James C. Harvell, Jr. THE ULTRASTRUCTURE OF RAT OVARIAN INTERSTITIAL GLAND CELLS DURING PREGNANCY: EFFECTS OF DENERVATION (Under the direction of Dr. Hubert W. Burden) Department of Biology, April, 1979.

The fine structure of the interstitial gland was studied in control and denervated ovaries on Days 6, 10, 14 and 18 of pregnancy. Prominent fine structural features of control ovaries are as follows: At Day 6, nuclei are large and spherical and have peripheral heterochromatin. Mitochondria are numerous, often bizarre in shape, and have tubular cristae. Smooth endoplasmic reticulum is abundant. Golgi zones, osmiophilic lipid droplets, and gap junctions are prominent. At Day 10, nuclei are similar to Day 6. Mitochondria have both tubular and lamellar cristae, Golgi zones are compressed, and lipid droplets are less osmiophilic. At Day 14, nuclei are large, irregularly shaped and contain dense heterochromatin. Mitochondria are small, the Golgi complex extensive and lipid droplets electron lucent. At Day 18, nuclei are small and mitochondria have lamellated cristae. Lipid droplets are large and numerous. Fine structural features of denervated ovaries are as follows: At Day 6, nuclei are small and irregular with dense heterochromatin. Mitochondrial cristae are lamellated and tubular. Numerous annular gap junctions are present. Denervation at this stage of pregnancy results in increased release of lipid droplets from interstitial gland cells. At Day 10, nuclei are larger than Day 6 and spherical. The Golgi complex is dilated. Lipid droplets are osmiophilic and associate closely with mitochondria and agranular endoplasmic reticulum. At Day 14, cytoplasmic features include small mitochondria with lamellar cristae and numerous scattered, free

ribosomes. Intercellular space is pronounced. At Day 18, nuclei and mitochondria are reduced in size. Gap junctions are prominent, particularly between cells which border adrenergic nerve terminals. Collectively, these observations suggest that interstitial cells of controls are highly stimulated during the first half of pregnancy and undergo regression during the last half of pregnancy. Denervation at all stages of pregnancy studied, caused a qualitative and quantitative increase in the fine structural features which are characteristic of regressing steroid-secreting cells. Cutting the ovarian nerves during early pregnancy results in an augmented release of lipid droplets from interstitial cells. These responses of interstitial gland cells to denervation provide additional morphological evidence of a functional role for the adrenergic nerves in this ovarian compartment.

THE ULTRASTRUCTURE OF RAT OVARIAN INTERSTITIAL GLAND CELLS DURING PREGNANCY: EFFECTS OF DENERVATION

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OF RAT OVARIAN INTERSTITIAL GLAND CELLS

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THIS THESIS IS DEDICATED TO MY PARENTS

MR. AND MRS. JAMES C. HARVELL

AND MY BROTHER

MR. CECIL S. HARVELL

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INTRODUCTION

The mammalian ovary contains groups of glandular cells interspersed between follicular elements and luteal tissue. Collectively, these groups of glandular cells are referred to as the interstitial gland (Brambell, 1956; Guraya, 1973; Mossman and Duke, 1973*a*). Before puberty, interstitial gland cells are derived directly from the differentiation of fibroblast-like stromal cells (Davies and Broadus, 1968; Mossman and Duke, 1973*a*). After the initiation of cyclic activity, most interstitial gland is derived from hypertrophy and luteinization of the theca interna of atretic follicles (Guraya, 1966*a*, *b*; 1967*a*, *b*, *c*; 1968*a*, *b*; 1972*a*; Guraya and Greenwald, 1964*a*, *b*; 1965).

Interstitial gland cells have been recognized in the mammalian ovary for over a century (Pflüger, 1863) and are invariably present in the ovaries of young and adult mammals with some variation in the amount and distribution (Brambell, 1956; Harrison, 1962; Mossman *et al.*, 1964; Mossman and Duke, 1973*a*; Baille *et al.*, 1966; Guraya, 1966*a*; 1967*a*; 1968*a*). Since the maturing follicle and the corpus luteum display conspicuous cyclic alterations, the morphological and functional significance of the less conspicuous interstitial gland has been largely overlooked by anatomists and reproductive physiologists (Lawrence *et al.*, 1977; Guraya, 1979). Nevertheless, ovarian interstitial gland cells show changes in both quantity and quality which may be correlated with the reproductive age and the physiological state of the ovary (Brambell, 1956; Harrison, 1962; Guraya, 1973).

Interstitial gland cells possess fine structural, histochemical and biochemical features of steroid-secreting cells (Guraya, 1973;

Mossman and Duke, 1973*a*, *b*). These cells have an extensive network of agranular endoplasmic reticulum and polymorphic mitochondria with cristae which are predominately tubular (Fawcett *et al.*, 1969; Davies and Broadus, 1968; Christensen and Gillim, 1969; Gillim *et al.*, 1969; Guraya, 1971). Histochemical features include an abundant distribution of lipoproteins, and numerous cholesterol-containing lipid droplets of variable size (Guraya, 1971). The well-developed agranular endoplasmic reticulum of ovarian interstitial gland cells probably derives from the many diffuse lipoproteins evident in the cytoplasm (Guraya, 1971 1972*a*). Morphological and biochemical studies indicate that the membranes of the smooth endoplasmic reticulum play an important role as active sites for enzymes involved in the biosynthesis of steroid hormones (Christensen, 1965; Christenson and Fawcett, 1966; Goodman *et al.*, 1968; Fawcett *et al.*, 1969; Gillim *et al.*, 1969; Christenson and Gillim, 1969; Guraya, 1968*d*, *f*; 1969*c*).

The adrenergic innervation of the rat ovary is generally sparse (Owman and Sjöberg, 1966; Burden, 1972; Lawrence and Burden, 1976) except for interstitial gland cells which receive numerous preterminal and terminal adrenergic fibers (Burden, 1972; Lawrence and Burden, 1976). The significance of the adrenergic innervation to this compartment is not known; however, it has been suggested that local neural stimuli may result in modulation of the structure and function of this ovarian component (Lawrence and Burden, 1976).

The fine structure of the ovarian interstitial gland cells responds to gonadotrophins. Ultrastructural alterations occurring subsequent to hypophysectomy in this ovarian component include a

reduction in nuclear and cytoplasmic volume, a reduction in the extent of agranular endoplasmic reticulum and the Golgi complex, reorganization of mitochondrial cristae from tubular to lamellar forms, and a loss of lipid droplets (Carithers and Green, 1972*a*, *b*; Capps *et al.*, 1978; Guraya, 1979). Treatment of interstitial gland with various gonadotrophins such as human chorionic gonadotrophin (HCG), pregnant mare serum gonadotrophin (PMSG), and luteinizing hormone (LH) affects a reversal of these ultrastructural changes (Carithers and Green, 1972*b*; Flérkó *et al*, 1967).

During pregnancy, both quantitative and qualitative morphological changes are observed in the fine structure of the interstitial gland (Lawrence *et al.*, 1977). Highly stimulated steroidogenic cells appear active until Day 14 of pregnancy. At Day 14, regressive morphological changes in the ultrastructure of the interstitial gland are noted with marked regression characteristic of Day 18 cells (Lawrence *et al.*, 1977). As pregnancy progresses, increases in ovarian interstitial gland norepinephrine and apparent increases in the density and intensity of interstitial fluorescent adrenergic nerves occurs (Lawrence and Burden, 1976).

The presence of a network of monamine-containing fluorescent nerves in the interstitial gland (Unsicker, 1970; Burden, 1972; Unsicker, 1974; Svensson *et al.*, 1975; Lawrence and Burden, 1976) suggests that, in addition to gonadotrophic hormones, nerves may influence the structural and functional integrity of interstitial gland cells. Therefore, the purpose of this research was to study the morphology of ovarian interstitial gland cells at the fine structural level subsequent to

denervation at different stages of pregnancy.

METHODS AND MATERIALS

Animals. Nulliparous female Sprague-Dawley rats weighing 165 to 225 g were housed in a room with a 14-h light (0500 to 1900h) and 10-h dark regimen. Estrous cycles were monitored daily between 0800 h and 1000 h by microscopic evaluation of aqueous vaginal lavages. Females in proestrus were housed with males of proven fertility. The day on which spermatozoa were found in the vaginal lavage was designated as Day 1 of pregnancy.

Denervations. Animals were anesthetized with chloral hydrate (300 mg/kg) and laparotomized on Days 2, 6, 10 and 14 of pregnancy for denervation or sham surgery. Animals were sacrificed 96 hours later, *viz.* on Days 6, 10, 14 and 18 of pregnancy. Figure 1 details the reproductive organs and reference structures in the rat including innervation and blood supply to the ovary. The ovarian plexus and vessels enter via the mesovarium. The left ovary of some animals was denervated by sectioning the mesovarium and the superior ovarian nerve (Burden, 1979) with a Birtcher Hyfrecator (Fig. 1). Since there are extensive anastomoses between utero-ovarian and ovarian vessels (Shröder, 1978), sectioning of the ovarian artery does not adversely affect the blood supply to the ovary (Hill, 1962). The right ovary was lifted with forceps, replaced in the abdominal cavity, and served as a control. In other animals, the left ovarian adrenergic nerves were destroyed by injection of 6-hydroxydopamine (6-HD; 10 mg in 0.05 ml 0.9% NaCl containing 0.3% ascorbic acid) into the periovarian bursa (Fig. 1). This treatment effectively eliminates all sympathetic nerve terminals (Malmfors and Thoenen, 1971). The

right periovarian bursa was injected with the vehicle and served as a control. All denervations were performed 4 days prior to sacrifice.

Electron Microscopy. For fixation, a thoracotomy was performed. A trocar was placed through the left ventricle and into the ascending aorta. The animal was perfused preliminarily with cold (4° C) Earle's balanced salt solution. After a brief wash with the salt solution, the animal was perfused with cold 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3). The ovaries were dissected, minced and immersed in fixative to achieve a total fixation time of one and one-half to two hours. After a wash in phosphate buffer, the tissue was post-fixed in 1% osmium tetroxide, stained en bloc with uranyl acetate (Karnovsky, 1967), dehydrated rapidly through a series of graded alcohols, and embedded in Durcupan. Thin sections (800-1000Å) were cut on a Porter-Blum MT 2-B Ultramicrotome and stained with lead citrate for 5-7 minutes (Reynolds, 1963). The tissues were viewed with a Philips EM 201.

Evaluation. Control ovaries were initially evaluated for proper fixation. In a situation where questionable fixation was evident, the entire sample including the experimental ovary was eliminated from the study. On Days 6, 10, 14 and 18 of pregnancy, 2-8 tissue blocks were sampled. A minimum of 25 electron micrographs were used to evaluate the possible effects of denervation on the morphology of ovarian interstitial gland cells. Electron micrographs of experimental ovaries were coded, read blind, and then subsequently compared with micrographs of control ovaries. Measurements of the diameter of

nuclei and mitochondria of cells from the various groups were made. For purposes of measurement, nuclei and mitochondria were assumed to be in cross section when a uniform double membrane could be discerned around the perimeter of these organelles. Numerical data were analyzed by one-way analysis of variance. Subsequently, group comparisons between days of pregnancy were analyzed by the Neuman-Keul analysis (Weiner, 1972).

RESULTS

Quantitative and qualitative changes are observed in the fine structure of interstitial gland cells as pregnancy progresses. The first half of pregnancy is characterized by nuclear and cytoplasmic hypertrophy, whereas during the last half of pregnancy, ultrastructural features suggest a functional regression of this ovarian component. All membranous organelles and inclusions show some degree of response during the course of pregnancy.

Ultrastructure of interstitial gland cells from control ovaries on Day 6 of pregnancy.

Interstitial gland cells from control ovaries on Day 6 of pregnancy possess nuclei which are large (Table 1) and spherical. The nuclei are characterized by heterochromatin along the inner membrane. A distinct, eccentrically located nucleolus is usually present (Fig. 2). Mitochondria are small (Table 1) and numerous. Most mitochondria appear rod-shaped, although some are attenuated and others appear cup-shaped or annular. The annular mitochondria encircle one another and form whorls of membranes within the cytoplasm (Fig. 3). Mitochondria have dense matrices packed with tubular cristae; lamellar forms are infrequent. Most matrices contain small, dense granules. The Golgi complex is large and consists of dilated cisternae. Profiles of Golgi are usually peripherally located, close to the plasmalemma (Fig. 4). Lipid droplets are numerous and most appear uniformly osmiophilic, although some droplets are partially leached (Figs. 2, 3). Individual lipid droplets are large and closely associated with multilaminar

profiles (Fig. 4). Agranular endoplasmic reticulum dominates the cytoplasm of steroidogenic interstitial gland cells (Fig. 3) and associates closely with lipid droplets (Figs. 2, 3). Scattered, free ribosomes and few polysomes are present. Specialized membranous contacts (gap junctions or nexuses) are common (Fig. 2). Filopodia interdigitate within the intercellular space (Fig. 3).

Ultrastructural changes in interstitial gland cells from control ovaries as pregnancy progresses.

The nuclei of interstitial gland cells at Day 10 of pregnancy in control ovaries are similar in size and shape to those observed at Day 6 (Table 1). During this stage of pregnancy, mitochondria are increased in size ($p < 0.05$) from those observed on Day 6 (Table 1). Mitochondria are numerous and rod-shaped or elongate. Cristae are both tubular and lamellar. The Golgi complex now consists of stacks of thin saccules close to membrane bound osmiophilic lipid droplets. Dense, whorled membranous bodies containing free ribosomes, vesicles and/or a single mitochondrion are present (Fig. 12).

On Day 14, the nuclei of interstitial gland cells are smaller ($p < 0.05$) than those observed in early pregnancy (Table 1) and highly irregular in shape. The amount of nuclear heterochromatin has greatly increased and clusters of perichromatin granules are present throughout the nucleoplasm. Mitochondria are smaller ($p < 0.05$) than on Day 10 of pregnancy (Table 1). The Golgi zones are extensive, with widely dilated stacks of saccules and associated vesicles. Lipid droplets are smaller than in Day 6 (Fig. 16). Most droplets are electron lucent and display a tendency toward dissolution and fusion. Cilia, with

companion basal bodies, often project into the intercellular space (Fig. 17).

Interstitial gland cells at Day 18 of pregnancy have nuclei which are small (Table 1). Mitochondria are larger than on Day 14 ($p < 0.05$). The mitochondrial matrix is characterized by lamellar cristae. An increase in the number and size of lipid droplets occurs. Lipid droplets are slightly osmiophilic (Fig. 20). Many areas formerly occupied by masses of convoluted tubules of agranular endoplasmic reticulum are now occupied by large lipid droplets. Distinct profiles of agranular endoplasmic reticulum are prominent (Fig. 21). The Golgi complex is reduced in complexity and fewer profiles are present. During this stage of pregnancy, there is frequently close association between large lipid droplets, sac-like profiles of agranular endoplasmic reticulum and large elongate mitochondria near the surface membranes of interstitial gland cells (Fig. 20).

Ultrastructure of interstitial gland cells from experimental ovaries on Day 6 of pregnancy.

The nuclei are small (Table 1) and irregular with heterochromatin around the nuclear perimeter. Mitochondria are small (Table 1) and generally rod-shaped. Cristae of the mitochondria are predominately lamellar and reduced in number. Golgi cisternae are flattened and numerous vesicles are apparent. Lipid droplets are predominately osmiophilic and exhibit a tendency toward dissolution and fusion (Fig. 5). Lipid droplets are frequently in close association with the surface membranes of the cells (Fig. 6). In some cells, there is an apparent dissolution of the thin strand of cytoplasm between

the membrane surrounding the droplet and the plasmalemma (Figs. 7, 8). In such cases, the lipid droplet appears to be released into the intercellular space (Fig. 8). This space has numerous "secretory ghosts" that apparently derive from the remnants of discharged lipid droplets (Fig. 9). Few profiles of agranular endoplasmic reticulum are present except in close proximity to lipid droplets. Annular gap junctions with membranes of pentalaminar configuration are numerous (Figs. 10, 11). Polysomes are common but many ribosomes are dispersed singly (Fig. 10).

Ultrastructural changes in interstitial gland cells from experimental ovaries as pregnancy progresses.

Interstitial gland nuclei from denervated ovaries on Day 10 of pregnancy are larger than nuclei from denervated ovaries on Day 6 of pregnancy (Table 1, Fig. 13). Mitochondria are larger ($p < 0.05$) than on Day 6 (Table 1) and their cristae are both lamellar and tubular (Fig. 14). The Golgi complex consists of dilated stacks with associated vesicles (Figs. 13, 15). The profiles of agranular endoplasmic reticulum are often dilated. Lipid droplets associate closely with mitochondria and agranular endoplasmic reticulum (Fig. 13). Occasionally, the mitochondrial membrane and the thin, single droplet membrane breakdown and the lipid is discharged on cristae and into adjacent cytoplasm (Fig. 15). Numerous polysomes are present in the cytoplasm.

On Day 14 of pregnancy, nuclei are similar in size to those of Day 10 (Table 1). Mitochondria are smaller ($p < 0.05$) than those on Day 10 (Table 1), have lamellar cristae, and occupy peripheral regions of most cells (Figs. 18, 19). Lipid droplets increase in

number and seem to polarize toward one end of the cell (Fig. 18). There is little tendency in these cells for lipid droplets to dissolve and fuse. Scattered, free ribosomes are profuse throughout the cytoplasm (Fig. 19). Intercellular space is pronounced (Fig. 18).

By Day 18 of pregnancy, nuclei are smaller ($p < 0.05$) than those in Day 6 interstitial cells (Table 1). Mitochondria are smaller ($p < 0.05$) than those in Day 10 interstitial cells (Table 1) and have lamellate cristae. Lysosome-like bodies are present. Lipid droplets are small and electron lucent. Gap junctions are frequently seen between interstitial gland cells. Polysomes and free ribosomes are present. The amount of intercellular space is reduced (Fig. 22).

Table 1. Diameter (μm, mean ± s. e. m.) of nuclei and mitochondria from control and denervated interstitial gland cells on different days of pregnancy.

	Treatment	Day 6	Day 10	Day 14	Day 18
Nuclei[1]	Control	9.67 ± 0.75^{a}	11.91 ± 1.44^{a}	4.72 ± 0.39^{b}	5.27 ± 0.39^{a}
	Denervated	6.38 ± 0.59^{a}	9.72 ± 0.89^{a}	$9.29 \pm 0.87^{a*}$	$3.17 \pm 0.14^{b\dagger}$
Mitochondria[2]	Control	0.42 ± 0.06^{a}	0.93 ± 0.04^{b}	0.24 ± 0.01^{a}	0.63 ± 0.02^{b}
	Denervated	0.31 ± 0.03^{a}	0.68 ± 0.04^{b}	0.27 ± 0.02^{a}	$0.27 \pm 0.01^{a*}$

Means in same horizontal row not bearing identical superscripts differ significantly ($p < 0.05$).

[1] $n = 10$

[2] $n = 30$

*Significantly ($p < 0.05$) different from controls

†Not significantly different from controls at $p < 0.05$
Significantly different from controls at $p < 0.1$

DISCUSSION

In the present study, the fine structure of the interstitial gland cells in the ovaries of control animals changes both qualitatively and quantitatively throughout pregnancy. During the first half of pregnancy, the nucleus and cytoplasm hypertrophy indicating a high degree of stimulation. During the latter half of pregnancy, the fine structural changes suggest a functional regression of interstitial gland cells.

On Day 6 of pregnancy, interstitial cells from intact ovaries possess an enlarged, spherical nucleus which is characteristic of active steroidogenic cells. A reduced, peripherally disposed heterochromatin present along the inner nuclear membrane and a distinct, eccentric nucleolus which persists into Day 10 are indicative of protein synthesis (Carithers and Green, 1972*b*; Lawrence *et al.*, 1977). In contrast, nuclei of interstitial gland cells from denervated ovaries during early pregnancy are small and irregular and contain large aggregates of heterochromatin. The coarse pattern of heterochromatin may indicate a reduced metabolic activity.

Nuclei of interstitial cells from both control and denervated ovaries of late pregnancy are decreased in volume and possess conspicuous peripheral heterochromatin and perichromatin granules. Similar nuclear features were reported in regressing interstitial cells subsequent to hypophysectomy (Carithers and Green 1972*a*). In this case, nuclear regression was more rapid than cytoplasmic regression (Carithers and Green, 1972*a*).

Mitochondria exhibit a characteristic fine structure in most steroidogenic cells and have been implicated in particular steps in the biosynthesis of steroids by cell fractionation studies (Christensen and Gillim, 1969). During early pregnancy, mitochondria in control tissues are often large and bizarre in shape with an electron dense matrix and tubular cristae. In contrast, mitochondria in denervated ovaries at this time are smaller with predominately lamellar cristae. Presumably, the organization of cristae into tubular structures greatly increases the effective surface area for energy-producing oxidations and important conversions in steroidogenesis (Carithers and Green, 1972b)

In late pregnancy, mitochondria from intact and denervated ovaries become small and rod-like with a dense matrix and lamellate cristae. Similar mitochondrial forms have been described in rat interstitial gland cells which have undergone regression following hypophysectomy (Carithers and Green, 1972*a*; Carithers, 1976; Capps *et al.*, 1978).

During the first half of pregnancy, agranular endoplasmic reticulum is abundant in control ovaries. Such large increase in membrane surface area results from hypertrophy of the diffuse lipoproteins or tubular smooth endoplasmic reticulum (Guraya, 1976a; 1979). Numerous enzymes which participate in steroid secretion are associated with the endoplasmic reticulum (Christensen and Gillim, 1969). Thus, the sites available for steroidogenesis are greatly increased.

Throughout pregnancy, agranular endoplasmic reticulum is much less in denervated ovaries. The few distinct profiles of smooth

reticulum are mainly in close association with cytoplasmic lipid droplets. By Day 14 of pregnancy, agranular endoplasmic reticulum is virtually absent from the interstitial gland of denervated ovaries. Christensen (1975) has investigated the sequential quantitative involvement of various organelles and critical enzymes in the synthesis of steroid hormones. In confirming the work of others (Fawcett *et al.*, 1969; Christensen and Gillim, 1969; Guraya, 1971, 1976*a*, *b*; Neaves, 1975), he correlated the presence and distribution of diffuse lipoproteins or agranular endoplasmic reticulum, mitochondria and lipid droplets with intense activity of enzymes known to be involved in steroidogenesis. The results of the present study show that interstitial gland cells from ovaries denervated during pregnancy contain substantially less agranular endoplasmic reticulum and it is suggested that steroidogenic activity may be decreased.

The increase in size and complexity of the Golgi complex through Day 14 of pregnancy in control ovaries suggests that this organelle may perform a significant role in steroidogenesis. Carithers and Green (1972*a*) reported a rapid increase in the size and number of Golgi lamellae and vesicles 48 hours following treatment of hypophysectomized rats with pregnant mare serum (PMS). Proliferation of Golgi vesicles and lamellae of interstitial gland after treatment with luteinizing hormone (LH) has also been noted (Carithers, 1976). Long and Jones (1967) have suggested that Golgi may be a site of steroid conjugation. As well, later steps in the steroidogenic pathway, classically assigned to smooth endoplasmic reticulum, may, in fact, occur in the Golgi zone since Golgi tubules are often indistinguishable from neighboring

profiles of smooth reticulum (Carithers and Green, 1972*b*). At Day 18 of pregnancy in control tissues, however, the extent of the Golgi complex is reduced. In denervated ovaries from early pregnancy, also, diminution of the vesicular elements of the Golgi complex is noted. These latter observations of the Golgi are characteristic of regressing steroidogenic interstitial gland cells (Carithers and Green, 1972*b*; Capps *et al.*, 1978).

Interstitial gland cells of intact ovaries from Day 6 through 10 of pregnancy are characterized by the presence of numerous lipid droplets. The matrix of the lipid droplets varies in osmiophilia, although during early pregnancy there is a tendency for all droplets within a given cell to be homogenously electron dense. Osmiophilic density may reflect variability in content of the different droplets. Since osmium tetroxide used in post-fixation is specific in its reaction with double bonds, a pale droplet suggests the presence of lipids with relatively saturated fatty acids or more cholesterol (Christensen and Gillim, 1969). Other factors such as the kind of buffer used with the fixative, or the extent of the lipid extraction during tissue preservation may also determine the density of the lipid matrix (Bjersing, 1967; Christensen and Gillim, 1969). These latter factors were carefully controlled in this study however, so that different lipid droplet densities may indicate different lipid droplet composition.

During early pregnancy, the individual lipid droplets of interstitial gland cells from control tissues are closely associated with agranular endoplasmic reticulum, mitochondria and/or Golgi zones. In denervated ovaries, there is dissolution of both the droplet-

cytoplasmic interface and the mitochondrial membrane. In this manner, lipid droplet contents are emptied into the surrounding cytoplasm. Based on ultrastructural and biochemical data, it has been hypothesized that a high degree of exchange occurs between lipid droplets, mitochondria, agranular endoplasmic reticulum and Golgi zones (Bjersing, 1967; Rhodin, 1971). Christensen and Gillim (1969) have postulated that this association may permit deposition or mobilization of lipid. In spite of the fact that open systems of communication do not exist between these organelles, the close association observed between their membranes makes it plausible that exchange of molecules of intermediate products in the pathways of steroid biosynthesis occurs.

In the interstitial gland cells of denervated ovaries on Day 6 of pregnancy, lipid droplets are in close association with the plasmalemma. In some cells, the thin strand of cytoplasm present between the droplet boundary membrane and the surface membrane opens to the intercellular space and the contents of the lipid droplet are released. The intercellular space has numerous "secretory ghosts" apparently derived from the remnants of discharged lipid droplets. The release of lipid droplets observed in interstitial gland cells under these experimental conditions is similar to the endoplasmocrine secretion reported by Rhodin (1971) in the adrenal cortex following stimulation with adrenocorticotrophic hormone. Thus, it appears that denervation during early pregnancy causes increased release of lipid droplet contents from ovarian interstitial gland cells.

In control and denervated ovaries from late pregnancy, lipid droplets in the interstitial gland are numerous. It has been suggested

that cells which are more active in steroid biosynthesis show fewer and/or smaller lipid droplets and that relatively inactive cells accumulate lipid droplets (Guraya, 1967*a*; 1976*a*). Carithers and Green (1972*a*) noted massive accumulations of large lipid droplets in regressed interstitial cells following hypophysectomy. Reduction in the number of lipid droplets occurs in rabbit interstitial gland following administration of HCG (Guraya, 1976*a*; 1973). Thus, the increase in number of lipid droplets observed in interstitial gland cells during late pregnancy suggests relative inactivity in these steroidogenic cells.

Throughout pregnancy, the interstitial gland cells of control and denervated ovaries contain close membrane contacts which have been identified as gap junctions. Several workers (Bennett, 1973; McNutt and Weinstein, 1973; Albertini and Anderson, 1975) have postulated that gap junctions act as specialized forms of cell contact which enable adjoining cells to exchange ions and compounds of low molecular weight. Compounds of molecular weights similar to cyclic AMP have been shown to pass through gap junctions (Sheridan, 1971), and endogenous levels of cyclic AMP may influence steroidogenesis (Butcher *et al.*, 1972). Thus, the biosynthetic activity of interstitial gland cells during pregnancy may be coordinated through gap junctions by the interchange of intercellular messengers such as cyclic AMP.

Gap junctions are often seen between interstitial cells which are associated with adrenergic nerve terminals. Those cells adjacent to the nerve terminus may be influenced directly by the adrenergic neurotransmitter. A cell so affected might furthermore evoke a

similar change in a neighboring cell via information transfer over gap junctions. Thus, gap junctions may provide a morphological basis for coordination of neurally evoked changes between steroidogenic interstitial gland cells during pregnancy.

In the interstitial cells from denervated ovaries, gap junctions assume a spherical configuration during early pregnancy. Such circular profiles enclose free ribosomes or fragmented cytoplasmic organelles. Acid phosphatase activity has been reported within the matrix of gap junctional vesicles and this enzyme activity is probably indicative of a stage in the specific degradation of gap junctional membranes (Larsen and Hai-Nan Tung, 1978). The occurence of similar annular gap junctions in interstitial cells from denervated ovaries correlates with other morphological features which are associated with less active interstitial cells.

There have been few ultrastructural studies concerned with the influence of nerves on cellular morphology. In the limb blastema, a neurotrophic dependence has been recognized in macromolecular synthesis (Geraudie and Singer, 1978). In parotid acini cells, morphological changes following denervation included a decrease in size of acinar cells and a depletion of secretory granules (Garrett and Thulin, 1975*a*) while stimulation resulted in nuclear enlargement and dilation of the granular endoplasmic reticulum (Garrett and Thulin, 1975*b*). Thus, it seems that organelles involved in synthesis and metabolism can be influenced by nerves. In the present study, the ultrastructure of interstitial gland cells subsequent to denervation is characteristic of regressed steroidogenic cells at all stages studied. Interruption

of the nerve supply to this important ovarian component results in qualitative and quantitative increase in the fine structural features that are characteristic of regressing steroid secreting cells. During early pregnancy, cutting the ovarian nerves caused an augmented discharge of lipid droplets, which is interpreted as a type of denervation hypersecretion. Thus, this study provides additional morphological evidence of a functional role for adrenergic nerves that innervate interstitial gland cells in the rat ovary.

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FIGURES

Figure 1. Schematic representation of the reproductive organs and reference structures in the rat. The dashed line at 1 indicates where the nerves were cut to denervate the ovary. The solid arrow at 2 indicates the site of injection of 6-HD into the periovarian bursa to effectively eliminate all sympathetic nerve terminals.

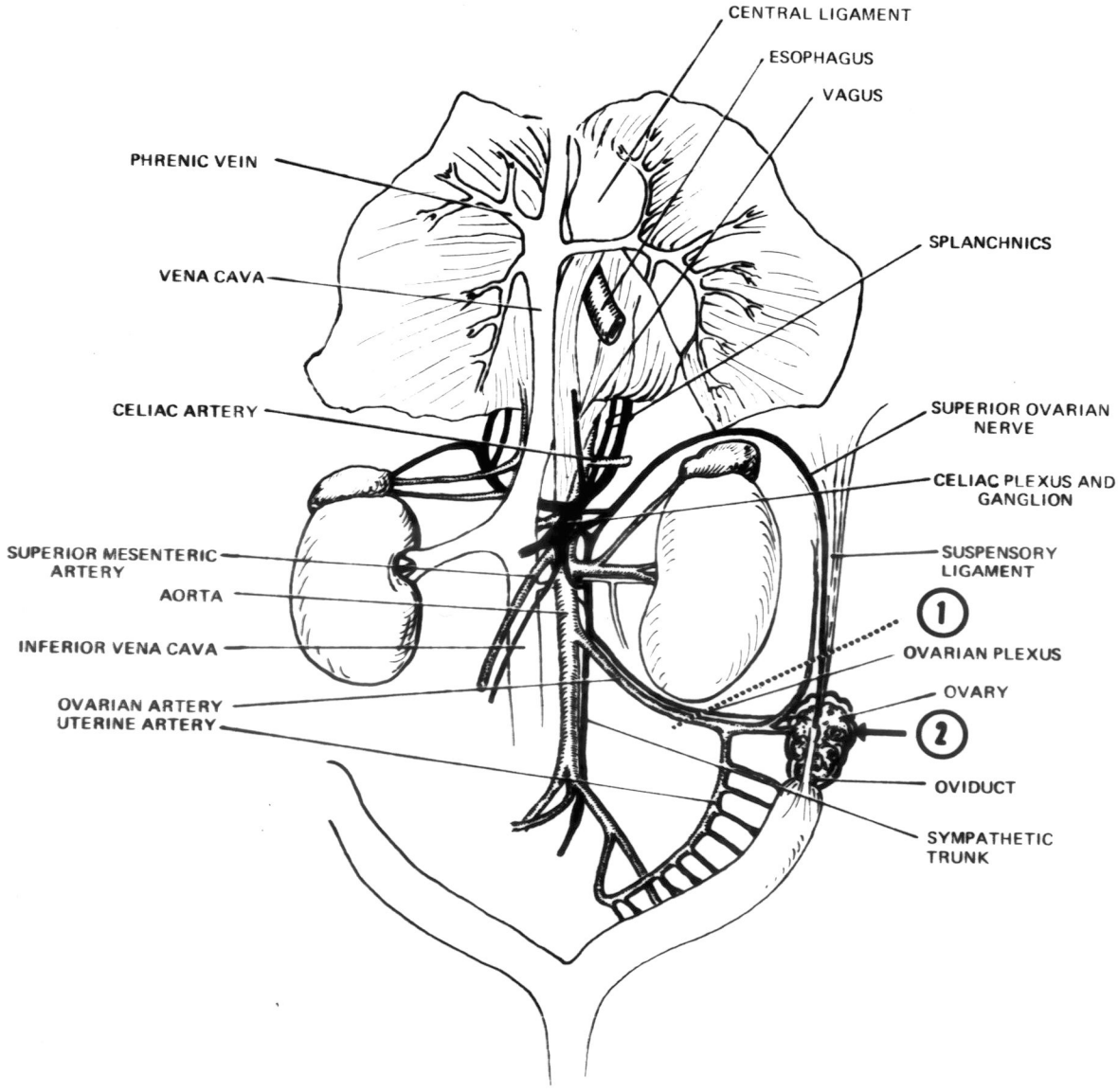

CENTRAL LIGAMENT
ESOPHAGUS
VAGUS
PHRENIC VEIN
SPLANCHNICS
VENA CAVA
CELIAC ARTERY
SUPERIOR OVARIAN NERVE
CELIAC PLEXUS AND GANGLION
SUPERIOR MESENTERIC ARTERY
SUSPENSORY LIGAMENT
AORTA
1
INFERIOR VENA CAVA
OVARIAN PLEXUS
OVARIAN ARTERY
OVARY
UTERINE ARTERY
2
OVIDUCT
SYMPATHETIC TRUNK

Figure 2. Portions of interstitial gland cells from control ovaries on Day 6 of pregnancy. The nucleus is large and spherical. Nuclear heterochromatin (H) is present. A distinct, eccentric nucleolus (N) is present. Mitochondria (M) have a dense matrix. Individual lipid droplets (L) are large. Smooth endoplasmic reticulum (SER) dominates the cytoplasm and associates closely with lipid droplets (arrowheads). A gap junction (arrow) is present between the plasmalemmae of two adjacent cells. x25,000.

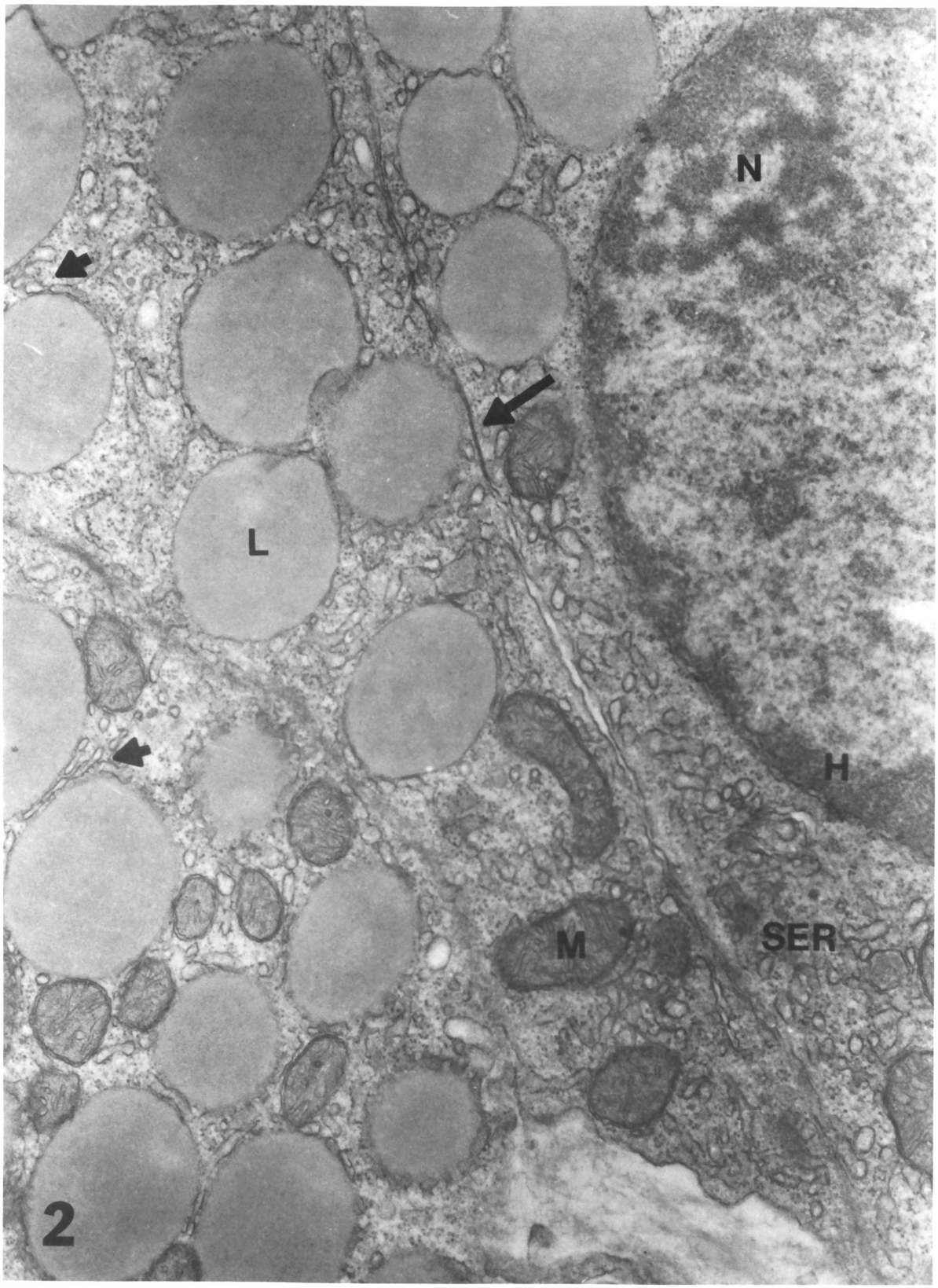

N
L
H
M
SER
2

Figure 3. Interstitial gland cells from control ovaries on Day 6 of pregnancy. Mitochondria (M) are bizarre in shape and have tubular cristae. Some mitochondria (MC) appear to surround a cytoplasm-like substance; other mitochondria encircle mitochondria and appear annular (AM). At this stage of pregnancy, there is a close association between mitochondria, agranular endoplasmic reticulum (arrowheads) and lipid droplets (L). Most lipid droplets appear partially leached. Filopodia (F) interdigitate within the intercellular space. x20,200.

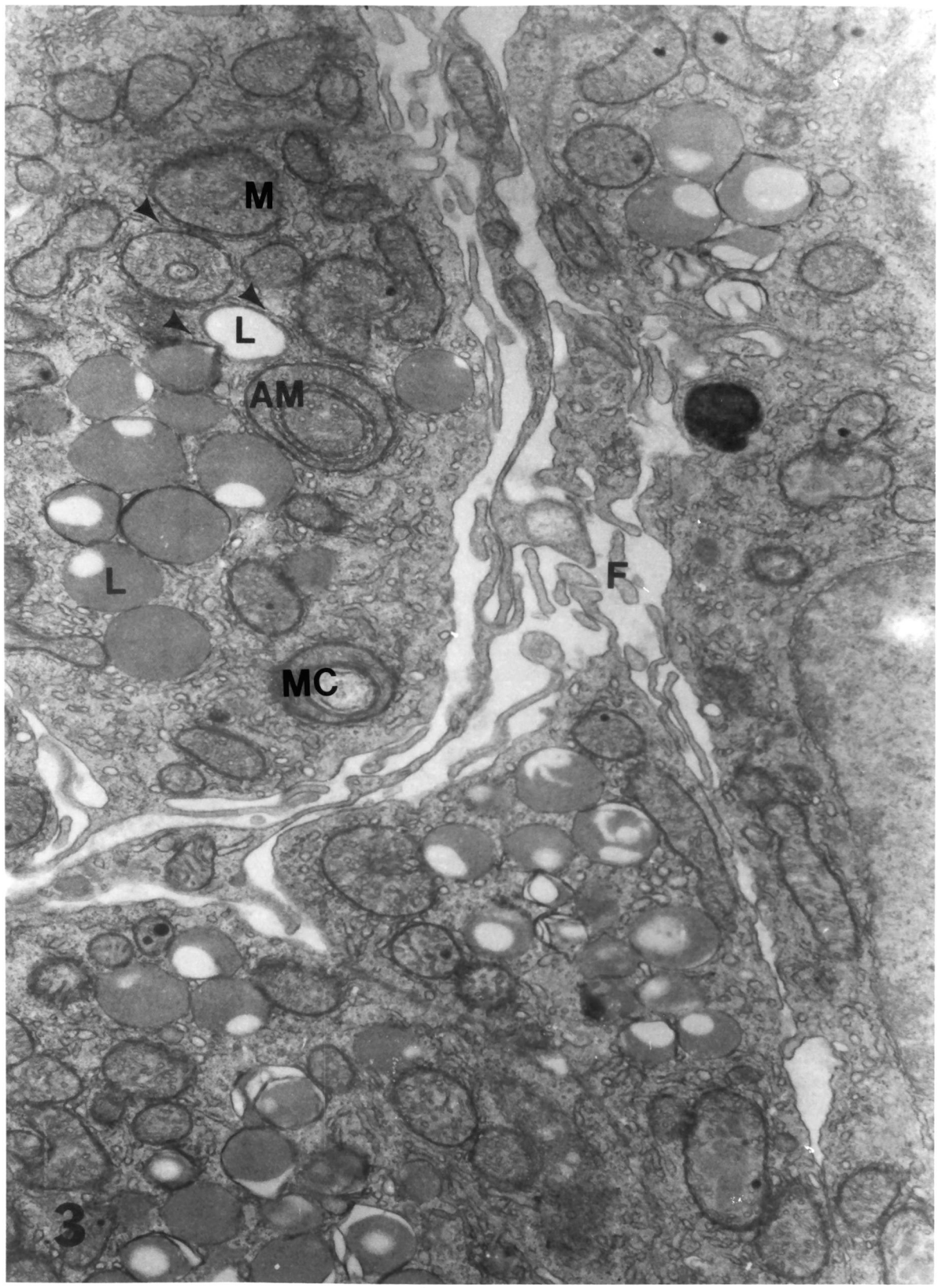

M
L
AM
L
F
MC
3

Figure 4. Portions of interstitial gland cells from control ovaries on Day 6 of pregnancy. Large, osmiophilic lipid droplets (L) associate closely with multilaminar membranous profiles (arrowheads) that appear to derive from the mitochondria. The Golgi (G) apparatus is enlarged with stacks of saccules and vesicles. Scattered, free ribosomes and a few polysomes (circles) are present. x25,000.

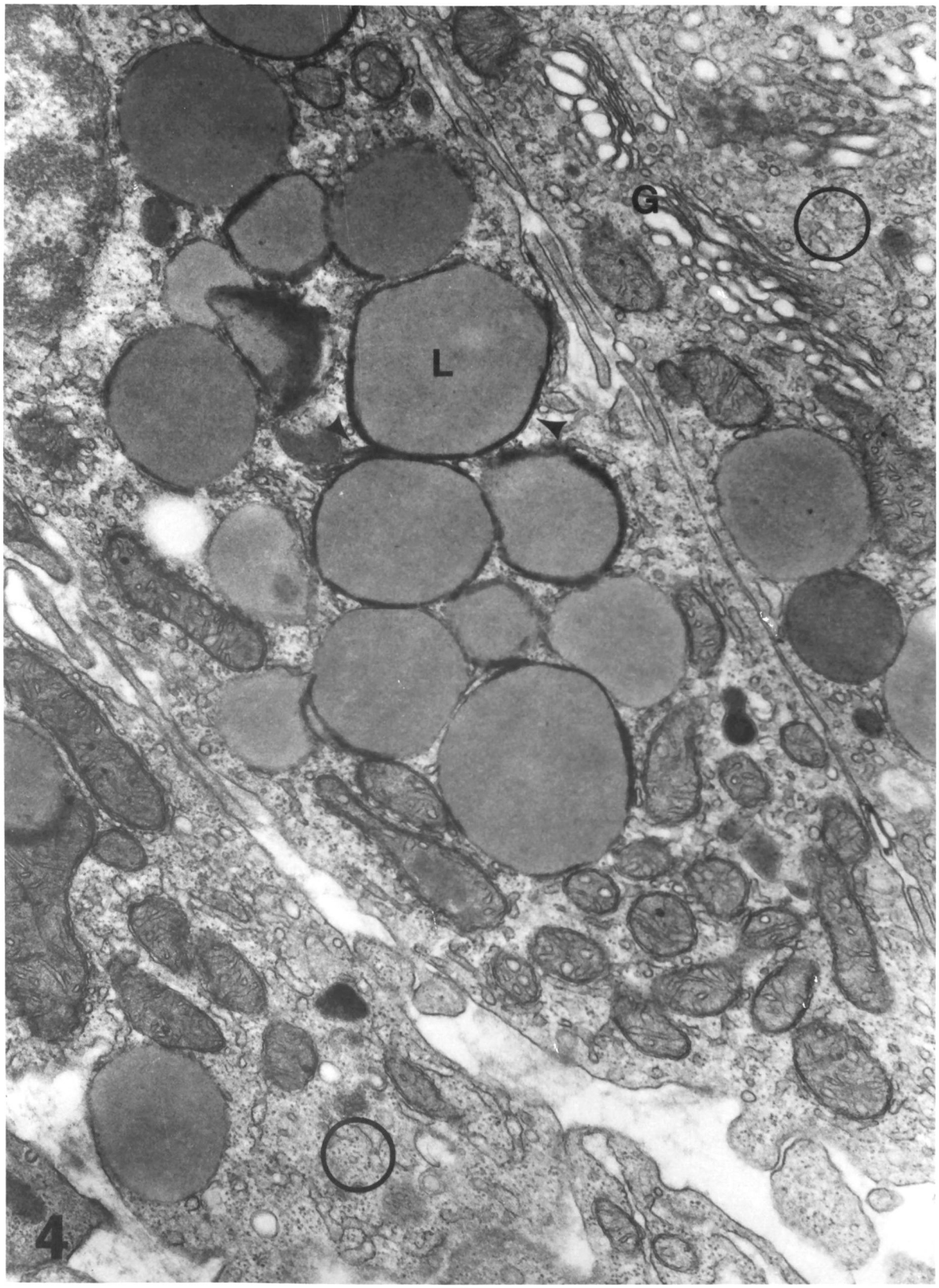
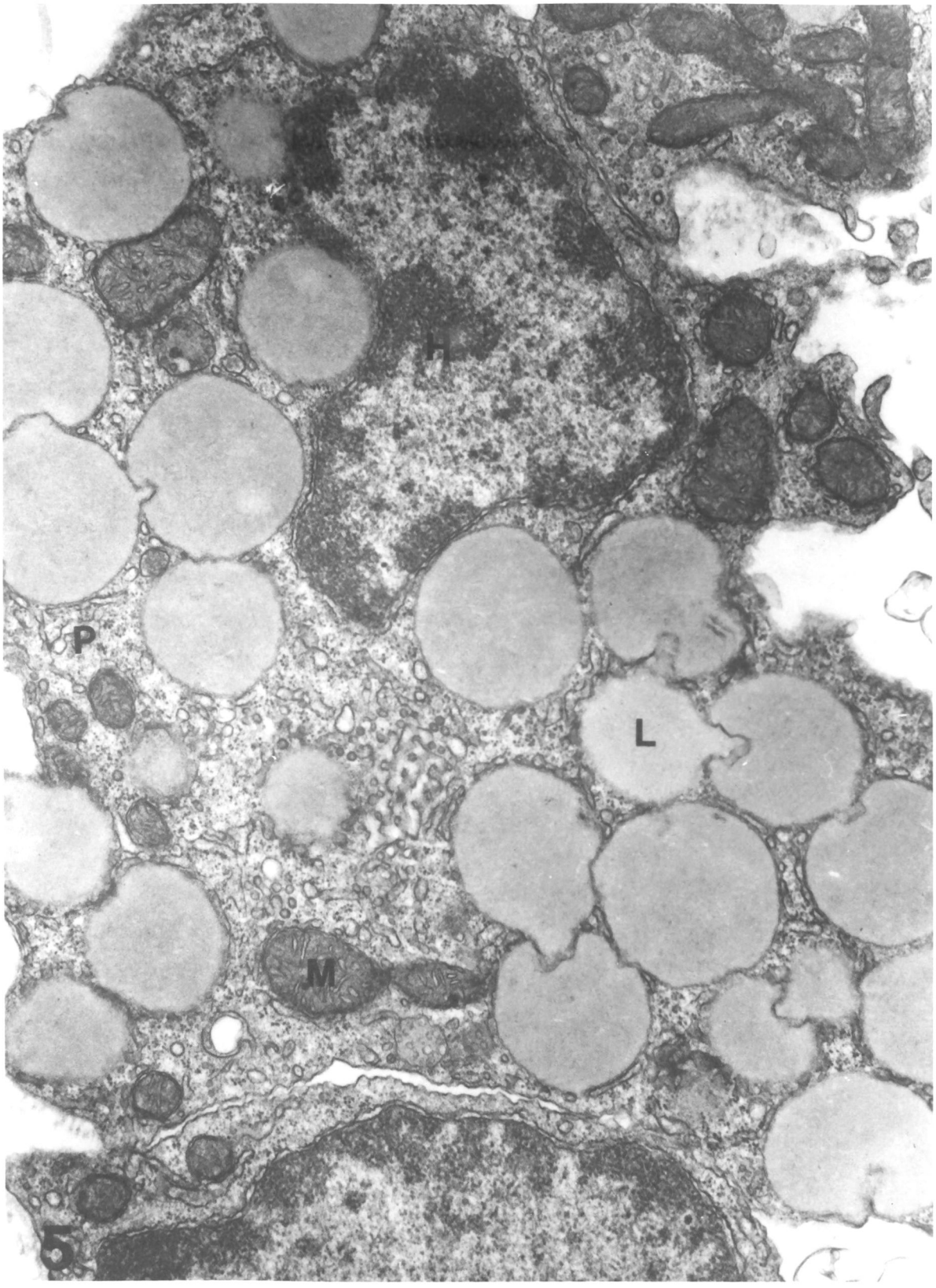

G
L
4

Figure 5. Interstitial gland cells from denervated ovaries on Day 6 of pregnancy. Nuclei are small and irregular. Dense clumps of heterochromatin (H) are present. Mitochondria (M) are rod-shaped with lamellate cristae. Lipid droplets (L) exhibit a tendency toward dissolution and fusion. Cytoplasmic polysomes (P) are numerous. x25,000.


H
P
L
M
5

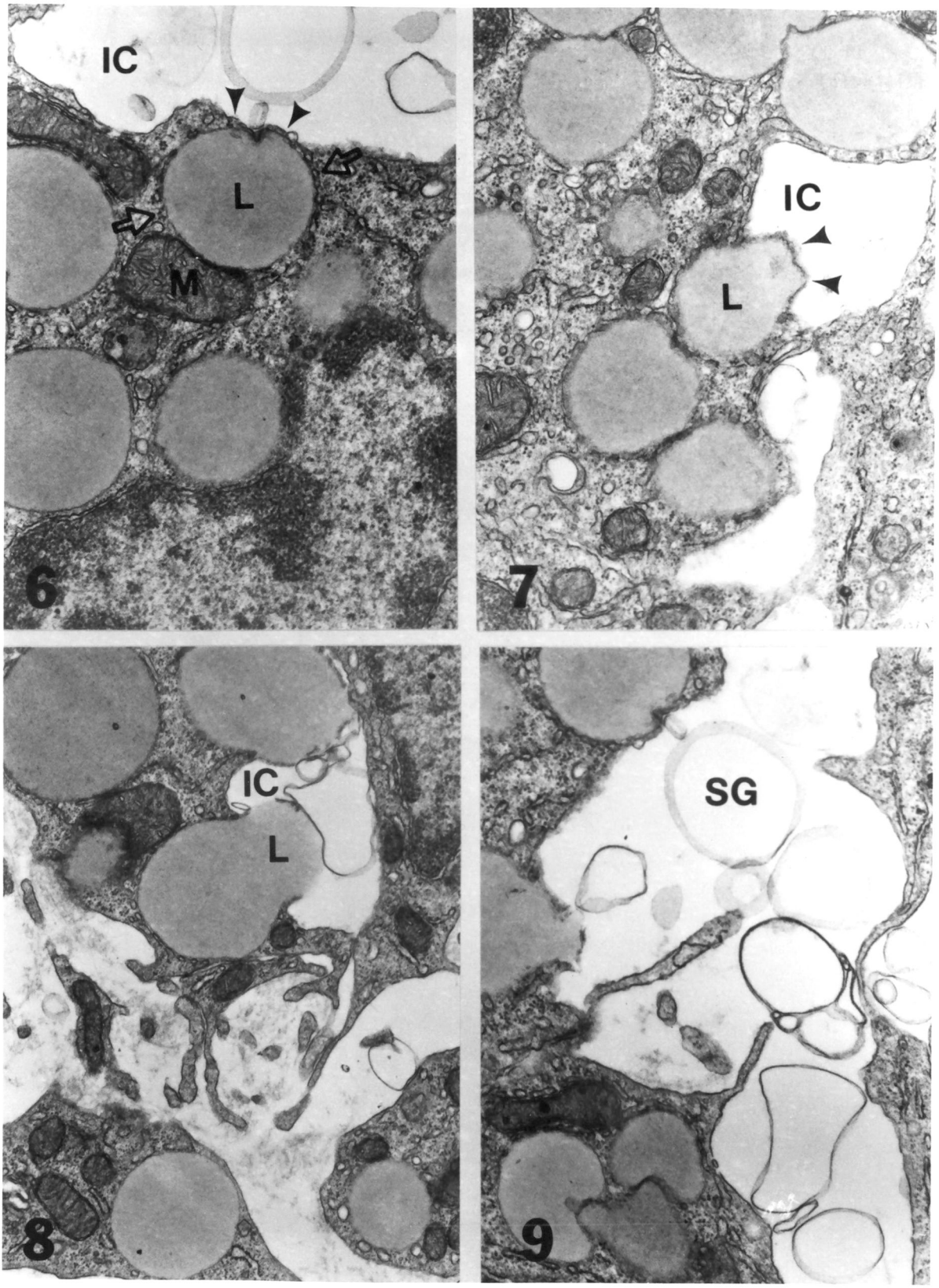
Figures 6 - 9 represent the stages of lipid droplet release in the interstitial gland of denervated ovaries on Day 6 of pregnancy. The sequence is arranged to simulate an assumed discharge mechanism.

Figure 6. The lipid droplet (L) is in close apposition to the surface membrane of the cell. Profiles of smooth endoplasmic reticulum (arrows) form a complete membranous casing around the lipid droplet. A thin strand of cytoplasm (arrowheads) is present between the droplet boundry membrane and the intercellular space (IC). A mitochondrion (M) is in close proximity to the lipid droplet being discharged. x25,000.

Figure 7. The lipid droplet (L) protrudes into the intercellular space (IC) forcing a portion of the surface membrane and cytoplasm (arrowheads) into the intercellular space. x25,000.

Figure 8. There is an apparent dissolution of the surface membrane and cytoplasm as the contents of the lipid droplet (L) is expelled into the intercellular space (IC). x25,000.

Figure 9. The content of lipid droplets, including their boundry membranes, have disappeared leaving membranous "secretory ghosts" (SG) behind. x25,000.


IC
L
M
6
IC
L
7
IC
L
8
SG
9

Figures 10 - 11 are interstitial gland cells from denervated ovaries on Day 6 of pregnancy.

Figure 10. Annular gap junctions (arrows) are prominent. Mitochondria (M) are elongate. Mitochondrial granules (arrowheads) or inclusions are present. Polysomes and single ribosomes are present (circles). Details of the annular gap junction outlined in the rectangle is shown in Figure 11. x25,000.

Figure 11. A profile of the annular gap junction. The pentalaminar nature of the membranous complex is barely discernable (arrows). x70,100.

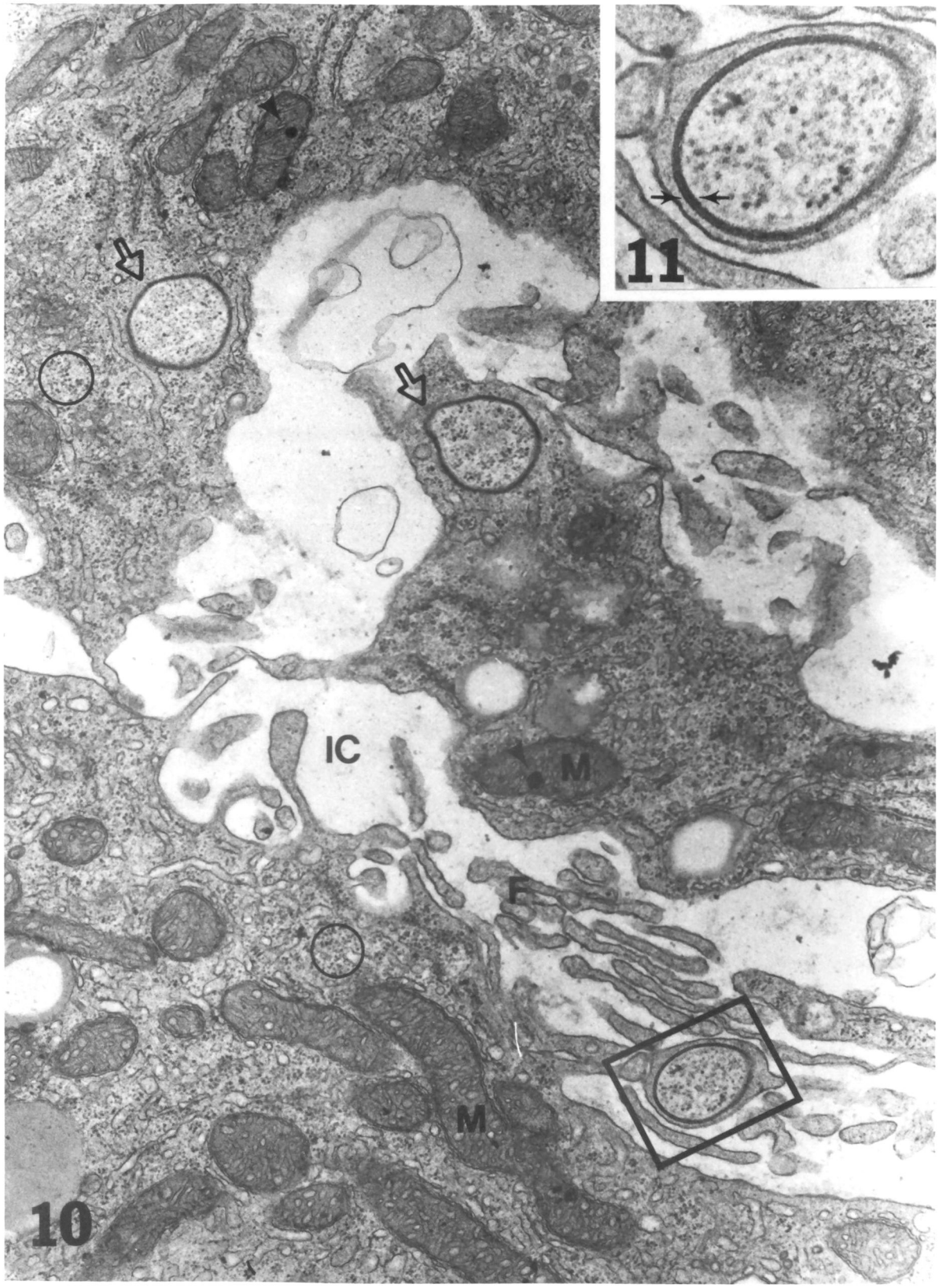
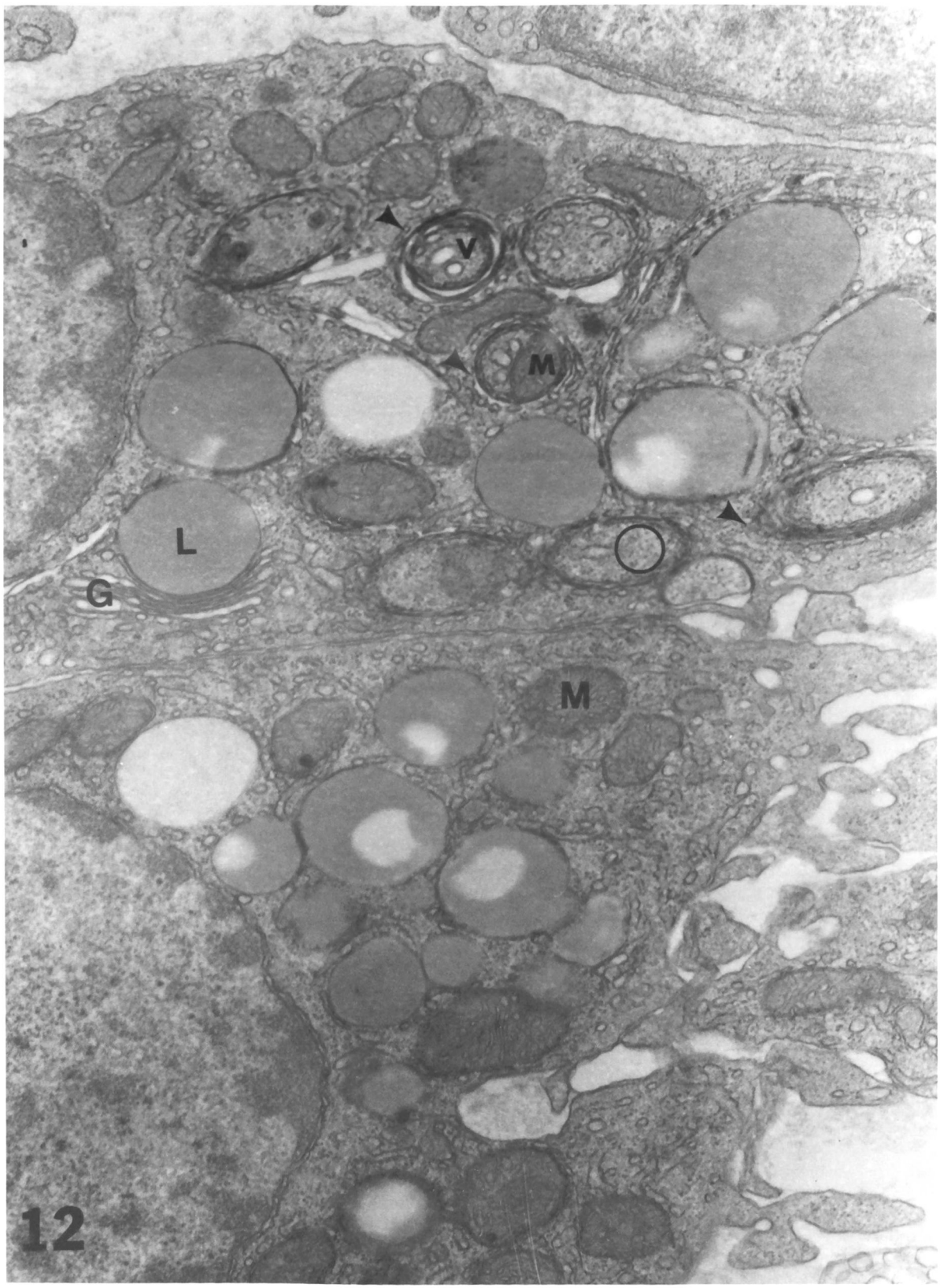

11
IC
M
F
M
10

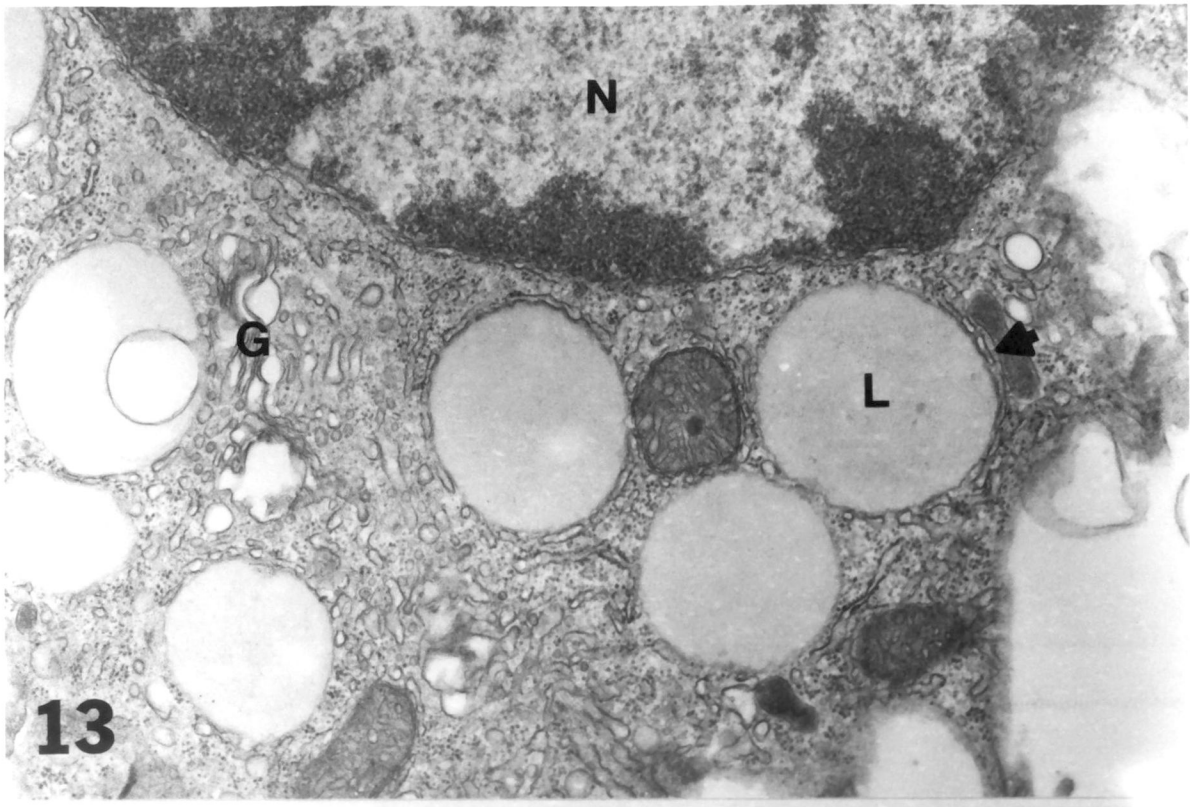
Figure 12. Interstitial gland cells from control ovaries on Day 10 of pregnancy. Cytoplasmic features include mitochondria (M) which are larger than Day 6. The mitochondrial matrix is dense and contains tubular and lamellar cristae. The Golgi (G) complex consists of thin saccules which associate closely with lipid droplets (L). Areas of the cytoplasm are enclosed in dense, whorled membranes (arrowheads). These membranous bodies contain free ribosomes (circles), vesicles (V) and/or a single mitochondrion (M). x25,000.


V
M
L
G
M
12

Figures 13 - 14 are interstitial gland cells from denervated ovaries on Day 10 of pregnancy.

Figure 13. Nuclei (N) are large and spherical. The Golgi (G) complex is dilated. Lipid droplets (L) associate with agranular endoplasmic reticulum (arrowhead). x25,000.

Figure 14. Mitochondria (M) are large and have lamellar cristae. Numerous polysomes (P) and scattered, free ribosomes (circle) are present. x25,000.


N
G
L
13

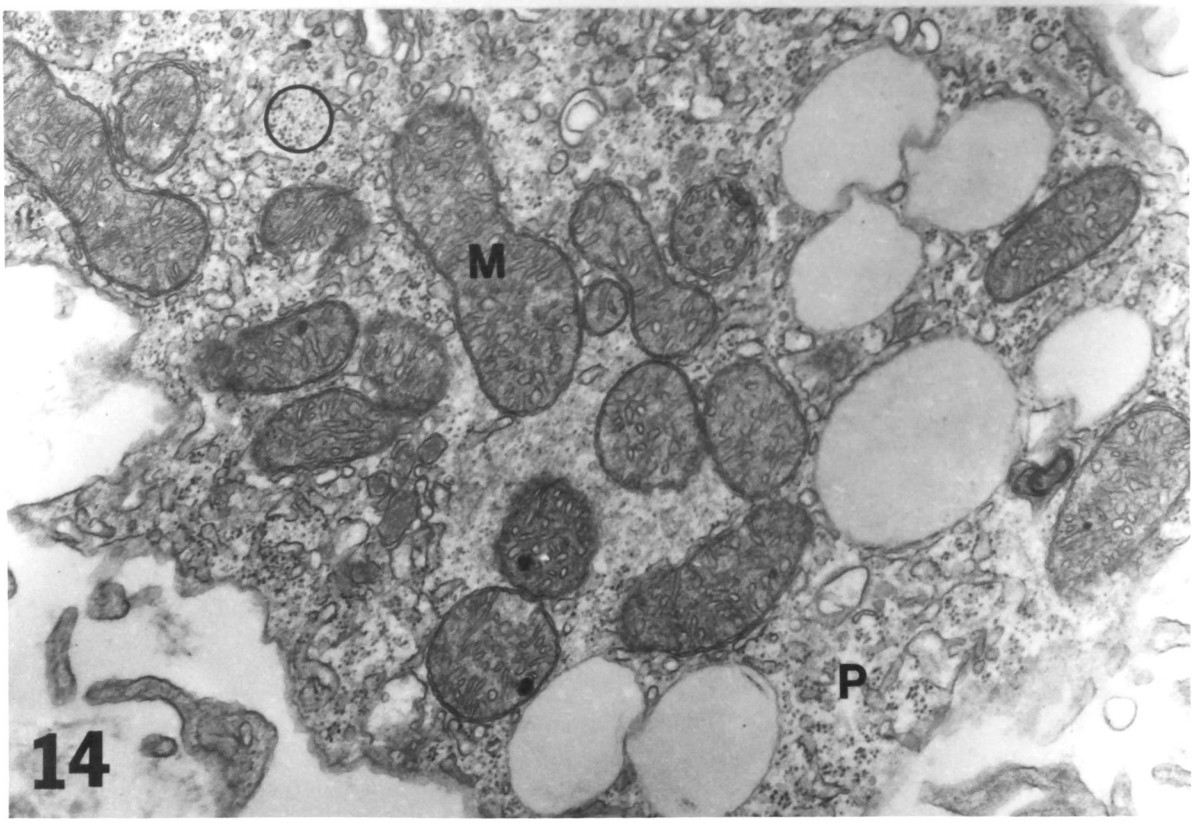
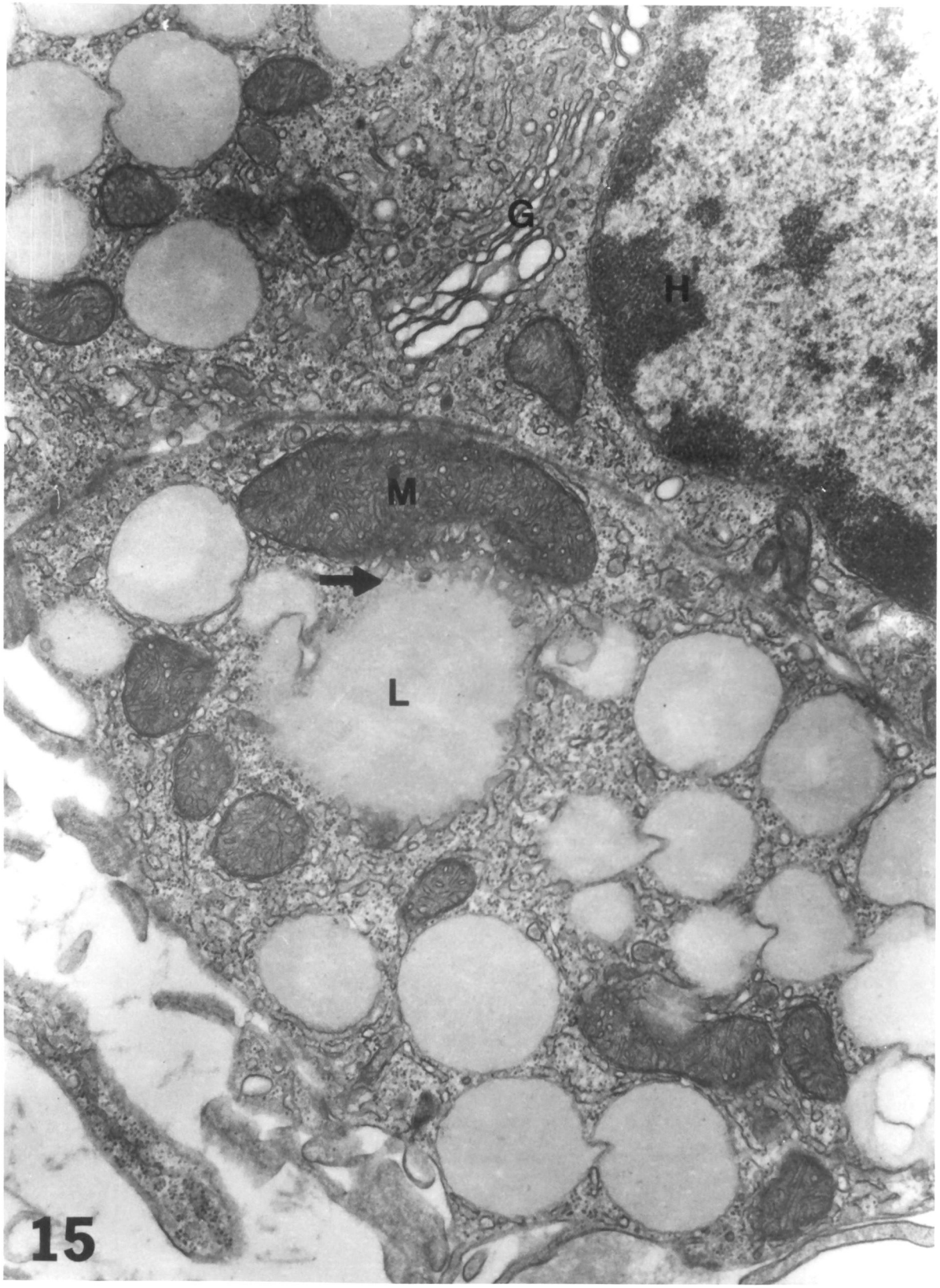

M
P
14

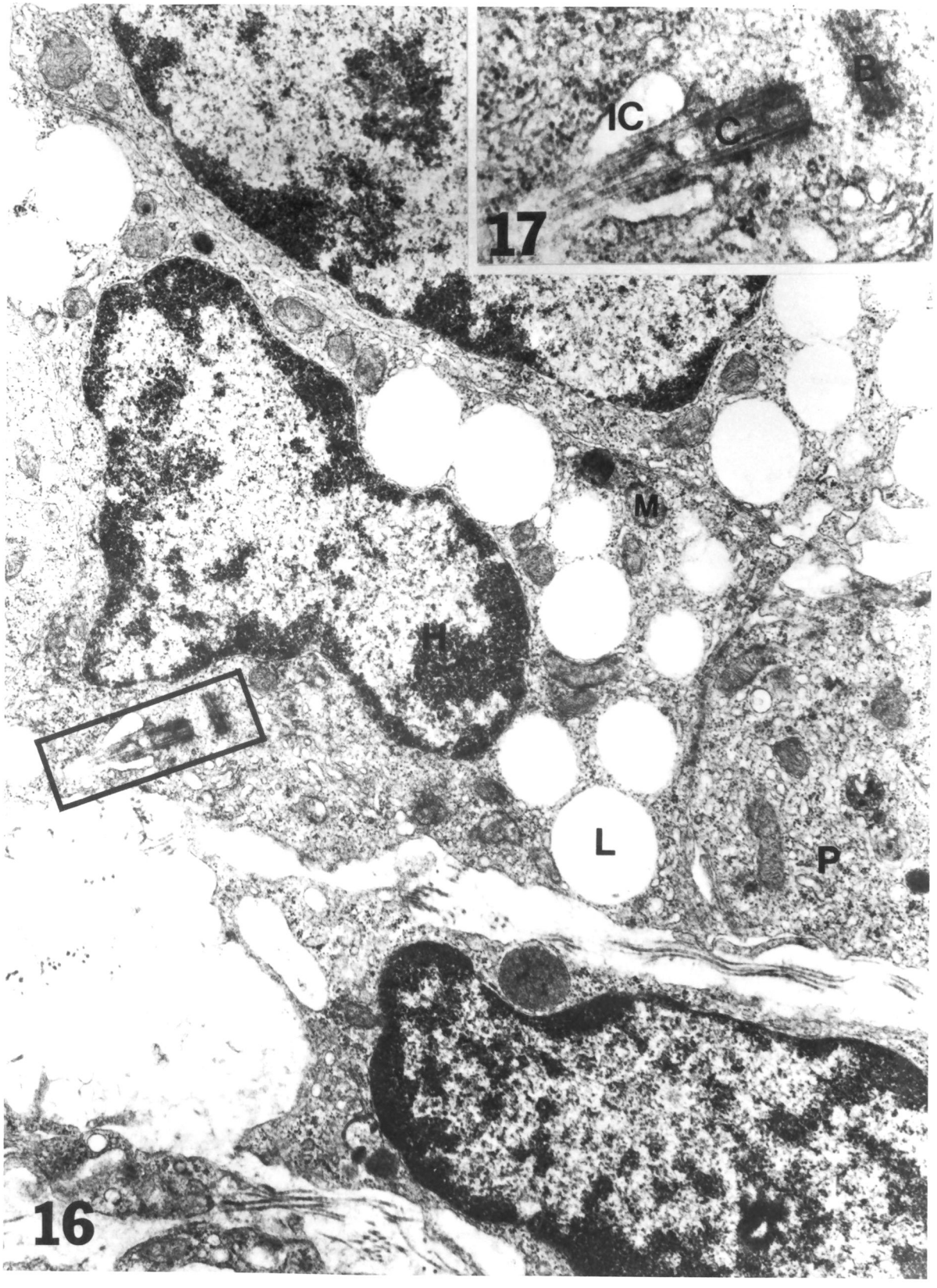
Figure 15. Interstitial gland cells from denervated ovaries on Day 10 of pregnancy. Nuclei are large with dense clusters of heterochromatin (H). Mitochondria (M) are large and possess lamellar and tubular cristae. The Golgi (G) complex consists of widely dilated saccules and vesicles. Lipid droplets (L) are large and tend to dissolve into closely associated mitochondria (arrow). Lipid droplets are predominately osmiophilic. x25,000.


G
H
M
L
15

Figures 16 - 17 are interstitial gland cells from control ovaries on Day 14 of pregnancy.

Figure 16. Nuclei are irregular in shape and the amount of heterochromatin (H) is increased. Mitochondria (M) are rod-shaped and smaller than during early pregnancy. Lipid droplets (L) are uniform in size. Polysomes (L) are present. The area outlined in the rectangle is shown at a higher magnification in Figure 17. x17,500.

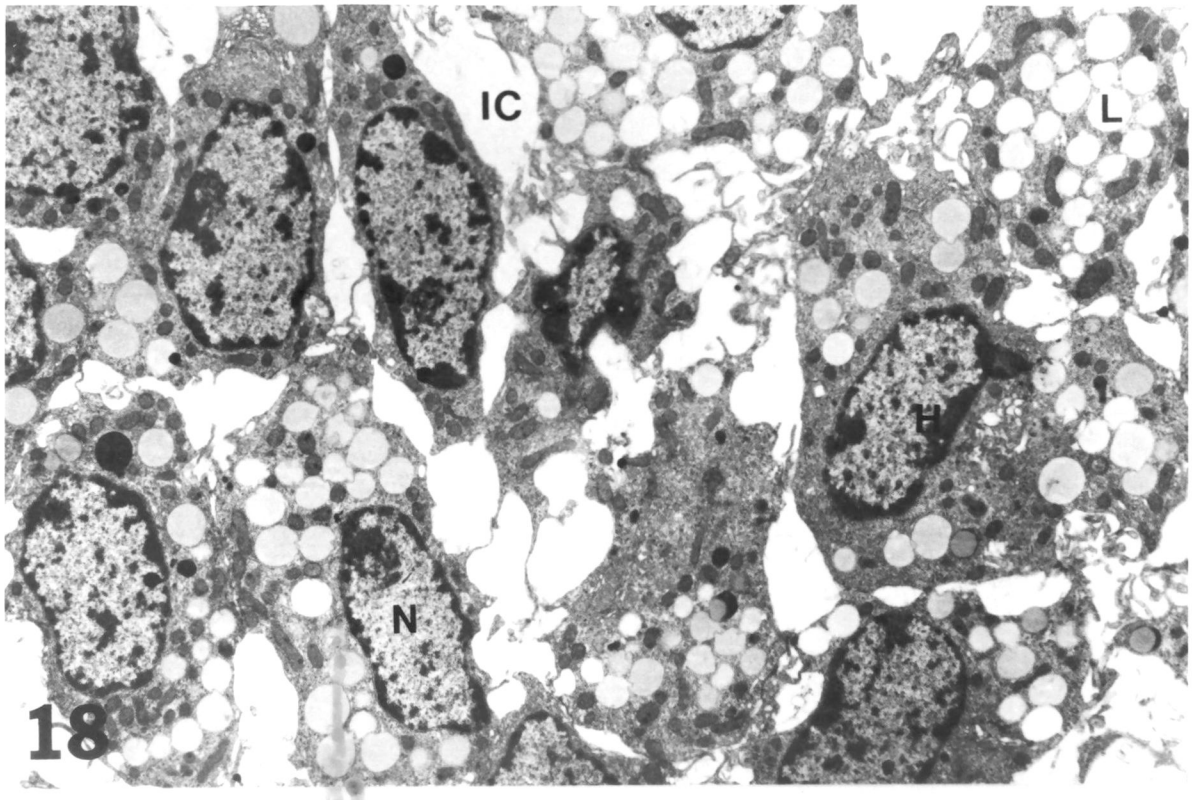
Figure 17. A cilium (C) and its companion basal body (B) project into the intercellular space (IC). x72,500.


IC
C
B
17
M
L
P
16

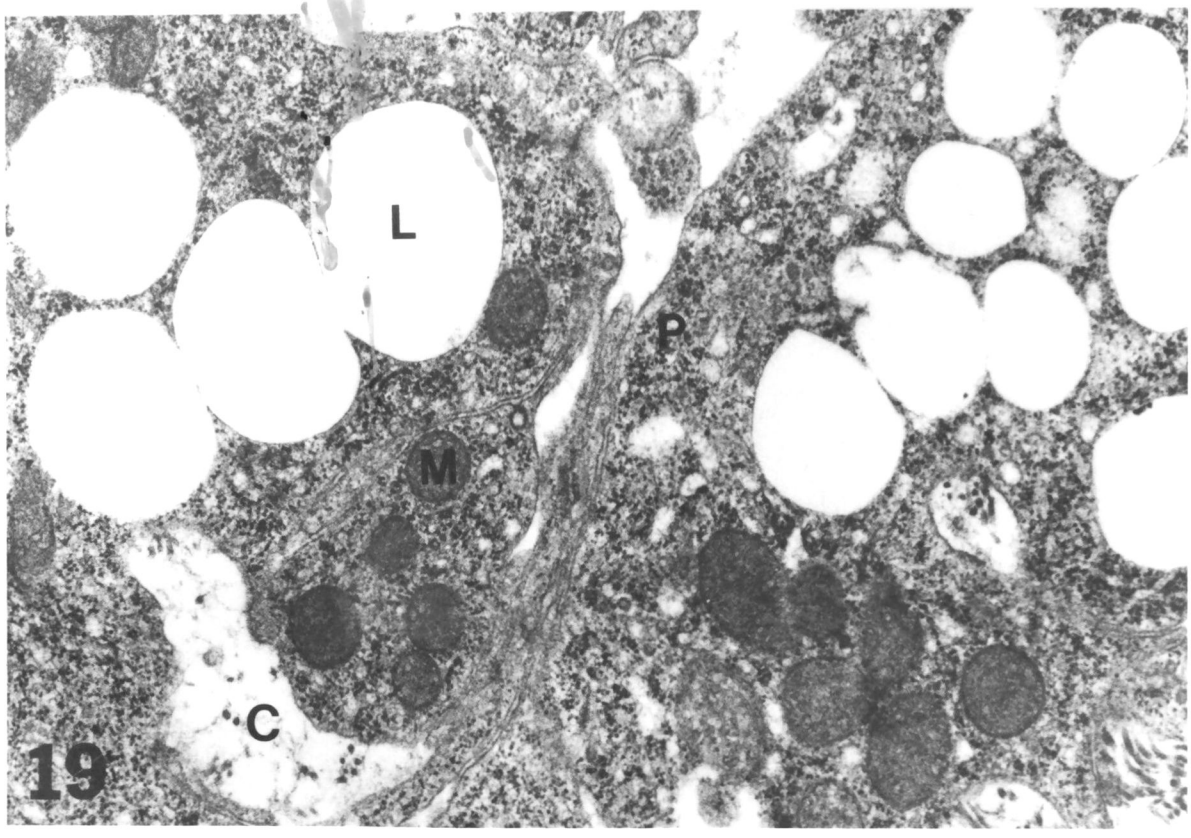
Figures 18 - 19 are interstitial gland cells from denervated ovaries on Day 14 of pregnancy.

Figure 18. Intercellular space (IC) is pronounced. Nuclei (N) are elongate with a dense complement of heterochromatin. Lipid droplets (L) are numerous and are located toward one end of the cell. x5,250.

Figure 19. Cytoplasmic features include mitochondria (M) which are small and rod-shaped, have an electron dense matrix and lamellar cristae. Lipid droplets (L) are electron lucent. Polysomes (P) and free ribosomes fill the cytoplasm. Collagen (C) fibers occupy some of the intercellular space. x25,000.



18

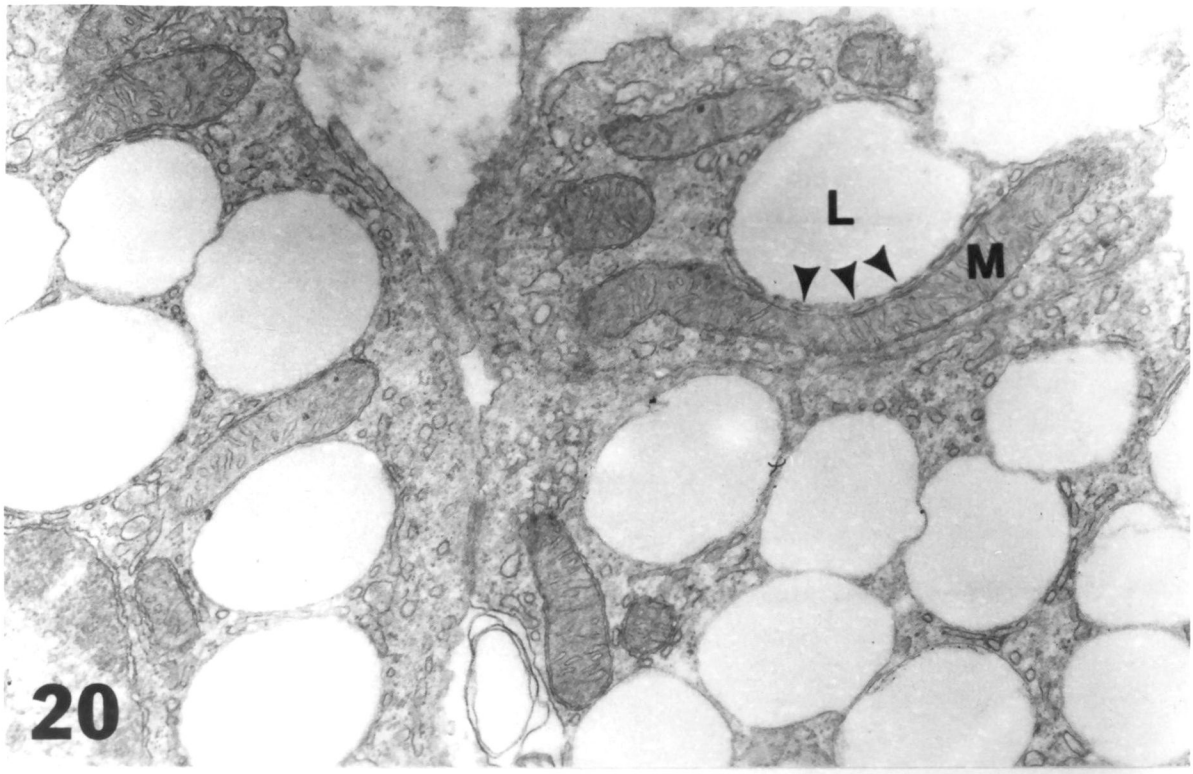


19

Figures 20 - 21 are interstitial gland cells from control ovaries on Day 18 of pregnancy.

Figure 20. Mitochondria (M) are elongate and associate closely with profiles of agranular endoplasmic reticulum (arrowheads) and lipid droplets (L). Lipid droplets are homogenous and electron lucent. x25,000.

Figure 21. Smooth endoplasmic reticulum (SER) is abundant. Numerous vesicles (arrowheads) are present. Lysosome-like bodies (LL) are present. x25,000.


L
M
20

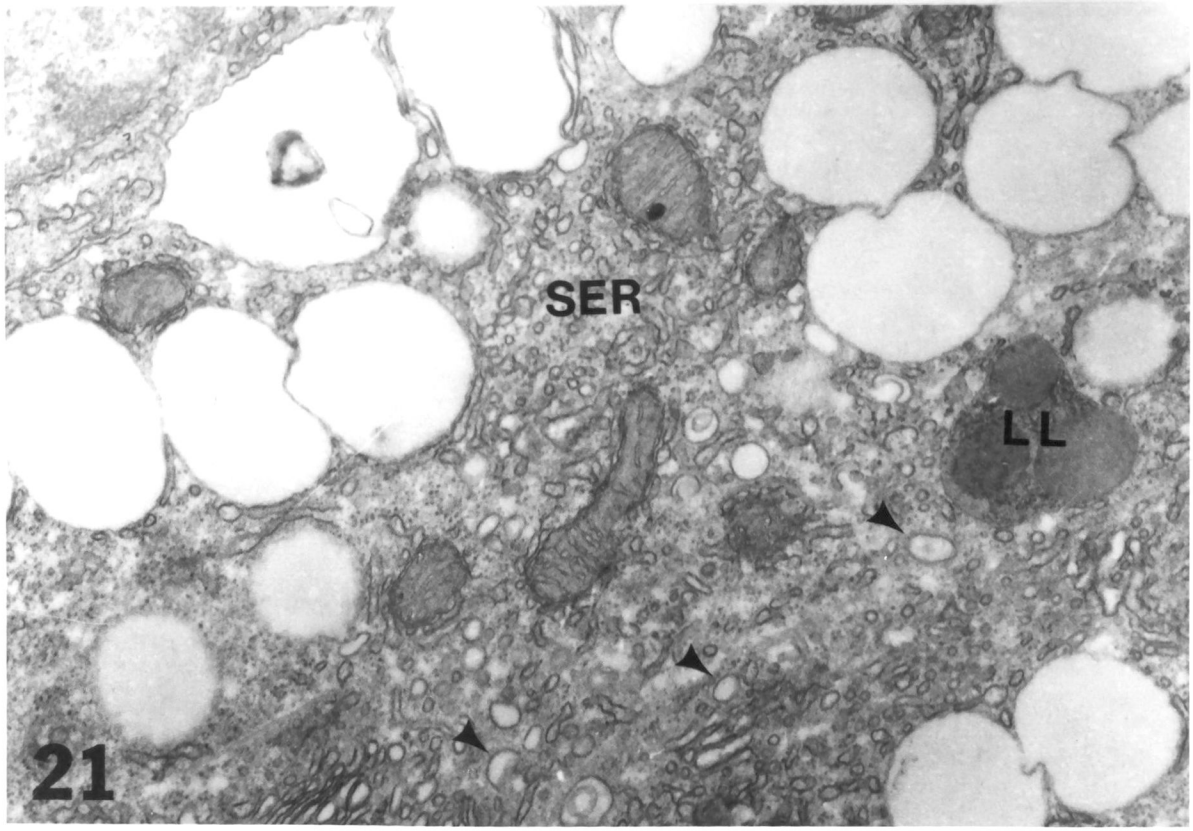
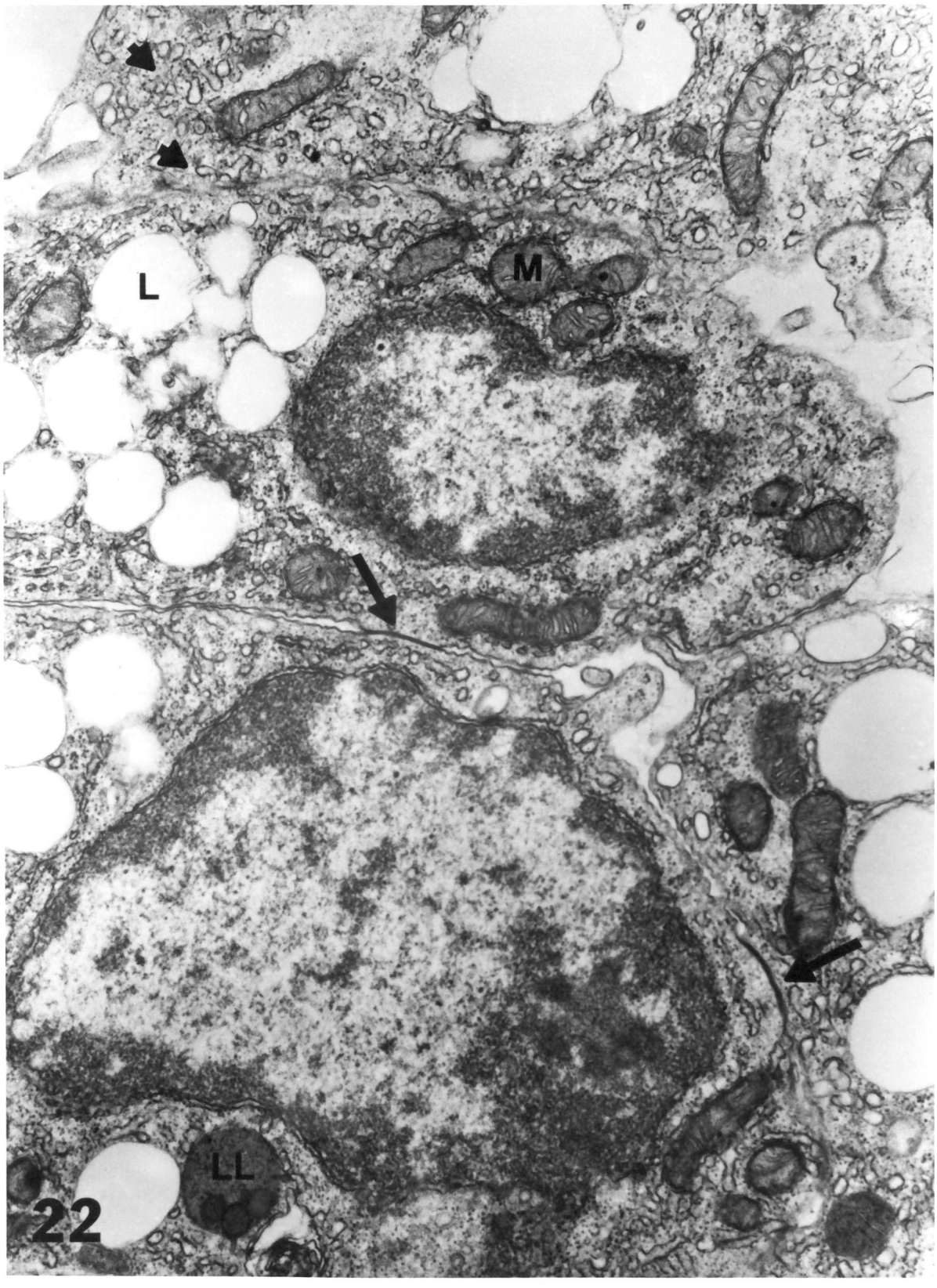

SER
LL
21

Figure 22. Interstitial gland cells from denervated ovaries on Day 18 of pregnancy. Mitochondria (M) are small. Lipid droplets (L) are small and completely leached. Numerous profiles of smooth endoplasmic reticulum (arrowheads) occupy the cytoplasm. Lysosome-like (LL) structures are present. Gap junctions (arrows) are present between interstitial cells. x17,500.


L
M
LL
22