Observations on the Cytoplasmic Membranes of Testicular Cells, Examined by Phase Contrast and Electron Microscopy*

BY DON W. FAWCETT, M.D., AND SUSUMU ITO, PH.D.

(From the Department of Anatomy, Cornell University Medical College, New York)

PLATES 63 TO 68

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ABSTRACT

In freshly isolated cells of the guinea pig germinal epithelium examined with phase contrast, dark contours are seen in the cytoplasm that appear to be optical sections of the cisternae of the endoplasmic reticulum. These increase in contrast, in number, and in linear extent with increasing time up to 4 hours after isolation of the cells from the testis. During this period, cisternae originally present in the cells are extended and new ones appear to be formed by coalescence of tubular and vesicular elements of the reticulum. The cisternae become associated in parallel array and ultimately form elaborate concentric systems resembling structures that have often been interpreted as intracellular "myelin figures." Until now our knowledge of the endoplasmic reticulum has been based largely upon electron micrographs. The observation that the cisternae are visible in certain cell types under phase contrast optics opens the way for experimental investigations on the behavior of this class of cytoplasmic membranes in living cells.

Among the most significant results of the application of the electron microscope to the study of cells has been the description by Porter and Palade (21, 15) of a new cytoplasmic organelle now commonly called the *endoplasmic reticulum*. In its most typical form it consists of membrane-limited tubules and canaliculi that branch and anastomose to form a more or less continuous system of channels extending throughout the cytoplasm. Tubular elements generally predominate, but these may be expanded into broad sinuses called the *cisternae* of the endoplasmic reticulum (15, 16). In electron micrographs of thin sections, the tubules usually appear as round or oval contours, while the cisternae present long profiles limited by two membranes that run parallel 200 to 400 A apart. The membranes of the cisternal profiles are continuous with one another at their ends and thus, in three dimensions, they enclose a broad, flat cavity. The endoplasmic reticulum is highly variable in its organization. In cells active in protein synthesis, the cisternae outnumber the tubular components of the reticulum, and they tend to be arranged parallel. Early doubts as to the existence of these structures in the living cell were largely eliminated by the observation that the Nissl bodies of neurons, which appear in electron micrographs as parallel arrays of cisternae (17), are also visible by phase contrast as irregular dark bodies in the perikarya of living neurons (18).

The observations of Porter and Palade on the form and disposition of the components of this pleomorphic organelle have been abundantly confirmed, and most of their interpretations have been accorded widespread acceptance. Their concept of the reticulum as a continuous canalicular network within the cell has, however, been viewed with skepticism in some quarters (22). Owing to the small size of the structures involved, neither the reticular nature of the system nor its continuity have been demonstrated *in vivo* by phase contrast microscopy, and to date, there have been few direct observations on the endoplasmic reticulum of living cells to substantiate the belief that its form and distribution change in different functional and environmental conditions. The findings reported

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here are part of a correlated light and electron microscope study on spermiogenesis in the guinea pig. Cells of the germinal epithelium were isolated from seminiferous tubules and examined in the fresh condition with the phase contrast microscope as a guide to the interpretation of electron micrographs. Living Sertoli cells, spermatocytes, and spermatids in different stages of differentiation were easily identified, and the form and distribution of their major cytoplasmic components studied. The tubular elements of the endoplasmic reticulum could not be resolved with the phase microscope, but it was possible to see optical sections of the cisternae. This paper records preliminary observations on the disposition, linear extent, and stability of the cisternae of the endoplasmic reticulum in living testicular cells, and describes their reorganization into concentric lamellar systems of a kind that have been interpreted heretofore as "*myelin figures*."

Materials and Methods

Phase Contrast Studies.—Testicular biopsies were taken under ether anaesthesia. A few 1 mm. lengths of seminiferous tubules were teased in a drop of Tyrode solution on a slide, and cells were expressed from the tubules by gentle pressure on the coverslip. The latter was then sealed with petroleum jelly and the preparation was examined with Zeiss-Winkel oil immersion, phase contrast objectives. Photomicrographs were made with a Leitz, Micro-Ibso, 1/3 x tube, using Wratten #58 (green) filter, and Adox KB 14 film, processed with FR X-22 developer. The light source was an electronic flash tube mounted in an adjustable Spencer model 370 microscope lamp housing. An American Speedlight Corporation model B-501 power supply was used. This provided an effective light duration of 1/1500 second. The long exposures usually used for phase photomicrography were thus avoided, and sharper negatives were obtained by eliminating the slight blurring of the image that normally results from thermal agitation within the cytoplasm and active movement of the organelles. Centration of the electronic light unit was facilitated by the use of a model M-403 continuous light adapter. However, this illumination was not used for scanning or photography because of its low intensity and flicker. For prolonged observation and selection of the fields for photomicrography, an auxiliary light source was used. This consisted of a Bausch and Lomb 100 watt, ribbon-filament microscope lamp. This light was placed perpendicular to the electronic flash source and directed to the microscope by means of a flip-out mirror mounted in a brass T tube interposed between the two light sources and the microscope.

Electron Microscope Studies.—Small fragments of guinea pig testis were fixed by immersion for 2½ hours in 1 per cent osmium tetroxide adjusted with veronal acetate buffer to pH 7.6. Following dehydration in a graded series of alcohols, the tissues were embedded in *n*-butyl methacrylate, sectioned on a Porter-Blum (Servall) microtome, and examined with an RCA model EMU-3B electron microscope at original magnifications of 2000 to 12,000 diameters.

Electron Microscope Observations

The germinal epithelium of the guinea pig is an unusually favorable material for studying the structural changes in cytoplasmic organelles during spermatid differentiation. The Golgi complex is particularly well developed, and its participation in the formation of the acrosome can be followed to better advantage than it can in other animals having a less prominent acrosomal system. The endoplasmic reticulum, which is rudimentary in the germ cells of many other species, is extensively developed in the spermatocytes and spermatids of the guinea pig. The fine structure of the germ cells and their supporting elements will be described here only in such detail as is necessary to provide a basis for comparison with phase contrast images of living cells isolated from the testis. A more detailed account of spermiogenesis in the guinea pig, as revealed by electron microscopy, will be published elsewhere.

The spermatocytes are recognized by their position, their size, and nucleocytoplasmic ratio. Their nuclei, which have a characteristic prophase pattern in histological sections, are less distinctive in electron micrographs. After osmium fixation they appear homogeneous except for the remnant of a nucleolus adhering to the nuclear membrane, and a few segments of the chromosomal cores described by Moses (13) and by Fawcett (8). Near the nucleus is a large Golgi complex, often containing several proacrosomal granules of small size. The mitochondria are short rods or spheres with a matrix of low density and internal membranes irregularly oriented. The cytoplasm is abundant, and its endoplasmic reticulum appears to be made up mainly of branching tubules, but in the peripheral cytoplasm there are also parallel arrays of cisternae.

The spermatids occur in the germinal epithelium in groups of four or more, all in the same stage of differentiation. It has been shown that intercellular bridges (Fig. 1) establish protoplasmic continuity between the individual members of these groups (6, 7). Different regions along the length of the seminiferous tubules contain spermatids in differ-

ent phases of the spermatogenic wave. The stage of differentiation of any particular group is easily judged by the stage of acrosome formation. Early spermatids have several large proacrosomal granules in their Golgi region. These subsequently fuse to form a single granule enclosed in a membrane-bounded vesicle. The vesicle becomes fixed to the nuclear membrane, while the Golgi complex remains closely applied to its surface. The granule in the interior of the acrosomal vesicle adheres to the limiting membrane of the vesicle at the site of its attachment to the nucleus. As differentiation proceeds, the acrosomal vesicle enlarges and its contents increase in density. Upon fixation, the material within the early vesicle is preserved in the form of a heavy flocculent precipitate around the acrosomal granule. The concentration of precipitable material in the vesicle increases as development progresses and, in micrographs of later stages, the spermatid nucleus is surmounted by a hemispherical acrosome, consisting of a homogeneous mass of dense material which is still enclosed by the membrane of the original acrosomal vesicle (Fig. 1). Prolongation of this membrane forms a narrow fold that extends from the margins of the acrosome (Fig. 2) to give rise to a thin head-cap, which ultimately covers much of the surface of the nucleus. Throughout this period, the Golgi complex is closely associated with the developing acrosome, but it subsequently migrates to the caudal end of the cell and is finally cast off in the residual cytoplasm. The spherical mitochondria of the spermatids are atypical in their internal structure, their cristae being displaced to the periphery, leaving an angular central cavity devoid of membranes and containing a matrix of very low density (Fig. 2). The endoplasmic reticulum is abundant, and its canalicular nature and net-like organization are more apparent in guinea pig spermatids than in those of other species. In well fixed specimens, the communicating system of tubules comprising the reticulum appears to be filled with an amorphous substance slightly more dense than the surrounding cytoplasm. As a consequence, the reticulum stands out very clearly in the micrographs. The greater part of the system consists of a network of meandering tubules, but long profiles of cisternae are also encountered. The latter are less abundant in spermatids than in spermatocytes. Ribonucleoprotein granules are plentiful and are generally distributed throughout the cytoplasmic matrix. Relatively few are associated with the membranes of the reticulum.

The Sertoli cells that envelope the spermatids are extraordinarily irregular in shape, and in electron micrographs of germinal epithelium one usually sees only small, polygonal areas of their cytoplasm occupying the interstices between the spermatids (Fig. 1). The cytoplasmic matrix is usually of low density. The endoplasmic reticulum is sparce, but long cisternal contours are seen occasionally. The mitochondria are very long and slender, with their long axes generally perpendicular to the basement membrane of the seminiferous tubule. In contrast to the mitochondria of the spermatids, these have the usual arrangement of internal membranes and a relatively dense matrix.

Phase Contrast Observations

Freshly Isolated Cells.—Upon isolation in physiological salt solution, the groups of germ cells joined by intercellular bridges either separate into individual cells or coalesce into binucleate, quadrinucleate, or multinucleate masses. The spermatocytes are easily recognized by their large size and by the distinctive pattern of prophase chromosomes in their nucleus (Fig. 12). The Golgi complex appears as a phase-dark, rounded mass adjacent to the nucleus. In secondary spermatocytes, several proacrosomal granules can often be seen in the interior of the Golgi complex. Spherical mitochondria are distributed at random in the cytoplasm. In addition to these organelles, fine dark lines are visible in the peripheral cytoplasm (Fig. 12). These are arranged concentrically and tend to run parallel to the cell surface. Some can be followed for half to two-thirds of the distance around the cell. As the microscope is focused from higher to lower levels these dark contours shift laterally, but their continuity and their relative position is maintained. Therefore, they cannot be interpreted as filaments, but must be regarded as optical sections of extensive sheets or lamellae. Inasmuch as electron micrographs of similar material show pairs of membranes disposed circumferentially in the cytoplasm, the dark lines observed at the periphery of the living spermatocytes are believed to correspond to cisternae of the endoplasmic reticulum.

One does not ordinarily expect to obtain a clear image of structures this small, with the light microscope. There is no doubt, however, that the cisternae are seen under the conditions described here. The explanation is the same as that which applies to the nuclear "membrane" which is clearly visible with the light microscope, although the

total thickness of its double membrane is no greater than that of the cisternae of the endoplasmic reticulum. In both instances one is dealing with an extensive sheet that has a curving course with respect to the plane of focus of the microscope. The depth of focus is sufficient to include an arc of the curving surface that subtends a line wide enough to be seen. Furthermore, the endoplasmic reticulum in the guinea pig germ cells has an amorphous content that is more dense than the surrounding cytoplasmic matrix, and this no doubt favors its visibility with phase contrast.

The isolated early spermatids are distinguished from spermatocytes by their smaller size, their nuclear characteristics, and their acrosomal system. The nucleus usually contains one or two conspicuous dense bodies that resemble nucleoli (Figs. 3 to 5, 10, and 11). However, in histological sections stained with the Feulgen reaction, it can be shown that these are not nucleoli, but dense aggregations of desoxyribonucleoprotein. Adhering to the nuclear membrane of the early spermatid, is a sharply outlined acrosomal vesicle having a fluid content of low refractive index. A small spherical acrosomal granule is found floating in the interior of the vesicle (Figs. 3 and 4). A phase-dark Golgi complex of variable shape is closely applied to the convex surface of the vesicle. As differentiation progresses, the area of contact between the acrosomal vesicle and the surface of the nucleus is enlarged and the refractive index of its contents increases. The acrosomal granule later becomes attached to the nucleus and appears as a dark hemispherical body surrounded by a less dense outer zone (Figs. 5 to 7). The mitochondria are spherical in shape, few in number, and widely distributed in the cytoplasm. A dense mass of irregular shape, identified as the chromatoid body, is located near the nucleus (Fig. 6). The faint linear contours, representing the cisternae of the endoplasmic reticulum, are more loosely arranged than in spermatocytes. They frequently are not parallel to the cell surface but run a flexuous, meandering course in the cytoplasm (Figs. 4 to 6).

During preparation of the specimens for examination, the Sertoli cells are usually disrupted in the teasing and compression of the seminiferous tubules. A few are spared and can be distinguished from other cell types by their mitochondria which are long and slender, whereas those of the germ cells are generally spherical.

Changes in the Appearance of the Cells upon Standing.—As the preparations stand, the environmental conditions in the thin film of saline between the slide and coverslip become increasingly unfavorable for survival of the cells, and peripheral areas of the cytoplasm gradually undergo a change in their physical state that results in a lowering of the refractive index. This causes the cisternal contours of the reticulum to stand out in higher contrast then they do in the freshly isolated cells (Figs. 7 and 8). The alterations in the cells appear to involve imbibition of water and solation of a portion of the cytoplasm. In the resulting fluid areas of the cytoplasm, mitochondria and other particulates exhibit lively Brownian motion, while the same components in the perinuclear cytoplasm are relatively quiescent. The long profiles of the cisternae are most conspicuous in the solated portions of the cells, and there they are observed slowly to change their arrangement. At first, the long profiles run a sinuous course independent of one another, but as time passes they tend to become laterally associated in curving, multilayered systems, and finally they form hollow concentric spheres that appear, in optical section, as dark rings composed of three to fifteen closely spaced concentric lamellae (Fig. 8).

The total surface of the membranes in these lamellar systems appears to become greater with increasing time after isolation of the cells from the testis. It was pointed out in a previous section of this paper that if one examines electron micrographs of seminiferous tubules that have been fixed immediately after their removal from the animal, the spermatids, in the acrosome phase of differentiation show relatively few cisternae. The endoplasmic reticulum consists mainly of tubular elements (Fig. 1). Thus it is not possible to account for the extensive lamellar systems seen with phase contrast 30 to 90 minutes after isolation of the cells, simply by assuming that changes in the refractive index of the cytoplasmic matrix have rendered preexisting cisternae more clearly visible. One is obliged to conclude that, under the conditions of observation employed here, the cisternae originally present have been greatly extended, and that new ones have been formed by coalescence and reorganization of the tubular elements that normally make up the bulk of the reticulum. It is also possible that Golgi membranes are also drawn upon to extend the cisternae, or that phospholipide in the cytoplasmic matrix may contribute to the formation of new membrane.

If the cells are allowed to remain in saline for 30 to 240 minutes and are then fixed and examined

with the electron microscope, concentric lamellar systems are easily identified and are found to be composed of pairs of membranes that differ in no essential respect from the cisternae seen in promptly fixed tissue (Fig. 15). A few ribonucleoprotein granules adhere to the membranes, and larger numbers are free in the surrounding cytoplasm. Although there is evidence of imbibition of water and a consequent decrease in density of the cytoplasm, there appears to have been little loss or dilution of the amorphous content of the endoplasmic reticulum. The narrow space between the cisternal membranes is still occupied by a substance of appreciable density. Numerous vesicular and tubular elements of the reticulum are found in close proximity to the free margins of the cisternae in a relationship suggesting that a process of coalescence may have been interrupted by fixation.

The changes in the organization of cytoplasmic membranes which have just been described are not confined to the germ cells, but are seen also in the sustentacular elements. As the preparations age, large, clear blebs form on the Sertoli cells, and some of these are pinched off as anuclear cell fragments. These have limiting membranes and maintain their integrity for long periods. Their origin from Sertoli cells can be ascertained by their distinctive mitochondria. In blebs on intact Sertoli cells and in detached portions of these cells, the endoplasmic reticulum undergoes a reorganization that leads to the formation of concentric systems of lamellae that are even more elaborate than those found in the germ cells (Figs. 9 to 11, 13, and 14).

Although these structures develop in cells that are gradually succumbing to unfavorable environmental conditions, their appearance cannot be regarded as a terminal event in moribund cells, because the changes in the endoplasmic reticulum that lead to their formation are already far advanced before obvious regressive changes are detectable in the other organelles. The mitochondria are generally considered to be fairly sensitive indicators of cell viability; yet in Sertoli cells 1½ hours after isolation (Figs. 9 to 11), long filamentous mitochondria are still present and show no sign of the swelling and fragmentation which would be indicative of impending cell death.

DISCUSSION

The great variety of intracellular membranes that have been revealed in recent years by the electron microscope has greatly stimulated interest in biological membranes in general and particularly in the membranous systems of the cytoplasm. The work reported here demonstrates, for the first time, that the formation of parallel arrays of membranes can be studied in living cells by phase contrast, and thus raises the hope that experiments can be devised that will contribute to an understanding of the factors that determine the form and arrangement of the intracellular membranes.

Under the conditions of observation described here extensive lamellae or cisternae appeared to arise by a coalescence of the tubular and vesicular elements of the endoplasmic reticulum. The formation of lamellar systems by fusion of vesicles has been postulated heretofore from examination of electron micrographs of fixed cells of various types. For example, Fawcett (5) observed in the livers of rats refed after a prolonged fast that the first sign of restoration of the endoplasmic reticulum was the appearance of irregular masses of small vesicles. These were believed to fuse into a close meshed network, which subsequently reorganized to form the tubules and parallel dispositions of cisternae that are characteristic of the normal liver cell. Hodge *et al.* (11) reported that the endoplasmic reticulum of *Nitella* seemed to arise by confluence of small spherical vesicles, and the same applied to the formation of the closed double membranes in the developing chloroplasts in *Zea*. In the embryonic retina, De Robertis (1) found that an accumulation of small vesicles preceded the development of the lamellar structure of the rod outer segment. The concept of morphogenesis of multilayered lipoprotein systems by fusion of small vesicles thus can be supported by a number of examples. Therefore, in the present study, the fact that extensive parallel arrays of cisternae seemed to arise by coalescence of other components of the reticulum, should not be unexpected. The conditions under which they formed, however, are somethat surprising.

Organelles made up of lipoprotein membranes are considered to be particularly sensitive to changes in their physical environment. They swell in hypotonic media and even in physiological salt solution. In one of his original accounts of the endoplasmic reticulum, Porter (21) described the fragmentation of the system into isolated vesicles in cells undergoing cytolysis. Hodge *et al.* (12) found that the elements of the endoplasmic reticulum of isolated liver cells rounded up into large vacuoles or broke up into a population of small

vesicles indistinguishable from those seen in electron micrographs of the microsome fraction. Thus it would have been expected that the reticulum of cells isolated from the testis would also fragment. It may be that the changes in the form of the membranes that were observed, represent reaction to injury rather than stigmata of degeneration. It is pertinent to note that concentric dispositions of cisternae have been observed in electron micrographs of ascites tumor cells (3), hepatomas (4) (Fig. 16), and pituitary tumors (10).

Policard, Collet, and Pregermain (19, 20) have recently reported concentric systems of membranes in electron micrographs of lung tissue from experimental silicosis. These authors did not, however, recognize any relationship between these membranes and those of the endoplasmic reticulum. Instead, such structures were equated with the "myelin figures" studied with the light microscope by Nageotte (14), Frey-Wyssling (9), and Dervichian (2). According to their interpretation, degenerative changes in the cytoplasm induced by the silica caused a liberation of phospholipide, which underwent hydration to form osmiophilic lipide films separated by layers of water. The layered systems were thus regarded as having essentially the same origin and structure as the "myelin forms" that arise *in vitro* upon hydration of lecithin.

The observations reported here clearly establish that the concentric systems previously called "myelin figures" and therefore dismissed as artifacts or pathological breakdown products of cells can be traced back directly to the membranes of the endoplasmic reticulum. The concentric systems formed intracellularly are not identical in their fine structure with the concentric systems formed by lecithin *in vitro*, but nevertheless, it is apparent that the physicochemical analogy of smectic paracrystalline systems (myelin forms) may be very useful in interpreting the mode of elaboration of parallel arrays of membranes in cells and the changes in their form under different environmental conditions.

Although concentric formations of membranes are usually found in pathological tissues or in normal cells exposed to an unfavorable environment, they are also seen on rare occasions in tissues that appear to be normal. Thus whorls of membranes have been seen in the perikarya of neurons (17), the eggs of *Spisula* (23), and in the liver and intestinal epithelium of *Triturus*. Future studies may reveal that the form assumed by the reticulum depends upon the physical consistency or state of hydration of the cytoplasm. Indeed, the present findings suggest that imbibition of water and solation of the cytoplasm favor the formation of extensive cisternae, while the maintenance of a canalicular reticulum may require the participation of a structured cytoplasmic matrix. In continuing these studies the influence of various suspending media and of surface-active agents on isolated testicular cells will be explored in an effort to define the conditions of the intracellular and extracellular environment which promote the development of lamellar systems of membranes.

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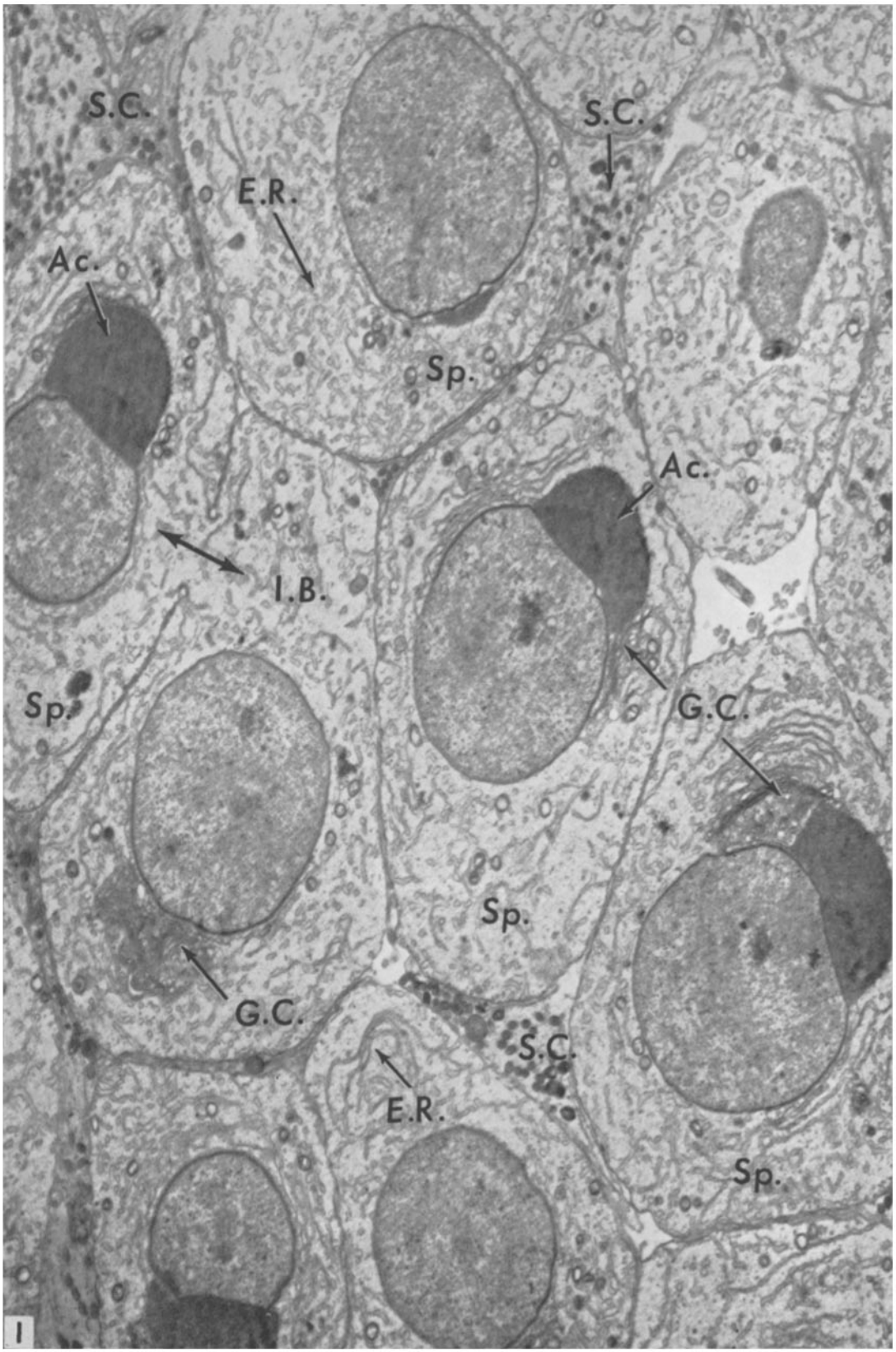
Explanation of Plates

Abbreviations

Ac., Acrosome.
A.G., Acrosomal granule.
A.V., Acrosomal vacuole.
C., Centriole.
C.B., Chromatoid body.
E.R., Endoplasmic reticulum.
G.C., Gogli complex.
H.C., Head cap.
I.B., Intercellular bridge.
M., Mitochondria.
N., Nucleus.
S.C., Sertoli cell.
Sp., Spermatid.

Plate 63

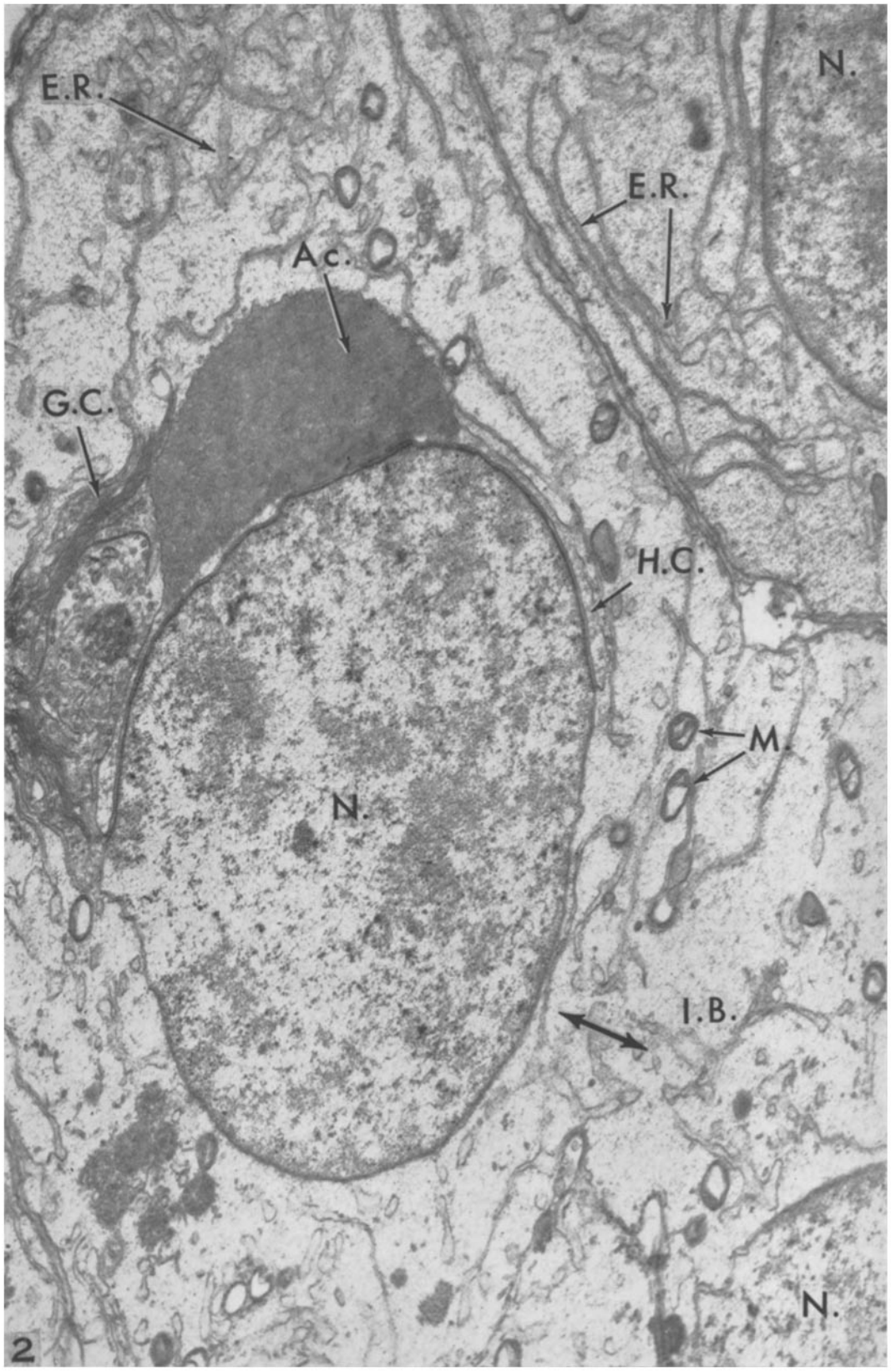
Fig. 1. An electron micrograph of guinea pig germinal epithelium at relatively low magnification, showing several spermatids (*Sp.*) and polygonal areas of Sertoli cell cytoplasm (*S.C.*) in the interstices between them. The cytoplasm of the Sertoli cells is easily distinguished from that of the germ cells by the smaller size and greater density of its mitochondria. The endoplasmic reticulum (*E.R.*) is very well developed in the spermatids (*Sp.*). It consists, for the most part, of a close meshed network of tubular elements, but there are also long profiles of cisternae. The spermatids are joined together in clusters by intercellular bridges (*I.B.*), like the one shown in this figure at the arrow. × 12,000.



(Fawcett and Ito: Testicular cytoplasmic membranes)

PLATE 64

FIG. 2. Electron micrograph of a guinea pig spermatid in approximately the same phase of spermiogenesis as the living spermatids illustrated in Figs. 5 and 6. The acrosome (*Ac.*) at this stage is composed of a dense substance that almost completely obscures the outline of the original acrosomal granule. A thin fold of the limiting membrane of the acrosomal vesicle has spread radially from the margin of the acrosome to form the head-cap (*H.C.*). The Golgi complex (*G.C.*) is lodged in the angle between the acrosome and head-cap. In the cytoplasm are a number of mitochondria (*M.*) and numerous elements of the endoplasmic reticulum (*E.R.*). The long sinuous profiles at the upper right (*E.R.*) and elsewhere in the micrograph are sections of broad flat sheets or cisternae of the endoplasmic reticulum. × 22,500.



(Fawcett and Ito: Testicular cytoplasmic membranes)

PLATE 65

All figures are photomicrographs of living cells under phase contrast optics.

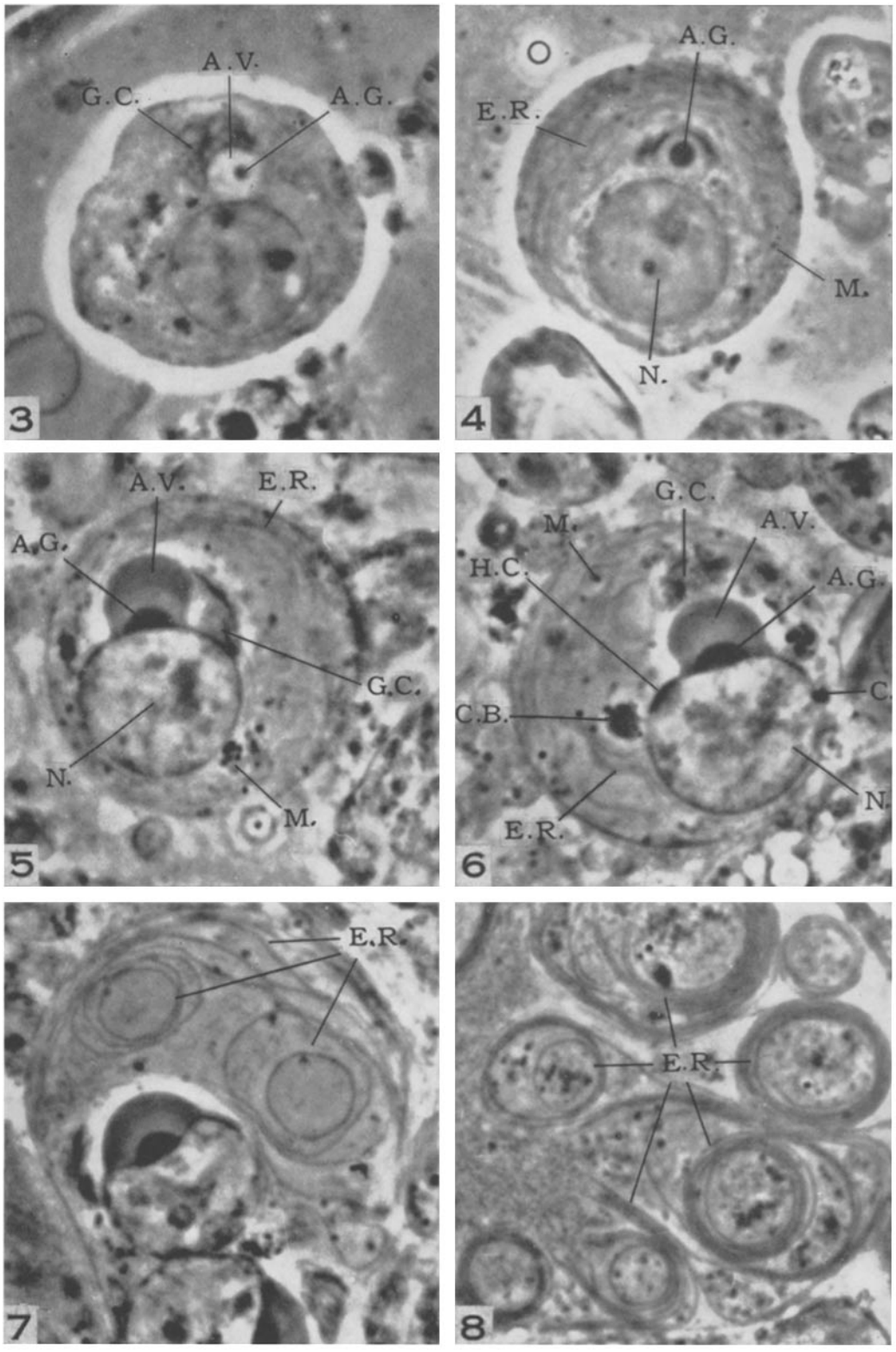
FIG. 3. A living spermatid freshly isolated from a seminiferous tubule. The acrosomal vesicle (*A.V.*) is identifiable as a clear juxtanuclear sphere containing a dense acrosomal granule (*A.G.*). A dark area associated with the surface of the vesicle is the Golgi complex (*G.C.*). Several clusters of dense spherical mitochondria are seen in the moderately dense, faintly mottled cytoplasm. × 2500.

FIG. 4. A spermatid slightly more advanced in its development than that of Fig. 5, as indicated by the larger size of its acrosomal granule (*A.G.*). At this stage, the granule (*A.G.*) appears to be floating in a fluid content of the vesicle, for in the living preparation, it can be seen to move freely from one side of the vesicle to the other. The cytoplasm shows a few mitochondria (*M.*) and indistinct linear contours (*E.R.*) that are interpreted as optical sections of cisternae of the endoplasmic reticulum. × 2500.

FIGS. 5 and 6. Spermatids in a later stage of differentiation. The acrosomal granule (*A.G.*) has become fixed to the nuclear membrane, and the content of the vesicle has increased in refractive index. A few cisternal profiles can be made out in Fig. 5 (*E.R.*). In Fig. 6, they can be seen more clearly. Other structures that are visible include the mitochondria (*M.*), Golgi complex (*G.C.*), chromatoid body (*C.B.*), a centriole (*C.*) and the developing headcap (*H.C.*). × 2500.

FIG. 7. A binucleate spermatid from a preparation kept under observation for more than an hour. The cell has imbibed water, and an area of cytoplasm at the upper part of the figure has liquefied. The accompanying change in refractive index of the cytoplasmic matrix has caused the linear profiles of the cisternae to stand out in better contrast. The cisternae are quite extensive and tend to roll up, so that in optical section they appear as closed circular profiles, one within the other. × 2500.

FIG. 8. An area of the peripheral cytoplasm of a syncytial mass of spermatocytes. The surface area of the cisternal membranes appears to have increased greatly, probably by coalescence of tubular elements of the endoplasmic reticulum. The resulting membranes have become rearranged to form multilayered concentric arrays, seen here in optical section. × 2500.



(Fawcett and Ito: Testicular cytoplasmic membranes)

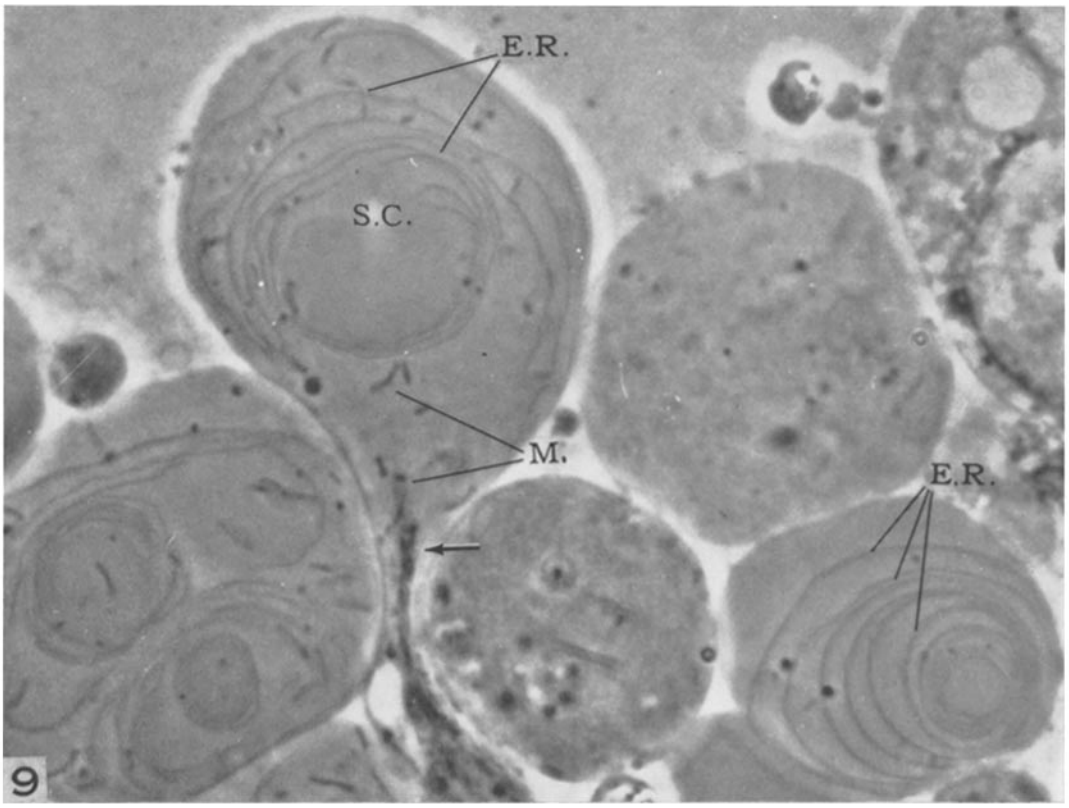
PLATE 66

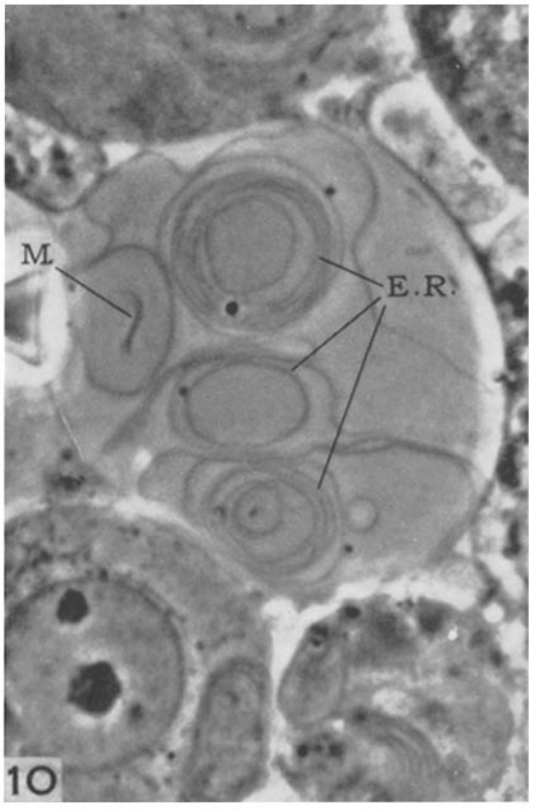
All figures are photomicrographs of living cells taken under phase contrast optics.

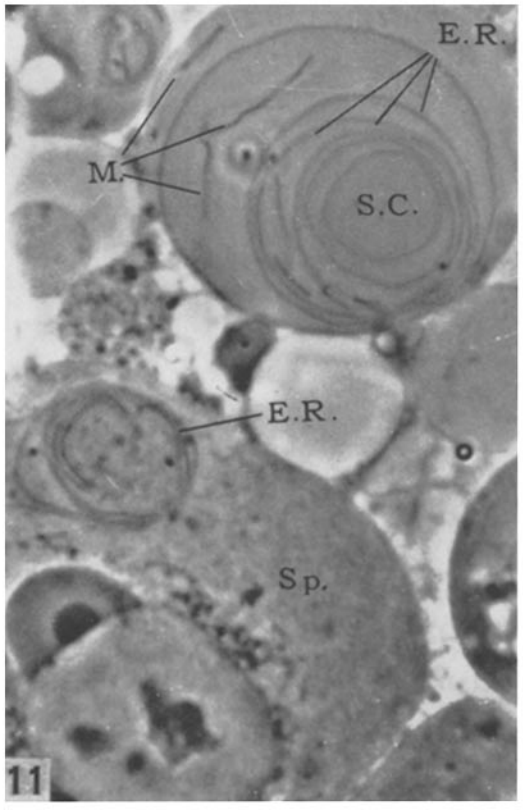
FIG. 9. A preparation of free cells 1 hour after their isolation from the testis. Included in this field are three fluid-filled blebs or vesicular expansions of Sertoli cells (*S.C.*). The continuity of one of these with a narrow process of an intact Sertoli cell is shown at the arrow. The cytoplasm in the slender process is crowded with organelles and is apparently gelated for the mitochondria were relatively immobile. The cytoplasm in the expanded tip is apparently solated, for the mitochondria there were in lively Brownian motion. Numerous cisternal contours are visible in the fluid portion (*E.R.*). At the lower right is an anuclear fragment of Sertoli cell containing membranes in concentric array. × 2500.

FIG. 10. A further example of the formation of concentric lamellar systems within fluid areas of Sertoli cell cytoplasm. × 2500.

FIG. 11. At the upper right of the figure is an isolated portion of a Sertoli cell, containing membranes (*E.R.*) which are arranged concentrically. The long dense rods are mitochondria (*M.*). At the lower left is a group of concentric membranes in the peripheral cytoplasm of a spermatid (*Sp.*). × 2500.







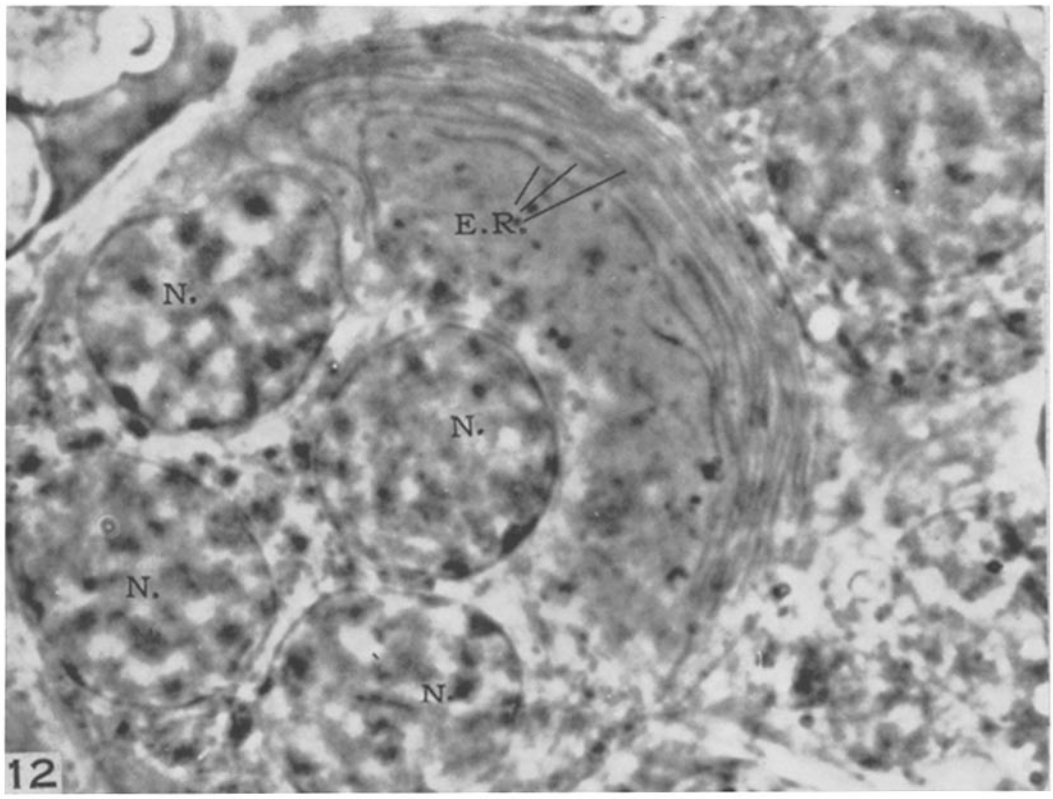
(Fawcett and Ito: Testicular cytoplasmic membranes)

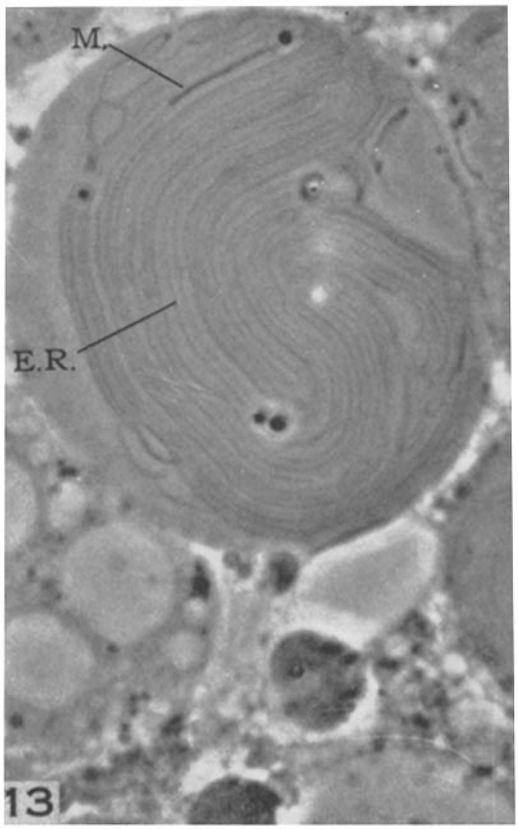
PLATE 67

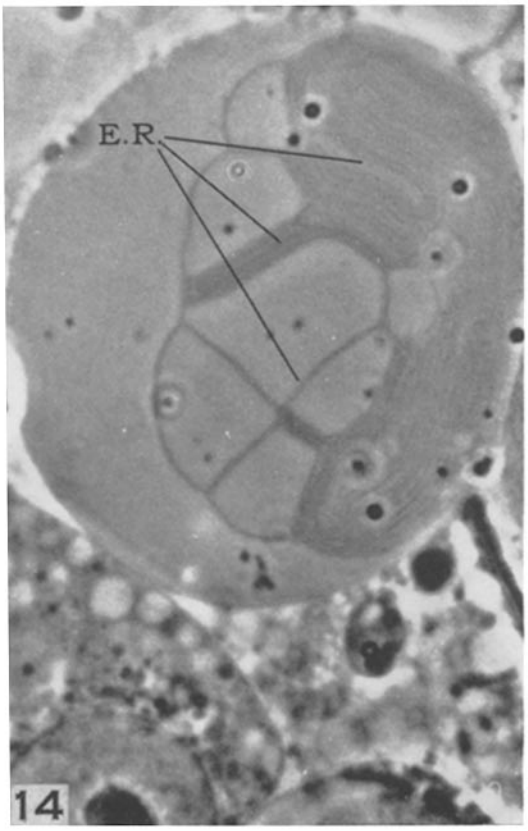
All figures are photomicrographs of living cells under phase contrast optics.

FIG. 12. A quadrinucleate mass formed by the coalescence of four conjoined spermatocytes. The nuclei (*N.*) are all in the same stage of prophase. The endoplasmic reticulum has reorganized into extensive cisternae arranged parallel (*E.R.*). In the right upper quadrant of the multinucleate mass ten to twelve layers can be counted. × 2500.

FIGS. 13 and 14. Isolated portions of Sertoli cells 4 hours after removal from the testis. The endoplasmic reticulum (*E.R.*) has formed extraordinarily elaborate masses of closely packed parallel membranes resembling myelin figures. It is of interest to note that after 4 hours under these unfavorable conditions, the mitochondria (*M.*) show no notable change in their form. × 2500.





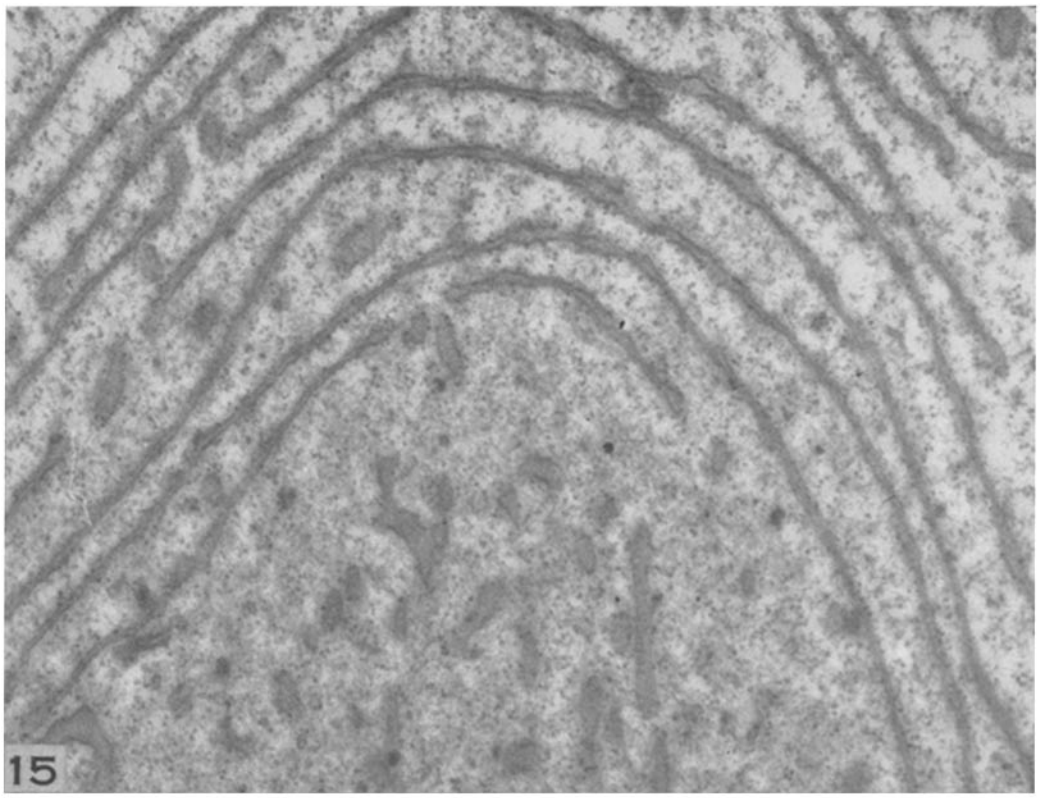


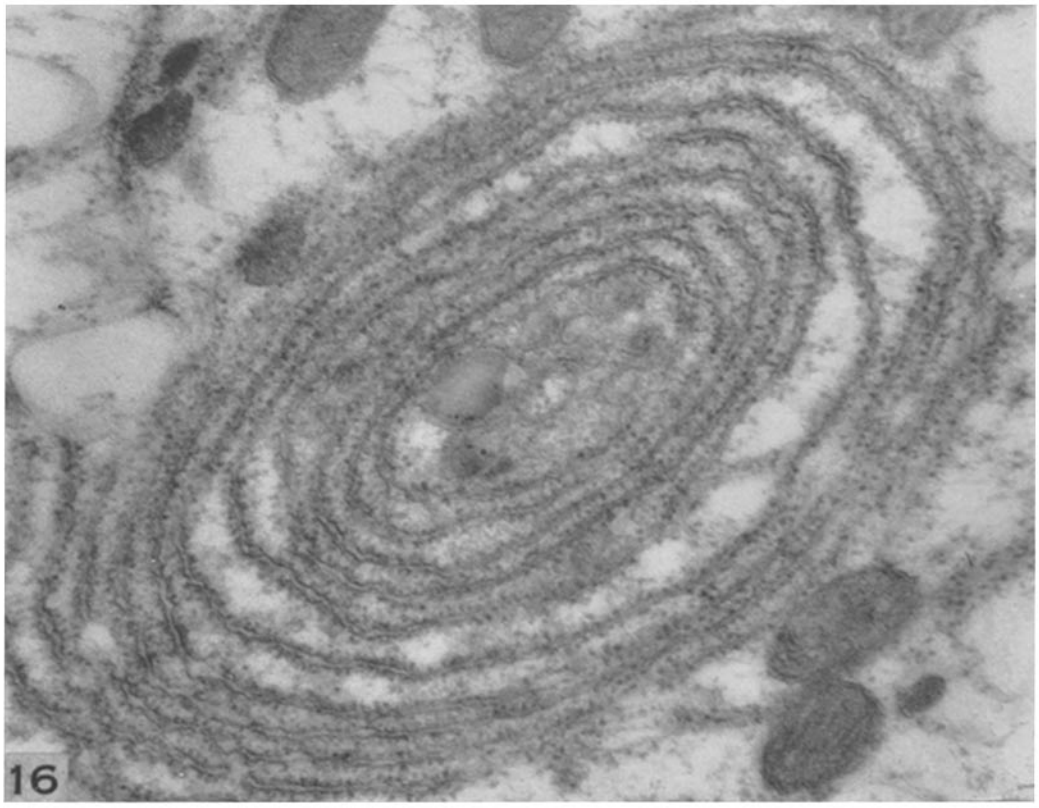
(Fawcett and Ito: Testicular cytoplasmic membranes)

PLATE 68

FIG. 15. An electron micrograph of an area of cytoplasm from one of the germ cells in a block of testicular tissue allowed to stand 4 hours in saline at room temperature before being fixed. Many long cisternal profiles are present, and the cavities bounded by the pairs of membranes still show a moderately dense amorphous content. Small granules, presumed to be ribonucleoprotein, are abundant throughout the cytoplasmic matrix. This degree of stability of the cisternae was unexpected. If allowed to stand longer, they ultimately break up into small vesicles, but only after many hours. × 36,000.

FIG. 16. A small area of a cell from a mouse hepatoma included to illustrate that concentric lamellar systems, resembling those described here in isolated cells, also may occur in the intact animal. The pairs of membranes form closed ovals arranged one within the other. Small ribonucleoprotein granules adhering to their surface serve to identify them as elements of the endoplasmic reticulum. × 60,000.





(Fawcett and Ito: Testicular cytoplasmic membranes)