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ULTRASTRUCTURAL ASPECTS OF FOLLICULAR GROWTH AND ATRESIA IN THE OVARY OF THE BAT, *Myotis grisescens*

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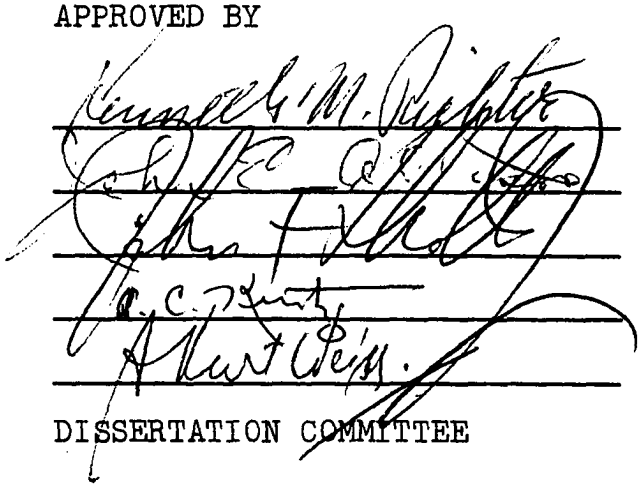

DISSERTATION COMMITTEE

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My professor, Dr. Kenneth M. Richter, 'talks to cells'. He uses the complex technology of the electron microscope for meaningful discoveries and opens challenging frontiers for those bold enough to abandon complacency.

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CHAPTER I

INTRODUCTION

Since Regnier de Graaf's (1641-1673) first general description, numerous studies have been devoted to the structural and functional characterization of the ovarian follicle. A complete characterization of the processes of follicular growth and follicular atresia has not been achieved to date. In this regard, Noyes *et al.* (1961), state that:

> Follicles are maturing and undergoing atresia concommitantly in the mammalian ovary and there is no way to distinguish a growing from an atretic follicle by inspecting the ovary at any particular time in the cycle.

Precisely why this state of uncertainty exists is not clear. Serious consideration of the literature reveals that this may be due to differences in points of view, objectives and interpretations by investigators. The nature of the material often allows only subjective interpretations (Ingram, 1962). Species differences manifested as physiological and morphological variations in the reproductive cycle add to the complexity. Differences in methods and sometimes an

overdependence on the reliability of these methods and the design of the studies, may be partially responsible for the state of uncertainty. Necessary information relating to the genesis, origin and longevity of the ovum is incomplete. Also, the basic processes of functional and structural cellular modulation of the ovarian tissue complex is largely unsettled.

While Noyes' et al. (1961) summation may be an overstatement, it is clear from the literature that definitive studies on follicular growth and atresia have not been made.

There are broad aspects of follicular growth and atresia which appear to be generally established. Follicular growth occurs throughout the active reproductive period of the individual. Follicles in all phases of development may undergo atretic transformation (Van Beneden, 1880; Mjassojedoff, 1923; Guthrie and Jeffers, 1938, 1950; Kingsbury, 1939; Wimsatt, 1944; Hisaw, 1947; Mandl and Zuckerman, 1950; Williams, 1955; Knigge and Leathem, 1956; Richter, 1958; Guraya and Greenwald, 1964; Odor, 1965; Adams et al., 1966; Guraya, 1965, 1968; Zerbian and Goslar, 1968). Atresia is the predominant fate of ovarian follicles produced in the lifetime of the individual (Ingram, 1962). In the bat, Myotis grisescens, only four follicles reach maturity during the female's lifetime (Guthrie and Jeffers, 1938). Follicular atresia may be described as the process whereby oocytes are lost or disappear from the ovary other than by ovulation

(Ingram, 1962). The lost oocytes may or may not be replaced by connective tissue elements, depending on the developmental age of the follicle in which the atretic alteration occurs.

The present study is an attempt to characterize the processes of follicular growth and atresia by an objective analysis of the total range of variation in the ultrastructure of the cells and tissue complexes of defined age groups of follicles. These age groups are the primordial, primary, secondary and tertiary follicular stages (Maximov and Bloom, 1930; Guthrie and Jeffers, 1938; Kingsbury, 1939; Wimsatt, 1944; Richter, 1958; Odor, 1965). Particular emphasis is placed on the ultrastructural changes occurring in the ovum and granulosa components of the follicle, their interrelations with each other, with the pellucid membrane and the follicular basement membrane.

CHAPTER II

MATERIALS AND METHODS

Female bats, Myotis grisescens, were collected from northeastern Oklahoma caves on the following dates: July 24, 1968; August 12, 1968; October 7, 1968; September 11, 1969. It was of particular interest to collect the animals in the late summer and early fall, since the processes of follicular atresia reach a maximum level during this prehibernation interval of the annual cycle. The bat ovary was chosen because its small size (Richter, 1958) obviated any cutting of the organ and allowed a whole ovary to be used. Much of the early material was used in testing for the most desirable methods.

The bats were kept in a cold storage room (11°C) on wet wads of paper in styrofoam boxes, sometimes as long as four to five days.

They were briefly anesthetized with ether and the ovaries removed under the dissecting microscope. In each case, one ovary was fixed immediately in cold (4°C) 1% osmium tetroxide, phosphate buffered to pH 7.5, while the other ovary was fixed in Lillie's 10% neutral buffered formalin.

Electron Microscopic Techniques

Fixation

The ovary to be used for electron microscopic examination was fixed in buffer 1% osmium tetroxide. Using a test range of pH 7.2-7.8, it was determined that the most satisfactory buffering was at the mid point. All fixing solutions were then buffered to pH 7.5.

Preparation of buffer solutions (Barka and Anderson, 1963). Two stock solutions are necessary. Stock solution A, a 0.2 M solution of monobasic sodium phosphate (NaH_2PO_4) consists of 27.58 gm of the salt made up to 1000 ml with distilled water. Stock solution B, a 0.2 M solution of dibasic sodium phosphate (Na_2HPO_4) consists of 28.38 gm of the salt made up to 1000 ml with distilled water. To obtain a 0.1 M working buffer (pH 7.5), 16 ml of Solution A and 84 ml of Solution B are mixed and diluted to 200 ml.

Preparation of the fixative. 0.5 g of crystalline osmium tetroxide are dissolved in 25 ml of the above buffer solution, overnight at $4^{o}C$. The next day, another 25 mg of the buffer is added to result in a phosphate buffered (pH 7.5) 1% osmium tetroxide fixative. The ovaries were fixed for two hours in this solution.

Embedding

Araldite (Cargille) was used as the embedding mixture. 27 ml of Epoxy Casting Resin A (Araldite 6005) was mixed with 23 ml of Epoxy Hardener (Dodecenyl Succinic Anhydride). About

1 to 2% of Epoxy Accelerator B (N-Benzyl Dimethylamine) is added after thorough mixing of the above. It is convenient to store the embedding mixture in syringes in the refrigerator till ready for use. The embedding procedure is a modified version of Luft, 1961.

Dehydration. After fixation, the ovaries were washed in the buffer solution for 45 minutes with several changes. Then exposure to 35%, 50% and 80% ethanol for 15 minute periods followed. Two 20 minute dehydration periods in 95% ethanol and four 15 minute periods in absolute ethanol completed the dehydration.

Infiltration. The tissue was immersed in a 50/50 mixture of absolute alcohol and propylene oxide for 60 minutes with three interim changes. This was followed by 45 minutes in propylene oxide with 3 changes. The last rinse with propylene oxide was decanted and immediately replaced with a 1:1 mixture of propylene oxide and araldite. After one hour, infiltration was continued with a 1:2 mixture of propylene oxide and araldite for 12-16 hours at room temperature.

Embedding. Gelatin capsules were filled with the embedding mixture. The individual ovaries were placed on top of the embedding material and kept at room temperature until the specimen had sunk to the bottom of the capsule. The capsules were placed overnight into a 35°C oven for polymerization. The process was allowed to continue the next day in a

45°C oven. The final polymerization takes place in a 60°C oven where the capsules may remain indefinitely.

Preparation of Sections

The blocks were trimmed according to directions with the Porter- Blum Ultra- Microtome Mt-2 using glass knives prepared with the LKB Knife- Maker. Ultra-thin sections (400-500 A) were cut with the Porter-Blum Mt-1 and Mt-2. The sections were mounted on 200 mesh specimen screens.

Staining

The ultra-thin sections were double stained.

1. Stained for ten minutes at 37°C with a saturated (7%) solution of uranyl acetate in 50% ethanol. Subsequently washed in 50% alcohol baths and thoroughly rinsed with distilled water.

2. Stained for less than one minute in 0.2% lead citrate (Venable and Coggeshall, 1965). Washing in 0.01 NaOH was followed by several rinses in distilled water. The grids were then air-dried. The sections were examined with an RCA EMU-4A electron microscope at direct magnifications ranging from 1,644X to 101,560X. Photographic images were recorded on Kodak Electron Image Plates or Kodak Electron Microscope Film (Estar, thick base).

Light Microscopic Studies

Thick sections were also prepared from the araldite embedded ovaries. These ranged in thickness from about

250-500 mu. They were stained by the hematoxylin method of Shires, Johnson and Richter, 1969, and examined with the light microscope.

One ovary from each bat was fixed in Lillie's 10% Neutral Formalin for at least 24 hours. Dehydration and infiltration was carried out by the Auto-Technicon. The ovaries were embedded in paraffin and subsequently sectioned.

The sections were stained with Harris Alum Hematoxylin and Eosin (Armed Forces Institute of Pathology Manual, 1960), mounted in serial sections and studied under the light microscope.

Quantitative Studies

Surface areas and volumes were calculated for the oocyte, zona pellucida, granulosa and the total follicle. These were based on linear measurements from electron micrographs of exemplary follicles representing the primordial, secondary, early and late tertiary follicle age groups. Standard mensural formulae (Handbook of Chemistry and Physics, 1967) were used. The data were tabulated and graphed.

CHAPTER III

OBSERVATIONS

General Aspects of the Ovarian Cycle of the Bat, Myotis grisescens

Hibernating bats have one reproductive cycle per year. The bat, Myotis grisescens used in this study is in this category. Ovulation occurs in the spring, usually in April, and one egg is ovulated at that time. Follicular development continues throughout the year, but shows seasonal variations. In the late summer and fall, the prehibernation period, there is a marked increase in number of large follicles (124 microns in diameter and above) (Guthrie and Jeffers, 1950). One follicle is said to reach maturity or near maturity during this time and to persist with little change through the hibernation period (Witschi and Pfeiffer, 1935), which lasts from October to the following April.

The forepart of the hibernation period is characterized by an increase in the number of developing follicles, principally of the primordial and primary follicle ages. It is also marked by a relatively high rate of atretic change involving the larger follicles produced during the prehibernation period (Guthrie and Jeffers, 1950). Just prior to the

termination of the hibernation period in the spring, in the preovulatory phase, many of the primordial and primary follicles developed during the hibernation period undergo atretic change.

The bats used in this study were collected in August and September and October, which is the prehibernation period or phase of their reproductive cycle.

General Histologic Structuring of the Prehibernation Ovary

The ovary of the bat, Myotis grisescens, during the prehibernation period is an ellipsoidal body measuring about 1.5 mm in length and 1.3 in diameter.

Histologically, it shows a structurally differentiated superficial cortex and a deep medulla. Its peritoneal or cortical surface is covered by germinal epithelium and is comprised of somewhat flattened cells. These are characteristically separated by vesicular intercellular spaces at the level of their general basal poles (Plate VII, Figure 14). Their nuclei are disproportionally large as compared to their cytoplasmic mass and are deeply invaginated. The cytoplasm shows a limited number of conventionally structured mitochondrial, Golgi and endoplasmic reticulum components. The latter show what appears to be a conventional distribution and disposition in the cell. Occasional microvilli characterize their free, peritoneal surfaces (Plate VII, Figure 14). Their basal surfaces are associated with a basement membrane.

The latter closely reflects the contour form of the basal surfaces of the germinal epithelial cells. A subepithelial connective tissue complex, the tunica albuginea, comprises a system of delicate tissue strands containing capillaries, fibroblasts, smooth muscle cells (Brambell, 1956) and connective tissue fibroblasts and fibrils. The latter comprises the functional stromal component of the ovarian cortex. It is differentially organized about follicles as the theca folliculi. The cortical follicles are usually of the primordial and primary age groups. In addition to the follicles, epithelial nodules or cords of unsettled status are present in the cortex (Pflüger, 1863; Van Beneden, 1880; Van der Stricht, 1912; Gerard, 19 ; Guthrie and Jeffers, 1938; Muta, 1958; Guraya, 1967).

The ovarian medulla is characterized by larger and thus developmentally older follicles, usually of the secondary through mature follicle age groups. Such follicles are associated with characteristically structured and well developed connective tissue thecae. Interstitial cells of undefined origin, in both lipid storage and non-storage phases, occupy a large portion of the medulla. They occur as isolated cells or cell clusters (Plate XXV, Figure 57; Plate XL, Figure 97, Plate I, Figure 1). In addition, epithelial cords and medullary cords are present; their significance is not settled (Pflüger, 1863; Dawson and McCabe, 1957; Burkl and Kellner, 1954; Guraya, 1967). The follicles, epithelial cords and

interstitial cell clusters are separated by streamers of medullary connective tissue stroma containing hilar blood vessels and smooth muscle cells (Oswalda-Decima, 1970).

The Ultrastructure of the Primordial Follicle Age Group

The primordial follicle, situated in the ovarian cortex, is about 17-30 microns in diameter. It consists of a small oocyte, about 12-24 microns in diameter, which is enclosed by a single layer of flattened epithelial cells, the granulosa cells, having a mean height of 4 microns. These are in direct or close apposition with the egg surface. There is no intervening recognizable pellucid membrane element present (Plate I, Figure 1; Plate II, Figure 2). A basal lamina, measuring about 0.07 micron separates the granulosa cells from the ovarian connective tissue stroma. This is present in all primordial follicles examined.

In the exemplary follicle (Plate I, Figure 1) it has been calculated that the granulosa layer consists of about 8 cells, having a collective volume of about 4000 cubic microns. The average volume per cell is about 500 cubic microns, which would be equivalent to a cuboidal cell measuring 7.9 microns per side. Each granulosa cell is in contact with about 211 square microns of egg surface per cell. The entire egg surface has been calculated from standard mensuration formulae (Handbook of Chemistry and Physics, 1967) to approximate 1,691 square microns; and the egg volume is calculated to

approximate 6,538 cubic microns. The egg-granulosa junction is of a smooth contour over large areas; but in more restricted regions it shows fine pit-like depressions and fine irregularities (Plate I, Figure 1; Plate II, Figure 2) with involvement of both the granulosa cells and the egg cell surface. The entire primordial follicle, exclusive of basement membrane has a volume of about 15,901 cubic microns and surface of 3,054 square microns.

The primordial oocyte is spheroidal to ellipsoidal in gross form. It shows no overt microvilli. The nucleus is essentially spheroidal and presents the classical pale vesicular appearance. The karyoplasm is finely granular and shows only slight evidence of general and membrane associated condensations. Multiple prominent, but small, spheroidal to irregular nucleolar formations are present (Plate I, Figure 1).

The primordial oocyte cytoplasm is dominated by the mitochondrial complement. Within the same cell individual mitochondria range from exceedingly electron dense forms in which internal structure is barely discernible, through vesicular forms having an electron lucent matrix and an outer limiting membrane only, to uniquely structured ones showing lamellate cristae in multiple chevron-like patterns with an electron lucent matrix and a discrete double outer limiting membrane (Plate I, Figure 1; Plate VIII, Figures 15, 16). Their gross form is mainly spheroidal, but short rod-forms are present on occasion. The distribution appears to be a

random one. Individual mitochondria may be found just as closely associated with the nuclear membrane as with the plasma membrane, and other cytoplasmic components (Plate I, Figure 1; Plate VIII, Figure 15).

Prominent too, because of their electron density, rather than numbers, are irregularly shaped inclusions which may or may not be vesiculate. Their origin and composition have not been established, but are considered here for purposes of description, to be essentially lipid or lipc-protein bodies (Plate I, Figure 1). The endoplasmic reticulum is present in the primordial oocyte in highly variable form. Conventional type rough endoplasmic reticulum is of infrequent occurrence. It appears to exist as an extensive system of smooth membraned vesicles of varied sizes and shapes, which are randomly distributed and may be in close apposition with the plasma membrane. They are filled with slightly electron dense particulate material (Plate VIII, Figure 15). The ribosomal component of the endoplasmic reticulum system is predominantly distributed in the cytoplasmic matrix as free ribosomal particles. When associated with the membranous component of the endoplasmic reticulum, the ribosomal particles show no overt pattern of alignment. Irregularity is the key pattern and few ribosomal particles are associated with the membrane of any given endoplasmic reticulum vesicle (Plate VIII, Figure 15).

The Golgi unit of the primordial oocyte is largely structured of compact stacks of flattened vesicles with a

limited number of dilated ones. Other vesicles, and this may be peculiar to a plane of sectioning, appear largely to be loosely stacked and partially dilated (Plate VIII, Figure 15). The number of such units in this oocyte is not known. Their distribution seems to be a random one.

The granulosa cells of the primordial follicle are of a flattened somewhat squamous epithelial type. Their nucleus is flattened in conformation with the gross shape of the entire cell, and is characterized by deep, irregular incisions which give it a somewhat lobular appearance (Plate I, Figure 1; Plate V, Figure 11). The karyoplasm comprises electron dense condensations of chromatin principally associated with the nuclear membrane and a meshwork of fine filaments and granular material. The nuclear membrane consists of conventional type inner and outer membrane elements. It shows modifications as nuclear pores and blebs. The latter appear to involve only the outer nuclear membrane and the electron lucent substance between the two membrane components (Plate II, Figure 3).

The general cytoplasmic mass of the granulosa cell is of intermediate electron density. It contains numerous filamentous mitochondria of varied length and diameter (Plate I, Figure 1). The intrinsic structure of the mitochondria is basically typical of mitochondria in general. However, the cristae, of lamellate type, are of irregular disposition along the length of the unit. The mitochondrial matrix shows

variations in electron density along the length of the unit and in some instances virtually obscures all internal mitochondrial structure. The Golgi complement is comprised of multiple units of stacked flattened vesicles with numerous transitional forms of dilated vesicles. All are characteristically situated in a juxtanuclear position on the side nearest the granulosa oocyte junction (Plate II, Figures 2, 3). In addition, the full complement of Golgi units is characteristically ordered about the centriolar apparatus. The latter consists of a proximal and a distal centriole which bears a cilium having the 9+0 fiber pattern (Plate II, Figure 3) (Appley and Richter, 1970). The endoplasmic reticulum is not prominent and is present largely as dispersed free ribosomal particles and as vesicles with round or narrow, elongated profiles. These vesicles may be with or without attached ribosomes. The endoplasmic reticulum has a general distribution throughout the cytoplasmic area of the cell. The surface of the granulosa cells apposing the oocyte, may be discrete and smooth in parts and irregular and ambiguous in others (Plate II, Figures 2, 3). On its basal lamina side, the granulosa cell surface is generally smooth and in intimate association with this structure (Plate I, Figure 1; Plate II, Figure 2).

The Ultrastructure of the Primary Follicle Age Group

Primary follicles are situated in the ovarian cortex. All follicles of this age group are structurally characterized

as follows: They contain oocytes having a diameter ranging from approximately 25-30 microns through 70-80 microns. A single layer of granulosa cells surrounds the oocyte. These cells range in form from low cuboidal to low columnar. In this study, the transitional forms of granulosa cells have a mean height range of 2-3 microns through 10 microns. Each follicle shows a zona pellucida of transitional character which as a unit ranges from an ambiguously structured trace feature of 0.12 micron in thickness to a highly structured discrete one of approximately 4 microns thickness (Plate II, Figure 2; Plate X, Figure 22). A discrete, electron dense basement membrane is present which ranges from approximately 1000 angstroms (0.1 micron) through 1300 angstroms (0.13 micron) (Plate II, Figure 2; Plate X, Figure 22).

In the exemplary primary follicle (Plate X, Figure 22), the oocyte has a diameter of 48 microns. Microvilli on the surface of the egg have been calculated to approximate 46,844 in number. The calculated volume of the oocyte exclusive of microvilli is 57,908 cubic microns. Each microvillus has a volume of 0.001 cubic micron. The total volume including the microvilli is 57,955 cubic microns. The oocyte has a surface area, exclusive of microvilli, which measures approximately 7,240 square microns. Each microvillus has a surface of 0.07 square micron. The total surface of the oocyte, including the microvilli amounts to 10,519 square microns (Table I, III).

The zona pellucida is 4.0 microns thick and has an overt volume of 31,703 cubic microns. This includes the microvilli permeating it from the oocyte and the cell processes of the granulosa cells which traverse this region. The general zona pellucida--granulosa cell junction, has a surface of 9,854 square microns. Each cell of the granulosa is aligned with the pellucid membrane for an average distance of about 25 microns along this interface. It can be calculated that the single layered membrana granulosa is comprised of about 392 cells. Each cell has a volume of about 340 cubic microns. This would be equivalent to a cuboidal cell with each side measuring 6.98 microns. These cellular dimensions do not include the processes extending into the zona pellucida. The entire granulosa layer, excluding these processes, has a calculated volume of 133,248 cubic microns (Table 1).

The entire follicle, exclusive of the basement membrane, has a diameter of 76 microns; a volume of about 222,859 cubic microns; and a surface measuring about 18,151 square microns.

The primary follicle age group as noted in the preceding paragraphs is characterized by a general but extensive growth of its constituent parts: the oocyte, the granulosa and the newly formed zona pellucida (Plate IX, Figure 19, 20; Plate X, Figure 22).

The oocyte in these follicles shows the following intrinsic structural alterations: The nucleus acquires a

central positioning within the cell. A prominent nucleolus arises which is characteristically structured of filamentous elements (Plate X, Figure 22). The nuclear membrane shows characteristic undulations, similar to those seen in the primordial oocyte nucleus. The nuclear membrane is thicker than that of the primordial oocyte and measures about 500 angstroms. Except for a few short spans, a small fraction of a micron in length, the nuclear membrane shows no trilaminar structuring or nuclear pores (Plate X, Figure 22).

There is an increase in the number of mitochondria. Their distribution is a random one and their intrinsic structure is typical of this stage. Occasional mitochondria show a conversion of their cristae into a system of peripheral concentric lamellae (Plate VIII, Figures 17, 18). In addition, some mitochondria show a type of blebbing (Plate XI, Figures 23, 24). The Golgi component is increased and consists of numerous stacks of vesicles which are distributed generally throughout the cytoplasm. An orderly arrangement of Golgi structures close to the oocyte surface is spaced at intervals approximating 10 microns (Plate X, Figure 22; Plate XV, Figure 29). The endoplasmic reticulum complex is ambiguous. It appears to consist of a few smooth membraned vesicles and dispersed ribosomal particles (Plate VIII, Figure 17; Plate XI, Figure 23).

The egg surface shows the most extensive structural transformation. It is associated with the acquisition of

microvilli, each measuring 0.06 micron in diameter, by 0.37 micron in height (Plate X, Figure 22; Table 2). Desmosomal junctions are present between the egg plasma membrane and that of the granulosa cell processes (Plate X, Figure 22).

The granulosa cells show extensive change in gross form in the primary follicle age group. The main body of the individual cell changes from a flattened form, normal to the surface of the oocyte, to a low columnar form. Its basal aspect is essentially smooth and flat and is in close apposition with the basement membrane. On the side facing the oocyte, the granulosa cells change from a broad appositional association by desmosomal junctions to a labyrinthine system of coarse cytoplasmic processes retaining the desmosomal connections with the oocyte plasma membrane (Plate X, Figure 22). The granulosa cell transformation is concomitant with the progressive accumulation of zona pellucida matrix in the oocyte-granulosa interspace (Plate III, Figure 4; Plate IV, Figures 5, 6, 7, 8, 9; Plate IX, Figures 19, 20; Plate XV, Figure 33). Desmosomal junctions between granulosa cells are also differentiated and maintained during these cell transformations (Plate III, Figure 4; Plate IV, Figures 5, 6, 8, 9; Plate X, Figure 22).

The granulosa cells, during this process of gross transformation, show the following internal structural features and alterations: The nucleus becomes spheroidal and no longer possesses the deep incisures. The mitochondria are

prominent, filamentous units which sometimes show branching. Puffed, vesiculated areas, devoid of cristae are frequently seen in the mitochondria. The Golgi complex is prominent and polarized in location on the side of the cell nearest the oocyte and the emerging pellucid membrane (Plate IX, Figures 19, 20). The intrinsic structure is that of flattened vesicles accompanied by more dilated ones. It is usually juxtanuclear in position and organized about the centriolar apparatus which is sometimes observed to contain a cilium (Plate IX, Figures 20, 21). The endoplasmic reticulum appears as a system of rough surfaced elongated vesicles of general distribution in the cytoplasm.

The Ultrastructure of the Secondary Follicular Age Group

The secondary follicle age group is defined (Maximov and Bloom) as containing more than one layer of granulosa cells, but lacking a discrete antrum or antral spaces in the membrana granulosa. It must be recognized that this definition is based on light microscopic data and may not be entirely applicable to electron microscopic analysis. Nevertheless, occasional follicles do occur in the bat ovary whose basic structure and organization fits this defined follicle age group. In these follicles the granulosa cells are tightly packed. While forming a second row, some cells may still span the entire thickness from the basement membrane to the pellucid membrane zone (Plate XII, Figure 26).

In the exemplary secondary follicle (not entirely pictured on Plate XII, Figure 26) the entire follicle has a calculated volume of 1,098,141 cubic microns. This does not include the basement membrane. The follicle surface facing the basement membrane is 51,476 square microns. The radius of the follicle measures 63.5 microns and the diameter of the entire structure is 127 microns. The overt membrana granulosa has a thickness of 26 microns and has a volume of 877,276 cubic microns. The zona pellucida has a measured thickness of 4 microns and a calculated volume of 63,379 cubic microns which includes the microvilli and the granulosa cell processes which extend into it. The surface of contact with the granulosa measures 17,673 square microns (Table 1).

The oocyte of the secondary follicle has a projected diameter of 67 microns. The total number of microvilli has been calculated to be 208,600. The volume of the egg exclusive of microvilli is 157,486 cubic microns. The surface of each microvillus is 0.123 square micron; and the total effect of the microvilli is to increase the surface of the oocyte from 14,104 square microns to 39,688 square microns. Each microvillus has a length of 0.488 micron and a diameter of 0.08 micron (Table 2). The space between villi is approximately 0.179 micron.

The ultrastructural features of the oocyte, zona pellucida, and granulosa cells of the secondary follicle are as follows: The oocyte has an eccentrically situated nucleus.

It is spherical and has an undulating profile. The undulations also characterize the oocyte nucleus in primary and primordial follicles (Plate I, Figure 1; Plate X, Figure 22). The nuclear membrane has no discernible tri-laminar structuring and nuclear pores have not been observed. The nucleolus is prominent, eccentrically located as in the primary follicle, but appears to be more clearly comprised of a complexly coiled ribbon structure. The oocyte cytoplasm is dominated by increased numbers of mitochondria, which as in the primary and primordial follicle oocytes are spherical. The majority are vesiculate and this is referable to the conversion of the cristae into lamellae, concentric with the limiting mitochondrial membrane. Concurrently, the mitochondrial matrix becomes granular. The granules measure about 90 angstroms in diameter and in some instances, seem to form crystalline-like formations (Plate XI, Figure 25; Plate XIV, Figure 31). The Golgi component is comprised of numerous units consisting of stacks of flattened vesicles and numerous dilated electron lucent profiles. The latter in their alignment, appear to connect multiple Golgi units as in the primary follicle oocyte (Plate X, Figure 22). Golgi units are regularly spaced near the oocyte surface and are uniquely positioned relative to desmosomal junctions of the oocyte and the granulosa cell processes which span the entire thickness of the zona pellucida (Plate XIV, Figures 30, 31, 32). Some of the desmosomal

junctions comprise pit-like depressions in the egg surface which penetrate into the internum of associate Golgi units. They appear as isolated ring-shaped structures associated with Golgi units in some planes of section (Plate XIV, Figures 30, 31, 32). Endoplasmic reticulum components are not clearly demonstrable and identifiable. If present they appear to consist only of a diffuse system of free ribosomal particles (Plate XI, Figure 25).

The ultrastructure of the zona pellucida is essentially the same as that characterizing the primary follicle. Desmosomes, representing junctional connections between granulosa cell processes appear to be of more frequent occurrence and larger (Plate XII, Figure 26). Though occasional granulosa cell processes traverse the zona pellucida in a direct radial fashion, the great majority are canted. On the granulosa side they are parallel to the overt granulosa surface (Plate XII, Figure 26). The zona pellucida matrix, although relatively dense, has no clearly discernible intrinsic structuring (Plate XII, Figure 26).

Exclusive of the cell processes which penetrate the zona pellucida, the granulosa cell body is variable in gross form. This ranges from cuboidal to columnar (Plate XII, Figure 26). Adjacent to the zona pellucida, the granulosa cells are partially separated by broad intercellular spaces which affect interdesmosomal regions of these cells (Plate XII, Figure 26). Elsewhere the granulosa cells are closely

apposed and show numerous desmosomal junctions (Plate XII, Figure 26). The nuclei of these cells, though irregular in form, do not show the deep incisures characteristic of the primordial follicle (Plate I, Figure 1). Nucleolar structures are prominent. The chromatin material comprises irregular condensations of diverse sizes throughout the nucleus and at the nuclear membrane. The nuclear membrane shows conventional inner and outer membrane elements (Plate XII, Figure 26). The mitochondrial complement comprises numerous mitochondria which, unlike those found in primordial follicle granulosa cells, reach lengths of up to 4 microns and widths of 0.2-0.3 micron. The mitochondrial cristae appear as both lamellate and tubular structures and vary in disposition and clarity. The mitochondrial matrix may consist of electron lucent portions or may be completely filled with electron dense particles (Plate XII, Figures 26, 27; Plate XIII, Figure 28). In some mitochondrial cross-sectional profiles, only the limiting membrane is present (Plate XIII, Figure 28). The Golgi complex consists of one or more stacks of flattened vesicles and free dilated vesicles. The Golgi units are located in both the apical and the basal poles of the cells. In the apical pole, they are associated with a centriolar apparatus which may contain a cilium. The endoplasmic reticulum is abundant and distributed throughout the cytoplasmic mass and the cell processes penetrating the zona pellucida. It consists of flattened rough membraned vesicles, dilated cisternae and some free ribosomal particles.

Occasional cells show irregular accumulations of slightly electron dense material in the cytoplasmic matrix. These have a fine fibrous and granular structure (Plate XII, Figures 26, 27; Plate XIII, Figure 28). There is a structural similarity of these regions to histochemically characterized glycogen deposits (Revel et al., 1960; Revel, 1964). These areas may be surrounded by mitochondria, but are more frequently associated most closely with rough endoplasmic reticulum formations (Plate XII, Figures 26, 27; Plate XIII, Figure 28). The granulosa cell basement membrane junction is of smooth contour.

The Tertiary Follicle Age Group

The tertiary follicle age group is defined primarily by the status of the granulosa component, even though the final growth of the ooyte, preparatory to its meiotic maturation occurs in this age group.

The granulosa of this age group is a compound type of epithelium which ranges from a thickness of three cells to about fifteen cells. This follicle age group is specifically characterized by the presence of an intragranulosal antrum or space in multiple phases of formation. The antrum is filled with or comprised of a unique tissue fluid or liquor folliculi (Honore, 1900; Van der Stricht, 1911; Robinson, 1918; Mjassojedoff, 1923; Guthrie and Jeffers, 1938; Wimsatt, 1944; Brambell, 1956; Hadek, 1963). The most extensive changes at this stage are those associated with the granulosa cells,

reflecting their special involvement in the final differentiation of the zona pellucida and the genesis of the fluid filled antrum (Bjorkman, 1962; Hadek, 1963; Hope, 1965). In the bat, the antrum is comprised of multiple discrete antral spaces. As a group, they loosely divide the granulosa into cumulus oophorus, discus proligerus and parietal granulosa portions (Van Beneden, 1880, Guthrie and Jeffers, 1938; Wimsatt, 1944; Wimsatt and Kallen, 1957).

The tertiary follicle age group, in a premeiotic growth phase, includes follicles ranging from about 190 microns to 350 microns in diameter (Wimsatt, 1944). This size range is due largely to the increase in mass of the granulosa and its contained antral spaces (Table 1; Graph 1).

At the light microscopic level, the earliest tertiary follicles showing minute antrum spaces, have a diameter of 257 microns. The membrana granulosa of these follicles is about 84 microns thick and contains antrum spaces measuring about 8 microns in a direction perpendicular to two apposing granulosa cell surfaces. Essentially equivalent tertiary follicles, as observed with the electron microscope, reveal antral spaces as small as 2 microns in thickness (Plate XXVII, Figure 60). Late tertiary follicles have a mean diameter of 287 microns. The granulosa measures about 84 microns in thickness and contains fewer but larger antrum spaces of irregular form (Plate XXVIII, Figures 61, 62).

A. The early tertiary follicle: The oocyte of the early tertiary follicle has a mean dimension of 73 microns.

Its volume, exclusive of microvilli, is 173,119 cubic microns and the volume including the microvilli amounts to 173,952 cubic microns (Tables 1, 2; Graph 2). Each microvillus is 0.1 micron in diameter and 0.6 micron in length. A space of 0.18 micron exists between adjacent villi. The total number of microvilli per oocyte is calculated to be 177,228 (Table 2; Graph 3). The total volume of the microvilli per oocyte amounts to 833 cubic microns and the total surface to 33,673 square microns (Table 2; Graph 3).

The zona pellucida, 7.5 microns thick, has a volume of 183,716 cubic microns (Table 1; Graph 1). This includes the space occupied by the oocyte microvilli and the granulosa cell processes which extend into it.

The granulosa, 84 microns thick, exclusive of the granulosa cell processes penetrating the zona pellucida, has a volume of 8,428,134 cubic microns. Its surface, on the basement membrane side, is 205,946 square microns. Its surface on the zona pellucida side, exclusive of the extended cell processes, is 24,335 square microns (Table 1; Graph 2).

The entire follicle, with a mean diameter of 257 microns, has a calculated volume of 8,784,969 cubic microns (Table 1; Graph 1).

The ultrastructural aspects of the early tertiary follicle: Measurements recorded on Table 1, indicate that the

oocyte of the early tertiary follicle is only slightly larger than that of the secondary follicle. There is no measurable evidence of change in the amount per unit area of the Golgi, mitochondrial and other components of the oocyte relative to the secondary follicle oocyte. The mitochondria in the early tertiary follicle oocyte show the typical morphology for this stage (Plate XXVII, Figure 60) to an ever increasing extent. The mitochondrial membranes are folded into parallel peripheral layers. The granular interior of the mitochondria is free of cristae.

The zona pellucida is essentially unchanged in thickness from the previous stage, though its space defined volume shows a threefold increase over that of the preceding age group (Table 1). On its oocyte side, it is in close association with the oocyte plasma membrane and is penetrated by straight oocyte microvilli of increased size (Plate XV, Figures 34; Plate XVI, Figures 36; Plate XVII, Figures 60). The number of granulosa cell processes penetrating the entire thickness of the zona and in desmosomal junctions with oocyte intervillar plasma membrane, are reduced (Plate XXVII, Figure 60). On the zona's granulosa side, the labyrinthine system of granulosa cell processes is reduced in numbers and dimensions of the processes. Occasional processes closest to the principal granulosa cell body, show rough endoplasmic reticulum, mitochondrial elements and intercellular desmosomes (Plate XV, Figure 34; Plate XXVII, Figure 60).

The volume of the granulosa is increased ten-fold over that of the secondary follicle (Table 1; Graph 1). This increase is due to:

1. The increase in number of granulosa cells which are mitotically competent (Plate XXIII, Figure 42).
2. The hypertrophy of the granulosa cells.
3. Antrum formation.

Granulosa cells appear to be intimately involved in antrum formation. Ultrastructurally, the great majority of the granulosa cells of the tertiary follicle are in close apposition with each other, with a minimum of intercellular space. They show numerous desmosomal junctions (Plate XXIII, Figure 43; Plate XIX, Figure 44; Plate XX, Figure 46; Plate XXII, Figure 50; Plate XXVII, Figure 60). The intrinsic ultrastructural aspects of the nucleus, the Golgi components, the rough endoplasmic reticulum and the mitochondrial complement of the granulosa cells are essentially those of the preceding secondary follicle age granulosa (compare Plate XII, Figure 26 and Plate XXVII, Figure 60). The endoplasmic reticulum system, however is largely composed of small dispersed rough vesicles and free ribosomal particles. The granulosa cells differ from those of the secondary follicle in that they show in more pronounced and varied degrees two modifications which affect the general cytoplasmic matrix. One of these relates directly to antrum formation while the other appears to

relate to the accumulation of glycogen in these cells. It is possible that both modifications may be involved in antrum formation.

In the first modification, the granulosa cells show a progressive emergence of relatively electron lucent areas of irregular form and size. These are not membrane limited. They have a finely granular or fibrous intrinsic texture (Plate XVIII, Figure 43; Plate XIX, Figure 44; Plate XX, Figure 46; Plate XXI, Figure 48; Plate XXII, Figure 50). They are smaller in the deeper endoplasmic portion of the cell and largest in the more superficial ones, where they may comprise extensive cortical areas. They may or may not be associated with plasma membranes (Plate XXII, Figure 50). Antrum spaces when first clearly discernible, have morphological and dimensional features which closely correspond to these electron lucent cortical formations (Plate XVIII, Figure 43; Plate XIX, Figure 44; Plate XX, Figure 46; Plate XXI, Figure 48; Plate XXVII, Figure 60). It may be suggested that the electron lucent transformed material relates to antrum formation and that antrum formation involves a release of these cortical areas by an apocrine process.

In the second cytoplasmic modification, the granulosa cells show a progressive irregular accumulation of relatively electron dense material. These may be a fractional part of a micron to several microns in diameter and are not membrane limited (Plate XVIII, Figure 43; Plate XIX, Figures 44, 45;

Plate XX, Figures 46, 47; Plate XXI, Figures 48, 49; Plate XXII, Figure 50; Plate XXIII, Figure 51; Plate XXVI, Figure 58; Plate XXVII, Figure 60). They have an intrinsic structuring of fine particles and filaments which correspond precisely with that of reported and histochemically demonstrated glycogen depots in other cell types (Revel et al., 1960; Revel, 1964). On this structural basis, they are considered here to be glycogen accumulations (Plate XIX, Figures 44, 45; Plate XX, Figure 46, Plate XXI, Figure 48; Plate XXII, Figure 50). These areas may be distributed deep in the cell or in the cortical regions may be associated with the electron lucent material formations (Plate XIX, Figure 44; Plate XX, Figure 46; Plate XXI, Figure 48; Plate XXII, Figure 50).

B. The late tertiary follicle: The oocyte of the late tertiary follicle, with measurements derived by light microscopic means, has an average diameter of 90.3 microns. The volume of the oocyte, exclusive of microvilli is 383,704 cubic microns. With its villi included, the oocyte reaches a volume of 383,834 cubic microns (Tables 1, 2; Graph 1). The microvilli are 0.1 micron in diameter and 0.3 micron in length. The spacing between villi is approximately 0.6 micron. The calculated total number of microvilli per oocyte of the late tertiary follicle is 52,900 and these have a total calculated volume of 127 cubic microns. The total calculated surface of the microvilli amounts to 4,763

square microns. The surface of the oocyte, exclusive of its microvilli, is 25,530 square microns. The total surface with its microvilli included amounts to 30,293 square microns. As shown in Tables 1 and 2, the oocyte of the late tertiary follicle has increased two-fold in volume from the preceding stage. Its decrease of the total oocyte surface (Tables 1, 2) is largely due to the decrease in the number of microvilli. The zona pellucida has a thickness of 6.8 microns and an overt volume, including the microvilli and the penetrating granulosa cell processes, of 192,253 cubic microns (Table 1). The volume does not differ significantly from that of the early tertiary follicle.

The granulosa has a mean thickness of 84 microns. Its volume, exclusive of the granulosa cell processes in the zona pellucida, amounts to 11,798,337 cubic microns. On the basement membrane side, its surface is 258,741 square microns (Table 1). The entire follicle has a mean diameter of 287 microns and a total volume of 12,374,294 cubic microns (Table 1; Graph 1).

As shown on Tables 1 and 2, the oocyte of the late tertiary follicle has undergone a remarkable two to three-fold increase in volume and surface features over those of the oocyte of the early tertiary follicle. In contrast, the volume and surface aspects of the zona pellucida and granulosa of the late tertiary follicle are only 38-40% greater than those of the zona and granulosa of the early tertiary follicle (Table 1; Graphs 1, 2).

The ultrastructural aspects of the late tertiary follicle: It has not been possible, by electron microscopic analysis to relate the twofold increase in volume of the late tertiary follicle oocyte with specific increments of its cytoplasmic components. Random counts of mitochondria in the early (3.2 mitochondria per square micron of oocyte cytoplasm) and late tertiary oocytes (2.6 mitochondria per square micron of oocyte cytoplasm) show essentially 3 mitochondria per square micron of cytoplasm. As the oocyte volume is doubled between the early and late tertiary phases, it may be inferred that there must be an increase in the number of mitochondria to maintain this constant ratio. Similarly, it would seem likely that other cytoplasmic components also show some growth. These do not lend themselves to quantitations. The ultrastructural aspects of the late tertiary oocyte, in regard to the distribution and intrinsic structuring of its nuclear, mitochondrial, and Golgi elements are not significantly changed from those characterizing the oocyte of the early tertiary follicle. Noteworthy is the positioning of the nucleus in a very peripheral location (compare Plate XXVII, Figure 60 and Plate XXVIII, Figure 61). The oocyte surface shows the most pronounced structural alteration. This is characterized by a 3-fold increase in the intervillar spacing distance; a decrease by 2/3 in the number of microvilli; and a 50% reduction in the size of the individual villi (Table 2, Graph 3, Plate XXVII, Figure 60; Plate XXVIII, Figure 61).

The influence of microvillar change on the surface of oocytes of all follicle age groups is shown graphically in Table 1 and Graph 2.

The zona pellucida shows essentially very little change in volume (less than 4%) over that of the early tertiary follicle (Table 1, Graph 1), while its thickness is reduced 13-25%. On its oocyte side, the structural appearance largely reflects the alterations of the oocyte surface relating to the size, number and distribution of the oocyte microvilli (Plate XXVIII, Figure 61; Plate XXIX, Figure 64). In addition, there is a loss of desmosomal junctions between the oocyte and granulosa cell processes (Plate XXVIII, Figure 61; Plate XXIX, Figure 64). It is related to the absence of intact granulosa cell processes which penetrate the full thickness of the zona (Plate XXVIII, Figure 61). All that persists of each process in the zona of the late tertiary follicle are bits of protoplasmic debris without recognizable structure or order (Plate XV, Figure 35; Plate XXVIII, Figure 61; Plate XXIX, Figure 64). Similarly, on its granulosa side, the granulosa cell processes are reduced in number and size. The cells show a degenerative loss of intrinsic structure and structural order (Plate XV, Figure 35; Plate XXVIII, Figure 61).

The zona pellucida matrix, with increased electron density, is closely apposed to the oocyte plasma membrane. On its granulosa side, it may be variably separated from the granulosa plasma membrane by an electron lucent space or zone

(Plate XV, Figure 35; Plate XXVIII, Figure 61). The zona matrix shows a fairly granular and filamentous intrinsic structuring (Plate XV, Figure 35).

The granulosa of the late tertiary follicle, as noted earlier, reflects an increase of 38% in volume over that of the early tertiary stage without any change in its thickness (Table 1). As a unit, the granulosa shows regional differences in the packing and structural interrelations of its constituent cells. In the parietal portion, the majority of the cells are closely and broadly apposed to each other. They show a minimum of intercellular space and numerous desmosomal junctions (Plate XXVIII, Figure 63). Their intrinsic structure is like that of the early tertiary and secondary follicle granulosa cells (compare Plate XII, Figure 26 and Plate XXVIII, Figure 63). In its visceral portion, bounding the oocyte and in areas bounding the largest antral spaces, the granulosa cells are rounded, variably hypertrophic and loosely packed. Only small fractions of their plasma membranes are in close apposition. These sites frequently consist of desmosomal junctions (Plate XXVIII, Figures 61, 62; Plate XXXI, Figure 74). In many instances the interdesmosomal plasma membranes of apposing cells appear to be lacking altogether with open cytoplasmic continuity between cells (Plate XXVIII, Figures 61, 62; Plate XXXII, Figure 75). When these cells are not in close apposition, they are separated by antral spaces measuring up to 40 microns across (Plate XXVIII, Figure 62).

The intrinsic ultrastructural aspects of the nucleus, mitochondrial and Golgi complements of the visceral granulosa cells are like those of the early tertiary and secondary follicle granulosa cells (compare Plate XII, Figure 26, Plate XXVII, Figure 60 and Plate XXVIII, Figures 61, 62). The glycogen deposits in the cytoplasm are extensive (Plate XXVIII, Figures 61, 62, 63; Plate XXXIII, Figure 75). The electron lucent transformations or modifications of cytoplasmic matrix are characteristically common and involve massive portions of the cell, particularly the superficial or cortical areas (Plate XXVIII, Figures 61, 62, 63). These areas have a finely reticulated character and occasional particles found there are considered to be free ribosomes. They are devoid of mitochondrial and Golgi components. The electron lucent formations may not be surfaced or associated with a plasma membrane. In these instances they appear to be in open and broad continuity with existing antrum (Plate XXVIII, Figures 62, 63; Plate XXXI, Figures 73, 74; Plate XXXIII, Figure 75). Residual debris of granulosa cell processes of varied sizes occurs in formed antrum spaces. These are structurally equivalent to those residues of the granulosa cell processes penetrating the zona pellucida. They contain glycogen bodies; remnants of structurally disordered mitochondria, Golgi vesicles and ribosomal particles, and are usually without plasma membranes (Plate XXVIII, Figures 62, 63; Plate XXXIII, Figures 75, 76, 77; Plate XXXIII, Figures 78, 79). Quite

commonly they are associated with the largest antra (Plate XXVIII, Figure 62). Entire granulosa cells show this type of structural degradation (Plate XXXII, Figure 75; Plate XXXII, Figure 79).

In the preceding section, the ultrastructural features of the oocyte, the zona pellucida and the granulosa, as well as certain quantitative aspects and interrelations of these components have been detailed in defined follicle age groups ranging from the primordial through the tertiary follicle. The principal or common feature which characterizes these follicle age groups is the synchronized repetitious occurrence of identical structural features and structural trends in the oocyte, zona and granulosa components. This is exemplary of the accepted "principle of repetition" in the ordering of natural systems (Frey-Wyssling, 1955; Weiss, 1962; Schmitt, 1963; Du Praw, 1968). Repetitious structure and structural trends in the ovarian follicles of these age groups appear to be directed toward the maturation and maintenance of a functionally and structurally competent follicle, and are taken to represent normal structural order.

In the following section, although the data are incomplete at this time, demonstrable deviations away from the synchronized repeat pattern are detailed for several of the defined follicle age groups. These deviations are considered to be abnormal, in a sense, in that they relate to or are directed toward follicular destruction rather than preservation.

Structural Deviations in the Primordial Follicle Age Group

The oocyte is of a stellate form and is characterized by cytoplasmic processes of irregular size and shape. Its plasma membrane may be complete, incomplete or lacking, and shows areas of fusion with the plasma membranes of the adjacent granulosa cells. The cytoplasm contains residual Golgi vesicles, unidentifiable smooth membranes, dispersed ribosomal particles and lipid inclusions (Plate VI, Figure 13).

The granulosa cells present diffuse cytoplasmic processes which penetrate into the oocyte cytoplasmic remains or interdigitate with oocyte cytoplasmic processes, showing either fused adjoining membranes or no membranal separation. An actual blending of oocyte and granulosa cell cytoplasm occurs, involving a sequence of a) the fusion of the adjacent plasma membranes; b) an eventual dissolution of the fused membranes and c) free exposure of oocyte and granulosa cell cytoplasm. The fusion of the membranes can also be observed between adjoining granulosa cells (Plate V, Figures 10, 11; Plate VI, Figure 13). Except for lipid inclusions, the ultrastructural aspects of the granulosa cells are normal for this follicle age group. The basement membrane is altered and may be incomplete or absent (Plate VI, Figure 13).

Structural Deviations in the Primary Follicle Age Group

The oocyte shows large bulbous cytoplasmic processes measuring up to 5 microns across. These consist mainly of

clear cortical cytoplasm and shows fusion of the oocyte and granulosa cell plasma membranes. Occasional mitochondria, Golgi and endoplasmic reticulum elements may be present (Plate V, Figures 10, 11; Plate VI, Figure 12). Whether these cortical lobes relate to the genesis of microvilli is not determined. They are similar to elaborations which occur as deviations of the oocyte surface in the tertiary follicle age group (Plate XVI, Figure 38, 39; Plate XVII, Figures 40, 41). All other structural features of the oocyte are normal (compare Plate III, Figure 4, and Plate VI, Figure 12; Plate VII, Figure 14).

The granulosa cells appear normal except for altered cellular positioning which is reflected in irregularities in the basement membrane (Plate VII, Figure 14). A fusion of adjacent cell plasma membranes can be observed (Plate V, Figures 10, 11).

Structural Deviations in the Secondary Follicle Age Group

The oocyte shows extensive deviations in ultrastructure. The surface changes consist of:

1. a confluence of cortical cytoplasm forming bulbous pedicles for individual and groups of villi;
2. unusually large microvilli (0.114 micron wide and 0.39 micron long), curved and tilted in numerous planes;

3. an absence of oocyte and granulosa cell desmosomal junctions (Plate XVI, Figures 38, 39; Plate XVIII, Figures 40, 41).

The oocyte mitochondria have incomplete limiting membranes. The cortical Golgi units are dilated vesicular structures. These may form vesicular chains and extend into the cortical region containing the pedicles. They are closely packed, unlike the regularly spaced formations observed in the growing follicle oocytes. Some units appear to form concentrically lamellated whorls in the cell surface group (Plate XVII, Figures 40, 41). Data on the oocyte nucleus are lacking.

The zona pellucida on its oocyte side is devoid of granulosa cell processes. On the granulosa side an occasional granulosa cell process can be observed (compare Plate XII, Figure 26 and Plate XVIII, Figure 40).

The granulosa presents a wide range of structural deviations. 1) Granulosa cells located adjacent to the basement membrane present a normal ultrastructure (Plate XXIV, Figure 54; Plate XXV, Figure 57; Plate XXXV, Figure 88). 2) Some cells of the granulosa show a complete retrograde loss of intrinsic structural order. It is comparable to those of the granulosa cells of the late tertiary follicle related to the genesis of the Call - Exner bodies and final antrum formation. 3) Lipid depositions are seen in some cells, as the only sign of deviation. 4) Other cells are marked with the appearance of lipid myelin-like formations and electron lucent vacuolar

spaces (Plate XXXV, Figure 88). 5) Some cells show accumulations of electron lucent preantral substance which occurs normally in the tertiary follicle age group (compare Plate XXII, Figure 50 and Plate XXXV, Figure 87). 6) Membrane bound inclusions (Plate XXIV, Figures 52, 54, 55; Plate XXV, Figure 57), sometimes containing protoplasmic debris and lipid myelin formations (Plate XXXV, Figures 87, 88) are found in some of the altered granulosa cells. 7) The granulosa cells collectively lack structurally identifiable glycogen deposits. 8) Intercellular desmosomal junctions are either greatly reduced or absent (Plate XXV, Figure 57; Plate XXXV, Figures 87, 88).

The basement membrane is deeply convoluted, with granulosa cell processes following the convolutions (Plate XXV, Figure 57; Plate XXXV, Figure 87).

Structural Deviations in the Tertiary Follicle Age Group

Data on deviations of the tertiary follicle oocyte have not been obtained. The zona pellucida, unlike that of the normal tertiary follicle, is marked by a complete absence of granulosa cell processes, and the zona-granulosa junction is smooth (compare Figures 58 and 59 on Table XXVI).

Deviations in the granulosa are extreme. 1) Preantral material is present intracellularly as large rounded electron lucent inclusions (Plate XXVI, Figure 59; Plate XXIV, Figure 55). 2) Intercellular antral spaces are virtually

absent (Plate XXVI, Figure 59). 3) The plasma membranes of cells in apposition show a loss of their individual identity through fusion, forming a common, thick and diffuse membrane affecting a large segment of the cells' surfaces (compare Figures 58 and 59 on Plate XXVI). 4) Desmosomal junctions are reduced in number, size and electron density (compare Figures 58 and 59 on Plate XXVI). 5) The basal granulosa cells adjacent to the basement membrane have cytoplasmic processes which fill and conform with the convolutions of the basement membrane (Plate XXXVI, Figure 89). In some follicles, the basal granulosa cells have incomplete plasma membranes on those surfaces adjacent to the basement membrane. The cytoplasmic matrix of these cells is in open continuity with an electron lucent zone which may be described as a "supra-basement membrane zone" (compare Plate XXVIII, Figure 63 with Plate XXXVIII, Figure 95). 6) Internal structural deviations of the granulosa cells include: a) an overvesiculation and dilatation of Golgi units; b) the formation of endoplasmic reticulum cisternae, particularly in the basal granulosa cells; c) irregular deposits of lipid; d) the appearance of lipid myelin formations; and e) a shortening of the mitochondria and a concomitant loss of their normal cristae pattern. All of these can be observed in Plate XXXVI, Figure 89.

Deviations in Follicles of Unknown Age Group

Follicles in this category are reduced to diminutive traces of recognizable gross structural features. These consist of discontinuous segments or remnants of the basement membrane, which are convoluted and have a thickness of about 690-1600 angstroms (Plate XXXIX, Figure 96; Plate XL, Figure 97), a supra basement membrane space, and diminutive clusters of rounded and completely retrograde granulosa cells (compare Plate XXXIX, Figure 96 and Plate XL, Figure 97). Peripheral to the remnants of the basement membrane is a lobulated zone of collagenic fibrous tissue and occasional fibroblasts. The fibroblasts, in regions where the basement membrane shows discontinuities, may occupy a position within the supra-basement membrane space and be adjacent to the granulosal cell remnants in the center of the collagenic scar (Plate XXXIX, Figure 96; Plate XL, Figure 97). Other fibroblasts are positioned at the surface or periphery of the fibrous scar. The fibroblasts deep within the scar show a holocrine transformation related to collagen fibril formation, which are equivalent to those known to occur in other circumstances of connective tissue formation (Richter and Schilling, 1969) (Plates XXXIX, Figure 96; Plate XL, Figure 97).

In other instances only a small, characteristically lobulated fibrocollagenous scar structure is recognizable. This contains no discernible basement membrane, granulosa, zona pellucida or oocyte (Plate XL, Figure 97).

CHAPTER IV

DISCUSSION

In this study an attempt has been made to obtain data on the total range of ultrastructural changes which characterizes each defined follicle age group and to analyze such changes in order to determine the degree to which they bear out the "principle of repetition." The latter consisted of determining which specific ultrastructural features, trends or combinations of these occur repetitively in all of the follicular age groups studied and which do not. As recorded in the preceding observations, repetitive ultrastructural features are taken to be indicative of, or relating to, normal follicles in multiple growth phases. Deviations from a repetitive pattern are taken to be indicative of follicles in some phase of follicular atresia. This applies to follicles of all age groups.

This approach, tedious and time consuming, requires comprehensive data on the ultrastructural features of the ovum, the zona, the granulosa and the basement membrane components of follicles in all stages of maturation. It appears at present to be the only feasible way to characterize the

process of atresia by ultrastructural criteria. This approach was also indicated because reported information on the ultrastructure of normal as well as atretic follicles of all developmental stages is exceedingly fragmentary and incomplete (Moricard, 1958; Trujillo-Cenoz and Sotelo, 1958; Anderson and Beams, 1959, 1960; Chiquoine, 1959; Bjorkman, 1962; Hope, 1965; Odor, 1965; Blanchette, 1966; Adams et al., 1966; Baca and Zamboni, 1967; Weakley, 1967; Norrevang, 1968; Priedkalns et al., 1968; Priedkalns and Weber, 1968; Kessel, 1968, 1969; Odor and Blandau, 1969).

This study on the prehibernation bat ovary is complete concerning follicles which range in developmental age from the primordial to the late tertiary follicle age group. Fully mature follicles have not been observed in this material.

Functional Trends Indicated by Quantitative Interrelationships of the Oocyte, Granulosa and Zona Pellucida during Normal Follicular Growth

It is generally agreed from light microscopic approaches, that the follicle during its growth and maturation from the primordial through the tertiary follicle stage is characterized by four general processes: 1) a progressive growth or increase in oocyte cell size; 2) a transformation of the unilaminar granulosa into a multilaminar one; by the growth and mitosis of granulosa cells; 3) the development of an intra-granulosa space or antrum; 4) the progressive

development of a zona pellucida between the granulosa cells and the oocyte. The data presented here confirm these general characteristics of follicular growth.

While these transformations are concurrent ones, their interdependence as active processes, and their detailed ultra-structural interrelationships have not been completely characterized.

Previous reports, derived from light and electron microscopic approaches dealing with the growth of ovarian follicles have related oocyte growth and follicle growth to alterations in oocyte and follicle diameter (Guthrie and Jeffers, 1938, 1950; Wimsatt, 1944; Mandl and Zuckerman, 1951, 1952; Knigge and Leathem, 1956; Brambell, 1957; Hadek, 1958; Hope, Humphries and Bourne, 1963; Adams, Hertig and Foster, 1966; Baca and Zamboni, 1967; Kessel, 1968, 1969). As the oocyte is contained within a granulosa, and finally becomes also confined within a pellucid membrane, diametral changes comprise only a rough index of the growth changes involved. The two-dimensional approach may lead to erroneous interpretations of the growth, individually and collectively of the oocyte, the zona pellucida and granulosa components, and on their changing structural and functional interrelations during follicle maturation.

In this study, the general growth characteristics of the oocyte, the zona and the granulosa, considered as unit components, have been analyzed quantitatively in terms of

dimensionally defined space volumes. Their structural interrelations have been analyzed in terms of dimensionally defined surface areas (Tables 1, 2; Graph 2).

The diameters of the oocyte, the zona pellucida, the granulosa and of the total follicle, recorded here, are in agreement with all values reported by others for the bat. The other studies did not include all of the stages of follicle growth which have been included in this study (Guthrie and Jeffers, 1938, 1950; Wimsatt, 1944). The individual patterns of growth of the oocyte, the zona, the granulosa and the total follicle are compared graphically in Table 1, and are expressed as change in volume. (Note: If one assumes that the follicular components have at least the density of water, 1.0, the volume values can be converted into mass values, i.e. Volume x Density = Mass or $u^3 \times 10^{-12} \times 1.0$ = mass in grams).

The volume data presented here (Tables 1, 2; Graph 1) shows the four principal points :

1. The total follicle, during the development from the primordial to the late tertiary growth stages, undergoes a near logarithmic growth, showing a 775-fold increase in volume or in mass. In contrast, the total follicle shows only an 11-fold increase in diameter and illustrates the inadequacy of using simple diametral or planar changes to characterize fully, the three dimensional growth.

2. The growth pattern of the granulosa quantitatively parallels that of the total follicle. It approaches

a logarithmic type growth. The granulosa increases 3,000-fold in volume and mass between the primordial and tertiary follicle stages.

In the primordial follicle stage, the granulosa comprises about 1/4 of the total mass of the follicle, while in the tertiary stage, its total mass almost equals that of the follicle. The ratio of granulosa to the total follicle is 1:1.05.

3. The oocyte, in contrast to the granulosa, shows only a 59 fold increase in volume and mass from the primordial to the tertiary stage. In the primordial stage, the oocyte comprises approximately 1/2 of the total follicle, while in the tertiary stage it amounts to about 1/32 of the mass of the follicle. In the primordial stage, the oocyte has a volume or mass 1.6 times that of the granulosa. This relationship is progressively reversed during follicle growth. In the tertiary stage the granulosa has a volume or mass approximately 31 times larger than that of the oocyte.

4. There is no zona pellucida in the primordial follicle. During subsequent follicular development, the zona pellucida is developed and comes to comprise approximately 1/64 of the total volume or mass of the late tertiary follicle (Table 1, Plate I, Figure 1, Plate IV, Figures 5-9; Plate IX, Figures 19, 20; Plate XV, Figures 33, 34, 35; Plate XXVIII, Figures 61, 62, 63). In the tertiary stage, the zona pellucida has a volume and mass equal to about 1/61 of that

of the granulosa and about 1/2 that of the oocyte. Other workers have noted changes in thickness of the zona in the secondary and tertiary stages and have interpreted them to indicate a swelling or shrinkage of the zona (Guthrie and Jeffers, 1938; Ingram, 1962). As shown here, the zona increases in volume until the early tertiary phase. The changes in thickness do not relate to swelling or shrinkage, but to a redistribution of the mass of the zona, with little change in its volume (Table 1). The collective considerations of the individual curves of volume, or mass, changes in follicular development through the tertiary stage suggest that the metabolic activity involved in follicular growth is directed disproportionately toward increasing the mass, or volume, of the granulosa component of the follicle over those of all others. The disproportionate mass of granulosa relative to that of the oocyte, would suggest that the granulosa has special qualitatively and quantitatively geared roles to play

1) in the screening or modification of essential substances destined for the oocyte,

2) in the active transfer of essential substances between the theca and the oocyte,

3) in the establishment of necessary granulosa volume or mass requisite to the formation of the oocyte release mechanism, which functions at ovulation.

Attempts have not been made by other workers to characterize quantitatively the surface area changes of the

oocyte, the zona and the granulosa components during follicular development. The patterns of surface change a) for the total follicle on its theca or basement membrane side; b) for the zona pellucida on its granulosa side; and c) for the oocyte with and without microvilli during follicular development are compared graphically in Table 1.

The quantitative data (Tables 1, 2) on surface area change show the following general points:

1) The critical surfaces representing interfaces between the theca and follicle, between granulosa and zona pellucida and between the oocyte, the zona pellucida and granulosa show characteristic and synchronized quantitative patterns of increase in surface area. These surface area increases are greatest during the secondary and tertiary stages of follicle growth.

2) The granulosa-theca interface shows an 85 fold increase in surface area during follicular development through the late tertiary growth stage.

Including the granulosa surface on the zona pellucida side, the total granulosa surface is increased 70 fold by the tertiary stage. In comparing the total granulosa surface to the oocyte surface, the former is approximately 4 times greater at both the primordial and late tertiary stages.

3) Surface area change in the growing oocyte is primarily associated with the transient development of microvilli. A progressive genesis and an increase in numbers of

microvilli, as well as alterations in the dimensions of the individual villi, occur during the primary through secondary growth stages (Tables 1, 2) (Plate X, Figure 22; Plate XII, Figure 26; Plate XV, Figures 33, 34, 35; Plate XVI, Figures 36, 37; Plate XXVII, Figure 60; Plate XXVIII, Figure 61). Through the development of microvilli, the oocyte surface is increased 40 times by the late tertiary stage visualized here, and is equivalent to about 1/4 of the total granulosa surface area at the same stage. The change in oocyte surface area not referable to microvilli, is relatively small, amounting to approximately a 22-fold increase by the late tertiary. It is commonly assumed that microvilli disappear eventually during the very latest tertiary phase. There are however no data available on the presence or absence of microvilli in the meiotically mature oocyte (Anderson and Beams, 1959; Chiquoine, 1959; Hope, et al., 1963; Baca and Zamboni, 1967). As shown in Tables 1 and 2, the number of microvilli continually increases into the late tertiary stage though the individual microvilli decrease in size at the same time. One can envisage this progressive diminution in size of the microvilli to result eventually in their structural disappearance.

4) The relationship, structurally and quantitatively, of the oocyte surface to granulosa surface and to zona pellucida matrix is changed in a transitory but characteristic pattern during follicular development. In the primordial

follicle stage the oocyte and granulosa share a common interface. It is represented by their respective and closely apposed plasma membranes. There are no oocyte microvilli or desmosomal junctions present. There is no zona pellucida (Plate I, Figure 1; Plate II, Figure 2). In this stage, the contiguous surface areas of the oocyte and the granulosa approach a 1:1 ratio (Table 1). Total oocyte surface is structurally associated with the granulosa in the primary follicle stage. In the subsequent primary and secondary stages, oocyte surface is shared by a) granulosa cells, with desmosomal junctions which develop between the oocyte and granulosa cell processes (Plate X, Figure 22), and b) zona pellucida matrix which accumulates progressively between granulosa cells associated through desmosomal junctions with the oocyte. Direct association between oocyte and granulosa cell surfaces, comes to involve only a small fraction of the total oocyte surface at these stages. Measurements which have been attempted indicate that this contact is about 1% of the total oocyte surface. The remaining and by far the greater portion (about 99%) of the oocyte surface, which is increased and modified by microvilli, comes to impinge directly on the accumulating zona pellucida matrix (Plate X, Figure 22; Plate XV, Figure 33) (Tables 1, 2). This surface modification is strictly a functional one, related to increased absorption of, or exposure to, substances reaching the oocyte. These substances must penetrate the zona matrix and pass with or

without screening or modification through the granulosa. It is significant that the rate of oocyte growth is maximal at these stages and parallels the proportional increment of microvilli. In the tertiary stages, the late tertiary stages in particular, direct association of oocyte surface with granulosa surface is lost. The total oocyte surface becomes related structurally only to the zona pellucida matrix. This is due to specific concurrent changes: 1) the progressive loss and the disappearance of the oocyte-granulosa desmosomal junctions, and 2) the progressive structural degradation and lysis of granulosa cell processes which arose during the primary and secondary follicle stages (Plate X, Figure 22; Plate XII, Figure 26; Plate XXVII, Figure 60; Plate XXVIII, Figure 61). At this time, there is no further increase in the volume or mass of the zona (Table 1). The production of zona material appears to be dependent on the closely associated granulosa cells. The absence of further zona growth is coincident with an apocrine type of destruction of the granulosa cell processes. The granulosa cells, without the zona pellucida penetrating processes, now present a surface to the zona pellucida, which is only 25% greater than that of the oocyte surface without microvilli (Table 1). This is a permissible comparison, in that oocyte microvilli apparently disappear during tertiary stages later than those visualized in this study. The original ratio approaching a 1:1 relationship between the oocyte and granulosa surfaces in the

primordial follicle stage is again approached in the latest tertiary stages, when the ratio is of the order of 1:1.3 (Table 1). It seems significant that this approach to a 1:1 ratio should characterize the oocyte-granulosa cell relationship in both the primordial and late tertiary follicle stages. Both of these stages represent stable and essentially static growth phases. In the primordial follicle, oocyte growth has not begun, and in the late tertiary follicle, oocyte growth has ceased. Collectively, the changing quantitative and qualitative structural surface interrelationships during follicle development indicate the existence of essential and specific quantitative surface interrelationships between the oocyte and granulosa, by which the total growth, synthesis, differentiation and physiologic maturation processes of the follicle are regulated.

Functional Trends Indicated by Ultrastructural Changes in the oocyte, Granulosa and Zona Pellucida during Normal Follicular Growth

The detailed ultrastructure of the mitochondria, Golgi, endoplasmic reticulum and other structural features of the oocyte and granulosa cells during normal follicular growth in the bat, are in the main in accord with the observations on isolated follicle stages described for other mammalian forms (Anderson and Beams, 1959, 1960; Bjorkman, 1962; Franchi and Mandl, 1962; Hope et al., 1963; Hope, 1965; Odor, 1965; Zamboni et al., 1966; Weakley, 1967; Baca and Zamboni,

1967; Priedkalns and Weber, 1969; Norrevang, 1968; Odor and Blandau, 1969). The present study has demonstrated, in addition, that these basic protoplasmic components, as they occur in the oocyte and in the granulosa cells, show different spectra of structural transformations and trends during the course of normal follicle growth and maturation.

Ultrastructural Trends in the Oocyte

The quantitative increase in oocyte mass during development into the late tertiary stage, is paralleled by a progressive (unmeasured here) increase in mitochondrial mass (Plate I, Figure 1; Plate III, Figure 4; Plate X, Figure 22; Plate XXVIII, Figure 60). The intrinsic structural transformations shown by the mitochondria relate to a transient role of yolk condensation and storage within these organelles. The mitochondria function as yolk platelets (Plate XI, Figure 25; Plate XIV, Figure 31; Plate XXIV, Figures 64, 65, 66, 67, 68, 69; Plate XXX, Figures 70, 71, 72) during oocyte maturation and growth (Van der Stricht, 1906; Schwarz et al., 1960; Lanzavecchia, 1961; Ward, 1962; Balinsky and Devis, 1963; Appley, 1971).

An enzyme, protein phosphokinase, responsible for the condensation of soluble, partially phosphorylated yolk phosphoprotein (phosvitin) into insoluble, fully phosphorylated yolk phosphoprotein, has been found within mitochondria (Wallace, 1964). This involves ATP as the phosphate donor being changed to ADP. The reversibility of this enzyme

catalyzed reaction has been demonstrated (Rabinowitz and Lipmann, 1960). The relative ease with which the rephosphorylation of the ADP can proceed, indicates that yolk granules represent storage of high energy bonds which can subsequently be used for the anaerobic generation of ATP during early embryogenesis (Wallace, 1964).

During the cleavage stages, the mitochondria revert to their conventional, homeostatic, intrinsic ultrastructure (Lanzavecchia, 1960).

It seems indicated by these circumstances that some of the intrinsic metabolic activity of the oocyte during its development is utilized by the synthesis or formation of additional mitochondria which come to function as yolk protein storage depots and subsequently as normal metabolic energy source units (Wallace, 1964; Appley, 1971).

The general increase in oocyte mass is also paralleled by a progressive, unmeasured here, increase in the mass of its contained Golgi units (Plate VIII, Figures 15, 16; Plate X, Figure 22; Plate XI, Figure 25; Plate XIV, Figures 29-32; Plate SVII, Figure 60). These come to comprise an extensive system in which the Golgi units are spaced in an orderly fashion throughout the cytoplasm (Hope, 1956; Adams and Hertig, 1964). Near the oocyte surface, the Golgi units are spaced at regular intervals and topographically in close association with oocyte--granulosa cell desmosomal junctions (Plate X, Figure 22; Plate XIV, Figures 30, 31, 32; Plate

XVII, Figure 60)(Trujillo-Cenoz and Sotelo, 1958; Sotelo and Porter, 1959; Anderson and Beams, 1960; Baca and Zamboni, 1967; Glass and Cons, 1968).

While the total functional role of the Golgi complex is not established, it is generally agreed that it functions as a "segregation apparatus" in the absorption, isolation, concentration and separation of numerous chemical substances including secretion products, enzymes, salts and water (Nassanov, 1923; Richter, 1940, 1942, 1955; Dalton, 1961; Adams and Hertig, 1964; Neutra and Leblond, 1966). It therefore appears, that the remarkable synthesis of additional Golgi units during oocyte growth must be related to the increased functional levels of absorption and segregation of the growing oocyte.

The precise functional significance of desmosomal junctions is not clearly defined, but it is generally presumed that they are sites where an orderly and regulated transfer of substances is effected between the cells involved. The oocyte and the granulosa cells are fundamentally unlike cells. The oocyte-granulosa cell desmosomal junctions are of a transient occurrence and are present, and increased in number, only during the periods of pronounced oocyte growth. They disappear at the time oocyte growth ceases. They also occur quite uniquely in close association with oocyte cortical Golgi units and it therefore seems indicated that they relate to an increased level of transfer of essential

substances to the growing oocyte for absorption and segregation by the Golgi complex.

Microvilli are generally considered to be structural devices for increasing the absorptive surface of cells. The oocyte microvilli are of a transient nature. They arise and increase in total mass in parallel with rapid oocyte growth, and wane with the cessation of this growth. It seems indicated that the total pattern of changes the microvilli undergo, relates to a regulated level of transfer of substances into the growing and physiologically maturing oocyte.

Collectively, the structural patterns characterizing the Golgi complex, the mitochondrial complex, the system of microvilli and the oocyte-granulosa desmosomal junctions of the oocyte during its development, all relate in different ways to the regulated absorption and storage of essential substances by the oocyte. This is also indicated in a somewhat negative way, by the characteristic paucity or absence of clearly recognizable endoplasmic reticulum components. These are represented largely as dispersed ribonuclear particles in oocytes of all stages (Okada and Waddington, 1959; Wishnitzer, 1960; Anderson, 1964; Adams and Hertig, 1964; Wartenberg, 1964; Hadek, 1965; Zamboni and Mastroianni, 1966; Norrevang, 1968). It is well established that the mass of endoplasmic reticulum present in cells varies directly with the level of synthesizing activity. Therefore the growing oocyte is not to be characterized as an actively synthesizing

cell. This does not exclude the synthetic activities involved in the elaboration of essential protoplasmic organelles and other fundamental protoplasmic substances the oocyte must produce.

The functioning of the endoplasmic reticulum is dependent on RNA released from the nucleus. Nuclear pores and the trilaminar structuring of the nuclear membrane are involved in the RNA transfer (De Robertis et al., 1970). As shown here, nuclear pores and the trilaminar structuring of the nuclear membrane occur only in the oocytes of the very youngest primordial follicles and are absent by the end of the primary follicle stage (Plate I, Figure 1; Plate X, Figure 22). At this time the nuclear membrane is completely converted into a characteristic single membrane structure having a thickness approximately twice that of the earlier trilaminar membrane. This persists as such through the late tertiary stage (Plate X, Figure 22; Plate XXVII, Figure 60; Plate XXVIII, Figure 61).

The nuclear membrane transformation occurs and persists concurrently with the lack of defined endoplasmic reticulum components during the primary through tertiary follicle growth stages. It would thus appear that both the nuclear RNA transfer and the endoplasmic reticulum synthesizing activity of the oocyte are minimal during the principal growth stages. Oocyte growth and maturation is largely a complex storage process in which essential oocyte material is

acquired by absorption and stored in an orderly and regulated manner. Active oocyte synthesis relates only to the formation, growth and modification of intrinsic protoplasmic structural components which are quantitatively involved in different ways in the absorptive and storage process.

Ultrastructural Trends in the Granulosa Cells

The quantitative and structural data presented here indicate that granulosa development, in marked contrast to the oocyte, is characterized by multiple processes involving a high level of metabolic synthesis.

1. The most prominent feature of granulosa development unlike that of the oocyte, is the production of large numbers of cells. Quantitative data presented indicate that a disproportionately high level of synthetic activity is directed toward the total replication of daughter granulosa cells (Table 1) (Plate XVIII, Figure 42; Plate XXVII, Figure 60; Plate XXVIII, Figures 61, 62, 63).

2. In addition some synthesizing activity is directed toward the repair of granulosa cells adjacent to the oocyte which contribute a portion of their protoplasmic mass to the definitive pellucid membrane (Plate IX, Figure 20; Plate X, Figure 22; Plate XV, Figure 33). While the precise mechanism of origin of zona pellucida matrix is unsettled, the data presented here are consistent with reports that it is partly formed by an apocrine type of secretory process which

involves some loss of granulosa cell protoplasm and a repairing of the remaining cell portion.

3. Antrum formation, as demonstrated here and reported by others (Janosik, 1887; Alexenko, 1891; Van der Stricht, 1911; Mjassojedoff, 1923) involves the formation and release of pre-antral substance by ordered apocrine and holocrine types of secretory activity. In the apocrine process, synthetic activity is directed toward the repair of the participating granulosa cells and the production of pre-antral material. In the holocrine process the entire protoplasmic mass of individual granulosa cells degenerates and lyses and is contributed to the definitive antral fluid (Plate XVIII, Figure 43; Plate XXVII, Figure 60; Plate XXVIII, Figures 61, 62, 63; Plate XXXI, Figures 73, 74; Plate XXXII, Figures 75, 76, 77; Plate XXXIII, Figures 78, 79; Table XXXIV, Figures 80, 81, 82, 83, 84, 85, 86) (Mjassojedoff, 1923). These cells, as indicated by volume changes in the granulosa during the tertiary follicle stages, are replaced by the replication of additional granulosa cells through fundamental cell growth synthesizing processes (Table 1).

4. The granulosa cells during follicular development show progressive accumulations of glycogen (Wimsatt and Kallen, 1957) (Plate XII, Figures 26, 27; Plate XIII, Figure 28; Plate XIX, Figures 44, 45; Plate XX, Figures 46, 47; Plate XXI, Figures 48, 49; Plate XXII, Figure 50; Plate XXIII, Figure 51). While the precise functional significance of

glycogen formation and storage in these cells is speculative (Wimsatt and Kallen, 1957, Adams et al., 1966; Revel et al., 1960; Revel, 1964; Le Beux, 1969) it reflects a high level of synthesizing activity.

5. Antrum formation involves the destruction of whole granulosa cells by an holocrine type process (Plate XXXII, Figure 75; Plate XXXIII, Figure 79; Plate XXXIV, Figure 86). Aside from this exception, the detailed ultrastructural features and trends shown by the mitochondrial, Golgi and endoplasmic reticulum components of the granulosa cells during follicular growth and differentiation, are those which characterize cell types actively engaged in synthesis (Richter and Schilling, 1969).

The synthetic activities as demonstrated here and reported by others (Odeblad and Bostrom, 1953; Miller and Linnertz-Niklas, 1960; Bjorkman, 1962) involve: a) the maintenance of a complement of conventionally structured mitochondria, as well as an increase in mitochondrial size and number during follicular development (Plate I, Figure 1; Plate III, Figure 4; Plate IX, Figures 19, 20; Plate X, Figure 22; Plate XII, Figure 26; Plate XIII, Figure 28; Plate XIX, Figures 44, 45; Plate XXVII, Figure 60; Plate XXVIII, Figures 61, 62, 63). b) the maintenance of a complement of conventionally structured Golgi units with some increase in size and changes in intrinsic vesiculation (Plate II, Figures 2, 3; Plate IX, Figures 20, 21; Plate XII, Figure 26; Plate

XV, Figure 34, Plate XVIII, Figure 43; Plate XXIII, Figure 51). c) the maintenance of an extensive complement of endoplasmic reticulum. This, depending on specific physiologic states or specific cellular activities, consists either of conventionally structured rough-membrane systems or of mixed type with dispersed free ribosomal particles. Both types may be increased in amount during follicle development (Plate IX, Figures, 19, 20, 21; Plate X, Figure 22; Plate XII, Figure 26; Plate XVIII, Figure 43).

The major activities of the granulosa cells, as shown by the structural development and differentiation of the granulosa and pellucid membrane, and by the ultrastructural features and changes of the mitochondria, Golgi and endoplasmic reticulum during follicle growth, indicate metabolic processes directed toward a high level of synthesis.

This is in sharp contrast to the oocyte where the principal structural transformations during follicle development are expressions of metabolic activity directed toward high levels of absorption and storage and relatively low levels of synthesis. These contrasting functional activities, suggest that the oocyte is functioning as a special cellular repository for substances screened, modified or possibly synthesized by granulosa cells. The stored material is essential to subsequent aspects of fertilization and development.

The oocyte nuclear membrane undergoes a transformation which appears to be a mechanism for the temporary

isolation of oocyte nuclear material by the minimization or differential regulation of nucleo-cytoplasmic exchanges. This confirms the role of the oocyte as a storage phase, since cells involved in normal or high levels of synthetic activity, require nuclear pores and the trilaminar nuclear structure for the maintenance of necessary nucleocytoplasmic exchange rates.

Follicular Atresia

It has not been possible, in the course of this study, to completely characterize all structural aspects of the atretic transformation of follicles of all stages of development.

From the considerations of the morphological data recorded here, of normal follicular growth patterns and the deviations from these normal repetitive growth patterns, it appears that the character and complexity of the atretic process is directly proportional to the structural and functional complexity attained by the follicle at the onset of the atretic process. The proposal of the existence of two types of atretic processes is clearly an expression of this general relationship (Guthrie and Jeffers, 1938, Kingsbury, 1939, Knigge and Leathem, 1956). This approach is probably too limited in that it lumps atretic transformations into a) one group affecting the least structurally and functionally complex stages of follicular development, the pre-follicular and the primordial, and into b) a second group with progressively

increasing structurally and functionally complex stages of follicular development, the primary through the mature follicles.

The atretic process, as it affects follicles of all stages of development, is not characterized by any one specific alteration or histochemical feature as has been suggested (Deane, 1952, Arvy, 1960; Lobel et al., 1961; McKay et al., 1961; Adams et al., 1962, Guraya, 1964).

As shown here (Chapter III), the atretic transformation comprises a specific combination of structural alterations which are characteristic for each stage of follicular development. However, the atretic process is characterized by a common group of general features in and toward which there is convergence, at all follicular stages, of all specific structural deviations from the pattern of normal follicular development.

1. The atretic process is characterized by the involvement of three cell systems, namely: the oocyte; the pre-granulosa and granulosa; the ovarian fibro-vascular system and the theca. This is clearly expressed by the youngest affected follicles (Plate V, Figures 10, 11; Plate VI, Figures 12, 13; Plate VII, Figure 14) and all other ages of follicles in extreme or near terminal phases of atretic transformation in which the future follicle is reduced to a fibrocollagenous scar structure (Plate XXV, Figure 56, 57; Plate XXXVI, Figure 89; Plate XXXVIII, Figure 95; Plate XXXIX, Figure 96; Plate XL, Figure 97).

2. The atretic process is characterized by alterations expressed in multiple ways in the topographical interrelationships of the participating cell systems. This is shown in the youngest through the oldest follicles (Plate V, Figure 10, 11; Plate VI, Figures 12, 13; Plate VII, Figure 14; Plate XXIV, Figures 52, 53, 54; Plate XXV, Figures 56, 57; Plate XXVI, Figures 59; Plate XXXV, Figures 87, 88; Plate XXXVI, Figure 89; Plate XXXVIII, Figures 94, 95).

3. The atretic process is characterized by the progressive loss of cellular identity or individuality in the participating cell systems. This is expressed in multiple ways which vary in structural detail with each cell system and with the follicular developmental age.

In the oocyte, the atretic process is expressed as a loss of a) normal cell contour (Plate V, Figures 10, 11; Plate VI, Figures 12, 13; Plate VII, Figure 14), b) plasma membrane, c) microvilli, and d) the entire cell in a terminal process of destruction (Plate VI, Figure 13; Plate XVI, Figures 38, 39; Plate XVII, Figures 40, 41) (Mjassojedoff, 1923; Guthrie and Jeffers, 1938; Hisaw, 1947; Knigge and Leathem, 1956; Richter, 1958; Guraya, 1967).

In the granulosa cells, the atretic process is expressed as a loss of normal cell contours and discrete plasma membranes (Plate V, Figures, 10, 11; Plate VI, Figures 12, 13). A fusion of contiguous granulosa cell membranes occurs (Plate V, Figures 10, 11; Plate VII, Figure 14), as well as a

fusion with the oocyte plasma membrane (Plate V, Figures 10, 11; Plate VI, Figure 13, lower left arrow) and a blending of granulosa cell and oocyte cytoplasm (Plate VI, Figure 13). A broad spectrum of internal retrograde changes involving a total structural disordering of cell substance (Plate XXIV, Figures 52, 54, 55; Plate XXV, Figure 57; Plate XXVI, Figure 59; Plate XXXIV, Figures 80, 81, 82, 83, 84, 85, 86; Plate XXXV, Figures 87, 88; Plate XXXVI, Figure 89) is another important characteristic of the atretic process. In the granulosa cell, as well as in the oocyte, there is a terminal destruction and loss of individual and total granulosa population of cells (Plate XXXV, Figures 87, 88; Plate XXXVI, Figure 89; Plate XL, Figure 97) (Mjassojedoff, 1923; Guthrie and Jeffers, 1938, Kingsbury, 1939, Wimsatt, 1944, Knigge and Leathem, 1956).

In regard to the fibroblasts of the theca, loss of cellular identity occurs. The fate of the thecal cells has not been clearly established. In the follicular scar forming phase of the atretic process, loss of cellular identity is expressed by apocrine and holocrine transformations of the fibroblasts in active fibrillogenesis (Richter and Schilling, 1969; Richter et al., 1964; Porter and Pappas, 1959; Stearns, 1940). Transformation into other cell types has also been reported (Guraya, 1967).

4. The atretic process is characterized by a progressive deceleration of intrinsic metabolic activities

related to the maintenance of the structural and functional integrity of the cell systems. This is expressed most clearly by follicles in terminal phases of the atretic transformation. In the case of primordial follicles, the oocyte is reduced to traces of its former substance (Plate VI, Figure 13). In older follicles, the oocyte and granulosa cells are reduced to traces or are not present at all (Plate XXV, Figures 56, 57; Plate XXXV, Figures 87, 88; Plate XXXVI, Figure 89, Plate XL, Figure 97). In earlier phases of the atretic transformation, the deceleration of basic metabolic activity is expressed by specific deviations in the mitochondrial, Golgi, endoplasmic reticulum and nuclear components bearing on their intrinsic structuring, disposition and abundance relative to that of their counterpart in the cell system of normal follicles (Plate XVI, Figures 38, 39; Plate XVII, Figures 40, 41; Plate XXIV, Figures 52, 53, 54, 55; Plate XXV, Figures 56, 57; compare on Plate XXVI, Figures 58 and 59; Plate XXXIV, Figures 80, 81, 82, 83, 84, 85, 86; Plate XXXV, Figure 87, 88; Plate XXXVI, Figure 89) (Smuckler, 1964; Trump and Ericsson, 1965; Ericsson, et al., 1965; Kohn, 1965; Trump and Bulger, 1967).

5. The atretic process is characterized by alterations in specific cellular activities or roles played by the oocyte and granulosa cells, differing from those during normal follicular development. a) The quantitative and structural data recorded here indicate that the characteristic

functional role of the oocyte is one of absorption and storage. This activity is dependent on the progressive synthesis of mitochondrial and Golgi elements, and the development of an absorptive system of microvilli. This activity appears to be interrupted in the atretic transformation and this is indicated by several circumstances: Oocytes, or their remains, are smaller in size than their normal counterparts (Plate VI, Figures 12, 13; Plate XVII, Figures 40, 41) (Guthrie and Jeffers, 1938, Knigge and Leathem, 1956). Yolk platelets are reduced in number or lacking. Cortical Golgi elements are dispersed as vacuolar units rather than stacks or are completely altered or lacking (Plate XVI, Figures 38, 39; Plate XVII, Figures 40, 41). Microvilli are of bizarre form, size and distribution or are completely lacking (Plate XVI, Figures 38, 39; Plate XVII, Figures 40, 41) (Steiner and Carruthers, 1962). b) The functional role of the granulosa, in normal development appears to be one of "physiologically screening" or regulating of the transport of substances to the oocyte. This is in accord with the results of experimental studies by Wallace, et al. (1970) on protein uptake by isolated oocytes.

Dye segregation by the granulosa cells has been considered indicative of phagocytic or macrophagic capabilities (Richter, 1958). It is at the same time an expression of the general "physiologic screening activity" of these cells during normal follicular development and oocyte growth. It

has been suggested that the oocyte in the atretic transformation is phagocytically removed by granulosa cells (Mjassojedoff, 1923, Guthrie and Jeffers, 1938). This has not been demonstrated to occur, nor be effected by the conventional structurally characterized process of phagocytosis.

The progressive orderly fusion and blending of normally structured granulosa and oocyte cell substances demonstrated here in primordial and primary follicles (Plate V, Figures 10, 11; Plate VI, Figures 12, 13) is considered to be a special, ultrastructurally characterized type of a phagocytic or resorption process by which the oocyte is removed by the granulosa cells. It clearly is an expression of the point that the atretic process is characterized by alterations in the functional activity of the oocyte and granulosa cells. For the granulosa, this is a change from the 'physiologic screening and oocyte directed transport system' to a 'system of resorption of oocyte substance.' For the oocyte, it is a change from an "absorption, storage and growth system' to a system of 'reserve substances available to the granulosa for resorption.' Studies on the resorptive process in follicles older than the primordial and primary stages have not been made. It is presumed that for them the process would be basically similar but modified by the degree of differentiation and development of the intervening membrana pellucida, which has been attained prior to the onset of the atretic transformation.

Antrum formation in the tertiary follicle stage, involves initially an apocrine activity of the granulosa cells and finally a destructive holocrine type secretion (Plate XVIII, Figure 43; Plate XXVIII, Figures 61, 62, 63; Plate XXXI, Figures 73, 74; Plate XXXII, Figures 75, 76, 77; Plate XXXIII, Figures 78, 79). Alteration in the apocrine activity is expressed by the inability of the granulosa cells concerned to shed their intracellularly produced and cortically accumulated pre-antral substance. These are retained intracellularly as characteristic large vacuolar formations (Plate XXIV, Figures 52, 54, 55; Plate XXV, Figure 57; Plate XXVI, Figure 59) (Zollinger, 1948). These vacuolar formations are also due to the fusion of the plasma membranes of the granulosa cells during the atretic transformation which obliterates all intergranulosa cell spaces (compare on Plate XXVI the Figures 58 and 59).

A third type of normal granulosa cell activity altered by the atretic transformation, is that of glycogen formation and storage. As demonstrated here and reported by others (Wimsatt and Kallen, 1956), granulosa cells of the bat ovary, particularly in the rapidly growing phases of the secondary and tertiary follicles, are characterized by extensive accumulations of glycogen (Plate XII, Figures 26, 27; Plate XIII, Figure 28; Plate XIX, Figures 44, 45; Plate XX, Figures 46, 47; Plate XXI, Figures 48, 49; Plate XXII, Figure 50; Plate XXIII, Figure 51; Plate XXVI, Figure 58).

In granulosa cells of equivalent age, but undergoing atretic transformation, glycogen accumulations are uniformly lacking (compare Figures 58 and 59, on Plate XXVI). This has been determined by others from histochemical data dealing with injured or dying tissues (Caulfield and Klionsky, 1959).

6. The atretic process is characterized by a regression in the structural stability of the pellucid and basement membranes, and the ultimate fibrocollagenous scar.

In the basement membrane, the atretic process is expressed by the development of discontinuities or breaks (Plate V, Figures 12, 13; Plate XXXV, Figure 57; Plate XXXVI, Figure 89) and by the progressive aquisition of a corrugated appearance, showing a highly irregular outline (Plate XXV, Figure 57; Plate XXVI, Figure 59; Plate XXXV, Figure 87, Plate XXXV, Figure 89; Plate XXXVIII, Figures 93, 94, 95). In primordial aged follicles, the basement membrane may be completely lacking during the atretic transformation (Plate V, Figure 11). In older follicles in the terminal phases of atretic transformation, the normal smooth and close junction between the granulosa cells and the basement membrane is progressively converted into a broad, electron lucent interzone. This interzone is designated here as the supra-basement membrane space and is crossed by numerous irregular processes of the granulosa cells (Plate XXXVIII, Figure 95, Plate XXXVI, Figure 89). This zone together with highly folded remnants of the basement membrane persists until scar

formation is virtually complete and there are no remaining traces of either the oocyte or the pellucid membrane (Plate XXXIX, Figure 96; Plate XL, Figure 97). This complex, the residue of basement membrane and the supra-basement membrane interzone, appears to be what light microscopists have considered erroneously to be a remnant of the pellucid membrane. The so-called "glassy membrane" (Gm) is a collagenous layer which thickens soon after onset of the atretic transformation. It is formed between the theca and the basement membrane (Plate XXV, Figure 57; Plate XXXVI, Figure 89; Plate XXXVIII, Figure 95). It persists and enlarges during the atretic process and forms the main portion of the mature scar (Plate XXXIX, Figure 96; Plate XL, Figure 97). The scar is eventually absorbed, but the details of this process are unknown.

The data concerning the structural alteration of the zona pellucida during the atretic transformation are incomplete. In the primordial and early primary follicle, there is no recognizable zona pellucida. It is represented in these follicles by an oocyte-granulosa intercellular space, which may contain villi of different sizes (Plate IV, Figures 6, 7, 8, 9; Plate V, Figure 10, at arrow; Plate IX, Figure 19, at arrow). In secondary and early tertiary follicles the zona is normally characterized by matrical material and granulosa cell processes extend across it terminating in desmosomal junctions at the oocyte. In the late tertiary stage, the zona

is comprised of matrical material and no discernible remains of the granulosa cell processes. In the atretic secondary and tertiary follicles, the zona is altered by a loss of oocyte -granulosa cell junctions and a complete loss of granulosa cell processes (Plate XVII, Figure 40).

In summary, the atretic process is attended and characterized by specific structural alterations at all follicle ages, which together

1. involve the oocyte, the granulosa and thecal cellular elements of the follicle,

2. relate to changes in the spatial or steric interrelationship of the follicular cell systems,

3. relate to the loss of individuality of the oocyte, granulosa and thecal fibroblast cells, as structural and functional units,

4. relate to a deceleration of intrinsic metabolic activity requisite to the maintenance of structural and functional cellular and tissue integrity,

5. relate to alterations in specific activities of the oocyte, granulosa and thecal fibroblasts as functional units, and

6. relate to alterations in the structural stability of the pellucid membrane, basement membrane and the follicular scar.

CHAPTER V

SUMMARY

The processes of growth and atresia have been characterized by an objective analysis of the total range of variations in ultrastructure of the cells and tissue complexes of the primordial, primary, secondary and tertiary follicular developmental age groups. Emphasis was placed on ultrastructural aspects of the oocyte and granulosa components of the follicle, their interrelations with each other, with the zona pellucida and the follicular basement membrane.

General growth characteristics of the oocyte, the zona and the granulosa, considered as unit components, have been evaluated quantitatively in terms of their dimensionally defined space volumes and surface areas. The results revealed manifold increases and dynamic relative changes of the follicular components not realized by the conventional approach of using diametral increase.

Nuclear and cytoplasmic structural changes, recorded for the oocyte and the granulosa cells during development, clearly outline their contrasting functional roles. The role of the oocyte is expressed by the metabolic activities geared

toward high levels of absorption and storage, and low levels of synthesis. The oocyte functions as a special cellular repository for substances screened, modified and possibly synthesized by the granulosa cells. The granulosa cells of bat ovarian follicles, involved in transport and synthesizing activities, have the additional role of glycogen storage.

Normal growth emerges as a synchronized, repetitious occurrence of identical structural features and trends in the follicular components. It demonstrates the "principle of repetition" in the ordering of natural systems. Repetitious structural trends appear to be directed toward the maturation and maintenance of a functionally and structurally competent follicle.

Deviations from the synchronized repeat pattern can be considered to be abnormal in that they relate to, or are directed toward follicular destruction. The atretic structural deviations involve the oocyte, granulosa and thecal components of the follicle. The character and complexity of the atretic transformation is directly proportional to the structural and functional complexity attained by the follicle at the onset of the atretic process.

The atretic process, involving all components of the follicle, is marked by changes in steric relationships, loss of cellular individuality and a deceleration of intrinsic metabolic activities required to maintain structural and functional cell and tissue integrity. The roles of the

oocyte and granulosa, as functional units, are altered and lost, and the follicle is eventually replaced by a fibro-collagenous scar.

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APPENDIXES

APPENDIX A

TABLE 1

SPACE DEFINED VOLUMES (V) AND SURFACES (S) OF THE OOCYTE, ZONA PELLUCIDA, GRANULOSA AND FOLLICLE

Follicle Age Groups	Oocyte				Zona Pellucida	
	Minus Villi		Plus Villi			
	$V\text{-}u^3$	$S\text{-}u^2$	$V\text{-}u^3$	$S\text{-}u^2$	$V\text{-}u^3$	$S\text{-}u^{2*}$
Primordial	6,538	1,169	-------	------	-------	------
Primary	57,908	7,240	57,955	10,519	31,703	9,854
Secondary	157,486	14,104	158,272	39,688	63,379	17,673
Early Tertiary	173,119	14,229	173,952	47,902	183,716	24,355
Late Tertiary	383,704	25,530	383,831	30,293	192,253	33,449

*Surface on zona pellucida side.

**Surface on granulosa side.

***Surface on basement membrane side.

TABLE 1--Continued

Granulosa		Total Follicle	
$V-u^3$	$S-u^{2**}$	$V-u^3$	$S-u^{2***}$
4,000	3,054	15,901	3,054
133,248	18,151	222,859	18,151
877,276	51,476	1,098,141	51,476
8,428,134	205,946	8,784,969	205,946
11,798,377	258,741	12,374,294	258,741

TABLE 2

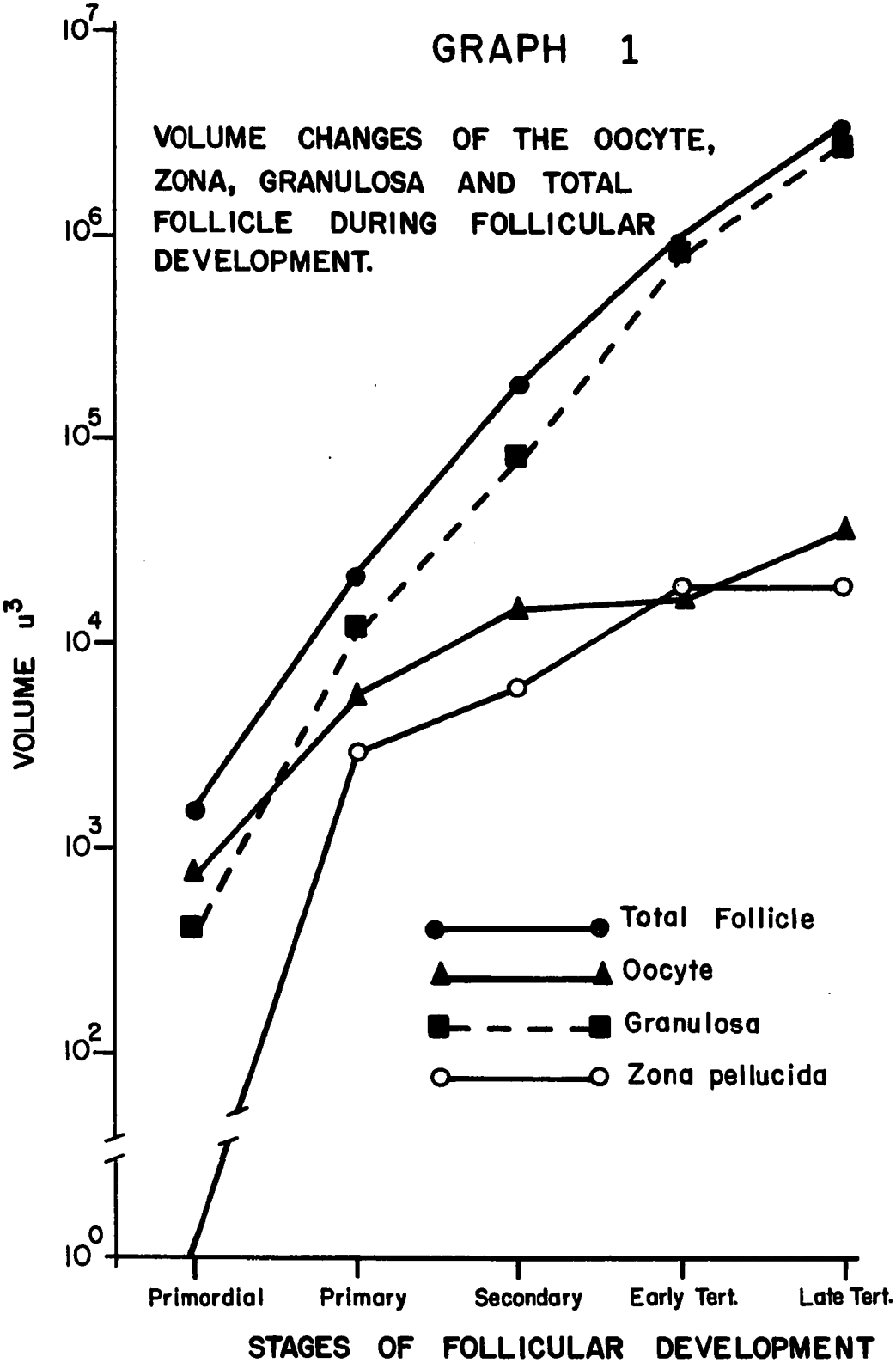
DIMENSIONS OF THE OOCYTE AND MICROVILLI

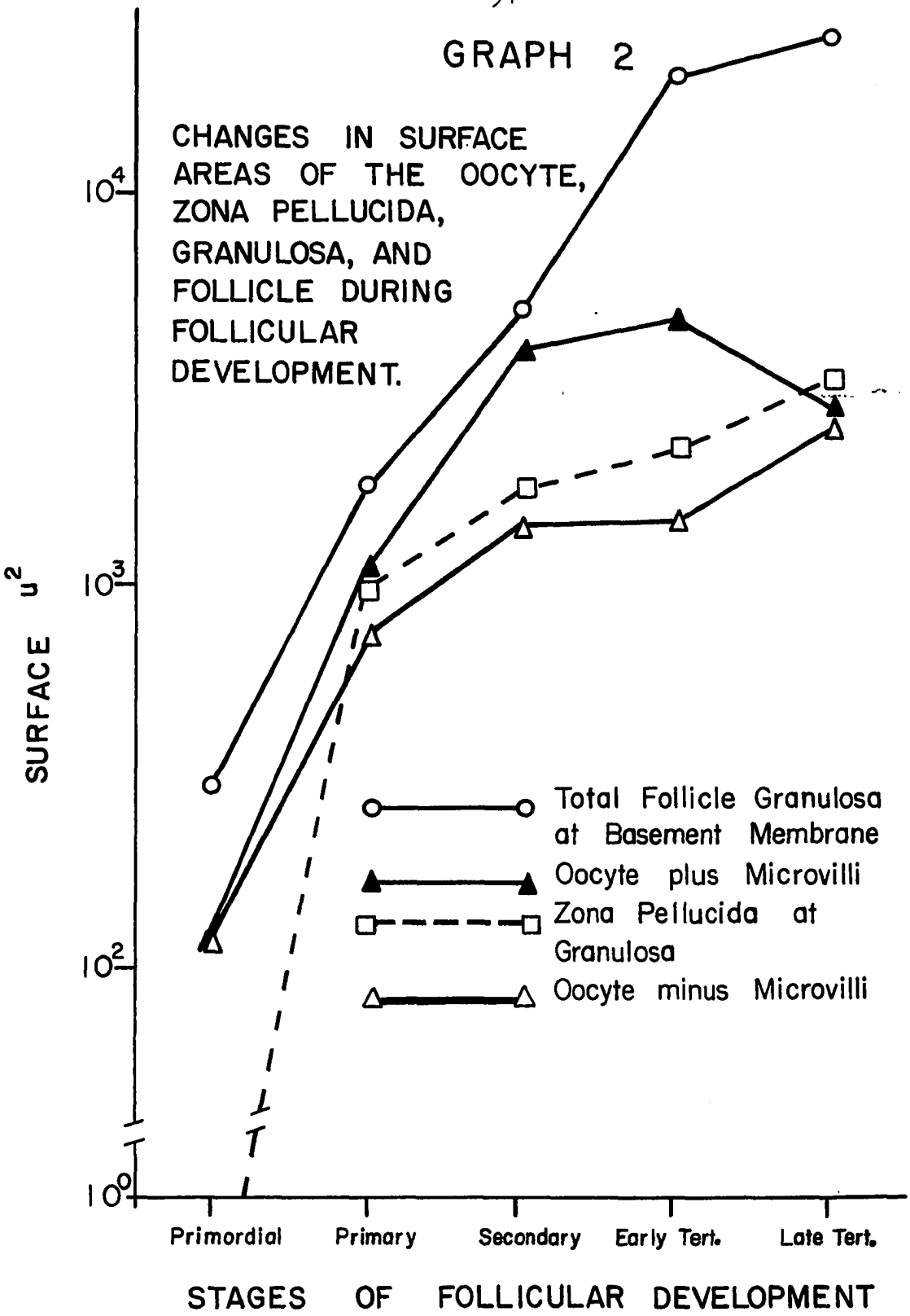
Follicle Age Group	Oocyte Diameters u	Number of Oocyte Villi	Dimensions of Individual Villi		Volume and Surface Measurements of Individual Villi		Volume and Surface Measurements of Total Villi	
			Diameter u	Length u	$V\text{-}u^3$	$S\text{-}u^2$	$V\text{-}u^3$	$S\text{-}u^2$
Primordial	23.2	-----	-------	-----	-------	------	--------	---------
Primary	48.0	46,544	0.06	0.370	0.001	0.07	47	3,279
Secondary	67.0	208,600	0.08	0.488	0.0024	0.123	786	25,584
Early Tertiary	73.0	177,228	0.10	0.600	0.0047	0.188	833	33,673
Late Tertiary	90.3	52,920	0.10	0.300	0.0029	0.09	127	4,763

APPENDIX B

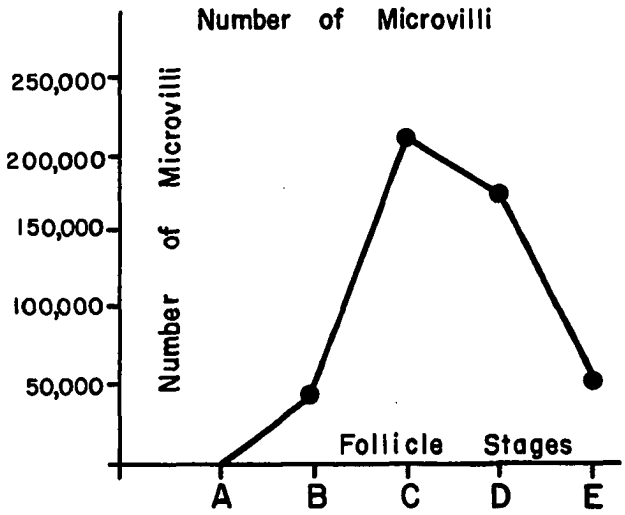
GRAPH 1

VOLUME CHANGES OF THE OOCYTE, ZONA, GRANULOSA AND TOTAL FOLLICLE DURING FOLLICULAR DEVELOPMENT.




GRAPH 2
CHANGES IN SURFACE AREAS OF THE OOCYTE, ZONA PELLUCIDA, GRANULOSA, AND FOLLICLE DURING FOLLICULAR DEVELOPMENT.
SURFACE u²
10⁴
10³
10²
10⁰
Total Follicle Granulosa at Basement Membrane
Oocyte plus Microvilli
Zona Pellucida at Granulosa
Oocyte minus Microvilli
Primordial
Primary
Secondary
Early Tert.
Late Tert.
STAGES OF FOLLICULAR DEVELOPMENT

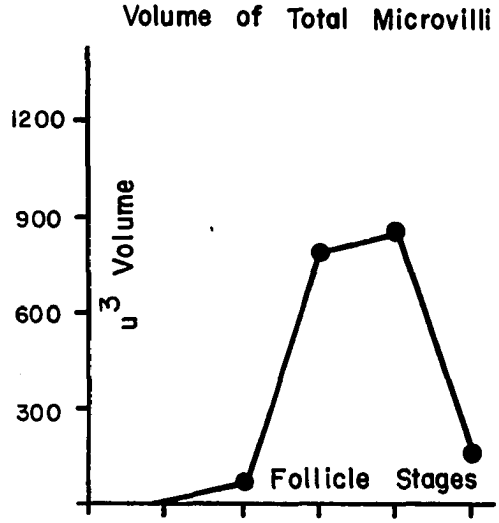
GRAPH 3

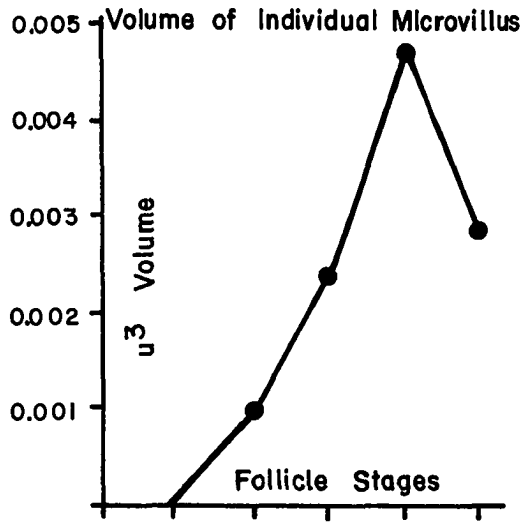


Follicle Stages

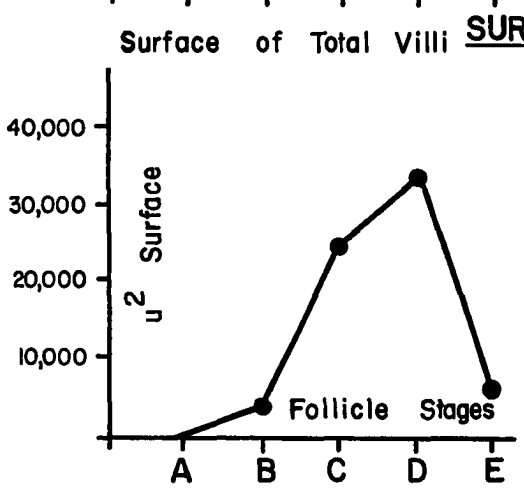
A - Primordial
B - Primary
C - Secondary
D - Early Tertiary
E - Late Tertiary

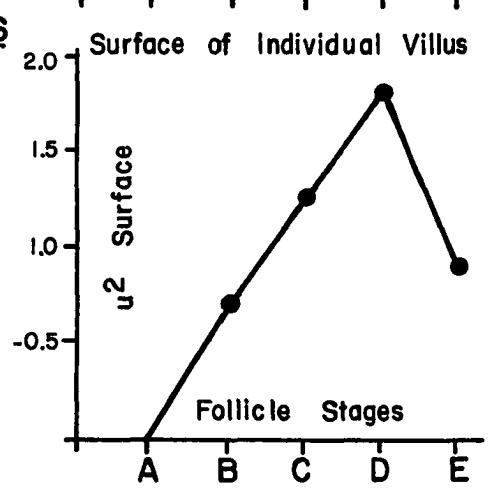
VOLUMES





SURFACES





APPENDIX C

List of Abbreviations

An	antrum
Bm	basement membrane
C	cilium
CC	corona radiata cells
Chr	chromosomes
Coll	collagenic layer (glassy membrane)
Cp	cellular processes
Ct	centriole
Cys	cytosegrosome
D	desmosome
DA	dense area at the base of the cilium
Cc	distal centriole
ER	endoplasmic reticulum
Es	extracellular space
F	filaments
Fb	fibroblast
FNB	field of nuclear pores
G	Golgi
GC	granulosa cells
GE	germinal epithelium

Gm	glassy membrane
Gv	Golgi vesicles
IC	interstitial cells
Ld	lipoid droplets
M	mitochondria
Mac	macrophages
Mv	microvilli
N	nucleus
O	oocyte, ovum
Oa	opaque areas, indicative of glycogen storage
Pc	proximal centriole
Pm	plasma membrane
Pr	procentriole
Pyk. N	pyknotic nucleus
Ri	ribosomes
S	pericentriolar satellite
Sh	sheath of cilium
TC	thecal cells
TA	tunica albuginea
V	villi
ZP	zona pellucida

PLATE I

Figure 1. A cortical area of a prehibernation bat ovary showing the major portion of a primordial follicle, consisting of an oocyte (O), oocyte nucleus (N), granulosa cells (GC) and thecal cells (TC) forming a thecal layer. A group of interstitial cells (IC) can be observed among the follicles.

Note: The arrow at the top of the page is pointing to a granulosa cell of an early primordial follicle which contains a pair of centrioles and a cilum arising from the distal member of the pair. An enlarged view of this area can be seen on Plate II, Figure 2.

5600X

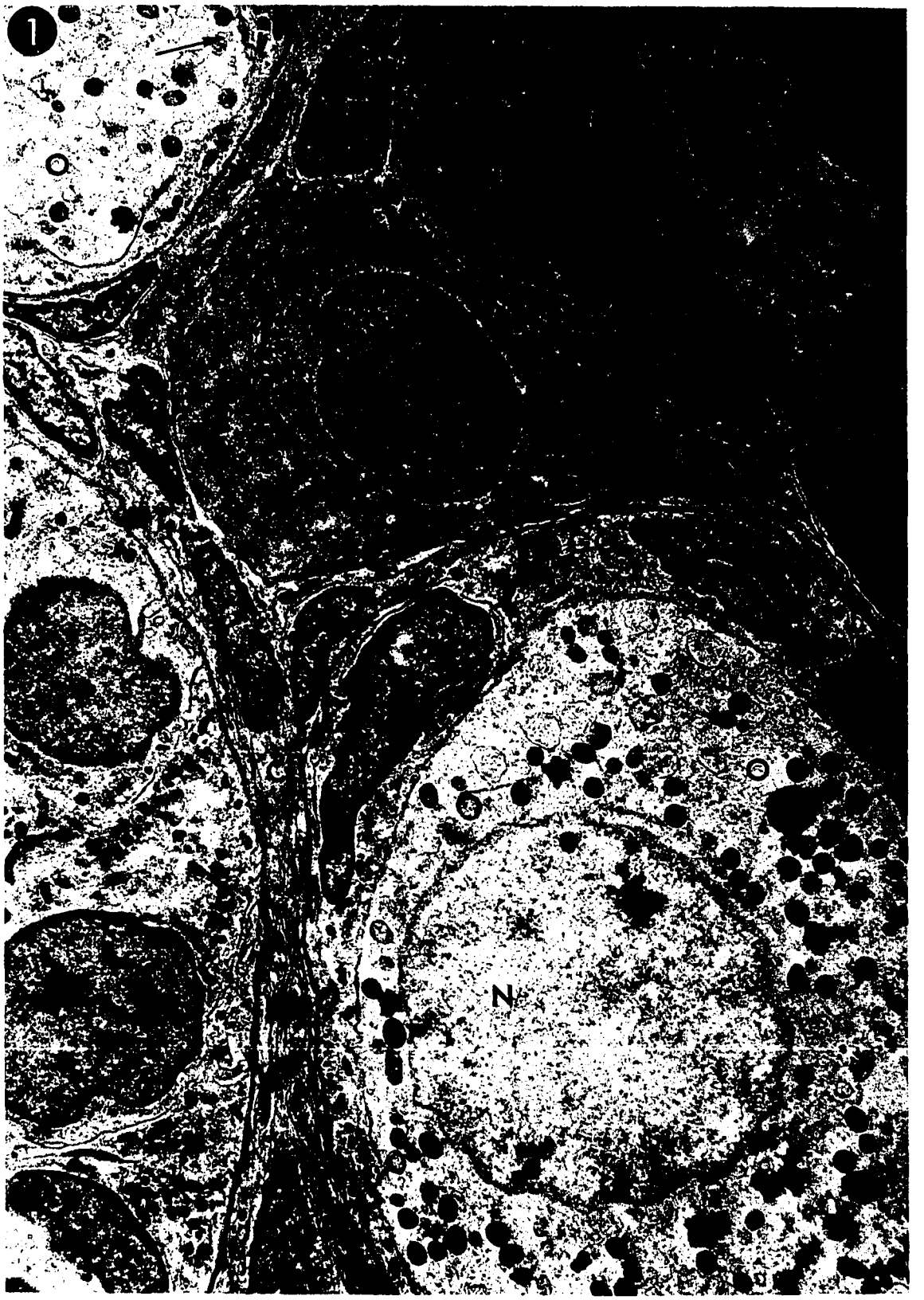

1
N

PLATE II

Figure 2. Details of the primordial follicle shown on Plate I, Figure 1. Sections of the oocyte (O), a ciliated granulosa cell and the basement membrane (Bm) measuring about 0.1 micron, can be observed. The opacity of the regions at the arrows, between the adjoining oocyte and granulosa cell, signifies the earliest stage of zona pellucida formation.

Note: The cilium (C) is attached to the distal centriole (Dc); the proximal centriole (Pc) is shown in cross-sectional view; the pericentriolar satellite (S) and the nearby Golgi (G) found in the centrosomal area of the cell.

Figure 3. Details of a primordial follicle showing portions of the ovum (O) and a granulosa cell containing an ensheathed cilum (C) in a cross-sectional view. There are two layers of plasma membrane (Pm) around the cilium, one closely aligned to the axoneme and the outer on representing a reflection of plasma membrane (arrows). Extracellular space (Es) is found between the two layers of plasma membrane.

Note: The central pair of filaments is lacking in the cilium which contains an atypical 9+0 filament pattern.

60,000X


2
S
Pc
Dc

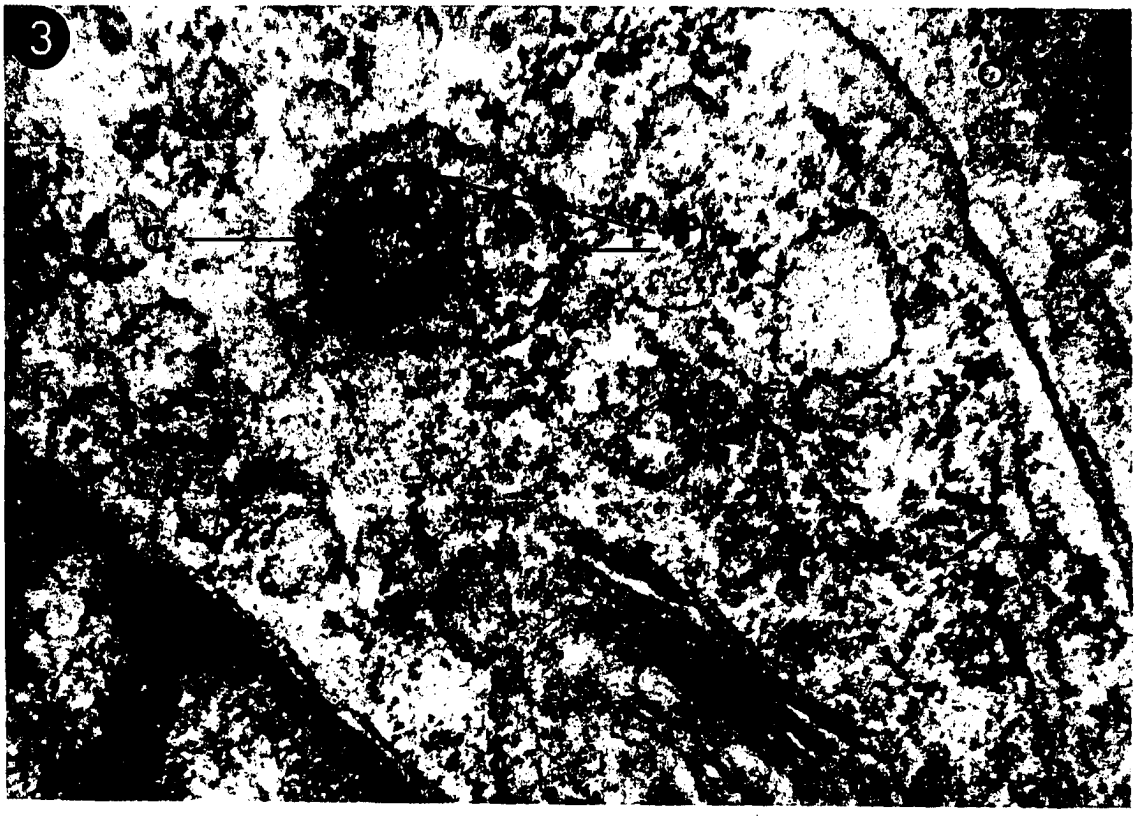

3

PLATE III

Figure 4. An overview of an early primary follicle containing an oocyte and one layer of cuboidal follicle cells. The cytoplasm of the ovum appears much more electron lucent than that of the granulosa cells (GC). The plasma membranes of the oocyte and follicle cells show desmosomal attachments. Aspects of zona pellucida formation, shown here, can be seen at higher magnifications on Plate IV.

5,300X

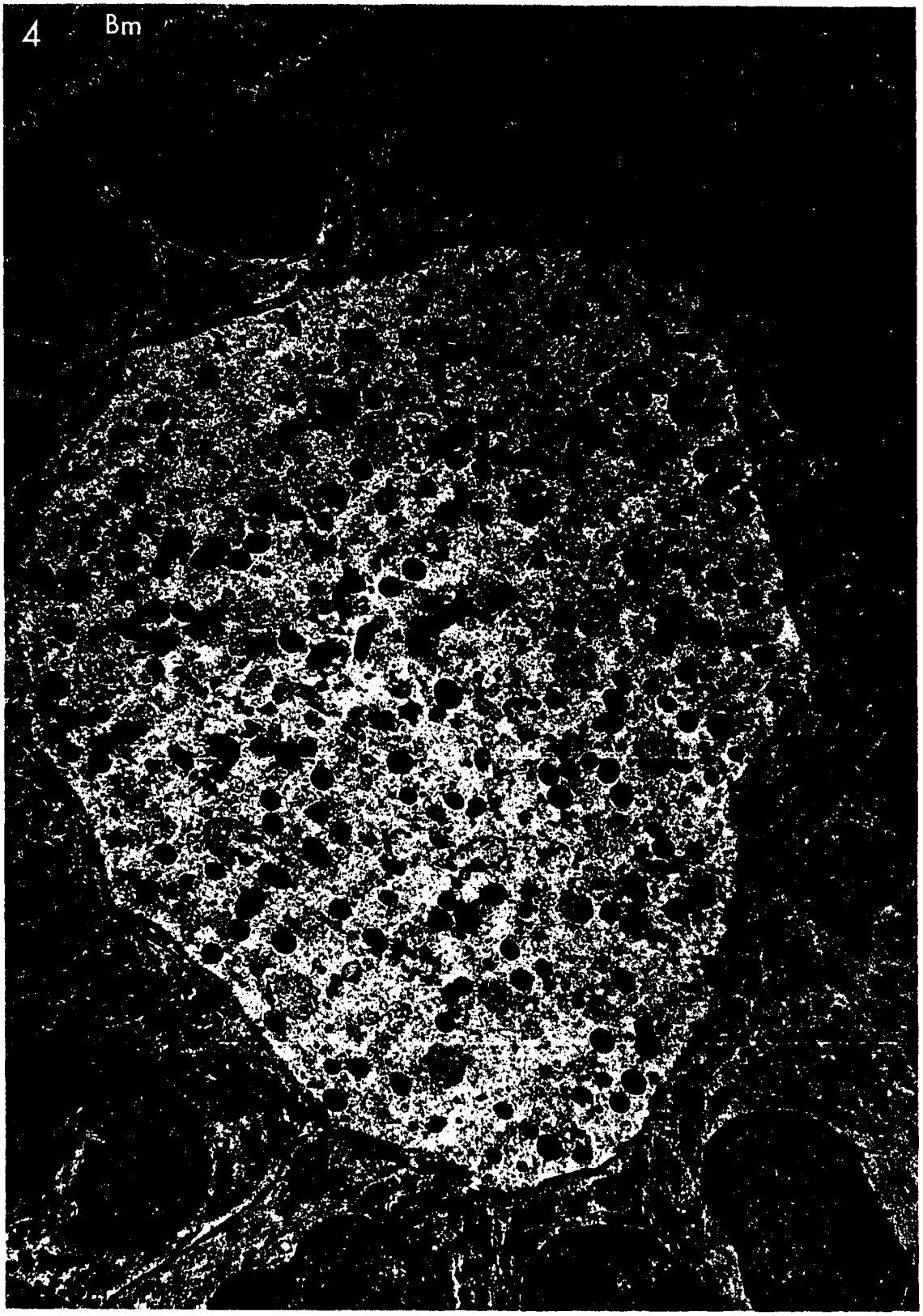

4
Bm

PLATE IV

Figures 5-9 represent details of the oocyte-granulosa interface from the primary follicle shown on Plate III, Figure 4. Pictured here, at higher magnifications, are the structural modifications of the plasma membranes and adjoining cortical cytoplasm of the oocyte and granulosa cells. These modifications also include projections, in the form of villi, into the intervening intercellular spaces which are formed. All these changes are related to zona pellucida formation.

Figure 5. Desmosomes, the simplest interaction, are shown in the adjacent plasma membranes of the oocyte (O) and the granulosa cells (GC), and between adjoining granulosa cells.

Figure 6. Desmosomes and microvilli (Mv) are shown between the oocyte and granulosa cells.

Figure 7. A region of microvilli containing uniformly dense material.

Figure 8. An area of closely aligned, long processes is shown. Note the intense desmosomal attachments.

Figure 9. Desmosomes and microvilli are shown closely associated with an area which contains accumulated deposits of opaque material.

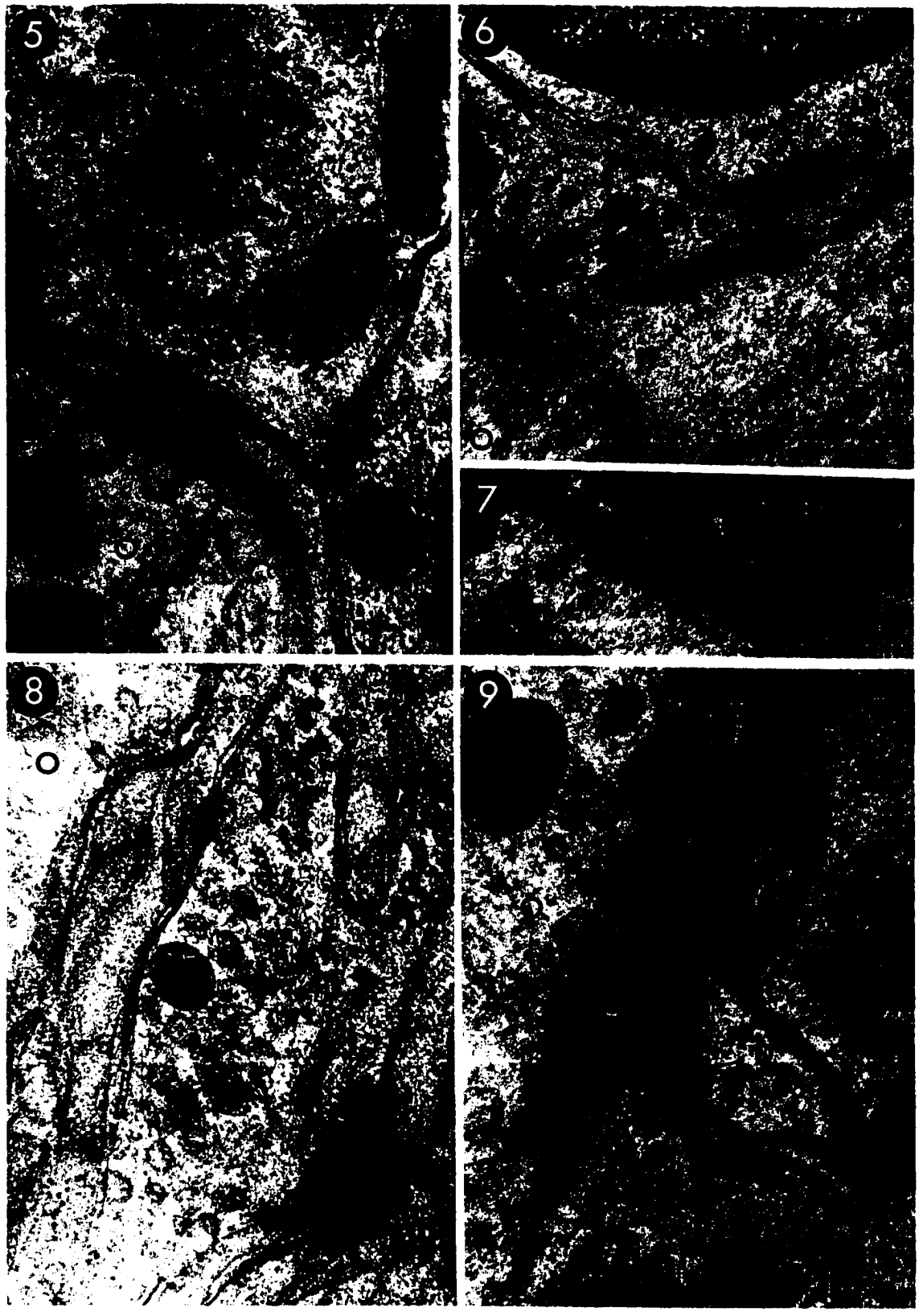

5
6
7
8
9

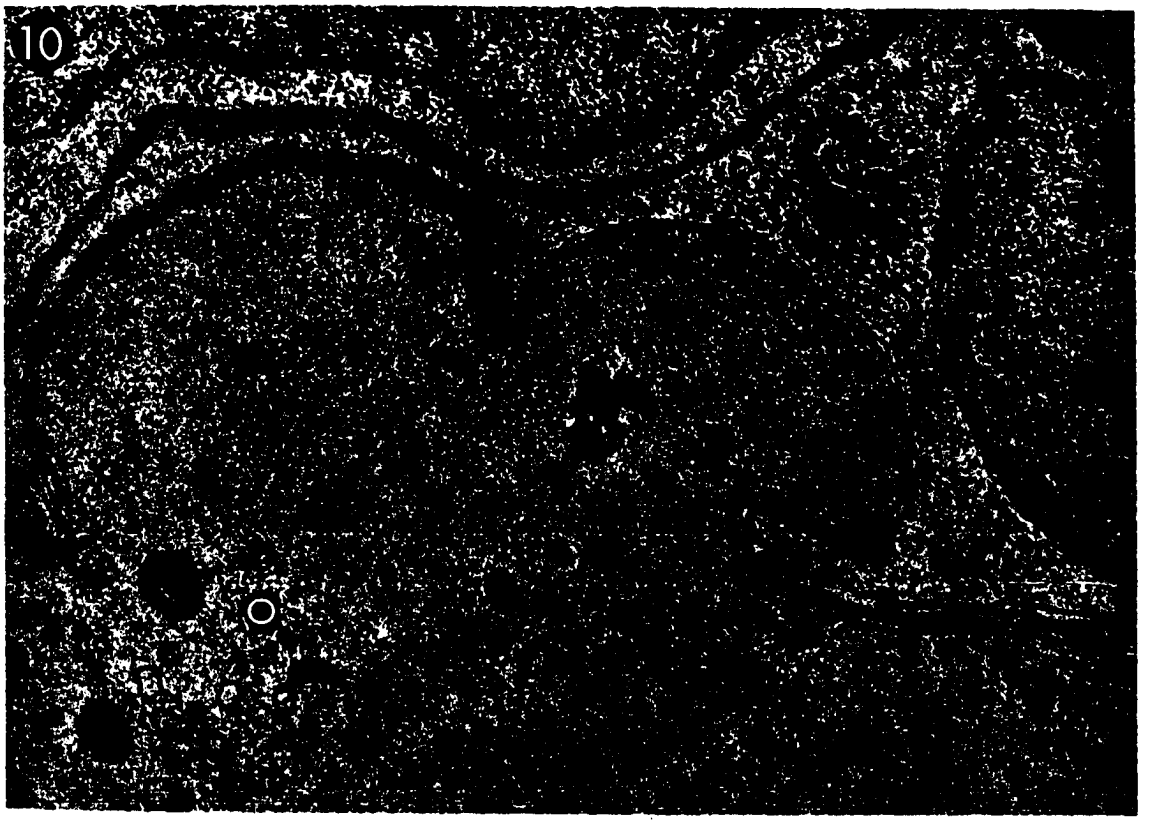
PLATE V

Figure 10. Distinct variations in the shape of the oocyte can be observed in primordial and primary follicles undergoing atretic transformations. Broad massive processes of oocyte cortical cytoplasm, sparsely seeded with organelles, and delimited by a plasma membrane are closely apposed to the plasma membranes of the granulosa cells. A fusion of the apposed plasma membranes of the oocyte processes and the granulosa cells can be seen. There is also fusion of the plasma membranes of adjacent granulosa cells.

Long and slender oocyte processes can be seen extending into an intercellular space formed between the oocyte and the granulosa cells (arrow).

Figure 11. The broad processes of the oocyte may be encircled to varying extents by the adjacent granulosa cells. In this figure, the processes are shown partially or totally enclosed. The total enclosing may be due to perpendicular planes of sectioning. The plasma membrane fusions mentioned above, can be observed here. Thecal cells (TC) surround this portion of the follicle.

14,300X


10
O


11
GC
O

PLATE VI

Figure 12. An early primary follicle undergoing atretic transformation. The oocyte cytoplasm is now partially distributed into processes encircled by granulosa cells (Arrows). The processes have reduced amounts of, or completely lack, organelles.

The above changes are superimposed on those normally occurring during zona pellucida formation (see Figures 5-9).

The follicle has become distorted and the granulosa cells are unevenly distributed (compare to Figure 4).

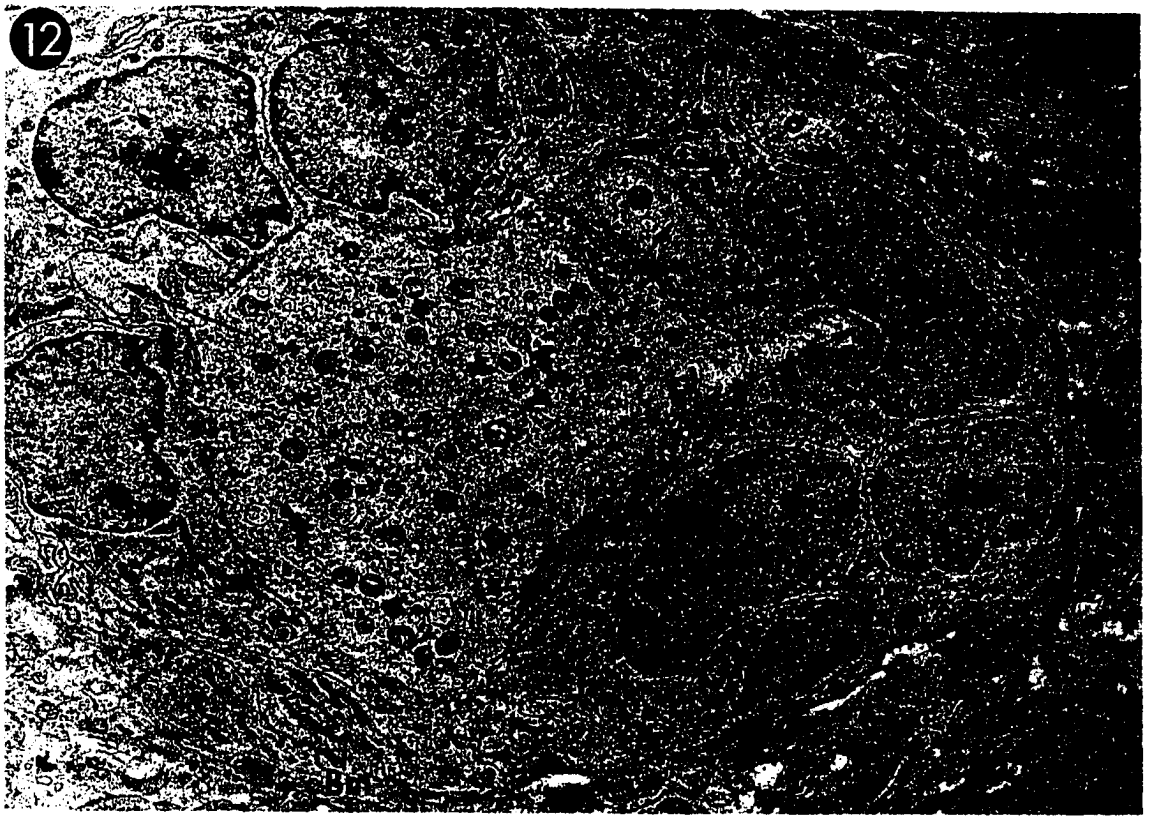
The central portion of the oocyte contains conventional cytoplasmic structures and organelles.

4,200X

Figure 13. The ovum of this primordial follicle during the atretic process, has completely lost its former morphology and is almost devoid of cytoplasmic structures and organelles. Remnants of the oocyte can be traced into long processes (Arrows) separated or continuous with the central portion. Membrane fusion of adjacent plasma membranes can be observed. In other areas one or both of the plasma membranes are lacking and oocyte cytoplasm is exposed to that of the granulosa cells.

The basement membrane is barely discernible.

7,500X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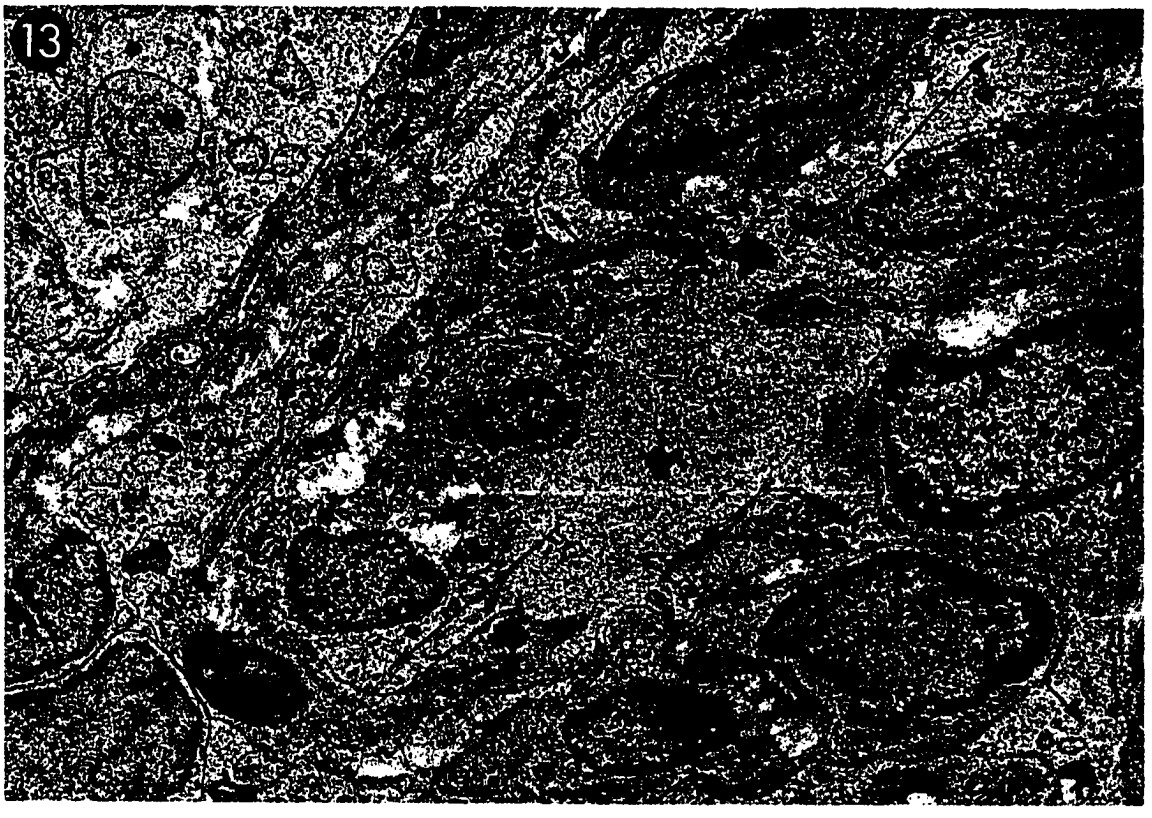

13

PLATE VII

Figure 14. A peripheral section of the bat ovary, showing the cells of the germinal epithelium (GE). Traces of microvilli (MV) can be seen on the free border of these epithelial cells. The germinal epithelium rests on a tortuous basement membrane (Bm). The subepithelial tunica albuginea (TA) is described in detail in the text. Interior to the tunica albuginea lies the cortical region of the ovary, containing the follicles described in Figures 12 and 13.

5,300X

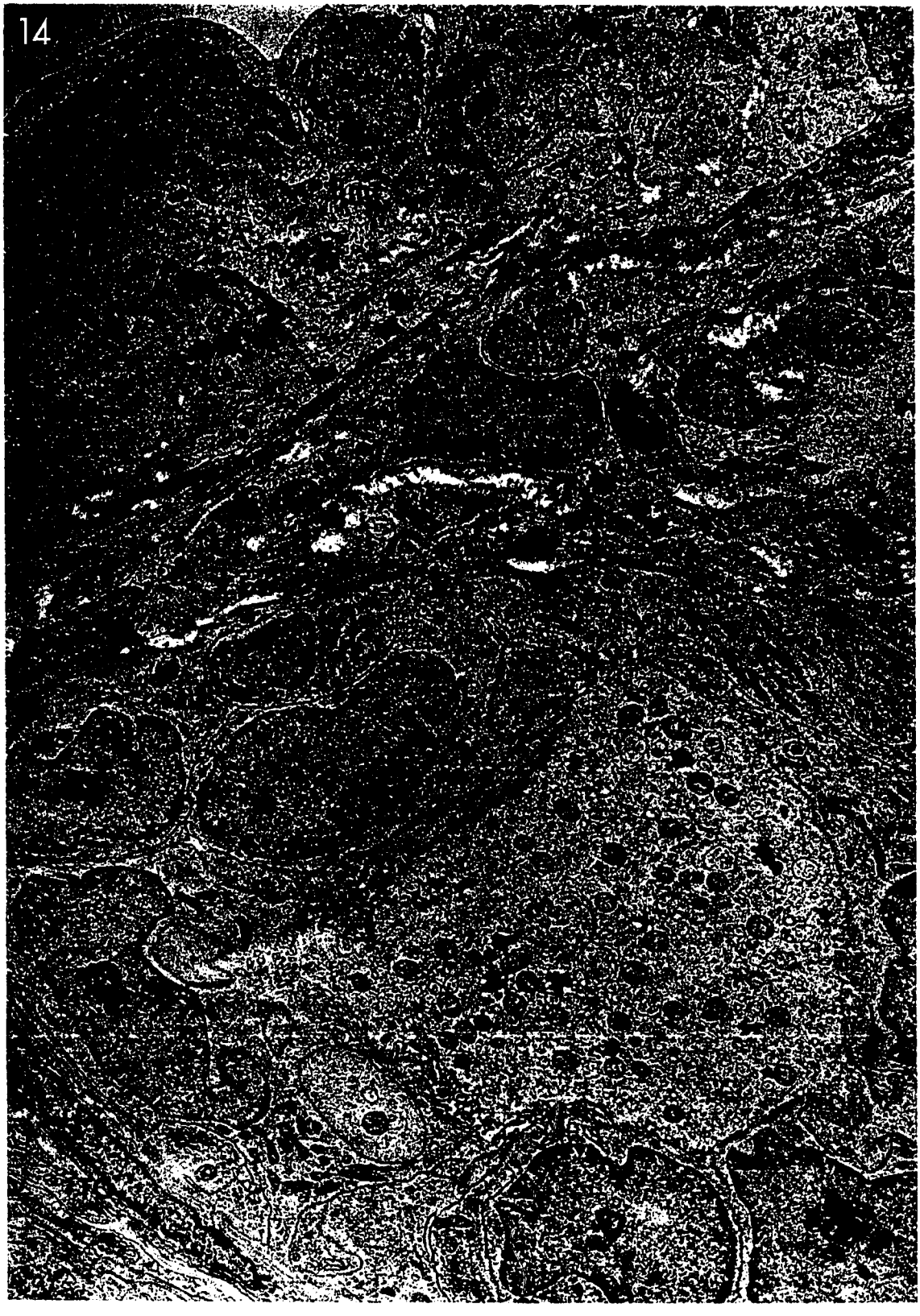

14

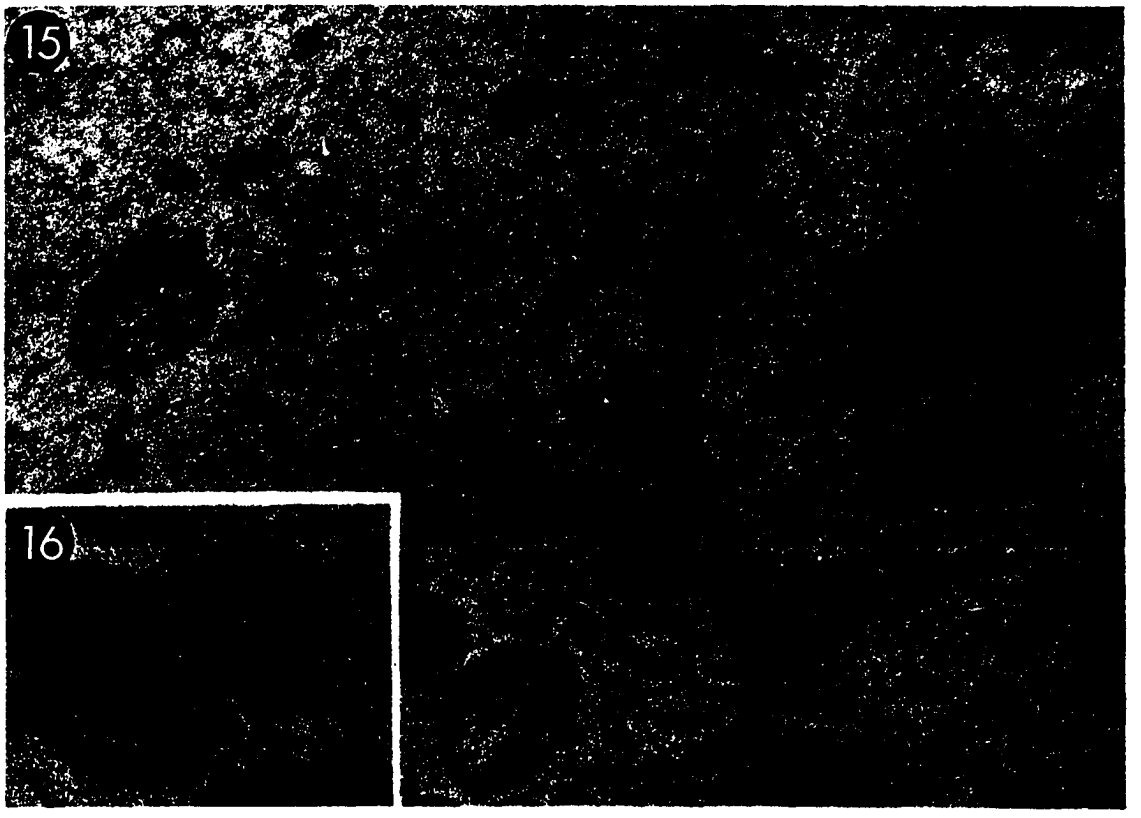
PLATE VIII

Figure 15. Cytoplasmic area from an oocyte of a primordial follicle. The mitochondria characteristically contain few cristae and are often closely associated with endoplasmic reticulum vesicles. The Golgi structures are well developed and distributed throughout the cytoplasm. Large and small endoplasmic reticulum vesicles are seen. Some are sparsely seeded with ribosomes on their cytoplasmic side (Arrows).
30,000X

Figure 16. Mitochondria enlarged to a magnification of 42,000X.

Figure 17. Cytoplasmic area of a primary follicle oocyte. The granulosa cells of the follicle are in the transition between cuboidal to columnar morphology. The zona pellucida is produced at this stage and the Golgi (G) structures can be observed in very peripheral positions. The mitochondria have increased considerably in number.

Figure 18. A mitochondrion of this stage with an internal structure typically found in more mature follicle oocytes.
30,000X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16


17
18

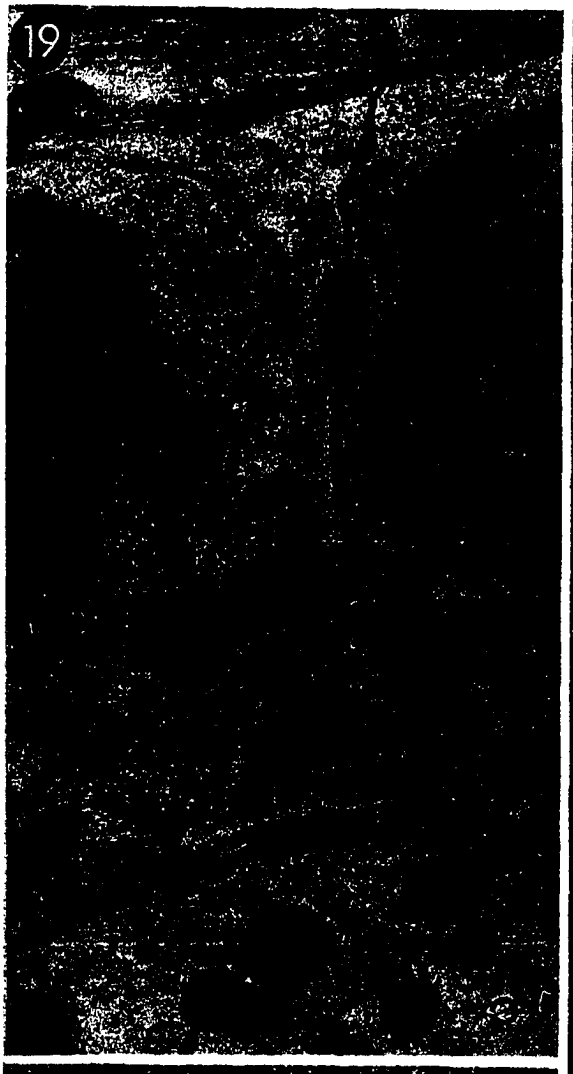
PLATE IX

Figure 19. A section of a primary follicle containing a single layer of cuboidal granulosa cells (GC). The granulosa cells are linked by desmosomes. They are delimited on one side by a basement membrane (Bm), approximately 0.1 micron thick and on the other side by the oocyte (O). Villous processes (V) associated with zona pellucida production are found at the junction of the oocyte and granulosa cells.
14,000X

Figure 20. A section of a later primary follicle which contains one layer of columnar granulosa cells and a zona pellucida (ZP). Microvilli from the oocyte and cytoplasmic processes (Cp) of the granulosa cells are seen extending into the zona pellucida. Note the position of the nuclei in the columnar cells as compared with the cuboidal cells in Figure 19.

A cilium (C) is found in the conventional position, pointing away from the basement membrane and toward the ovum.
14,600X

Figure 21. An enlarged view of the centrosomal area of the cell in Figure 20. The ensheathed cilium (Sh) (C) extends from the distal centriole (Dc). The proximal centriole (Pc) is at right angles to the distal one. The Golgi (G) structures are closely associated with the centrosomal area.
25,000X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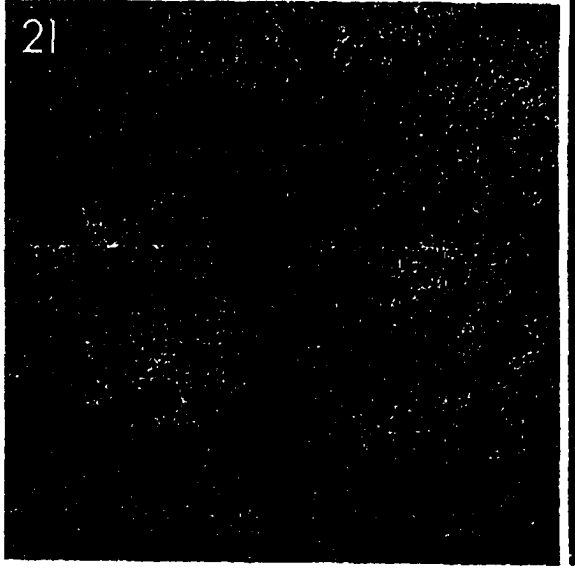
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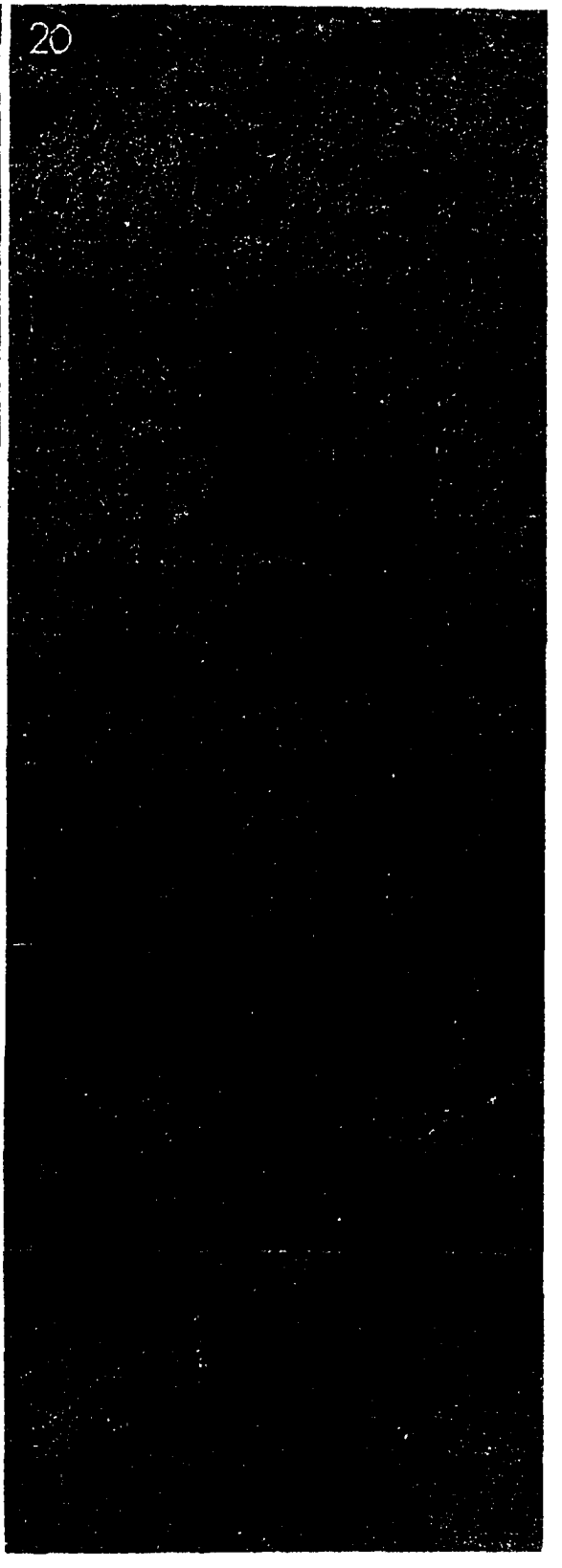

20

PLATE X

Figure 22. A primary follicle. In the ovum, note the nucleus with its slightly undulating outline and prominent nucleolus. Details of the oocyte cytoplasm are shown in Figure 23. Surrounding the ovum is the zona pellucida and the single layer of granulosa cells resting on a basement membrane. The basement membrane is approximately 0.13 micron thick. Typical thecal cells (TC) and lipid laden interstitial cells (IC) surround the follicle. 5,400X

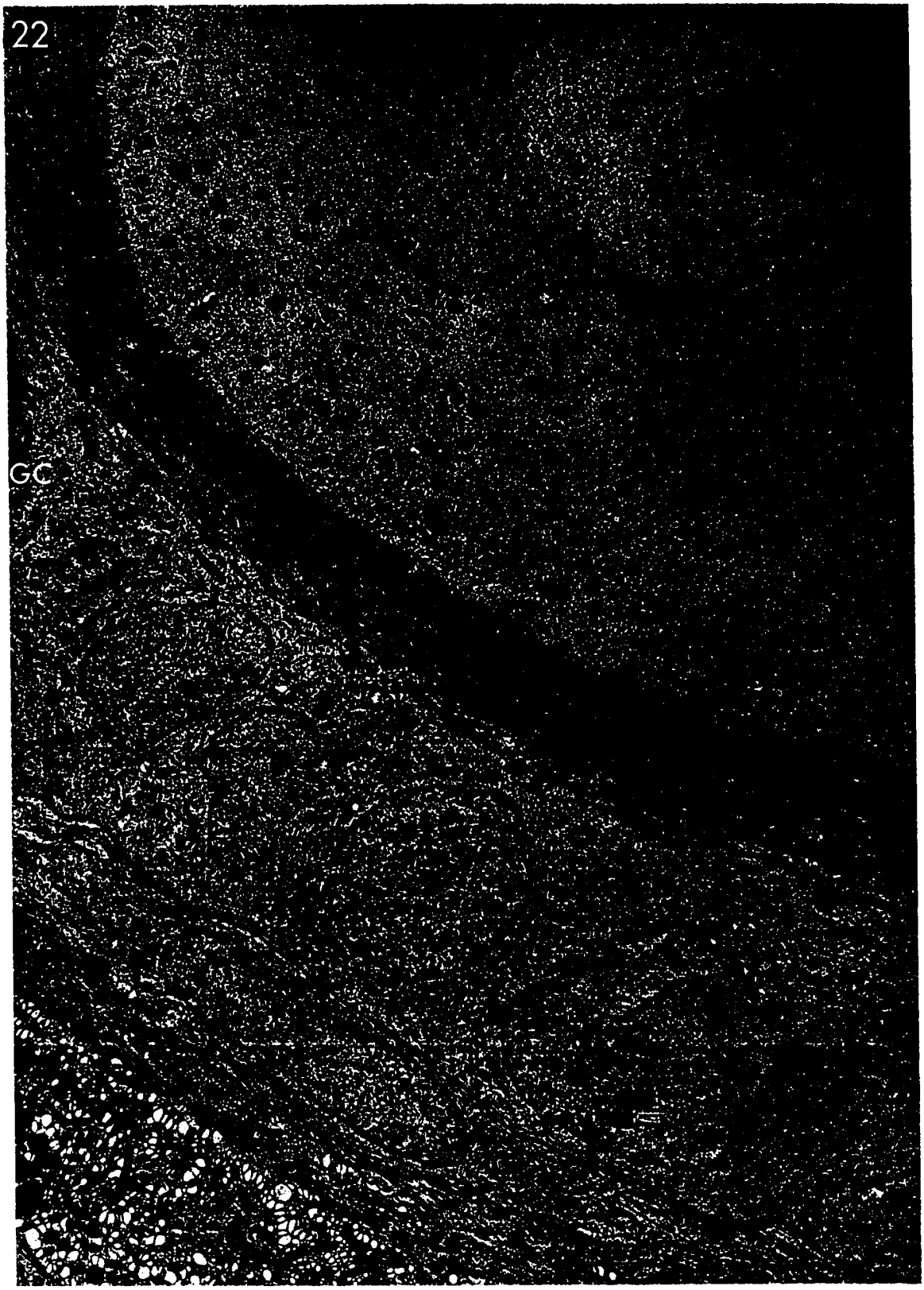

22
GC

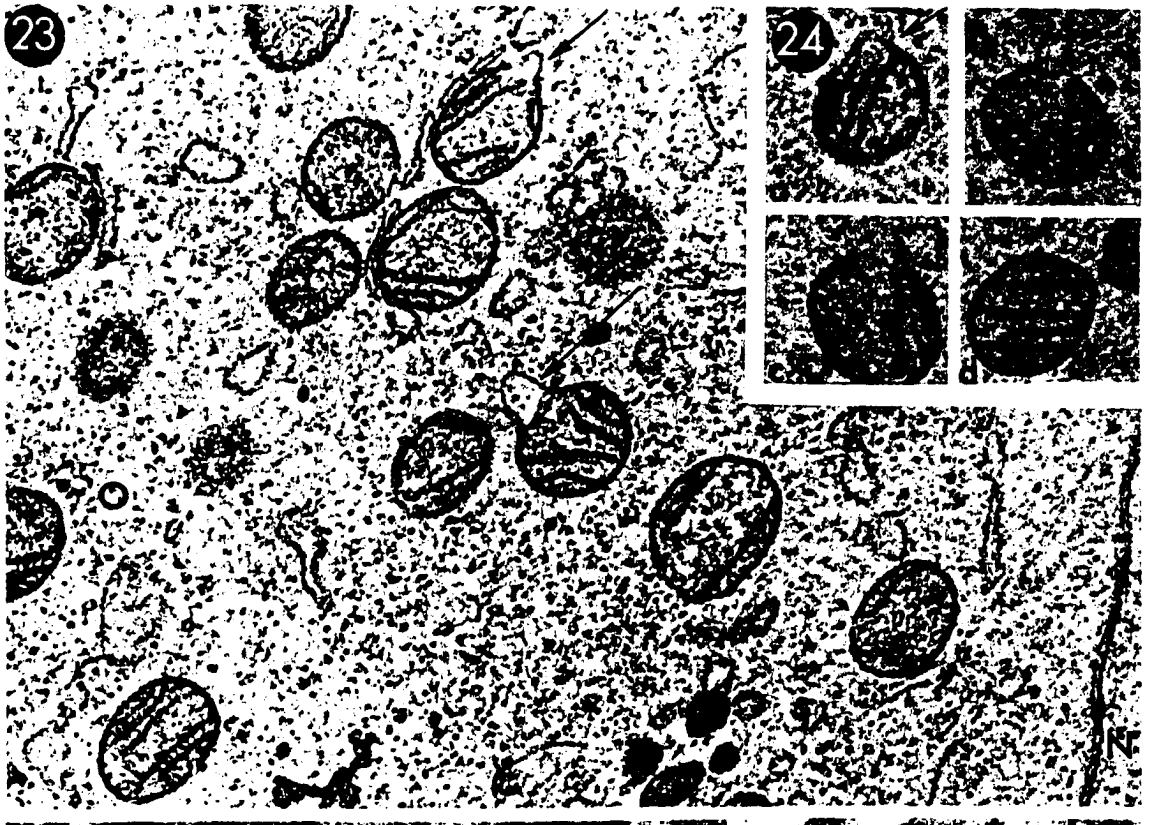
PLATE XI

Figure 23. A cytoplasmic portion of the oocyte from the primary follicle shown in Figure 22. The mitochondria appear uniformly rounded. Arrows indicate electron lucent protrusions or extensions of the mitochondrial matrix. Smooth membraned cytoplasmic vesicles are often closely associted with mitochondria.

Figure 24. The mitochondria are arranged (a, b, c, d) to show the formation (arrow) of electron lucent blebs (a, b) and their possible release into the cytoplasm (c, d).

Figure 25. Cytoplasmic detail of oocyte from an early secondary follicle. Electron dense granules are arranged in a semicrystalline pattern within the mitochondrial matrix. These granules are presumed to be yolk and the mitochondria can be referred to as yolk precursors. Electron lucent vesicles are closely associted with these organelles. The Golgi (G) are seen in peripheral locations in the oocyte.

43,000X


23
24
N

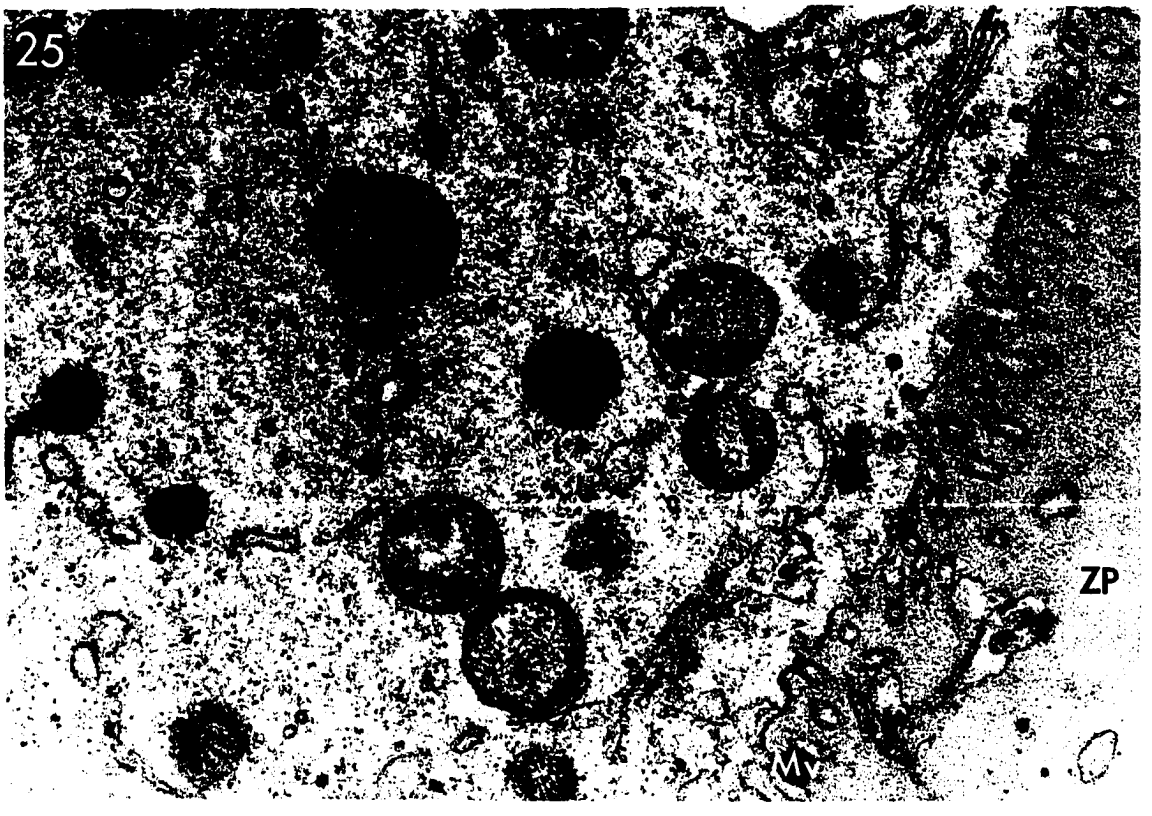

25
ZP
M

PLATE XII

Figure 26. A section of an early secondary follicle, showing the ovum (O), the zona pellucida (ZP) and the granulosa cells (GC). The granulosa cells have increased in number.

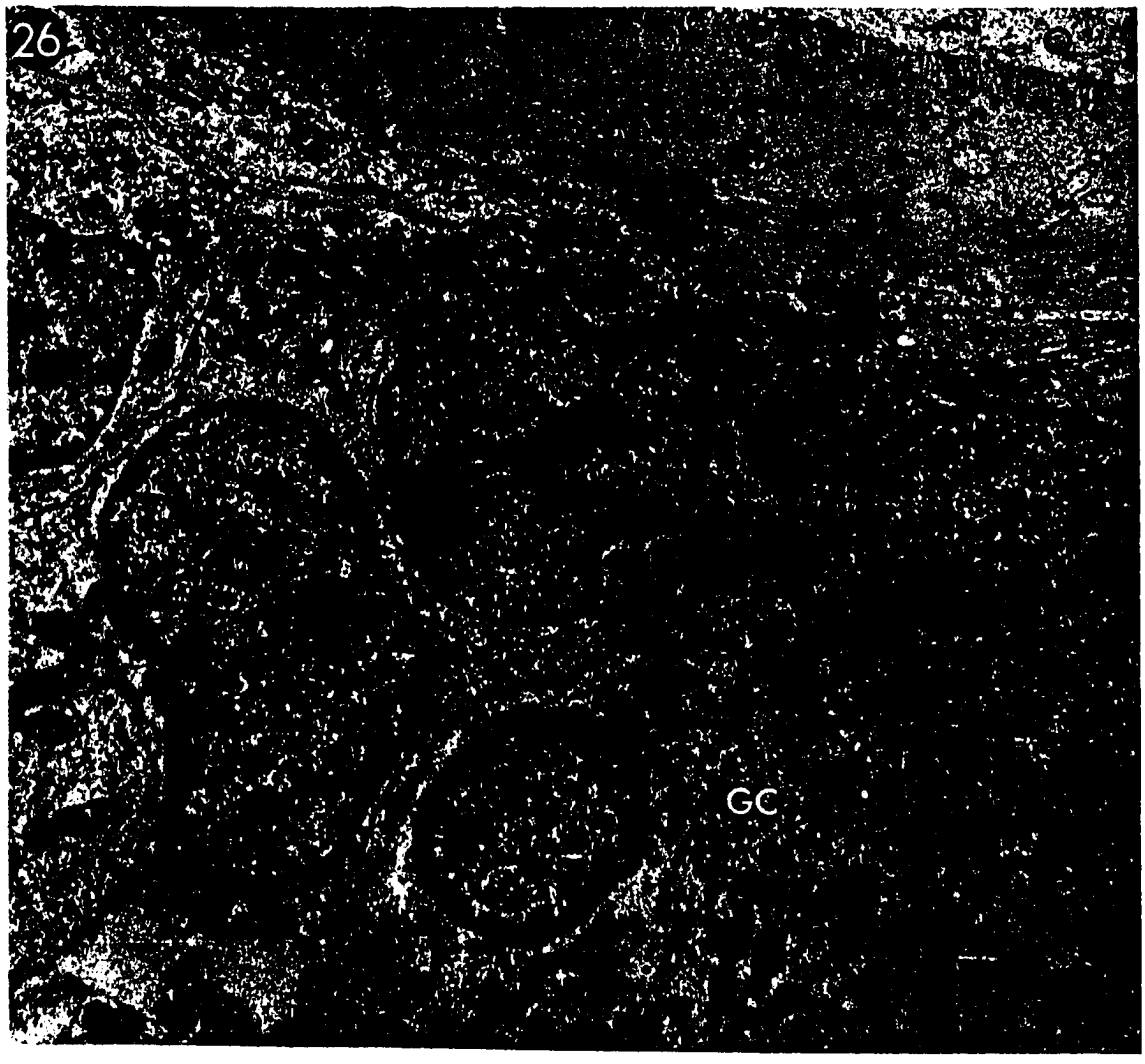
At the double arrows, relatively electron lucent intracellular areas can be observed.

Note the intercellular location of zona pellucida material among the granulosa cells.

10,000X

Figure 27. A higher magnification view of the area at the double arrows at (a) reveals a beaded array of filaments, with dark granules interspersed and peripherally.

25,600X


26
GC

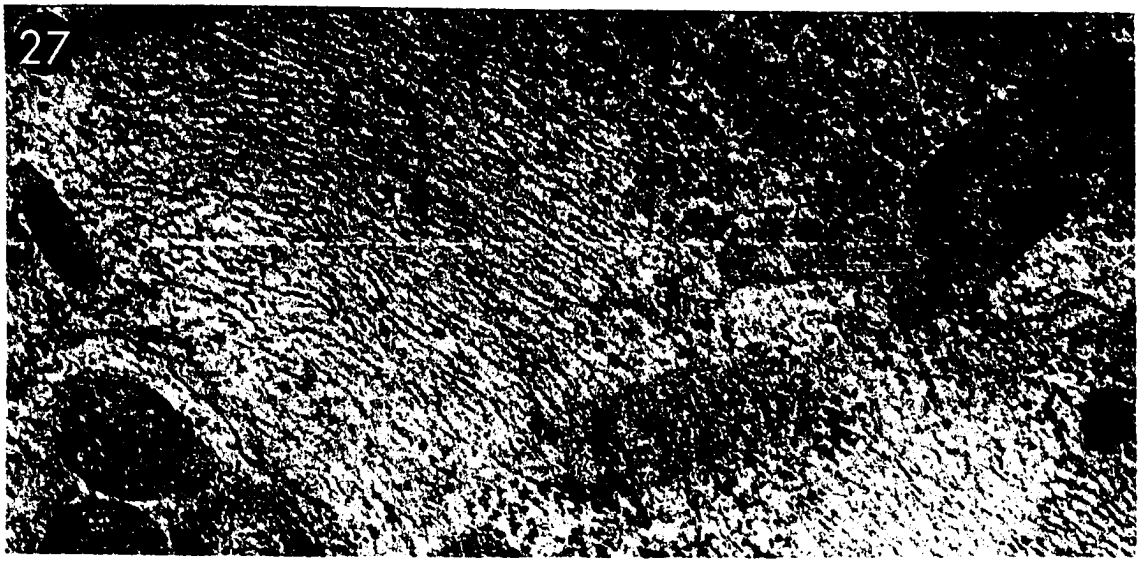

27

PLATE XIII

Figure 28. The area indicated by double arrow (b) in Figure 26 is shown here at a higher magnification. A whorl-like arrangement of beaded filaments can be seen. Dark granules, with dimensions of 150 angstroms by 80-90 angstroms are interspersed and peripheral.

The filaments show a repeat pattern of wider and narrower dimensions. The wider parts range between 65-70 angstroms and the narrow portions between 35-40 angstroms. This repeat pattern imparts the beaded appearance which is continuous along the length of the filament.

73,600X

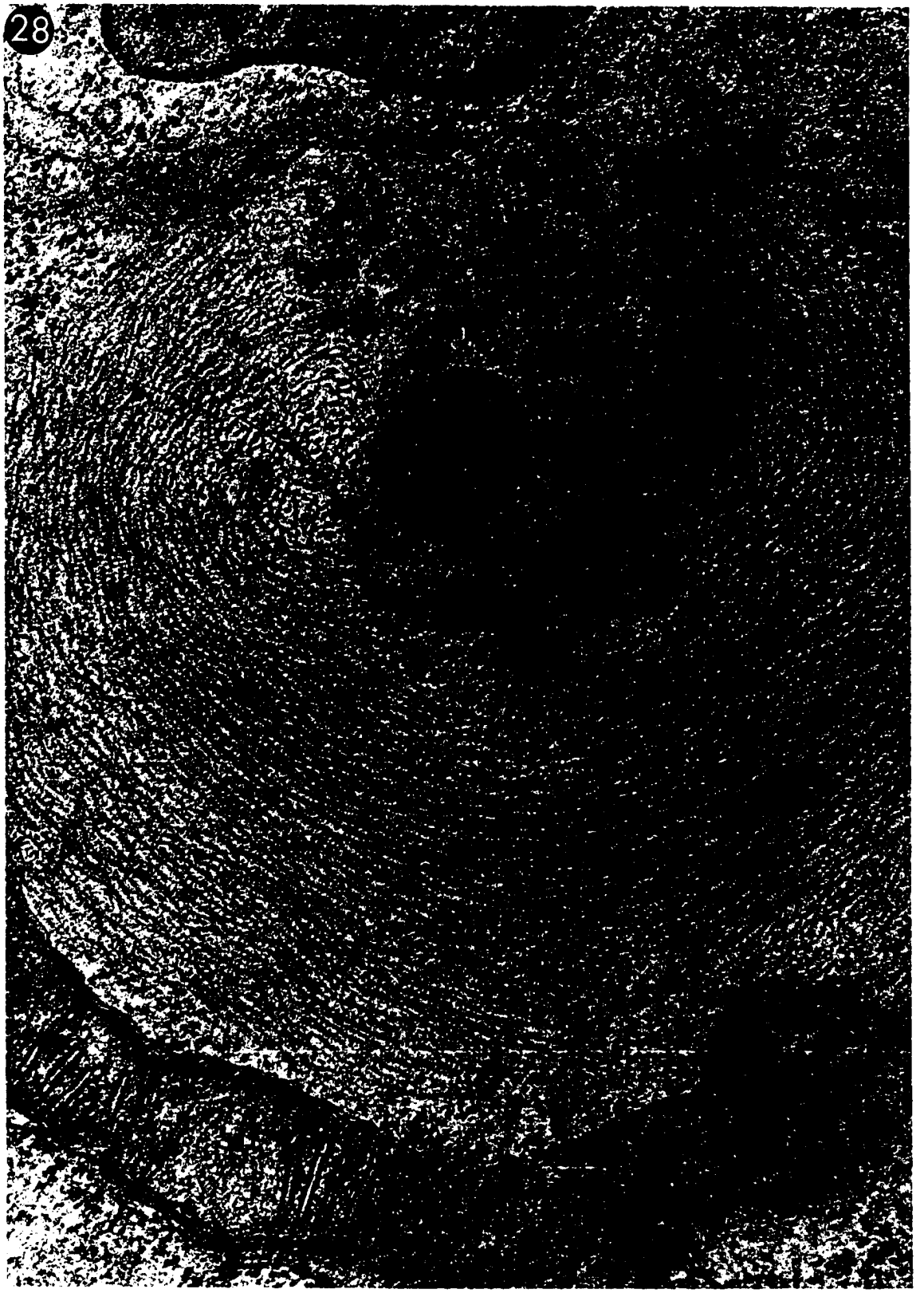

28

PLATE XIV

Variations in Golgi Structures and their Relations to the Oocyte Surface Features in Primary and Secondary Follicles

Figure 29. Golgi comples of a primary follicle oocyte. This peripherally located Golgi complex shows a widespread development. It consists of flattened stacks of vesicles in parallel and concentric arrangements. At the long arrow is a rosette configuration reminiscent of the contractile vacuole of a paramecium. An orderly row of small circular profiles is seen surrounding a large electron lucent vesicle.

Figure 30. Golgi structures of a secondary follicle oocyte. Three elements predominate: 1) flattened stacked vesicles, 2) large electron lucent ones, and 3) small round seemingly pinched off profiles of varying electron densities.

Note the close association of the Golgi with the desmosomes (arrow).

Figure 31. Cytoplasmic portion of a secondary follicle oocyte. Note the Golgi, the characteristic mitochondria and the closely associated desmosomes.

Figure 32. Golgi from a bilaminar secondary follicle oocyte. Note the Golgi and the desmosomes at the arrow.

30,000X

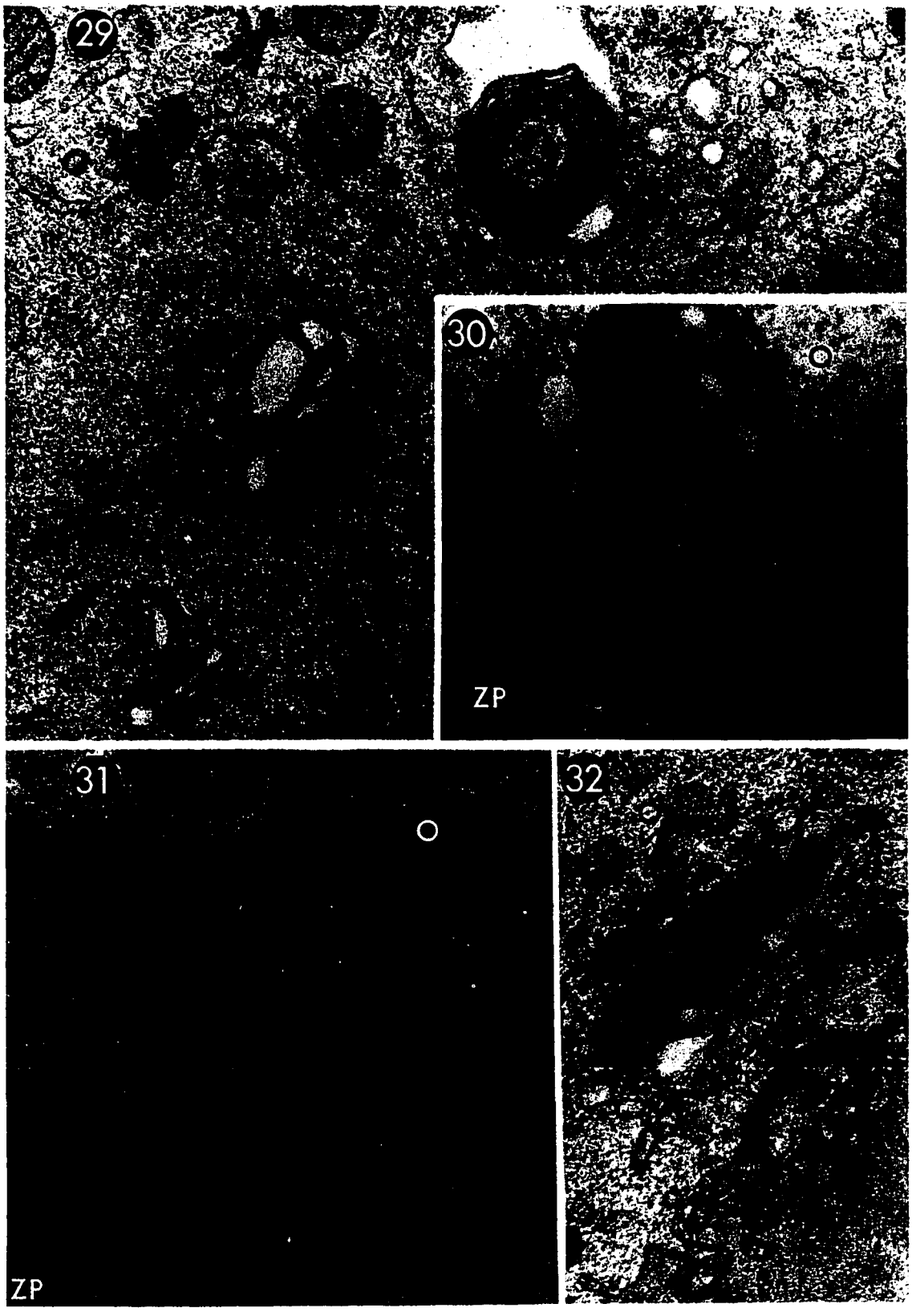

29
30
ZP
31
ZP
32

PLATE XV

Changes in the Zona Pellucida and Associated Structures

Figure 33. The oocyte, zona pellucida and granulosa cell of a primary follicle. The zona at this stage measures about 4.4 microns.

Figure 34. The zona pellucida in an early tertiary follicle. At this stage the zona measures about 10.4 microns.

Note: The cilium of the granulosa cell extending into the extracellular space adjoining the zona pellucida. The cilium is characteristically oriented toward the ovum. The dense area (DA) at the base of the cilium; the pericentriolar satellites (S) and the procentrioles (Pr) can be seen. A multitude of Golgi vesicles (Gv) are found in the adjacent cytoplasm.

Figure 35. In the late tertiary follicle, the zona pellucida is reduced to about 8.3 microns diameter.

Note: The plasma membrane of the oocyte has flattened and the villi have become smaller in size and sparser in distribution (see Table 2).

15,300X

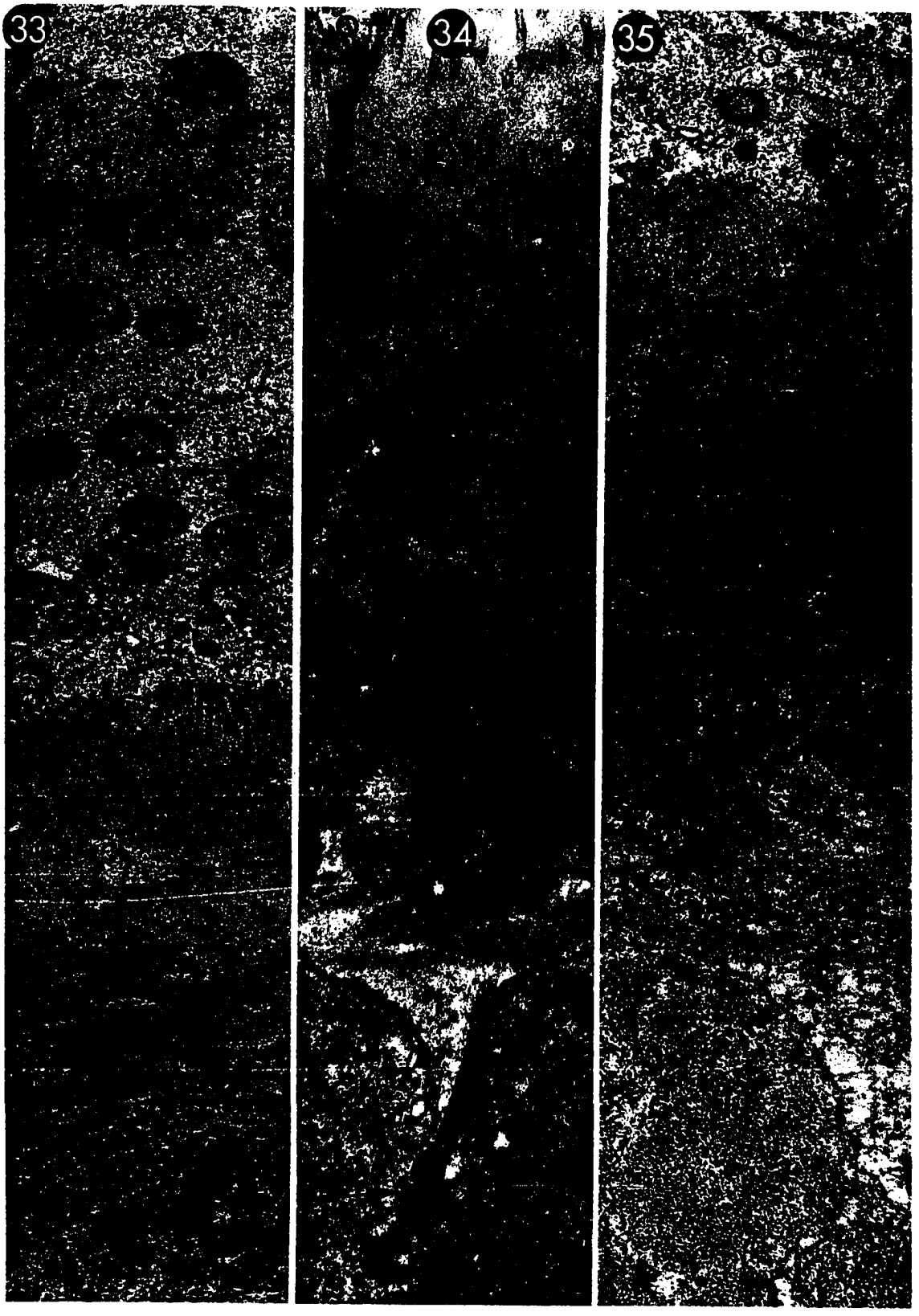

33
34
35

PLATE XVI

Changes of the Peripheral Cytoplasm and Plasma Membrane Structures of the Ovum

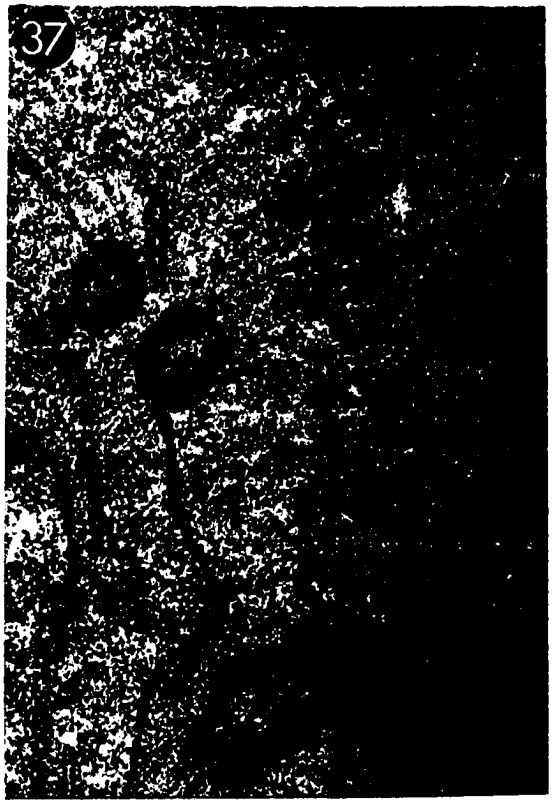
Figure 36. Cytoplasm and villi of an early tertiary follicle ovum. Note the length and regularity of the villi (see Table 2 and text for details).

Figure 37. Cytoplasm and villi of a late tertiary follicle ovum. Note the shorter and wider spaced villi (see Table 2 and text for details).

Figure 38. A much altered peripheral region of an atretic secondary follicle ovum. Note the lack of conventional villi and discontinuities in the plasma membrane. The mitochondria (M) have the appearance of mitochondria of later stages.

Figure 39. Atretic secondary follicle ovum. See text for detailed descriptions of structures.
12,500X


36


37


38
M


39

PLATE XVII

Figure 40. A section of a secondary follicle during atretic transformation, showing the altered ovum (O), zona pellucida (ZP) and granulosa cells (GC).
7,300X

Figure 41. The ovum from the previous figure is shown at higher magnification. Note the clusters and bead-like arrangement of small vesicles. Some organelles, like the mitochondrion (M) at the arrow, seem intact, while others are in the process of disintegration.
40,200X


40

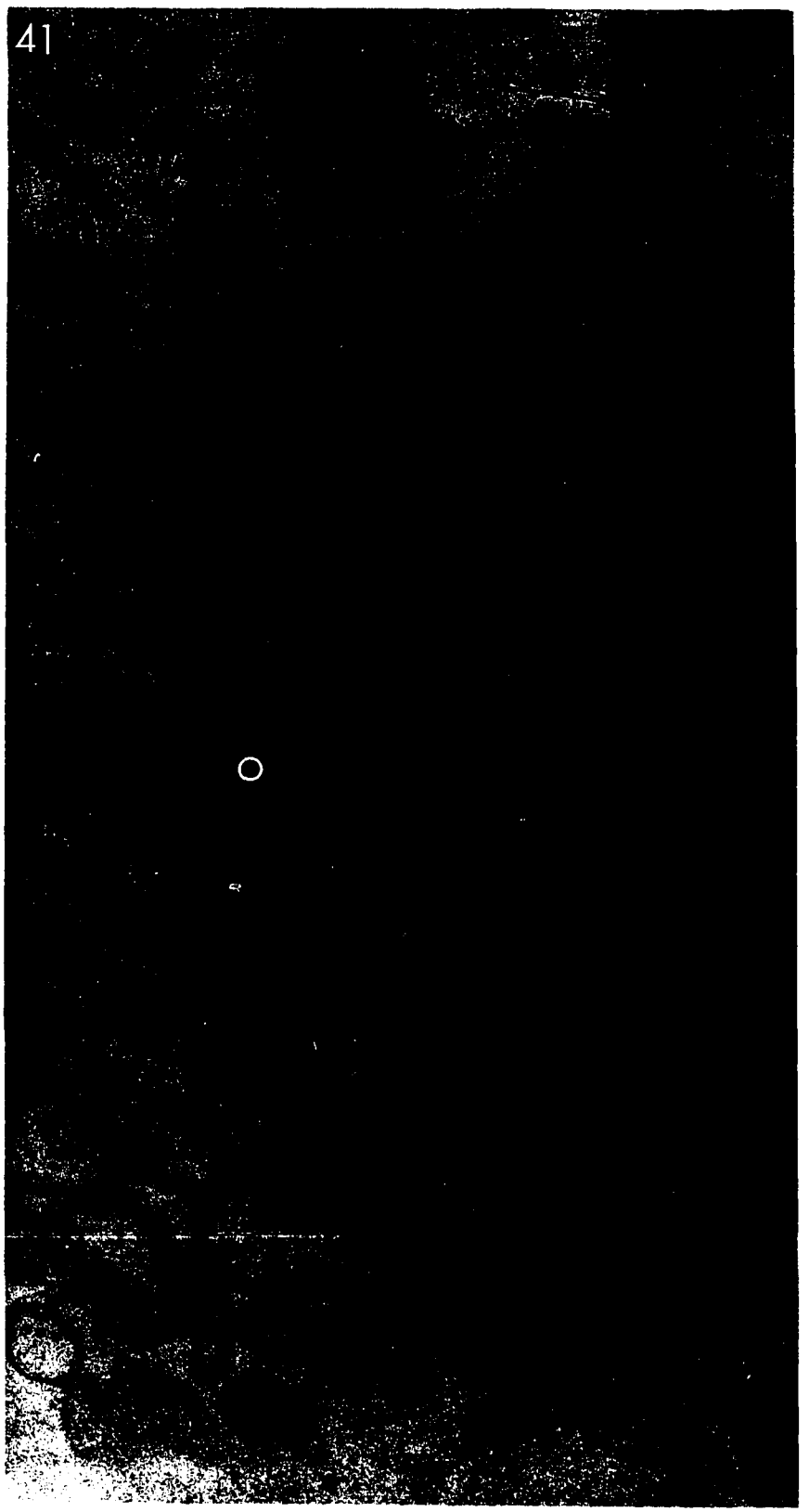
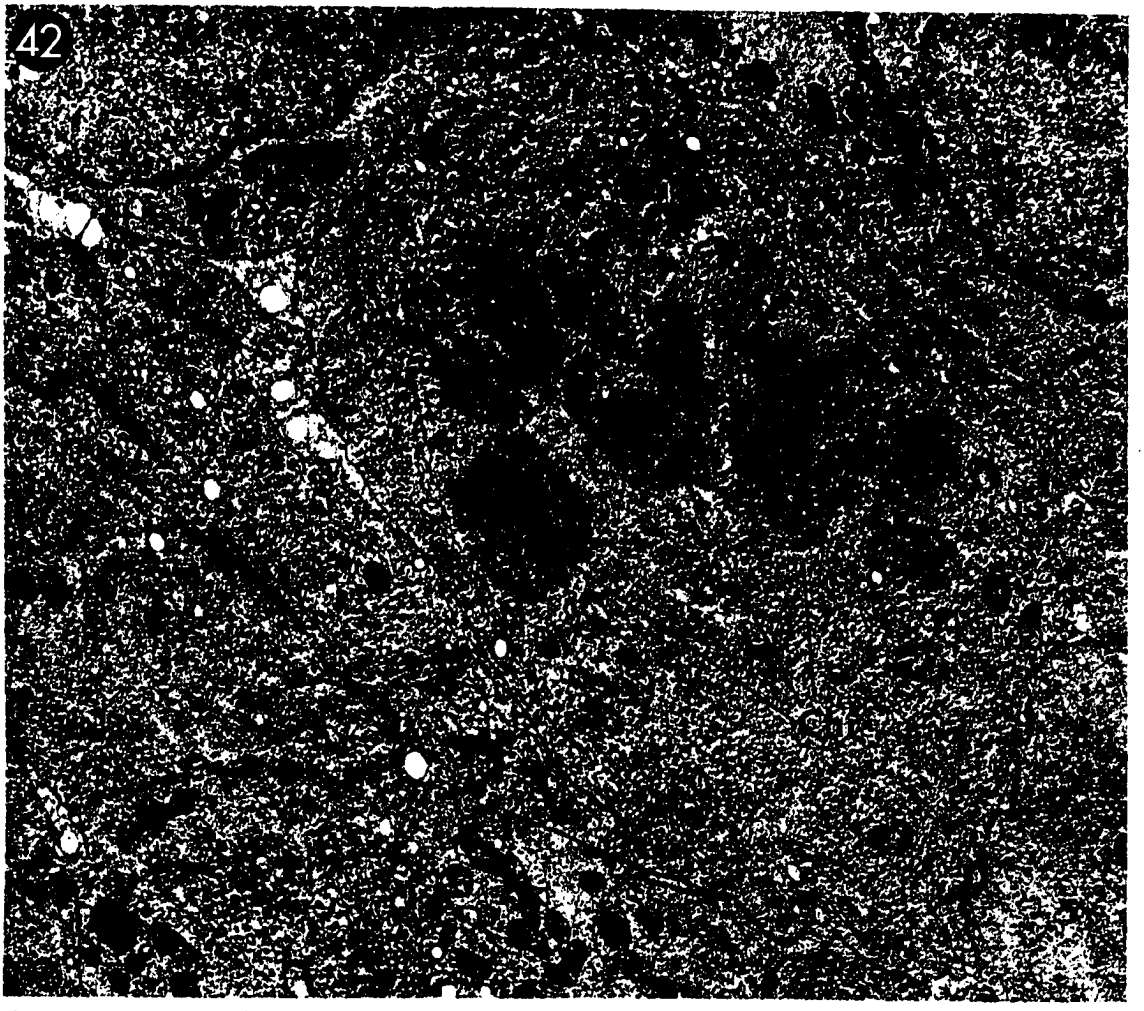

41

PLATE XVIII

The Granulosa Cells of the Tertiary Follicle

Figure 42. Tertiary follicles are marked by large increases in granulosa cells. This is accomplished by mitosis. Note the chromosomes (Chr) of the tertiary follicle granulosa cell in mitosis.

Figure 43. A portion of a tertiary follicle showing granulosa cells in the process of antrum formation. 9,300X

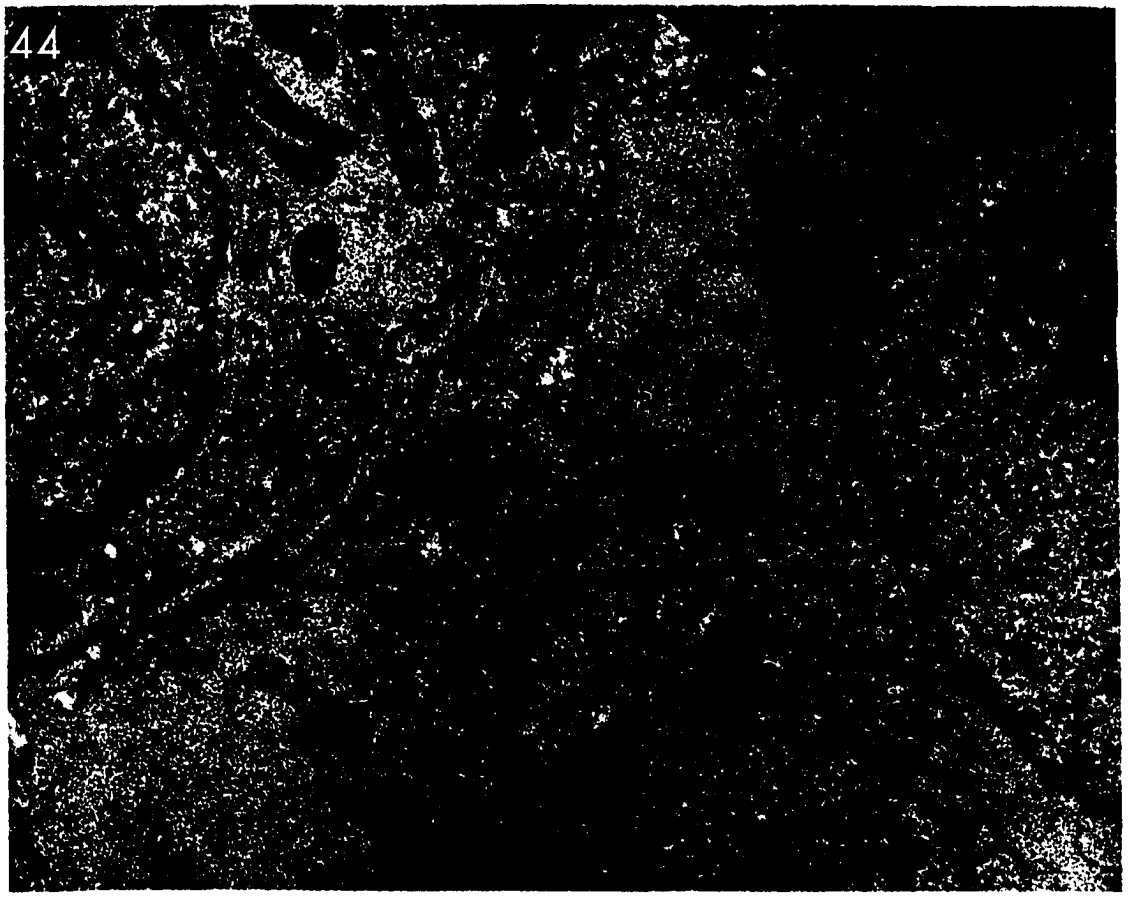

42


43
E

PLATE XIX

Figure 44. Areas of glycogen storage in tertiary follicle granulosa cells. Note that these areas are devoid of organelles.
18,700X

Figure 45. Higher magnification view of an area such as shown in Figure 44. Note the vacuolization in the adjacent mitochondria.
56,200X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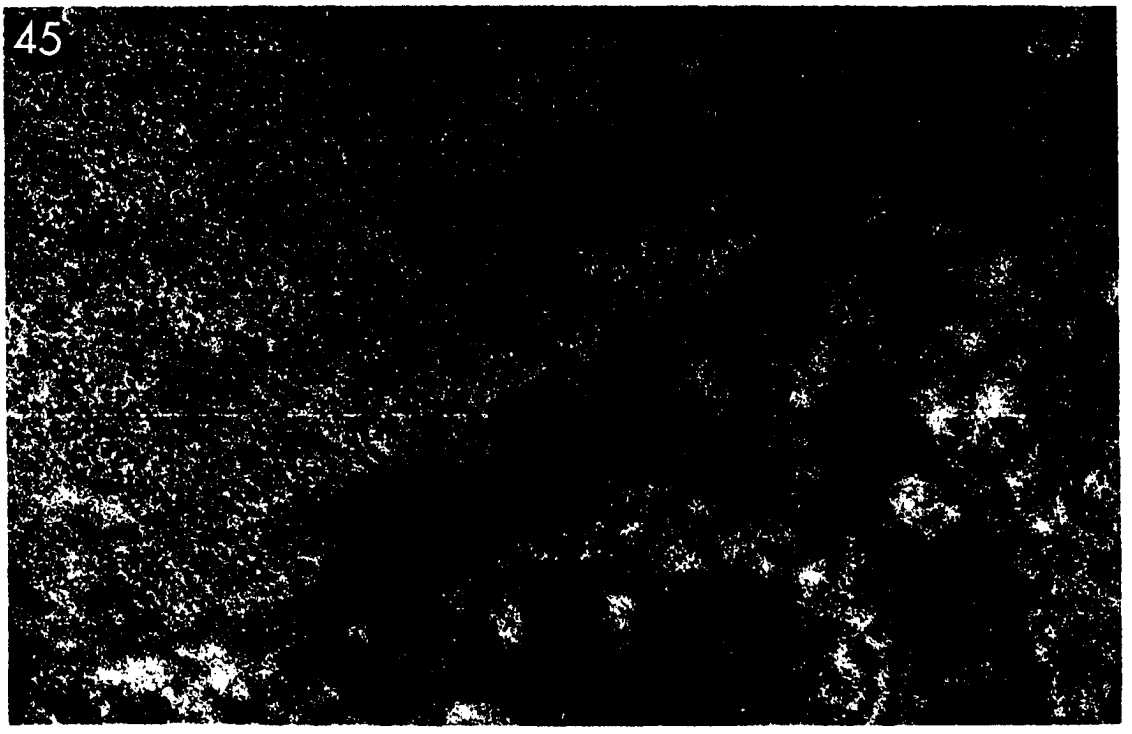

45

PLATE XX

Figure 46. A portion of a tertiary follicle. Antrum formation and glycogen deposition occurs in all parts of the follicle. The granulosa cells and their characteristic transformations are shown here comprising an area adjacent to the zona pellucida (ZP). Note the opaque areas (Oa), representing glycogen storage areas; the extracellular spaces (Es) related to antrum formation; and the desmosomal attachments between granulosa cells (C).
8,000X

Figure 47. The arrow in Figure 46 points to the area shown here at higher magnification. Note the fibro-granular appearance of the area, and the partial disintegration of the mitochondria (M) at the arrow.
23,000X

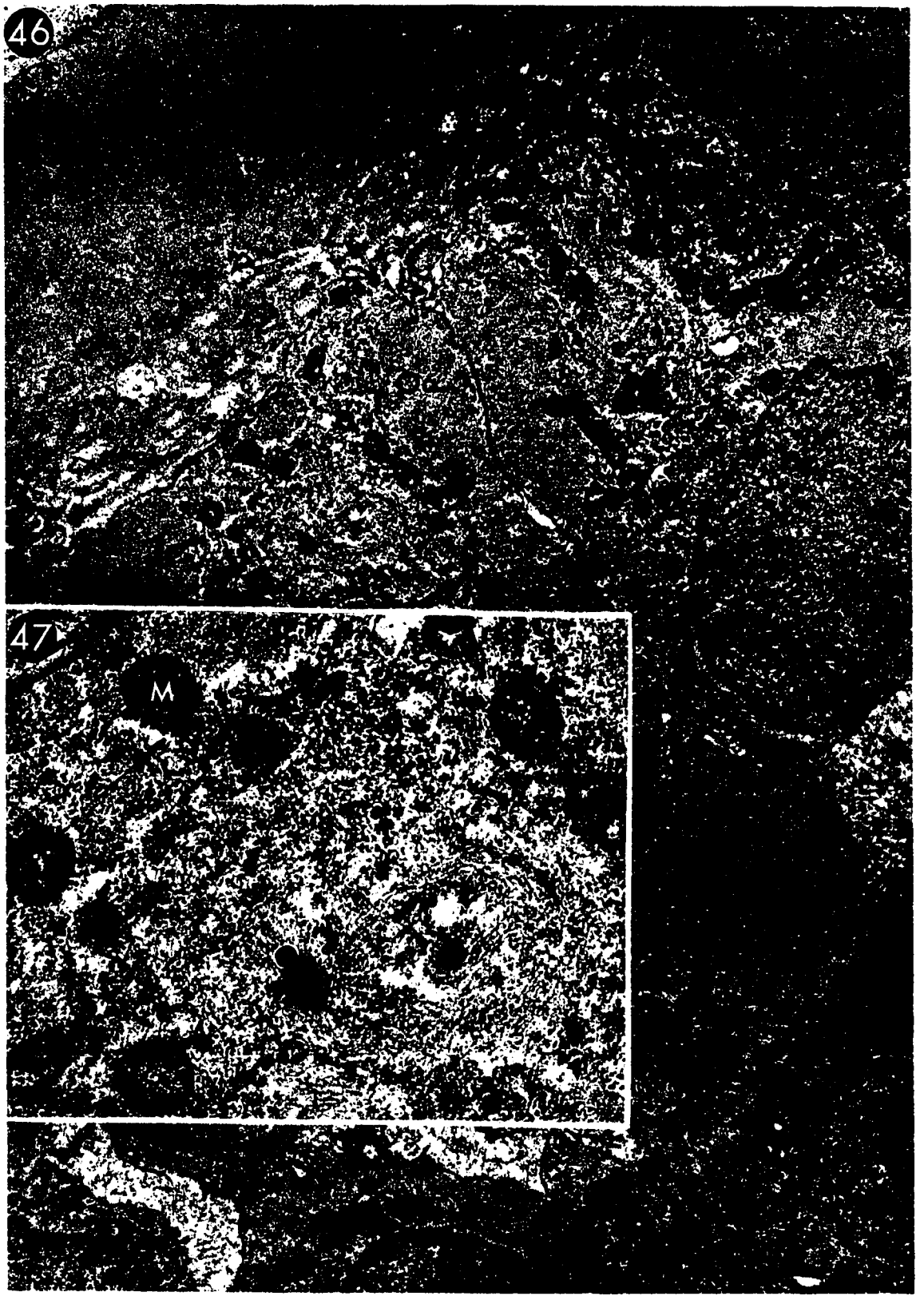

46
47
M

PLATE XXI

Figure 48. A portion of a tertiary follicle adjoining the basement membrane (Bm). Antrum forming cells and their characteristic transformations are shown in this area. The opaque areas (Oa) are glycogen depots. 14,200X

Figure 49. The area indicated in Figure 48 at arrows is shown here at higher magnification. Various structural manifestations of cytoplasmic transformations into glycogen storage areas are shown. (See text for details) 74,000X

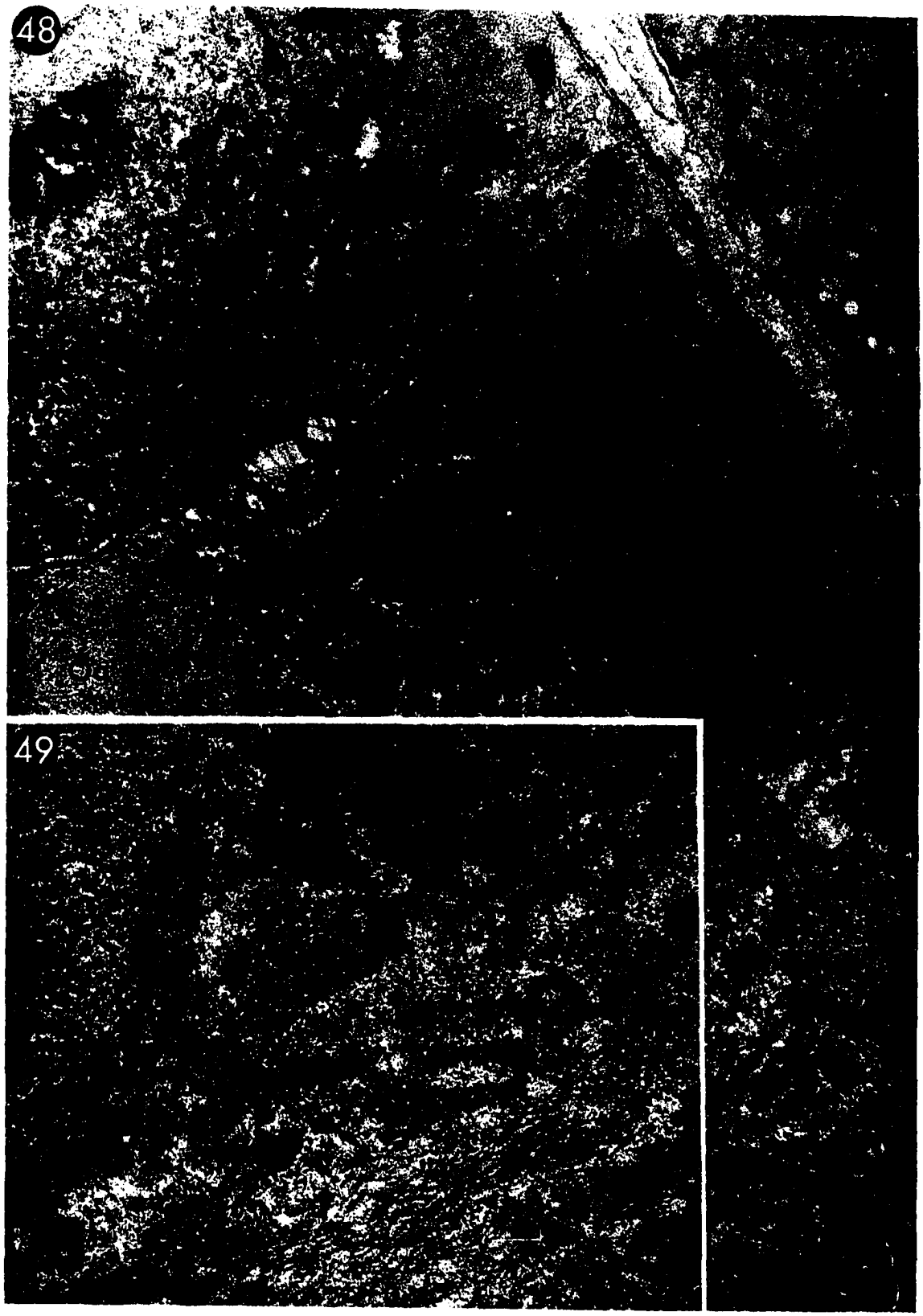

48
49

PLATE XXII

Figure 50. A section of a granulosa cell showing pre-antrum changes and glycogen deposition.

Note the desmosomes (D) and the lipoid droplets (Ld).

59,200X

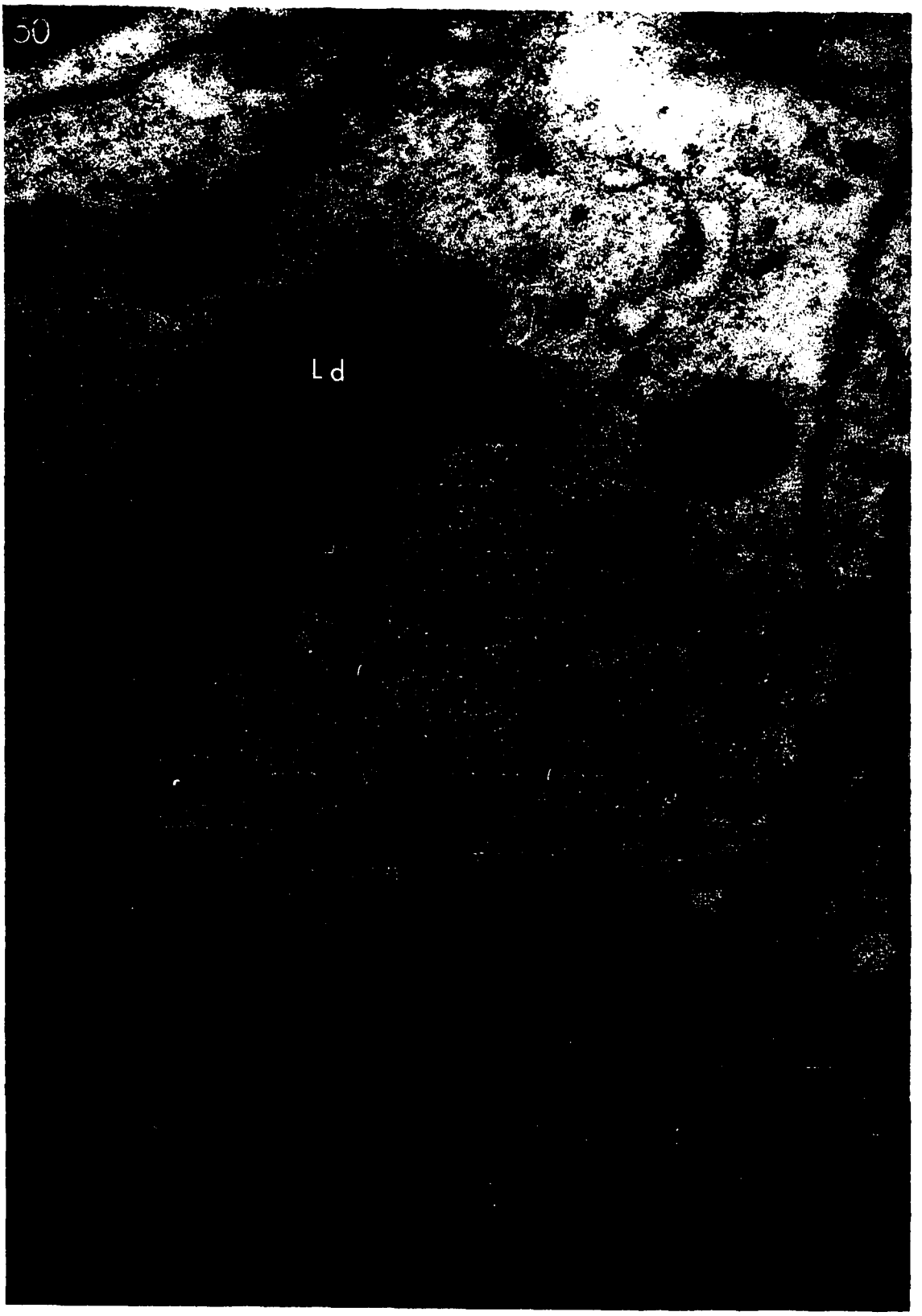

50
Ld

PLATE XXIII

Figure 51. Tertiary follicle. A perinuclear portion of a granulosa cell. The cell is involved in antrum formation and glycogen storage.

Note the nucleus (N), the centriole (Ct), the Golgi (G) and mitochondria (M).

84,200X


51
N
G

PLATE XXIV

Figure 52. Early changes in the granulosa cells (CG) of an atretic secondary follicle. The concomitant changes in the oocyte are pictured in Figure 38 and 53.
6,800X

Figure 53. The ovum of the follicle partially shown in Figure 52.
6,800X

Figure 54. A portion of an atretic secondary follicle. Note the vesicle formation at the arrow.

Figure 55. The rounded vesicles of the atretic tertiary follicle in Figure 59, are shown here at higher magnification. Note the double membranes surrounding these intracellular formations.
26,700X

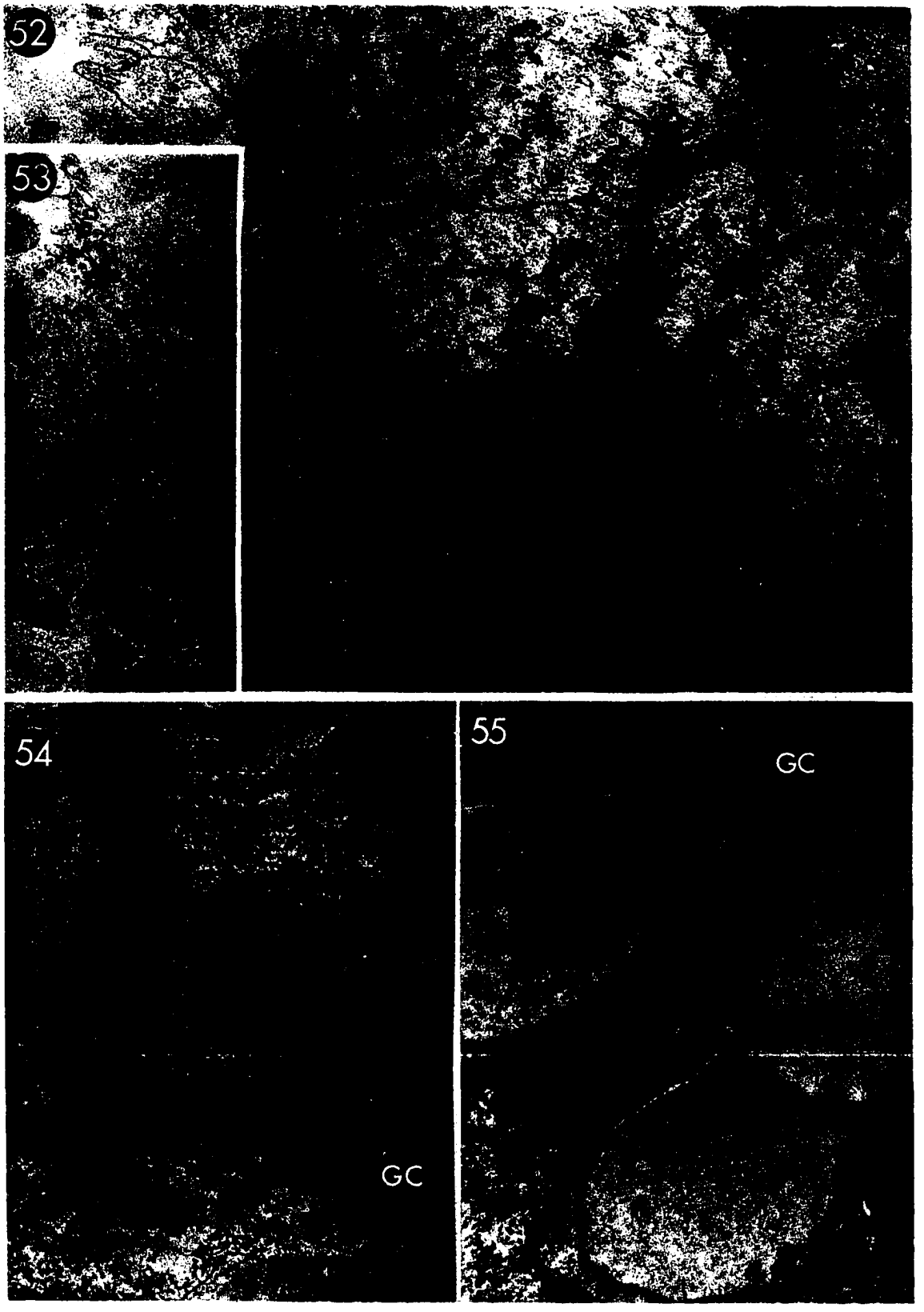

52
53
54
GC
55
GC

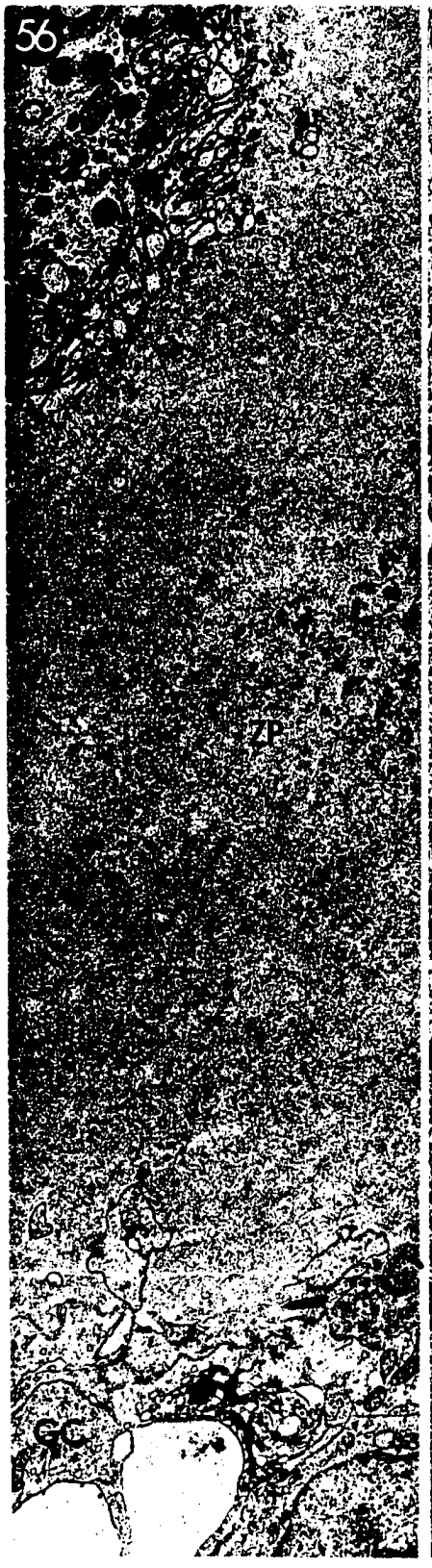
PLATE XXV

Figure 56. A section of an atretic secondary follicle, showing extreme atretic transformations in the oocyte (O), the zona pellucida (ZP) and the granulosa cells (GC).

Figure 57. A portion of the granulosa of the same atretic secondary follicle. The changes appear to be more pronounced in the cells bordering the zona pellucida.

The basement membrane has taken on a corrugated appearance. An extensive development of the glassy membrane (Gm) can be seen. Note the hypertrophied thecal cells (TC) and the lipid laden interstitial cells (IC).

7,300X


56
ZP

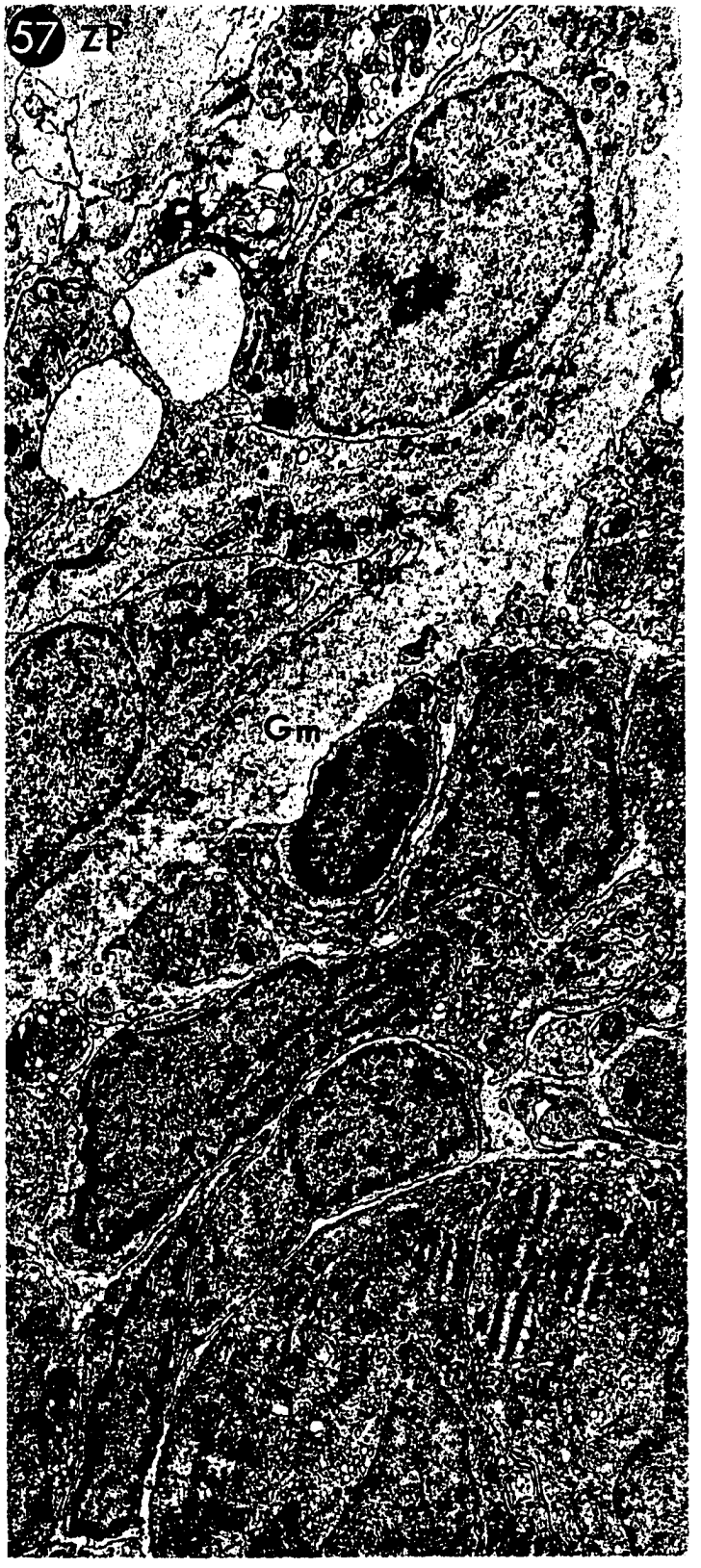

57
ZP
Gm

PLATE XXVI

Figure 58. A growing tertiary follicle. The zona pellucida (ZP) shows interlacing of granulosa cell processes. In the granulosa cells (GC), areas of glycogen deposits are indicated by the arrows. Antrum formation is in process, with the many irregularly shaped intercellular spaces in appearance.

Figure 59. An atretic follicle of the same developmental age as that in Figure 58. In comparing the two follicles, several changes can be observed. The electron lucent intracellular areas are no longer present. The intercellular spaces have disappeared and large, balloon-like intracellular vacuoles have formed. Changes at the zona pellucida and the basement membrane are seen and the broad, collagenous "glassy membrane" (GM) has developed.
8,300X

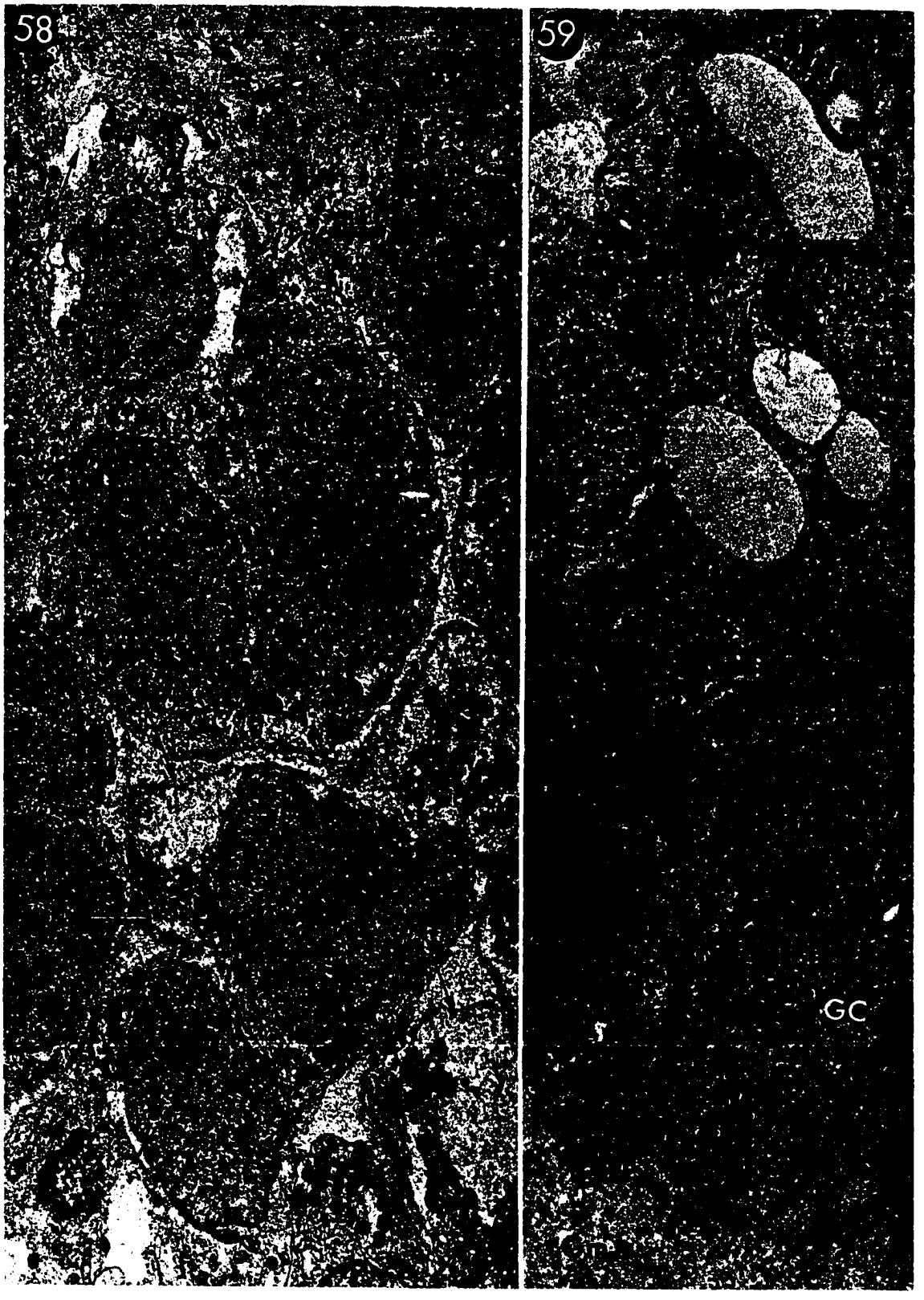

58
59
GC

PLATE XXVII

Figure 60. An early tertiary follicle portion. In the ovum, the nucleus (N) has an undulated outline. The plasma membrane of the ovum displays long and regularly shaped microvilli. Evidence of a few remaining granulosa cell processes can still be found in the zona pellucida (ZP). The granulosa cells (GC) are involved in antrum formation.

5,300X

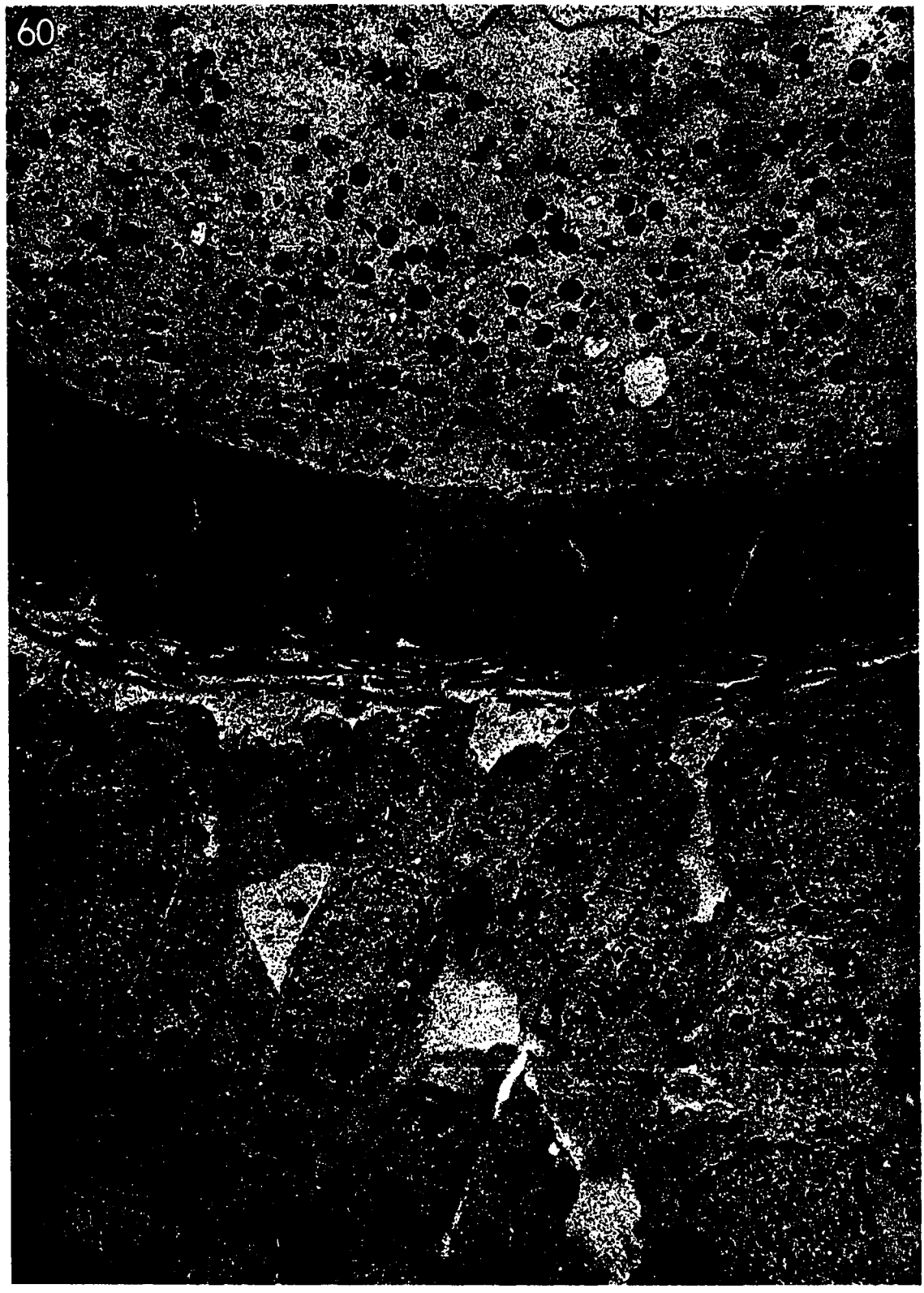

60°

PLATE XXVIII

A Composite View of a Mature Tertiary Follicle

Figure 61. The nucleus (N) is in an offcentered position and its outline is fairly regular. The microvilli at the oocyte surface, are decreased in size and spaced further apart.

The innermost layers of the granulosa cells, now that an antrum (AN) has formed are now called the corona cells (CC).

Figure 62. The middle portion of the follicle, showing the lowest portion of the corona cells, the antrum and the granulosa cells (GC). These cells appear to be most actively involved in the apocrine process leading to the formation of the secondary antral fluid (Arrow).

Figure 63. The outermost portion of the follicle consisting of layers of granulosa cells and the limiting basement membrane.

5,400X

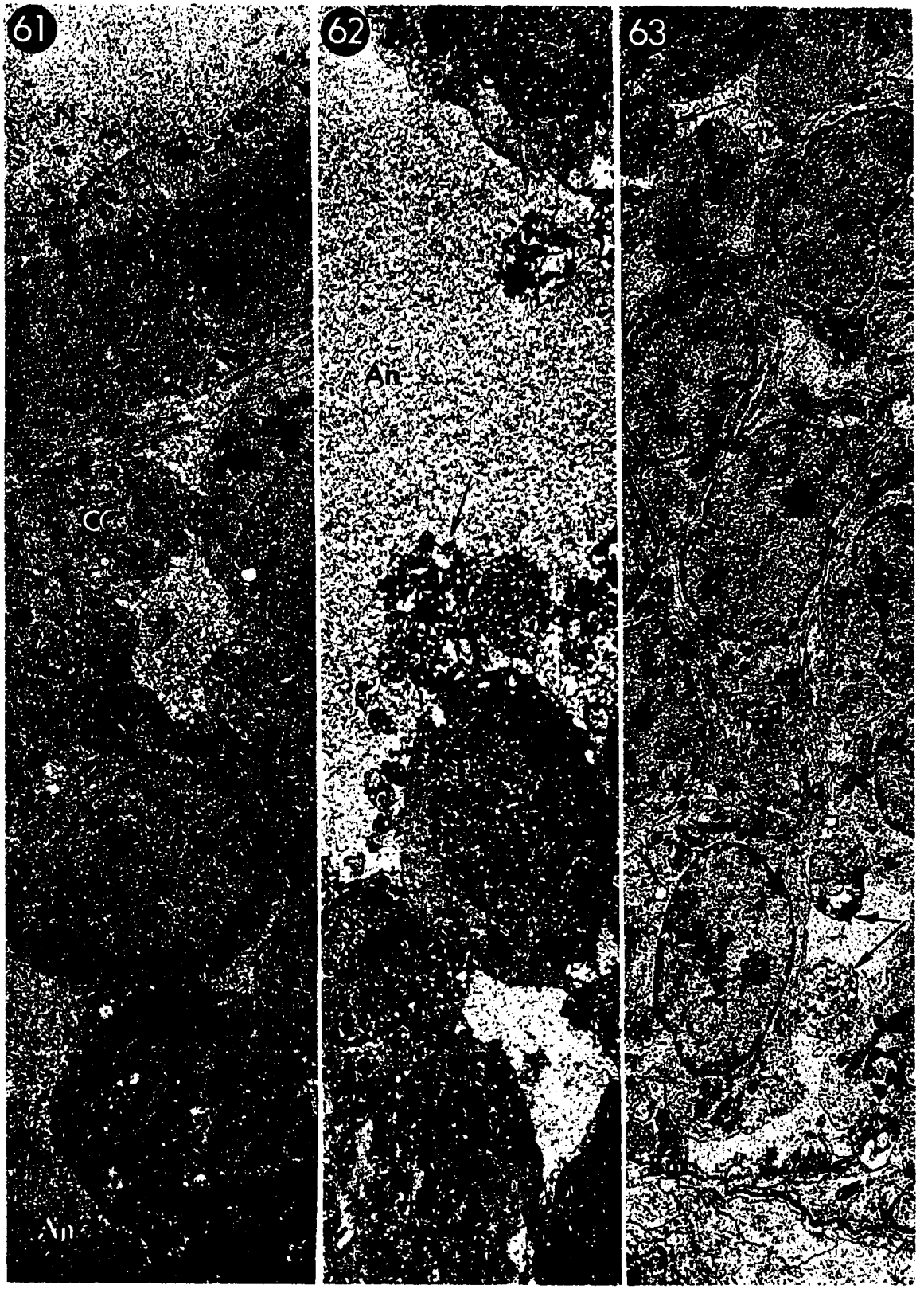

61
N
CC
An
62
An
63

PLATE XXIX

Figure 64. A cytoplasmic section of the oocyte of a tertiary follicle. The mitochondria are uniformly exhibiting the morphology typical for this stage. Many smooth membraned vesicles are dispersed in the cytoplasm. Some of these are closely aligned to the mitochondria. Note the Golgi (G) and the lipoid droplets (Ld).

Figures 65-69. These figures show the developmental process of the formation of mitochondrial electron lucent protrusions. These protrusions appear to be in continuity with the interior of the mitochondria, yet are distinctly more electron lucent. Figure 69, shows an invaginated area which may be the site of an ejected vesicle.

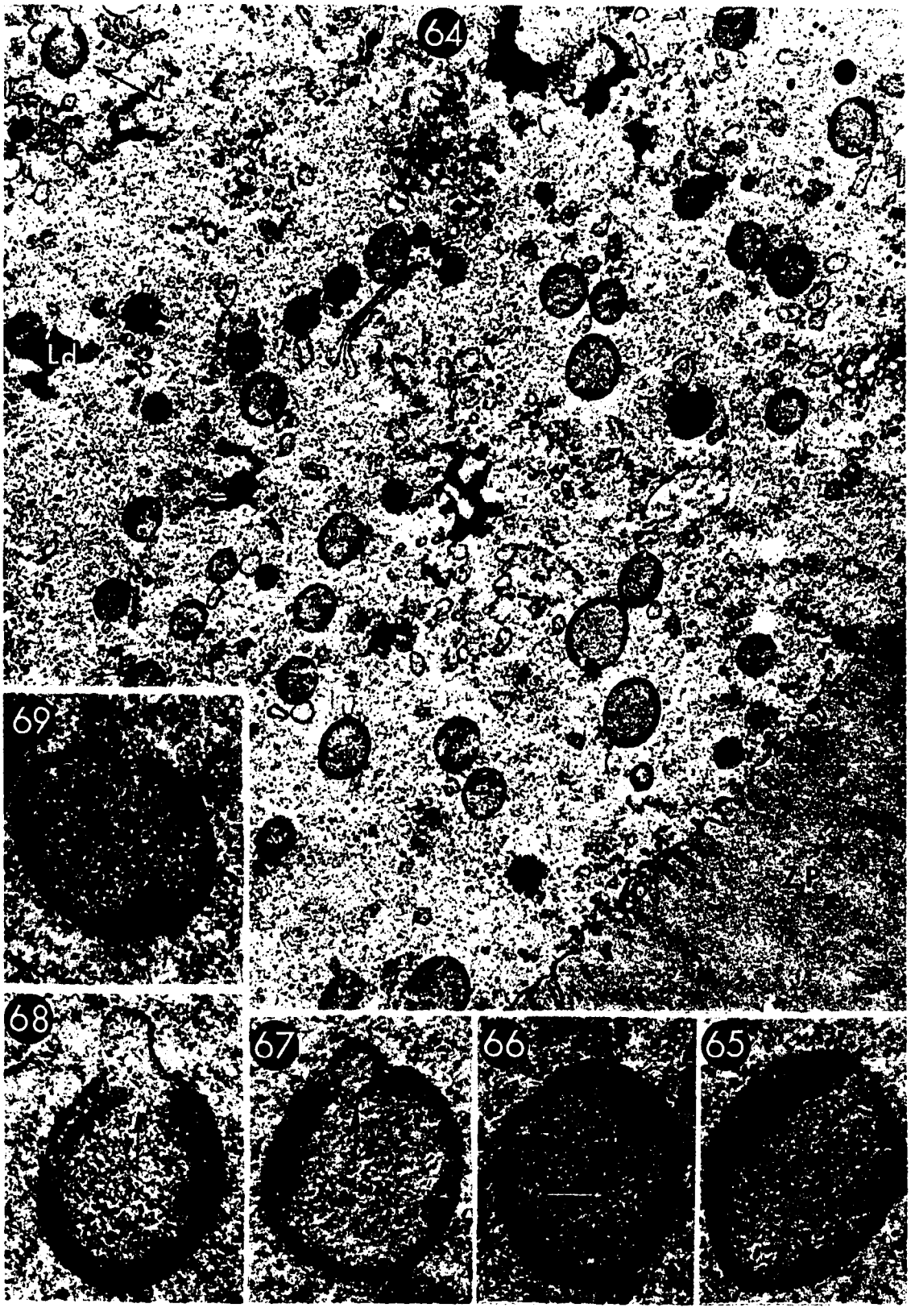

64
Ld
ZP
69
68
67
66
65

PLATE XXX

Figures 70, 71, 72. The mitochondria of the tertiary follicle can now be referred to as "yolk platelets." Their entire membrane system is arranged as peripheral concentric lamellae. Some of these have condensed to form electron dense structures. The central portion shows the semi-crystalline arrangement of the olk granules.

Note the vesicles, some closely associated with the mitochondria.

70,000X

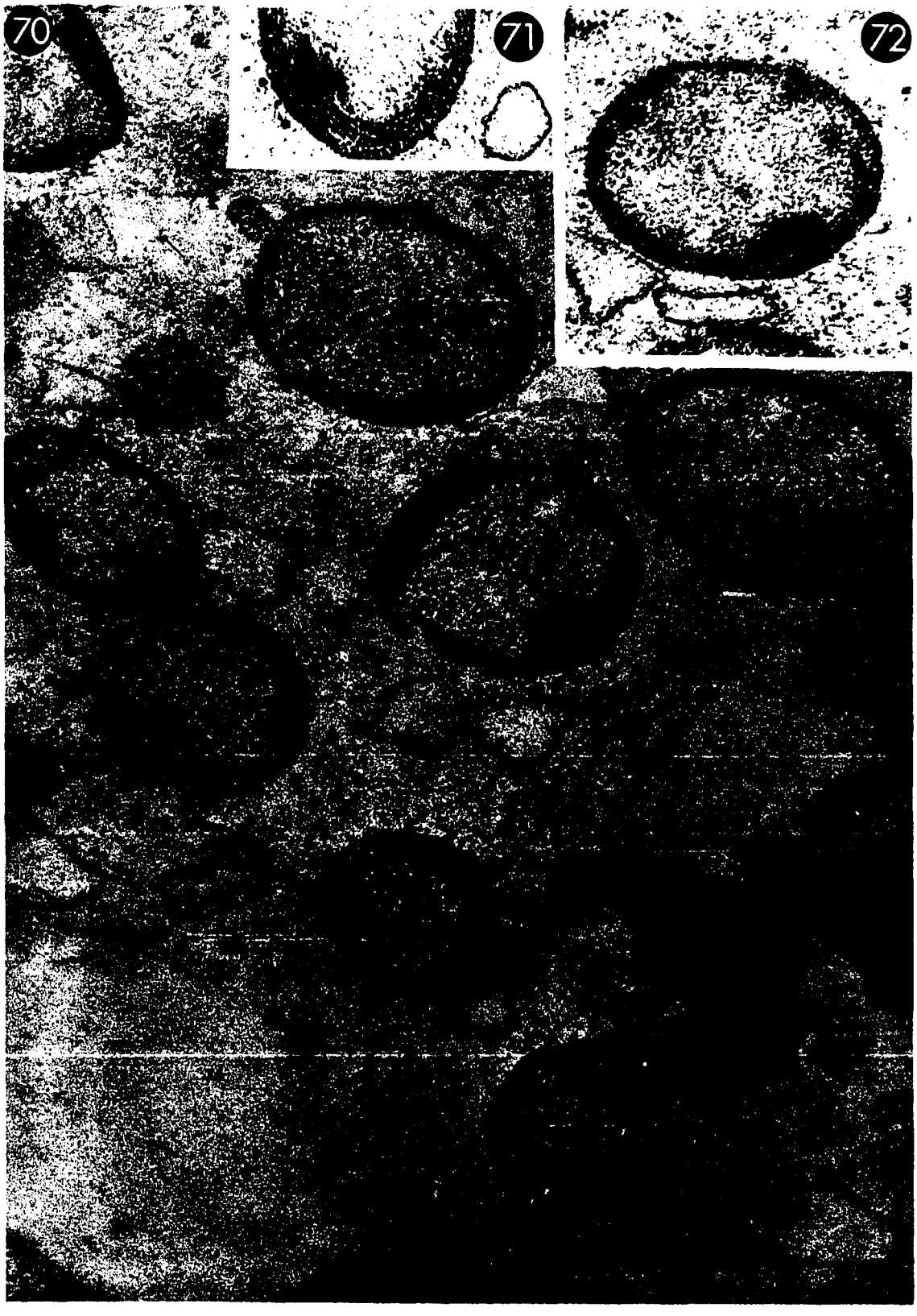

70
71
72

PLATE XXXI

Figures 73 and 74. As antrum production continues in the tertiary follicle granulosa, an apocrine and/or holocrine process is involved. The granulosa cells become further separated. They characteristically remain attached by desmosomes (D) and the areas surrounding these structures.

34,000X


73

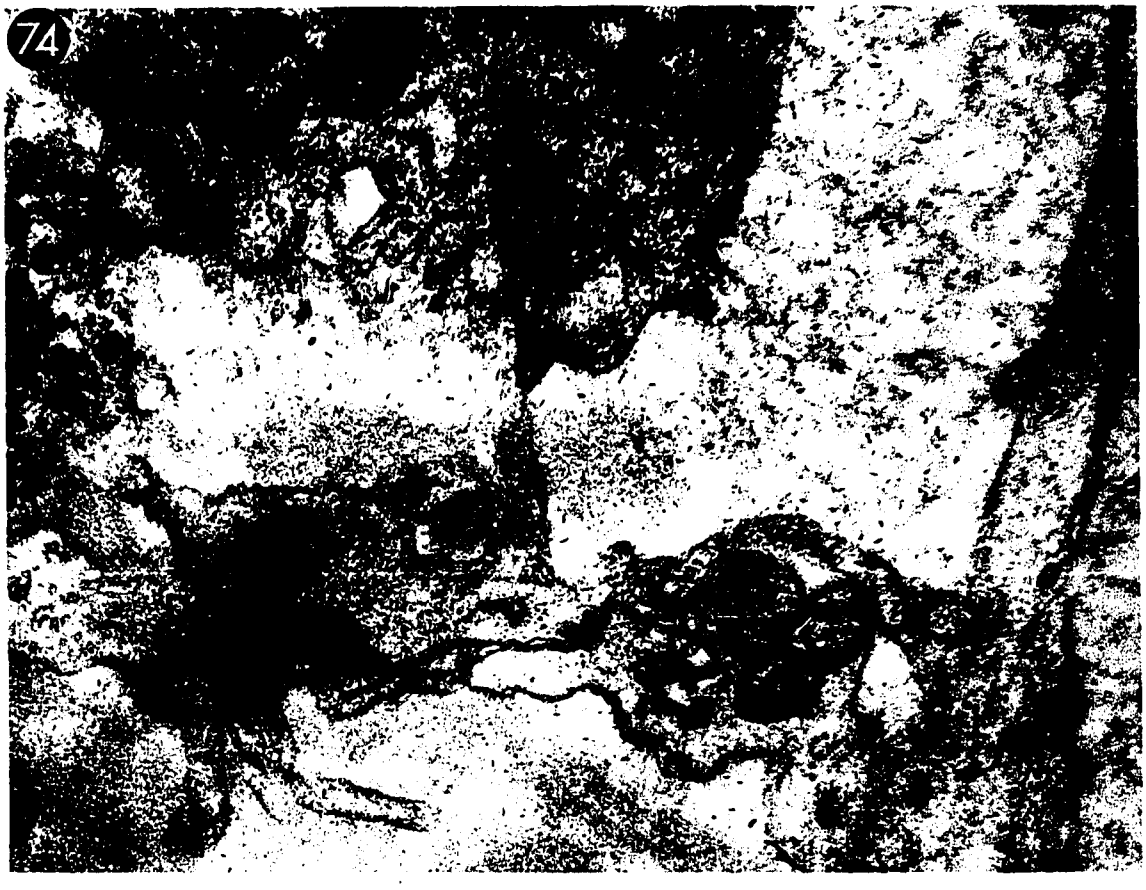

74

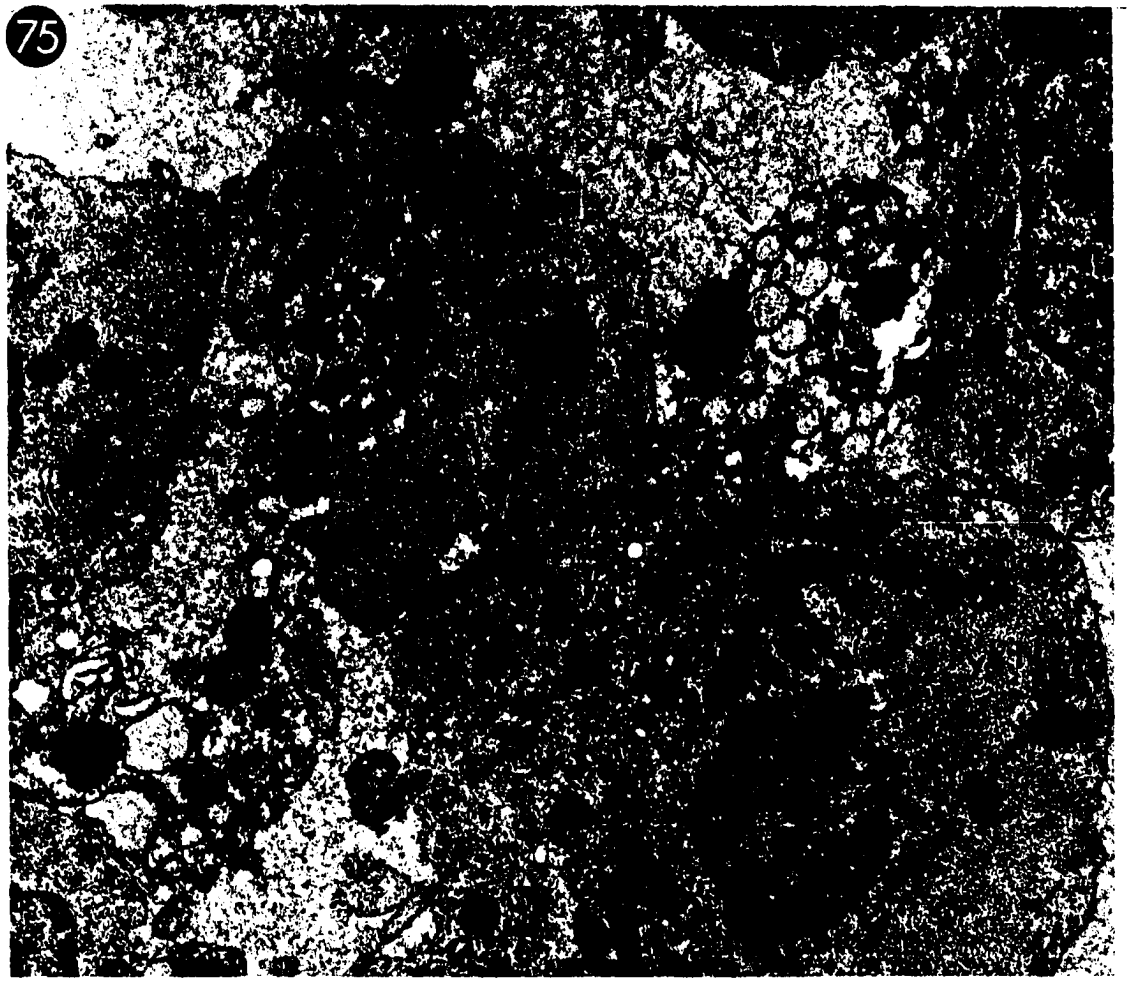
PLATE XXXII

Figure 75. Portions of granulosa cells of a tertiary follicle. The cells are actively involved in the final stages of antrum production, which entails apocrine and/or holocrine processes. The presence of a pyknotic nucleus (Pyk. N) points into the direction of the holocrine process.

Large aggregates, sometimes membrane bound, of vacuolated and partially digested organelles and cell components are seemingly shed into the existing antrum spaces. These bundles of cell debris, take on different staining. Lying in the antral spaces, they have been referred to as Call-Exner Bodies.

Figures 76 and 77. More examples of these socalled Call-Exner Bodies.

10,600X


75


76


77

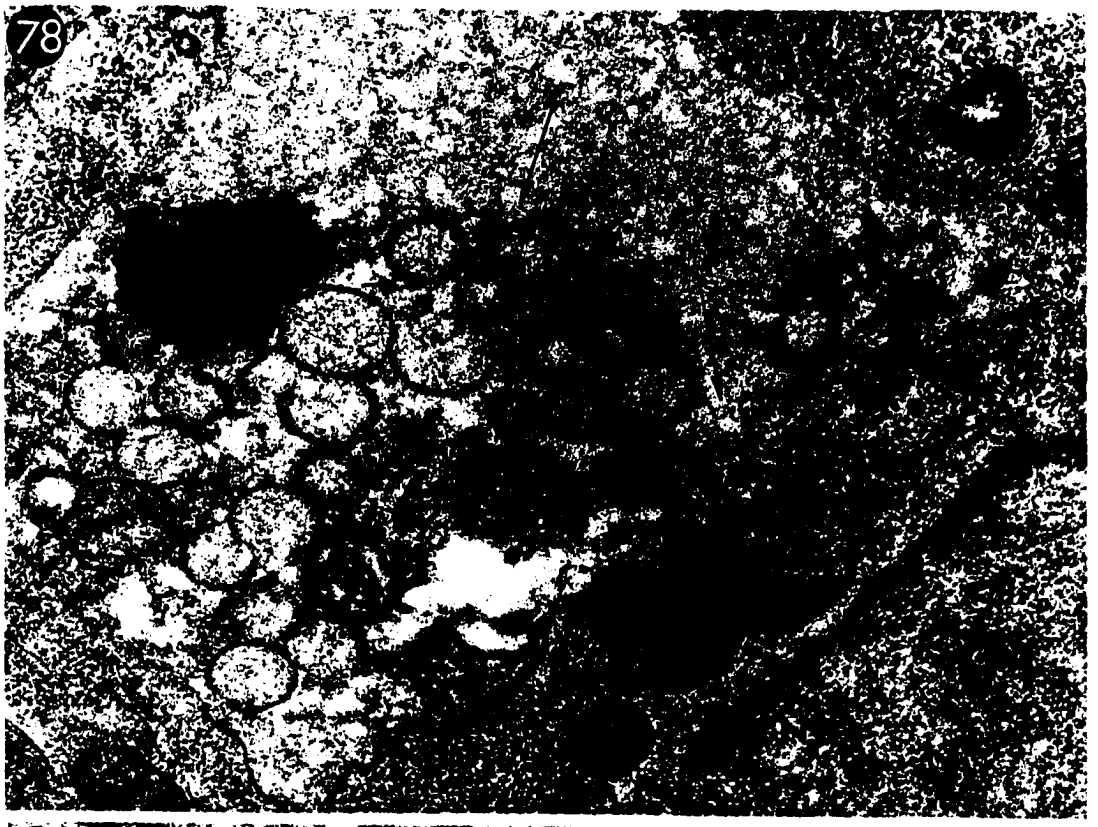
PLATE XXXIII

Figure 78. The socalled Call Exner Bodies, eventually break apart. The organelles undergo self digestion and blend with the follicular fluid.

Figure 79. A field of nuclear pores (FNP) can be observed within the cellular debris area. This points to a holocrine process involved in the formation of the secondary follicular fluid.

Note the mitochondria in various stages of disintegration.

26,000X


78

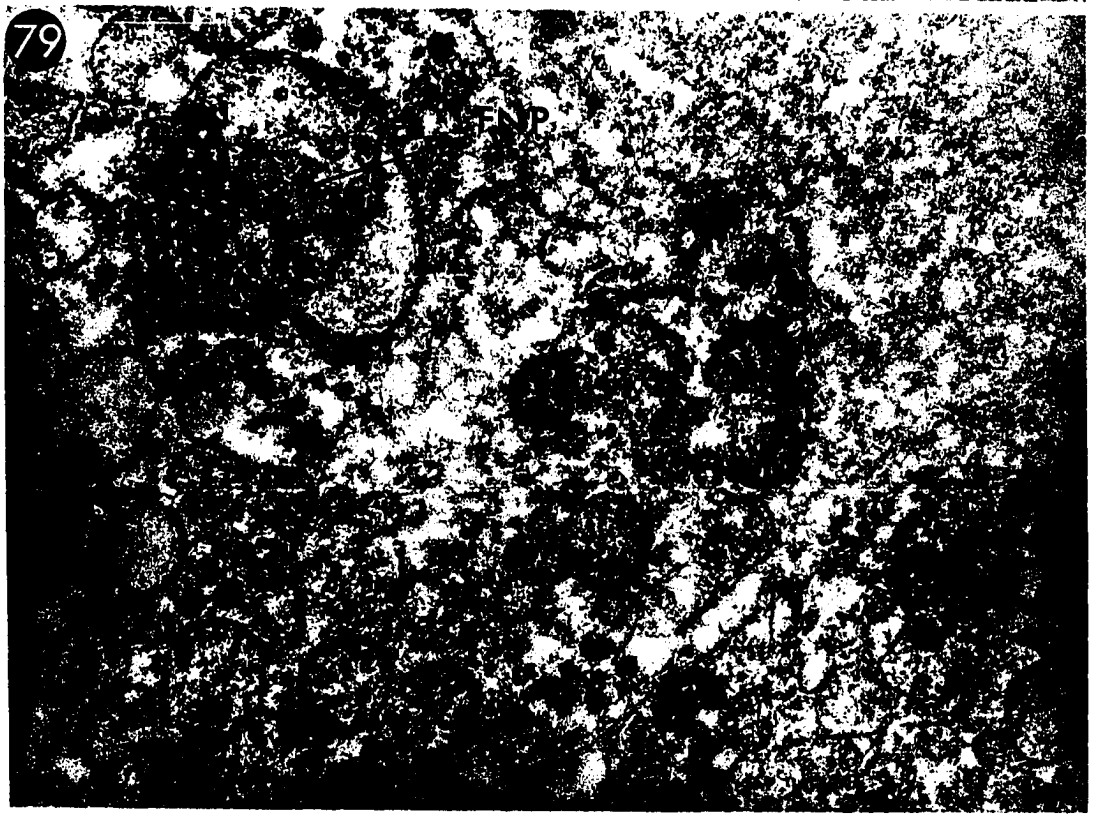

79
N P

PLATE XXXIV

Figures 80, 81, 82, 83, 84, 85, 86. Portions of the granulosa cells become sequestered by membranes, forming the so-called cytosegrosomes (Cys) or autophagic vacuoles. Digestion of organelles occurs intracellularly, to form vacuoles. These figures trace the course of this process.

Note in Figure 86, the field of nuclear pores (FNP), attesting to the extent of whole cell destruction in this case.

26,000X

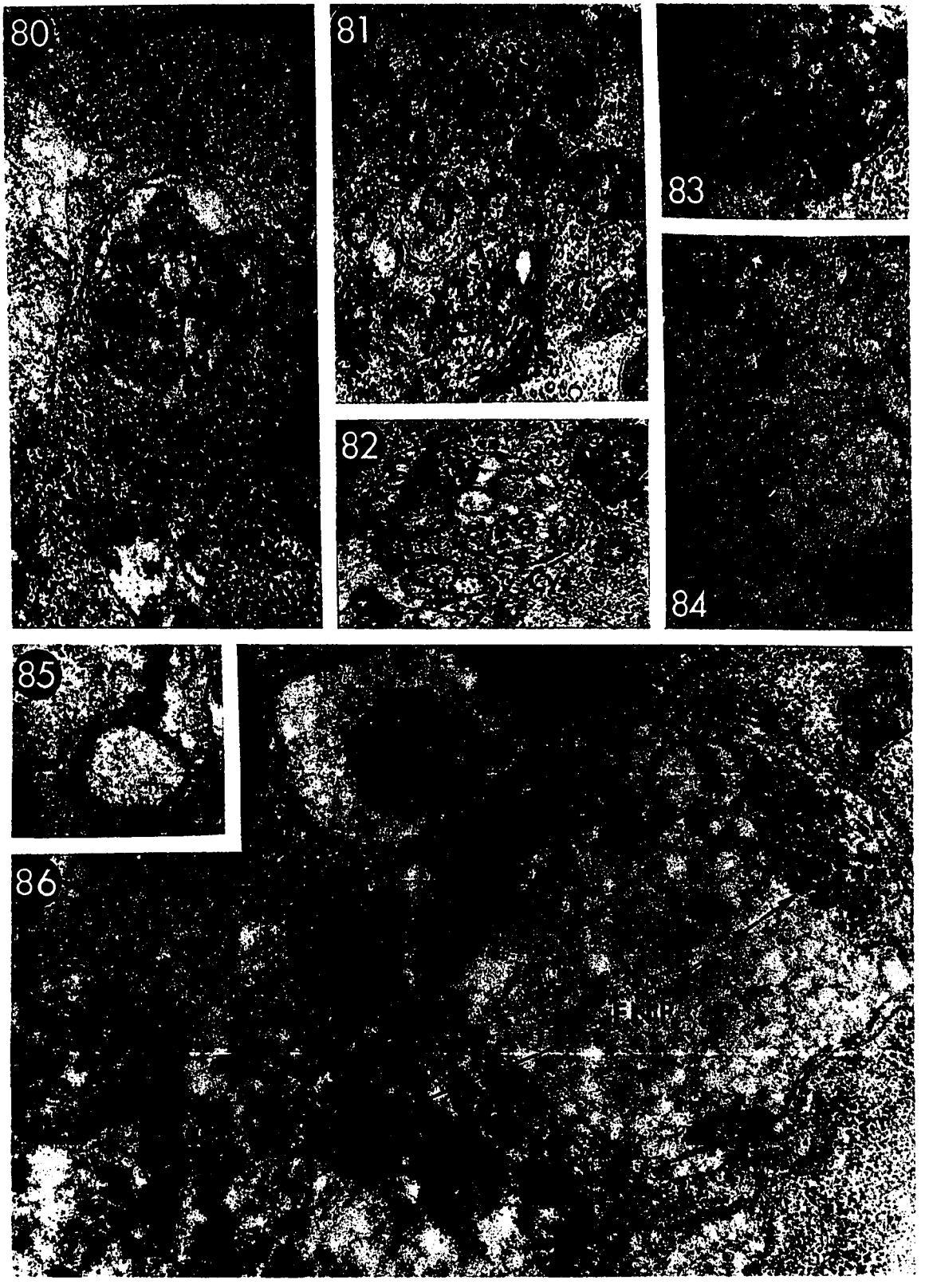
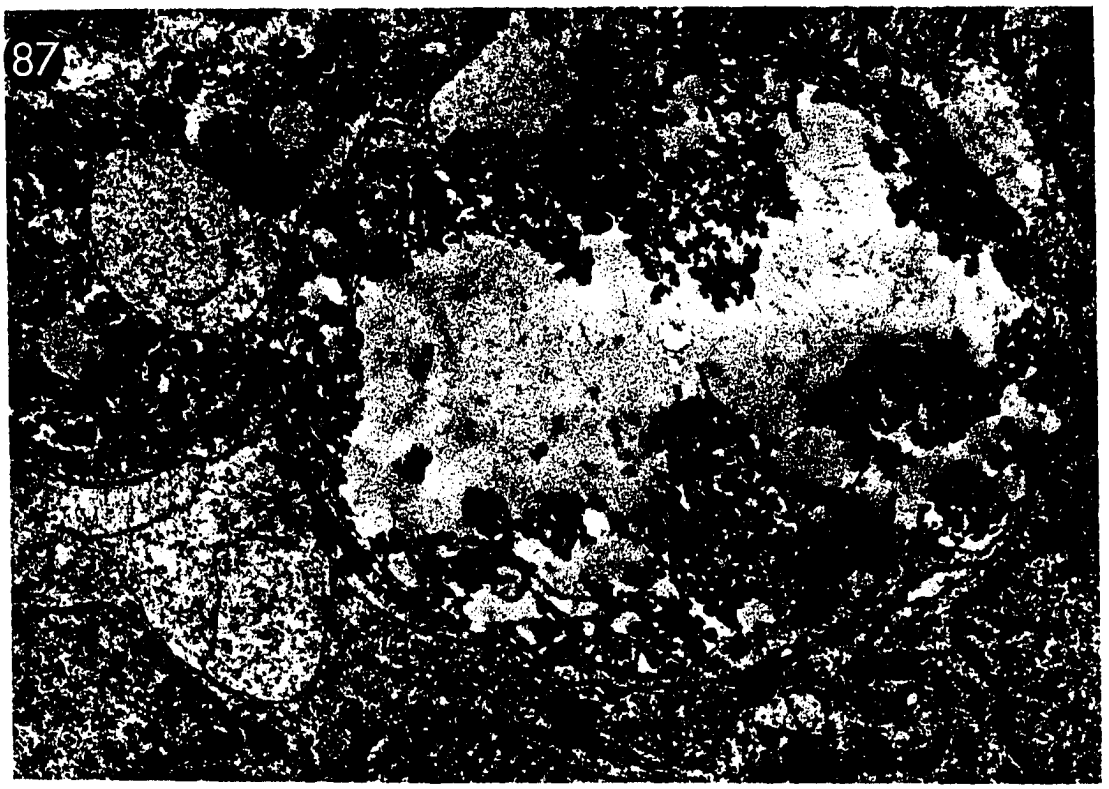

80
81
83
82
84
85
86

PLATE XXXV

Figure 87. A portion of a secondary follicle, showing granulosa cells undergoing atretic changes. Adjacent cells may respond to the atretic process in very different fashions, with some cells completely lysed and adjacent cells retaining much of their conventional structuring.
3,500X

Figure 88. Granulosa cells of a secondary follicle undergoing atretic transformations. Note the myelin-type formations and the accumulation of lipid material. Cells adjacent to the basement membrane sometimes appear more refractory to atretic changes.
8,600X


87

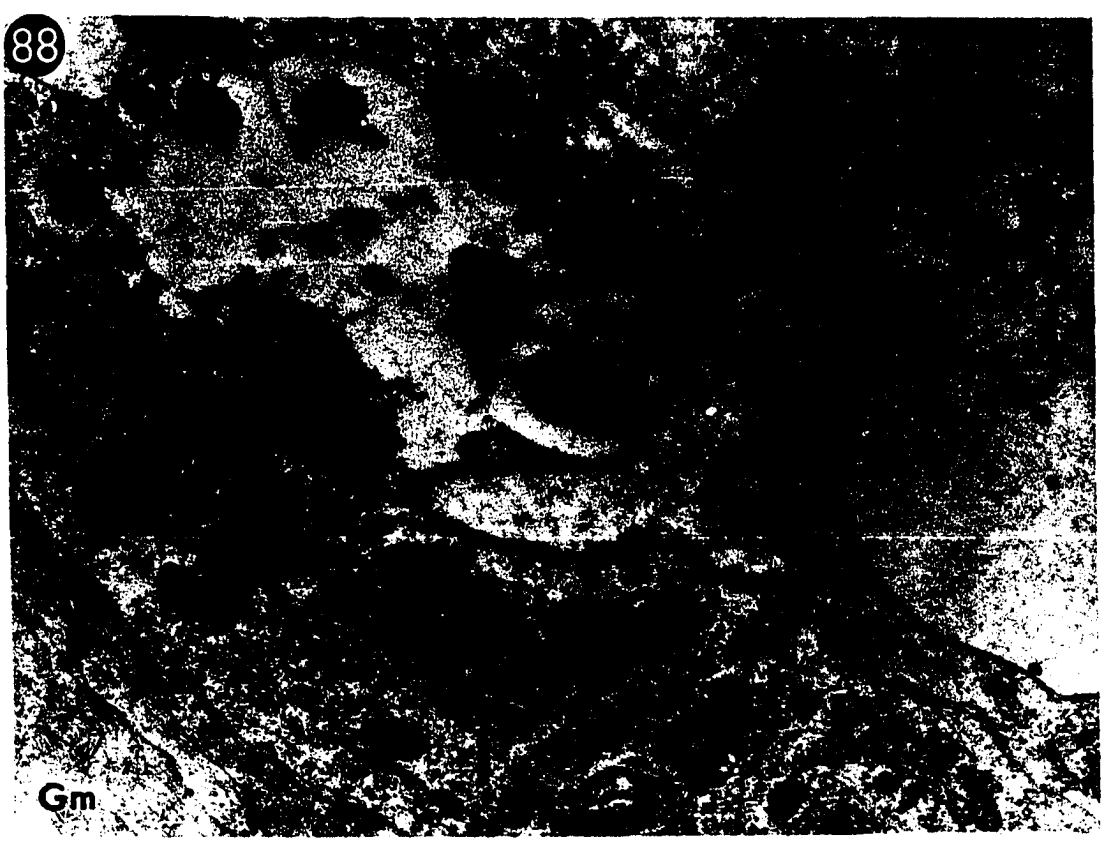

88
Gm

PLATE XXXVI

Plate 89. A portion of the granulosa of an atretic tertiary follicle. Note the corrugated basement membrane (Bm), the formation of the supra-basement membrane and the collagenic "glassy membrane" (Gm).

17,000X


89
Ld
Bm

PLATE XXXVII

Figure 90. A high magnification view of a helical formation of ribosomes (Ri) from the granulosa cell shown in Figure 91.
220,000X

Figure 91. A section of a granulosa cell from a tertiary follicle. Note the nucleus (N) and the double layered nuclear membrane (Nm). Vesicles (V) in the cytoplasm appear to extend to the basement membrane (Bm) and one such vesicle is deforming the basement membrane in an outward direction. Near the nucleus the helical formation of ribosomes is pointed out by the arrow.
46,500X

Figure 92. A vesicle of indistinct outline, seems to be moving inwardly through the basement membrane, deforming the plasma membrane of the granulosa cell (GC).
244,000X


90


92

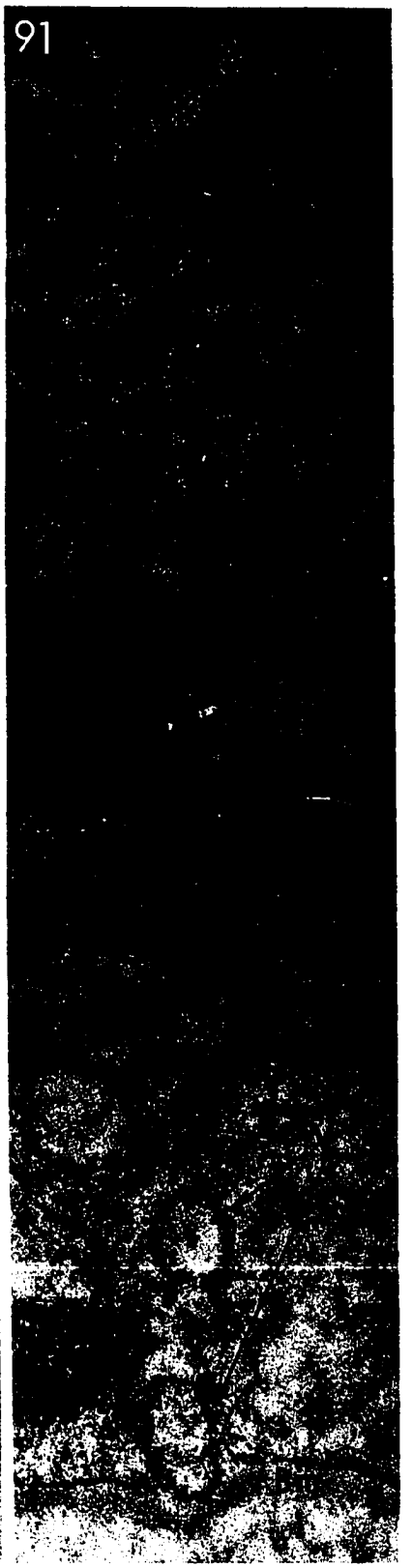

91

PLATE XXXVIII

Figure 93. An inward buckling of the basement membrane can be observed for a tertiary follicle. This is indicated by the arrows.

9,300X

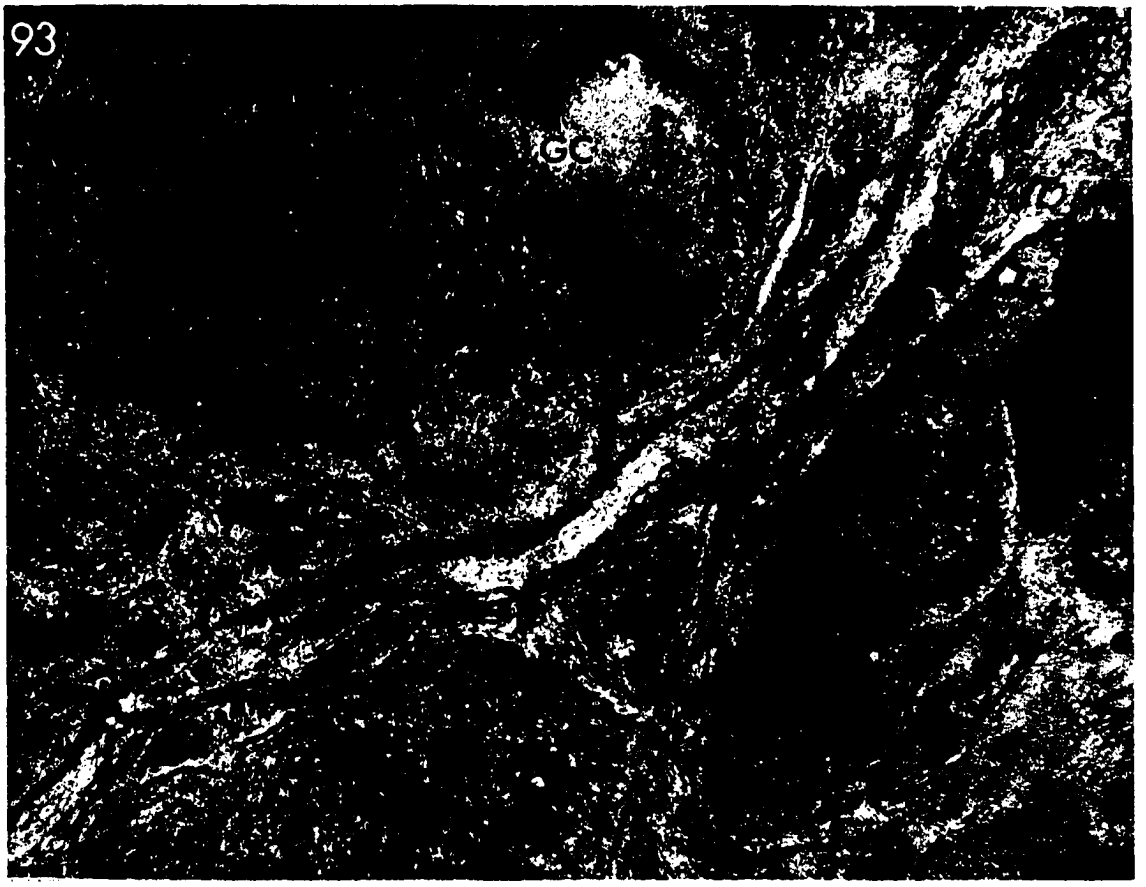
Figure 94. A magnified view of the area in Figure 93, designated by the arrows. The buckling of the basement membrane can be observed at higher magnification.

40,800X

Figure 95. A more advanced stage of basement membrane alteration. The basement membrane has assumed a corrugated appearance and has separated from its close alignment with the granulosa cells. The intervening material is referred to as "supra-basement membrane." It persists into the scar formation phases, together with the "glassy membrane" (Gm).

Note the adjoining thecal cells (TC).

5,800X


93
GC


94


95

PLATE XXXIX

Figure 96. Details of scar formation. Macrophages (Mac.) as well as fibroblasts (Fb) can be seen within the area of the "supra-basement membrane" (see Figure 95). The collagenic (Coll.) structure of the surrounding "glassy membrane" can be observed.
10,000X


96
Vac
Coll
IC

PLATE XL

Figure 97. The scar. The "supra basement membrane" has now been almost completely obliterated, leaving only remnants of itself and the surrounding basement membrane, within the scar. Fibroblasts (Fb) can also be found there. The scar now consists almost entirely of the collagenic "glassy membrane."

Note the surrounding interstitial cells (IC) and fibroblasts.

6,100X


97
IC
Fb