# Electron Microscopic Studies on Ovarian Oocytes and Unfertilized Tubal Ova in the Rat

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ABSTRACT

Developing rat ova have been studied with the electron microscope. Special attention was paid to relations of ova to the granulosa cells, the developmental stages of ovarian follicles, and the cytology of the unfertilized tubal ova. The relationship of the oocyte to the surrounding granulosa cells was found to change from one of a simple apposition of the plasma membranes to a complex interdigitation of microvilli from the ovular surface and processes from the granulosa cells extending into the matrix of the zona pellucida. This complex interrelation is maintained until the formation of the first polar body is initiated. At this time no microvilli are found and the oolemma presents a gently undulating outline. Also at this time, a perivitelline space forms and the granulosa cell processes retract. In the unfertilized tubal ova no microvilli are present and the processes of the follicular cells are completely withdrawn.

The cytoplasmic elements of the oocyte in various stages of development are described in some detail. Of particular interest is the change noted in position and degree of aggregation of the Golgi complex as maturation proceeds. The distribution and structural characteristics of the mitochondria, ergastoplasm, dense particles, and multivesicular bodies are described.

## INTRODUCTION

Many interesting problems relative to the development of the ovarian follicles have been raised by studies using the light and phase microscopes. Some of these concern the relationships between the developing oocyte and its surrounding granulosa cells; the possible means of nutrition of such oocytes; the formation of the zona pellucida; the cytoplasmic modifications occurring during follicular development; and the fine structure of developing ovarian oocytes as compared to that of unfertilized tubal ova. It was anticipated that an electron microscope study of the developing rat ovarian follicles and of unfertilized tubal ova would contribute additional information on these problems.

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## *Methods*[1, 2]

For the study of the early developmental stages of ovarian follicles, tissue was removed from Sherman strain rats varying in age from less than 1 day to sexual maturity. Much of this tissue was obtained from prepuberal animals. The earlier stages of follicular development, up to the appearance of multilaminar follicles with small antra, may be found much more frequently in such ovaries. Fixation was also better and sectioning easier than with ovaries from adult animals where the abundance of luteal and interstitial tissue create problems. The sequence of follicular development in prepuberal rats has been reported by Vincent and Dornfeld (52) and the quantitative aspects by Slater and Dornfeld (45).

For the study of follicles in the preovulatory period,

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young, sexually mature females were used. Estrus was determined by positive response to manual manipulation of the pudendal region (9). The females found to be in heat were sacrificed before 6:30 a.m., and the largest follicles dissected out and fixed. A number of sections of the same ovum were examined. Unfertilized tubal ova were sectioned *in situ* in the ampulla of the oviduct.

In all, tissues from 85 rats were studied. Ovarian and tubal tissues were fixed by immersion for periods varying from 1 to 2½ hours in cold, 1 per cent buffered (pH 7.4) osmic acid to which sucrose (15.7 gm. per 100 ml.) was added. The tissues were dehydrated in a graded ethyl alcohol series, embedded in a mixture of 1.5 parts of methyl methacrylate to 8.5 parts of butyl methacrylate, and finally sectioned for electron microscopy with a Porter-Blum microtome. The sections were mounted on grids covered with formvar films or films coated with carbon. Some grids were covered with a second film according to the techniques of Watson (54).

In the latter part of the study sections on grids were stained by floating them, section down, on a 2 per cent solution of uranyl acetate, with subsequent rinsing in distilled water. The sections were examined with a RCA-EMU-3C electron microscope. The electron micrographs were taken at initial magnifications between approximately 1800 and 14,000 diameters. Thicker sections mounted, unstained, in amyl acetate were examined with the phase microscope.

## OBSERVATIONS

*1. Unilaminar Follicles.*—The earliest recognizable follicles are those in which a single layer of flattened granulosa cells is incomplete, frequently with a portion of the plasma membrane of one oocyte abutting directly against that of an adjacent ovum (Fig. 1). Later, numerous follicles are observed in which a continuous layer of flattened granulosa cells completely surrounds the egg. On many areas of the cell surface the plasma membrane of the ovum is parallel to that of the granulosa layer and is separated from the latter by a small, non-uniform, intercellular space (Fig. 2, *ic*). Occasionally, small projections of the oocyte membrane appear to indent the granulosa cell membrane. In other regions of the same egg, circular or ovoid profiles are seen intervening between the two membranes as if processes from the granulosa layer had been cut in cross-section (Fig. 4, *g*). Membranous profiles (Fig. 4, arrows) lying immediately adjacent to or in contact with the oocyte are noted and are similar to those seen, for example, along the surface membrane of mesothelial cells during particle uptake (35). The position and appearance of these structures suggests the possibility of micropinocytosis.

In the smaller unilaminar follicles the nucleus of the oocyte lies eccentrically (Figs. 2 and 4). The nuclear membrane consists of two dense lines with an intervening space. When the membrane is sectioned obliquely, circular profiles, similar to the "nuclear pores" in other cells, are observed (53). When present in the section the nucleolus appears as an irregular area composed of dense granules. The characteristically large Golgi complex is situated in close association with the nucleus (Figs. 2, 4, *gc*). This complex, as reported in numerous other cell types (17, 27), is composed of many paired, smooth surfaced membranes. The width of each paired unit, including the dense lines and interspace, averages approximately 38 mμ. In addition, there are many vesicles the diameter of which is approximately the same as that of the paired membranes. As seen in Fig. 4, several larger vacuoles are often associated with the complex.

The mitochondria, scattered at random throughout the cytoplasm, show a dense matrix containing relatively few cristae. Their outline frequently appears to bulge in regions where the cristae are absent (Figs. 4, 5, 9, *m*). The diameter of the mitochondria averages 340 mμ.

Another type of cytoplasmic complex, designated "multivesicular body" by Sotelo and Porter (46), is also present. It may consist of a relatively large vacuole surrounded by numerous vesicles and containing either a few or many small vesicles about 29 mμ wide (Fig. 4, *mvb* (1)). In some cases (Figs. 12, 20, *mvb* (2)) the vacuole also contains one or more very dense bodies of different sizes, called nucleoids by these workers. At this stage only a few multivesicular bodies are observed in a given section of an oocyte.

The remainder of the cytoplasm contains many granules of varying sizes and density (Fig. 4). Some are very small and are presumed to be precipitated protein of the matrix. Others (*dp*) which stain selectively with uranyl acetate and measure approximately 20 mμ are considered to be ribonucleoprotein particles of the type associated with ergastoplasmic membranes (39). Here these are usually scattered in small groups throughout the ooplasm and are only infrequently seen on membranous profiles. In addition to the granules, scattered vesicles and occasional vacuoles, not associated with the vesicles, are seen (Fig. 4).

In the oocytes of small unilaminar follicles certain cytoplasmic structures are observed which are not prominent at any later follicular stage.

These are considered to be indicative of degenerative processes since they are more numerous in obviously degenerating oocytes (45). The most frequent type is similar to myelin figures reported in other cells, showing a concentric arrangement of membranes separated from one another by a narrow space (Fig. 3, *mf*). The two membranes and the intervening space together measure approximately 20 mμ. Transitional stages suggest the possibility of their arising from mitochondria (Fig. 3, 1 to 5), although often the bodies are open at one side or the central region is not occupied by the membranes. Another unusual structure similar to the normal multivesicular bodies, but larger, is a vacuole within which lies a number of membranous inclusions, some of which are quite dense (Fig. 3, *v*). In addition, obviously abnormal follicles are observed. The nucleoplasm of the oocyte is clumped, and the nuclear membrane is thickened. Within the ooplasm there are also dense clumped regions and structures of the two degenerative types described above. Unlike follicular degeneration at later stages, in which degenerative changes appear first in the granulosa cells, the granulosa cells associated with these follicles appear normal (52).

*2. Initial Phases of Formation of the Zona Pellucida.*—The earliest stage of zona pellucida formation is observed in follicles where a single layer of low cuboidal granulosa cells completely encircles the oocyte. The substance of the zona is still not deposited in a layer of uniform width (Figs. 5 to 7). The oocyte and granulosa cell plasma membranes remain in intimate contact with one another in some regions, and in others become separate. The intervening spaces are filled with the finely granular substance of the zona, but the over-all density of the material does not appear as great as in later stages. The oolemma has begun to form a few short microvilli which are embedded in the forming zona (Figs. 5, 6, *mv*). As more of the zona pellucida is deposited (Figs. 8, 9, *zp*), the space intervening between the plasma membranes of the oocyte and granulosa cells widens and the microvilli of the oolemma become more numerous. Short, blunt processes of greater diameter than the ovular microvilli and extending from the surface of the granulosa cells into the zona make their appearance at this time (Figs. 8, 9, 6, *gp*, arrow).

Unilaminar or bilaminar follicles can be seen in which zona substance extends into the intercellular space between two adjacent granulosa cells of the most internal layer (Fig. 7, between *C* and *B*). Because of the complex topographical relationships between the granulosa cells and the oocyte at this stage, the granulosa cells may be seen in section to be in close contact with the oolemma in certain areas (*A*), while separated by an accumulation of zona material in others (*B*). An appearance such as that in Fig. 7 does not necessarily mean that the zona has been deposited by granulosa cells *A* and *B*. The small area of cytoplasm of cell *A* probably represents an extension of this cell in contact with the oocyte in the plane of this figure. At other levels this cell probably does not intervene between cell *B* and the oocyte membrane. It is not possible on the basis of this sort of configuration to conclude that the material of the zona is formed by the granulosa cells.

*3. Multilaminar Follicles, without Antra or with Antra but Prior to Preovulatory Swelling.*—No structural differences are noted in the normal follicles, in which the egg is surrounded by two or more layers of granulosa cells, whether from prepuberal or mature animals. The width of the zona pellucida varies between 600 and 3600 mμ and increases during the follicular growth period (compare Fig. 10 with Fig. 6, *zp*). It may be observed that there are no canalicular structures in the zona as postulated by light microscopists. The plasma membrane of the egg and granulosa cell processes is immediatey surrounded by the substance of the zona in every case.

The complex pattern of interrelationship between the oocyte and the granulosa cell layer is well established in these follicles (Figs. 10, 12 to 14, 17, *mv*, *gp*). Microvilli are more numerous than in previous stages and usually extend into the zona to about one-third to one-half of its width. In outer diameter the microvilli measure approximately 62 mμ, each membrane being about 8 mμ thick. Usually no formed ooplasmic components are seen within the microvilli; however, they often appear somewhat more dense (perhaps due to electron-scattering from closely adjacent membranes) than the ooplasmic matrix (Figs. 10, 13, *mv*).

The granulosa cell processes extending obliquely into the zona are quite irregular in their distribution and form (Figs. 10, 12, 17, *gp*). Their average diameter is 106 mμ. Some abut directly against the oolemma or the ovular microvilli. Many isolated segments appear within the zona pellucida, having been caught in the plane of section (Figs. 10, 12, 13, *gp*) and these often contain small

granules such as those seen in the body of the granulosa cell (Figs. 10, 17, *gp*).

In some of the oocytes at this time there lies, just internal to the oolemma, a narrow zone 60 to 70 mμ in width possessing a density greater than that of the ooplasmic matrix (Fig. 13, *ds*). It may be that this dense band is indicative of chemical activity involved in transport of substances into or out of the oocyte or is related to zona pelludica formation.

At these stages also occasional granulosa cells retain part of their initial direct contact with the oolemma. Also some areas of the zona directly adjoin the intercellular spaces with no covering granulosa cells present. The latter is likewise found in follicles undergoing the preovulatory swelling and in unfertilized tubal ova.

As early as the bilaminar follicular stage the disposition of cytoplasmic components differs from that of the unilaminar follicle. In general, this difference persists to the time of the initiation of the first polar body formation. The larger structures such as the mitochondria, Golgi complex, and multivesicular bodies are variably associated with one another in small groups (Figs. 10 to 13, 17, *m*, *gc*, *mvb*). Most of the oocytes show such groups located primarily near the periphery. In some eggs during the late stages of zona pellucida formation, the Golgi complex lies almost entirely at the periphery in the form of stacks of 4 to 6 paired membranes with associated vesicles (Figs. 10, 12, 13, *gc*). The mitochondria average 200 mμ in width. Sometimes very small vesicles and fine granular material are associated with the groups of the larger organelles. The areas between groups are occupied by the fine granules of the cytoplasmic matrix. A well defined ergastoplasm composed of membranes with attached dense particles, as seen in somatic cells, is observed infrequently (Figs. 11, 12, 17). When present, the membranes of the ergastoplasm often form an ovoid mass such as that in Fig. 11 (*er*). Most of the dense particles are scattered randomly throughout the ooplasm rather than upon the ergastoplasmic membranes. The multivesicular bodies (Fig. 12, *mvb*) are observed more frequently than in earlier stages, type *2* being proportionately more numerous.

*4. Multilaminar Follicles during the Period of Preovulatory Swelling and the Unfertilized Tubal Ova.*—Follicles obtained a few hours prior to ovulation have undergone the major part of the preovulatory swelling (10). The germinal vesicle of the egg has broken down and the first polar body has reached at least the stage of metaphase in its formation (34).

At this time interesting changes occur in the relationship between the oocytes and granulosa cells (Figs. 18 to 20, *ol*, *gp*). A perivitelline space (*pv*) of variable width appears with no sharp boundary between it and the substance of the zona pellucida. Its contents are somewhat less dense than the zona and include granular material and vesicles similar to those within the ooplasm. The fine microvilli previously present are withdrawn and only relatively blunt processes remain. These project in an irregular fashion from the surface of the oocyte and contain ooplasmic elements. Later in the preovulatory period only occasional blunt processes are observed, the remainder of the oolemma exhibiting a characteristic, irregular, and somewhat undulating outline (Fig. 18, *ol*). The processes of the granulosa cells, which formerly extended deep into the zona, are also being withdrawn (Fig. 20, *gp*). The larger ones are less frequently encountered and the relative numbers appear to be smaller.

Unfertilized tubal ova obtained from animals killed the morning following the heat period are still surrounded by the mass of cumulus cells (Figs. 15, 21, 22, *g*, *gn*). The width of the perivitelline space varies from 900 mμ around the periphery to 13,900 mμ at the site of the first polar body (Figs. 21, 22, *pv*). The width of the zona averages 2800 mμ, with a range of 1300 to 4600 mμ. The oolemma shows an undulating form, as in the oocytes in the late preovulatory follicles (Figs. 21, 22, *ol*). Cytoplasmic processes no longer extend from the internal surfaces of the granulosa cells into the zona pellucida. Instead the plasma membrane of the granulosa cells now appears smoothly opposed to the zona. In many areas of the outer surface of the zona a loosely organized, fairly dense substance appears between the granulosa cells and the zona proper and also between adjacent cells of the corona radiata itself (Figs. 21, 22, *gl*).

In oocytes present in follicles removed during the heat period and in unfertilized tubal ova the peripheral concentration of many cytoplasmic elements is greater than has previously been seen. Numerous ovoid membranous structures about 180 mμ in diameter are observed (Figs. 18, 20, *er*), only a few of which have been seen previously in the oocytes of follicles with small and medium sized antra. For the most part the membranes are smooth, but occasionally small dense particles are attached to them. Because of the occasional pres-

ence of such particles the structures are considered to be atypical ergastoplasm. The content of these elements is somewhat denser than the ooplasmic matrix. The multivesicular bodies appear especially numerous in the oocytes of large preovulatory follicles and in the unfertilized tubal ova. Both types are seen, but the type containing the nucleoids appears more frequently than in previous stages (Fig. 20, *mvb* (*2*)). The mitochondria are smaller (270 mμ in diameter), more uniform in shape, and contain a more dense matrix than in the unilaminar follicles. A vesicular complex consisting of numerous small membranous structures, varying in size from 15 to 150 mμ can now be seen (Figs. 16, 21, *vc*). The smaller units fall within the size range of the vesicles associated with the multivesicular bodies and of the Golgi membranes and vesicles but they are less numerous than the larger units of the complex. In sections examined with the phase microscope these ovoid complexes appear dense and homogeneous as viewed against the background matrix (Fig. 15, *vc*). It is interesting that as the vesicular complex appears the Golgi complex becomes unidentifiable. This raises the question as to whether or not the vesicular complex includes elements of the Golgi apparatus.

## DISCUSSION

Relatively few electron microscopic studies of developing oocytes have been reported and these have been primarily concerned with various invertebrate eggs (1–3, 12, 15, 19, 31, 42, 43, 47, 59). Of the lower vertebrates both frog (25) and *Triturus* (56, 57) oocytes have been studied. Several papers dealing with mammalian oocytes have appeared recently, including that of Yamada *et al.* (58) on early stages of mouse oocytes; that of Sotelo and Porter (46) on late ovarian oocytes and fertilized tubal ova in the rat; that of Chiquoine (14) on early stages of development in the rat; and that of Trujillo-Cenóz and Sotelo (49) on a few ovarian stages in the rabbit.

### *Relationships between the Oocyte and Granulosa Cells of the Corona Radiata:*

The relationship between the oocyte and the granulosa cells immediately surrounding it varies from the simple apposition of adjacent plasma membranes in the unilaminar follicles to the complex arrangement of the ovular and granulosa cell processes embedded within the zona pellucida in the large growing follicles. In the unilaminar follicle the plasma membranes of the oocyte and flattened granulosa cells are in close contact. The nutritional requirements of the growing oocyte are presumably adequately met by this simple contact of membranes. Vesicles such as those seen in other instances of micropinocytosis may partially mediate exchange of substances. The relationships in these early follicles have been noted briefly in the rat by Chiquoine (14) and in more detail in the mouse by Yamada *et al.* (58).

The zona pellucida has been of interest for many years. The time of its original appearance is not often mentioned in the literature. It is usually assumed to develop in the bi- or trilaminar follicles (18, 23, 30, 32, 60), but its initial appearance in the unilaminar stage was described by Van Beneden (50) in the bat as early as 1880 and by Chaudhey (13) in the fish. This has been confirmed in the mouse (58) and, in the present work, in the rat.

The relative roles of the oocyte and granulosa cells in the genesis of the zona pellucida have been investigated extensively. Some workers believe it to be a product of the oocyte; others, an exclusive product of the granulosa cells; and still others, the result of the participation of both the egg and the granulosa cells. Reviews of the literature on the origin of the zona pellucida are readily available (13, 16, 32, 46, 48). The fact that the zona originally appears in a layer of variable thickness over the surface of the developing egg has been noted only rarely in the light microscopy literature (32). This pattern of appearance was readily apparent in the electron microscopic studies on the mouse (58), rat (14, 36), and rabbit (49). Chiquoine (14) believes that the zona is a product of the follicle cells, basing his opinion on the appearance of the zona substance between two contiguous granulosa cells instead of between the granulosa cell and the oocyte as is the usual case. Similar images (Fig. 7) were observed in this study but are not considered to be conclusive proof of such a view, since the three-dimensional arrangements of the cells in question are not known. Trujillo-Cenóz and Sotelo (49) likewise postulate the origin of the zona from the follicle cells, since the peripheral parts of their cytoplasm are filled with a substance similar in density to that of the zona. In the present work such regions are also observed occasionally (Figs. 8, 9, 6, *gp*, arrow), but are considered to be only suggestive of the participation of the granulosa cells in the formation of the zona. That the oocyte may be involved in this process as well is indicated by the location of the Golgi complex closely adjacent to the forming zona, since this organelle has been

implicated in secretory activity in other cell types. At the present time, it would seem that strictly morphologic observations have failed to provide the answer to the question of the origin of the zona pellucida.

In the literature, attention has often been directed to the fact that the processes of the granulosa cells extend into the zona pellucida (6, 13, 16, 24, 32, 46). Invariably the question is raised as to whether there is direct continuity between such processes and the ooplasm. In the present study no such continuity was observed.

Ovular microvilli have been observed on the surface of some invertebrate (1, 15, 19, 31) and vertebrate eggs (13, 25, 36, 46, 58). Kemp (25) reported an extensive development of microvilli, 80 mμ wide and 170 mμ long, which were estimated to increase the absorptive surface 35 times in the oocyte of *Rana pipiens*. In the mouse, Yamada *et al.* (58) observed microvilli penetrating the zona. These are 60 to 120 mμ in diameter and are less numerous than in the frog. Sotelo and Porter (46) present detailed information about the relations of the granulosa cell processes and the ovular microvilli to the zona of the rat egg. Here the microvilli, approximately 80 mμ in diameter, extend to about the middle of the zona and are free of cytoplasmic elements, in contrast to the processes of the granulosa cells. They state that at about the time of ovulation the corona cell processes begin to retract and that once fertilization occurs the microvilli disappear, to reappear after the first segmentation division. In the present study stages intermediate to the large ovarian follicles and the fertilized tubal ova which Sotelo and Porter studied have been examined. The granulosa cells begin to retract their processes and the ovular microvilli are withdrawn at the time of the initiation of the formation of the first polar body within the ovary, rather than at the time of ovulation or fertilization. In unfertilized tubal ova the retraction of the granulosa cell processes is complete and the oolemma presents an undulating outline. The retraction of the granulosa cell processes has also been reported by light microscopists studying the ova of the mole (24), the sow (16), and the rat (4). A dissenting report is that of Braden (11) in which many processes are noted passing from the coronal cells into the zona in freshly ovulated rat ova examined with the phase microscope.

During the observed phases of first polar body formation, there is apparent a pinching off of bits of ooplasm at the site of constriction of the polar body. These may contribute the small vesicles and granules seen in the perivitelline space, since the ovular plasma membrane remained intact in all observed cases save one. A similar process has been described by Van der Stricht (51) as occurring in various mammalian ova from the beginning of the period of maturation and by Lams (28) as taking place after fertilization. The presence of globules and granules are noted in the perivitelline space of human follicular ova with the phase microscope (44).

A few investigators have noted also the deposition of a thin layer of substance interposed between the coronal cells and the zona pellucida (20, 24, 50). Such a layer is seen around unfertilized tubal ova with the electron microscope. This is composed of material of the same density as the intercellular substance between, and surrounding, cells of the cumulus mass. It has been reported that the intercellular substance of the cumulus cells is positively stained with the periodic-acid–leucofuchsin method and is partially extracted subsequently by treatment of the sections with hyaluronidase (11, 23). Since the rat egg is fertilized before the dispersal of the cumulus cells (4, 29, 37), it is possible that this outer granular layer is penetrated by the sperm in the same way that the latter passes through the intercellular substance of the coronal cells.

Contrary to the impression one obtains with the phase microscope, it can be seen that the entire surface of the zona is not covered completely with a closely packed layer of coronal cells. The coronal cells of unfertilized tubal ova exhibit dense cytoplasmic bodies, perhaps indicative of a functional change leading to degeneration. Blandau (8) has noted that while granulosa cells obtained from ovarian follicles will grow in tissue culture, such cells from tubal eggs fail to survive.

*Cytoplasmic Components:*

The distribution of mitochondria and the Golgi complex in the oocyte has been repeatedly reviewed (7, 16, 21, 33, 46, 51). In general, the distribution of mitochondria at all stages observed and of the Golgi complex in the primary and maturing follicles prior to preovulatory changes observed here agrees with this literature. However, the location or even the existence of the Golgi complex in the cytoplasm of the oocyte during the first maturation division and of the unfertilized tubal ovum is not clear. In the present work the characteristic Golgi complex (17) is not seen in oocytes at these times. In no electron microscopic

studies is this complex describing during these stages, and observations made with the light microscope are not specific in this regard either. Kulesch (26) was unable to demonstrate the Golgi apparatus in eggs in graafian follicles. Nihoul (33) states that the Golgi elements disappear in the rabbit egg at about the time of ovulation and then aggregate in the center of the tubal egg shortly after sperm penetration. Gresson (22) states that the Golgi bodies are smaller in the tubal than in the ovarian ova of the mouse.

The obvious increase in the number of multivesicular bodies in the large ovarian follicle reported by Sotelo and Porter (46) is confirmed in the present study. Relatively large aggregates of small vesicles, the vesicular complex, appear at the time of polar body formation. Whether the complex includes the Golgi elements at this stage is open to question. Sotelo and Porter label similar aggregates in fertilized ova "a mass of centrosomal vesicles" which, they believe, may originate from the multivesicular bodies.

It is interesting that the size of the mitochondria decreases and their shape becomes more uniform in ova within the preovulatory follicles and within the oviduct in comparison to those in unilaminar follicles. Yamada *et al.* (58) and Chiquoine (14) also note the somewhat atypical appearance of the mitochondria in the unilaminar follicles.

Cytoplasmic basophilia in various stages of follicular development has been studied in the rat with histochemical techniques (5, 52). Sotelo and Porter (46) discuss this in some detail in connection with the ribonucleoprotein-containing particles which, in other cell types, may or may not be associated with the membranous elements of the ergastoplasm. Most of the basophilia of the egg to the two-cell stage is considered by them to be due to the free particulate form which, in general, conforms to the observations reported here. However, membranous elements are seen more frequently in the multilaminar follicles prior to preovulatory changes than previously reported. Although typical ergastoplasmic membranes (38–41, 55) are rarely seen either in ova undergoing polar body formation or in unfertilized tubal ova, membranous elements with only occasional particles attached are very plentiful and are considered to be atypical ergastoplasm.

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## Explanation of Plates

*Key to Abbreviations*

*c*, centriole
*db*, dense body in granulosa cell
*dp*, dense particles (of Palade)
*ds*, dense substance of ooplasm
*er*, ergastoplasmic membranes
*g*, granulosa cell
*gc*, Golgi complex
*gl*, granular external layer of the zona pellucida
*gn*, granulosa cell nucleus
*gp*, granulosa cell process
*ic*, intercellular boundary
*l*, lipid inclusion
*m*, mitochondrion
*mf*, myelin figure
*mv*, microvillus
*mvb*, multivesicular bodies; types 1 and 2
*nc*, nucleolus
*o*, oocyte
*ol*, oolemma (plasma membrane of oocyte)
*on*, nucleus of oocyte
*pv*, perivitelline space
*v*, vacuole
*vc*, vesicular complex
*zp*, zona pellucida

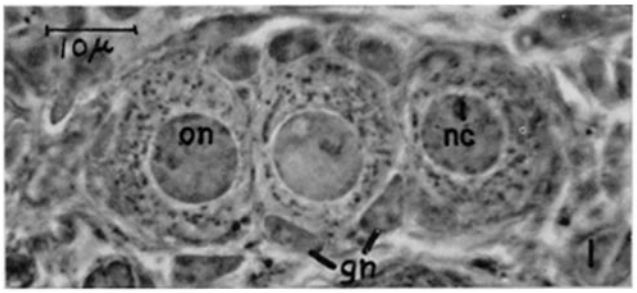
Except where otherwise indicated the scale line represents 1 micron.

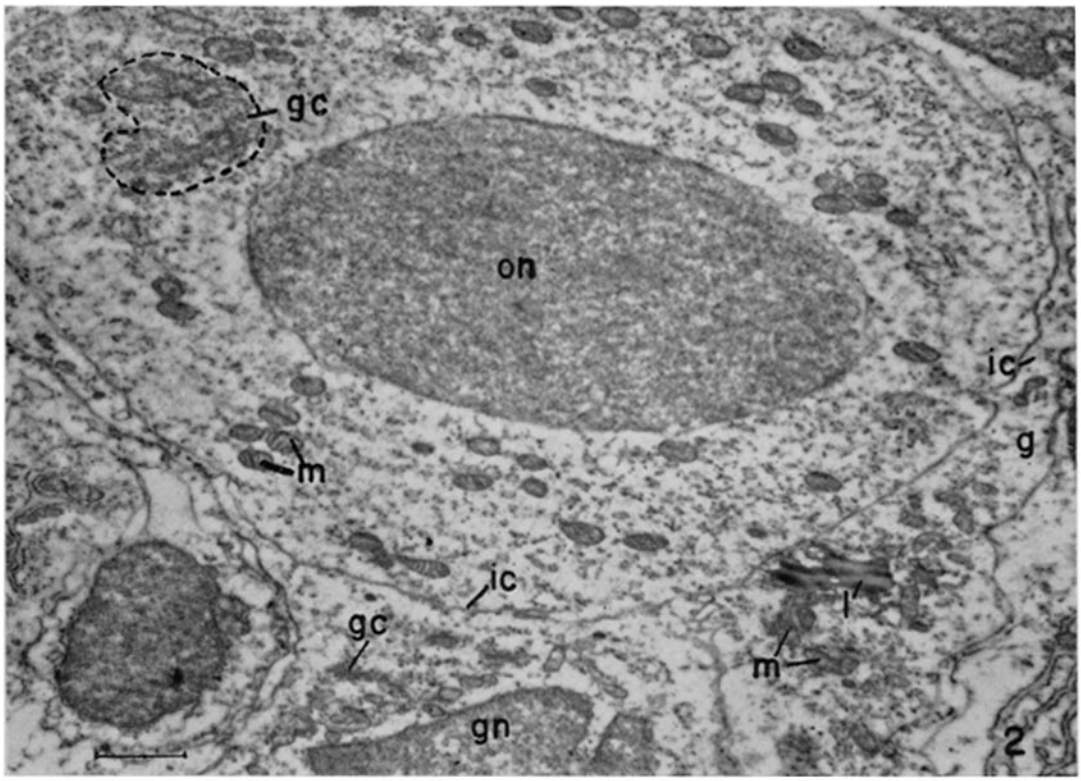
### Plate 307

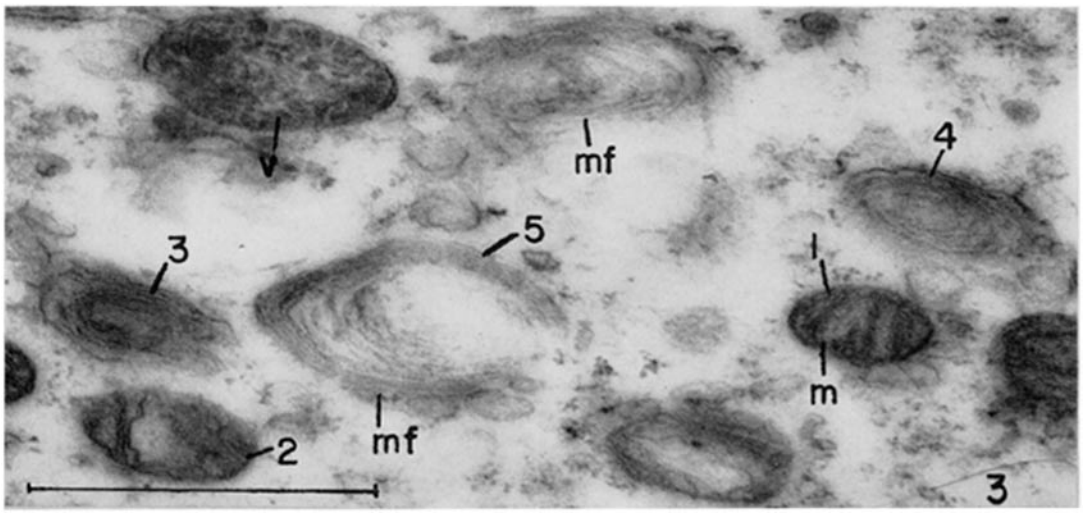
Fig. 1. Unstained section, as seen with the phase microscope, of three unilaminar follicles in which the oocytes are surrounded by an incomplete layer of flattened granulosa cells (*gn*). × 1170.

Fig. 2. Unilaminar follicle in which the oocyte is completely surrounded by a layer of granulosa cells (*g*). The Golgi complex (*gc*) lies aggregated at one pole of the nucleus (*on*). Mitochondria (*m*), vesicles, and granules are scattered throughout the ooplasm. Lipid inclusions (*l*), such as those seen in one of the granulosa cells, are not often observed. × 11,700.

Fig. 3. Cytoplasm of an oocyte of an unilaminar follicle. Early degenerative changes are noted in this and many other oocytes in such follicles obtained from prepuberal rats. In contrast to atresia in multilaminar follicles, in these the degenerative changes appear first in the oocyte. The myelin figures (*mf*) consist of a series of membranes separated by narrow interspaces. Possible transitional steps from mitochondria (*m*) may be noted in this figure, going from *1*, a normal mitochondrion, to *5*, a myelin figure. A large vacuole, enclosing many smaller membranous elements, is seen at *v*. This body is similar, though larger than the multivesicular bodies normally present. × 46,900.



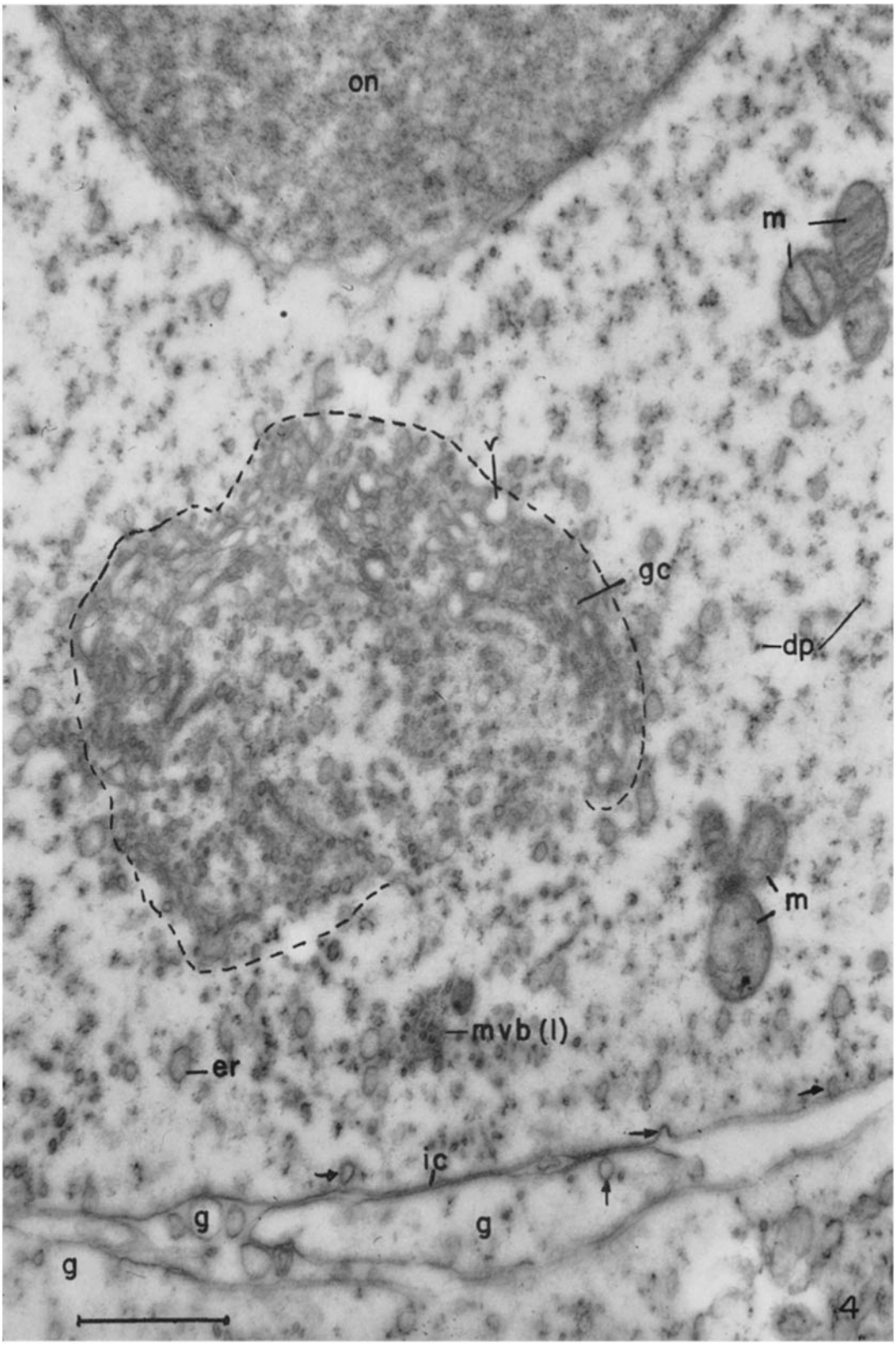




(Odor: Ovarian oocytes and unfertilized tubal ova)

PLATE 308

FIG. 4. Unilaminar follicle in which the oocyte is surrounded by a single, but overlapping layer of granulosa cells. The aggregated Golgi complex (*gc*) lies adjacent to the nucleus. The large mitochondria (*m*), with an abundance of matrix, are visible. Note the double nature of the nuclear membrane. Note also the vesicular elements ( ↑ ) near the plasma membranes of the oocyte and of the granulosa cells (*g*). Numerous dense particles (*dp*) are scattered in the ooplasm and are infrequently associated with ergastoplasmic membranes (*er*). Ovary of 2-day rat. Section stained with uranyl acetate. × 24,000.



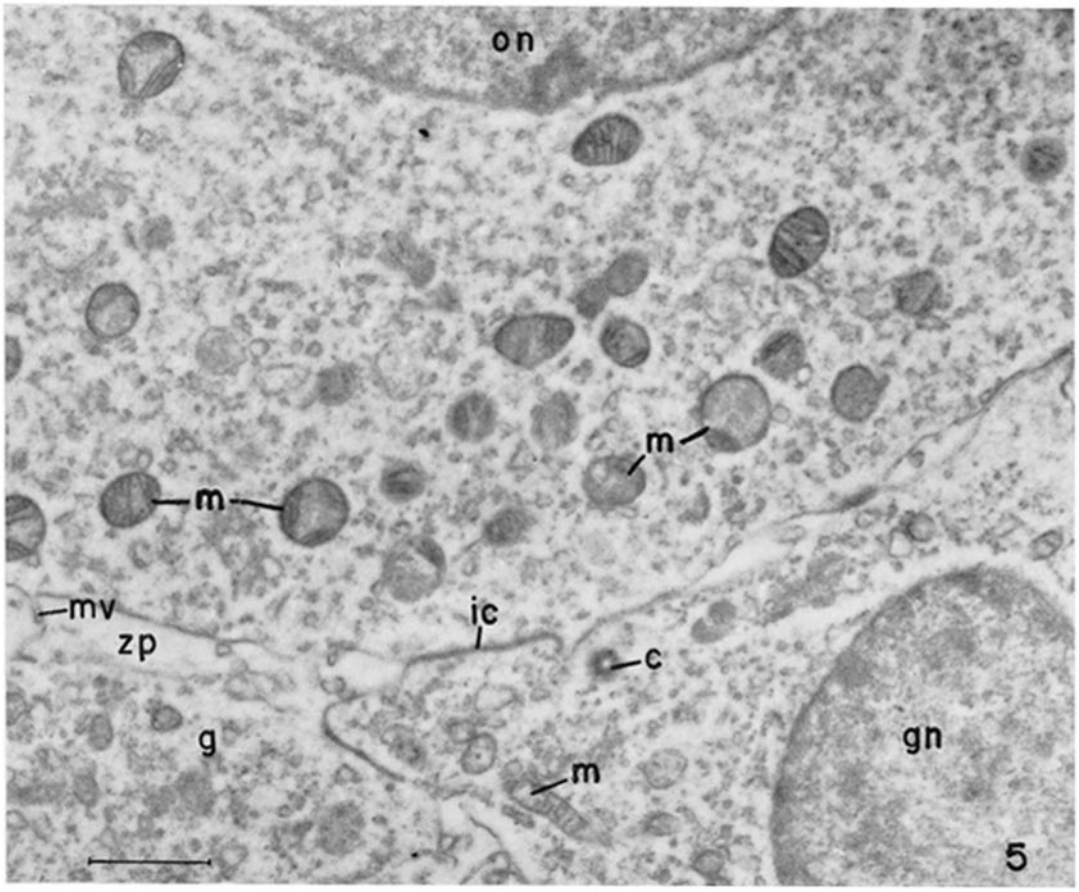

(Odor: Ovarian oocytes and unfertilized tubal ova)

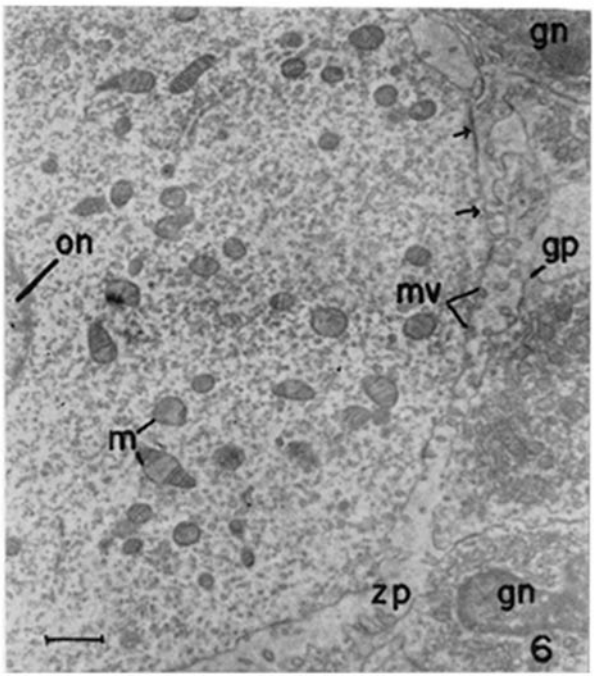
PLATE 309

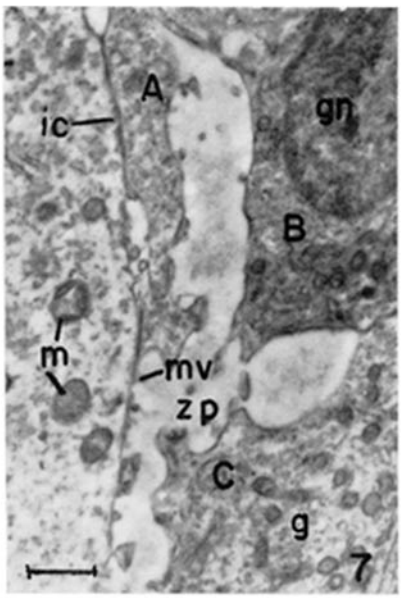
FIG. 5. Unilaminar follicle with a single layer of low columnar follicular cells (*g*). Zona pellucida (*zp*) formation has begun and a few microvilli (*mv*) are visible. Most of the oocyte's surface is still in direct contact with the granulosa cells. The mitochondria (*m*) have relatively few cristae and much matrix. Ovary of 8-day rat. × 15,900.

FIG. 6. Low power view of unilaminar follicle in which zona pellucida formation had progressed somewhat farther than that seen in Fig. 5. Note that there is more zona substance (*zp*) and a somewhat greater number of microvilli (*mv*) present. In some areas, however, the oolemma and granulosa cell membranes are still in intimate contact ( ↑ ). Ovary of 8-day rat. × 7200.

FIG. 7. Unilaminar follicle in which the oocyte is surrounded by a layer of cuboidal cells and in which zona pellucida formation is progressing. Note that some of the zona material (*zp*) lies between granulosa cells *B* and *C* and some between cells *B* and *A*. Images such as these suggest, but are not conclusive evidence for, the origin of the zona substance from the granulosa cells. Ovary of 11-day rat. × 9200.



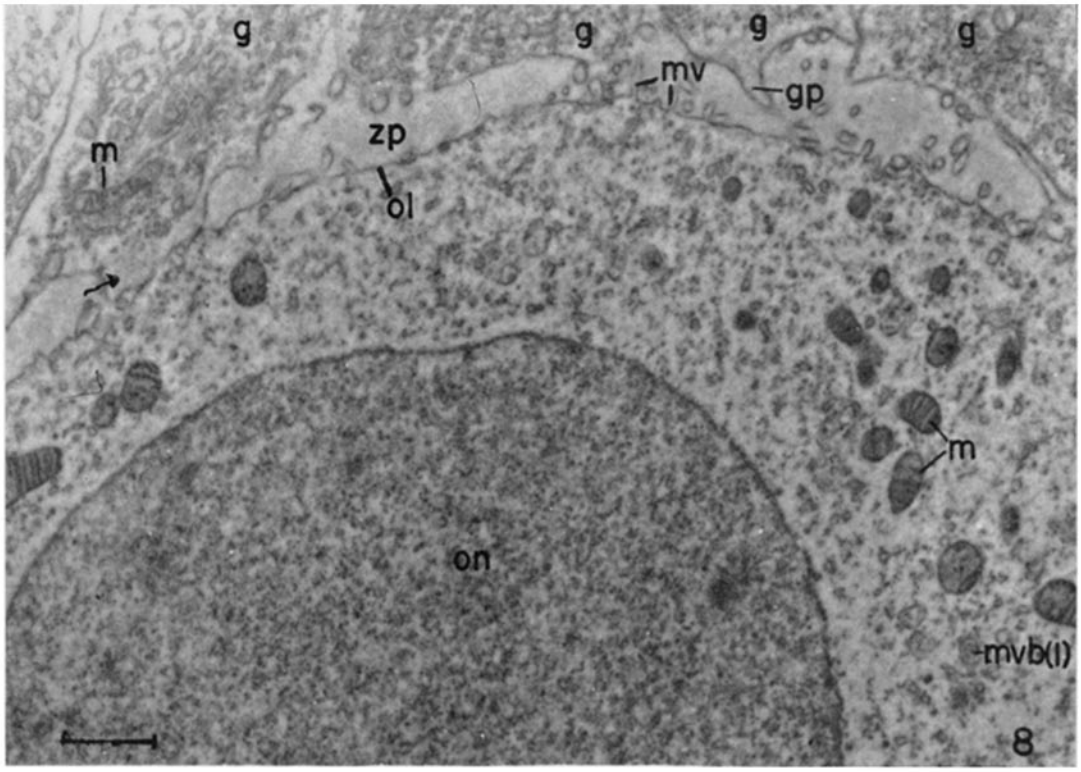



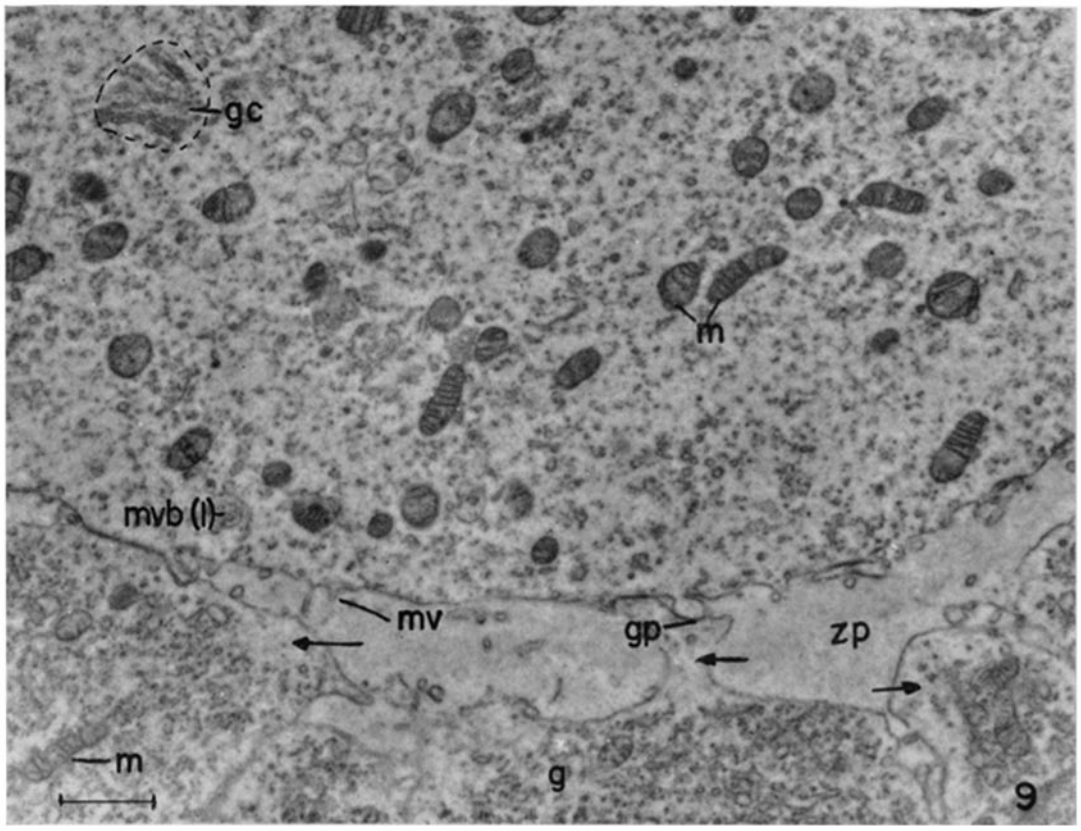




(Odor: Ovarian oocytes and unfertilized tubal ova)

PLATE 310

FIGS. 8 and 9. Unilaminar follicle during early stages of zona pellucida formation. The figures represent two areas of the same oocyte. The unequal deposition of the substance of the zona (*zp*) is shown very well. The granulosa cells (*g*) are still in contact with the oocyte in a number of places. Note the agranular appearance and low density of the peripheral areas of the cytoplasm of the granulosa cells as indicated by arrows. These appear somewhat similar to the material of the zona. A small Golgi unit (*gc*), no longer in close apposition to the nucleus, may be seen in Fig. 9. Only a few ovular microvilli (*mv*) are present. Ovary of 8-day rat. × 12,400.

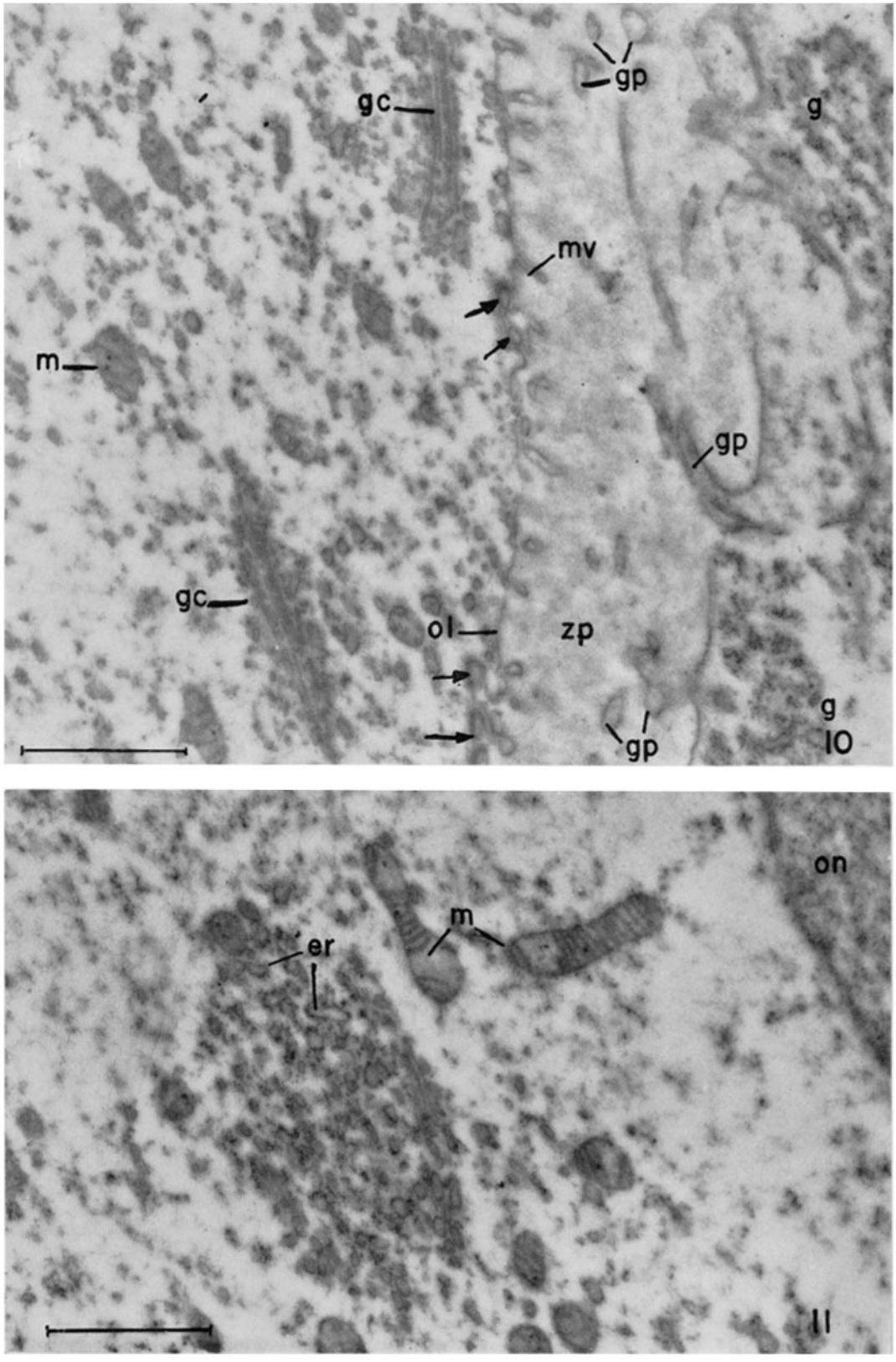



(Odor: Ovarian oocytes and unfertilized tubal ova)

PLATE 311

FIGS. 10 and 11. Different areas of the same oocyte are shown in these two figures. The tissue was obtained from a 15-day-old rat. The egg is surrounded by one to two layers of granulosa cells (*g*), no antrum being present. Note the peripheral location of the Golgi (*gc*) units in Fig. 10. The zona (*zp*) has increased in width. The processes of the granulosa cells (*gp*) extend into it from the exterior and the microvilli (*mv*), from the interior. There is close apposition of presumed cross-sections of granulosa cell processes to the oolemma where indicated by the arrows. In Fig. 11 ergastoplasmic membranes (*er*), with attached dense particles, are visible. × 26,200.

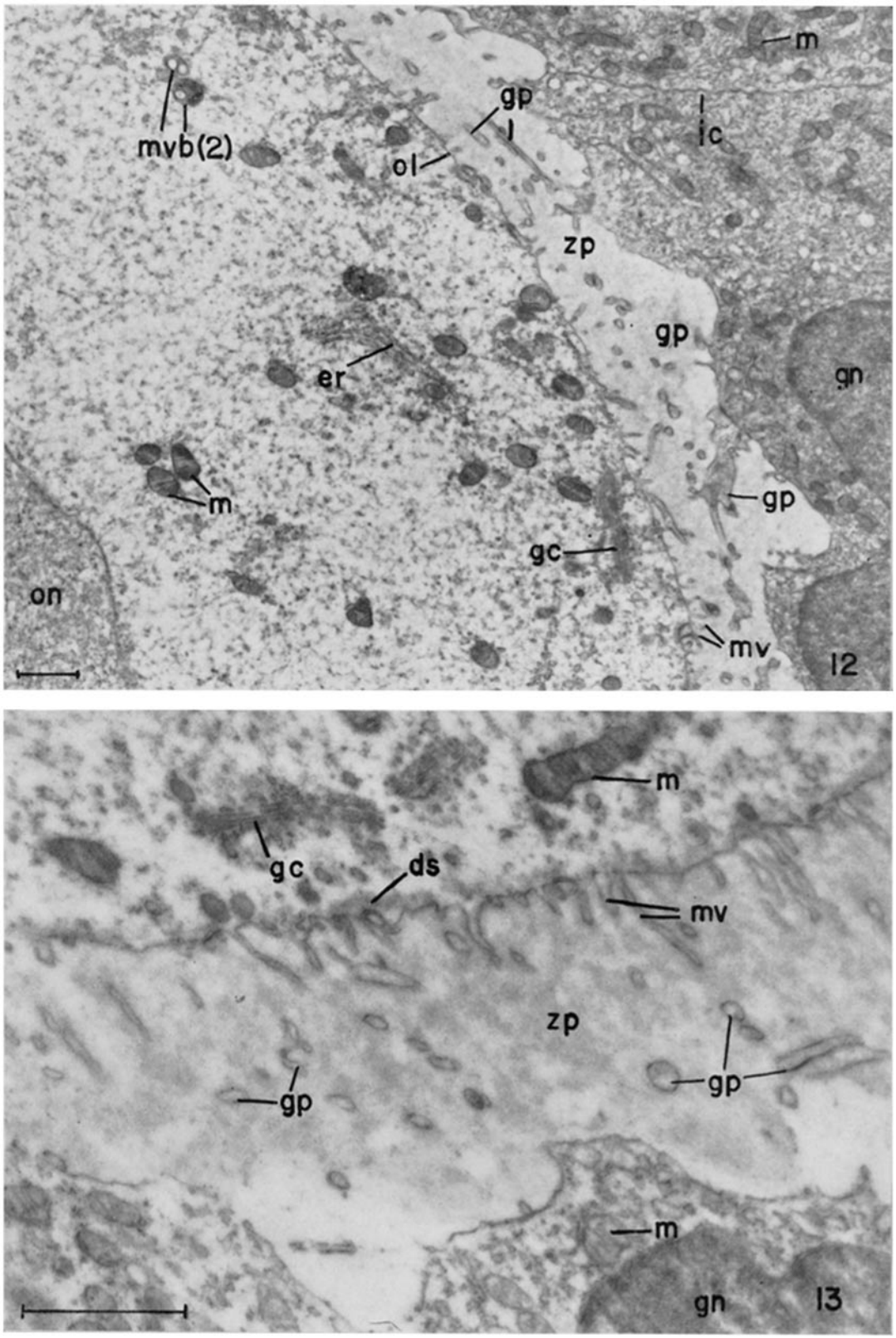



(Odor: Ovarian oocytes and unfertilized tubal ova)

PLATE 312

FIG. 12. Bilaminar follicle with no antrum, from 63-day-old rat. A small Golgi unit (*gc*) lies peripherally, adjacent to the zona pellucida (*zp*). A group of ergastoplasmic membranes (*er*) with attached particles is visible. Such membranes are infrequently observed. The zona pellucida has not attained its maximum width and has both ovular microvilli (*mv*) and granulosa cell processes (*gp*) extending into it. × 9600.

FIG. 13. Multilaminar follicle with discontinuous antral spaces. Again the peripheral locus of the Golgi elements (*gc*) is apparent. The microvilli (*mv*) are shown especially well in this figure. They do not contain formed elements of the cytoplasm, but are of somewhat higher density than the matrix of the ooplasm. Note the dense substance (*ds*) lying just within the ooplasm, bordering on the oolemma. The granulosa cell processes (*gp*) are present mainly in oblique or cross-section. Ovary of 15-day-old rat. × 26,000.




(Odor: Ovarian oocytes and unfertilized tubal ova)

PLATE 313

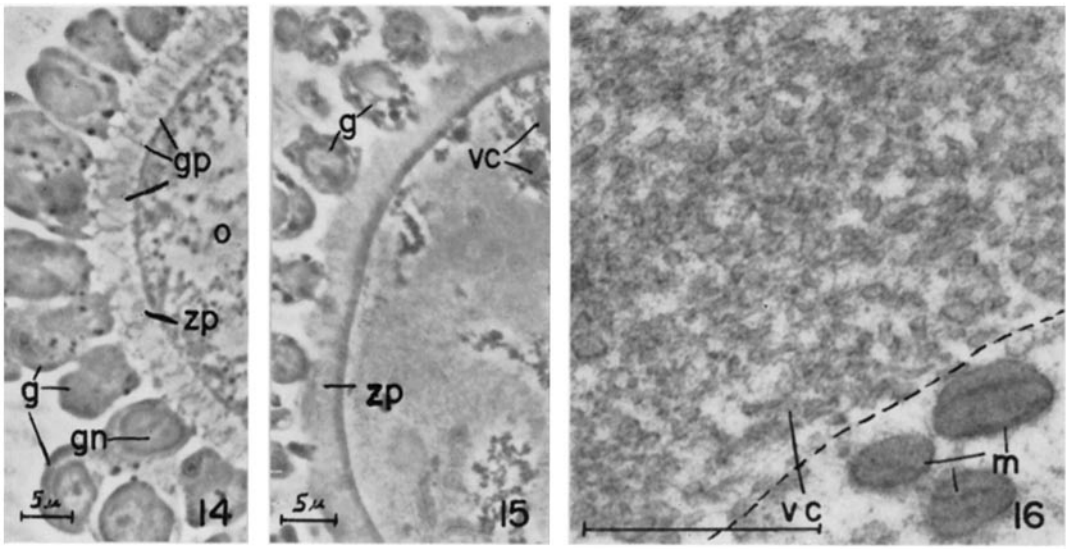
FIG. 14. Unstained section of an oocyte and its corona radiata observed with the phase microscope in a multilaminar follicle with an antrum. Note the numerous processes (*gp*) extending from the bodies of the granulosa cells (*g*) into the zona (*zp*). × 1500.

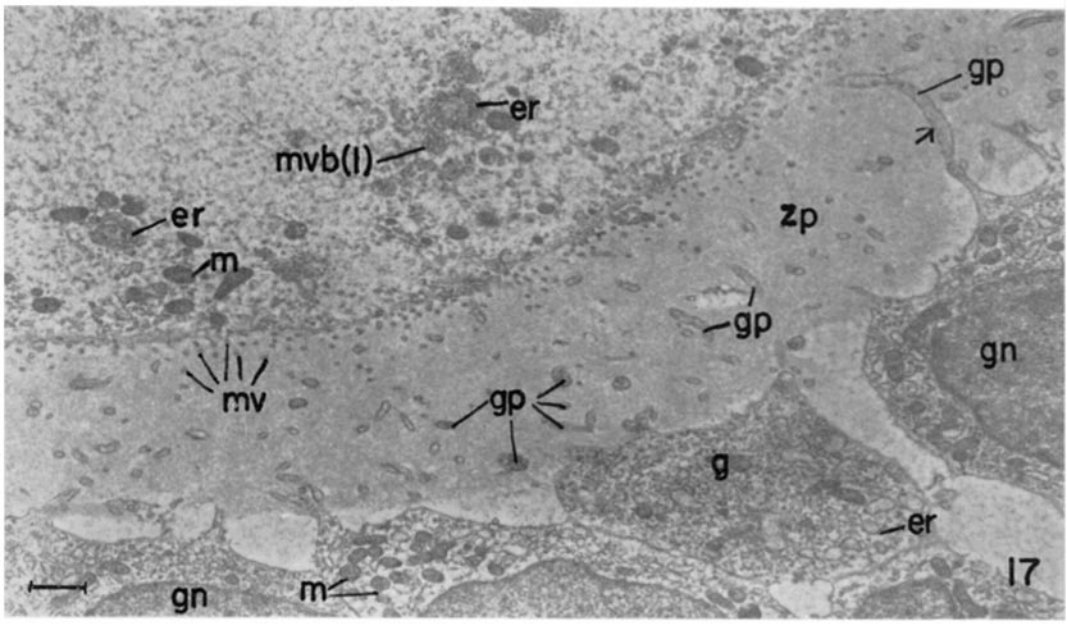
FIG. 15. Unstained section of an unfertilized tubal ovum as observed with the phase microscope. Note the lack of granulosa cell processes in the zona (*zp*) as compared to the oocyte in Fig. 14. The dense body (*vc*) in the ooplasm is considered to be comparable to the vesicular complex (*vc*) seen in the electron micrographs of ova at this stage. × 1500.

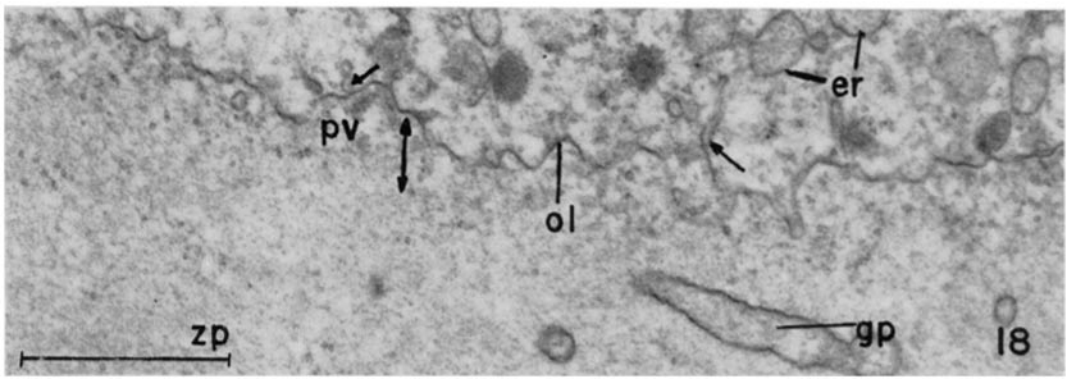
FIG. 16. High power view of the vesicular complex (*vc*) of an egg which contains a first metaphase maturation spindle. Note the considerable range in size of the membranous elements which make up this complex. The ovum is present in a large preovulatory follicle. Tissue from sexually mature rat. × 31,100.

FIG. 17. Multilaminar follicle with discontinuous antral spaces. The numerous granulosa cell processes (*gp*) are shown especially well. Most of them are seen as short isolated segments within the zona pellucida (*zp*), but to the right ( ↑ ) is one in continuity with the cell body (*g*). The microvilli (*mv*) are cut primarily in cross-section, so appear as small profiles in the zona. Ovary from 63-day rat. × 7200.

FIG. 18. Peripheral area of cytoplasm of oocyte in a multilaminar preovulatory follicle from a sexually mature rat. The character of the ovular plasma membrane (*ol*) is undulating in outline, the microvilli having disappeared. The arrows indicate several membranous profiles in close association with the oolemma. A narrow perivitelline space (*pv*) is filled with material similar to the matrix of the ooplasm. × 29,700.



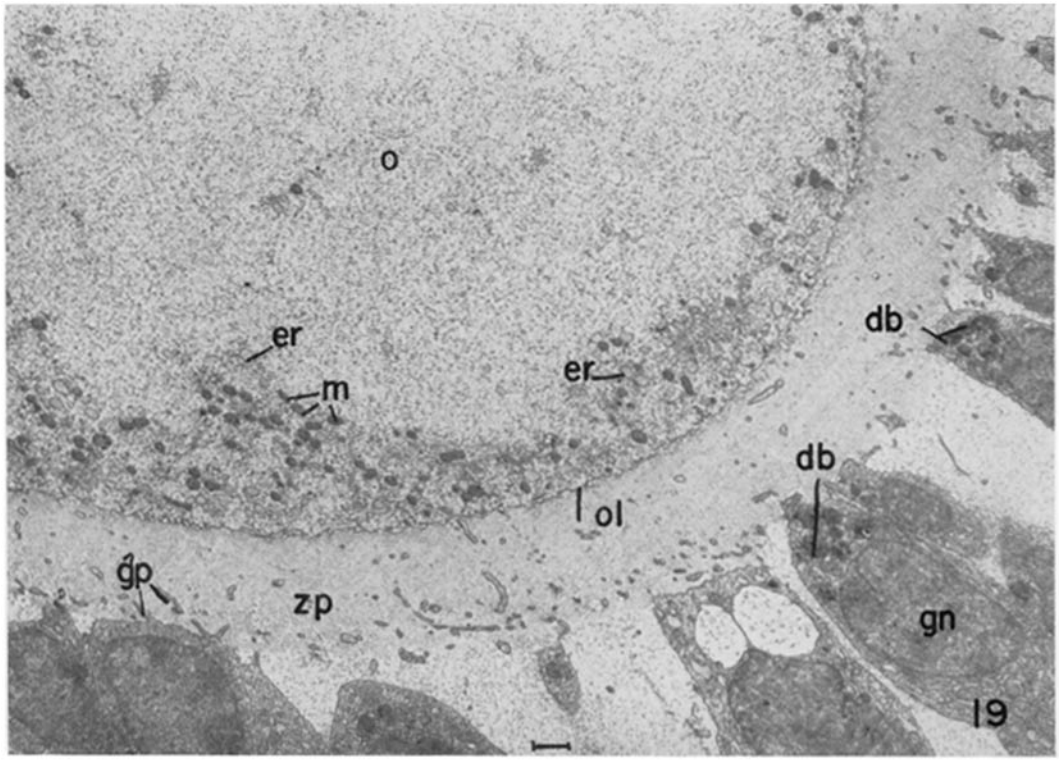



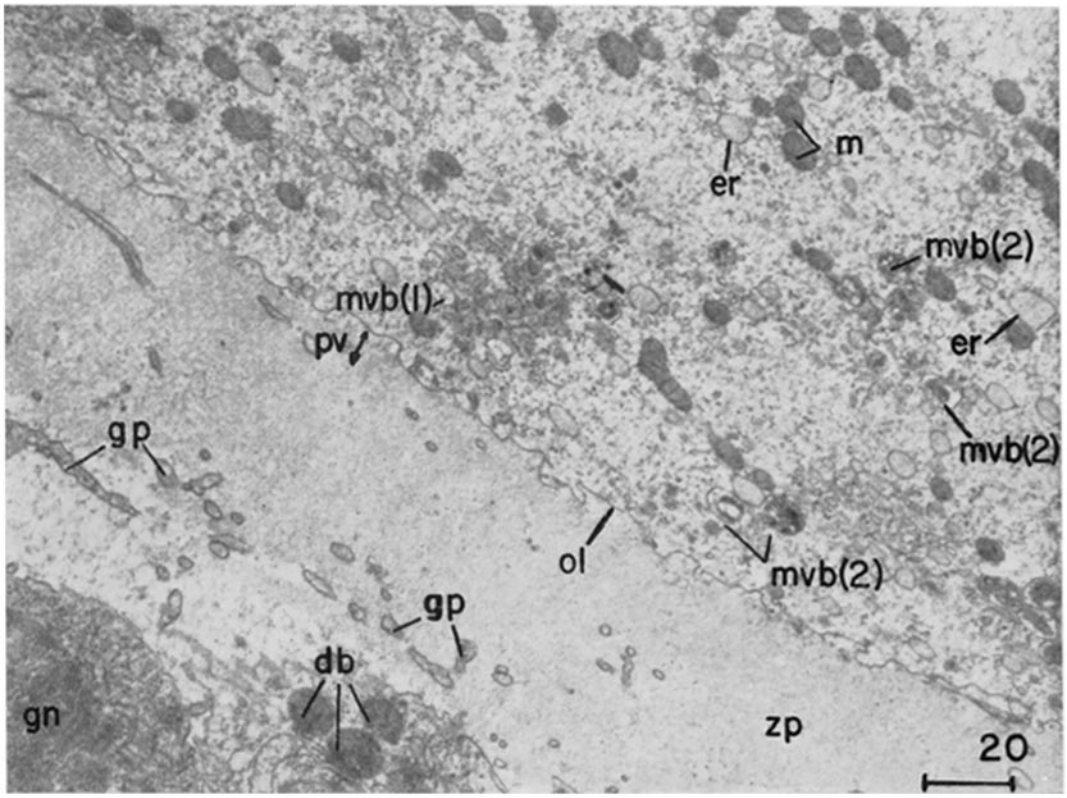




(Odor: Ovarian oocytes and unfertilized tubal ova)

PLATE 314

FIGS. 19 and 20. The figures show two different areas of the same oocyte. It lies in a multilaminar follicle from a sexually mature rat killed shortly before ovulation. The fact that the microvilli have disappeared to be replaced by an irregularly undulating plasma membrane (*ol*) is apparent. There appear to be fewer granulosa cell processes (*gp*) in the internal part of the zona pellucida (*zp*). The electron-dense bodies (*db*) in the granulosa cell cytoplasm are not noted prior to the preovulatory stage and are believed indicative of degenerative changes. Fig. 19, × 4700; Fig. 20, × 11,700.

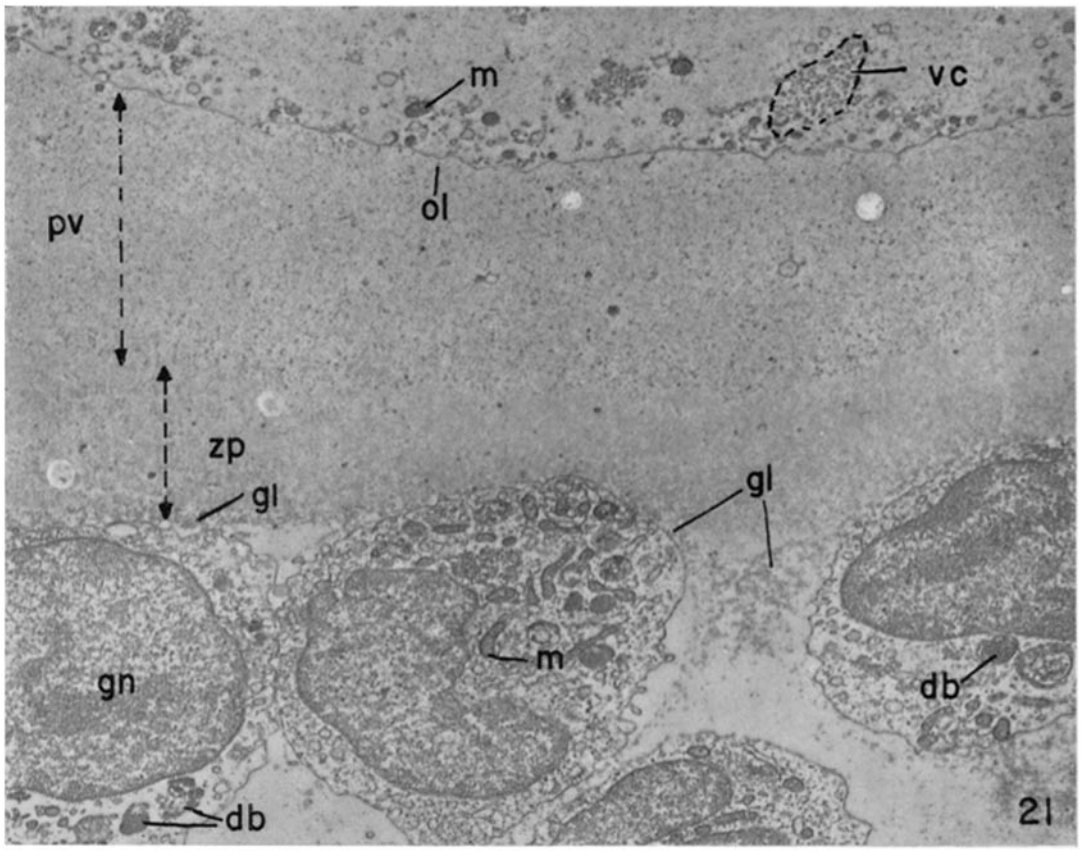


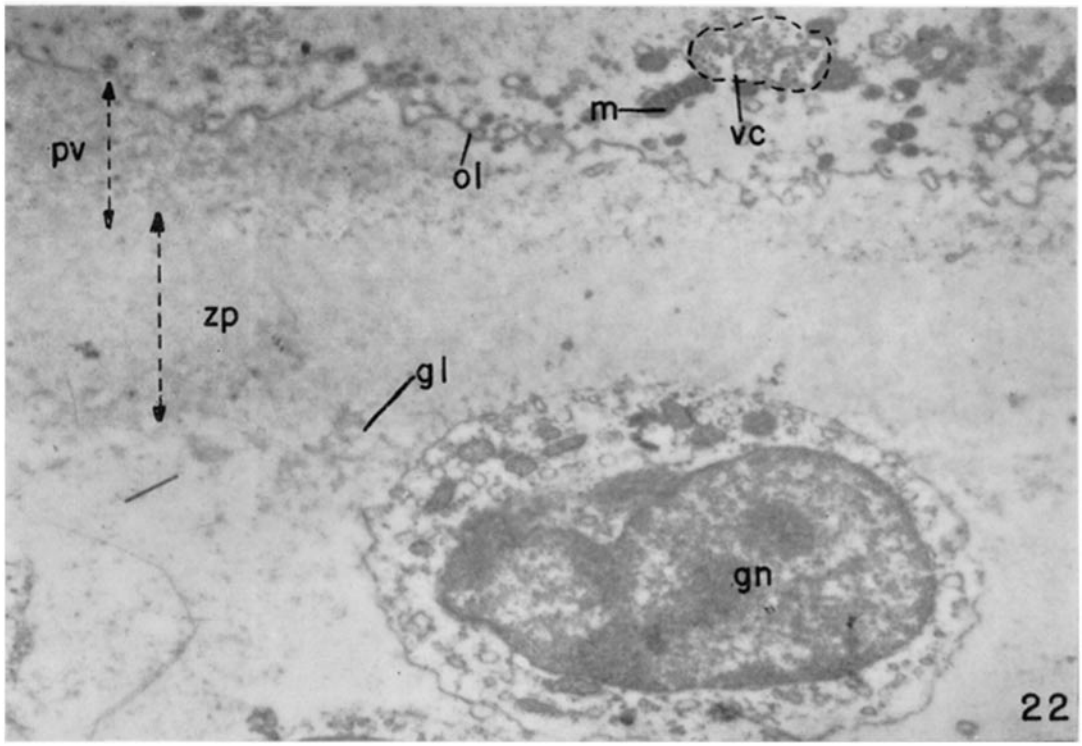




(Odor: Ovarian oocytes and unfertilized tubal ova)

PLATE 315

FIGS. 21 and 22. Unfertilized tubal ova. Note the smooth apposition of the plasma membranes of the granulosa cells to the zona pellucida (*zp*), with no processes extending inward. The ovular microvilli have disappeared, an undulating outline of the oolemma (*ol*) being evident. The perivitelline space (*pv*) appears granular in contrast to the more homogeneous appearance of the zona. An outer thin granular layer (*gl*) interposed between the granulosa cells and the zona and between adjacent corona cells is visible at this, but not at earlier, stages. In Fig. 21 degenerative bodies (*db*) are present within the cytoplasm of the granulosa cells. Fig. 21, × 6000; Fig. 22, × 9600.




(Odor: Ovarian oocytes and unfertilized tubal ova)