STUDIES ON THE FINE STRUCTURE OF THE MAMMALIAN TESTIS

I. Differentiation of the Spermatids in the Cat (Felis domestica)*

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Although spermatogenesis has been studied intensively by classical cytological methods for the past fifty years, many details of the complex cytological transformations involved have remained obscure because of the limited resolving power of the light microscope. The present electron microscope study of spermatogenesis in the cat provides new information bearing upon several of the points left unsettled by the light microscope. These include the relation of the Golgi complex of the spermatid to the idiosome and its role in the formation of the acrosome; the origin of the acrosomal vacuole and its contribution to the head cap; the nature of the caudal sheath or manchette; and the relationship of the developing spermatids to the Sertoli cells.

The first applications of the electron microscope to the study of thin sections of the testis were the works of Watson (32) and of Challice (3) on spermatogenesis in rodents. The pictures they obtained represented a marked improvement in resolution over the best attainable with the light microscope and their studies yielded valuable information. Since the publication of their papers, however, significant advances have been made in methods of fixation and microtomy (23, 26) which have made it possible, in the present study, to extend these earlier observations considerably with electron micrographs of higher magnification and better resolution.

Materials and Methods

Three adult male cats of mixed breed were used in the study. Small blocks of testis (2 $mm.^3$) were fixed for electron microscopy in 1 per cent osmium tetroxide buffered to pH 7.4–7.8 with the Michaelis veronal-acetate buffer as recommended by Palade (23). After 2½ hours' fixation the tissue was washed briefly in distilled water (15 minutes), dehydrated

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in increasing concentrations of ethyl alcohol (60, 80, 95, and 100 per cent), and infiltrated (3 hours) with three changes of *n*-butyl methacrylate monomer containing 2 per cent luperco CDB[1] as a catalyst. Polymerization was promoted by exposure to mild heat (45°C.) in an oven for 24 hours. Sections 25 to 35 mμ in thickness were cut on a Servall thin sectioning microtome (26) and examined without removing the plastic. Electron micrographs were taken with an RCA electron microscope model EMU-2E at original magnifications of 2000 to 8000 and were enlarged photographically to the desired size. The magnifications at the various settings of the microscope were determined by calibration with a replica of a diffraction grating ruled 30,000 lines to the inch.

Blocks of tissue from the same testes were fixed for light microscopy in Bouin's or Orth's fluid. These were embedded in paraffin and 5 μ sections were stained by hematoxylin and eosin or by the periodic acid–Schiff reaction counterstained with hematoxylin.

OBSERVATIONS

Spermatids.—The spermatid nucleus is round or oval in electron micrographs and the karyoplasm is comprised of closely packed granules of intermediate density 100 to 150 A in diameter, dotted here and there with coarser aggregates of denser granules (Figs. 3 and 4). With buffered osmium fixation the nucleolus is preserved and is easily recognizable but there are no local variations in density within the nucleus which are comparable to the chromatin pattern that is seen with the light microscope after the use of acid fixatives. The nucleus is enclosed by a pair of membranes, the inner one of which is considered to be the karyotheca proper while the outer one is regarded as the limiting membrane of the cytosome. In various somatic cell types, these two membranes are continuous with one another around the margins of pore-like openings connecting the karyoplasm and cytoplasm (33). Although such pores are fairly common in the nuclear membrane of spermatogonia in the cat, they are rarely if ever seen in the spermatids.

The cytoplasm is moderately dense and finely granular in character. The ergastoplasm or endoplasmic reticulum is represented by an extensive system of membrane-bounded canaliculi and flattened vesicles which run a serpentine course in the cytoplasm. The elements of this system are often expanded into sinuses of irregular shape which have a content of lower density than the surrounding cytoplasm (Figs. 2 and 6). The small (150 A) particles of ribonucleoprotein, generally associated with the outer surface of the endoplasmic reticulum in other cell types, are not found adhering to the membranes but are scattered individually or in small clusters throughout the cytoplasm of the spermatid.

The mitochondria are rather uniformly distributed in the cell and show no tendency to congregate at the periphery immediately subjacent to the plasma membrane as they do in the spermatids of the rat (22, 32). The internal structure of the mitochondria is less orderly than usual. The internal folds or ridges are irregular in outline and may lie parallel instead of perpendicular to the

[1] Luperco CDB a preparation of 2,4-dichlorobenzoyl peroxide manufactured by the Novadel-agene Corp., Buffalo.

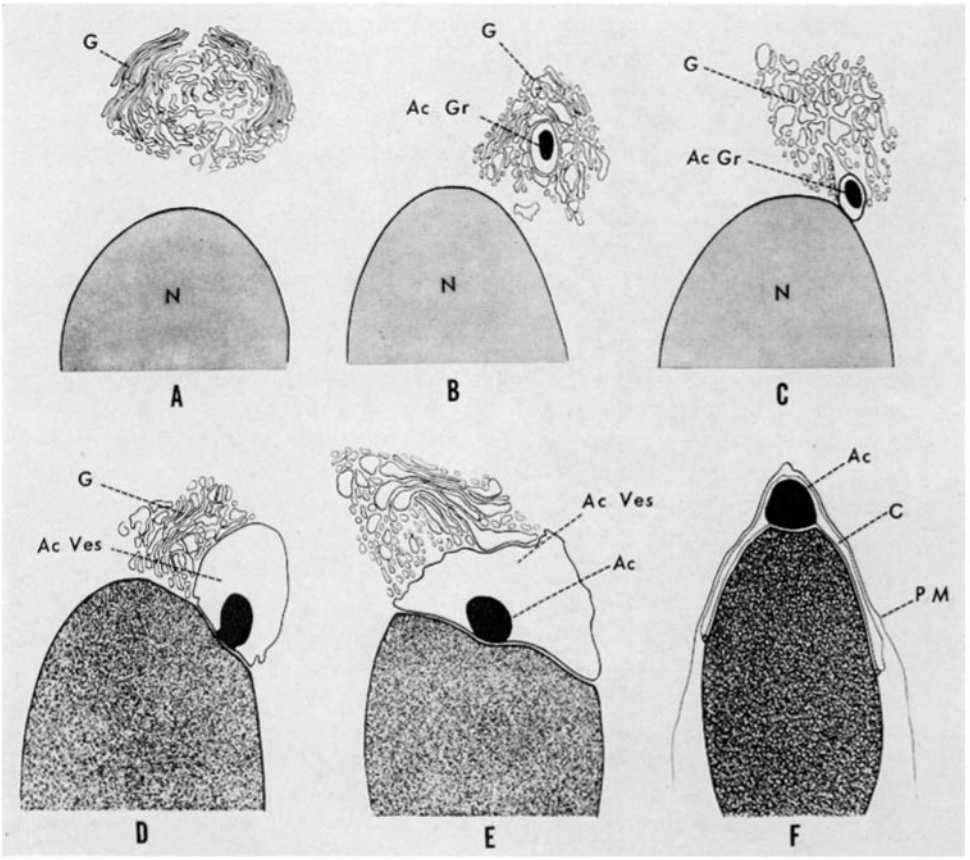
mitochondrial surface. Thus the center of the organelle is often free of membranes and occupied by a matrix of relatively low density (*M* in Figs. 1, 3, and 6). This atypical appearance of the mitochondria was also noted in rat spermatids by Palade (22) and does not appear to be a consequence of poor preservation for it is observed in specimens which, by other criteria, seem very well fixed.

The Golgi complex of the early spermatid is a conspicuous spherical or reniform body consisting of parallel membranes and large numbers of small vacuoles. On close inspection, the membranes are found to limit extremely thin, flattened vesicles (see Text-fig. 1 A) arranged in parallel array around the periphery of the complex while the small vacuoles tend to be clustered in the center (Fig. 3). It is possible that the small vacuoles arise from the flattened vesicles by budding or pinching off from their margins. The dense osmiophilic granules described as a component of the Golgi complex in certain other cell types (4, 7) are not observed in the spermatid.

An irregular mass of osmiophilic granular material is frequently found in the cytoplasm near the Golgi complex (Fig. 6). This structure apparently corresponds to the "chromatic body" or "accessory body" of light microscopy. It is composed of closely aggregated dense granules of unknown origin. It migrates back to the caudal pole of the nucleus later in the development of the spermatid, but it is not yet known whether it participates in the formation of any of the structural elements of the middle piece.

Differentiation of the Acrosome.—As development of the spermatids proceeds, the Golgi complex becomes irregular in outline, and vacuoles develop between its membranes disrupting their parallel arrangement. Gradually the membranes and vacuoles come to be randomly intermingled, with little vestige of their original concentric organization remaining. While the bulk of the vacuoles remains exceedingly small, a few in the center of the complex become distinctly larger and one or more of these come to contain a moderately dense granule, the proacrosomal granule of light microscopy (Fig. 4). The granule is round or oval in outline and is surrounded by a clear space which, in life, is probably occupied by a fluid of which the only detectable residue in the electron micrographs is a faint flocculent precipitate in the vacuole. The proacrosomal granule appears to be a secretory product of the Golgi complex and grows within its enlarging vacuole by accretion of new material to its surface. The number of Golgi vacuoles in each spermatid that develop proacrosomal granules varies with the species, one or two being reported in rats and mice, and ten or more in the guinea pig (25, 14). Two or three are said to occur in the cat (16) but, in the present study, one appeared to be the rule. Two were seen on only a few occasions. Inasmuch as the sections employed here were one-hundredth the thickness of those used for light microscopy, the chances of two vacuoles being included in the same section are correspondingly reduced. Nevertheless, it seems unlikely that more than two is a common occurrence, otherwise two

would have been observed in the same section somewhat more frequently. If two vacuoles arise they apparently soon coalesce to form a single sizable vacuole, and the separate granules also fuse into one. The vacuole then approaches



TEXT-FIG. 1. Diagrammatic representation of the sequence of stages (A to F) in the formation of the acrosome (*Ac*) and head cap (*C*) from the Golgi complex (*G*). The acrosomal granule (*Ac Gr*) arises within a small Golgi vacuole (B), which becomes fixed to the anterior pole of the nucleus (*N*) and there enlarges (C, D, and E) as the acrosomal vesicle (*Ac Ves*). When the Golgi complex moves posteriorly in the elongation of the spermatid, the acrosomal vesicle collapses over the pole of the nucleus to form the closed head cap (*C*), and the plasma membrane (*P M*) becomes closely applied to its surface (F). Drawings based upon electron micrographs of Figs. 3 to 8.

the anterior pole of the nucleus where it becomes adherent to the nuclear membrane, constituting the acrosomal vesicle with its contained acrosomal granule (Figs. 5 and 6). The granule becomes fixed to that portion of the wall of the vesicle which is adherent to the nuclear membrane and continues to grow.

The Golgi complex remains closely applied to the surface of the acrosomal vesicle and the electron micrographs suggest that as differentiation proceeds small vacuoles arise continually within the Golgi complex and coalesce with the vesicle, thus bringing about its progressive enlargement.

At this stage the normal rounded contour of the nucleus is slightly flattened by the growing vesicle, as though the fluid contents of the latter were under sufficient tension to deform the underlying nucleus (Fig. 6). With further expansion of the vesicle and extension of its area of adherence to the nuclear membrane, the deformation of the nucleus becomes more marked until it is quite flat or even slightly concave (Fig. 7).

At this stage a major change in the distribution of the cytoplasm takes place, resulting in the elongation of the developing spermatid. The cytoplasm shifts away from the anterior end of the cell and, associated with this, the Golgi complex leaves the acrosomal region and moves back along the side of the nucleus to a new position in the caudal portion of the cell. Soon thereafter, the lumen of the acrosomal vesicle is reduced to a narrow cleft and its wall takes on the form of a double-layered cap extending well down over the pole of the nucleus (Fig. 8). Gresson and Zlotnik (10) attribute this change of form to a process of active growth of the anterior region of the nucleus into the vesicle. The fine structure of the parts as revealed in electron micrographs, however, leads us to believe that after departure of the Golgi complex from the surface of the acrosomal vesicle, further contributions to its maintenance cease, and through gradual loss of fluid contents the vesicle becomes flaccid, collapsing over the pole of the nucleus to form the head cap. At the same time the nucleus resumes its rounded contour and participates in the general elongation of the spermatid.

Thus, the head cap of the advanced spermatid is not a single membrane growing downward over the nucleus from the acrosomal granule as is generally supposed (14), but instead it is a two-layered structure derived directly from the acrosomal vesicle. The granule situated at the apex of the head is enclosed between its outer and inner membranes which are continuous with one another at the lower margin of the cap (Fig. 9). When the cytoplasm of the spermatid has all been displaced to the postnuclear region, the plasma membrane of the anterior part of the cell is brought into close apposition with the outer membrane of the head cap. The plasma membrane in turn is in very close contact with the membrane of the supporting Sertoli cell (Fig. 8). In electron micrographs of suitably thin, longitudinal or transverse sections through the head region of late spermatids (Figs. 9 and 10), five distinct membranes can be distinguished. From outside inward, these are: the plasma membrane of the Sertoli cell and that of the spermatid, the outer and inner membranes of the cap, and the nuclear membrane. In addition to these five which are regularly present, there may be other thin membranous differentiations of the Sertoli cytoplasm closely associated with the heads of developing spermatids (Figs. 11 and 12). These are tenuous, and discontinuous, paired membranes and no doubt repre-

sent elements of the endoplasmic reticulum, although they lack the small granular component (24) which is usually an identifying characteristic of this cytoplasmic organelle. Not uncommonly the mitochondria of the Sertoli cell are clustered around the tip of the developing spermatids.

Nuclear Changes.—Little change is observed in the character of the nucleus up to the formation of the head cap but soon thereafter it begins to elongate and, associated with this change of shape, there is a progressive condensation of the nuclear contents. The finely granular ground-substance of the nucleus becomes compacted into coarse and extremely dense granules first seen at the periphery next to the cap, but later throughout the nucleus. In the progressive condensation of nuclear substance the number of the larger dense granules appears to increase at the expense of the less dense background material as though the former arose by aggregation of the latter. The nucleus thus gradually comes to consist entirely of coarse, extraordinarily dense granules (Fig. 14). There is some reason for believing that in the late stages of spermatogenesis the nucleus may lose its granular character, becoming more homogeneous and less osmiophilic. Further observations upon late spermatids and mature sperm are needed before this can be stated with conviction. Such a change, however, is quite consistent with the changes in appearance of the nucleus that have been observed with the light microscope. With iron hematoxylin the staining of the nucleus is said to become increasingly intense up to a certain point, but later much of this affinity for the stain is lost again and the nucleus takes on a light bluish gray appearance (21).

The Caudal Sheath or Manchette.—After the formation of the acrosome and head cap and the caudal migration of the Golgi complex, a number of delicate filaments make their appearance in the cytoplasm around the caudal pole of the nucleus. These seem to arise from a ring-like structure which differentiates just behind the caudal margin of the head cap (Fig. 14). This structure undoubtedly corresponds to the so called nuclear ring observed with the light microscope by Zlotnik (34, 10). Although its origin is still somewhat obscure, it seems to originate as a differentiation of the cytoplasm or of the cell membrane and not of the nucleus. In section the ring has a C-shape with the opening directed posteriorly (Fig. 14). The filaments comprising the manchette arise from it along either side of the opening. These filaments are more slender and less dense than those of the tail flagellum and are perfectly straight as though they possess a considerable degree of rigidity. In transverse sections through the postnuclear region of the spermatid (Fig. 13), large numbers of cross-sections of these filaments are disposed in a circle around the sperm flagellum. They seem to be held in this relation by their common attachment to the ring encircling the nucleus rather than by any close lateral bonding between neighboring filaments. In micrographs of high resolution the filaments have a hollow appearance with an osmiophilic exterior and a center of relatively low density (Fig.

13 *A*). In this respect they resemble the longitudinal filaments of cilia and flagella which also have a tubular aspect in cross-section (7). In both instances it is difficult to tell whether this is due to a difference in composition between the exterior and interior which is reflected in a different degree of binding of osmium oxides or whether this appearance is the result of incomplete penetration of the filaments by the fixative. The caudal sheath becomes more conspicuous as the filaments increase in number and become more closely packed, but the electron micrographs studied to date, fail to disclose any evidence of a coalescence of the filaments into a continuous sheet as has been reported in earlier studies with the light microscope (18, 12).

The ultimate fate of the manchette is not known. It is generally regarded as a transient structure which disappears as abruptly as it arises but some investigators have contended that it persists and becomes an important component of the sperm (21). The present study leaves this point in doubt, but it is expected that further observations with the electron microscope on larger numbers of late spermatids and mature sperm will provide a definite answer to the problem.

Within the caudal sheath of the late spermatid are found the proximal centriole and the intracellular portion of the flagellum. The centriole occupies a shallow depression in the base of the nucleus and is not spherical, as it is usually depicted, but is cylindrical, having its long axis perpendicular to the longitudinal filaments of the tail. The elongated form of the centriole and its relation to the tail were correctly described by Kölliker (12). In thin, transverse sections the centriole is round in contour and has a central cavity containing material of very low density (Fig. 14). Its thick osmiophilic wall is inhomogeneous and, in section, appears as a dark ring having light spots regularly spaced around its circumference. When these light spots are distinct enough to be counted they number about nine. In its hollow, cylindrical form and in the character of its wall, the centriole thus bears a strong resemblance to the basal corpuscles of certain mammalian cilia (6). The longitudinal filaments of the flagellum take origin near the proximal centriole but are not continuous with it. The number and arrangement of the tail filaments are the same as have been reported for cilia and flagella throughout the animal kingdom (6). The dense ring that encircles the flagellum a short distance from the base of the nucleus is often called the "ring centriole" (Fig. 14), but it is quite different from a centriole in its fine structure, and it is likely that it arises independently. It is oval in section, rather dense and homogeneous throughout, except for a small oval structure of considerably greater density situated on its posteromedial aspect quite near the tail filaments. The function of this ring and its role in spermatogenesis are poorly understood. It apparently moves away from the nucleus as differentiation proceeds and is found later on, at the posterior end of the middle piece where it may possibly play a role in formation of the dense filaments or strands

which arise at that point and are deposited circumferentially around the full length of the principal piece of the sperm tail. On superficial examination this wrapping appears to be a spiral and it has therefore been named the "cortical helix," but, as indicated in a previous paper, the successive turns frequently branch and rejoin and thus this cannot properly be considered a true spiral (8). The details of development of the complex postnuclear region of the spermatozoon are being studied further and will be the subject of a subsequent paper.

Conjoined Spermatids.—The second spermatocyte division is generally assumed to be a typical mitosis giving rise to two separate spermatids. Electron micrographs with their sharp definition of cell limits now cast some doubt upon the validity of this assumption. In the present study several examples of binucleate spermatids were encountered in which there was no evidence of constriction of the cytoplasm even though an acrosome was beginning to differentiate in relation to each of the nuclei. Pairs of differentiating spermatids connected by a narrow protoplasmic bridge were a very common occurrence (Fig. 1). The plasma membrane enclosing the connection was thickened locally by deposition of osmiophilic granular material on its inner aspect, and therefore it often stood out clearly as a dark ring around the intercellular bridge. No persisting remnants of the spindle were seen and the cytoplasm of the two cells communicated freely. Indeed, strands of ergastoplasm were sometimes seen passing through from one to the other. These findings could perhaps best be accounted for by postulating that karyokinesis in the second spermatocyte division proceeds normally, but the daughter nuclei are fully reconstituted without division of the cell body. In the resulting binucleate spermatid a deep constriction develops later, separating the cell body into two portions which, however, remain connected by a narrow isthmus to an advanced stage of their differentiation into spermatozoa. In a few instances three spermatids were seen to be joined by two bridges, and it is not unlikely that a fourth cell was so connected in another plane of section. The question is thus raised as to whether all four cells resulting from the two spermatocyte divisions may not remain connected for a time in this manner. This question cannot readily be answered by the methods used in this study, for even if this were a regular occurrence the chances of all three bridges falling within the plane of a 300 A section would be very small indeed. The possibility that the intercellular bridges observed were abnormal structures cannot be ruled out but the frequency with which they were seen in these thin sections is so great, that the possibility of this being a normal condition merits serious consideration.

DISCUSSION

In tissues prepared by cytological methods for examination with the light microscope, the Golgi substance of the spermatid is usually described as occurring in the form of dark staining rods or crescents closely applied to the surface

of a central spherical body of different tinctorial properties which is variously known as the *sphere*, *archoplasm*, or *idiosome*. Outer and inner components of this juxtanuclear body have also been distinguished in fresh material examined with the phase contrast microscope. Thus, Austin and Sapsford (1) describe the Golgi complex of the early rat spermatid as consisting of short dark rods at the periphery of a lighter zone and these coalesce in later stages to form an inverted U-shaped structure. Oettlé (20) noted two distinct zones in the Golgi complex of unfixed human spermatids. It is apparent from the study of electron micrographs that the appearance of cortical and medullary regions with the light microscope is a reflection of the segregation of the flattened membranous vesicles and the spherical vacuolar components of the Golgi complex which exist at this stage. When examined with phase contrast the close parallel membranes around the outside seemingly produce a greater light retardation than the minute vacuoles in the center. So also when stained by osmium or silver in the classical methods for demonstrating the Golgi apparatus, it is the closely approximated membranes at the periphery which are most heavily impregnated. Parallel membranes and small vacuoles are regular constituents of the Golgi complex of all tissues examined to date with the electron microscope (4, 31, 7). The only unusual features of the Golgi complex of the spermatid which set it apart from that of other cells are its large size and the tendency for the two components to be segregated with the membranes arranged around the outside of a central aggregation of submicroscopic vacuoles. This concentric organization does not persist in later stages of spermatogenesis. There seems no longer to be any advantage in preserving a distinction between these two zones of the Golgi complex, and the terms *idiosome* and *archoplasm* and their several synonyms might well be abandoned. With the elimination of this distinction the persisting dispute as to whether the proacrosomal material is a product of the *archoplasm* (*idiosome*) or of the Golgi elements (*dictyosomes*) becomes pointless. The electron micrographs suggest that the lamellar vesicles of the Golgi complex give rise to the associated small vacuoles and it is quite clear that the material forming the acrosomal granule arises within these vacuoles.

Oliver (21) referred to the clear zone, seen with the light microscope around the developing proacrosomal granule, as a *hyaline area* or *hyaline body*, but the majority of other investigators have described it as a vacuole probably containing fluid (18, 2, 5, 10). The acrosomal vacuole has been clearly seen by phase contrast microscopy in living spermatids of lumbricus, rat, mouse, and man by Price *et al.* (27), Gresson (11), and Oettlé (20). In spite of these observations Leblond and Clermont (14) in their recent study of spermatogenesis in several rodent species concluded that the acrosomal vacuole is an artifact of fixation. Thus, they also rejected Duesberg's suggestion that the acrosomal vacuole might enter into formation of the head cap. Instead they believed the cap was derived from the substance of the acrosomal granule which gradually spread out

and grew down over the anterior segment of the nucleus. Lake and Smiles (13) and Challice (3) also expressed the belief that the acrosomal vacuole is a result of poor preservation. Watson (32), on the other hand, apparently did not consider it an artifact and described its origin correctly. The results of the present investigation with the electron microscope fully substantiate his findings and those of earlier investigators with respect to the existence of an acrosomal vacuole and establish beyond doubt that this structure has an important role in the formation of the head cap. The changing distribution of the substance of the acrosomal granule observed by Leblond in the cap phase of spermatogenesis is confirmed, but the flattening of the granule and its extension downward over the nucleus take place within the confines of the preexisting membranous cap formed earlier by the collapse of the acrosomal vacuole or vesicle.

The relationship of the heads of the developing sperm to the Sertoli cells has been variously interpreted. Some have thought the heads were actually within the cytoplasm of the sustentacular cells, others have considered them as occupying deep recesses in the cell surface. This problem that seemed incapable of solution with the limited resolving power of the light microscope, has been clarified by high resolution electron micrographs in which two distinct limiting cell membranes can always be distinguished. Thus, it appears that the spermatids develop in the most intimate relation to the surface of the surrounding Sertoli cells and are lodged in deep indentations of their surface, but are never actually within their cytoplasm. In fact, at least four membranes are interposed between the nucleus of the late spermatid and the cytoplasm of the sustentacular cell. The substance of the acrosome is separated from the surface of the Sertoli cell by two membranes. If we are justified in assuming that these membranes persist on the fully differentiated sperm, their presence would render unlikely Leblond's suggestion that the viscosity of the polysaccharide substance of the cap plays a role in holding the spermatids in the seminiferous epithelium, and may also be responsible for the adherence of sperm heads in rouleaux in the epididymis of the guinea pig (14).

The caudal sheath of the spermatid was described by Kölliker (12) and considered by him to be an outgrowth of the nuclear membrane. Renson (28) described it in the rabbit as a sort of "hyaline tube" extending backward from the posterior segment of the nucleus. It was restudied in the rat by Lenhossek (15) and given the name *manchette* (*Schwanzmanchette*). Mèves (18) gave an account of its formation in the guinea pig from delicate filaments of cytoplasmic origin, having their anterior end inserted upon the nuclear membrane and their posterior end terminating free in the cytoplasm. The filaments were arranged in more or less parallel fashion and were said to increase in number and finally fuse into a continuous tube. Several later authors criticized Mèves' interpretation and continued to consider the manchette as a product of the nucleus. Among these Schoenfeld (30) studying spermatogenesis in the bull, and von

Mollé (19) studying it in the squirrel, depicted the manchette as a double-layered membranous structure and thought of it as arising from a circular fold of the nuclear membrane which grew backward to form a cylinder. Oliver (21), working with the fur seal, fully confirmed Mèves' (18) account of the development of the caudal sheath and its filamentous nature was reaffirmed in the recent electron microscope studies of Watson (32) on the rat testis. In this latter work the origin of the filaments was by no means clear, but they appeared to spring from the posterior margin of the acrosome. The present study of the cat has shown that the delicate filaments of the caudal sheath arise neither from the nuclear membrane, nor the rim of the acrosome, but from a distinct ring of unknown provenience which encircles the nucleus just behind the posterior margin of the head cap. This structure undoubtedly corresponds to the so called "nuclear ring" originally described by Zlotnik (34) with the light microscope, in dog spermatids and later identified in several other species by Gresson and Zlotnik (10).

One of the most puzzling problems raised by the present observations concerns the correct interpretation of the protoplasmic bridges found connecting spermatids in pairs or groups of four. Owing to the thinness of the sections used for electron microscopy and the restriction of the field, it is not possible to determine with any certainty whether there is always a delayed separation of the daughter cells of the spermatocyte divisions or whether this is simply a very common abnormality. It is known that degeneration of a considerable number of cells is a normal occurrence in spermatogenesis (29) and it might be argued that failure to complete spermatocyte division is merely an early event in a sequence of regressive changes leading ultimately to degeneration of the cells concerned. Against this interpretation is the fact that no signs of degeneration were detected in any of the conjoined spermatids. It is said to be possible to cause coalescence of spermatids by pressure or rough handling of the tissue prior to fixation. It is unlikely that the intercellular bridges and binucleate spermatids reported here were artifacts produced in this manner for there was a minimum of manipulation of the fresh tissue during specimen preparation and the sections revealed little evidence of tissue damage.

The observation of intercellular connections between pairs of spermatocytes and early spermatids is not entirely new, but it does not appear to have been made heretofore in any study of mammalian spermatogenesis. In his account of spermatogenesis in amphiuma, McGregor (17) described and illustrated "spindle remnants" which persisted as delicate bridges between the daughter cells. These were formed in the division of secondary spermatogonia, primary and secondary spermatocytes and were observed still connecting spermatids up to the stage of differentiation of acrosomes and tail flagella. According to McGregor this was the first instance in which "intermediate bodies" had been observed in spermatids later than telophase.

Study of electron micrographs of the protoplasmic connections between spermatids in the cat reveals that they depart in certain respects from the usual descriptions of intermediate bodies (*Zwischenkörper*). The latter are formed in telophase of mitotic division when the deepening cleavage furrow encounters a bundle of persisting interzonal spindle fibers and the abstriction of the daughter cells is temporarily arrested. It may be that the protoplasmic connections between cat spermatids arise in the same manner, but the frequent occurrence of binucleate spermatids and the absence of spindle remnants in the intercellular bridges suggest a different explanation, namely that karyokinesis in the second spermatocyte division proceeds normally, but the constriction of the cell body is delayed until after the reconstitution of the daughter nuclei and initiation of acrosome formation. In this case a spindle remnant would not be involved in formation of the intercellular bridges. Typical intermediate bodies have a very transient existence, whereas in the cat the protoplasmic connections are still present between spermatids which are elongating after the formation of the acrosome and the caudal migration of the Golgi remnant. It is not clear just when the communication between cells is finally broken or why it persists so long, but it is conceivable that the close association of maturing spermatozoa in clusters at the luminal end of the Sertoli cells is a consequence of their having been held together in pairs or in groups of four up to a relatively late stage of spermatogenesis.

In addition to our observation of such bridges in the cat, they have recently been observed in human testis (9) and a reexamination of the figures in Watson's paper on the rat discloses two or more clear examples of spermatids connected by a bridge, but no mention was made of this in the text (32). It is expected that future studies of other species with the electron microscope will reveal that intercellular connections between developing germ cells are a rather general phenomenon.

SUMMARY

The differentiation of cat spermatids was studied in thin sections examined with the electron microscope. The Golgi complex of the spermatid consists of a central aggregation of minute vacuoles, partially surrounded by a lamellar arrangement of flattened vesicles. In the formation of the acrosome, one or more moderately dense homogeneous granules arise within vacuoles of the Golgi complex. The coalescence of these vacuoles and their contained granules gives rise to a single acrosomal granule within a sizable membrane-limited vacuole, termed the acrosomal vesicle. This adheres to the nuclear membrane and later becomes closely applied to the anterior two-thirds of the elongating nucleus to form a closed bilaminar head cap. The substance of the acrosomal granule occupies the narrow cleft between the membranous layers of the cap.

The caudal sheath is comprised of many straight filaments extending back-

ward from a ring which encircles the nucleus at the posterior margin of the head cap.

Attention is directed to the frequent occurrence of pairs of spermatids joined by a protoplasmic bridge and the origin and possible significance of this relationship are discussed.

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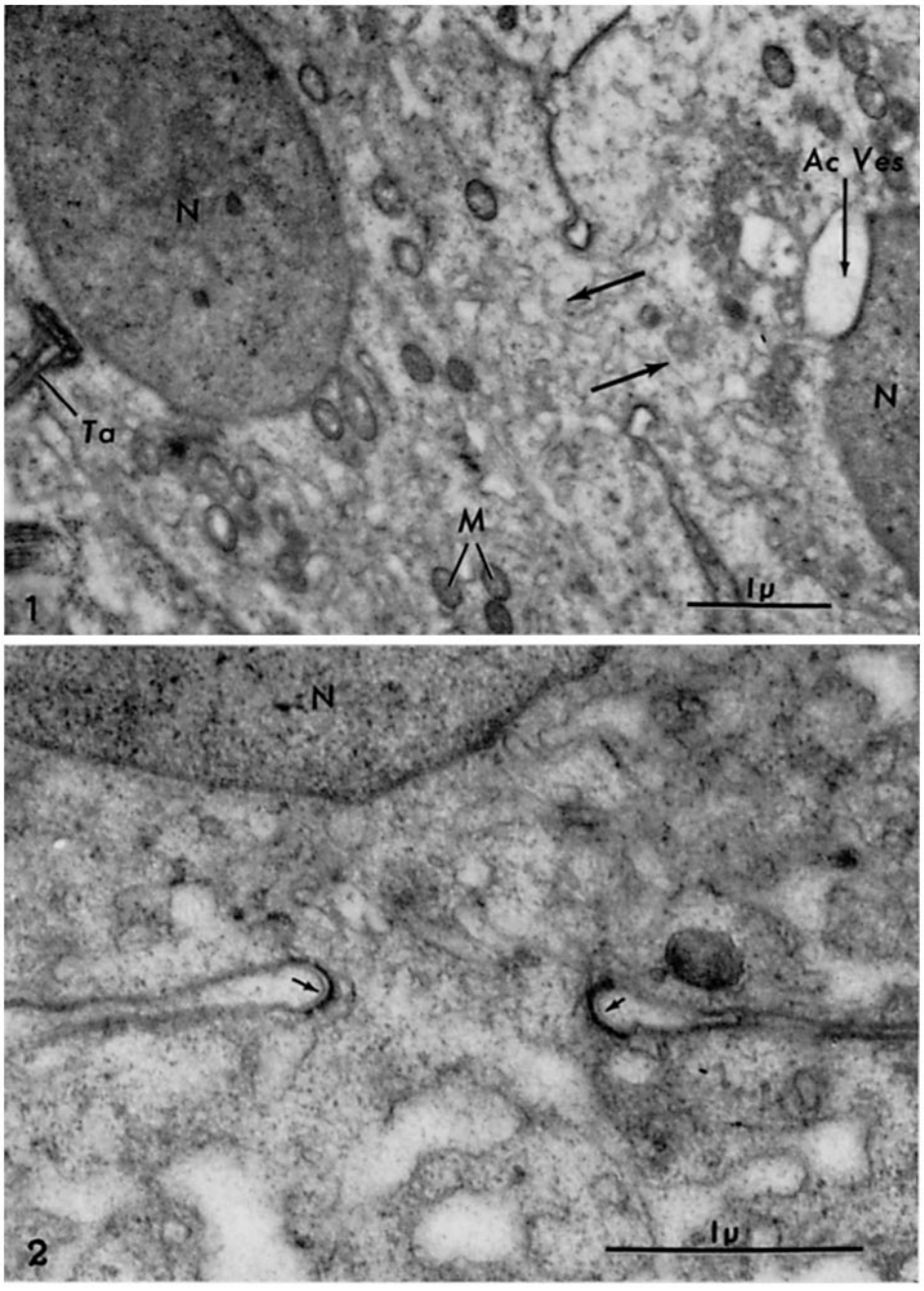
EXPLANATION OF PLATES

Ac, acrosome.
Ac Gr, acrosomal granule.
Ac Ves, acrosomal vesicle.
Cap, head cap.
Ch B, chromatic body.
Er, endoplasmic reticulum (ergastoplasm).
Go C, Golgi complex.
M, mitochondria.
Ma, manchette.
N, nucleus.
Pn C, postnuclear cap.
Pr C, proximal centriole.
Pr G, proacrosomal granule.
Ri, nuclear ring.
Ri C, ring centriole.
Se C, Sertoli cell.
Sp N, sperm nucleus.
Ta, tail flagellum.
Ta F, tail filaments.
Va, vacuole.

PLATE 79

FIG. 1. Electron micrograph of a pair of spermatids connected by a broad intercellular bridge (at arrows) through which there is an open communication between the cytoplasm of one cell and the other. Beginning differentiation of the tail flagellum (*Ta*) is seen in the spermatid at the left of the figure and an acrosomal vesicle (*Ac Ves*) is forming in relation to the nucleus (*N*) at the right. × 19,318.

FIG. 2. Intercellular bridge connecting two early spermatids. A portion of the nucleus (*N*) of the upper cell is included, but that of the lower cell is out of the field. Both were typical interkinetic nuclei. Unlike the so called intermediate body or spindle rest which may connect the daughter cells for a short time after anaphase of mitotic division, the intercellular bridge depicted here contains no vestige of the spindle apparatus and it persists long after the reconstruction of the nuclei following the second spermatocyte division. Notice that the cell membrane of the spermatid is thickened to form a darker ring surrounding the bridge. An attenuated portion of the enveloping Sertoli cell extends deeply into the furrow between the spermatids, and its thin cell membrane can be seen (at arrows) in close apposition to the thickened region of the spermatid membrane which surrounds the bridge. × 37,045.



(Burgos and Fawcett: Fine structure of mammalian testis. I)

PLATE 80

FIG. 3. Electron micrograph of a typical early spermatid including a portion of the nucleus (*N*), several mitochondria (*M*), and the Golgi complex (*Go C*). The Golgi complex consists of membranes arranged in more or less concentric layers around the periphery of an aggregation of small vacuoles. × 23,854.

FIG. 4. As differentiation progresses, the Golgi complex loses its concentric organization and certain of its vacuoles become considerably enlarged (*Va*). One or two of these come to contain a dense spherical body, the proacrosomal granule (*Pr G*). × 23,854.

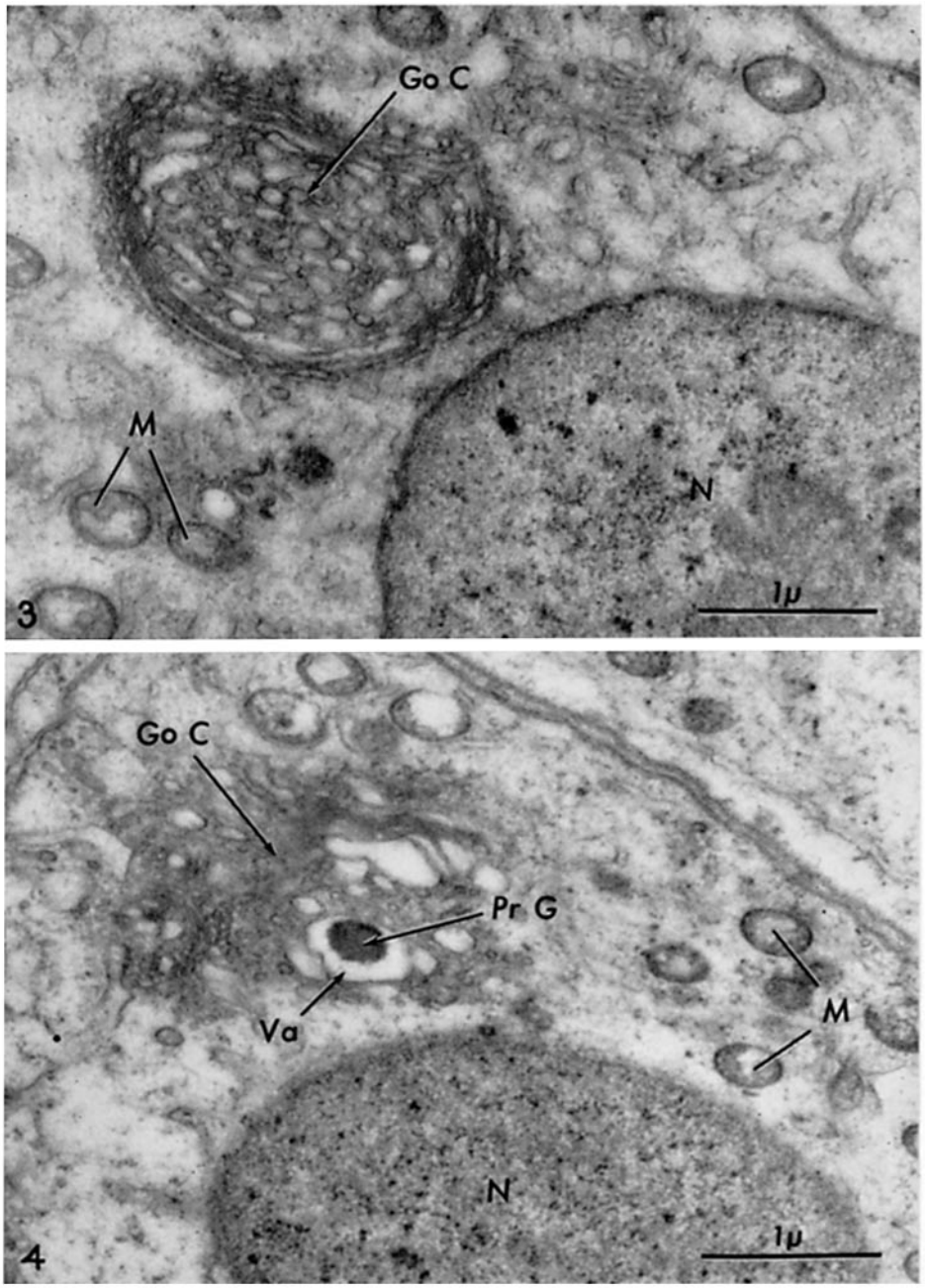


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PLATE 81

FIG. 5. The vacuoles and their proacrosomal granules fuse into a single vacuole containing one acrosomal granule (*Ac Gr*). This vacuole approaches the anterior pole of the nucleus and becomes fixed to the nuclear membrane as the acrosomal vesicle (*Ac Ves*). The Golgi complex (*Go C*) remains closely associated with its surface. × 22,590.

FIG. 6. The acrosomal vesicle (*Ac Ves*) is progressively enlarged, apparently by addition of vacuoles which arise in the adjacent Golgi complex (*Go C*) and coalesce with it. At the same time, the acrosomal granule grows by accretion to its surface and becomes adherent to the wall of the acrosomal vesicle where the latter is in contact with the nuclear membrane. The chromatic body (*Ch B*) or accessory body is often situated near the developing acrosome at this stage. × 21,084.

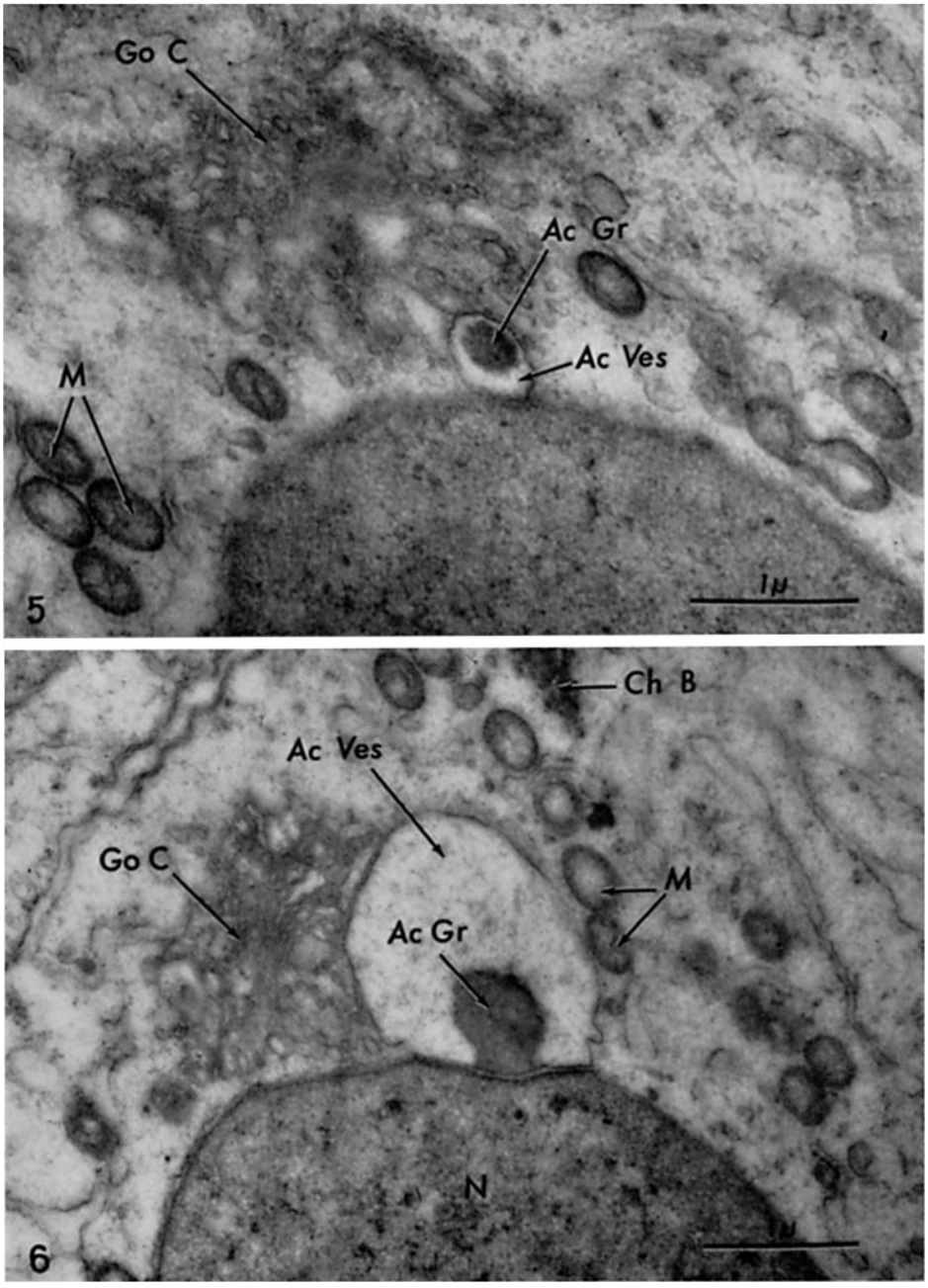

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PLATE 82

FIG. 7. The anterior pole of the nucleus is flattened by the expanding acrosomal vesicle which suggests that the fluid contents of the vesicle are under considerable pressure. × 24,242.

FIG. 8. In the elongation of the spermatid, the Golgi complex leaves the side of the acrosomal vesicle and migrates to the caudal part of the cell. The vesicle then becomes flaccid, possibly through loss of part of its fluid content, and it collapses over the pole of the nucleus which at the same time regains its rounded contour. Thus at this stage the head cap is a closed membrane-bounded structure enclosing a narrow cavity which contains the acrosome (*Ac*). The cell membrane of the spermatid is closely applied to the cap with no intervening cytoplasm. The plasma membrane of the Sertoli cell (*Se C*) is closely applied to the outer layer of the cap. × 26,060.

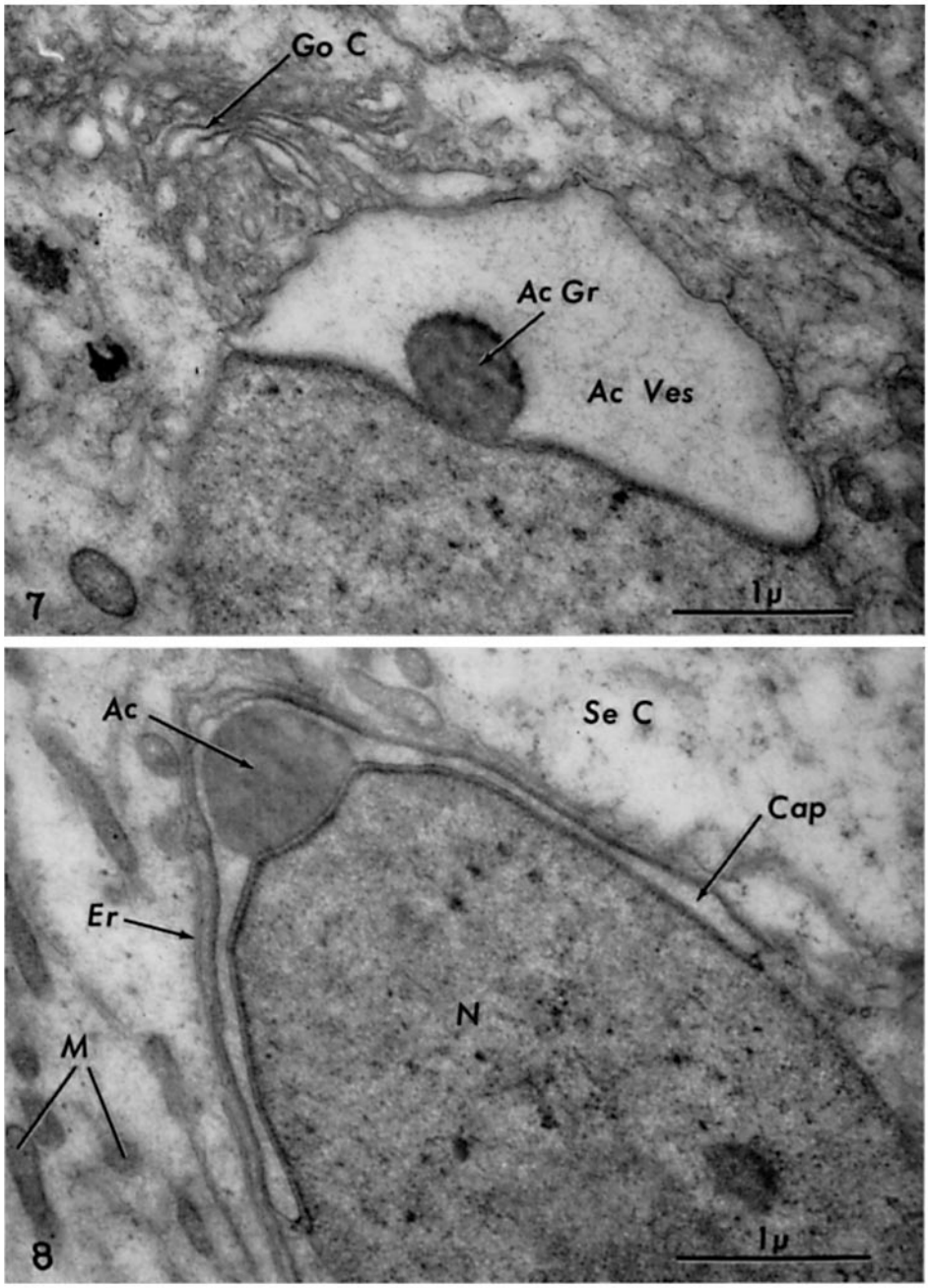

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PLATE 83

FIG. 9. Electron micrograph showing clearly at higher magnification the several membranes investing the head of the spermatid. These are, from outside inward: (*a*) plasma membrane of the Sertoli cell, (*b*) plasma membrane of the spermatid, (*c*) outer layer of the cap, (*d*) inner layer of the cap, (*e*) nuclear membrane. The outer and inner layers of the head cap (*c* and *d*) are continuous with one another at the posterior border of the cap (*) at the lower right corner of the figure. Discontinuous paired membranes visible in the Sertoli cytoplasm (*Se C*) around the spermatid are elements of the endoplasmic reticulum (*Er*) of that cell. × 60,144.

FIG. 10. Transverse section through the head of a more advanced spermatid with an elongated, condensed nucleus. (For orientation see Fig. 12.) The same enveloping membranes shown in Fig. 9 can be identified here (*a*, *b*, *c*, *d*, and *e*). × 52,465.

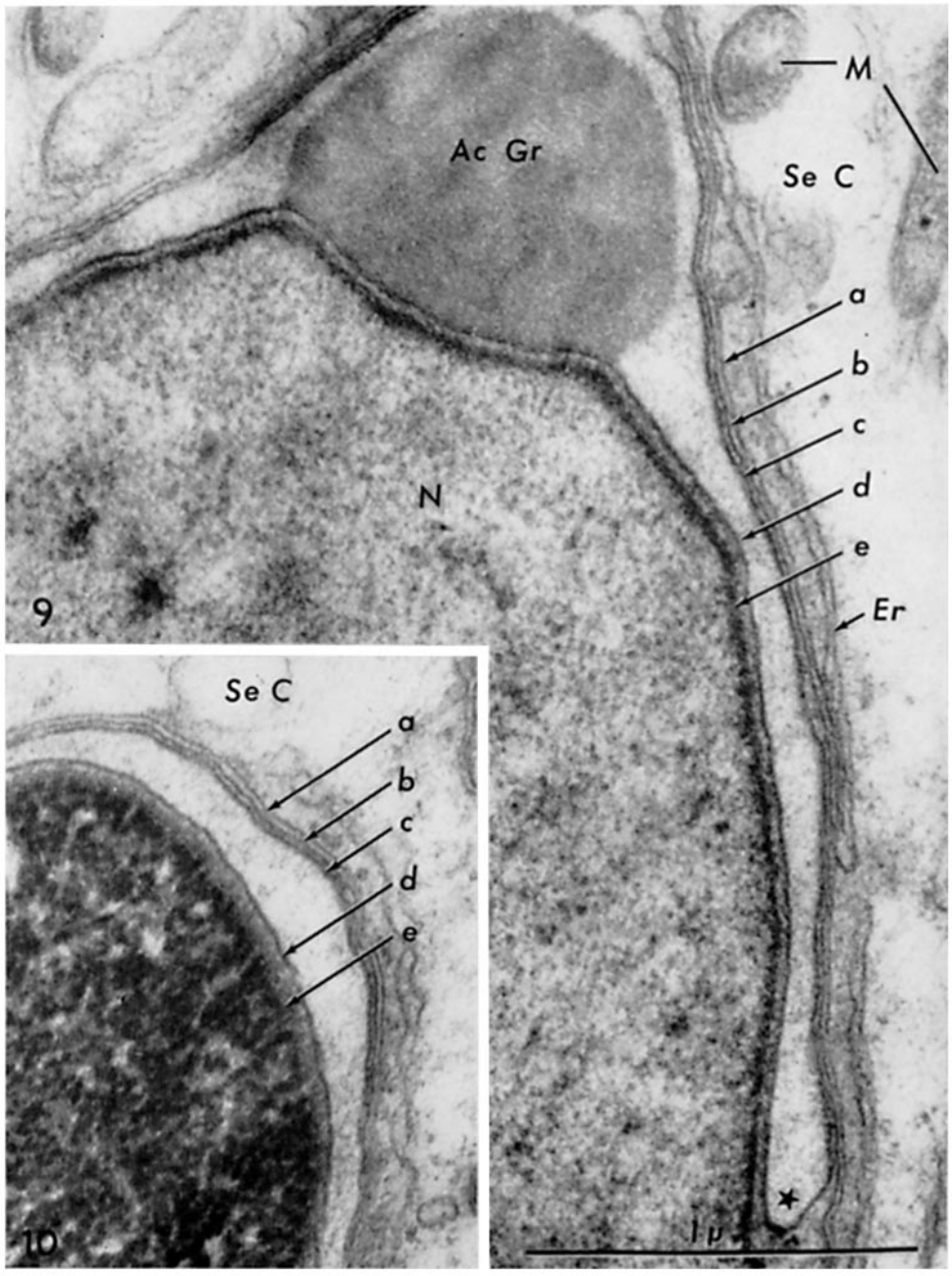

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PLATE 84

FIG. 11. A vertical section through a Sertoli cell including part of two late spermatids and showing the intimate relation of the developing heads to the surrounding Sertoli cell. Observe the beginning condensation of the nuclear material into coarse osmiophilic granules, and the clustering of Sertoli cell mitochondria around the head of the spermatid. × 25,699.

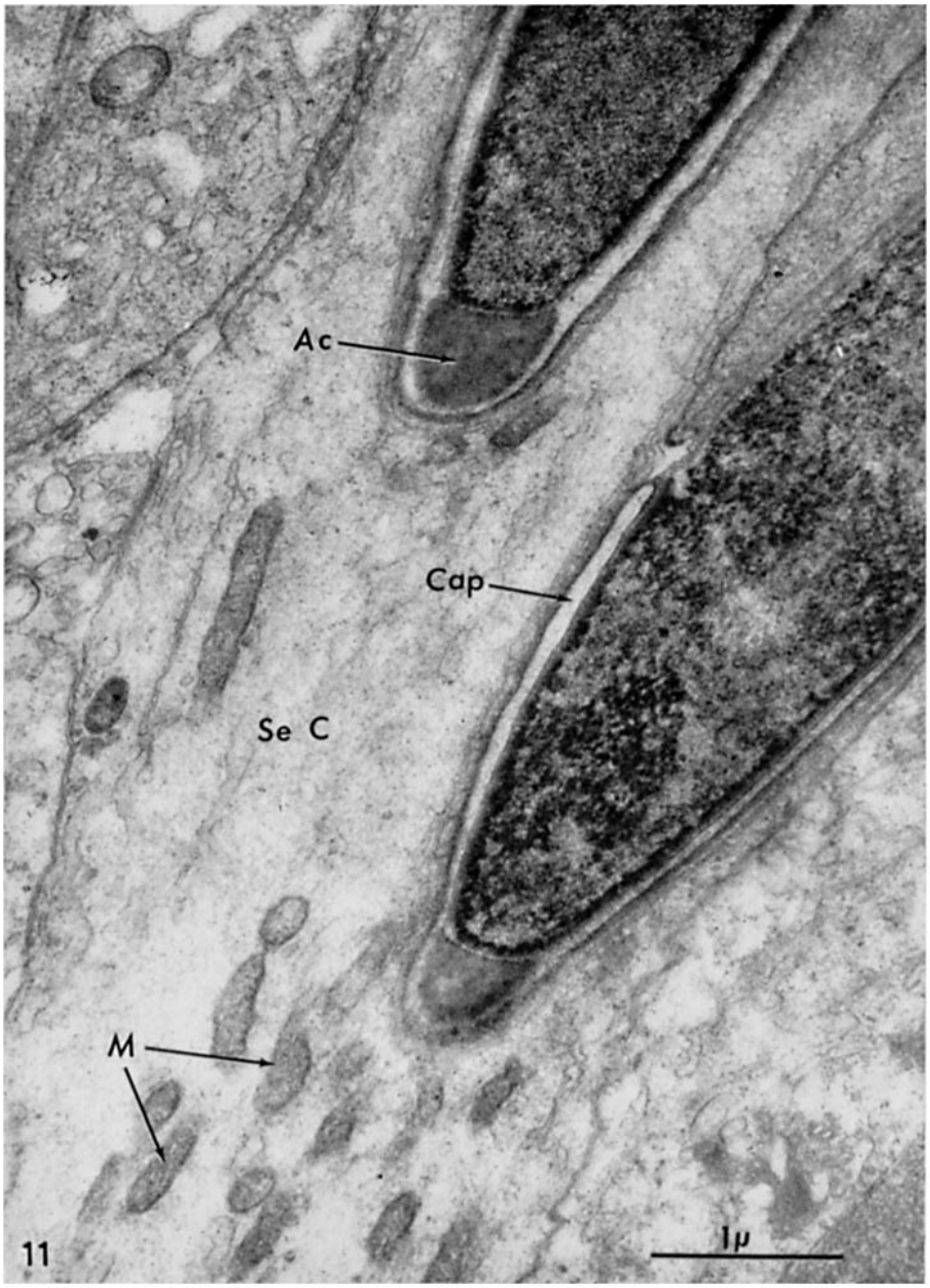

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PLATE 85

FIG. 12. A section of a Sertoli cell including five developing sperm heads cut transversely or obliquely. Although the sperm heads appear to be within the cytoplasm of the Sertoli cell, examination of an area such as that included in the rectangle (see Fig. 10) clearly reveals a continuous Sertoli cell membrane between the sperm head and the cytoplasm of the surrounding sustentacular cell. × 13,147.

FIG. 13. A transverse section through the postnuclear region of an advanced spermatid showing in the center of the figure a cross-section of the tail flagellum surrounded by a pair of membranes. The larger ring in the spermatid cytoplasm is the so called caudal sheath or manchette. This is not a continuous membrane, as often described, but a cylindrical aggregation of delicate filaments. × 29,506. When examined at very high magnification these appear to be tubular. (See inset *A*.) × 65,000.

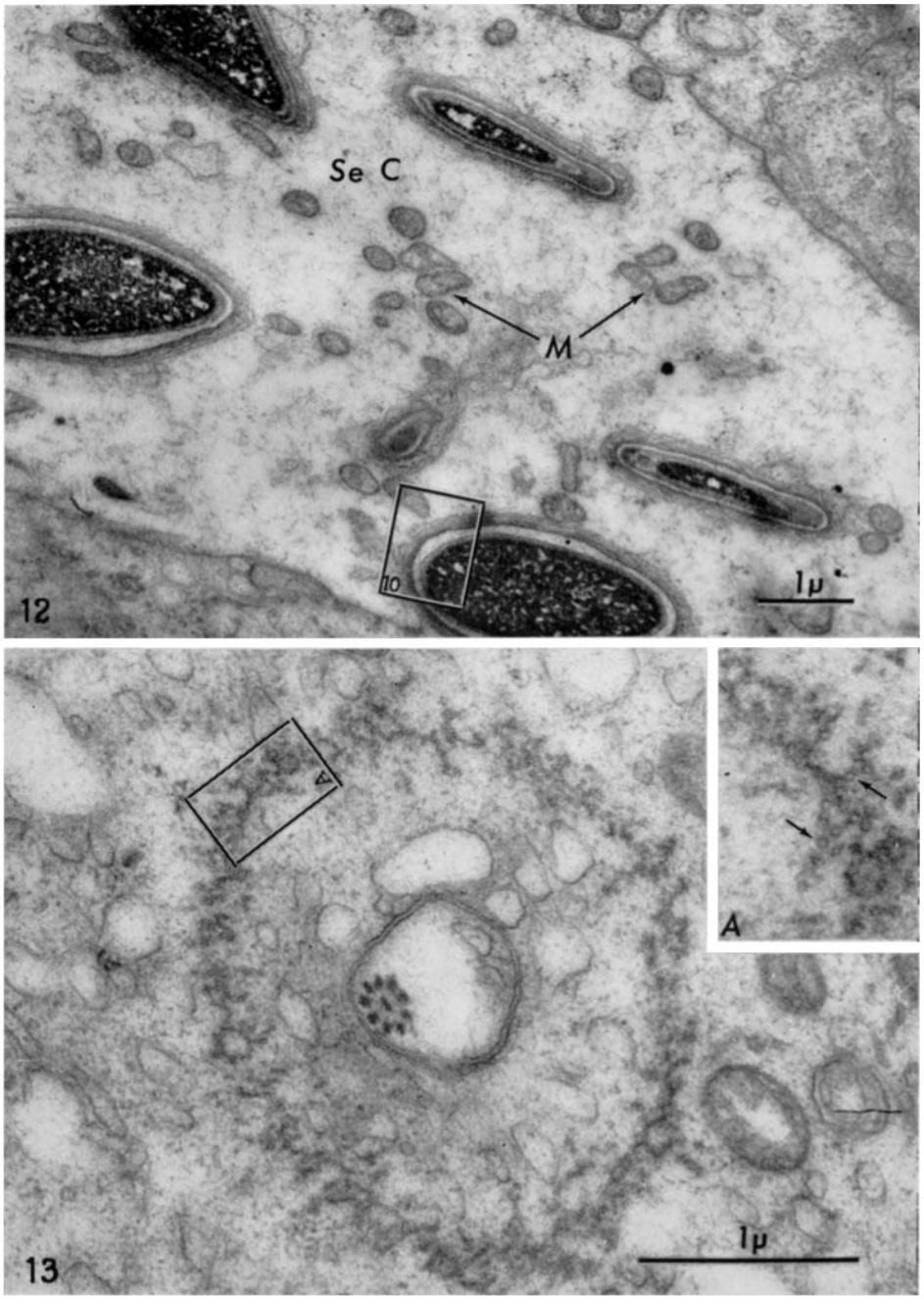

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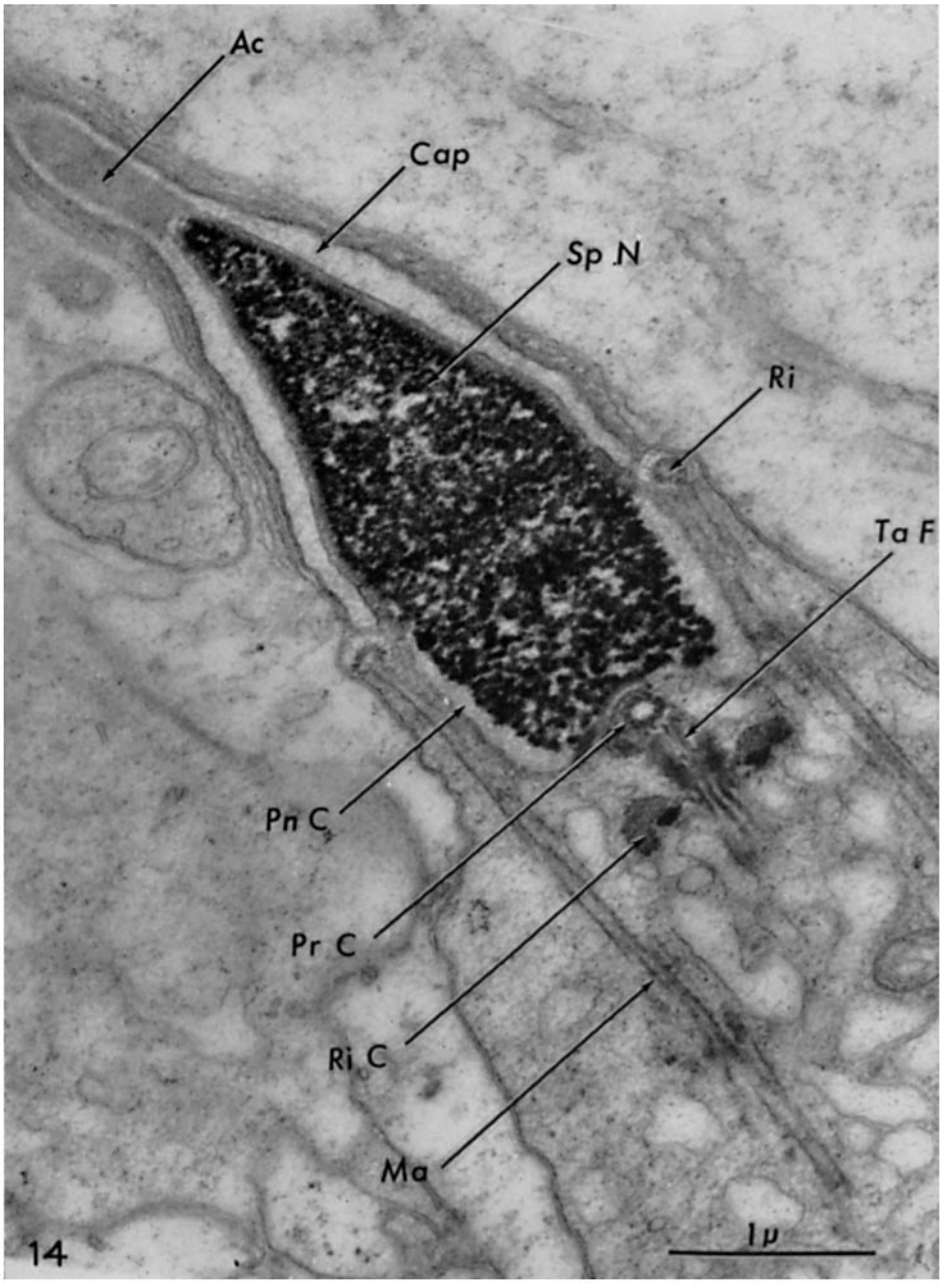
PLATE 86

FIG. 14. A longitudinal section of the head region of a late spermatid showing the arrangement of the various components of this complex cell including: the sperm nucleus (*Sp N*), acrosome (*Ac*), head cap (*Cap*), nuclear ring (*Ri*), manchette (*Ma*), postnuclear cap (*Pn C*), proximal centriole (*Pr C*), ring centriole (*Ri C*), and tail filaments (*Ta F*). The so called postnuclear cap (*Pn C*) appears here as a space between the nuclear membrane and the condensed chromatin of the posterior segment of the nucleus. Its origin and significance require further study. × 26,891.



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