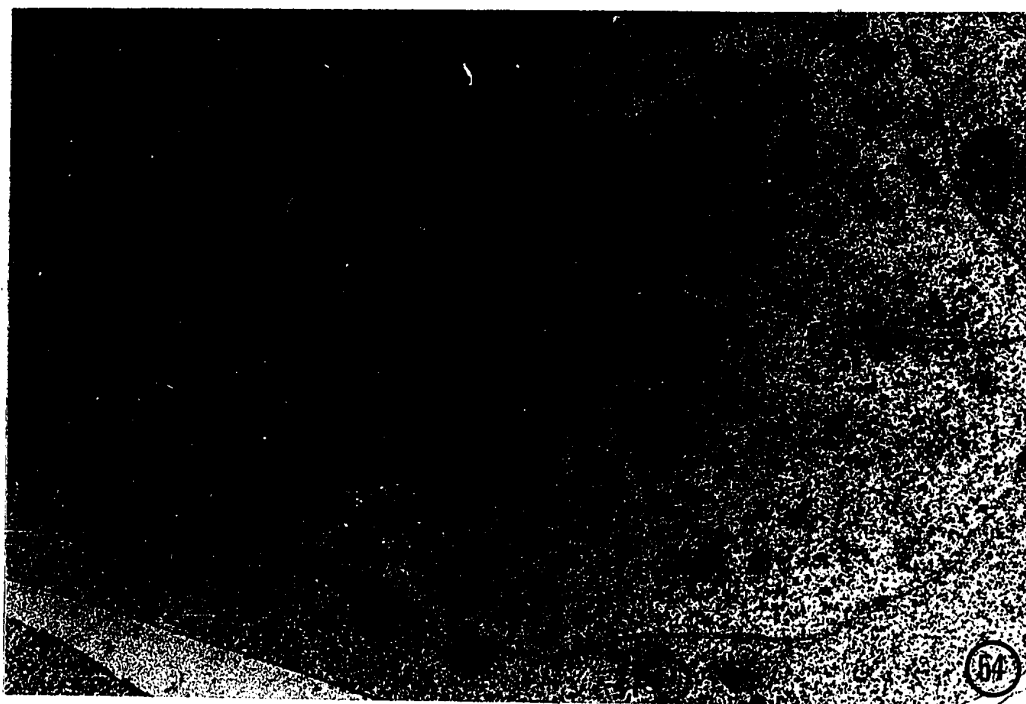
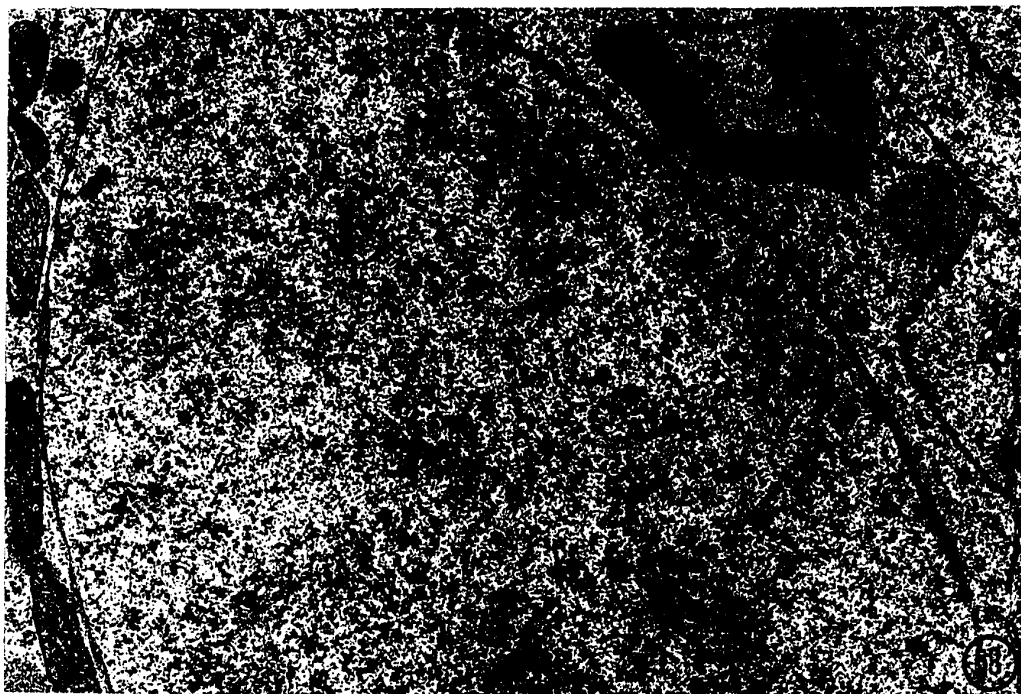


FIGURE 55. The lower end of the testes shows much accumulations of sloughed membranes as cytoplasmic debris(CD). The testicular wall (TW) is along the bottom of the figure.

x 19,000

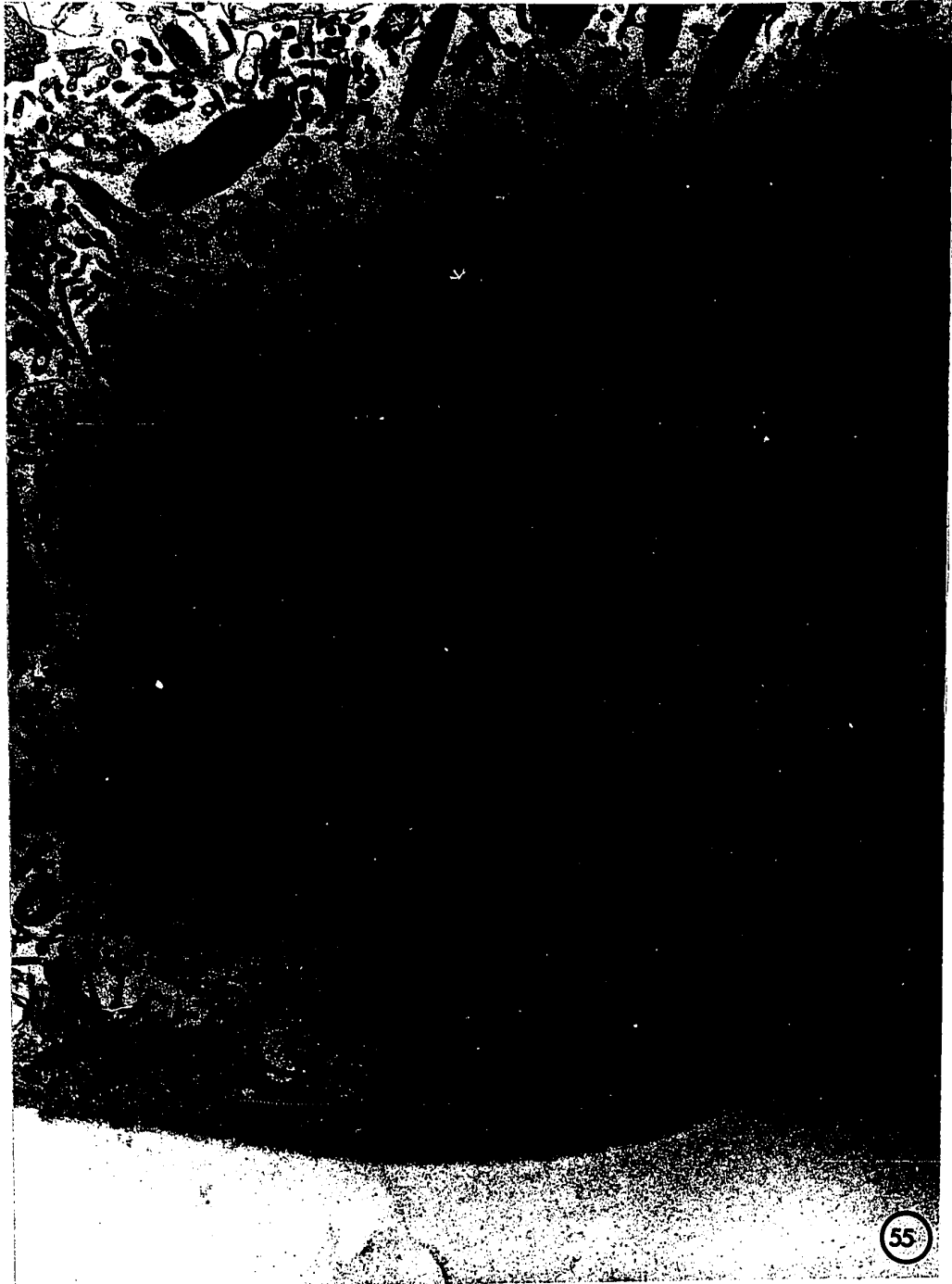


FIGURE 56. Note the condensation of the chromatin material into short fibres as in the normal.

x 21,000

FIGURE 57. Note the apparent loss of nuclear material from some of the heads (LH) while other heads are dark. Note also mitochondrial derivatives (MD), and the vesicular substructure of the centriole adjunct (CA).

x 24,000

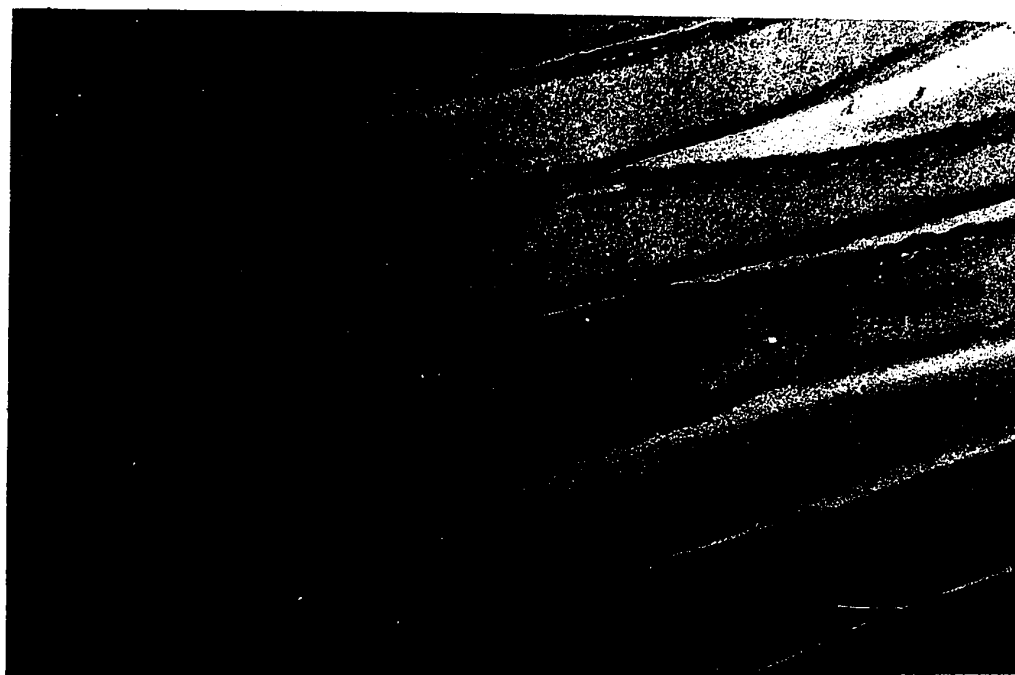
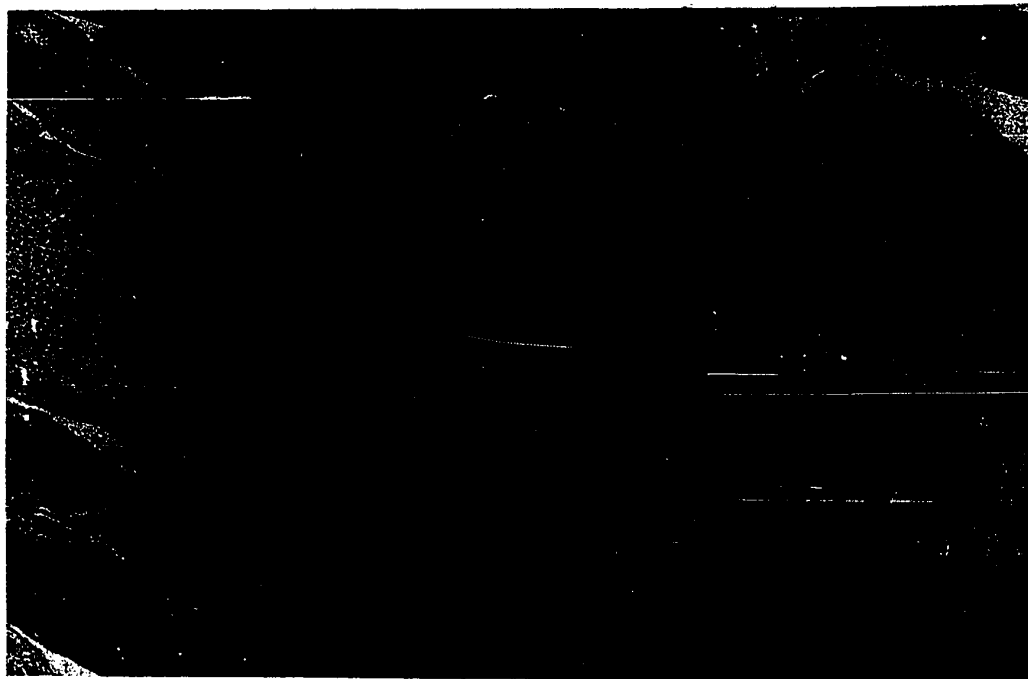


FIGURE 58. The formation of the nebenkern body (NB)
has the appearance identical to the nebenkern
in the normal strain.

x 16,000

FIGURE 59. Note two developing axial filaments (AF)
from a common cytoplasm.

x 19,000

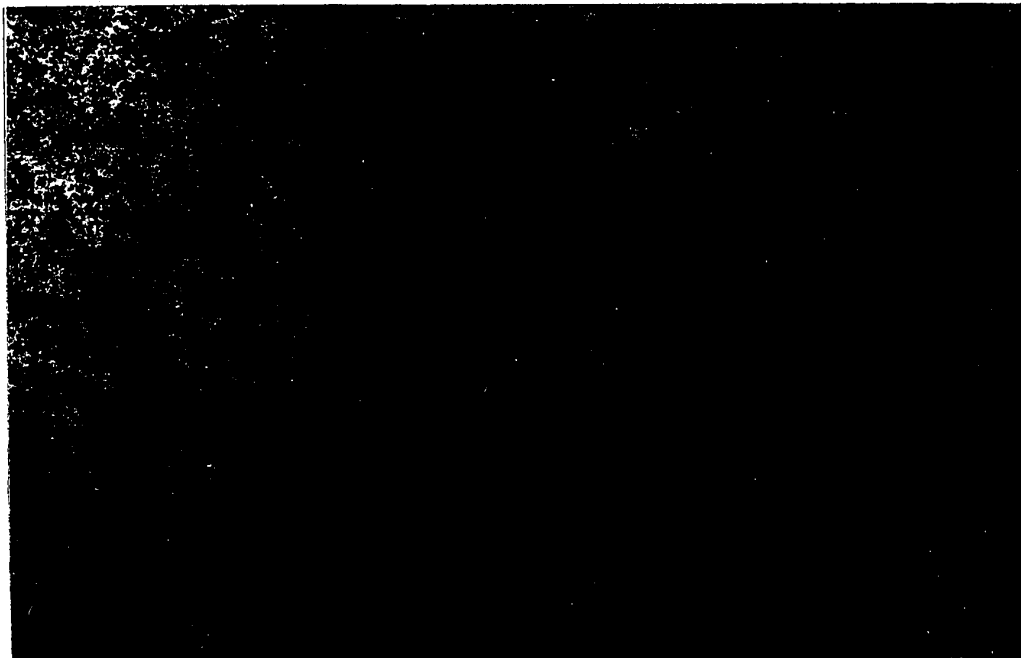
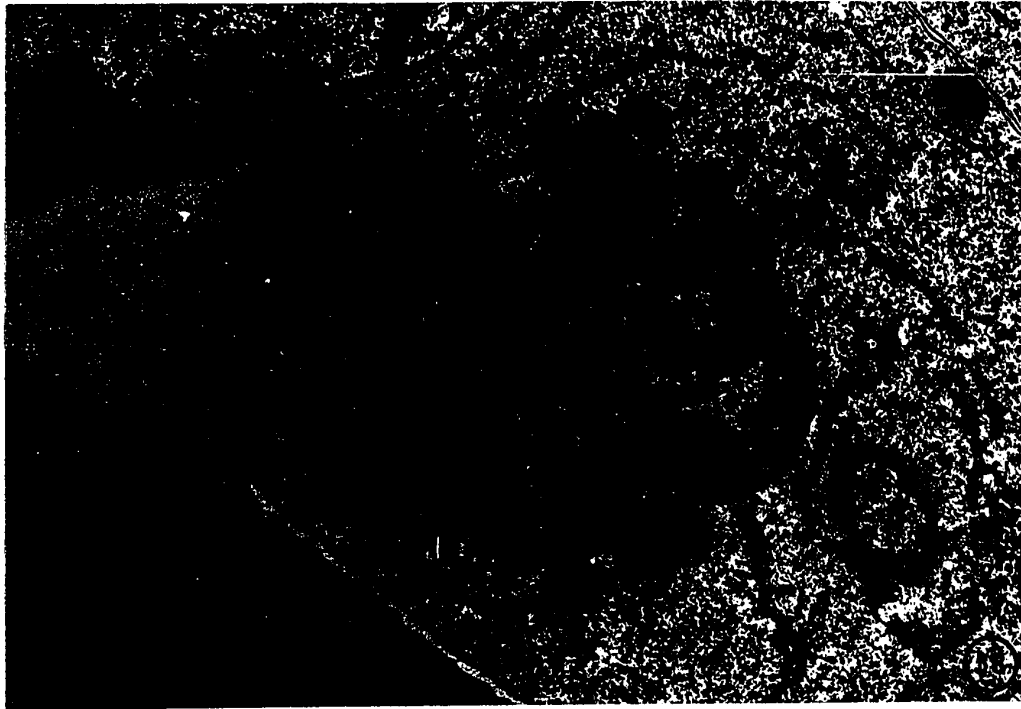


FIGURE 60. Note the occurrence of bundles of axial filament and mitochondrial derivatives. Some axial filaments are also "free" in the lumen and many are disrupted indicated by arrows. Many of the filament elements show central fibres and radial linkages.

x 19,000



FIGURE 61. Note the occurrence of several bundles of
axial filament and various cytoplasmic bodies (CB).

x 11,000



FIGURE 62. Note a bundle of axial filaments in longitudinal section.

x 12,000

FIGURE 63. Note the occurrence of tails with two axial filaments (U) and the occurrence of normal tails (V).

x 24,000

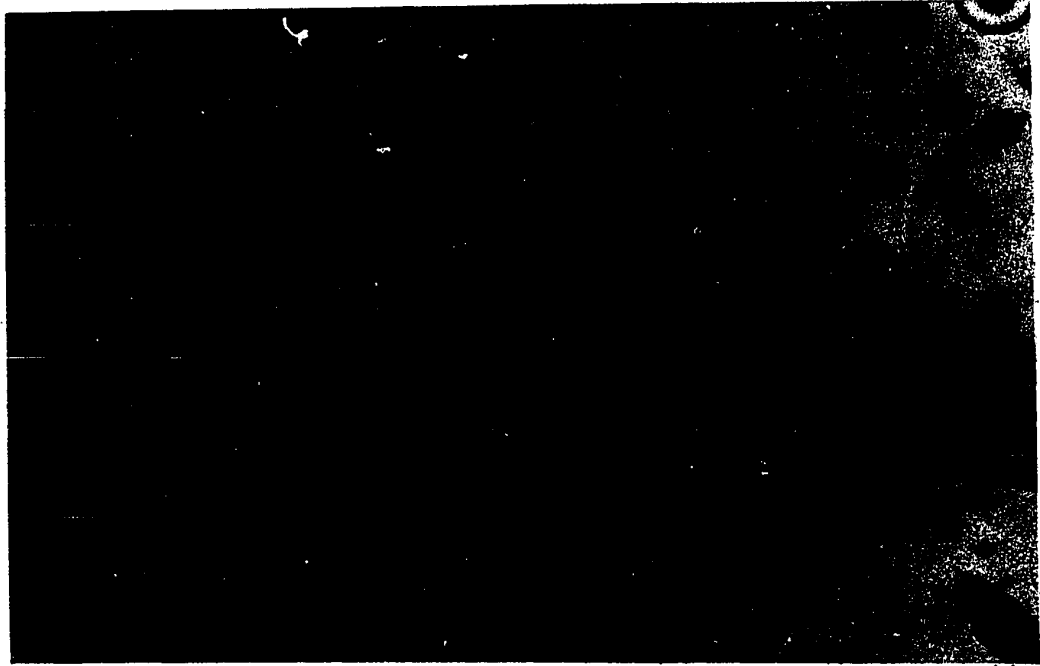
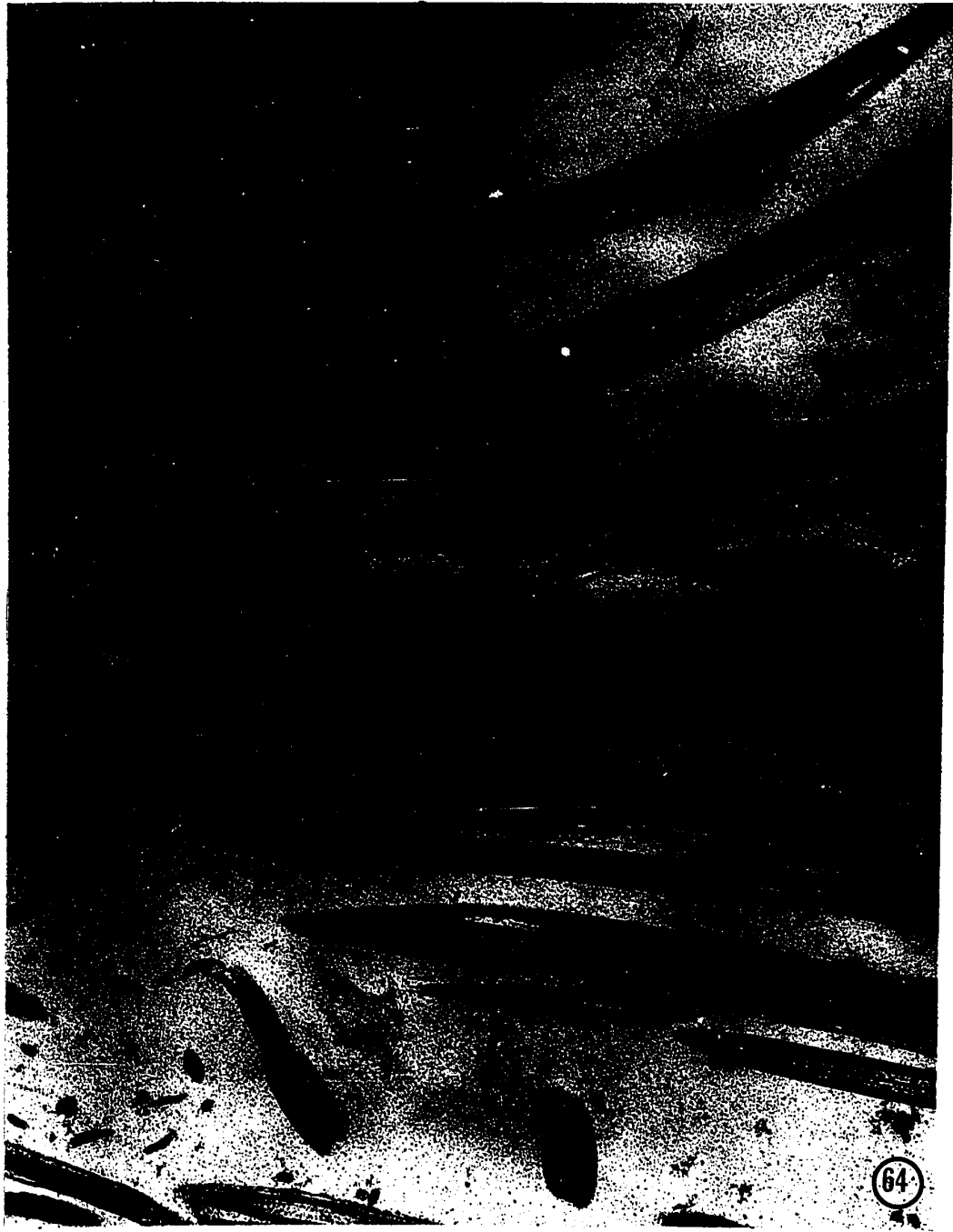


FIGURE 64. The axial filament of several spermatids are frayed from the main tail piece.

x 14,000



FIGURES 65 and 66. Note the large cytoplasmic bodies
(CB) in the lower testes. Many show myelin figures
(MF) and fibrous areas (F).

Fig. 65 x 14,000

Fig. 66 x 20,000



FIGURE 67. Note the occurrence of many axial filament
in a cytoplasmic body (CB) as well as "free" filament
units.

x 13,000



FIGURES 68 and 69. Many of the cytoplasmic bodies (CB) are phagocytized by the testicular wall (TW). Note the occurrence of a fibrous area (F).

x 12,000

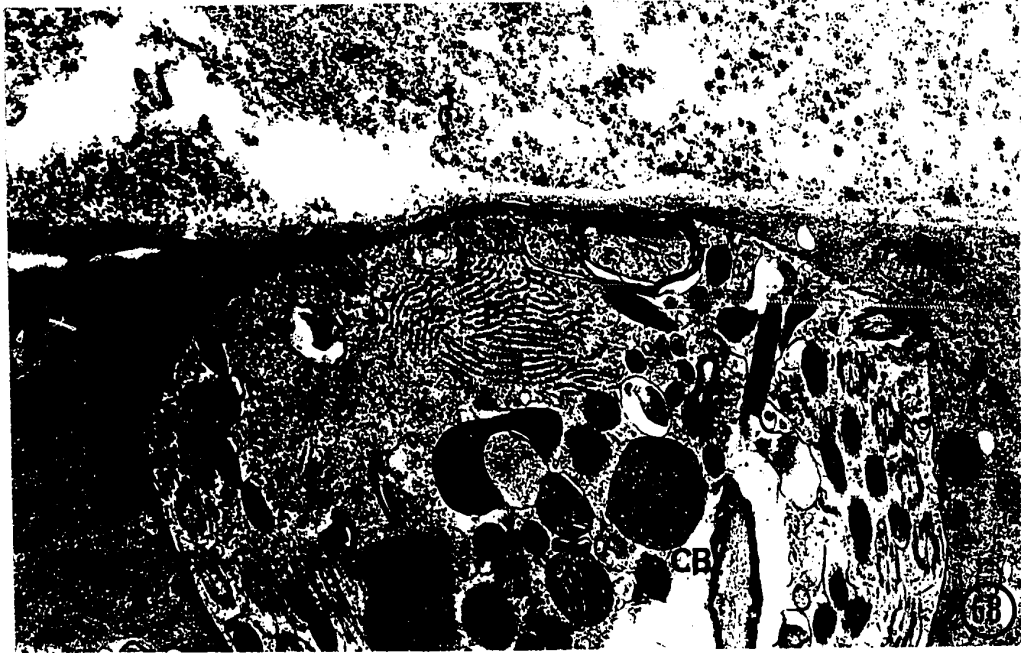


FIGURE 70. Note the hemocyte (H) among the other cytoplasmic bodies (CB). Portions of axial filament (AF) are contained in one end of the hemocyte.

x 14,000

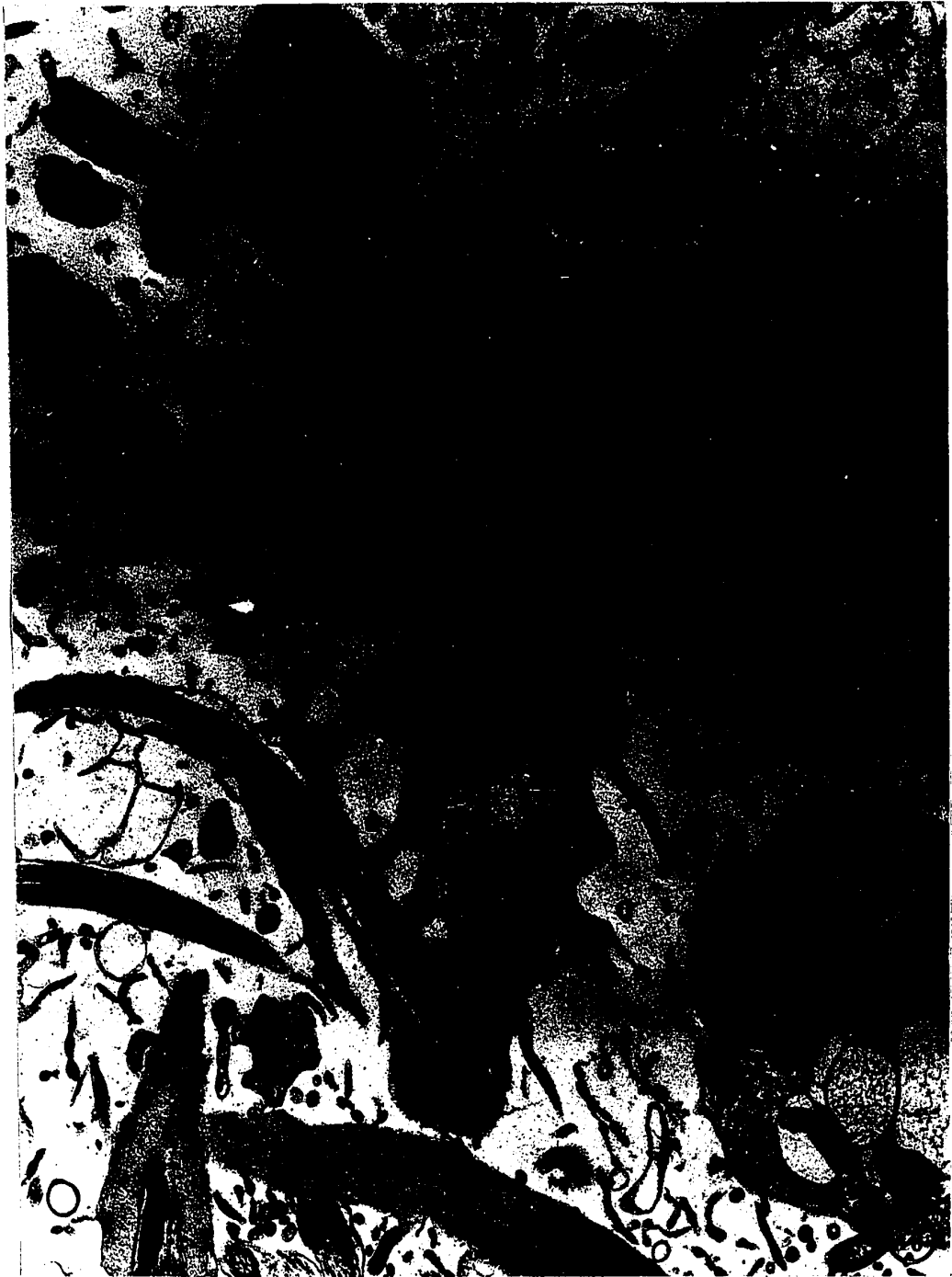


FIGURE 71. One of the cytoplasmic bodies found in the normal strain showing mitochondria (M) and vesicular appearing material (J).

x 24,000

