OBSERVATIONS ON THE FINE STRUCTURE OF THE FETAL RAT ADRENAL GLAND AT TERM AND DURING PROLONGED GESTATION

by

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TO

Mary Ann

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INTRODUCTION

Information pertaining to the effects of prolonged pregnancy on the physical condition of the fetus is rather extensive. However, light microscopic investigations concerning the effects of prolonged pregnancy on the various organ systems are very limited (1, 2), and ultrastructural studies are nonexistent. The objectives of this investigation are, therefore:

1. to describe the ultrastructure of the fetal adrenal gland at term and during prolonged pregnancy.
2. to correlate the above morphological findings with known biochemical data pertaining to adrenal gland function, fetal stress, and placental dysfunction.

GENERAL CONSIDERATIONS

Postmaturity is defined as a pregnancy which is prolonged to the 43rd week of gestation or longer (3-7). Using this criterion, postmaturity occurs in 5-6% of all pregnancies and accounts for 30% of all perinatal deaths (3,4). Many postmature fetuses do not exhibit any adverse effects as a result of the prolongation of pregnancy. However, a significant number do display a very striking clinical picture associated with prolonged pregnancy known as the "postmaturity syndrome". This syndrome is characterized by the loss of the vernix caseosum, meconium staining, and a thin physical appearance associated with loose, wrinkled parchment-like skin (3, 4, 8-12).

The "postmaturity syndrome" is believed to be the result of aging or dysfunction of the placenta. During the normal course of

gestation, the placenta is active in the synthesis, metabolism and transport of steroids to the fetus (13-26), and in the transport of amino acids, carbohydrates (27-32), minerals (33-40) and antibodies (41-50) from maternal to fetal circulation. As gestation approaches term, changes in many metabolic activities and morphology of the placenta reflect a regression of placental activity.

A variety of metabolic changes have been observed at the light microscopic level by histochemical methods. These studies demonstrated a decrease in glycogen content (51-53), cytoplasmic RNA (53, 54), iron concentration (53, 55) and sodium transfer (56) as well as a reduction in the concentration of metabolic enzymes such as lactic, malic, and succinic dehydrogenases (54). An increase in fatty necrosis (12) and calcification (8, 12, 57) was also observed in term placentae. However, Clayton (58) has observed several cases of prolonged gestation in which the degree of calcification was markedly less than in placentae of normal term pregnancies and cautioned against the use of increased calcification as an index to placental dysfunction.

At the light microscopic level, the morphological changes noted at term were an increased concentration of glycoprotein, collagen and mucopolysaccharides (51, 53), and the occurrence of small fibrotic and poorly vascularized placental villi (10, 51, 53, 59). Similar observations have been reported in the placenta during

postmaturity (10, 51, 57, 60). In postmature placentae, McKay (53) reported a thickening of the basement membrane underlying the fetal capillary endothelium. McKay (53) as well as Ballantyne (10) also observed an attenuation of the syncytial trophoblast. Ultrastructural studies of term human (59) and rat (61, 62) placentae have provided evidence suggestive of syncytial degeneration which was characterized by fibrinous deposition, cytoplasmic vacuolization, mitochondrial ballooning and an increase in connective tissue elements. In prolonged pregnancy studies, Connell (62) described a further attenuation of the syncytium, an increase in connective tissue elements, especially between trophoblast III and fetal capillaries, and degenerative changes in the fetal capillary endothelium. He also noted, as compared to preterm and term placentae, a further reduction of adenosine tri-, di-, and monophosphatase activities in trophoblast layers II and III.

McKiddie (11) has suggested that placental dysfunction during postmaturity may result in a reduction of oxygen supply to the fetus which would cause fetal stress and, in many cases, fetal death. The observations of Barcroft et al., (63) who studied postmaturity in rabbits support this concept. These investigators found a decrease in placental oxygen saturation from 30% at term to 20% in prolonged gestation, as well as a decrease in brain oxygen saturation from 50% to 17%. In humans displaying the postmaturity syndrome, Walker (64)

and others (65) reported a decrease in oxygen concentration in the umbilical circulation. This concentration fell from 46% to 30% in the umbilical vein, and from 25% to 4% in the umbilical arteries (43rd week). However, Sjostedt et al., (66) and other investigators (67, 68, 69) pointed to the fact that oxygen concentration does not decrease significantly in those fetuses not displaying the postmaturity syndrome in prolonged gestation.

Furthermore, Walker et al., (65) and Marks (70) noticed that the hemoglobin concentration increased from 16 gm/100 ml at term to 20 gm/100 ml in postmature infants which was interpreted as indicating fetal hypoxia. According to Cook, et al., (71) those infants exhibiting the postmaturity syndrome in prolonged pregnancy had an increase in hemoglobin concentration. However, when reticulocyte smears were made no significant increases over the normal term count were observed. On the basis of this observation, Cook et al., (71) concluded that increased hemoglobin concentration was due to fetal dehydration rather than hemopoesis.

Several investigators (see Sjostedt(66) for review of literature) have noted increases in fetal plasma pentoses, protein bound hexoses, non-protein nitrogen, urinary protein and glucose in those fetuses exhibiting advanced stages of the postmaturity syndrome. The increases in plasma pentoses, protein bound hexoses, and non-protein nitrogen were interpreted as an indication of placental tissue

destruction. Proteinuria and glycosuria were interpreted as being indicative of possible disturbances in fetal renal and pancreatic function.

On the basis of the foregoing observations of placental dysfunction and fetal stress, a response by the fetal adrenal cortex during prolonged pregnancy would be anticipated since it is known that the fetal adrenal cortex is functional during the latter part of gestation (72-76). However, before discussing fetal adrenal function, it is important to note that fetal adrenal cortical activity is primarily under the control of fetal rather than maternal pituitary hormones. Experimentation in several species has revealed that hypophysectomy of fetal rats (77), rabbits (78) and mice (79) in utero resulted in atrophy of the fetal adrenal cortex. Jost (80) reported similar findings in hypophysectomized rat fetuses of adrenalectomized mothers. On the other hand, when pregnant female rats were adrenalectomized, the fetal adrenal cortices of intact fetuses exhibited hypertrophy (80, 81) which was subsequently reversed by the administration of glucocorticoids to the adrenalectomized females (82). Fetal adrenal hypertrophy was also reported by Noumura (83) in intact fetuses of females which had undergone simultaneous hypophysectomy and adrenalectomy. Davis et al., (82) reported that an excess of corticoids injected into intact pregnant female rats caused a reduction in the size of the fetal adrenals. This phenomenon was also observed when the concentration

of circulating maternal corticoids were elevated as a result of ACTH injections (83) or subjection to a stress, such as hypoxia (84).

As was briefly mentioned earlier, the fetal adrenal cortex is functional during the latter stages of gestation. Jost (72) and Jacquot (73) have reported that the fetal adrenal cortex is involved in the deposition of glycogen in the fetal liver. Their studies revealed that a significant reduction of fetal liver glycogen occurred as the result of fetal hypophysectomy in rabbits and rats. In the same experiments, it was noted that maternal adrenalectomy (performed only in rats) caused a further reduction of glycogen in livers of hypophysectomized fetuses. Moreover, fetal liver glycogen stores returned to normal values upon subsequent corticoid administration to either the adrenalectomized females or hypophysectomized fetuses.

The fetal adrenal cortex, as well as pituitary gland, has also been implicated in the synthesis of adrenalin from noradrenalin. Studies in fetal rats by Margolis et al., (74), Roffi (75) and Parker et al., (76) have revealed that the enzyme phenylethanolamine-N-methyl transferase (PNMT) which is necessary for the conversion of noradrenalin to adrenalin appeared to be under the control of the adrenal cortex. In these studies, PNMT activity and adrenalin concentration decreased as a result of fetal hypophysectomy but returned to normal values when ACTH or glucocorticoids were administered. Similar observations have been reported in the adrenal medulla of

the adult rat (85, 86). Since the adrenal medulla is not well developed in the fetus, as evidenced by the presence of only a few cells, it is generally considered to be non-functional during gestation (87).

Morphological studies on the effects of prolonged pregnancy on the fetal adrenal gland or, for that matter, on any other organ of the fetus are very limited. Boe (2) has described morphological changes in several endocrine glands of the rat fetus in prolonged pregnancy. These changes were noted as an enlargement of the thyroid gland, which contained distended follicles and congested capillaries, and a reduction in the size of the pancreatic islet cells, which exhibited pyknotic nuclei and a paucity of cytoplasm. Boe (2) also noted an enlargement of the adrenal gland which displayed marked congestion of the cortical capillaries and medullary sinusoids. No cytological changes were noticed in the cells of the cortex. However, the medullary cells were reported as exhibiting very little cytoplasm and small pyknotic nuclei. Boe (2) concluded from his observations that alterations in the above mentioned organs were due indirectly to advancing regressive placental changes which he described as marked attenuation of villi, congestion of the maternal blood spaces and a marked increase in the quantity of stromal elements. The observations of Eguchi et al., (1) on the adrenal cortex of the fetal rat during prolonged pregnancy contradict those presented by Boe (2). These investigators observed marked cortical cell hypertrophy and a decrease in the ascorbic acid content,

a factor which is used as an index of adrenal cortical activity (88). From their observations, Eguchi et al., (1) concluded that the fetal adrenal cortex remained active during prolonged pregnancy. These investigators, however, offered no explanation as to the possible function of the fetal adrenal cortex during prolonged pregnancy.

Since the studies of Boe (2) and Eguchi et al., (1) were limited by the resolution of the light microscope, the present investigation was designed to describe ultrastructural changes in the fetal rat adrenal cortex and medulla during prolonged pregnancy and to discuss the significance of observed changes with respect to adrenal cortical and medullary function.

MATERIALS AND METHODS

Animals and Handling of Tissues

Twenty adult Sprague Dawley rats were used for this study. Virgin females weighing 180-220 gm and exhibiting pre-estrous vaginal smears were placed at random in cages with males for 2 hours in an inverted light cycle room. The presence of spermatozoa in vaginal smears dated the time of mating to within ± 1 hour. The first day of pregnancy was designated as 24 hours after spermatozoa were found in vaginal smears. Using this method, the females delivered on the 22nd day of gestation (term).

The experimental groups consisted of adrenal glands from fetuses taken from 12 females whose gestational periods were prolonged 1, 2, and 3 days beyond term. Prolonged gestation was achieved by the daily subcutaneous injection of 7.0 mg progesterone in sesame oil beginning on the 20th day and daily thereafter through the 24th day of gestation. These adrenal glands were compared with the adrenal glands from fetuses taken from 4 normal term females.

In order to evaluate any influence exogenous progesterone might have on the morphology of the fetal adrenal gland, 4 pregnant females were given 7.0 mg progesterone in sesame oil daily from the 14th through the 21st day of gestation. These adrenals were compared with controls which consisted of the adrenal glands from fetuses taken from normal term females. Under nembutal anesthesia (5 mg/100 gm body weight), pregnant females were autopsied on the 22nd,

23rd, 24th, and 25th days of gestation - 8 animals on the 22nd day and 4 animals on each of the remaining 3 days. Immediately upon removal from the uterus each fetus was decapitated, thus severing the pituitary-adrenal axis. Laparotomy was performed on each fetus for the removal of both adrenal glands which were subsequently immersed in a drop of cold fixative (6.25% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4). Once in the fixative, each gland was cut into two equal halves.

Fixation and Embedding

The halved adrenals were transferred to vials containing 6.25% glutaraldehyde in 0.1 M cacodylate pH 7.4 for one hour at 3-4°C. After initial fixation, the specimens were rinsed in 0.1 M cacodylate buffer (pH 7.4) containing 0.2 M sucrose for 24 hours at 3-4°C (89). The specimens were then postfixed in 2% osmium tetroxide buffered in 0.1 M sodium cacodylate (pH 7.4) containing 0.2 M sucrose for 4 hours at 3-4°C.

After postfixation the specimens were rinsed in distilled water and dehydrated in a graded series of alcohols as follows:

1. 70%, for 10 minutes
2. 95%, two changes for a total of 15 minutes
3. 100%, three changes for a total of 25 minutes
4. Propylene oxide, for 10 minutes

Following dehydration, the specimens were infiltrated for 3 hours in

a 1:1 mixture of propylene oxide and complete epoxy resin mixture. Tissues were embedded in Epon 812 according to the method of Luft (90).

The plastic was initially polymerized at room temperature for 24 hours and subsequently at 60°C for 24 hours.

Sectioning and Staining

For orientation purposes, one micron thick sections were cut with glass knives on a Porter-Blum MT2 microtome (Sorvall), mounted on glass slides and stained for 30 seconds with Richardson's stain (91). Thin sections were cut using a diamond knife (duPont) on a Porter-Blum MT2 microtome (Sorvall) and mounted on naked 300 mesh copper grids. To enhance contrast the tissue sections were stained first with a saturated aqueous solution of uranyl acetate for 20 minutes (90) and then with 0.1-0.2% aqueous solution of lead citrate for 4 minutes (92).

All thin sections were viewed and photographed in an RCA, model EMU 3F, electron microscope. In order to avoid the large sampling error inherent in electron microscopy, the adrenal glands of 4 fetuses from each pregnant female were examined.

RESULTS

Light microscopy (figs. 1-3)

The fetal adrenal gland at term consists of cortical and medullary cells which are as well developed as they are in the adrenal gland of the adult. On the other hand, zonation of the gland at term (fig. 1) is not as distinct as it is in the adult. While the zona glomerulosa and zona fasciculata are fairly well delineated at term, the zona reticularis is poorly defined as is the medulla which is not yet fully formed. The outermost region of the gland, which is surrounded by a thin connective tissue capsule, is the zona glomerulosa. It consists of clusters of small light and dark cells exhibiting scant amounts of cytoplasm. The region lying beneath the zona glomerulosa is the zona fasciculata, which consists of large light and dark cells arranged in cords. Since the innermost region of the gland consists of an intermingling of large light and dark cortical cells of the future zona reticularis with light and dark cells of the undeveloped medulla, it is referred to as the "zona reticulo-medullaris" in the current investigation. The medullary cells are small and exhibit less cytoplasm than the cortical cells of the zona fasciculata and zona reticulo-medullaris.

During prolonged gestation (days 24 and 25), the fetal adrenal gland exhibits hypertrophy of the cortical cells of the zona fasciculata and zona reticulo-medullaris (compare fig. 3 with fig. 2).

Electron Microscopy

Term (Day 22)

Capsule (fig. 4)

Ultrastructurally, the capsule consists of several closely opposed layers of elongated spindle shaped connective tissue cells which are frequently attached by desmosomes and separated by a loose mesh network of collagen fibrils (fig. 4). The nucleus of the capsular cells is elongated and displays a single prominent nucleolus as well as heterochromatin clumping. The mitochondria, which are elongated and display typical lamellar cristae, are moderately abundant throughout the cell. The cytoplasm of these cells is also characterized by a well developed system of rough surfaced endoplasmic reticulum, polyribosomes, and Golgi membranes. Dense bodies and lysosome-like vesicles, however, are rarely observed (fig. 4).

Zona glomerulosa - term (figs. 5-8)

The zona glomerulosa consists of tightly packed light and dark polymorphic cells which are frequently attached by desmosomes (fig. 6). Microvillous extensions of the cell are frequently observed in the intercellular spaces (fig. 6). The organelles and inclusions of both cell types appear identical in morphology and distribution. The dark cells, however, are less numerous and exhibit a more dense cytoplasmic and nuclear matrix.

The irregularly shaped nuclei of both cell types are

approximately 5-6 microns in diameter and exhibit a single prominent nucleolus. The nuclear matrix of these cells is rather heterogenous in appearance and exhibits areas of clumped heterochromatin (fig. 6).

Mitochondria, which are found in moderate numbers, are evenly distributed throughout the cytoplasm of these cells. They vary both in shape and size (0.5 - 1.0 microns in diameter) and characteristically display tubular cristae and a dense matrix. The endoplasmic reticulum, which is composed of tubular structures, is primarily of the rough surfaced form (figs. 7, 8). Moreover, both the smooth and rough endoplasmic reticulum are sparsely distributed throughout the cytoplasm. Golgi membranes are well developed and are usually found near the nucleus.

Polyribosomes and lipid droplets, which occur either singly or in groups, are evenly distributed in the cytoplasmic matrix. However, dense bodies and lysosome-like vesicles are infrequently encountered (fig. 6).

Mitotic figures are commonly found throughout the zona glomerulosa but have been observed only in the light cells (fig. 5).

Capillaries are numerous throughout the zona glomerulosa. The capillary endothelium, which is attenuated and displays numerous fenestrations (arrow fig. 5), is separated from the cortical cells by a thin basal lamina (fig. 6). The nuclei of the endothelial cells are polymorphic in shape and display marked clumping of the

heterochromatin (fig. 4, 6). The cytoplasm of the endothelium exhibits a variety of organelles such as mitochondria, Golgi, and well developed granular endoplasmic reticulum (fig. 4).

Zona intermedia - term (figs. 9-12)

The zona glomerulosa is separated from the zona fasciculata proper by an intervening layer of cells, the zona intermedia. The zona intermedia actually comprises the outermost portion of the zona fasciculata. As in the zona glomerulosa, the zona intermedia contains both light and dark cells which are polymorphic in shape, tightly packed, and exhibit numerous microvilli (fig. 9). The typical nucleus in this zone is spherical in shape, exhibits a prominent nucleolus and a homogenous nuclear sap with little heterochromatin clumping (fig. 10). Mitochondria, which are numerous and spherical in shape, exhibit vesicular as well as a few tubular cristae (fig. 12). The endoplasmic reticulum occurs in a far greater concentration than in the cells of the zona glomerulosa. The smooth-surfaced endoplasmic reticulum predominates and appears as branching tubular structures (fig. 12), a few of which are dilated (fig. 10). Well developed Golgi membranes, which are similar to those previously described in the cells of the zona glomerulosa are commonly observed as are numerous polyribosomes and lipid droplets. Dense bodies and lysosome-like structures, however, are infrequently encountered (figs. 11, 12).

Mitotic figures are also present in the light cells of the zona

intermedia but in fewer numbers than in the zona glomerulosa (fig. 9).

Zona fasciculata - term (figs. 13-15)

Although the majority of the cells in the zona fasciculata are of the light form (fig. 13), one periodically observes dark cells of differing densities. The general morphology and arrangement of both light and dark cells are similar to that described in the zona intermedia. Mitochondria, which are spherical in shape and display vesicular cristae, are numerous and measure approximately 1.0 - 1.5 microns in diameter. The tubular arrangement of the mitochondrial cristae observed previously in the cells of the zona intermedia are rare in these cells (fig. 14). The smooth endoplasmic reticulum exhibits branching tubular profiles which are very abundant in both light and dark cells (figs. 14, 15). A few of these profiles are dilated (figs. 14, 15). Elements of the smooth endoplasmic reticulum are frequently observed surrounding mitochondria and lipid droplets (figs. 14, 15). Rough-surfaced endoplasmic reticulum, however, is rarely observed. Well developed Golgi membranes and numerous free polyribosomes are readily discernable throughout the cytoplasm (fig. 14). Cytoplasmic vacuoles, which represent extracted lipid inclusions and are commonly found in the cells of the zona glomerulosa, are infrequently encountered in the zona fasciculata. Lipid inclusions, on the other hand, are numerous in both light and dark cells and are frequently associated closely with mitochondria and membraneous

profiles that resemble smooth endoplasmic reticulum (fig. 15). This association will henceforth be referred to as the "LERM" complex. Dense bodies and lysosome-like structures are encountered only occasionally (fig. 15).

Zona reticulo-medullaris - term (figs. 16-27)

A well defined zona reticularis and fully developed medulla are not present in the adrenal gland of the fetus. What does exist is an intermingling of the cells of both of these zones which are not fully developed at this time. As a result of this arrangement, the present author has combined these two nondistinct regions into a single zone which will be referred to as the "zona reticulo-medullaris". The cells of this zone are either grouped as small clusters or as finger-like protrusions partially surrounded by sinusoids (figs. 16-18, 24).

The sinusoidal endothelium is attenuated and displays numerous fenestrations (figs. 16, 21). Its cytoplasm characteristically exhibits elongated mitochondria with lamellar cristae, rough-surfaced endoplasmic reticulum, widely dispersed polyribosomes and an occasional dense body (fig. 17). The nucleus of the endothelium is polymorphic in shape and characteristically displays marked clumping of the heterochromatin (fig. 18).

A. Cortical cells

The dark cortical cells of the zona reticulo-medullaris, which occur in greater numbers than the light cortical cells, typically

display a spherical nucleus with a prominent nucleolus and finely dispersed chromatin (fig. 16). The numerous mitochondria are spherical in shape and exhibit primarily vesicular cristae (fig. 19).

Smooth surfaced endoplasmic reticulum is abundant and appears as branching tubular profiles (fig. 20). A few of these profiles are dilated (fig. 19). The smooth endoplasmic reticulum is frequently observed surrounding mitochondria and lipid droplets (fig. 20). Golgi membranes and polyribosomes are well developed and randomly distributed throughout the cytoplasm (figs. 18, 19). Cytoplasmic vacuoles and lipid inclusions of varying densities are common and are observed at random. The "LERM" complex is frequently seen in these cells (fig. 16). The dark cells of this zone differ from those described in the zona fasciculata only in that they occur in far greater numbers.

The light cell type in this zone, however, differs quite markedly from the light cell of the zona fasciculata. The mitochondria, which are numerous, appear more polymorphic in shape and possess a denser matrix than the mitochondria of the light cells of the zona fasciculata (figs. 17, 22). The mitochondrial cristae are primarily vesicular but tubular elements are frequently observed (fig. 22). The smooth-surfaced branching tubular endoplasmic reticulum is abundant and is found to closely envelop the mitochondria and lipid inclusions (fig. 17, 22). This peculiar arrangement is most readily apparent in the light cells of this zone, but as previously mentioned it is also

observed in the dark cells of this zone as well as in the cells of the zona fasciculata. The morphology and distribution of the remaining organelles and inclusions are similar to those previously described for the light cells in the zona fasciculata. The light cell type of the zona reticulo-medullaris was not detected in the zona fasciculata. Conversely, light cells of the zona fasciculata are occasionally observed in the zona reticulo-medullaris.

Mitotic figures in the cortical cells of the reticulo-medullaris are rare and were only found in the dark cell type (fig. 21).

B. Medullary cells

The medullary cells, which are predominantly the light cell type, are polymorphic in shape, tightly or loosely packed and exhibit few microvilli. The nuclei, 4-5 microns in diameter, are oblong in shape and display marked clumping of the heterochromatin and a prominent nucleolus (fig. 24). Mitochondria, which are polymorphic in shape and exhibit lamellar cristae, are moderately abundant and found throughout the cell (figs. 24, 25). Smooth and rough surfaced endoplasmic reticulum are present and occur as short narrow and often dilated tubules (figs. 24, 25). Well developed Golgi membranes and associated electron dense vesicles are evident as are numerous polyribosomes which are distributed diffusely throughout the cytoplasm (fig. 24).

Membrane-limited granules are numerous in both light and dark

cells. All of the dark cells observed and a majority of the light cells contain granules which are very electron dense (fig. 24). Some of the light cells contain a few moderately electron dense granules in addition to the very electron dense granules (fig. 25). According to Coupland (93), the very electron dense granules contain noradrenalin (NA) while the moderately electron dense granules contain adrenalin (A).

Non-myelinated axons are found either singly or in layers in the intercellular spaces between the medullary cells (fig. 26). These axons contain numerous filaments and tubules, smooth surfaced vesicles, small dense granules and an occasional mitochondrion.

Cytoplasmic fragments of both the cortical and medullary cells of the zona reticulo-medullaris are frequently observed in the sinusoidal lumen (figs. 22, 23, 27). These fragments appear to be derived from cytoplasmic projections of these cells which penetrate the endothelium and extend into the vascular lumen (figs. 23, 27).

Fetal Adrenals from Fetuses of Pre-term Progesterone treated Females

The morphological description of the adrenal glands from fetuses of the pre-term progesterone treated females was similar to that described for the term fetal adrenals.

Prolonged Gestation

Day 23 (figs. 28-35)

The fetal adrenal gland at day 23 of gestation exhibits no

apparent alterations in the morphology of the cells of the zona glomerulosa (fig. 28), zona intermedia (fig. 29), zona fasciculata (fig. 30), or of the cells of the zona reticulo-medullaris (figs. 31-35). The general arrangement of the cells, the numbers, distribution and size of the cytoplasmic organelles and inclusions are similar to those described for the cells of the term fetal adrenal gland. As was seen at term, cytoplasmic protrusions of the cortical and medullary cells of the zona reticulo-medullaris are found passing through the attenuated endothelium into the sinusoidal lumen (figs. 32, 24).

Day 24 (figs. 36-48)

At day 24 of gestation the cells of the zona glomerulosa (fig. 36) and zona intermedia (fig. 37) reveal no apparent alterations in morphology. The distribution of the light and dark cell types in both zones and their cytoplasmic organelles and inclusions remain similar to the description given for the cells of the zona glomerulosa and zona intermedia at term.

The majority of the light and dark cells of the zona fasciculata at day 24 are similar to those seen at term (figs. 38, 40). A number of others, however, exhibit ultrastructural changes in the mitochondria and smooth endoplasmic reticulum. The mitochondria display varying degrees of internal disorganization and a clumping of many of their cristae (figs. 39, 41). These cells also possess a few mitochondria which have doubled in size to that seen at term. The smooth

endoplasmic reticulum of these cells exhibits a significant increase in the numbers of dilated profiles (figs. 39, 41). Other cytoplasmic organelles such as the Golgi membranes, polyribosomes, dense bodies and lysosome-like structures are similar in arrangement and distribution to those previously described in the light and dark cells of the zona fasciculata at term.

The light and dark cortical cells described in the term zona reticulo-medullaris are present at day 24 of gestation but occur in fewer numbers (fig. 42). On the other hand, numerous dark (figs. 43, 44) and light (figs. 44, 45) cortical cells of this zone exhibiting morphological changes are frequently observed. Many of the morphologically altered dark cells exhibit numerous hypertrophied mitochondria in which the diameter often exceeds 2. 25 microns (fig. 43). The matrices of these mitochondria exhibit varying degrees of disorganization. The numerous hypertrophied mitochondria appear to compress the cytoplasm in these cells. An abundance of swollen smooth endoplasmic reticulum is apparent only in those dark cells which do not exhibit numerous hypertrophied mitochondria (compare fig. 43 with 44).

Hypertrophied mitochondria as well as an increased occurrence of swollen tubular profiles of the smooth endoplasmic reticulum are also present in the morphologically altered light cells of this zone (figs. 44, 45). The overall lipid content in this zone at day 24 of gestation is only slightly decreased over that observed at term. The

"LERM" complex is frequently observed in these cells. The protrusion of intracellular contents from the cortical cells through the endothelium into the sinusoidal lumen is occasionally observed during day 24 of gestation (fig. 44).

Medullary cells of the zona reticulo-medullaris at day 24 of gestation consist of both light and dark cells which are similar to those described at term (fig. 46). In addition, light medullary cells which exhibit morphological changes are frequently found at this time. These cells are characterized by the presence of swollen and rarefied mitochondria, a decreased number of membrane-limited catecholamine granules, and nuclei which occasionally appear rarefied. (figs. 47, 48). The granular endoplasmic reticulum, polyribosomes and Golgi complex, however, remain morphologically unchanged. Cytoplasmic fragments of the medullary cells which contain membrane-limited catecholamine storage granules, granular endoplasmic reticulum, and polyribosomes are often observed in the sinusoids (fig. 48).

Day 25 (figs. 49-64)

The cells of the zona glomerulosa (fig. 49) show no changes in morphology on day 25 of gestation and are similar to those described at term. Mitotic figures in the light cells at this stage of gestation are observed as frequently as they were at term.

The majority of the cells of the zona intermedia appear similar

to those described at term (fig. 50). In addition, the zona intermedia exhibits for the first time a few morphologically altered light cells (fig. 51) in which many of the mitochondria are hypertrophied and display varying degrees of matrical disorganization. Furthermore, there is an increase in the numbers of dilated tubular profiles of the smooth endoplasmic reticulum. Polyribosomes, Golgi membranes and lipid droplets have an appearance and distribution similar to the unaltered cells previously described in this zone.

The description of cytoplasmic organelles and inclusions in both morphologically altered and unaltered cells of the zona fasciculata (figs. 52-54) and zona reticulo-medullaris (figs. 55-64) is similar to the description given for these elements in the corresponding cell types of the fetal adrenal gland at day 24 of gestation. There is, however, an increase in the numbers of morphologically altered cells in both zones at day 25. The magnitude of the changes, however, is not increased over those observed at day 24. The increased numbers of morphologically altered cells is especially evident in the cortical and medullary cells of the zona reticulo-medullaris. The "LERM" complex is frequently observed at this stage of gestation (figs. 53, 54, 56, 57, 62, 64). The presence of cortical and medullary cytoplasmic fragments in the sinusoids is frequently seen (figs. 58, 59). On the other hand, the protrusion of cortical and medullary cellular elements through the attenuated sinusoidal endothelium was detected only occasionally (fig. 60, 61).

DISCUSSION

Morphology of the Fetal Adrenal Gland at the Light Microscopic Level

The adult adrenal gland of the rat (87, 94) and other mammals (87, 94) is divided into an outer cortical region which consists of the zona glomerulosa, zona fasciculata, and zona reticularis and an inner medullary region. There are different opinions as to when this zonation becomes discernable in the rat. Josimovich et al., (95) and Idelman (96) have stated that zonation is completed by term. However, the results of the current investigation, which are represented by the schematic drawing in figure #1, demonstrated that the fetal rat adrenal at term consists of a fairly well defined zona glomerulosa and zona fasciculata. The zona reticularis is not a distinct zone at this time nor is the medulla which is poorly developed and is represented by only a few cells. Furthermore, an intermingling of cells in these two areas occurs. As a result of this arrangement, the current author has combined the two into a single zone which is referred to as the "zona reticulo-medullaris". Mitchell (97), Lever (98) and Walaas et al., (99) have also reported a similar incomplete zonation of term and newborn rat adrenals. However, these authors did not refer to the intermingled cortical and medullary cells as a separate zone. Furthermore, Mitchell (97) reported that a distinct zona reticularis does not become apparent in the rat until two weeks after birth. The adrenal medulla, as studied by Eranko et al., (100) does

not become a separate entity until 10 days after birth.

Morphological Changes in the Fetal Adrenal Gland During Prolonged Gestation

Adrenal Cortex

Morphologically, ACTH administration in the human adult (101) and in both the adult (102) and fetal (103) rat has been reported to cause a hypertrophy of the zona fasciculata and reticularis, but not of the zona glomerulosa. Biochemically, other investigators have reported an increased corticosteroid production as a result of ACTH administration in adult rats (104), dogs (105) and humans (101, 106). Similar results have been reported in rats (107, 108) which have been subjected to a variety of ACTH-releasing stimuli such as ether anesthesia, scalding, immobilization, electrical shock, abdominal incisions, and in dogs (109) subjected to ether anesthesia and surgical trauma. In the current investigation, cellular hypertrophy was also observed during prolonged gestation in the zona fasciculata and reticulo-medullaris (cortical cells only). These findings, in view of the above mentioned observations of ACTH and stress, may be interpreted to indicate increased adrenal cortical activity in response to augmented ACTH release resulting from fetal stress. Fetal stress, in turn, would be due to placental dysfunction which is thought to occur in prolonged gestation (62, 66). A more detailed discussion of the relationship between adrenal function and fetal stress

will be presented in a subsequent section.

In order to determine whether exogenous progesterone has an effect similar to ACTH administration on the morphology of the fetal adrenal gland, adrenals from fetuses of the pre-term progesterone treated females were compared with those of the non-treated term females. Ultrastructurally, no significant differences were found between these two groups. These observations are further supported by studies of Kitchell and Wells (110) who reported that exogenous progesterone administered to rat fetuses prior to term caused no significant changes in adrenal gland morphology or weight. On the other hand, perfusion of the adult adrenal gland with progesterone was reported to result in the formation of increased amounts of corticosteroids (111). Maternal corticosteroids have also been shown to cross the placenta and cause atrophy of the fetal adrenal cortex (82). On this basis, it is reasonable to speculate that increased levels of maternal corticosteroids might also be produced as a result of the administration of exogenous progesterone during prolonged gestation. In turn, the increased levels of steroids produced could cross the placenta and cause some degree of atrophy in the fetal adrenal cortex. However, as was shown in this investigation, hypertrophy rather than atrophy was observed during prolonged gestation. This suggests that exogenous progesterone probably had no significant effect on the corticosteroid production in the maternal adrenal cortex. The studies

of Eguchi et al., (1) support this hypothesis. They reported that the adrenals of progesterone treated gravid females during prolonged pregnancy exhibited no changes in morphology or ascorbic acid content, a factor which is used as an index of adrenal cortical activity (88). On the basis of the above observations it may be concluded that the exogenous progesterone used in this study had no apparent morphological effect on the adrenal gland of the fetus.

The ultrastructural changes observed in the morphologically altered cortical cells included an increase in the quantity of dilated smooth endoplasmic reticulum and the presence of swollen mitochondria which exhibited varying degrees of internal disorganization. Several of the hydroxylating enzymes, such as 11 β -hydroxylase and 18-hydroxylase, which are needed for the synthesis of steroids are exclusively located in the mitochondria (112-116). In view of this fact, it is suggested that mitochondrial hypertrophy may reflect an increase in activity of these enzymes. This hypothesis is supported by the studies of Koritz (113) and Griffiths et al., (117). The latter investigators (117) reported that ACTH administration in the rat stimulated the 11 β -hydroxylase activity throughout the cortex but especially at the fasciculata-reticularis border. Koritz (113) noted not only the conversion of cholesterol to pregnenolone, but also a swelling of the mitochondria after the addition of ACTH to an incubation medium containing mitochondria, cholesterol, and essential co-factors. This

swelling phenomenon was reversed by the addition of pregnenolone to the medium. From these results he postulated that the swelling possibly represented an increase in mitochondrial permeability which could conceivably facilitate entry of cofactors and cholesterol and/or allow the pregnenolone formed to leave the mitochondria and enter the microsomal fraction for further metabolism.

Mitochondrial swelling and internal disorganization have also been reported by Holden et al., (118) in hepatic cells of rats subjected to shock. Fonnesu (119) reported similar observations in the liver cells of rats which had been given lethal doses of bacterial toxins. These authors interpreted this phenomenon as indicative of inhibited oxidative phosphorylation. On the basis of these studies, one might suspect that the mitochondrial swelling observed in the present investigation could be the result of fetal hypoxia, which is assumed to occur during prolonged gestation as a result of placental dysfunction (64-66). Further observations by Holden et al., (118) tend to rule out this possibility. These authors reported no significant ultrastructural changes in liver cell mitochondria of rats subjected to hypoxia (60 minute exposure to an atmosphere of 7% oxygen). Bassi et al., (120) reported similar observations in hepatic mitochondria of rats exposed to an atmosphere of 3% oxygen for 120 minutes. Based on the above studies, it is suggested that mitochondrial swelling and the increased dilatation of smooth endoplasmic reticulum observed in the morphologically

altered cortical cells during prolonged gestation may be indicative of increased cortical activity.

Morphological changes similar to those described in the present study have been reported by several investigators in the adrenal cortices of dogs (121), rats (96, 122, 123) and human adults (124) and fetuses (125) which had been treated with ACTH. It must be pointed out, however, that the magnitude of the morphological changes observed in those studies appears to be dependent upon the dose and duration of the stimulation. For example, Nishikawa et al., (123) and Ashworth et al., (122) noted no changes in the cortices of adult rats except for a decrease in the lipid content four hours after a single injection of 4-5 IU of ACTH. Johannison (125) reported similar observations in the "fetal" zone of the human fetus which had been perfused for 100 minutes with a dose of 60 IU of ACTH. On the other hand, Johannison (125) noted striking morphological changes in both "light" and "dark" cells of the "fetal" zone after 7 hours of ACTH administration. These changes were reported as a further depletion of lipid, a marked dilation of the smooth endoplasmic reticulum, and an increase in the number of polyribosomes which are essential for the synthesis of the enzymes involved in steroidogenesis (126, 127). She also noted the presence of numerous hypertrophied mitochondria which exhibited relatively few cristae and varying matrical densities. Similar observations were reported in adult rats (122, 123) which were treated with

4-5 IU of ACTH for 7 days. In these studies it was observed that the smooth endoplasmic reticulum in the zona fasciculata and reticularis of these animals became markedly dilated. A further depletion of lipid as well as an increase in the size and numbers of mitochondria was also noted. Idelman (96) also reported similar results in his experiments with rats which had received 3 IU of ACTH twice a day for 21 days. These observations are in agreement with the findings of Carr (124) and Bloodworth (121) who studied the effects of prolonged ACTH treatment on the adrenal cortices of humans and dogs.

Schwartz et al., (128) reported similar changes in mitochondria and smooth endoplasmic reticulum in the adrenal cortex of rats subjected to stress, i.e., adrenalectomy. He further reported, however, that these changes were not nearly as great in magnitude as those observed in the adrenal cortices of rats which had been treated with ACTH. This observation again suggests that the pronounced changes in morphology are most likely due to dosage levels and duration of ACTH administration.

It should be emphasized, however, that the magnitude of the changes reported by the various investigators was not apparent during prolonged gestation. This may be due to several factors. It was noted, for example, that the quantity of lipid in the zona fasciculata and reticulo-medullaris during prolonged gestation had decreased only slightly over the quantity seen in the same zone at

term.* This is not too surprising when one considers the studies of Kamoun et al., (129) and Eguchi et al., (1). Kamoun et al., (129) found greater concentrations of corticosterone in the adrenals of the fetal rat (18-21 days) than in the adrenals of the newborn animals. Moreover, Eguchi et al., (1) noted that the lipid content of the adrenal in the newborn rat was greater than in the term fetus. These observations suggest that the fetal adrenal cortex is highly active prior to birth and that marked changes in morphology and steroid concentration would not be observed as a result of further "stimulation", e.g., placental dysfunction.

Furthermore, the striking changes reported by other investigators were not observed under "normal" physiological limits. The doses of ACTH given in all instances, in rats at least, greatly exceeded that which is found in plasma at the "resting" state (130) i.e., 23-46 pgm/ml, and even after acute ether stress (130) i.e., 1000 pgm/ml.

There appears to be considerable disagreement as to the significance of the "dark" cells in steroid producing tissues. Christianson (131) claims that in the testis "dark" cells represent artifacts of

*Eguchi et al., (1) reported similar results at the light microscopic level in their studies of the fetal rat adrenal in prolonged gestation. Furthermore they reported that the adrenals in prolonged gestation increased in size over term while those in the newborn had reduced in size and were smaller than the adrenals either at term or during prolonged gestation.

fixation. He bases his statement on the fact that when glutaraldehyde fixation is used prior to osmium tetroxide fixation the "dark" cell type is not detected. The present author as well as Johannison (125), however, used glutaraldehyde prior to osmium tetroxide and observed the presence of "dark" cells in the adrenal cortex. Moreover Christianson (131) studied the testis of two different species, the opossum testis which was fixed in osmium tetroxide alone (132) and the guinea pig testis fixed in glutaraldehyde and osmium tetroxide (131). This in itself may account for the difference.

Several investigators who reported observing "dark" cells in the ovary of the adult rat (133) and in the adrenal cortex of the adult rat (134), mouse (135) and human fetus (136) all claim that these cells are active in the secretion of steroids. Giacomelli (137) who studied the zona glomerulosa in the adult rat claimed that these are cells in a "resting" state i.e., they disappear as a result of subjecting animals to sodium restricted diets. Other investigators studying the adrenal cortex of newborn (138) and adult (123) rats suggested that these cells represent elements of degeneration. As a result of several observations in the current investigation, the present author favors a "metabolically active" role for these cells. During prolonged gestation, as many "dark" cells of the zona reticulo-medullaris responded to stress as "light" cells of the same zone. Furthermore, fewer numbers of "light" cells of the zona fasciculata

responded in a similar fashion during the same time interval i.e., days 24 and 25 (see table on page 35). It was also noted that the overall zonal response to stress advanced from the zona reticulo-medullaris to the zona fasciculata and eventually to the zona intermedia. Finally, a mitotic figure was detected in a "dark" cell which in itself would preclude degeneration (see fig. 21).

Adrenal Medulla

In the current study it was reported that numerous morphologically altered cells, which were characterized by swollen and rarefied mitochondria and a paucity of catecholamine storage granules, were seen during prolonged gestation (day 24 and 25) but not at term. This observation is interpreted as an attempt by the cells of the fetal adrenal medulla to respond to hypoxia and/or insufficient levels of blood glucose resulting from placental dysfunction. This hypothesis is supported by the biochemical studies of Hokfelt (139) who reported a decrease in the adrenalin but not the noradrenalin content of the adrenal medullae of rats subjected to hypoxia and insulin induced hypoglycemia. Histochemically, Hillarp et al., (140) reported similar observations in the medullae of rats and cats which were subjected to insulin induced hypoglycemia. These investigators interpreted their results as the response by the adrenal medulla to counteract the tendency of hypoxia and hypoglycemia to lower blood glucose levels.

ZONAL RESPONSE☆ TO STRESS DURING PROLONGED GESTATION

ZONE	DEGREE OF RESPONSE & DAY		
	DAY 23	DAY 24	DAY 25
Zona glomerulosa			
Light cells	0	0	0
Dark cells	0	0	0
Zona intermedia			
Light cells	0	0	+
Dark cells	0	0	0
Zona fasciculata			
Light cells	0	+	++
Dark cells	0	+	++
Zona reticulo-medullaris			
Cortical cells			
Light cells	0	++	++++
Dark cells	0	++	+++
Medullary cells			
Light cells	0	++	+++
Dark cells	0	0	0

0 = None
\+ = Few
++ = Moderate
+++ = Many
++++ = Majority

☆Zonal Response is measured in terms of numbers of cells in each zone undergoing ultrastructural modification in response to stress.

The ultrastructural observations of Yates (141) and D'Anzi (142) were also interpreted in a similar manner. These authors noted the preferential depletion of adrenalin storage granules in the adrenal medullary cells of adult hamsters and rats subjected to insulin induced hypoglycemia. Moreover, both investigators noted that these stressed cells exhibited large swollen mitochondria which were absent in the non-stressed cells.

However, in the current investigation, the majority of the cells of the term fetal rat medulla exhibited only the noradrenalin storage granules, although a few cells exhibited adrenalin as well as noradrenalin granules. Cells which contain adrenalin exclusively and are known to be the predominate cell type in the medulla of the adult rat (143) were not observed at term or, for that matter, during prolonged gestation. Similar observations were reported by Elfvin (144) who studied the development of catecholamine granules in the adrenal medulla of the fetal rat. However, he reported observing the adrenalin type cell of the adult in the medulla of the 2 day old neonate. These observations are in keeping with Hokfelt's (139) studies on the catecholamine content in the term fetal rat medulla which was found to contain a greater concentration of noradrenalin than adrenalin.

From the preceeding observations, it would appear that during prolonged gestation the medullary cells which responded to hypoxia and/or hypoglycemia were probably destined to become the adrenalin

type cells in the adult, even though they contained primarily, if not exclusively, noradrenalin.

Function of the Fetal Adrenal Gland during Prolonged Gestation

As previously discussed at the biochemical and morphological levels, various experimental stimuli resulted in the stimulation of the cortex and medulla, with a concomitant increase in the elaboration of their secretory products. It was also mentioned that the effects of these stimuli showed similar ultrastructural changes in the cortex and medulla. Although no ultrastructural studies on the effects of hypoxia and starvation on the adrenal gland have been published, it is reasonable to suspect that similar ultrastructural changes would occur, in view of the fact that hypoxia as well as starvation do cause an increase in corticoid (145) and catecholamine (146) elaboration.

Assuming that in some cases of prolonged gestation fetal hypoxia and inanition do occur as a result of placental dysfunction and on the basis of a functioning pituitary-adrenal axis which does exist in the fetus, it is reasonable to speculate that the adrenal gland of the postmature fetus responds in a similar fashion. The ultrastructural changes which were described during prolonged gestation in the current investigation would tend to support this hypothesis.

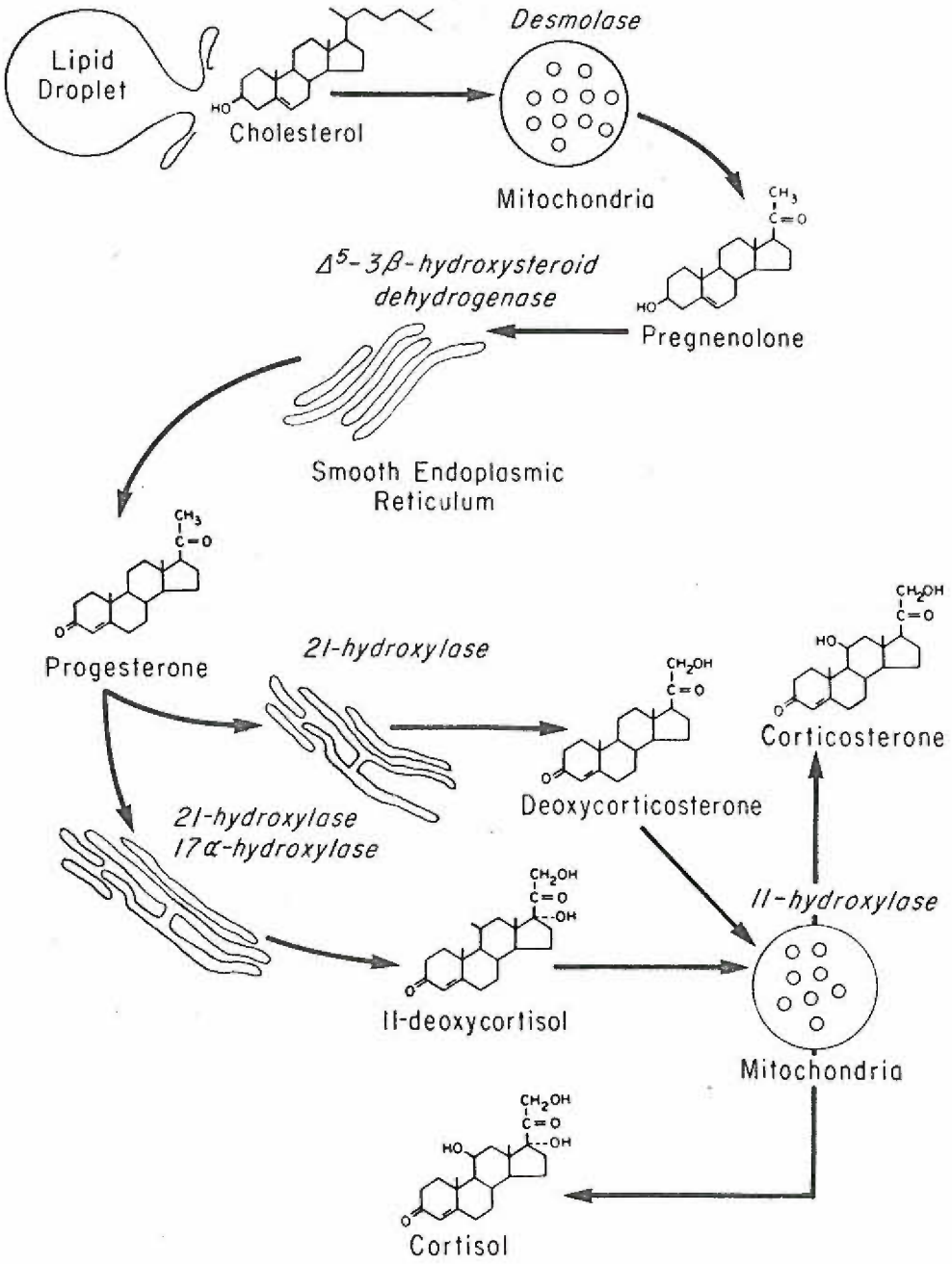
Morphological changes similar to those previously discussed in the zona fasciculata and reticularis of the adult, such as

mitochondrial hypertrophy and dilatation of the smooth endoplasmic reticulum, were also reported in the zona glomerulosa of the adult rat subjected to sodium deprivation (137). On the other hand, morphological changes were not observed in the zona glomerulosa during prolonged gestation in the current investigation. This suggests that the zona glomerulosa in the fetus is non-functional during prolonged gestation, or for that matter during the normal course of gestation. This hypothesis is supported by the fact that the electrolyte balance in the fetus of the rat (81) as well as in the human (147) is regulated by the maternal system via placental transport during gestation and even after the mother has been subjected to a low sodium diet. The above hypothesis may further be supported by the fact that the close association between mitochondria, lipid droplets and what appears to be "elements of the smooth endoplasmic reticulum", which is thought to be related to the steroid synthesizing activity of the cell, was observed only in the zona fasciculata and reticulo-medullaris both at term and during prolonged gestation. Moreover, Giacomelli et al., (137) reported the presence of a similar structure in the zona glomerulosa of the adult rat. This association of organelles which has been designated as the "LERM" complex in the current investigation has also been reported in the sow ovary (148) and in the adrenal cortex of the adult rat (96) and human (149). It should be noted, however, that Yates (150) and Long et al., (151, 152) did not observe this

association in the adrenals of the hamster, opposum and human respectively. Idelman (96) mentioned the possibility that this association may be myelinic artifacts produced by prefixation with aldehydes. Giacomelli et al., (137) reported his findings in tissue which was fixed only in osmium tetroxide. Moreover, Hashida et al., (149) noted the presence of this association in tissues which were fixed either in osmium tetroxide alone or in glutaraldehyde followed by osmium tetroxide.

The present investigator interprets this association as a possible morphological representation of the steroid synthesizing activity of the cell and is schematically diagramed on page 40. This scheme takes into account the known biochemical pathways which require both the mitochondrial and microsomal fractions for the synthesis of steroids in the adrenal cortex (112, 113, 114, 116). This scheme suggests that steroid precursors such as cholesterol, which are found in the lipid droplets (153), pass to the mitochondria via the smooth endoplasmic reticulum for conversion to pregnenolone (desmolase system) (112, 113). The pregnenolone then returns to the smooth endoplasmic reticulum for conversion to progesterone (Δ^5 3β -hydroxysteroid dehydrogenase) (154). Progesterone in turn is converted to either deoxycorticosterone (21-hydroxylase) or 11-deoxycortisol (17α-hydroxylase, 21-hydroxylase) in the smooth endoplasmic reticulum (155). Deoxycorticosterone and 11-deoxycortisol then pass back

SCHEMATIC REPRESENTATION OF THE PATHWAY OF STEROIDOGENESIS INCLUDING THE SUBCELLULAR LOCALIZATION OF THE PARTICIPATING ENZYME SYSTEMS



to the mitochondria and undergo 11β -hydroxylation for conversion into the end products cortisol and corticosterone (114, 116). The above scheme of events is consistent with the views presented by Bjersing (148), Idelman (96) and Giacomelli et al., (137) who, as mentioned above, also reported observing a similar association of organelles.

In the review of the literature, fetal inanition was denoted as one of the characteristics of the postmaturity syndrome which in the human is thought to result from placental dysfunction. Clifford (3) and Sjostedt (66) theorized that fetal weight loss reflected the failure of the placenta to provide the fetus with adequate nourishment which would result in the fetus having to depend on its own resources for survival. The studies of Eguchi et al., (1) revealed that fetal weight loss in the rat during prolonged gestation did not occur. This may be due to the fact that gestation in the rat can be prolonged no longer than 3 days and fetal viability still maintained. However, based on the morphological changes found in the current investigation, it is of interest to speculate whether the weight loss in humans during prolonged gestation is associated in large part with the metabolic function of the adrenal cortex and medulla of the fetus during this period.

As previously discussed, Jacquot (73) and Jost (72) have reported that fetal glycogen appears to be controlled by the hormonal activity of the fetal pituitary and adrenal cortex.

It has also been reported that the fetal livers of humans,

monkeys, and rats contain large quantities of glycogen which is thought to be utilized in the newborn for the maintenance of adequate blood glucose levels until suckling is established (156). Assuming that placental dysfunction exists in prolonged pregnancy, these glycogen stores could conceivably be drawn upon to maintain adequate blood glucose levels in the fetus during this period. This hypothesis is supported by the observations of several investigators (157-159) who reported that the fetal liver contains glucose-6-phosphatase, albeit in low amounts, which is required for the conversion of glycogen to free glucose. Since hepatic glycogenolysis in the adult is influenced predominately by pancreatic glucagon (160, 161), one may assume that the fetal pancreatic glucagon would be released during this period.

If, on the other hand, the fetal liver glycogen stores were to become depleted, which would cause a decrease in the blood glucose level, it is conceivable that the gluconeogenic effects of the fetal adrenal gland and pancreas would be initiated. The studies of Williams (161), Jacquot et al., (162) and Exton et al., (160) support this hypothesis. According to Jacquot et al., (162) the gluconeogenic effects of the fetal adrenal cortex and probably the pancreas are already active since the major precursor substances of fetal liver glycogen are amino acids rather than glucose. This observation may substantiate the high levels of corticosterone reported by Kamoun (129)

in the preterm rat fetus.

Gluconeogenesis in the adult liver, as reviewed by Exton et al., (160) and Williams (161) is stimulated primarily by glucagon and to a lesser extent by adrenal glucorticoids and catecholamines. Glucocorticoids and catecholamines exert their primarily gluconeogenic effect extrahepatically in that they mobilize glycerol from lipid stores (catecholamines and glucocorticoids) and amino acids from muscle protein (glucocorticoids). In turn these substrates, as well as muscle lactate, are converted in the liver to glucose which is released in the blood stream. Muscle glycogenolysis, on the other hand, is apparently stimulated only by the catecholamines (160, 161). In contrast to liver, muscle glucose is not liberated into the blood stream due to a lack of glucose-6-phosphatase and is subsequently metabolized in the tissue itself.

According to the review by Williams (161), episodes of starvation and/or hypoglycemia cause an increase in hepatic glycogenolysis as is shown by the rapid conversion of glycogen stores into free glucose.* Gluconeogenesis is also increased during these conditions in order to maintain adequate blood glucose levels. Moreover, Chowers (163) and Goldfien et al., (164) also reported elevated glucocorticoids and catecholamines in dogs which were subjected to

*Hypoxia also increases hepatic glycogenolysis but the mechanism involved is unknown (165).

starvation and insulin induced hypoglycemia.

On the basis of the evidence presented above, the morphological changes which were described in the stimulated cortical and medullary cells of the fetal adrenal gland during prolonged pregnancy are interpreted as reaction by the fetal adrenal to mobilize lipid and protein for conversion in the liver to free glucose. In this way sufficient levels of blood glucose could be maintained for survival. Fetal inanition which is observed during prolonged gestation would therefore result from lipid and protein mobilization by adrenal glucocorticoids and catecholamines.

A Possible Mechanism for the Release of Secretory Products in the Fetal Adrenal Gland

The mechanism for the release of cortical steroids and medullary catecholamines is to date not fully understood. According to Fujita (166) and Lever (167) the secretory products of the cortex are probably first secreted into the perivascular space and then passed into the vascular lumen. Other investigators have presented evidence that in the adrenal medulla of the rabbit (168), hamster (169) and rat (143) a merocrine type secretion release occurs. This is defined as the release of secretory products through the cell membrane with the cell remaining intact (170). Coupland (143), DeRobertis et al., (168) and Diner (169) reported that the smooth surfaced vesicles which contained granular material (catecholamines) coalesced with the cell

membrane and then voided their contents into the intercellular or perivascular spaces. Farquahar et al., (171) and Couch et al., (172) observed a similar mechanism of release from cells of the anterior pituitary gland of rats. In contrast to these observations, Yates (141) reported that a gradual reduction in the density of the membrane-limited granules occurred in the adrenal medulla of hamsters subjected to insulin induced hypoglycemia. Furthermore, he did not observe the membrane fusion phenomenon reported by the other authors. He interpreted his findings as evidence that catecholamines are released intracellularly without the actual disappearance of the granules.

In the current investigation, cytoplasmic protrusions extending through endothelial cell fenestrations into the sinusoidal lumina were occasionally observed at term and during prolonged gestation. Cytoplasmic fragments free in the lumina suggest that these protrusions become pinched off and thus released into the sinusoidal lumina. This phenomenon, which was observed in both cortical and medullary cells, is suggestive of an apocrine type secretory mechanism which is defined as loss of part of the cell cytoplasm along with the secreted products (170). Luse (173) and Zelander (174) have reported similar observations and interpretations in studies of the adrenal cortex of dogs and mice respectively.

This secretory mechanism described in the current study is not considered to be artifactual in nature. This is based on the

fact that on no occasion was the attenuated sinusoidal endothelium seen disrupted or fragmented as would be the case if inadequate preservation and/or mechanical traumatization occurred. However, it is unclear whether this process represents a mode of apocrine secretion or the release of degenerating cellular elements.

SUMMARY

The structure of the fetal rat adrenal gland is described by light and electron microscopy at term and during postmaturity i.e., gestation experimentally prolonged 1, 2, and 3 days beyond the normal time of parturition.

The cortical and medullary cells examined at all stages exhibit well developed cytoplasmic organelles and inclusions. On the other hand, zonation of the gland is not distinct at this time. The zona glomerulosa and fasciculata are fairly well delineated but the zona reticularis and medulla are poorly defined. In addition, the cells of the future reticularis and medulla are intermingled and therefore referred to as the "zona reticulo-medullaris" in this investigation.

A close association of mitochondria, lipid and membraneous structures morphologically similar to smooth endoplasmic reticulum is frequently observed in both light and dark cortical cells of the zona fasciculata and zona reticulo-medullaris. This association of organelles, referred to as the "LERM" complex, is interpreted as a possible morphological representation of the biochemical pathway of steroidogenesis.

During prolonged gestation, many of the light and dark cortical cells of the zona fasciculata and reticulo-medullaris exhibit swollen and rarefied mitochondria and increased dilatation of the smooth endoplasmic reticulum. In addition to swollen and rarefied

mitochondria, many light medullary cells also exhibit depletion of catecholamine storage granules. On the basis of known biochemical and morphological data, these ultrastructural changes are interpreted as a reflection of increased activity by the fetal adrenal gland to fetal stress (hypoxia and hypoglycemia) probably caused by the development of placental dysfunction during prolonged gestation.

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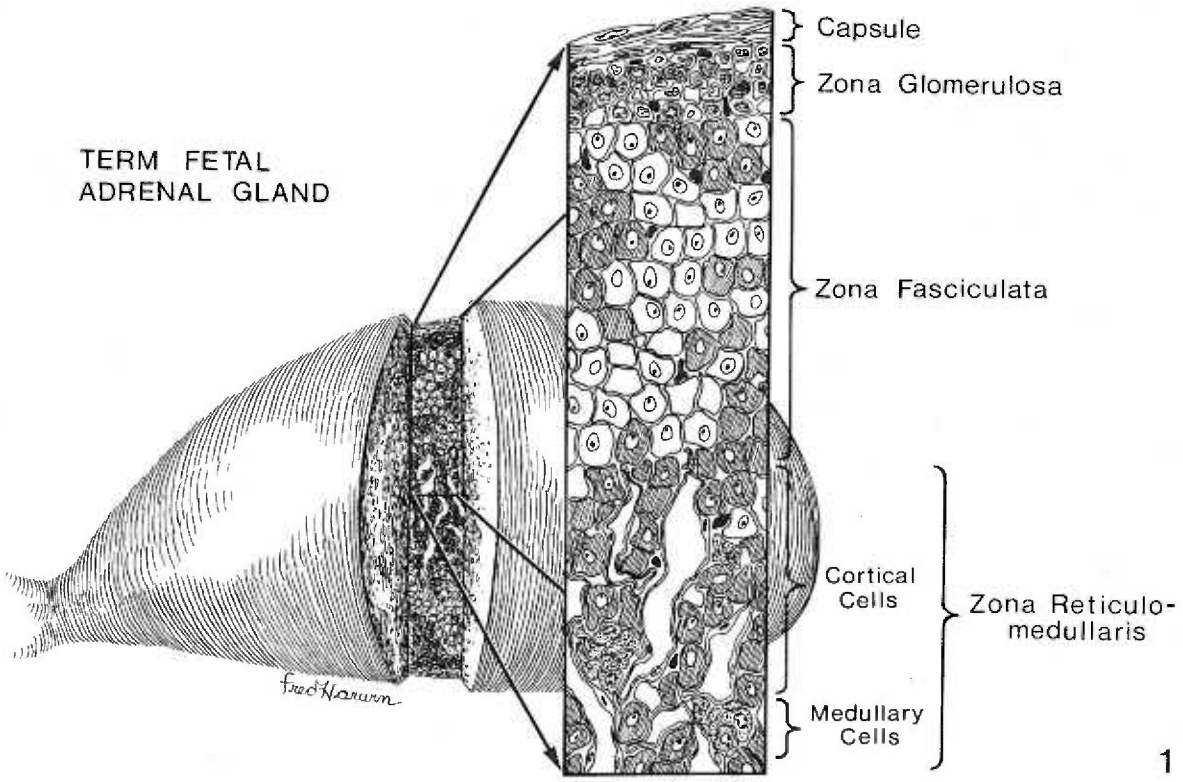
FIGURE 1

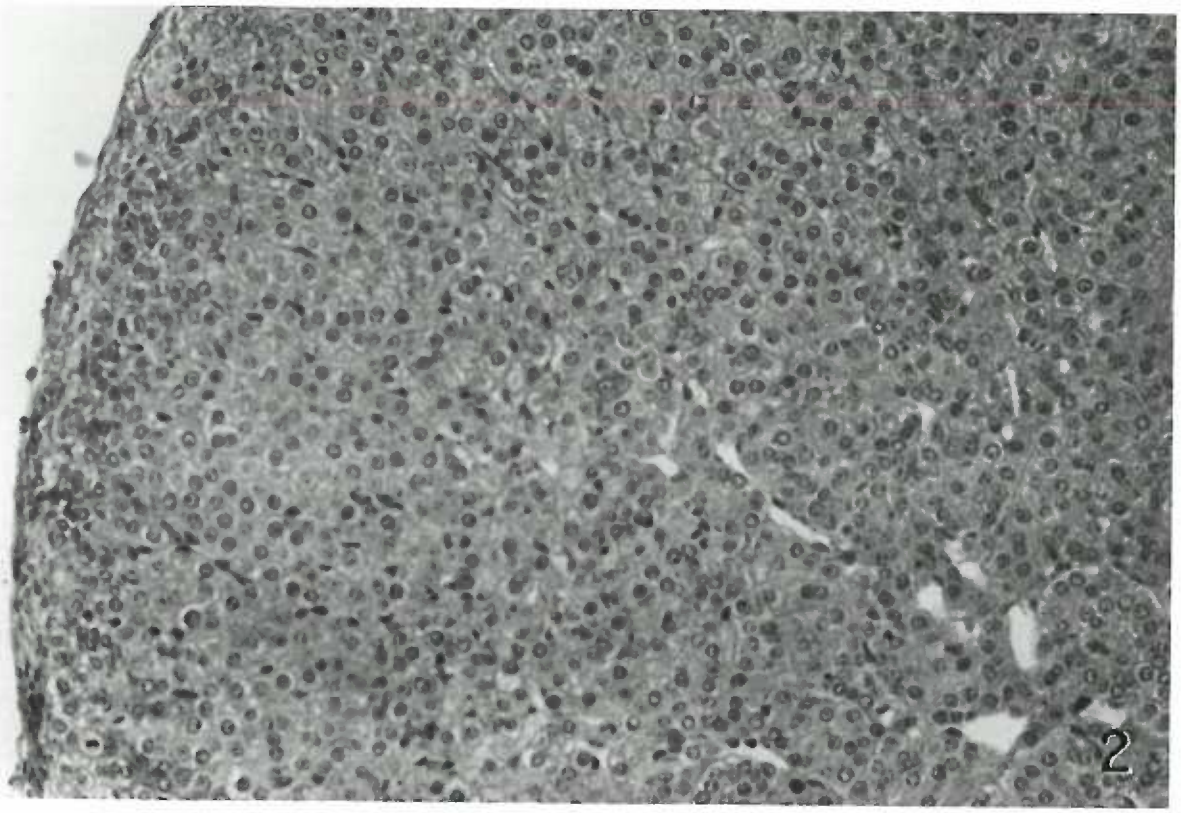
Schematic drawing of the fetal rat adrenal gland. Note the outer narrow zona glomerulosa which consists of small light and dark cells, the wide zona fasciculata consisting of large light and dark cells, and the innermost zone, the zona reticulo-medullaris which consists of large light and dark cortical cells intermingled with smaller light and dark medullary cells.


TERM FETAL
ADRENAL GLAND
Capsule
Zona Glomerulosa
Zona Fasciculata
Cortical
Cells
Zona Reticulo-
medullaris
Medullary
Cells
Fred Kamm

1

FIGURES 2 & 3

Cross sections of the fetal rat adrenal at term (fig. 2) and in prolonged gestation (fig. 3). Magnification 211 X. In contrast to term, the fetal adrenal gland in prolonged pregnancy shows a hypertrophy of the cortical cells in the zona fasciculata and zona reticulomedullaris.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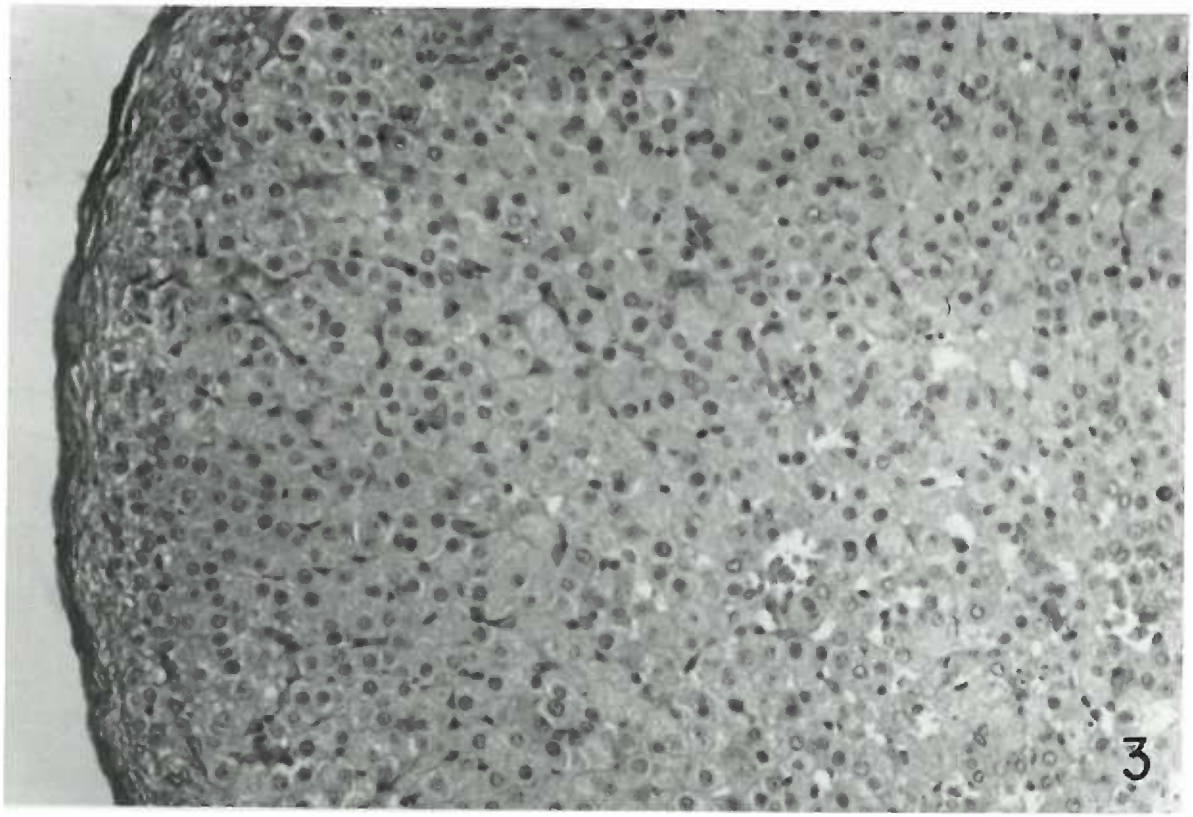

3

ABBREVIATIONS FOR FIGURES 4-64

A - adrenalin storage granules

Ax - axon

Bl - basal lamina

Co - collagen fibrils

CT - connective tissue cell

Db - dense body

DC - dark cell

Des - desmosome

End - endothelium

G - Golgi membranes

Gly - glycogen

ICS - intercellular space

L - lipid

LC - light cell

Lys - lysosome-like structure

M - mitochondria

MED - medullary cell

mv - microvilli

NA - noradrenalin storage granules

nf - neurofilaments

nt - neurotubules

Ps - polyribosomes

RER - rough endoplasmic reticulum

SER - smooth endoplasmic reticulum

SL - sinusoidal lumen

Vac - lipid vacuole

ZG - zona glomerulosa

ZI - zona intermedia

FIGURE 4

Cross section through the term fetal adrenal gland connective tissue capsule (CT). Magnification 12, 760 X. The connective tissue cells exhibit well developed rough endoplasmic reticulum (RER), elongated mitochondria (M) with lamellar cristae, polyribosomes (Ps), a well developed Golgi system (G) and an occasional dense body (Db) and lysosome-like structure (Lys). The connective tissue cells which are frequently attached by desmosomes (Des) are separated by a loose network of collagen fibrils (Co). The capillary endothelial cytoplasm (End) displays a prominent Golgi system (G), elongated mitochondria (M), and a well developed system of rough endoplasmic reticulum (RER).

Zona glomerulosa (ZG)

Intercellular space (ICS)

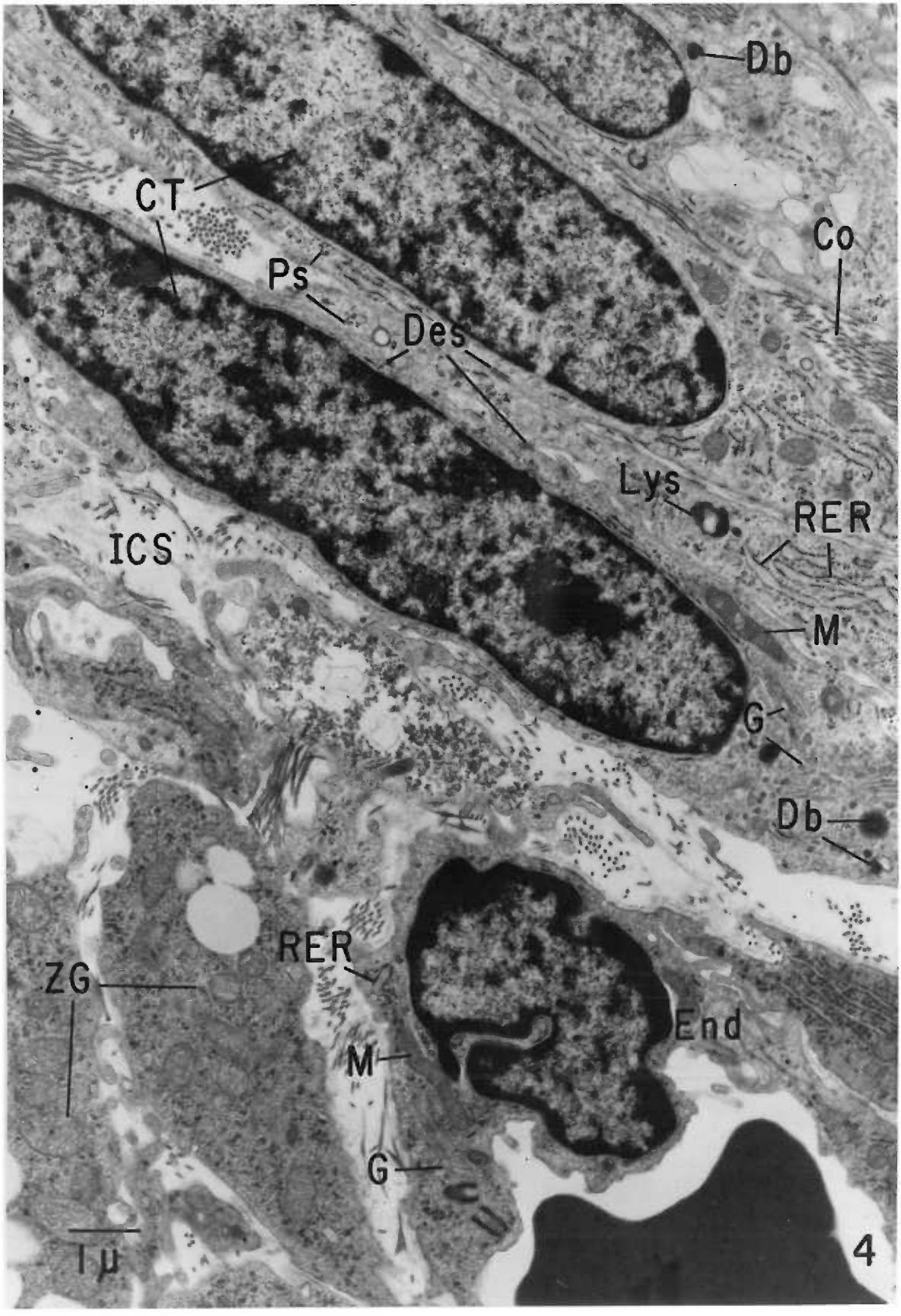

Db
CT
Co
Ps
Des
Lys
RER
ICS
M
G
Db
RER
ZG
End
M
G
1 μ
4

FIGURE 5

Term zona glomerulosa. Magnification 10,120 X. One of the light cells of the zona glomerulosa which lies beneath the connective tissue capsule (CT) is in an early stage of division (metaphase). Capillaries in this zone are characterized by an attenuated endothelium (End) which exhibits numerous fenestrations (arrows).

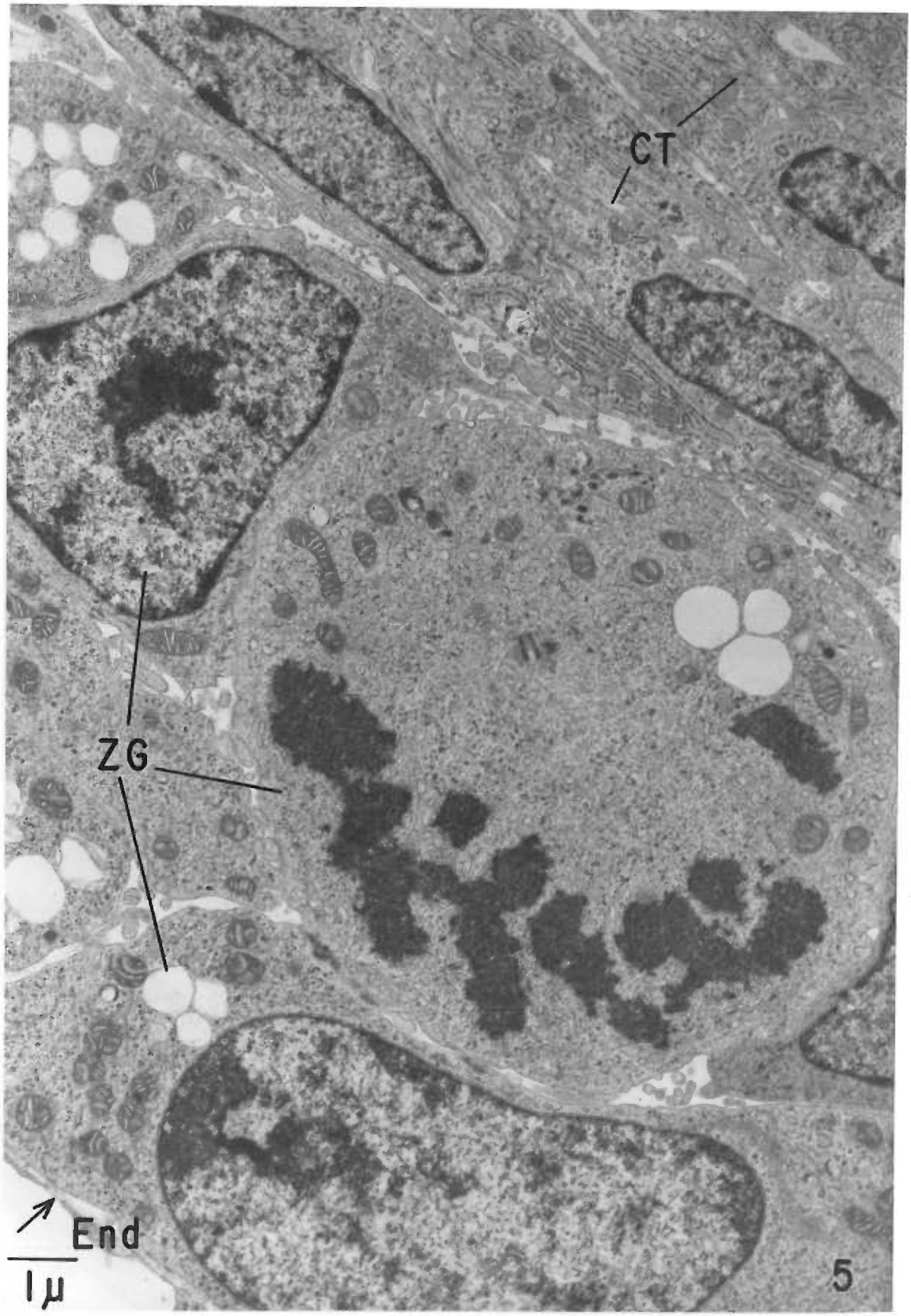

CT
ZG
End
1μ
5

FIGURE 6

Light (LC) and dark (DC) cells of the term zona glomerulosa. Magnification 11, 000 X. These cells exhibit polymorphic nuclei, dense bodies (Db), lysosome-like structures (Lys), and numerous cytoplasmic vacuoles (Vac) which represent extracted lipid droplets. Microvilli (mv) are frequently observed in both light and dark cells. The capillary endothelium exhibits a polymorphic nucleus and a well defined basal lamina (Bl).

Desmosomes (Des)

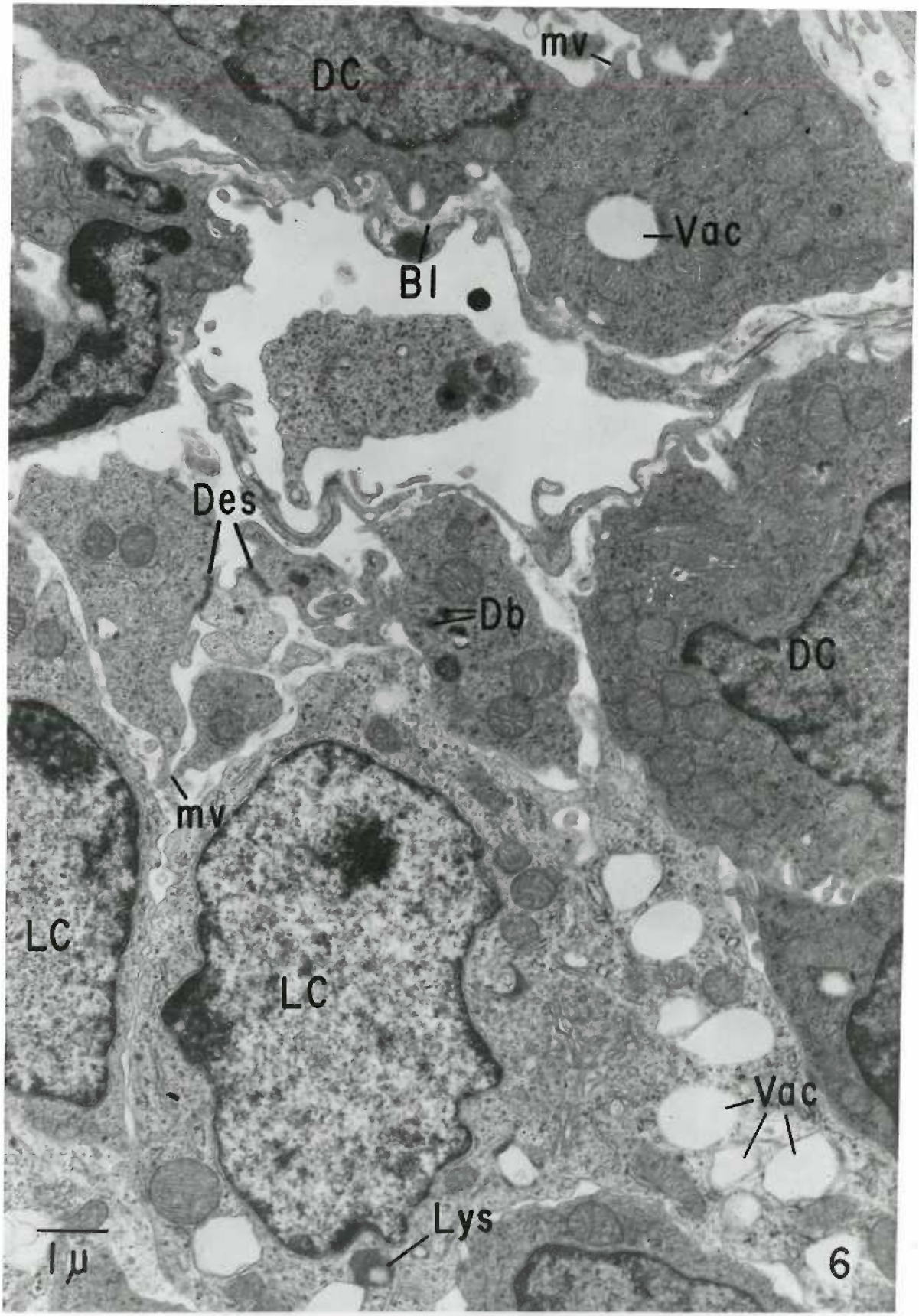

DC
mv
Vac
Bl
Des
Db
DC
mv
LC
LC
Vac
Lys
1 μ
6

FIGURE 7

Light cell of the term zona glomerulosa. Magnification 22, 440 X. This cell exhibits numerous polyribosomes (Ps), polymorphic mitochondria (M) with tubular cristae and a paucity of rough (RER) and smooth (SER) endoplasmic reticulum.

Desmosomes (Des)

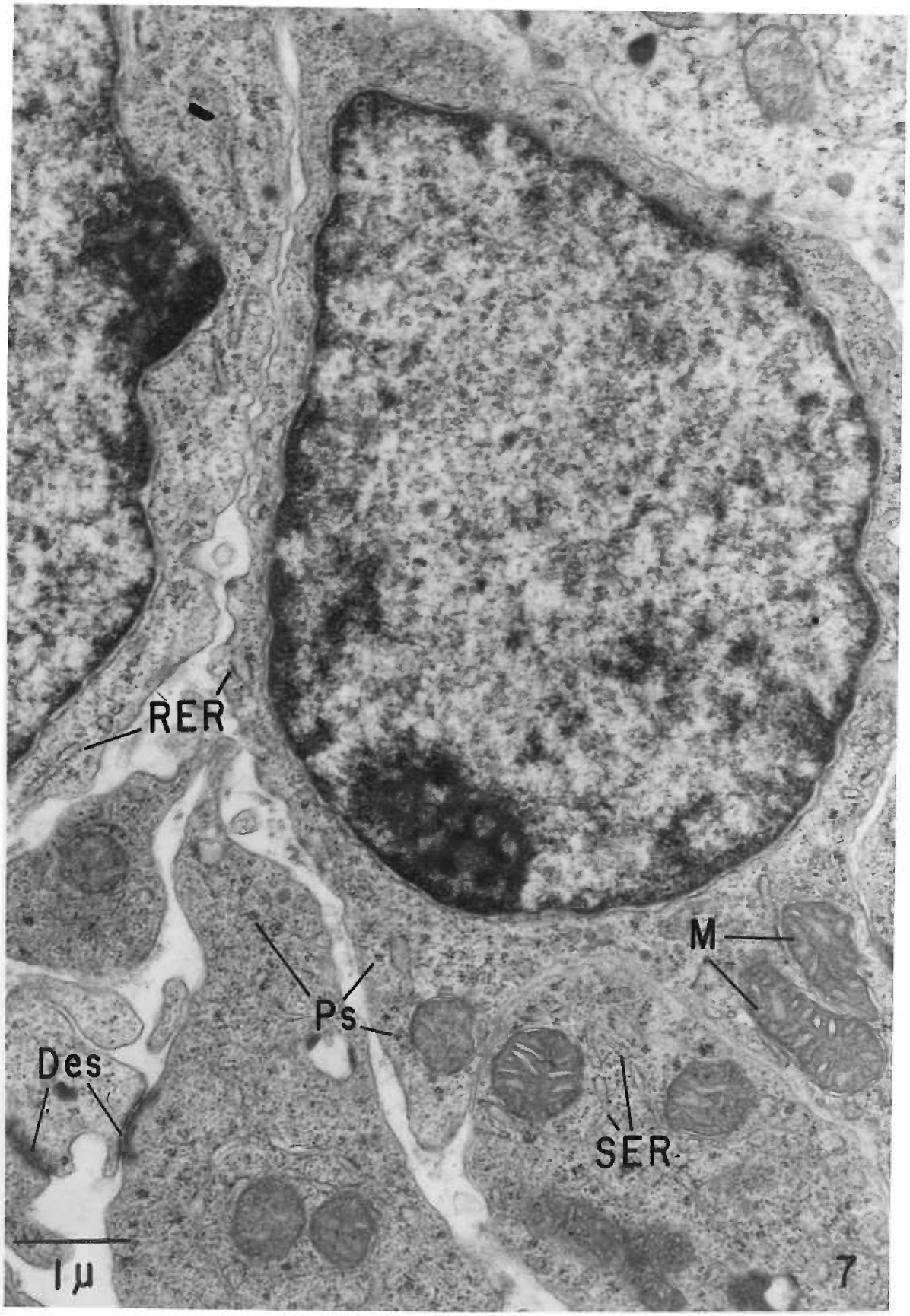

RER
Ps
M
Des
SER
1 μ
7

FIGURE 8

Enlarged portion of the light (LC) and dark (DC) cells in fig. 6. Magnification 22, 440 X. These cells exhibit a well developed Golgi system (G), numerous cytoplasmic vacuoles (Vac), and polymorphic mitochondria (M) with tubular cristae. Note the paucity of rough (RER) and smooth (SER) endoplasmic reticulum.

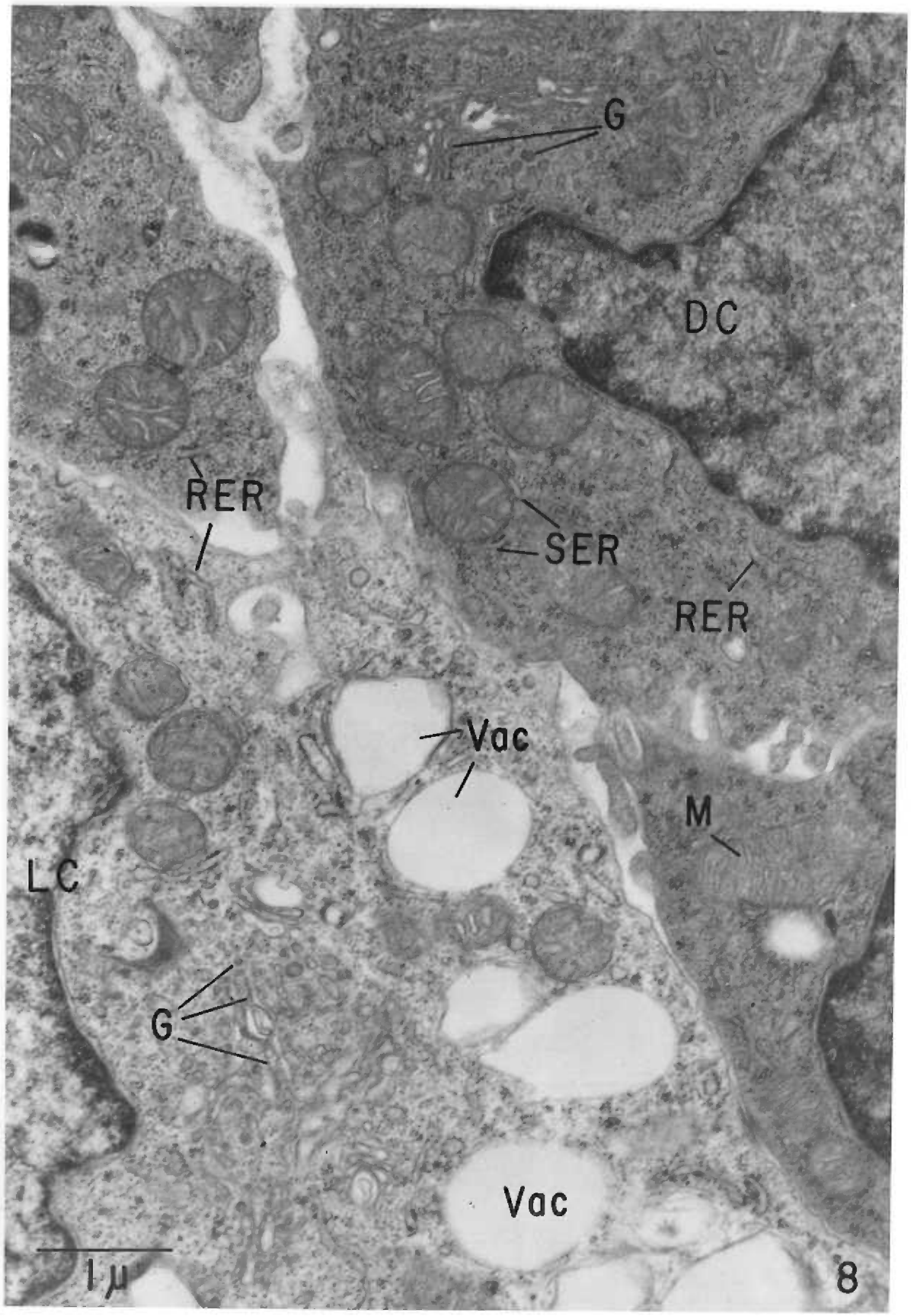

G
DC
RER
SER
RER
Vac
M
LC
G
Vac
1 μ
8

FIGURE 9

Light (LC) and dark (DC) cells of the term zona intermedia (ZI). Magnification 10, 686 X. These cells which are located between the zona glomerulosa and zona fasciculata exhibit numerous lipid droplets (L) and mitochondria (M) with tubular and vesicular cristae. Microvilli (mv) are commonly noted in these cells.

Zona glomerulosa (ZG)

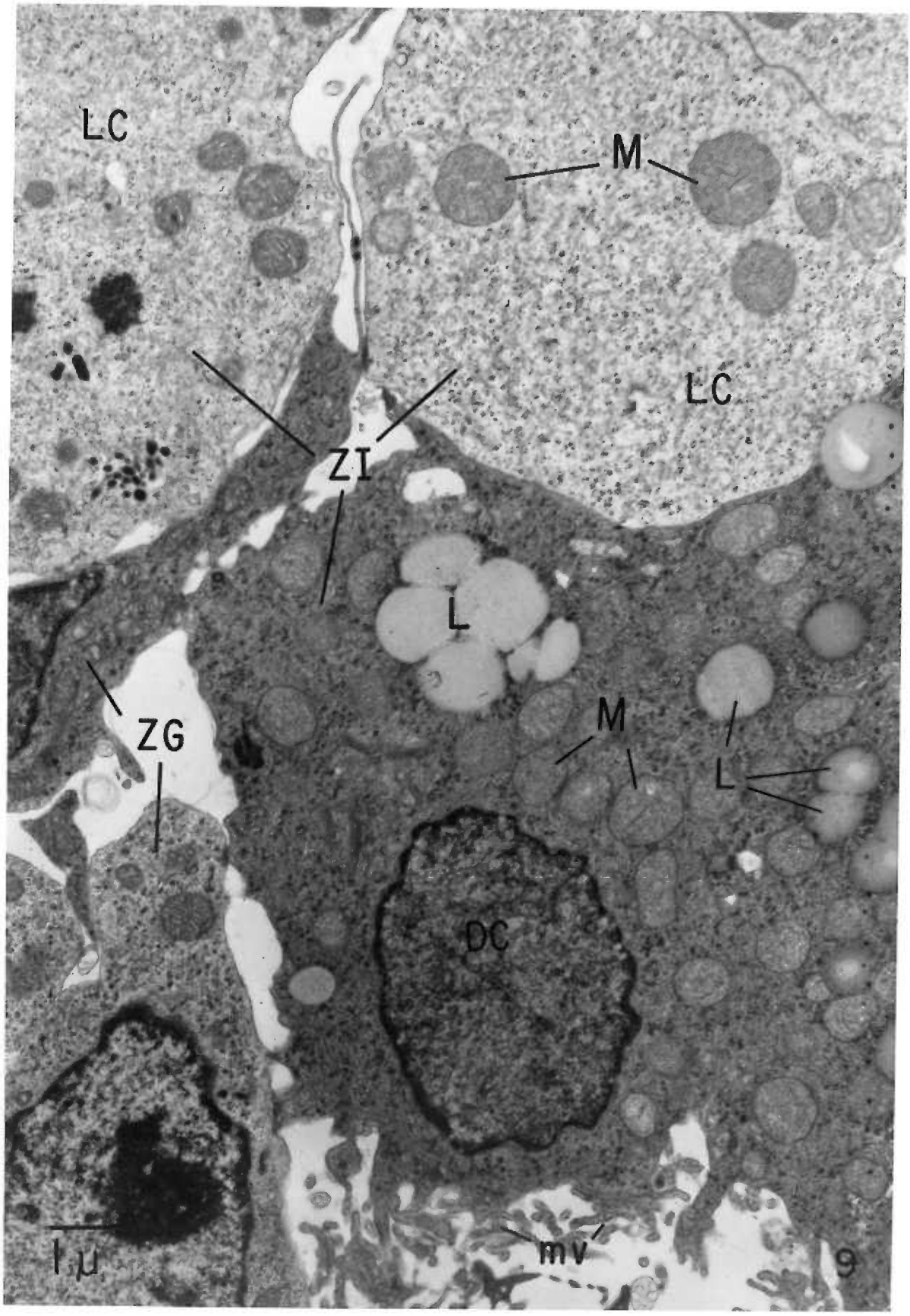

LC
M
LC
ZI
L
ZG
M
L
DC
1 μ
mv
9

FIGURE 10

Section through a light cell of the term zona intermedia. Magnification 22, 440 X. Cells of this zone are characterized by a well developed Golgi system (G), spherical mitochondria (M) with tubular and vesicular cristae, and an abundance of smooth endoplasmic reticulum (SER) which consists of tubular profiles. At this stage of gestation, only a few of the profiles are dilated (arrow).

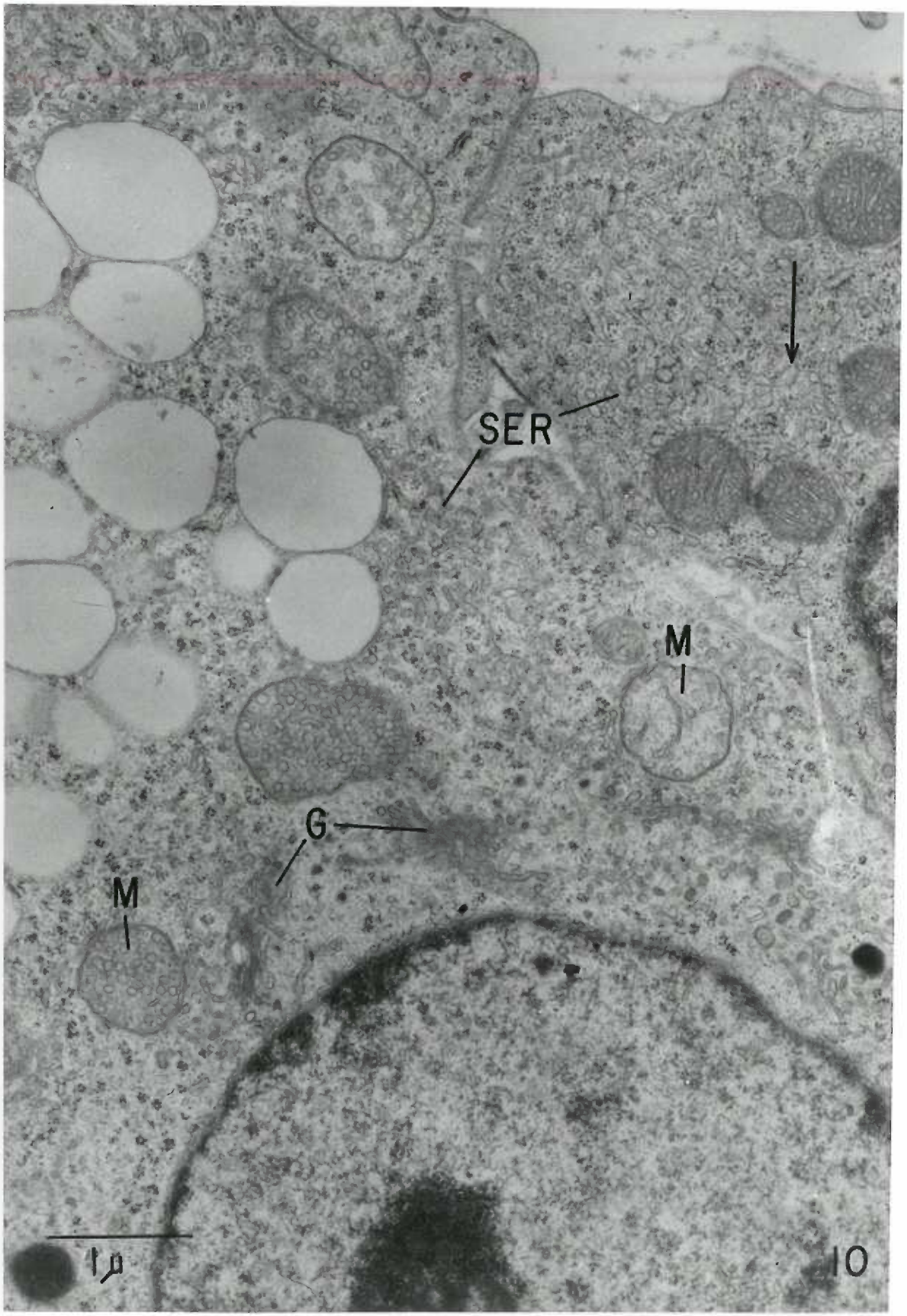

SER
M
G
M
1μ
10

FIGURE 11

Light (LC) and dark (DC) cells of the term zona intermedia. Magnification 9, 680 X. The cells of the zona intermedia display numerous mitochondria (M), lipid droplets (L) and vacuoles (Vac) representing extracted lipid. Microvilli (mv) are also numerous. Dense bodies (Db) and lysosome-like structures (Lys) are infrequently observed. Note that the attenuated endothelium (End) exhibits numerous fenestrations (arrows). A portion of the zona glomerulosa (ZG) is seen in the upper left hand corner.

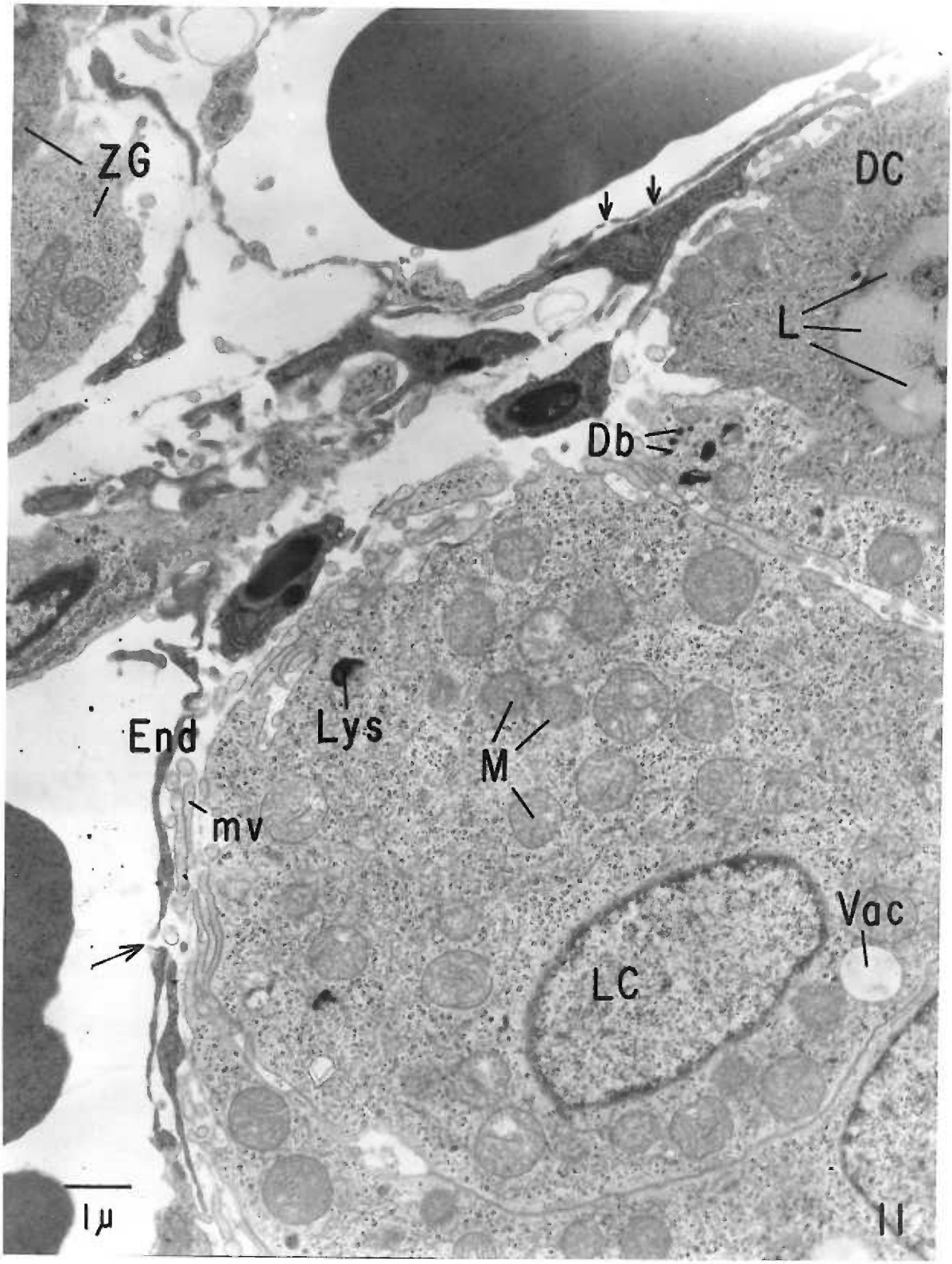

ZG
DC
L
Db
End
Lys
M
mv
Vac
LC
1μ
11

FIGURE 12

Enlarged portion of the light cell (LC) in figure 11. Magnification 22, 440 X. The cytoplasm of this cell is characterized by numerous polyribosomes (Ps) and spherical mitochondria (M) which exhibit tubular and vesicular cristae. The smooth endoplasmic reticulum (SER) is abundant and consists of branching tubular profiles. Rough endoplasmic reticulum (RER), dense bodies (Db) and lysosome-like structures (Lys) are not numerous in these cells.

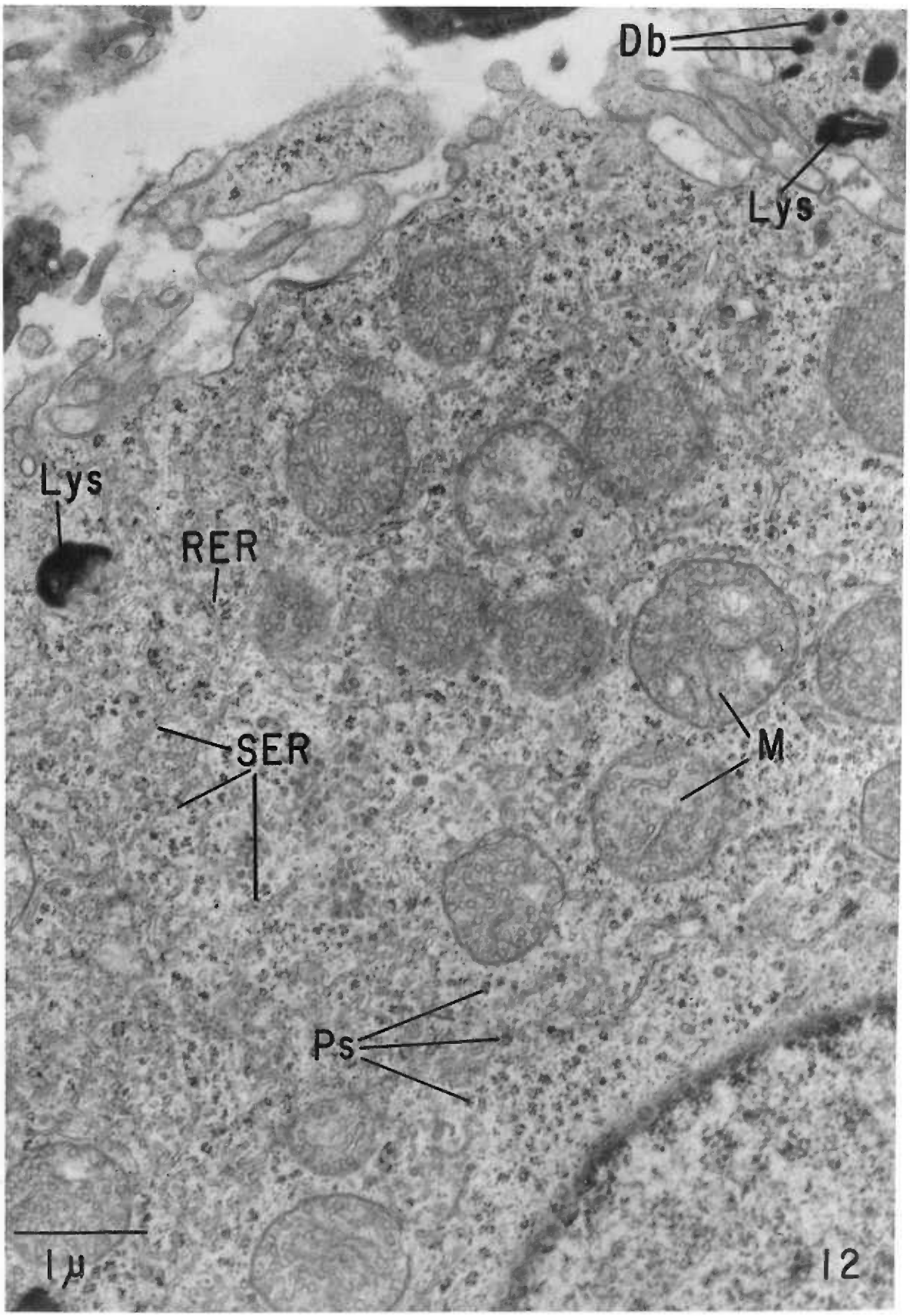

Db
Lys
Lys
RER
SER
M
Ps
1μ
12

FIGURE 13

Term zona fasciculata. Magnification 7, 124 X. The cells of this zone exhibit, in addition to light cells (LC), dark cells (DC) of varying densities. Lipid droplets (L) as well as spherical mitochondria (M) are numerous in both the light and dark cells.

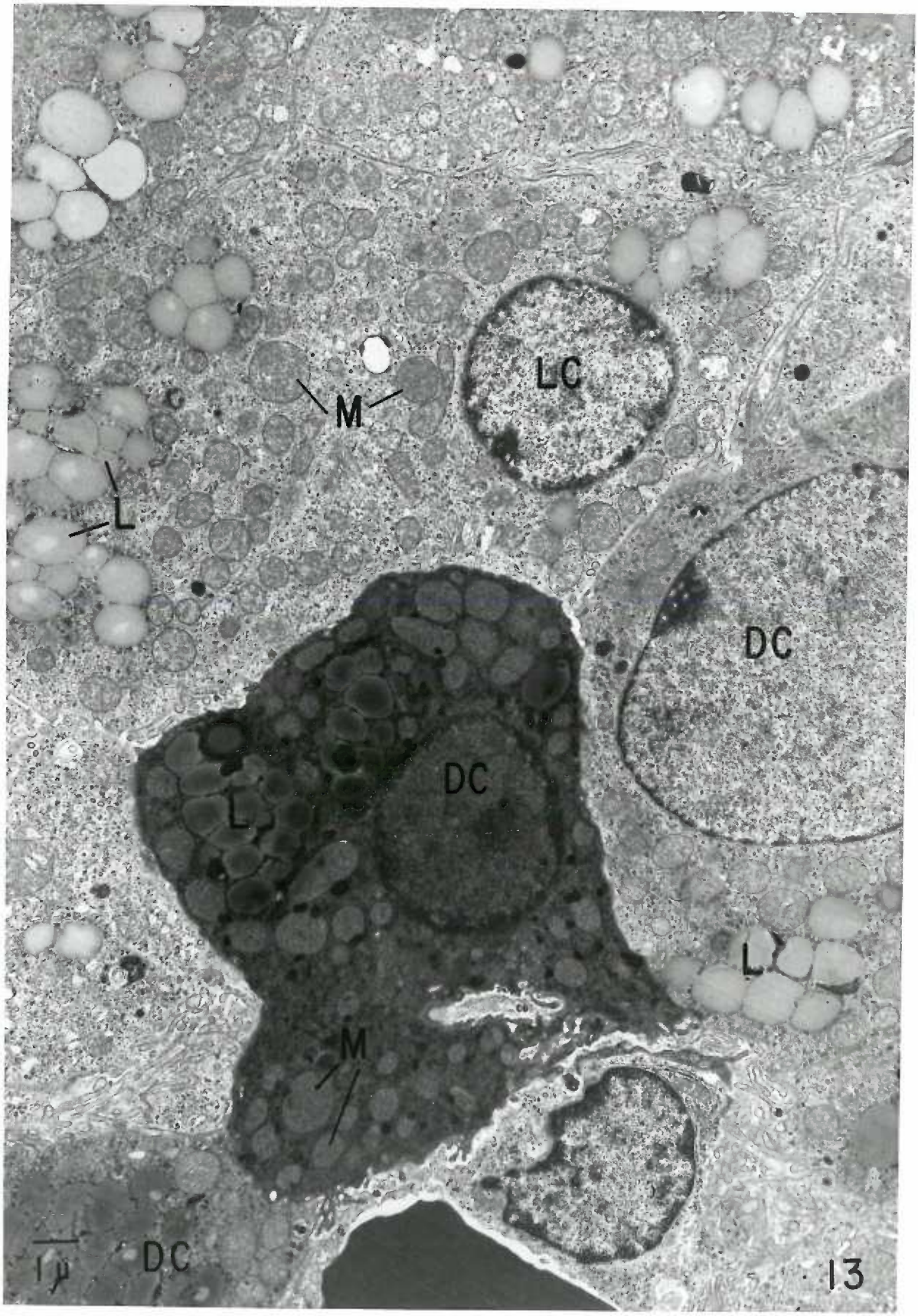

LC
M
L
DC
DC
L
L
M
1μ
DC
13

FIGURE 14

Light (LC) and dark (DC) cells of the term zona fasciculata. Magnification 11, 880 X. Both light and dark cells exhibit numerous spherical mitochondria (M) with vesicular cristae, lipid droplets (L) an abundance of polyribosomes (Ps), and a well developed system of Golgi membranes (G). The smooth endoplasmic reticulum (SER) consists of tubular profiles. At this stage of gestation, only a few of these profiles are dilated (short arrows). Many of the mitochondria and lipid droplets are surrounded by elements of smooth endoplasmic reticulum (long arrows).

Lysosome-like structures (Lys)

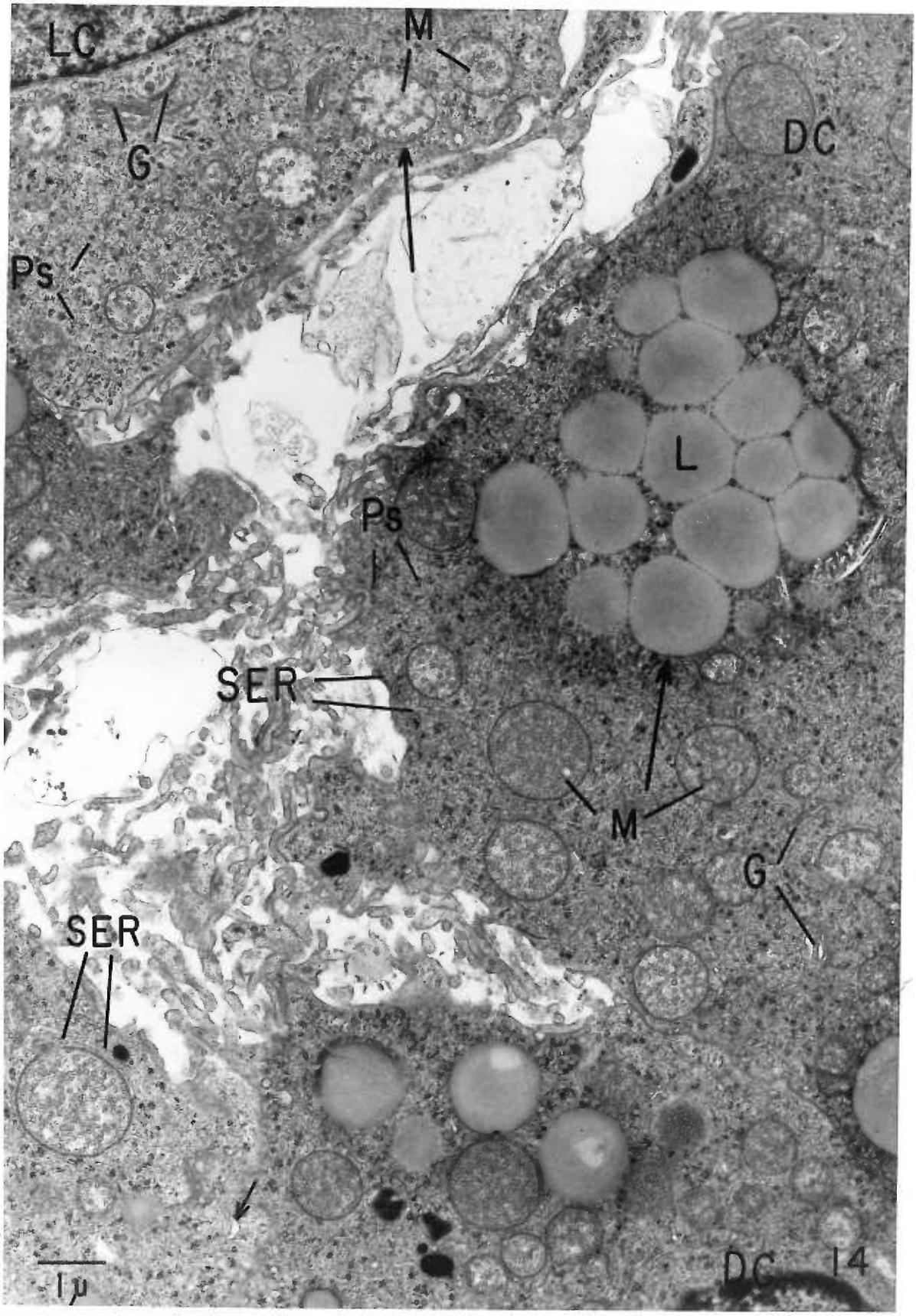

LC
M
G
DC
Ps
L
Ps
SER
M
G
SER
1μ
DC
14

FIGURE 15

Dark cells of the term zona fasciculata. Magnification 16, 720 X. A few tubular profiles of the smooth endoplasmic reticulum (SER) appear dilated (long arrows). Many mitochondria (M) and lipid droplets (L) are surrounded by elements of the smooth endoplasmic reticulum (short arrows). Dense bodies (Db) and lysosome-like structures (Lys) are occasionally seen in these cells. The attenuated endothelium (End) reveals numerous fenestrations. Note the close association between a lipid droplet, a mitochondrion and membraneous structures which resemble smooth endoplasmic reticulum (broad arrows). Henceforth, this association will be referred to as the "LERM" complex.

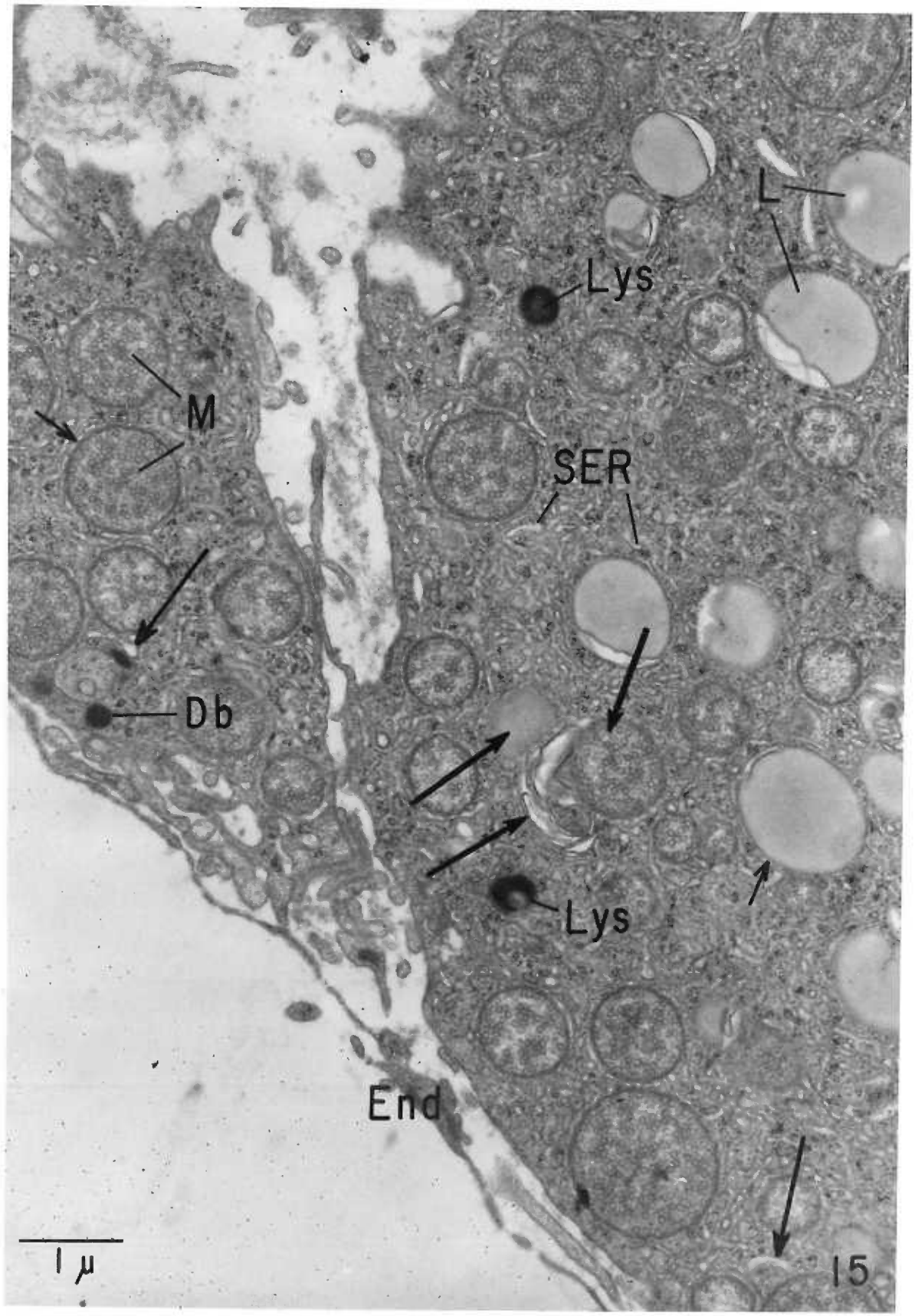

L
Lys
M
SER
Db
Lys
End
1 μ
15

FIGURE 16

Light (LC) and dark (DC) adrenocortical cells of the term zona reticulo-medullaris. Magnification 8, 494 X. Both light and dark cells exhibit numerous spherical mitochondria (M) and lipid inclusions (L). Note that in the light cell, the cytoplasmic matrix is less dense than the matrix of the mitochondria, a feature that is characteristic of the light cortical cells in this zone. The sinusoidal endothelium (End) is attenuated and displays numerous fenestrations (short arrows).

LERM complex (long arrows)

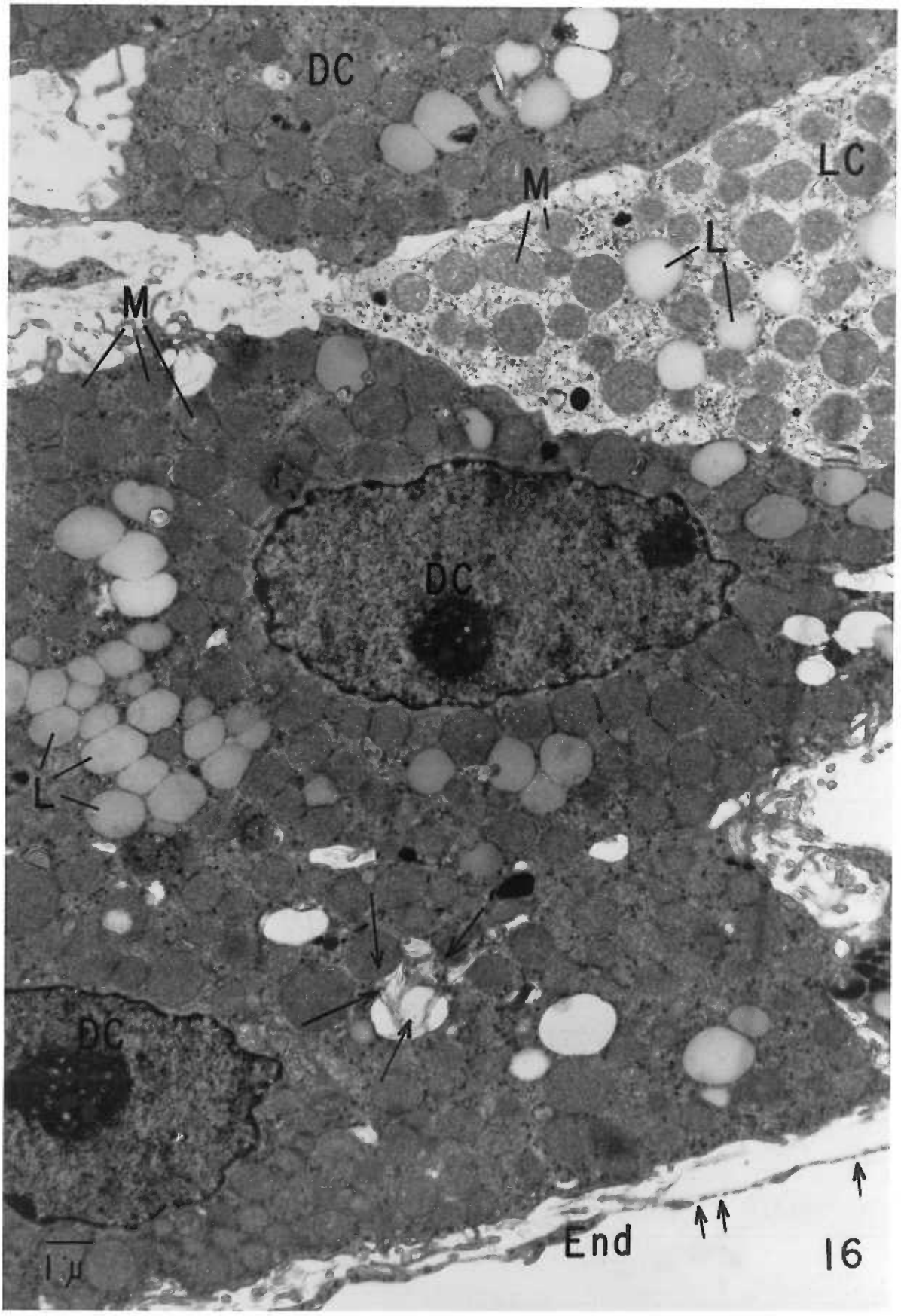
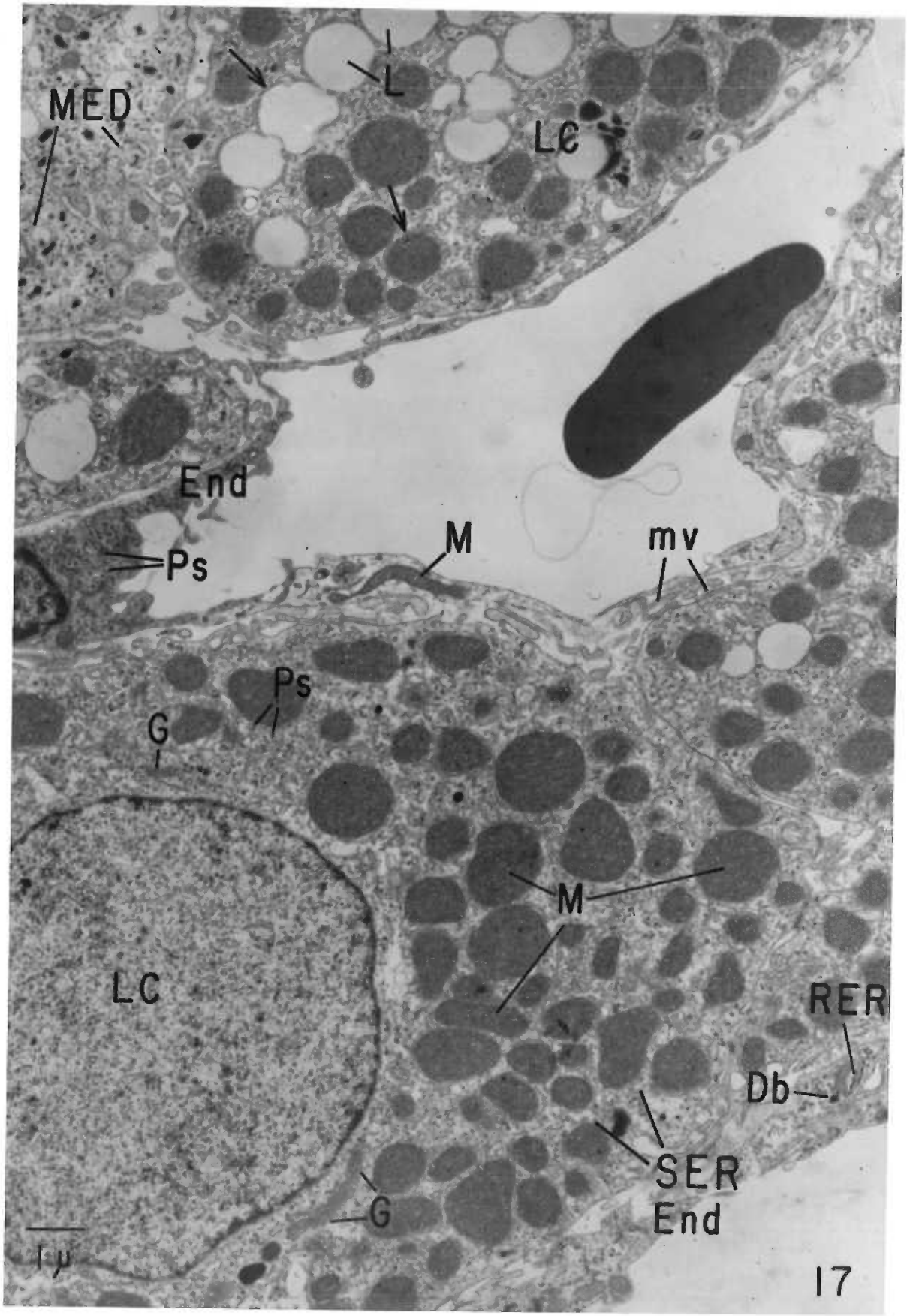

DC
LC
M
L
M
DC
L
DC
End
1 μ
16

FIGURE 17

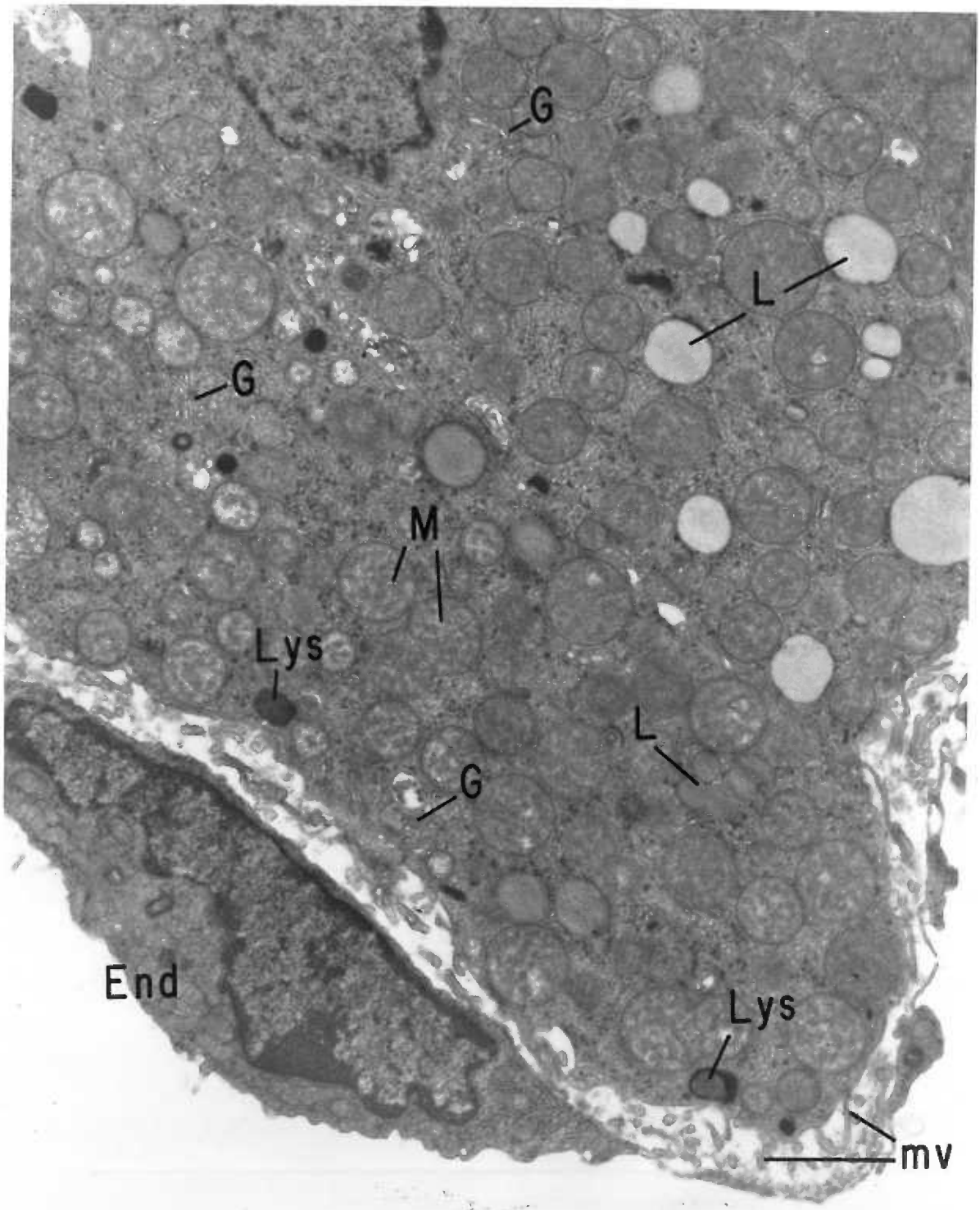
Light cortical cells (LC) of the term zona reticulo-medullaris. Magnification 10, 120 X. The light cortical cells exhibit well developed Golgi membranes (G) and tubular profiles of smooth endoplasmic reticulum (SER) which surround the mitochondria and lipid droplets (arrows). Polyribosomes (Ps) are abundant as are the lipid droplets (L). Polymorphic mitochondria (M) reveal a denser matrix than the surrounding cytoplasm. Microvilli (mv) are common. Note the attenuated endothelium (End) which displays elongated mitochondria (M) with lamellar cristae, rough endoplasmic reticulum (RER), polyribosomes (Ps) and an occasional dense body (Db). Portions of two light medullary cells (MED) are seen in the upper left hand corner.



17

FIGURE 18

Dark cells of the term zona reticulo-medullaris partially encompassed by a sinusoidal endothelium (End). Magnification 10, 120 X. The dark cells of this zone exhibit numerous spherical mitochondria (M), well developed Golgi membranes (G), numerous lipid droplets (L) and few lysosome-like structures (Lys). Microvilli are common (mv).



1 μ

FIGURE 19

Enlarged portion of the dark cell in the upper part of fig. 18. Magnification 50, 177 X. Note the well developed Golgi membranes (G), spherical mitochondria (M) with vesicular cristae, and polyribosomes (Ps). A few tubular profiles of the smooth endoplasmic reticulum are dilated (arrow).

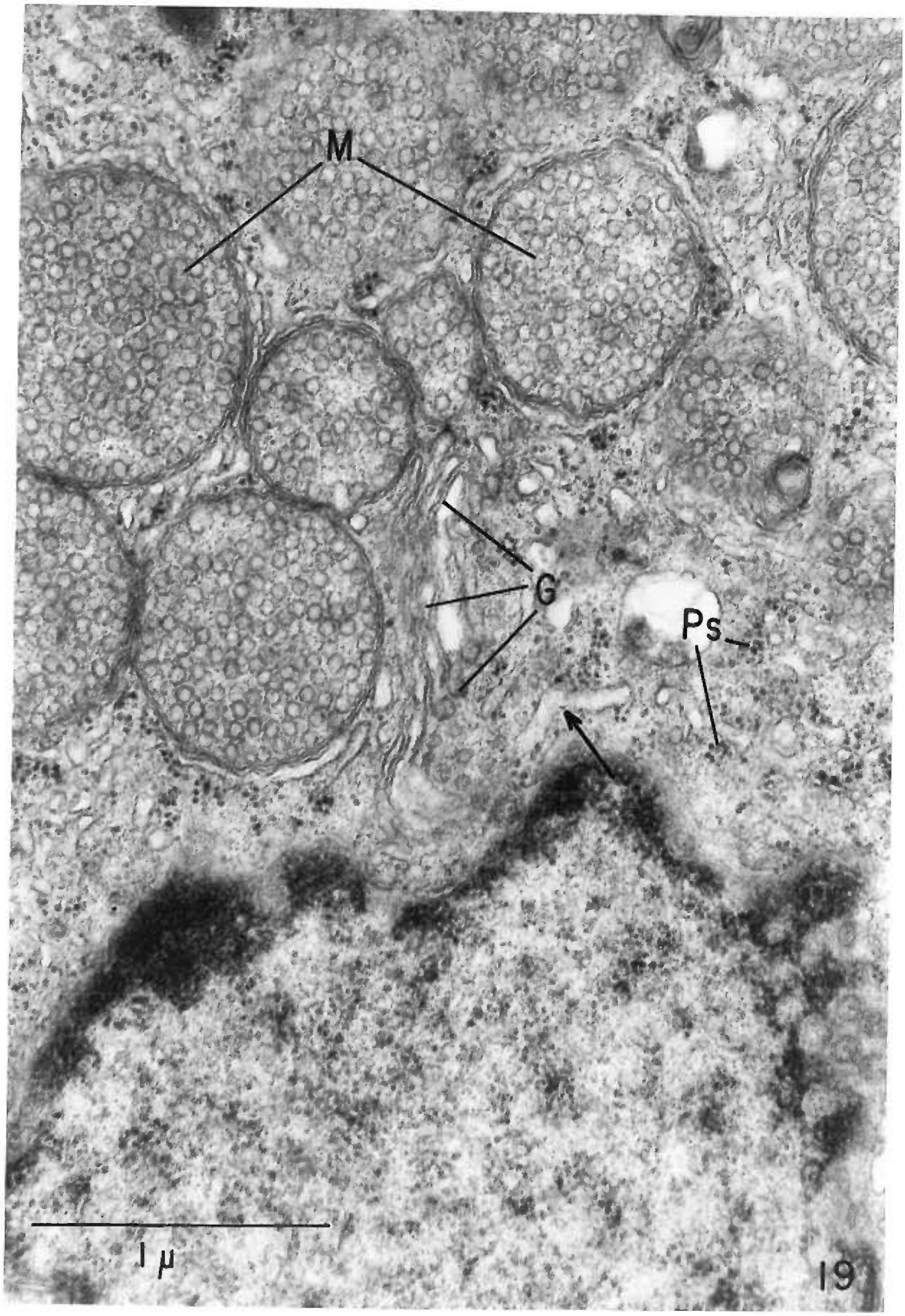

M
G
Ps
1 μ
19

FIGURE 20

Enlarged portion of the dark cell in the upper part of fig. 18. Magnification 50, 177 X. The partially extracted lipid droplets (L) are non-membrane-limited. The smooth endoplasmic reticulum (SER) appears as branching tubular profiles. Note that elements of the endoplasmic reticulum surround several mitochondria and the lipid droplets (arrows).

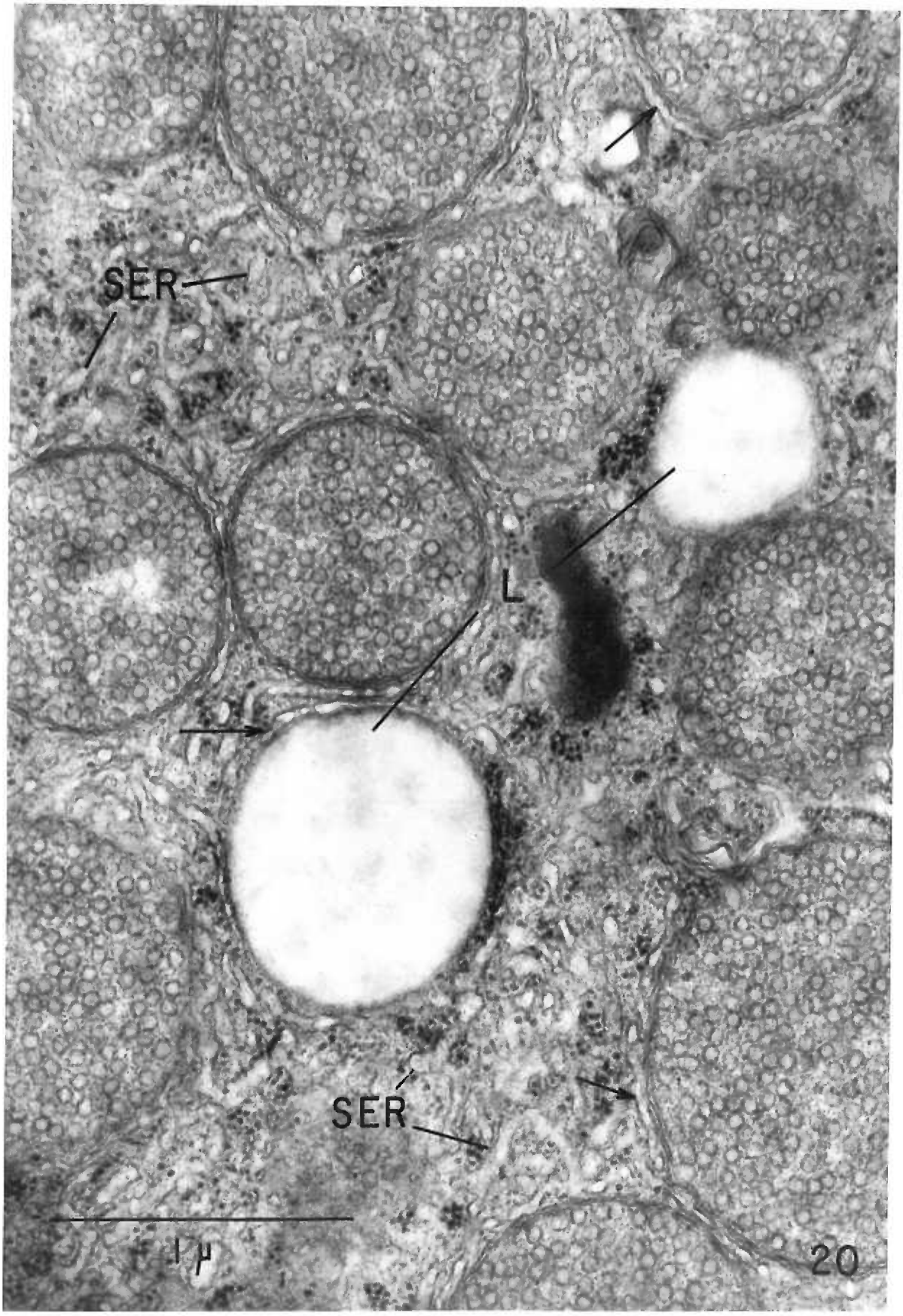

SER
L
SER
1 μ
20

FIGURE 21

Light (LC) and dark (DC) cortical cells of the term zona reticulo-medullaris. Magnification 14, 080 X. Mitotic figures, such as the one seen in the dark cortical cell, are rarely observed in the cells of the zona fasciculata and zona reticulo-medullaris. They are frequently detected, however, in the light cells of the zona glomerulosa.

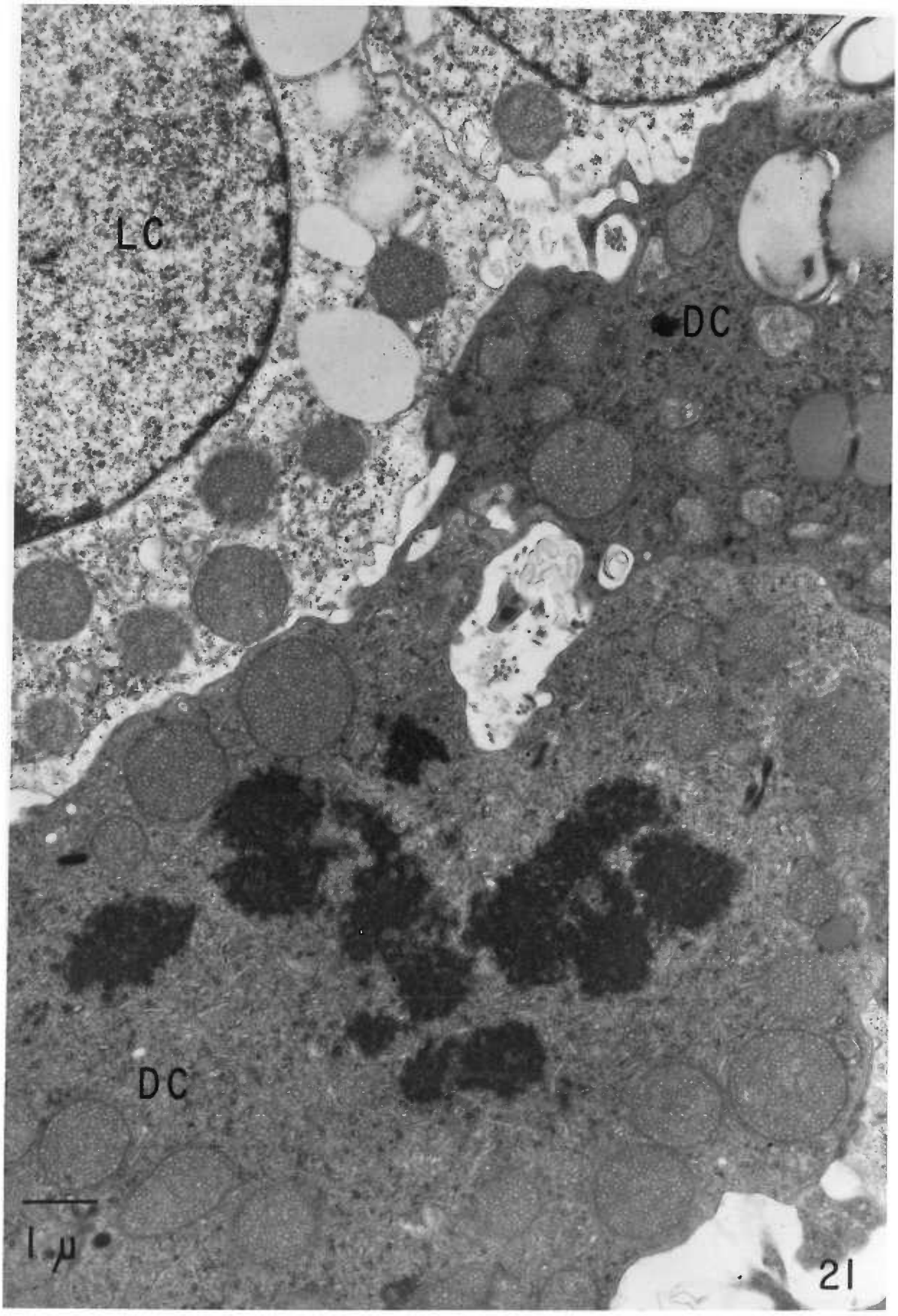
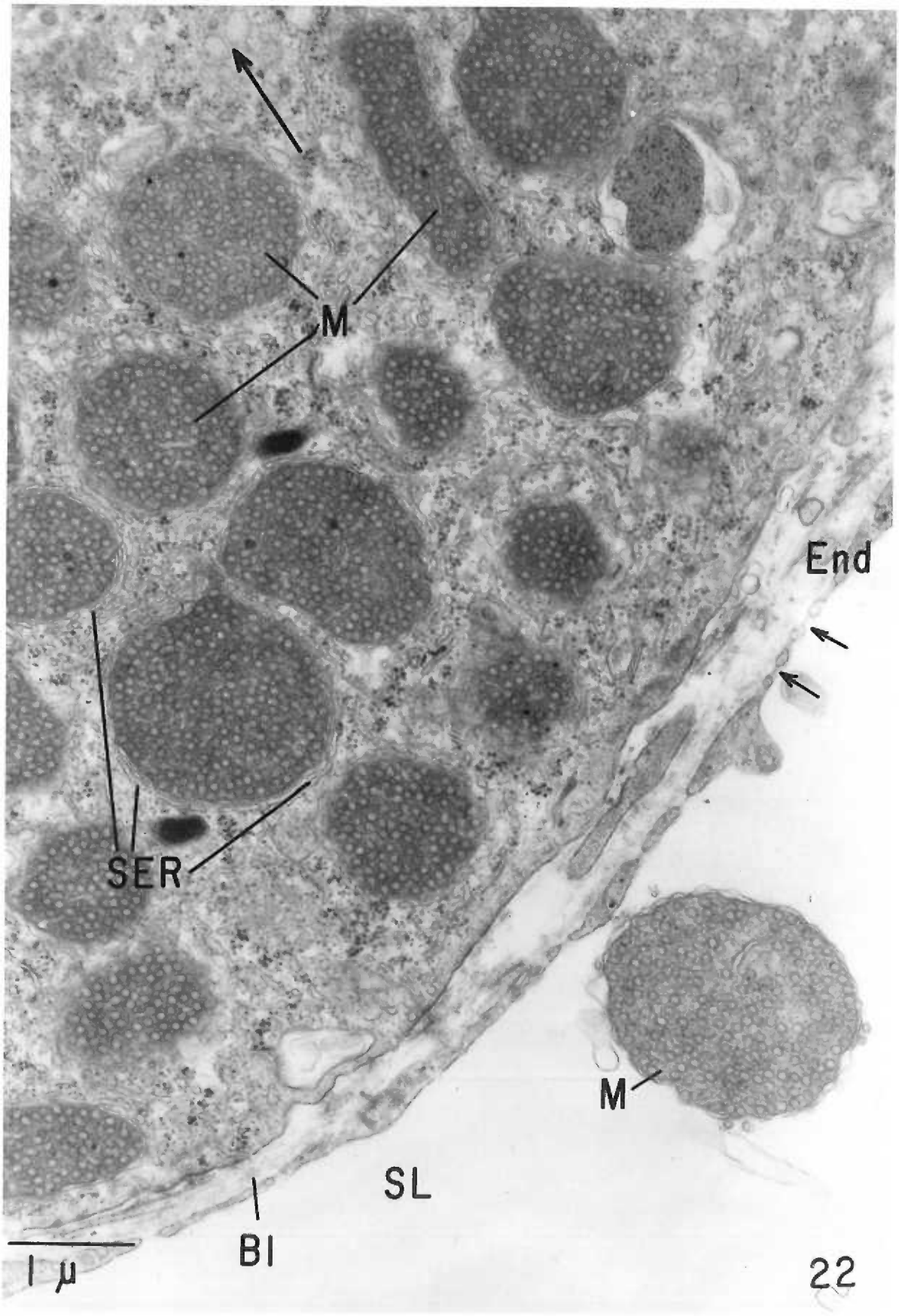

LC
DC
DC
1 μ
21

FIGURE 22

Light cortical cell of the term zona reticulo-medullaris. Magnification 21, 726 X. Mitochondria (M) of this cell type are primarily spherical but occasionally appear polymorphic in shape. All forms, however, exhibit tubular and vesicular cristae. Note the abundance of branching smooth endoplasmic reticulum (SER) which appears to closely envelop the mitochondria. A few elements of the smooth endoplasmic reticulum appear dilated (long arrows). The sinusoidal endothelium (End) reveals numerous fenestrations (short arrows) and is separated from the cortical cell by a well defined basal lamina (Bl). Cytoplasmic organelles of the cortical cells, such as the mitochondrion (M) shown here, are frequently observed in the sinusoidal lumen (SL).



22

FIGURE 23

Sinusoidal lumen (SL) of the term zona reticulo-medullaris. Magnification 20, 000 X. Note the evagination (arrows) of light cortical cell cytoplasmic organelles through the endothelium (End) into the sinusoidal lumen (SL).

M = mitochondrion

SER = smooth endoplasmic reticulum

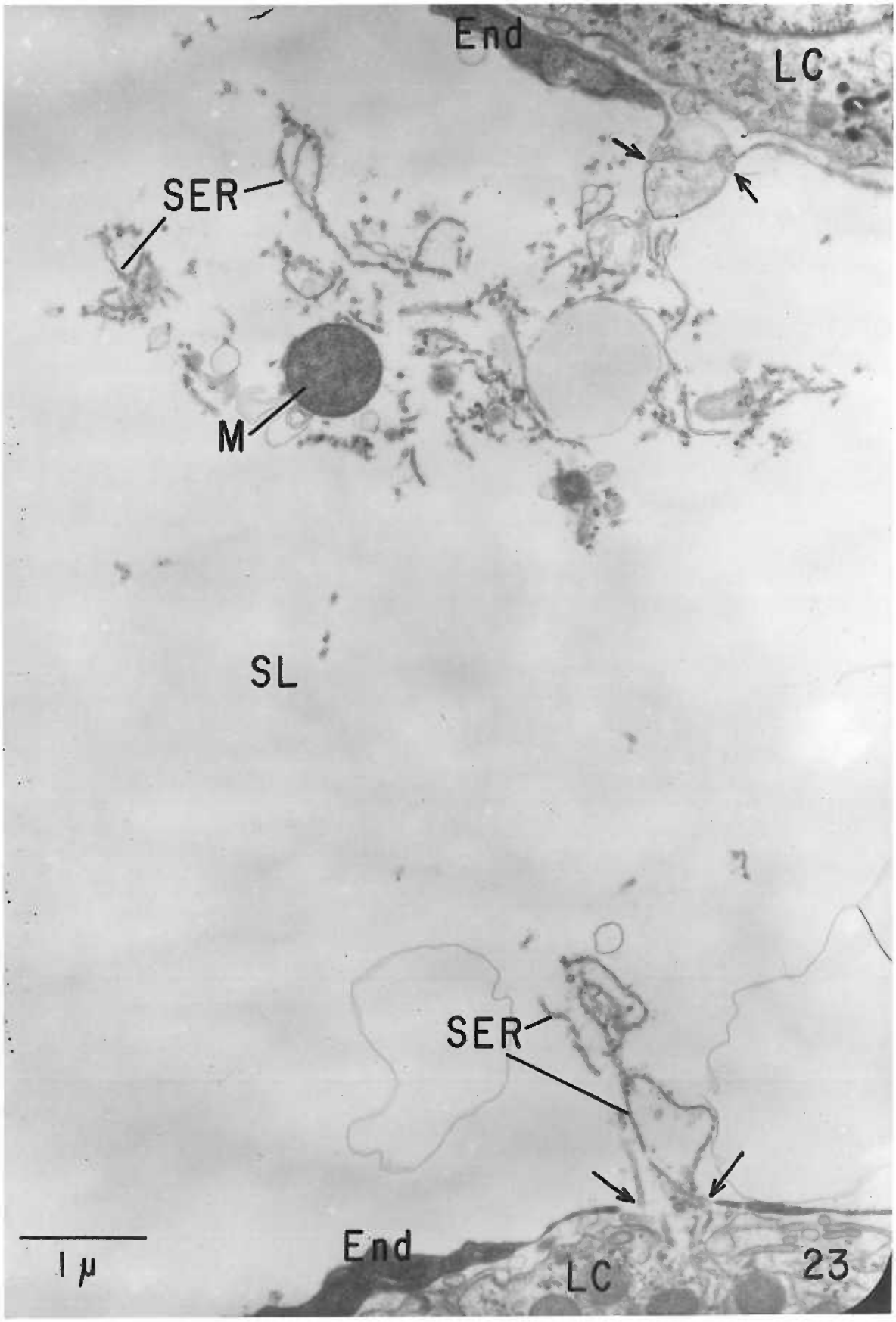

End
LC
SER
M
SL
SER
End
LC
1 μ
23

FIGURE 24

Light (LC) and dark (DC) medullary cells of the term zona reticulo-medullaris. Magnification 22, 444 X. Both light and dark cells contain polymorphic mitochondria (M) with lamellar cristae, numerous polyribosomes (Ps), and membrane-limited noradrenaline storage granules (NA). The light medullary cell in the center of the micrograph exhibits well developed Golgi membranes (G) and dilated tubules of the smooth endoplasmic reticulum (SER).

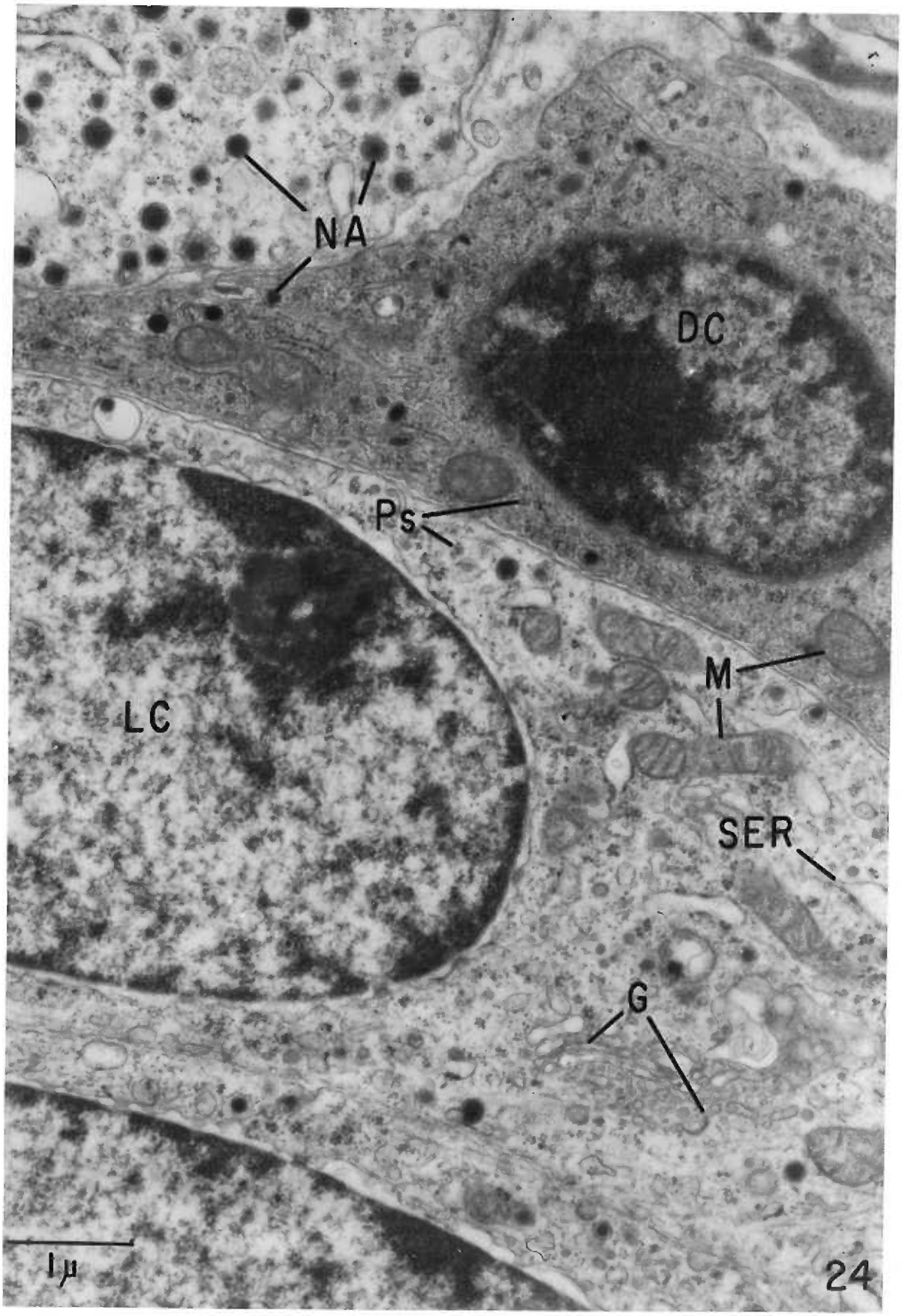

NA
DC
Ps
M
LC
SER
G
1μ
24

FIGURE 25

Light (LC) medullary cells of the term zona reticulo-medullaris. Magnification 22, 440 X. These cells contain both smooth (SER) and rough (RER) endoplasmic reticulum which occur as short narrow and often dilated tubules. The adrenalin storage granules (A) such as the ones seen here are infrequently observed at this stage of development.

NA = noradrenalin storage granules

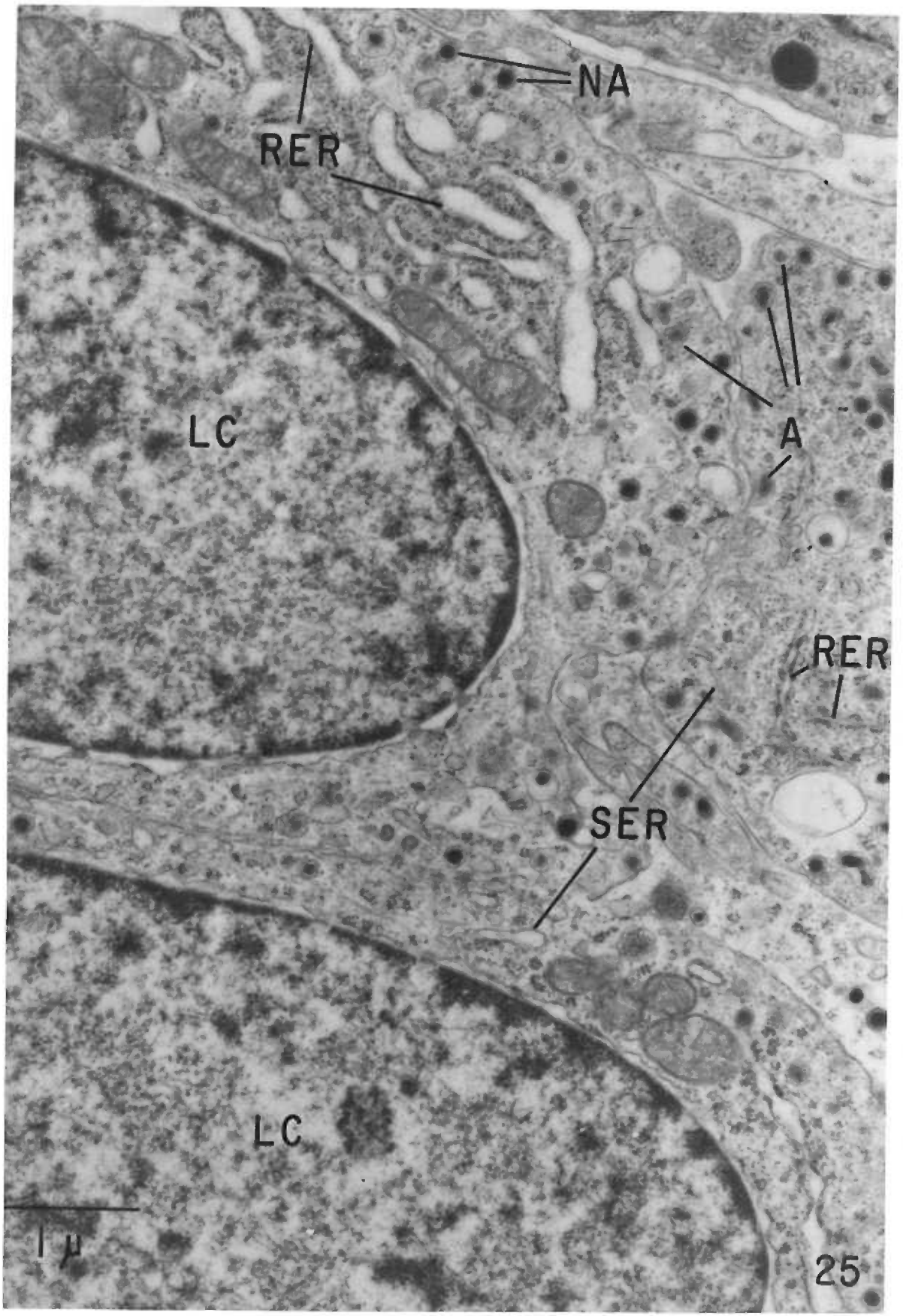

NA
RER
LC
A
RER
SER
LC
1 μ
25

FIGURE 26

Term zona reticulo-medullaris. Magnification 22, 440 X. Numerous non-myelinated axons (Ax) arranged in layers are found in the intercellular spaces of the medullary cells. These axons contain numerous filaments (nf), tubules (nt), small dense granules (Gr), and mitochondria (M).

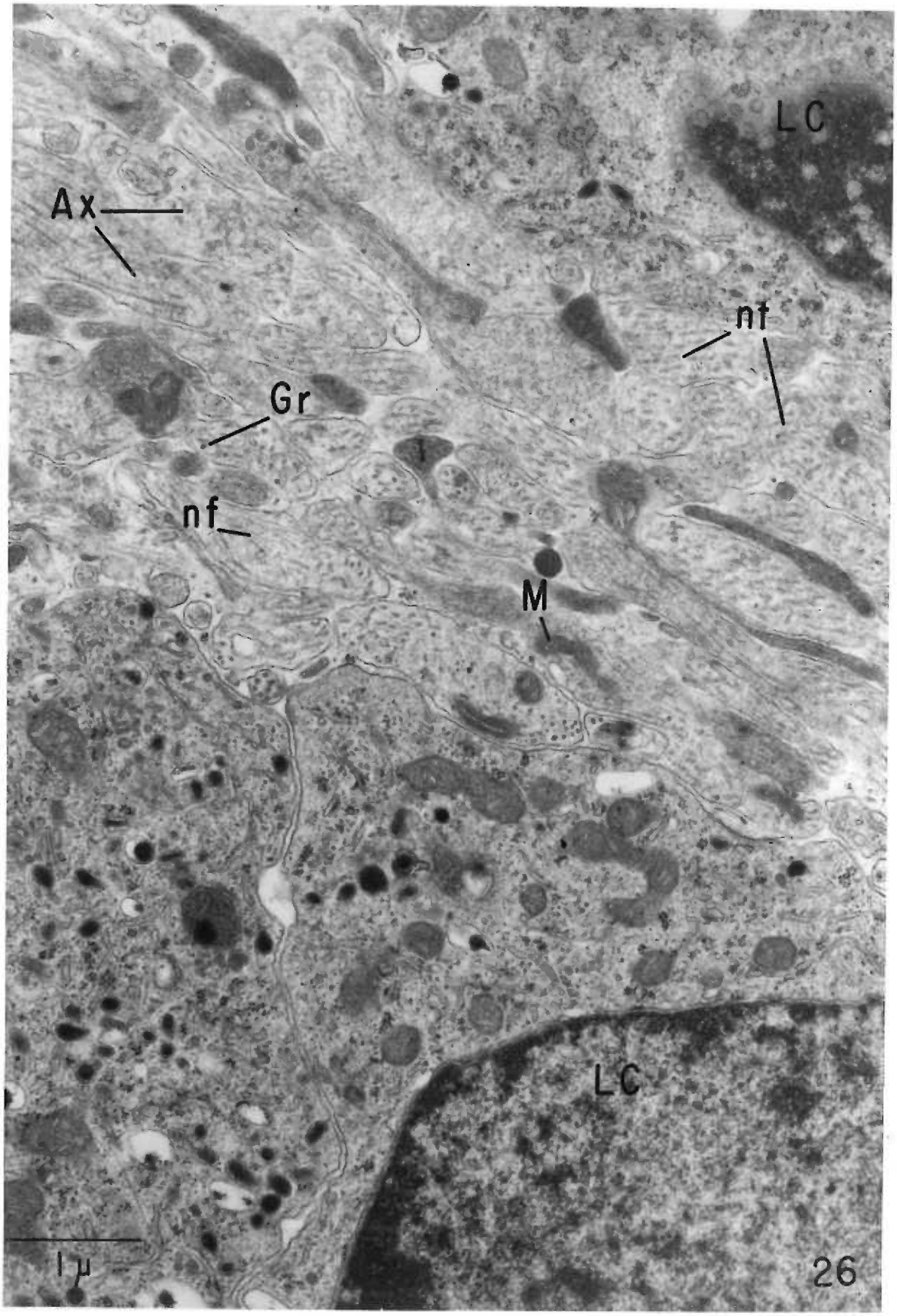

LC
Ax
nf
Gr
nf
M
LC
1 μ
26

FIGURE 27

Light (LC) medullary cell and sinusoidal lumen (SL) of the term zona reticulo-medullaris. Magnification 16, 280 X. Medullary (MED) cytoplasmic elements as well as elements of the cortical cells (arrow) are frequently found in the sinusoidal lumen (SL).

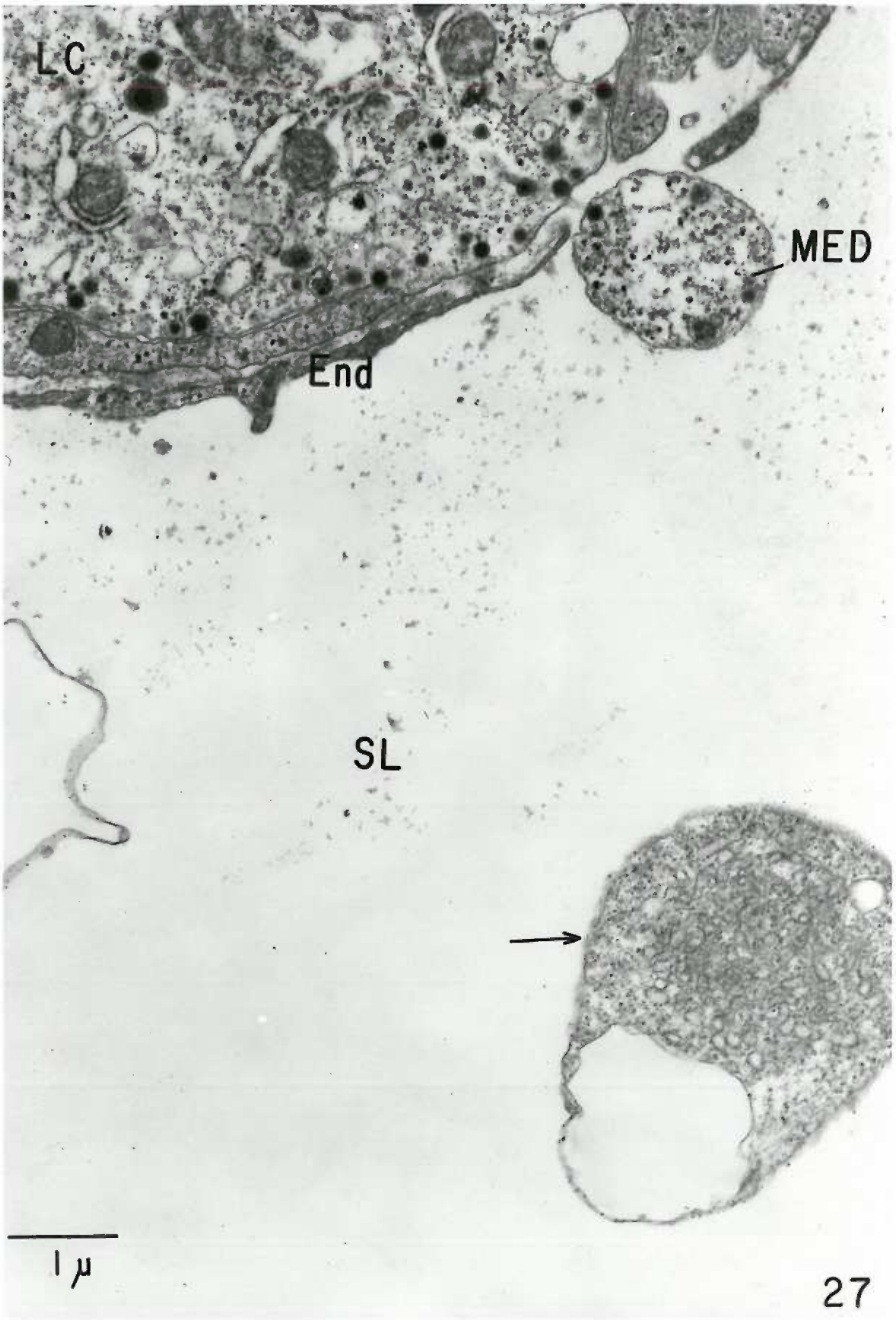

LC
MED
End
SL
1 μ

FIGURE 28

Light (LC) and dark (DC) cells of the zona glomerulosa at day 23 of gestation. Magnification 10, 560 X. Both light and dark cells exhibit well developed Golgi membranes (G), polyribosomes (Ps) and polymorphic mitochondria (M) with tubular cristae. Note the paucity of both smooth (SER) and rough (RER) endoplasmic reticulum in these cells. Microvilli (mv) and desmosomes (Des) are common. Mitotic figures such as the one shown in the lower right hand corner are frequently observed.

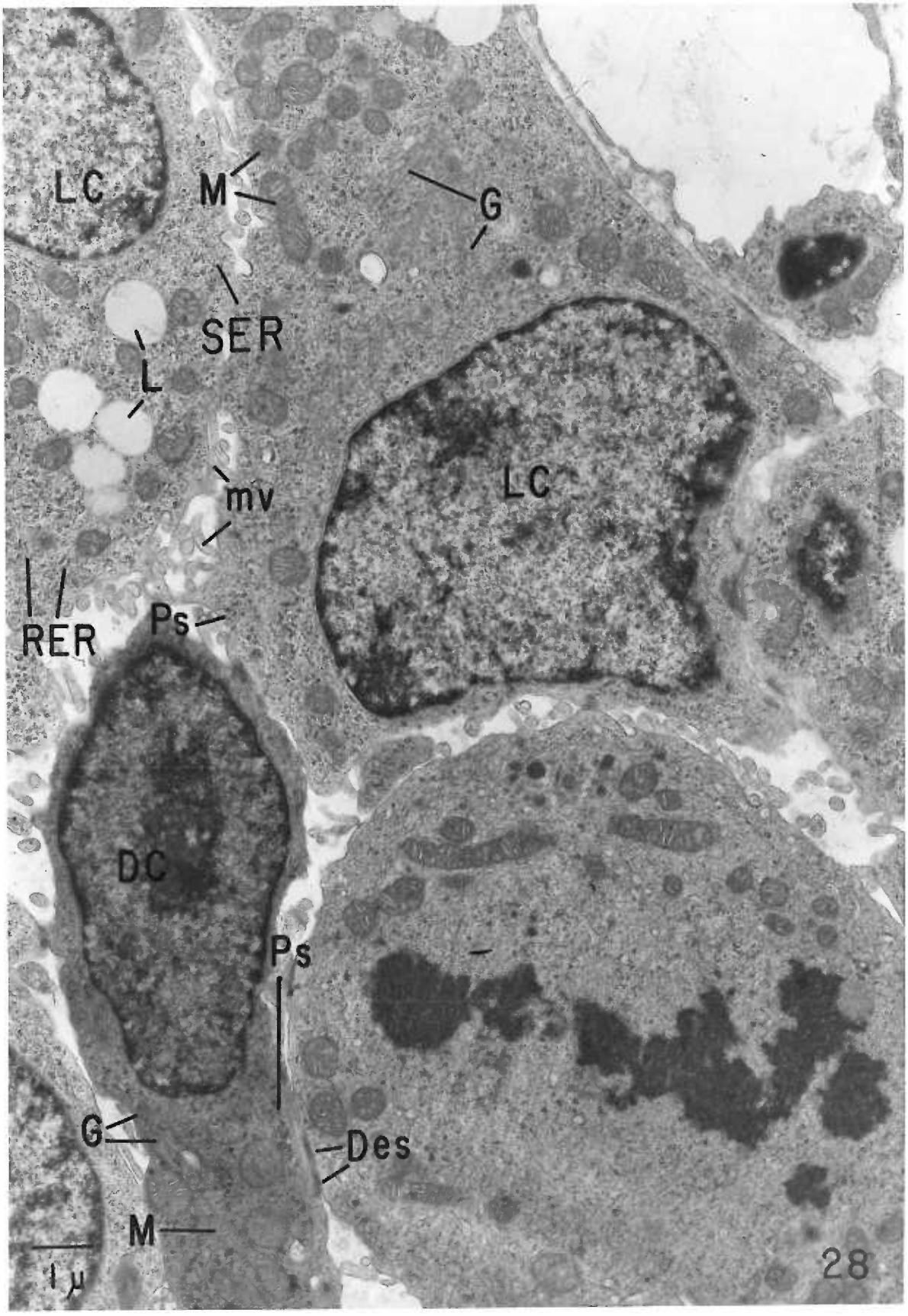

LC
M
G
SER
L
LC
mv
RER
Ps
DC
Ps
G
Des
M
1 μ
28

FIGURE 29

Light cells (LC) of the zona intermedia at day 23 of gestation. Magnification 9, 600 X. These cells contain numerous mitochondria (M) with both tubular and vesicular cristae, well developed Golgi membranes (G), polyribosomes (Ps), lipid droplets (L) and few lysosome-like structures (Lys). Note the abundance of smooth endoplasmic reticulum (SER) which is represented by tubular profiles. Some of these profiles appear dilated (arrows). Cells of the zona glomerulosa (ZG) are seen in the upper portion of the micrograph.

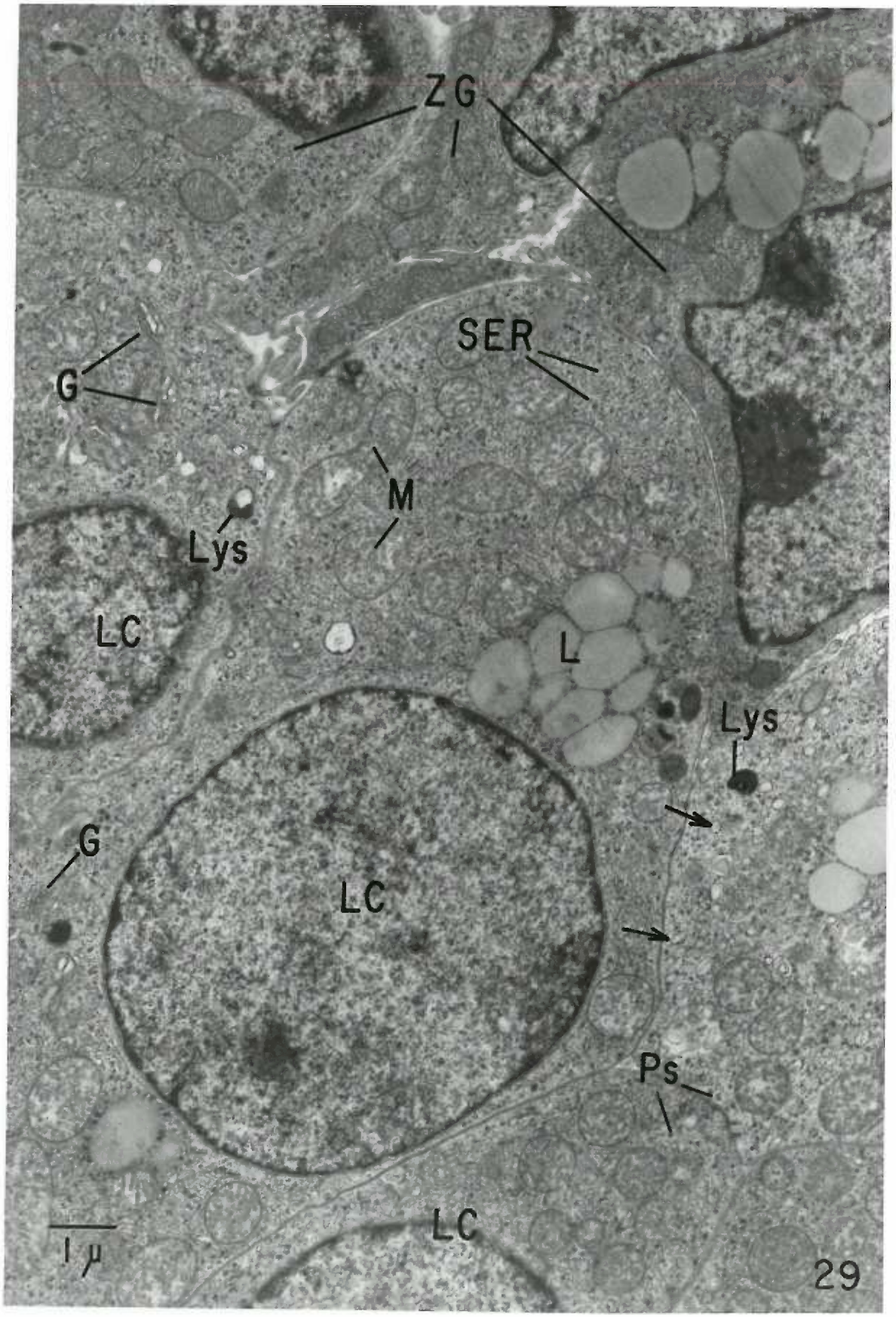

ZG
G
SER
M
Lys
LC
L
Lys
G
LC
Ps
LC
1 μ
29

FIGURE 30

Light (LC) and dark (DC) cortical cells of the zona fasciculata at day 23 of gestation. Magnification 10, 560 X. Both light and dark cells exhibit numerous spherical mitochondria (M) with vesicular cristae. Lipid droplets (L), polyribosomes (Ps), Golgi membranes (G) and an abundance of tubular profiles and smooth endoplasmic reticulum (SER) are shown. A number of these profiles are swollen (arrows).

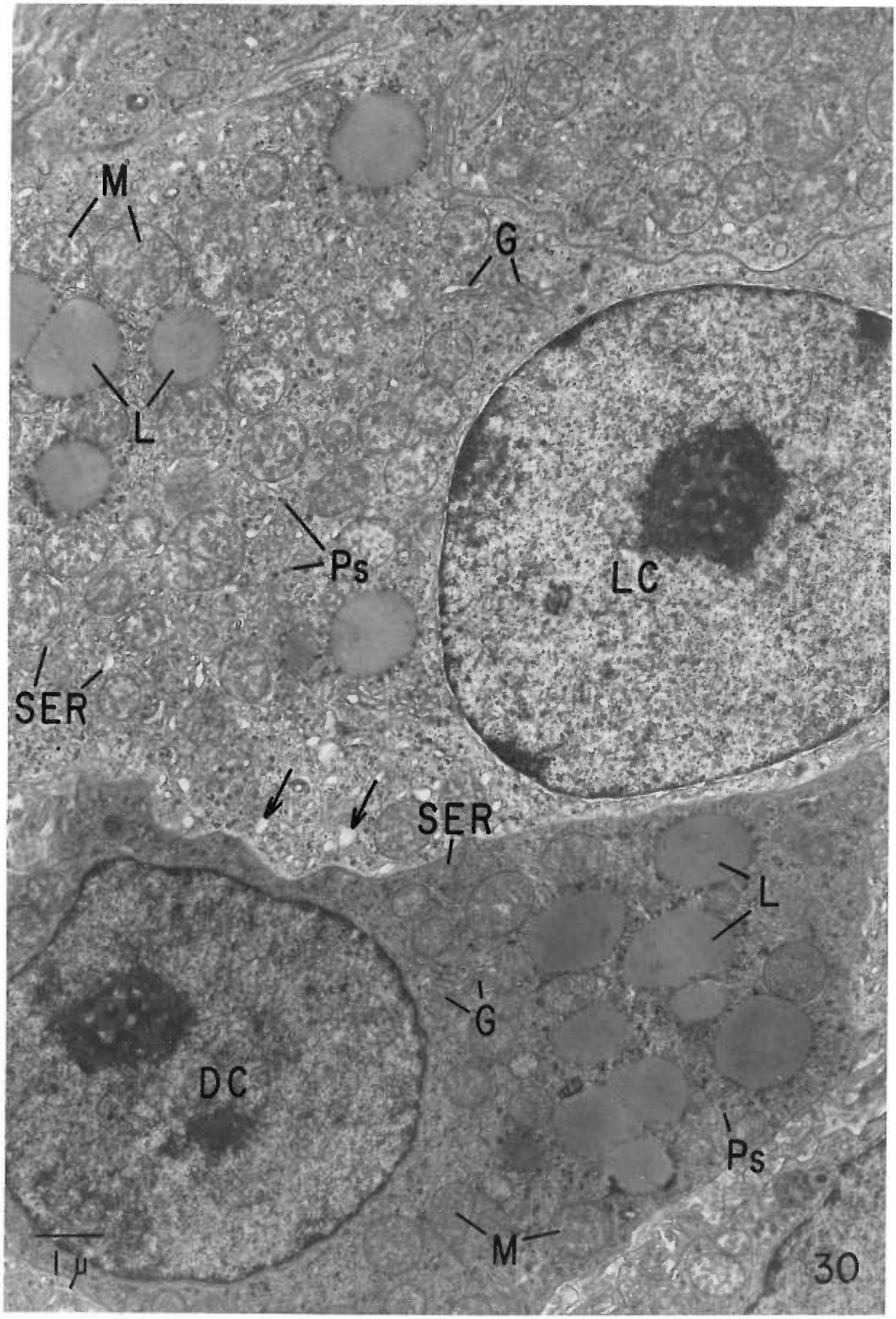

M
G
L
Ps
LC
SER
SER
L
G
DC
Ps
M
1 μ
30

FIGURE 31

Light cortical cells of the zona reticulo-medullaris at day 23 of gestation. Magnification 11, 000 X. These cells exhibit numerous mitochondria (M), lipid droplets (L), polyribosomes (Ps), and a few dense bodies (Db) and lysosome-like structures (Lys). Note that the smooth endoplasmic reticulum (SER) closely envelopes the mitochondria and lipid droplets (long arrows). The attenuated endothelium (End) reveals several fenestrations (short arrows).

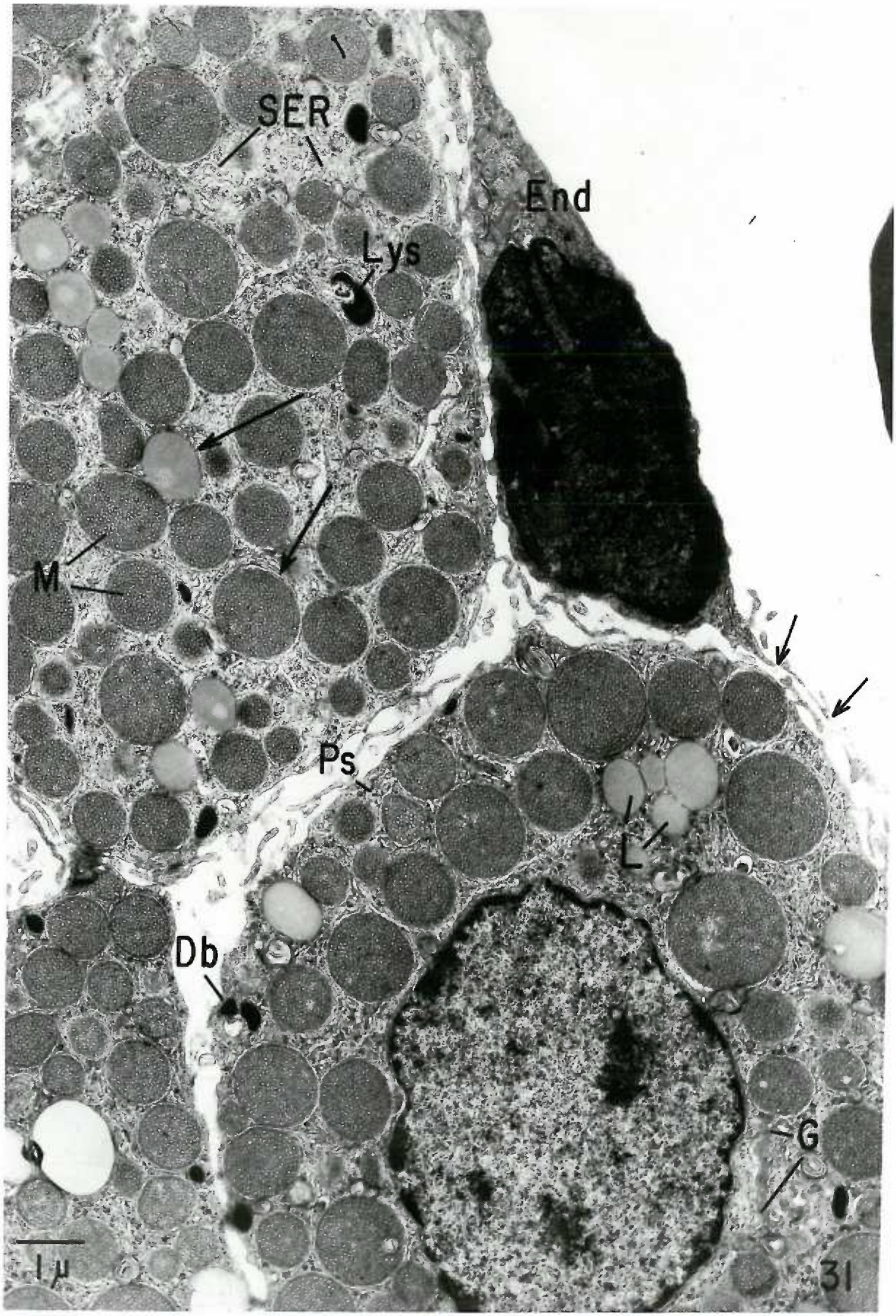

SER
End
Lys
M
Ps
L
Db
G
1 μ
31

FIGURE 32

Dark cortical cell of the zona reticulo-medullaris at day 23 of gestation. Magnification 17, 160 X. A cytoplasmic process (short arrows) containing various organelles is seen protruding through the sinusoidal endothelium (End).

LERM complex (long arrows)

Sinusoidal lumen (SL)

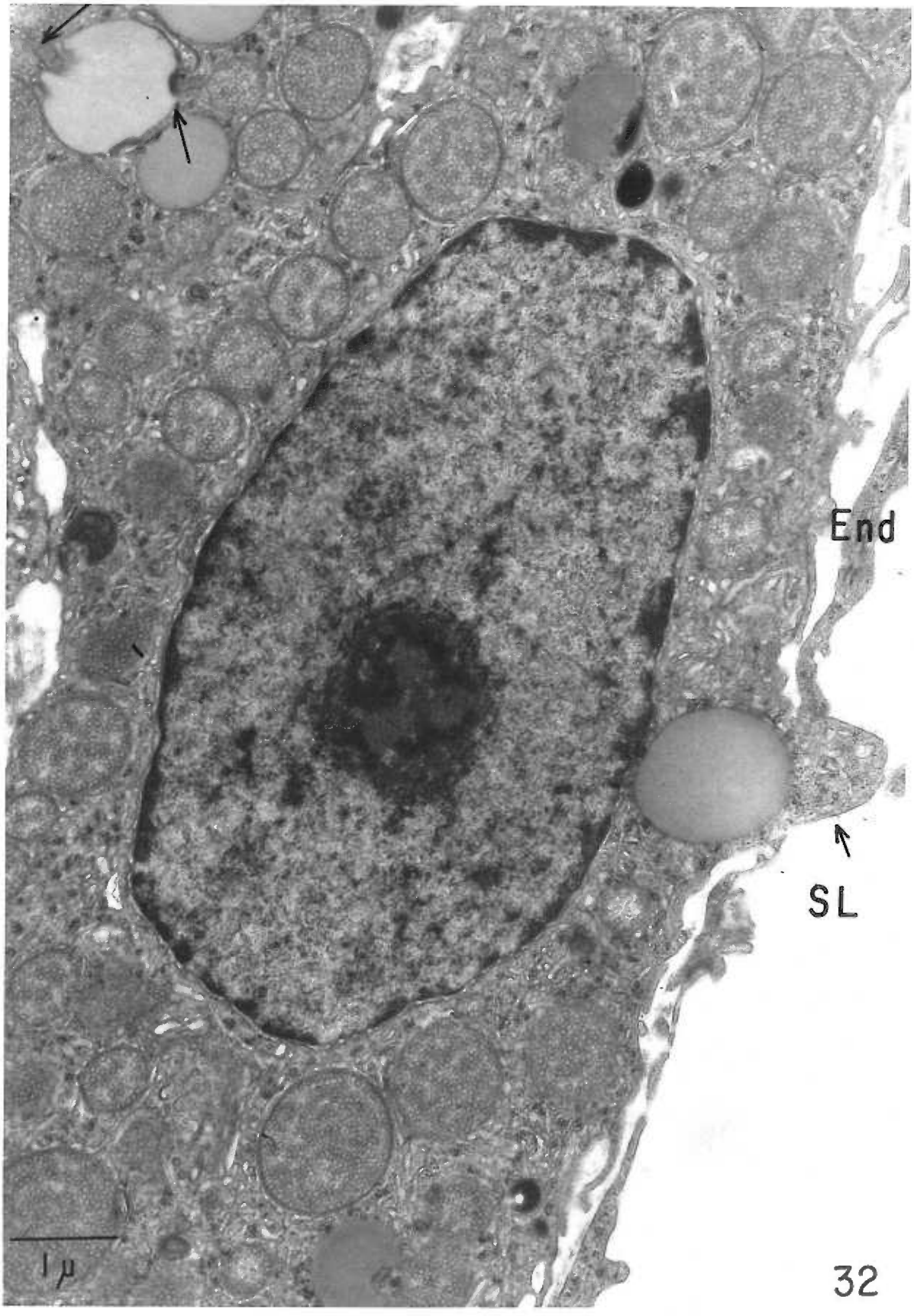

End
SL
1 μ

FIGURE 33

Light (LC) and dark (DC) medullary cells of the zona reticulo-medullaris at day 23 of gestation. Magnification 11, 000 X. These cells exhibit well developed Golgi membranes (G), elongated mitochondria (M) with lamellar cristae, and numerous membrane limited noradrenalin storage granules (NA). The adrenalin storage granules (A) are few in number.

Axons (Ax)

Terminal bouton (Tb)

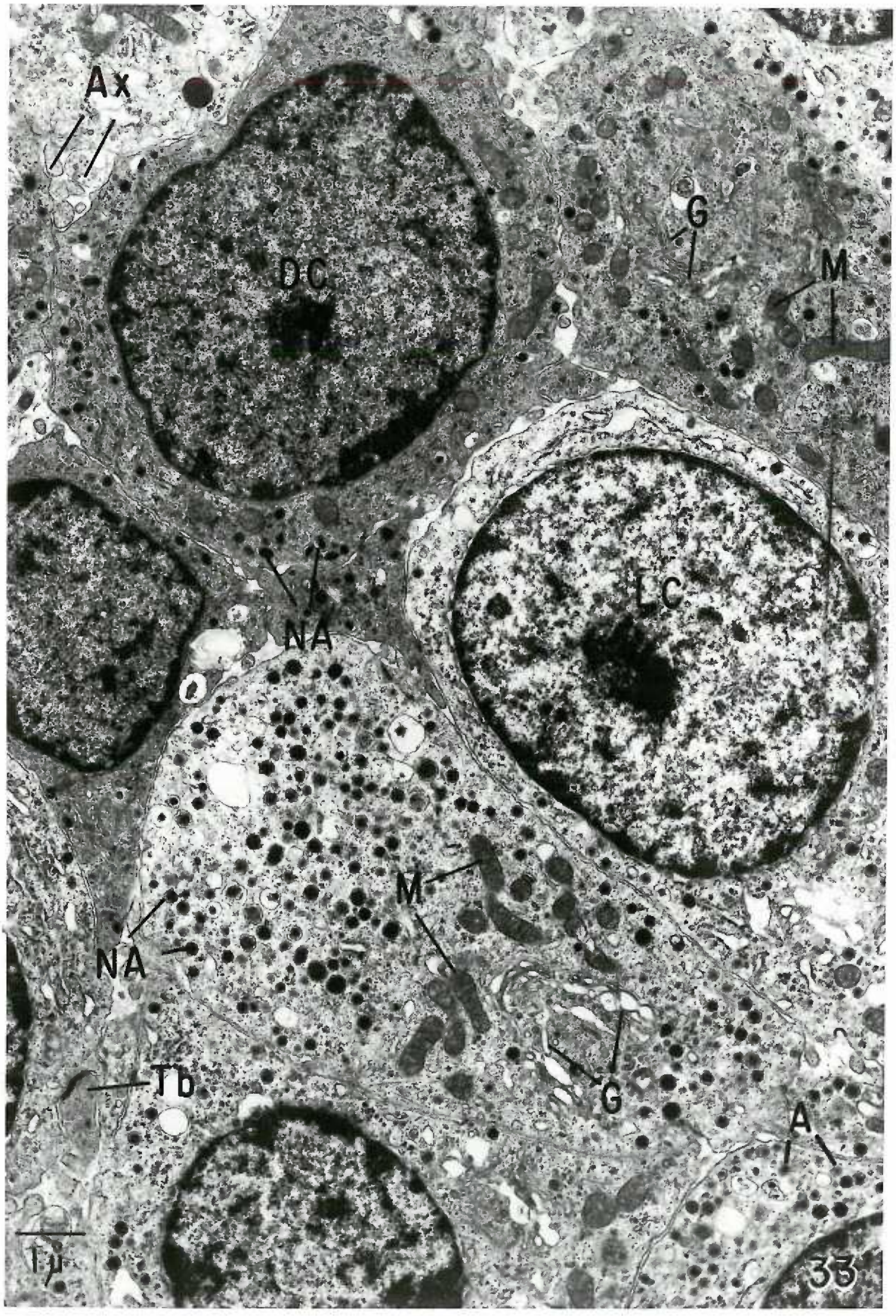

Ax
DC
G
M
LC
NA
NA
M
Tb
G
A
1μ
33

FIGURE 34

Light medullary cells (LC) and a sinusoid in the zona reticulo-medullaris at day 23 of gestation. Magnification 15, 400 X. Note the possible protrusion of a portion of the medullary cell through the endothelium (arrows) into the sinusoidal lumen (SL).

Endothelium (End)

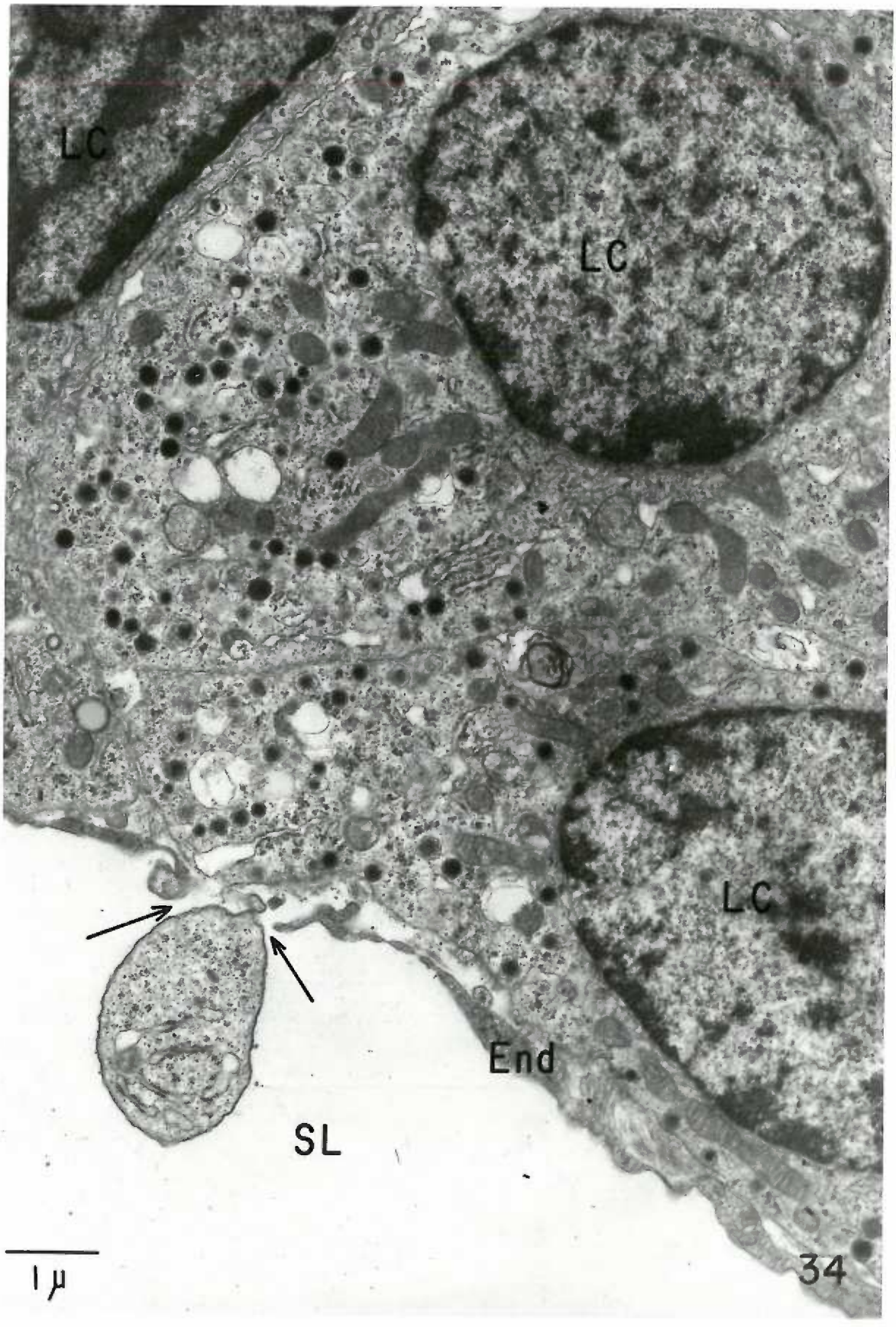

LC
LC
LC
End
SL
1 μ
34

FIGURE 35

Light medullary cell of the zona reticulo-medullaris exhibiting a mitotic figure at day 23 of gestation. Magnification 14, 760 X.

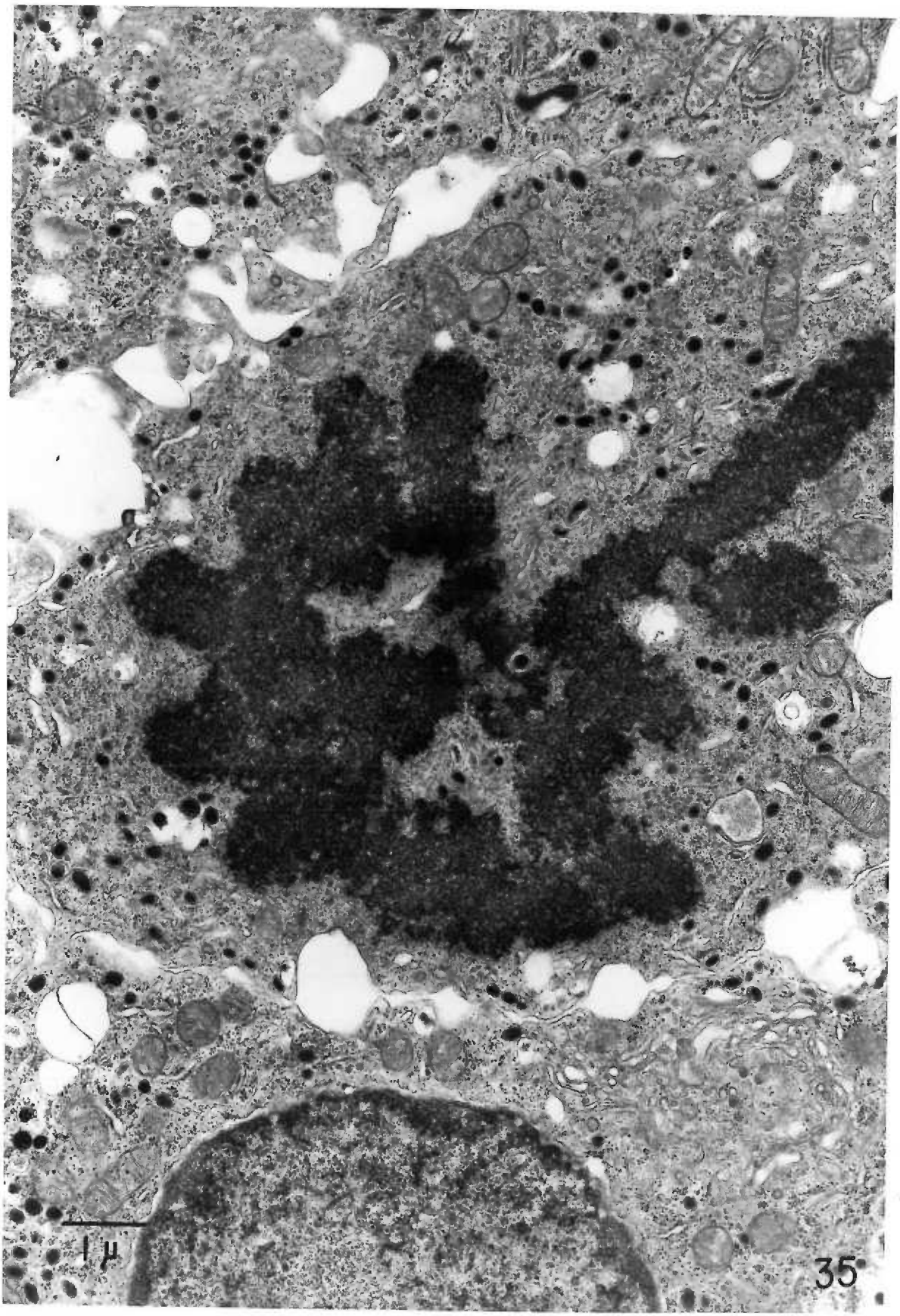

1 μ
35

FIGURE 36

Light (LC) and dark (DC) cells of the zona glomerulosa at day 24 of gestation. Magnification 10, 120 X. The morphological appearance of the cells in this zone is similar to that described for the zona glomerulosa at term.

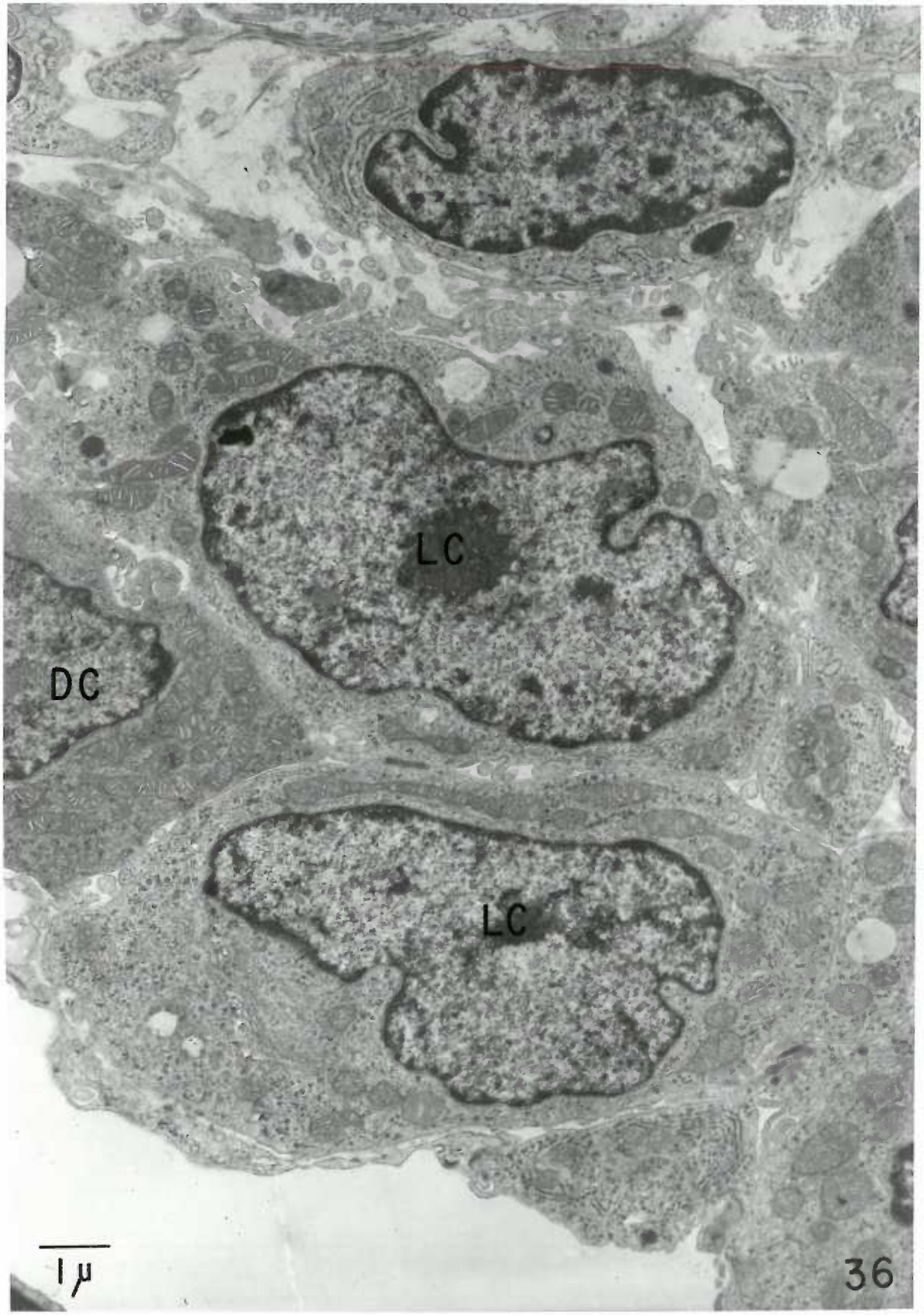

LC
DC
LC
1μ
36

FIGURE 37

Light (LC) and dark (DC) cells of the zona intermedia at day 24 of gestation. Magnification 14, 597 X. The cells of this zone reveal no apparent morphological changes and appear similar to the cells of the term zona intermedia. Cells of the zona glomerulosa (ZG) are seen in the lower left hand corner of the micrograph.

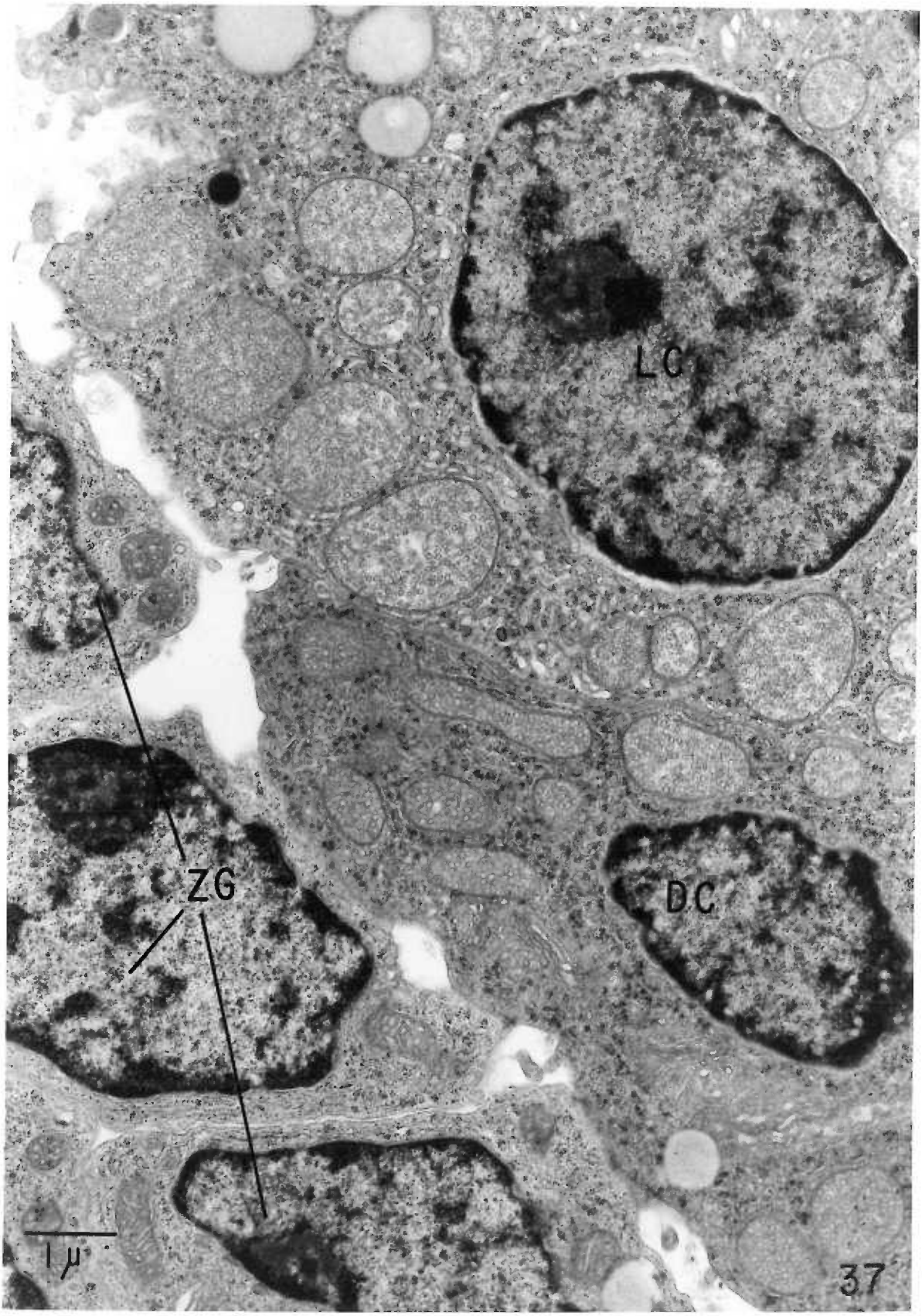

LC
ZG
DC
1 μ
37

FIGURE 38

Light (LC) and dark (DC) cortical cells of the zona fasciculata at day 24 of gestation. Magnification 11, 880 X. These cells are similar in morphological appearance to those described for the term zona fasciculata.

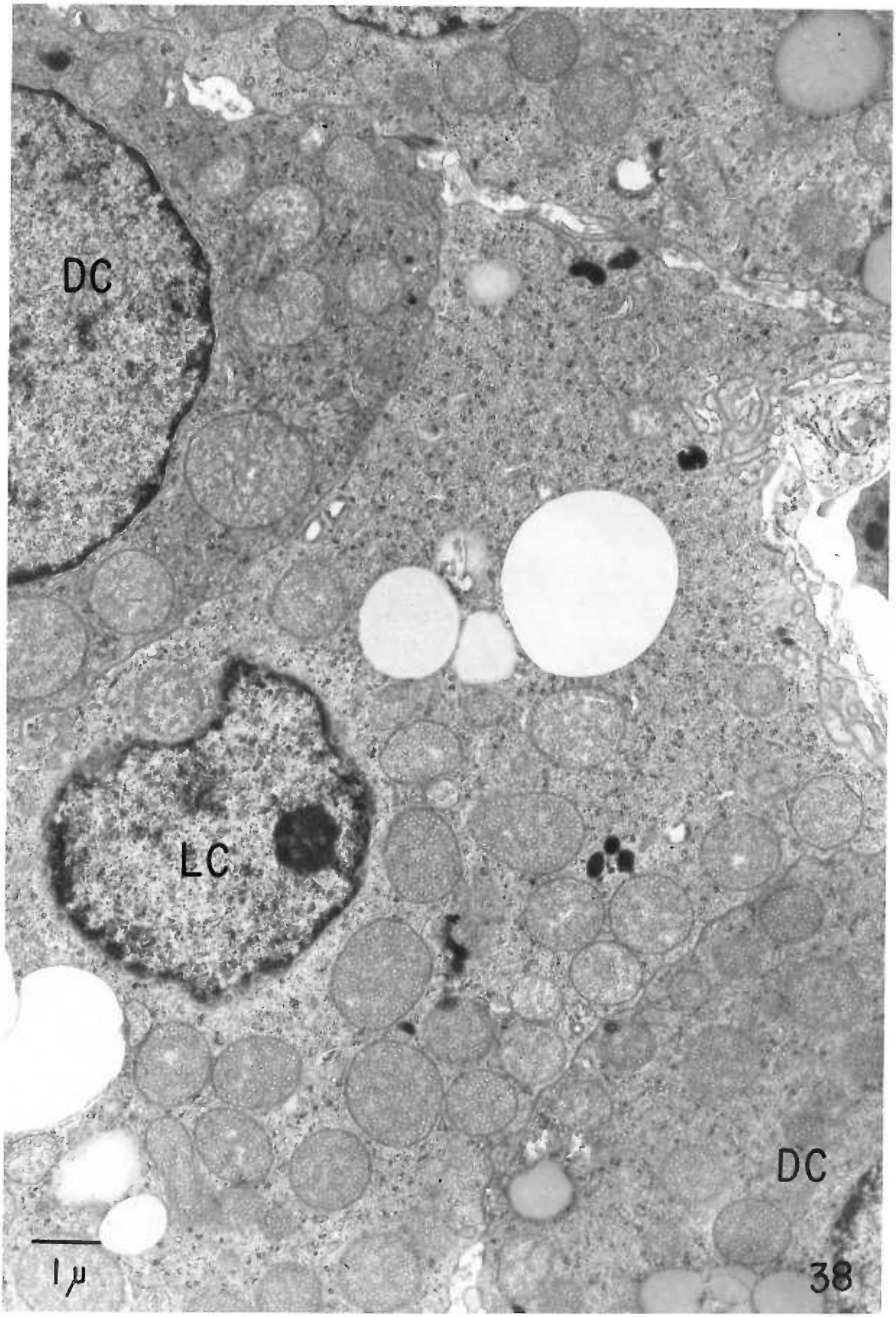

DC
LC
DC
1μ
38

FIGURE 39

Morphologically altered light (LC) cortical cells of the zona fasciculata at day 24 of gestation. Magnification 11, 000 X. In contrast to the cells of the term zona fasciculata, a number of the cells at this stage of gestation reveal mitochondria which exhibit internal disorganization (M_1) and hypertrophy (M_2). In addition there are greater numbers of dilated elements (arrows) of the smooth endoplasmic reticulum (SER).

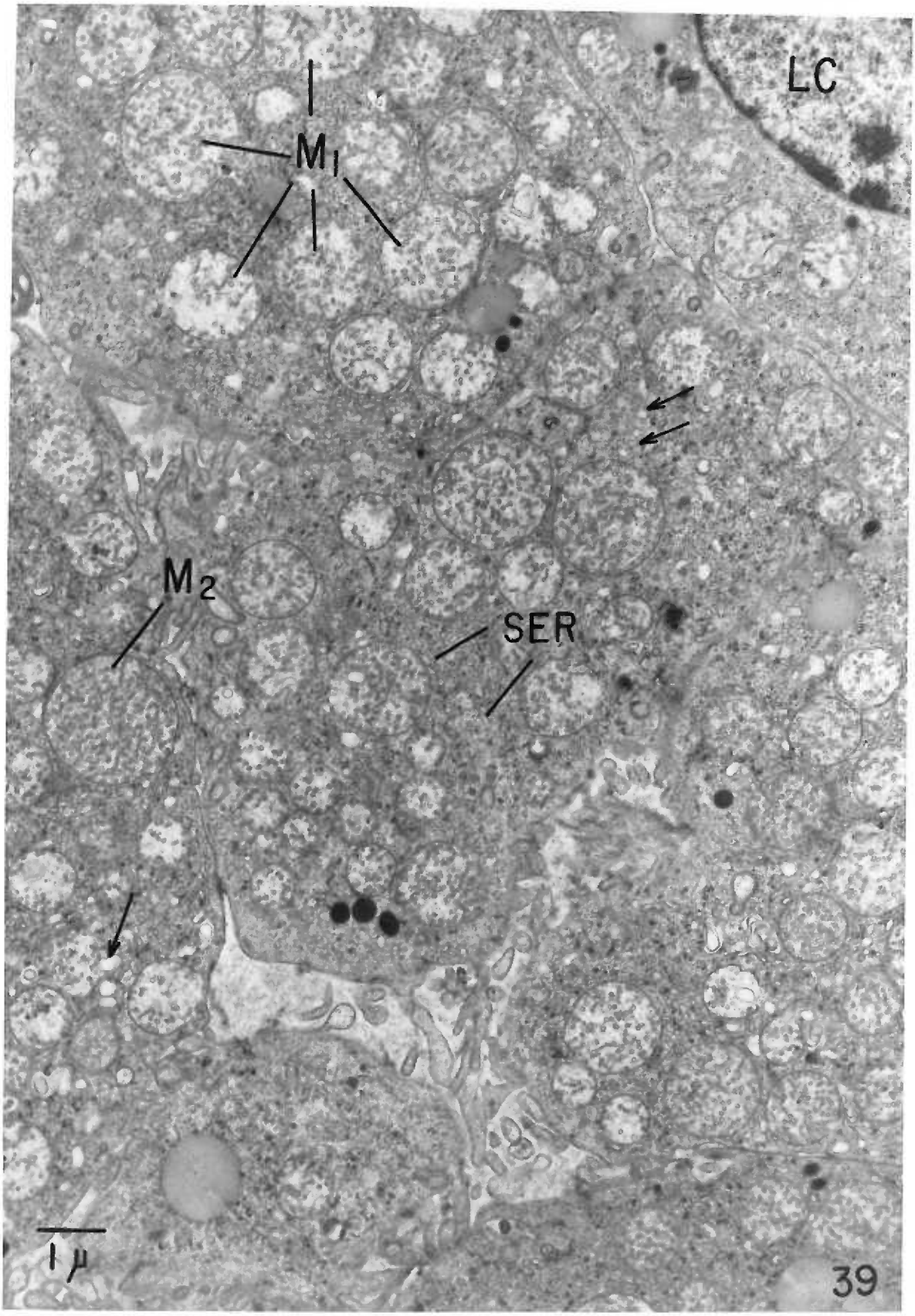

LC
M1
M2
SER
1 μ
39

FIGURE 40

Dark (DC) cortical cells of the zona fasciculata at day 24 of gestation. Magnification 11, 000 X. These cells appear morphologically similar to those described at term.

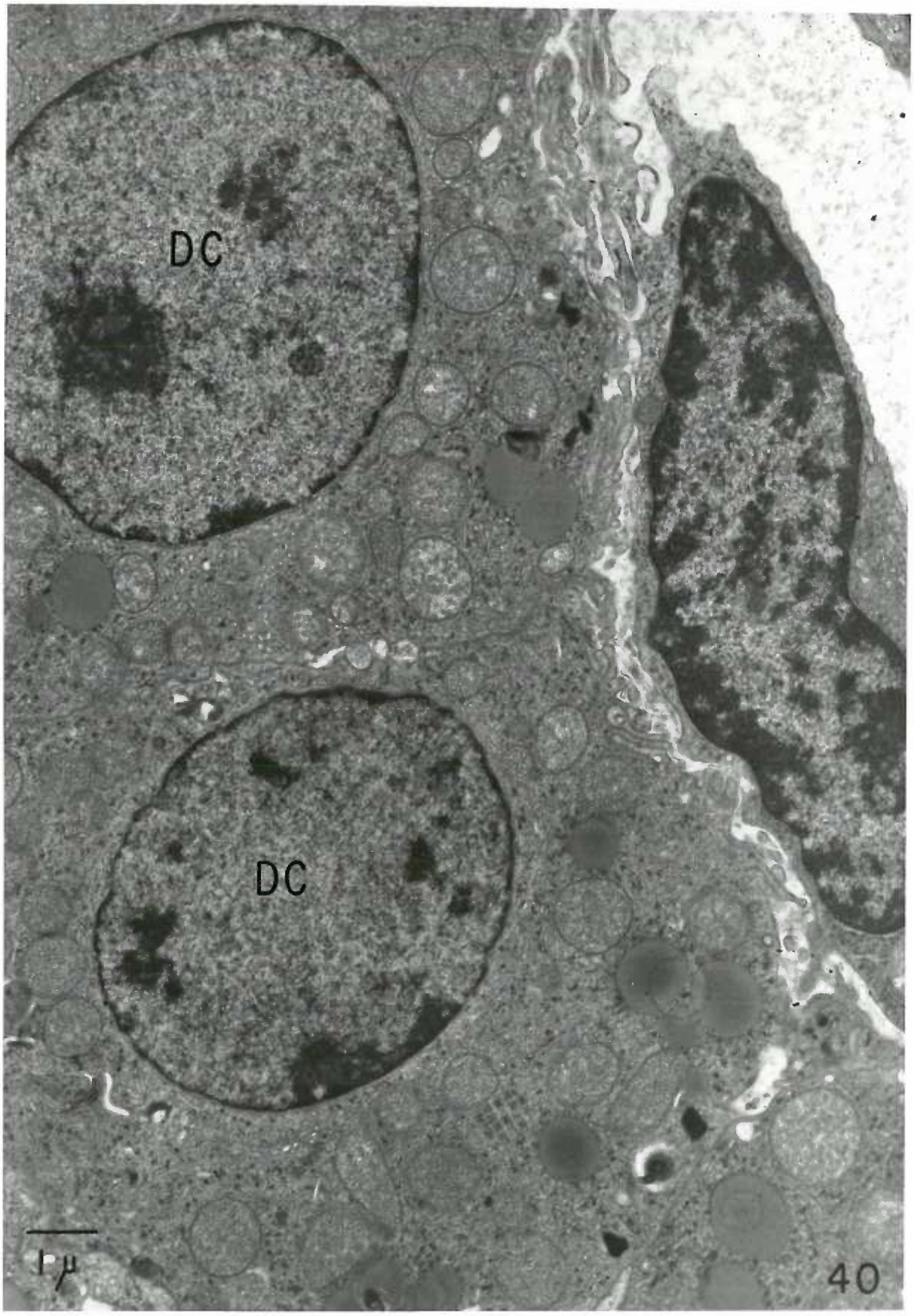

DC
DC
1μ
40

FIGURE 41

Morphologically altered dark (DC) cortical cells of the zona fasciculata at day 24 of gestation. Magnification 11, 000 X. Note that the mitochondria (M) of these dark cells, as compared with those seen in figure 40, are hypertrophied and exceed 2 microns in diameter. The cristae of these mitochondria exhibit varying degrees of disorganization. Numerous tubular profiles of the smooth endoplasmic reticulum (SER) are dilated (arrows).

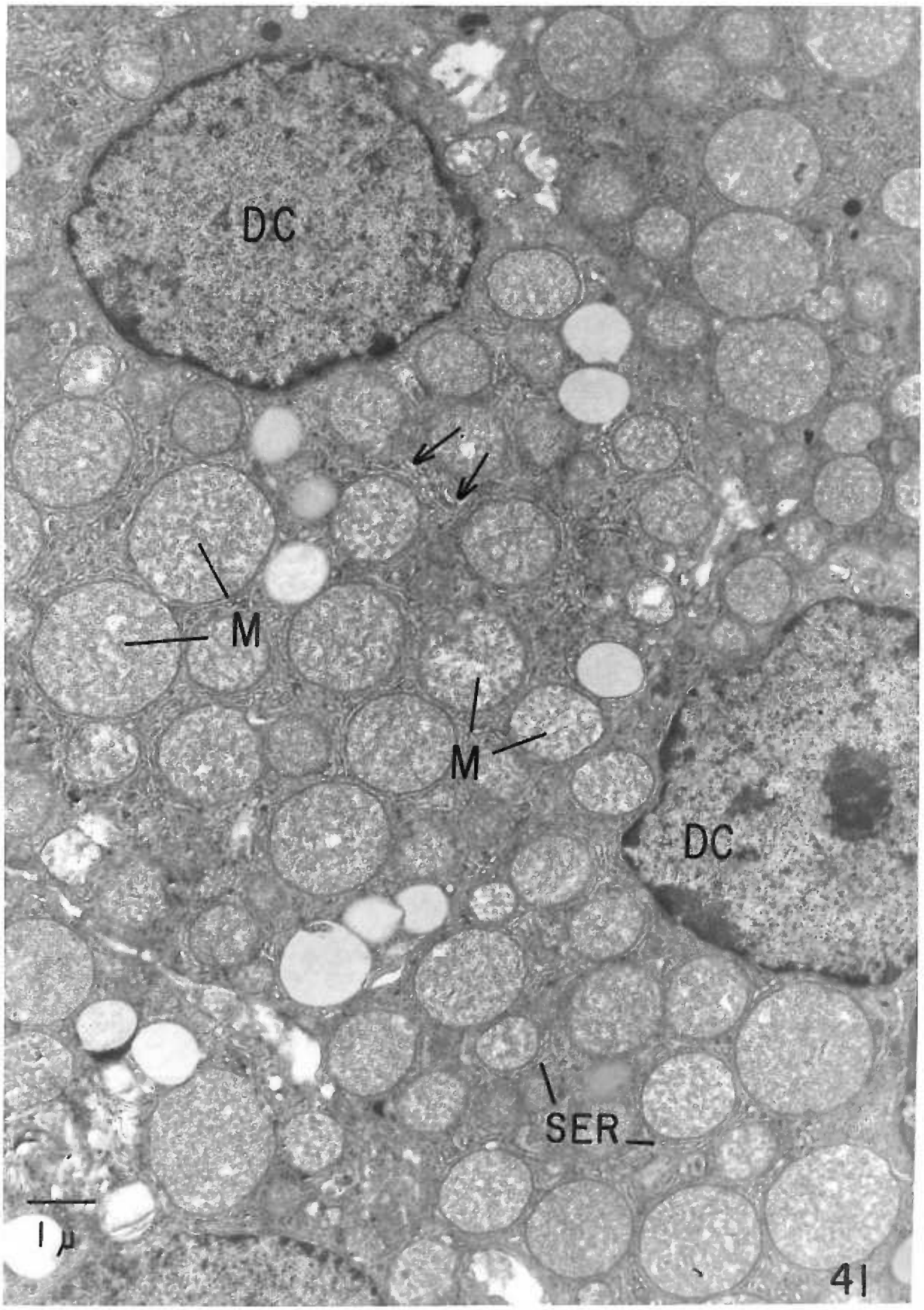

DC
M
M
DC
SER
1 μ
41

FIGURE 42

Light (LC) and dark (DC) cortical cells of the zona reticulo-medullaris at day 24 of gestation. Magnification 14, 597 X. Light and dark cortical cells which are similar to those described in the term zona reticulo-medullaris occur in fewer numbers at day 24 of gestation.

Endothelium (End)

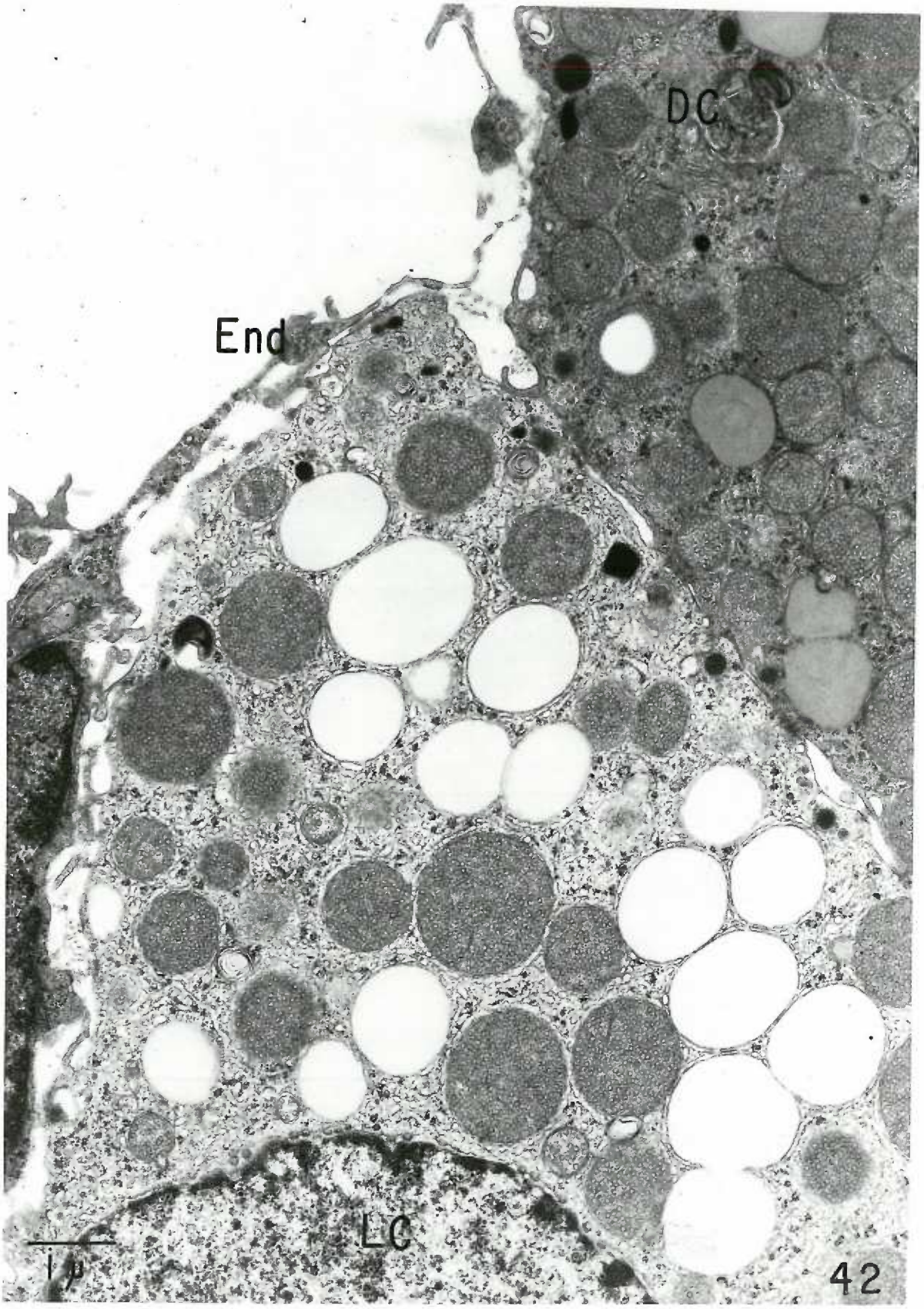

DC
End
LC
1 μ
42

FIGURE 43

Morphologically altered dark (DC) cortical cell of the zona reticulo-medullaris at day 24 of gestation. Magnification 12, 760 X. Dark cells exhibiting many hypertrophied mitochondria (M) are very numerous at this stage of gestation. The matrix in these mitochondria exhibit varying degrees of disorganization. There is a crowding of the cytoplasm due to the numerous hypertrophied mitochondria. Note the LERM complex (arrows).

Glycogen (Gly)

Smooth endoplasmic reticulum (SER)

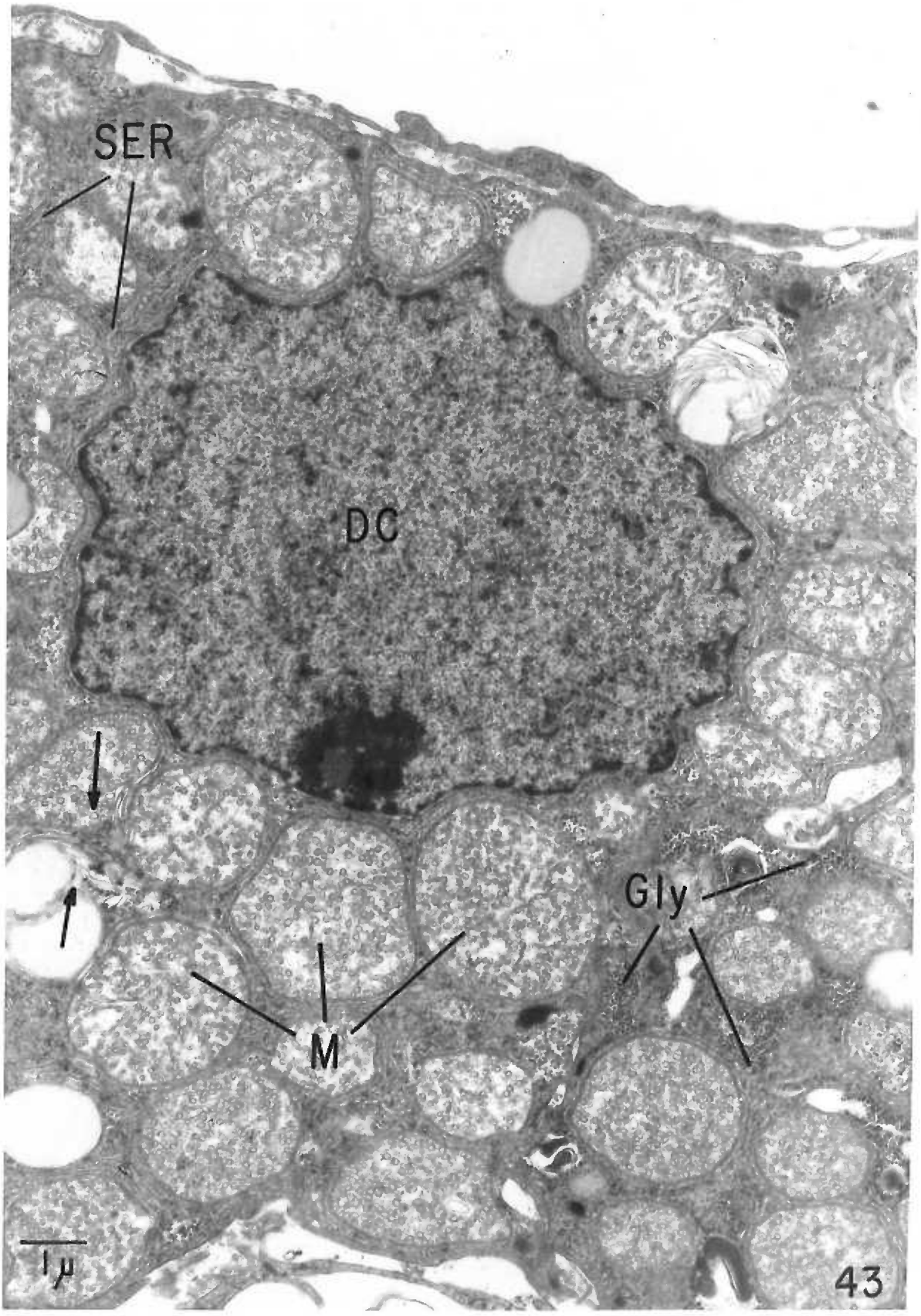

SER
DC
Gly
M
1μ
43

FIGURE 44

Morphologically altered light (LC) and dark (DC) cortical cells of the zona reticulo-medullaris at day 24 of gestation. Magnification 12, 760 X. Note the protrusion (arrows) of light cell cytoplasmic organelles through the attenuated endothelium (End) into the sinusoidal lumen (SL). Dilated tubular profiles of the smooth endoplasmic reticulum (SER) are a common feature in these cells at this stage of gestation.

LERM complex (arrows)

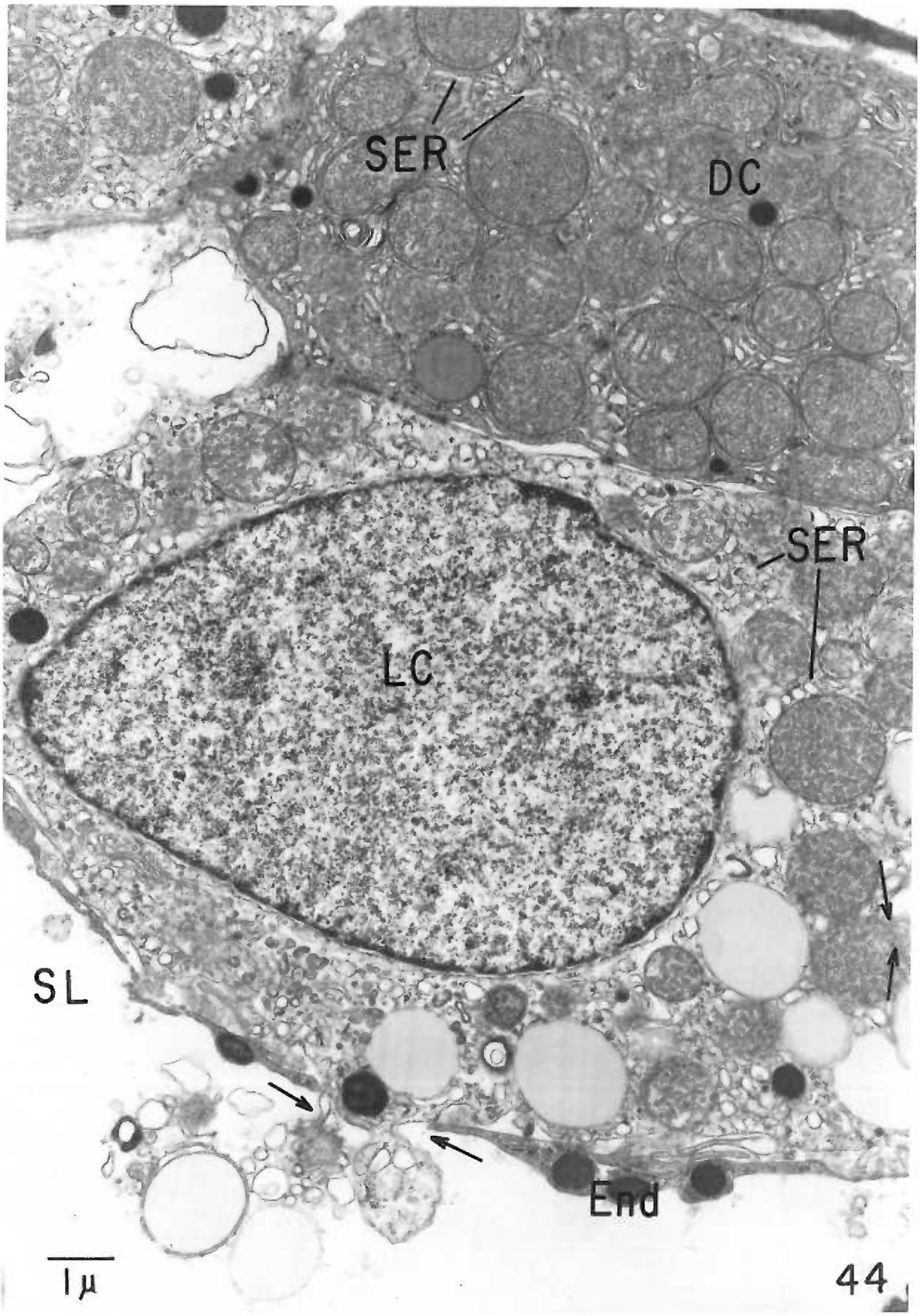

SER
DC
SER
LC
SL
End
1μ
44

FIGURE 45

Morphologically altered light (LC) cortical cell of the zona reticulomedullaris at day 24 of gestation. Magnification 16, 614 X. Hypertrophied mitochondria (M) and swollen tubular profiles of the smooth endoplasmic reticulum (SER) are abundant at this stage. Morphologically altered light cells, such as the one seen here, are very numerous at day 24 of gestation.

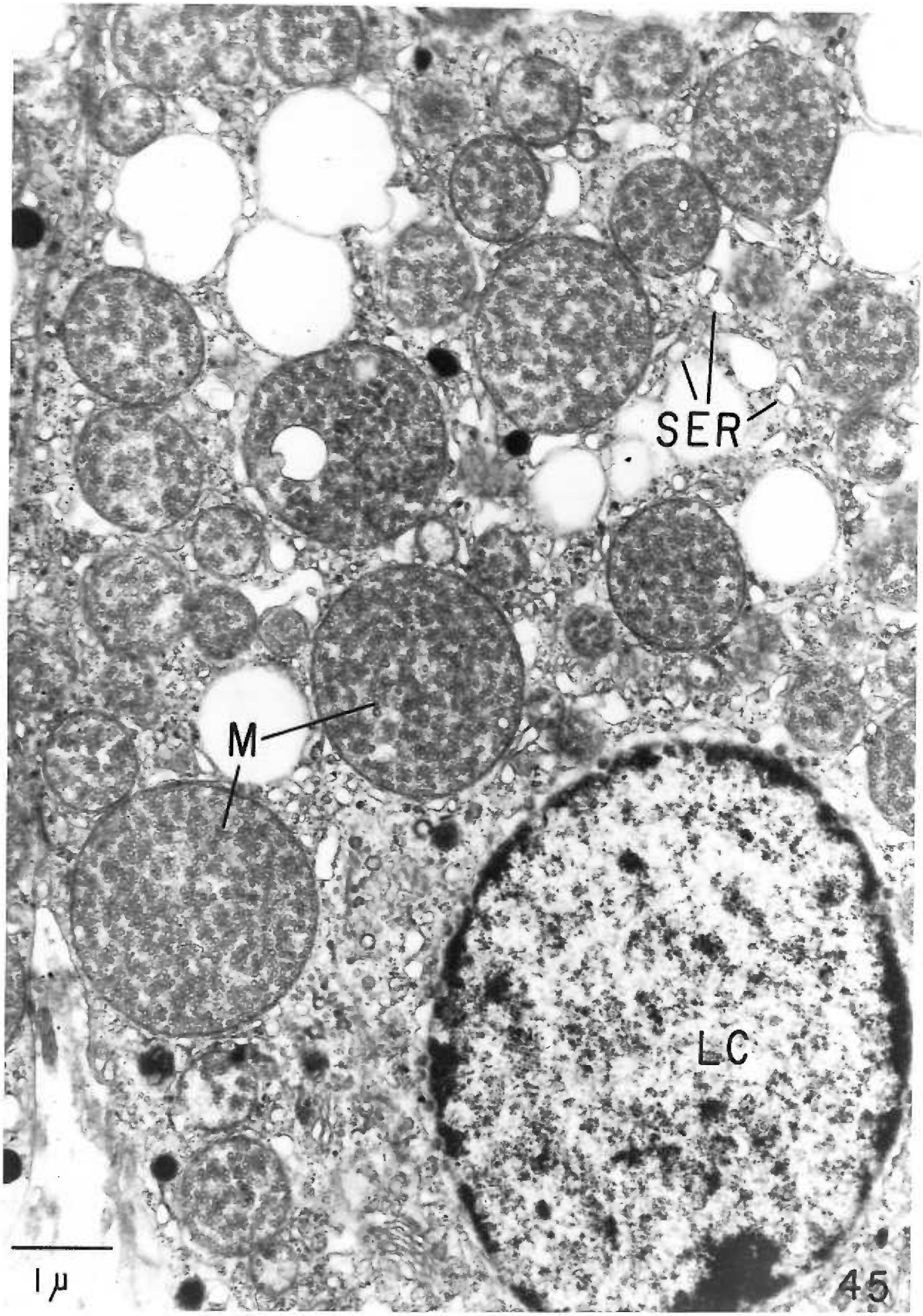

SER
M
LC
1 μ
45

FIGURE 46

Light (LC) and dark (DC) medullary cells of the zona reticulo-medullaris at day 24 of gestation. Magnification 7, 124 X. Although many of the light medullary cells at this stage are morphologically similar to those seen at term, they occur in fewer numbers.

NA = noradrenalin storage granules

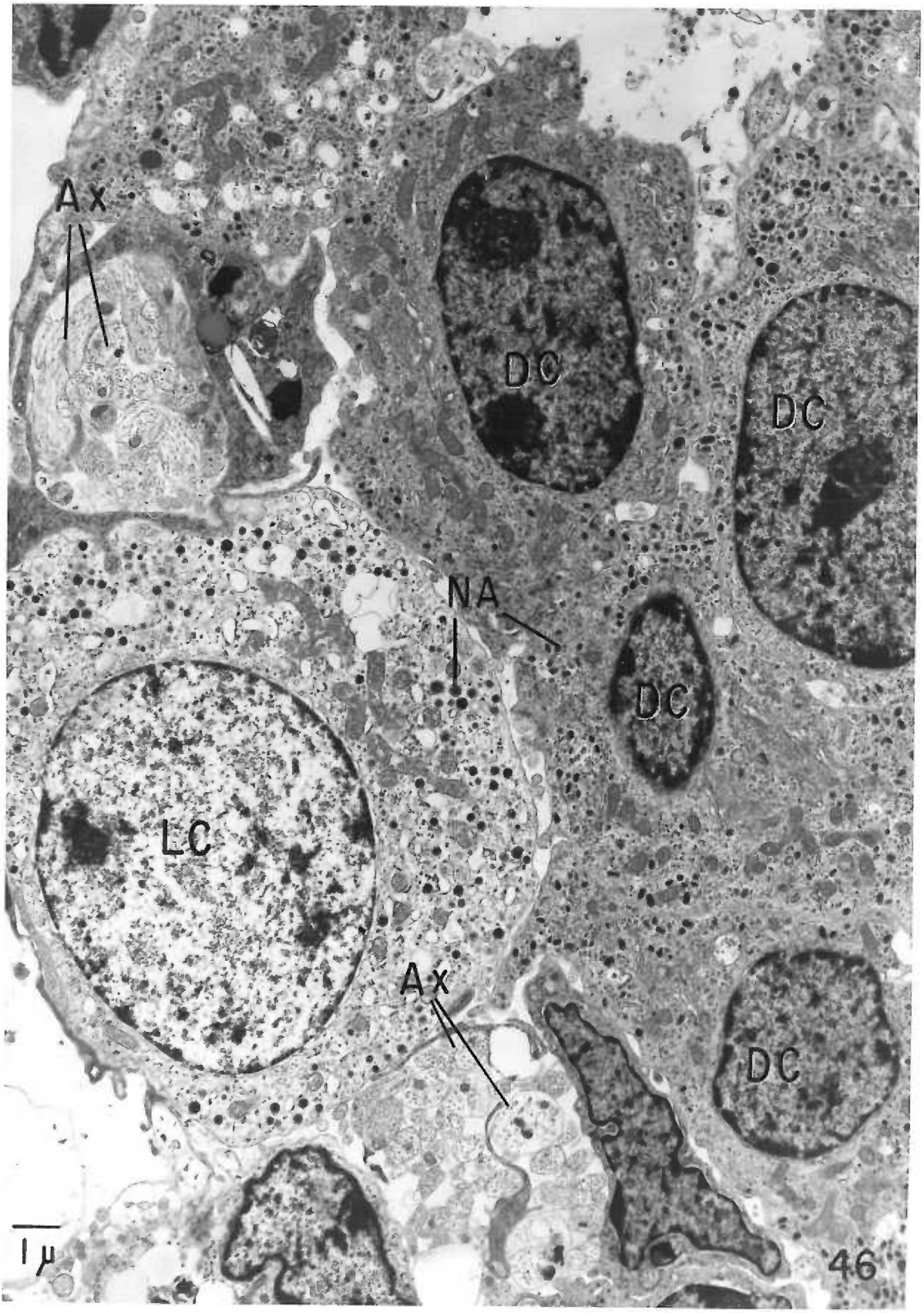
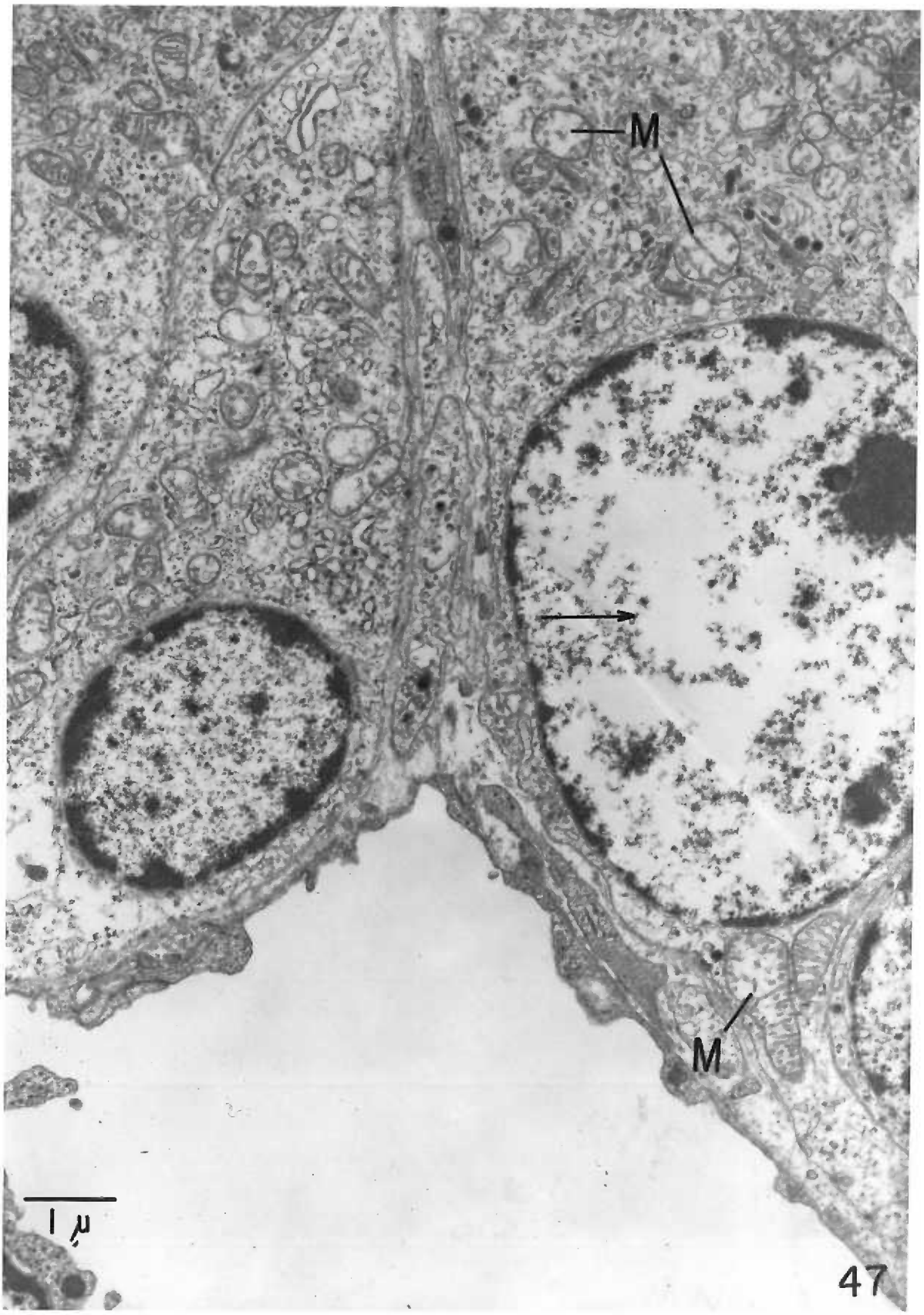

Ax
DC
DC
NA
DC
LC
Ax
DC
1 μ
46

FIGURE 47

Morphologically altered light medullary cells of the zona reticulo-medullaris at day 24 of gestation. Magnification 15, 336 X. These morphologically altered medullary cells exhibit swollen, rarefied mitochondria (M) and a paucity of membrane limited catecholamine storage granules. The nucleoplasm of one of the cells appears rarefied (arrow).



47

FIGURE 48

Morphologically altered light (LC) medullary cell and a normal appearing sinusoid of the zona reticulo-medullaris at day 24 of gestation. Magnification 15, 336 X. Note the paucity of membrane-limited catecholamine storage granules and the swollen and rarefied mitochondria (M). Portions of medullary cells (arrows) containing various cytoplasmic organelles are frequently seen in the sinusoidal lumen (SL).

Endothelium (End)

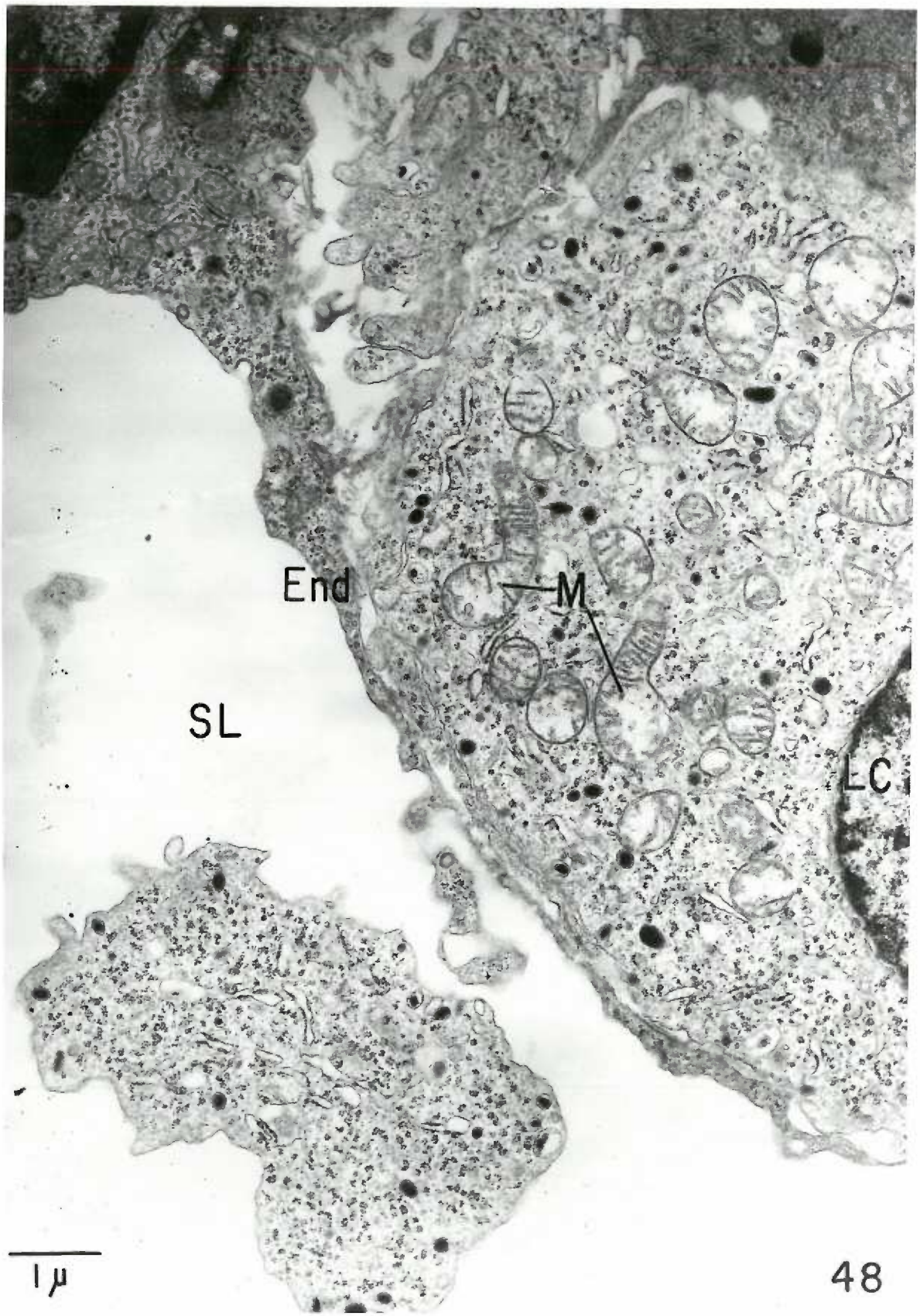

End
M
SL
LC
1 μ

FIGURE 49

Light (LC) cells of the zona glomerulosa at day 25 of gestation. Magnification 11, 000 X. The cells of this zone are morphologically similar to the cells of the term zona glomerulosa. Note the normal appearing endothelium (End) which exhibits fenestrations (arrows) and well developed cytoplasmic organelles.

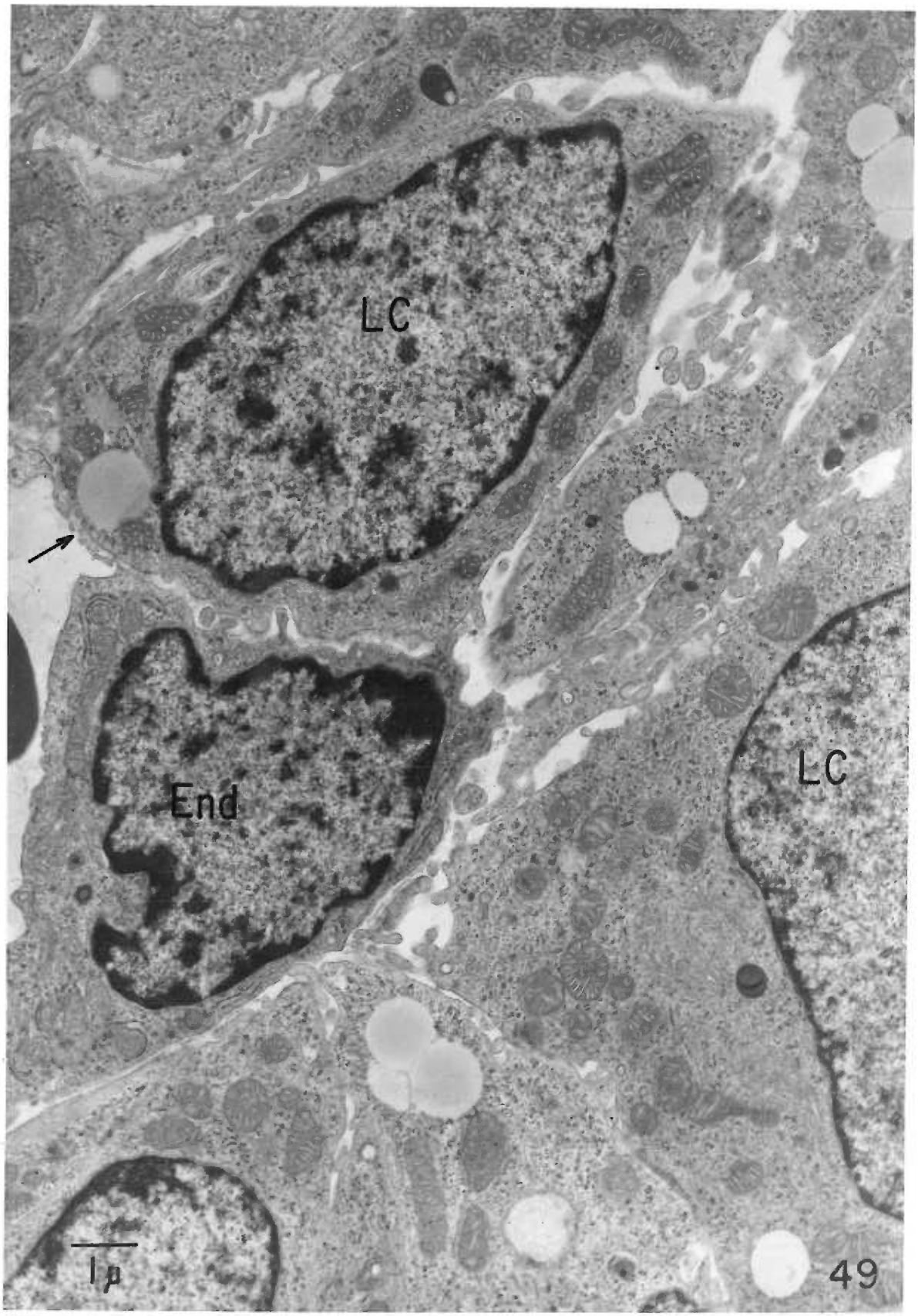

LC
End
LC
1μ
49

FIGURE 50

Light (LC) cells of the zona intermedia at day 25 of gestation. Magnification 11, 000 X. The majority of the cells in this zone appear morphologically similar to the cells of the term zona intermedia. Note the normal appearing light and dark cells of the zona glomerulosa (ZG).

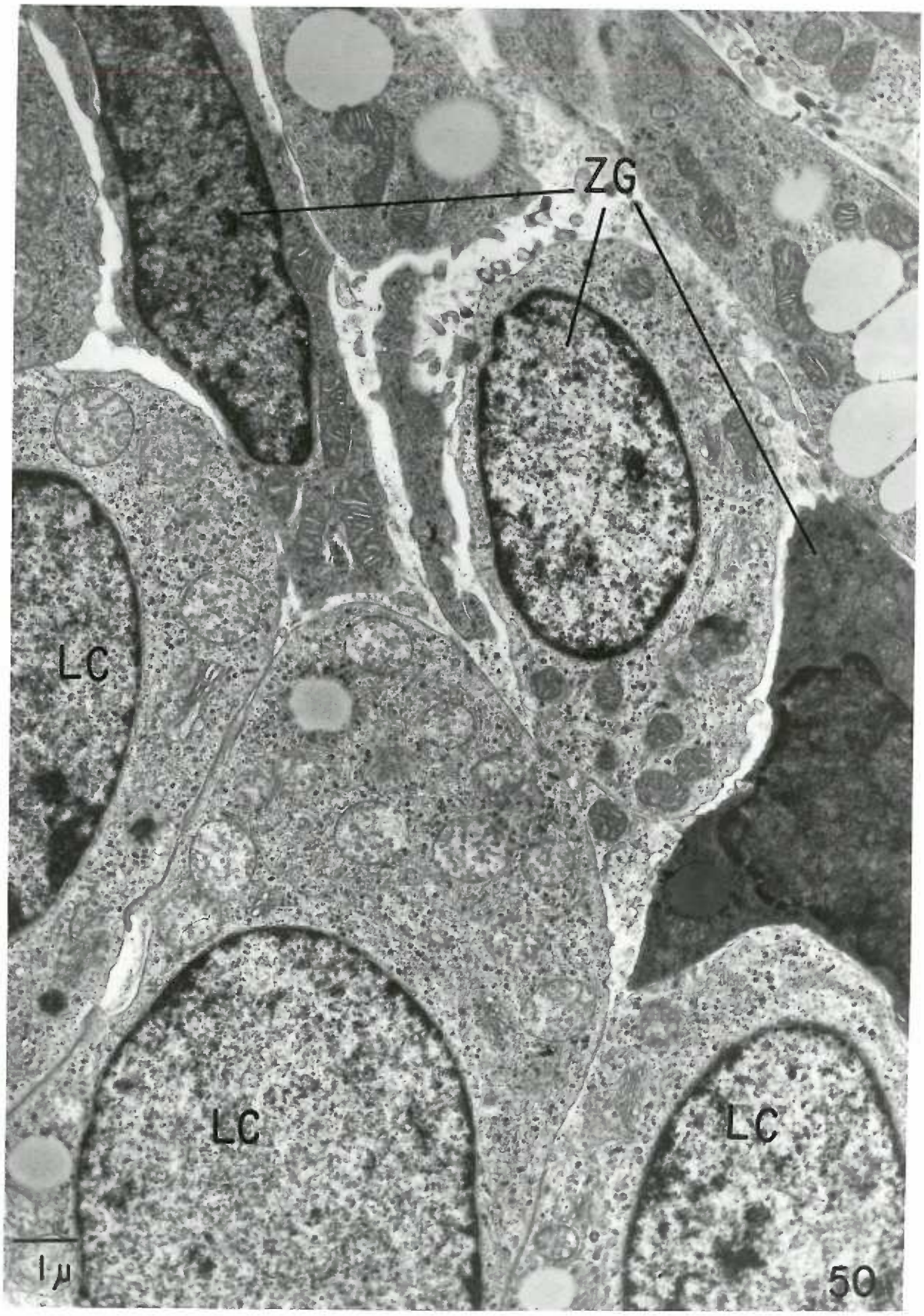

ZG
LC
LC
LC
1 μ
50

FIGURE 51

Morphologically altered light (LC) cortical cells of the zona intermedia at day 25 of gestation. Magnification 11, 000 X. A few light cells of this zone exhibit numerous hypertrophied mitochondria (M) in which the matrices are disorganized. Numerous tubular profiles of the smooth endoplasmic reticulum appear dilated (SER).

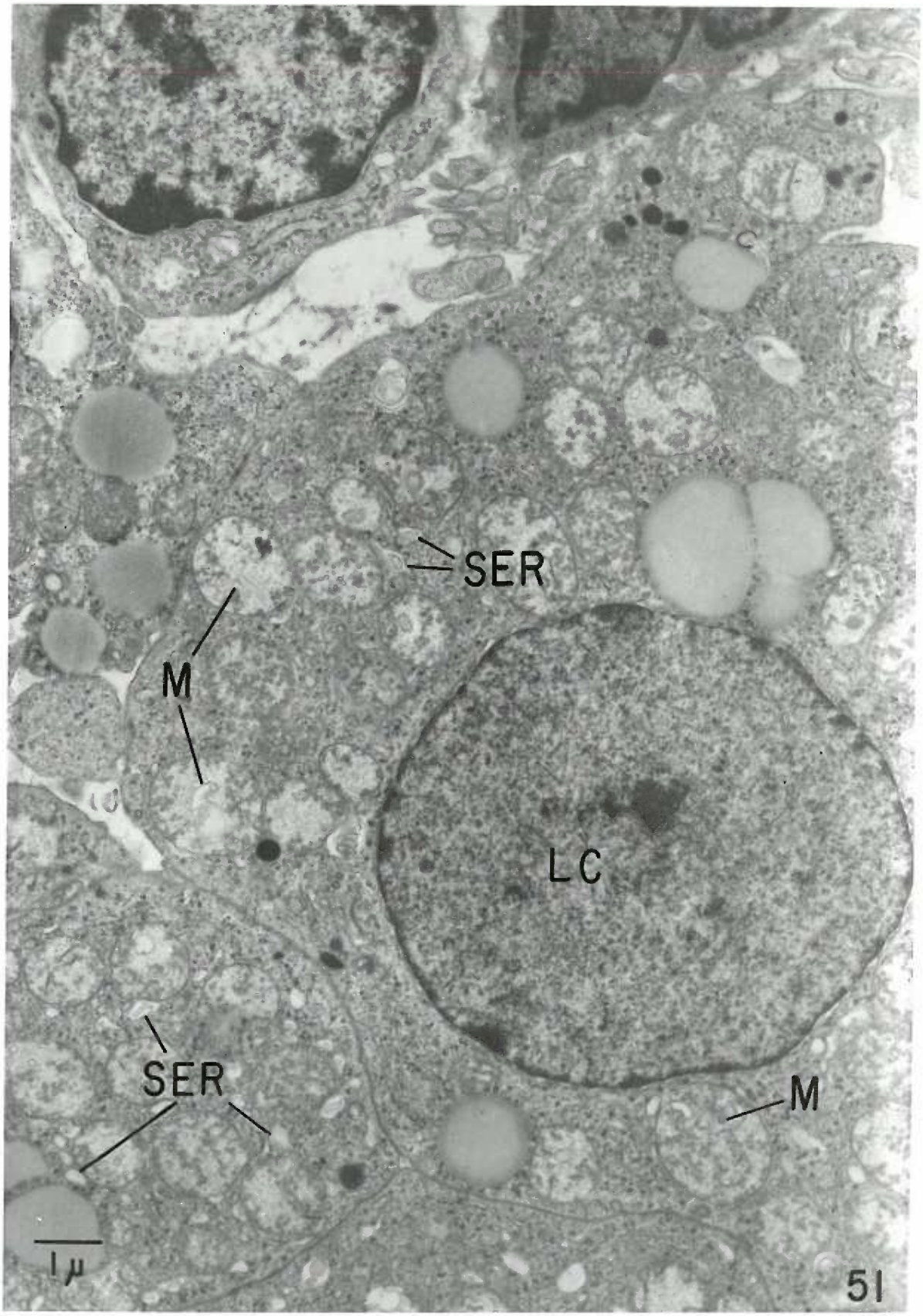

SER
M
LC
SER
M
1μ
51

FIGURE 52

Light (LC) cortical cells of the zona fasciculata at day 25 of gestation. Magnification 11, 000 X. The majority of the light cells at this stage are morphologically similar to those described at term.

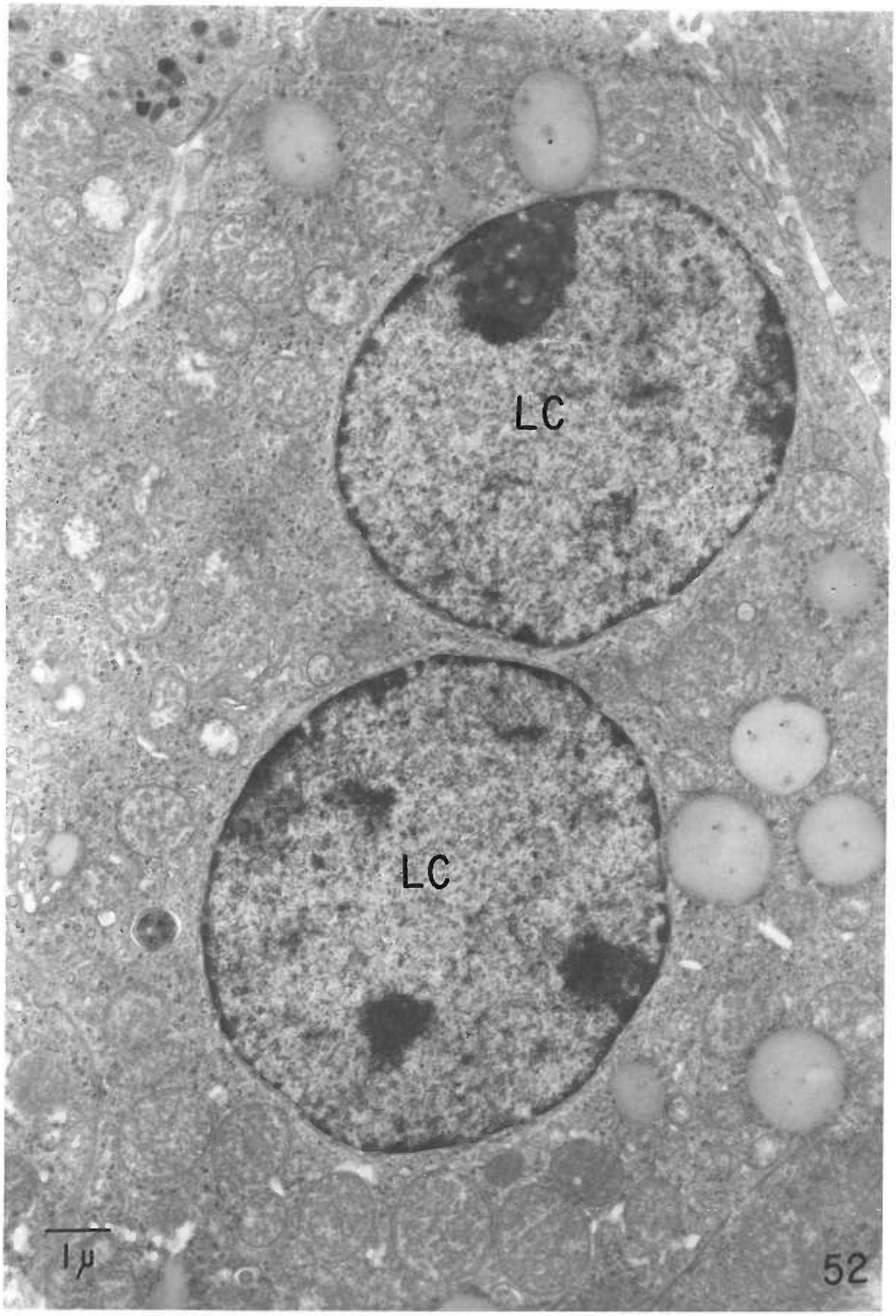

LC
LC
1 μ
52

FIGURE 53

Morphologically altered light (LC) cortical cells of the zona fasciculata at day 25 of gestation. Magnification 11, 000 X. Numerous dilated profiles of smooth endoplasmic reticulum (SER) and hypertrophied mitochondria (M) which exhibit internal disorganization are characteristic features of morphologically altered light cells at this stage of gestation. Note the LERM complex (arrows).

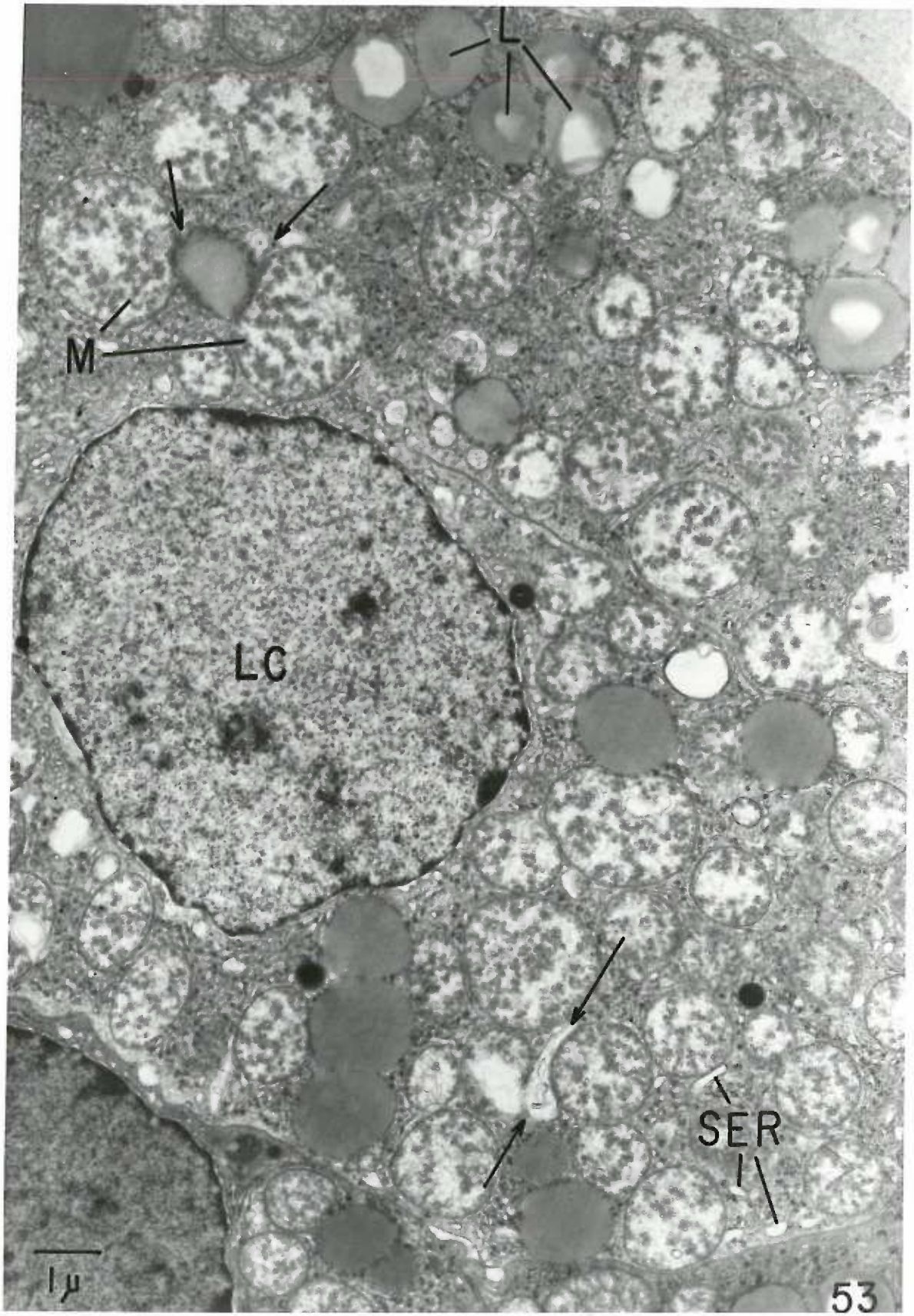
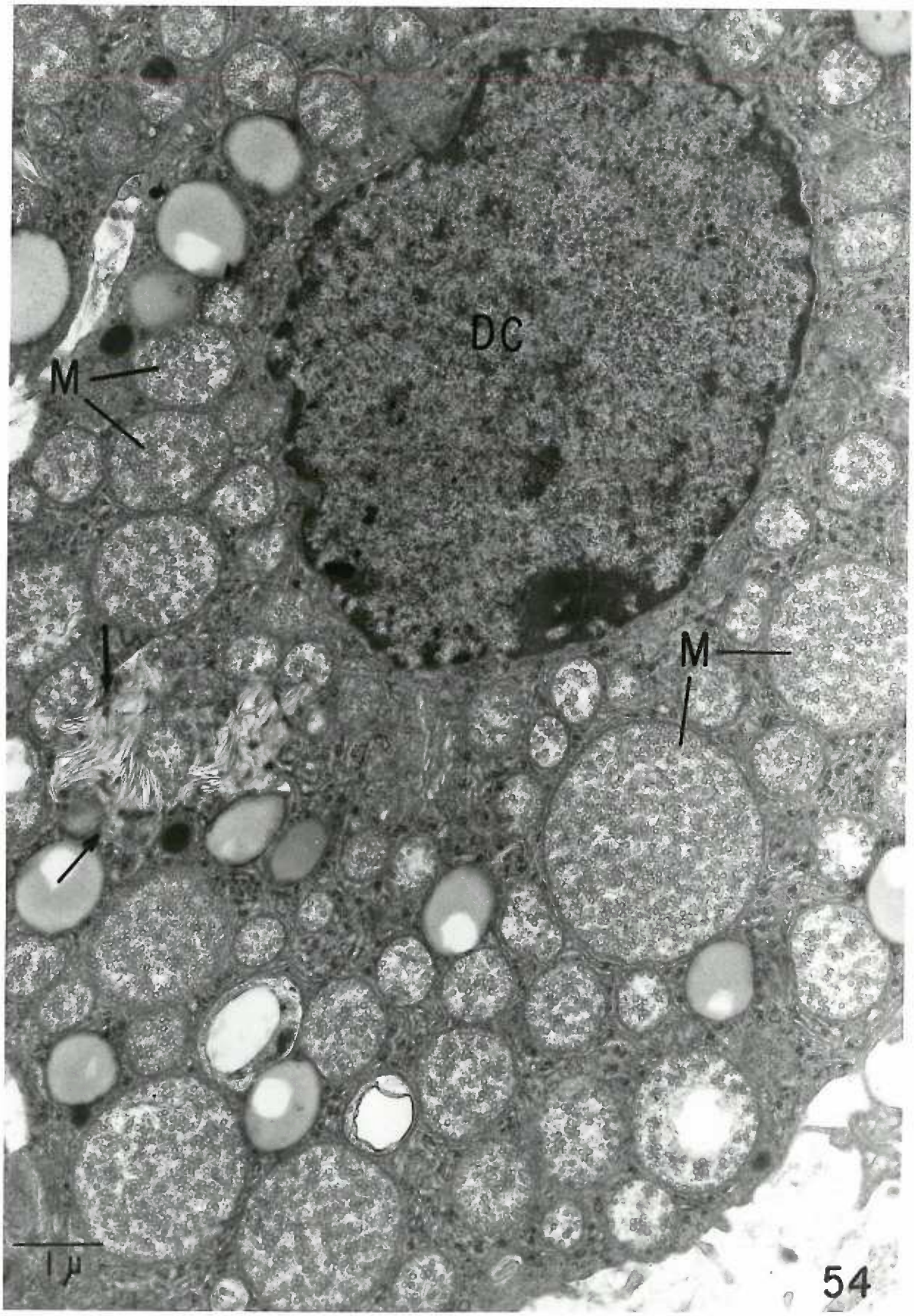

L
M
LC
SER
1μ
53

FIGURE 54

Morphologically altered dark (DC) cortical cell of the zona fasciculata at day 25 of gestation. Magnification 14, 520 X. The altered dark cells at this stage exhibit hypertrophied mitochondria (M) with varying degrees of internal disorganization. Note the close association of lipid, membranous structures and mitochondria (LERM complex - arrows).



54

FIGURE 55

Dark cortical (DC) and light medullary cells (LC) of the zona reticulo-medullaris at day 25 of gestation. Magnification 10, 560 X. The dark cortical cells and the light medullary cell, in the lower left hand corner of the micrograph, appear morphologically similar to those described at term. Note the morphologically altered light medullary cell (arrow) which exhibits swollen and rarefied mitochondria (M), as well as a paucity of membrane-limited catecholamine storage granules.

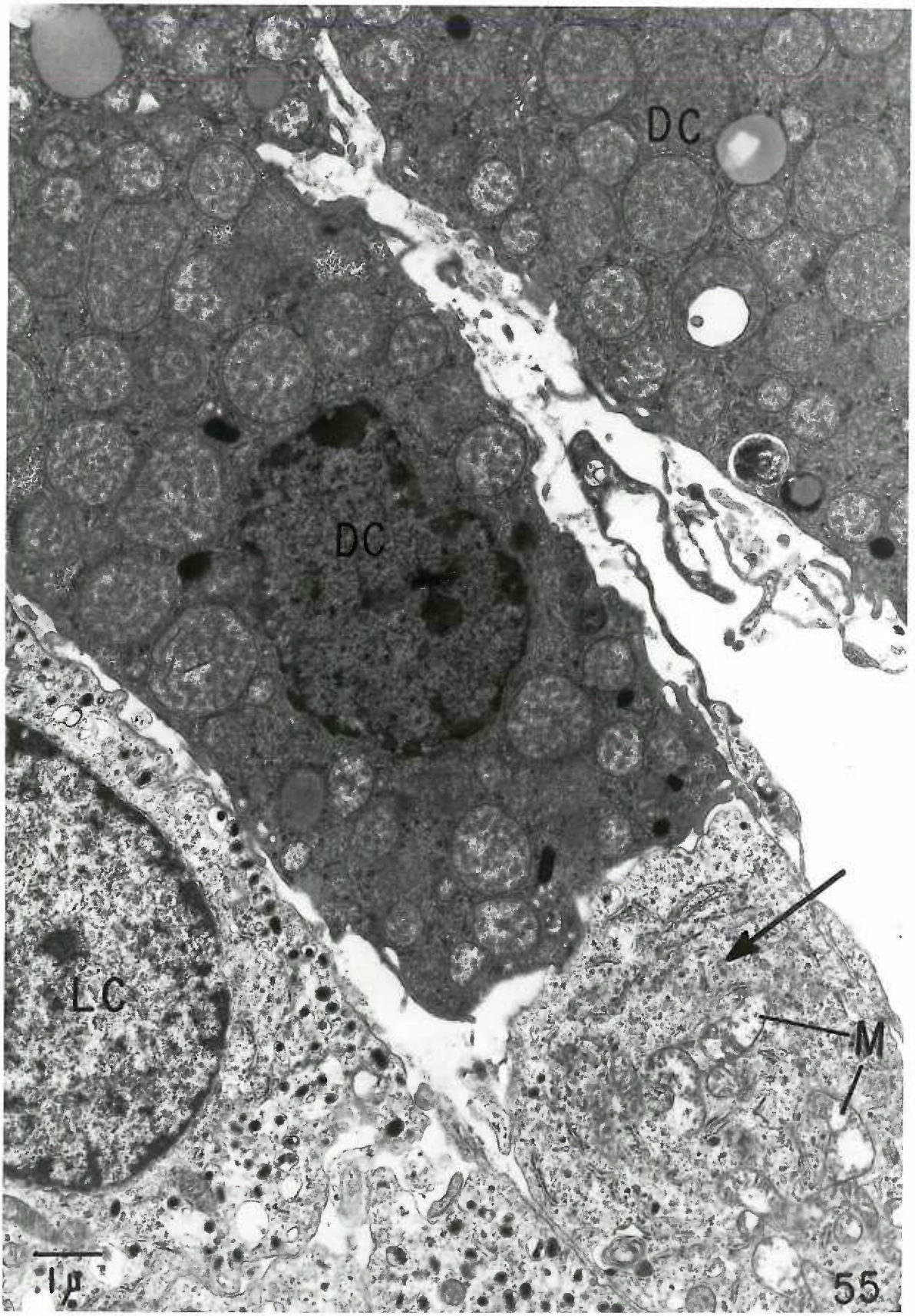

DC
DC
LC
M
1μ
55

FIGURE 56

Morphologically altered light cortical cell (LC) of the zona reticulomedullaris at day 25 of gestation. Magnification 15, 336 X. The majority of the mitochondria (M) in this cell are hypertrophied and exhibit disorganization of their matrices. The smooth endoplasmic reticulum (SER) consists of numerous swollen tubules in contrast to the predominately long, narrow tubular profiles described in the light cortical cells of this zone at term. Note the LERM complex (arrow).

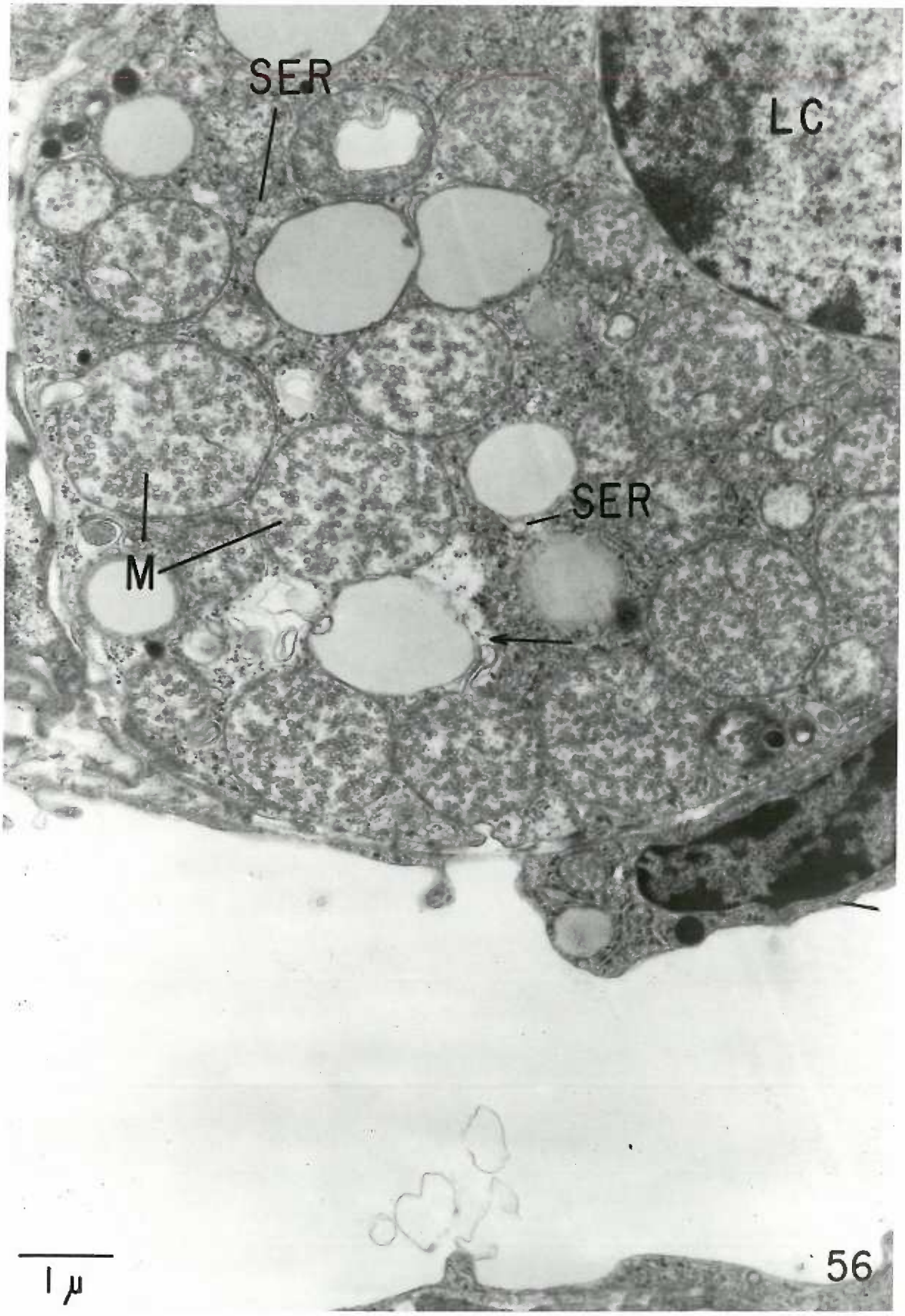

SER
LC
SER
M
1 μ
56

FIGURE 57

Morphologically altered dark (DC) cortical cells of the zona reticulo-medullaris at day 25 of gestation. Magnification 15, 336 X. These cells exhibit numerous hypertrophied mitochondria which cause a crowding of the cytoplasm.

LERM complex (arrows)

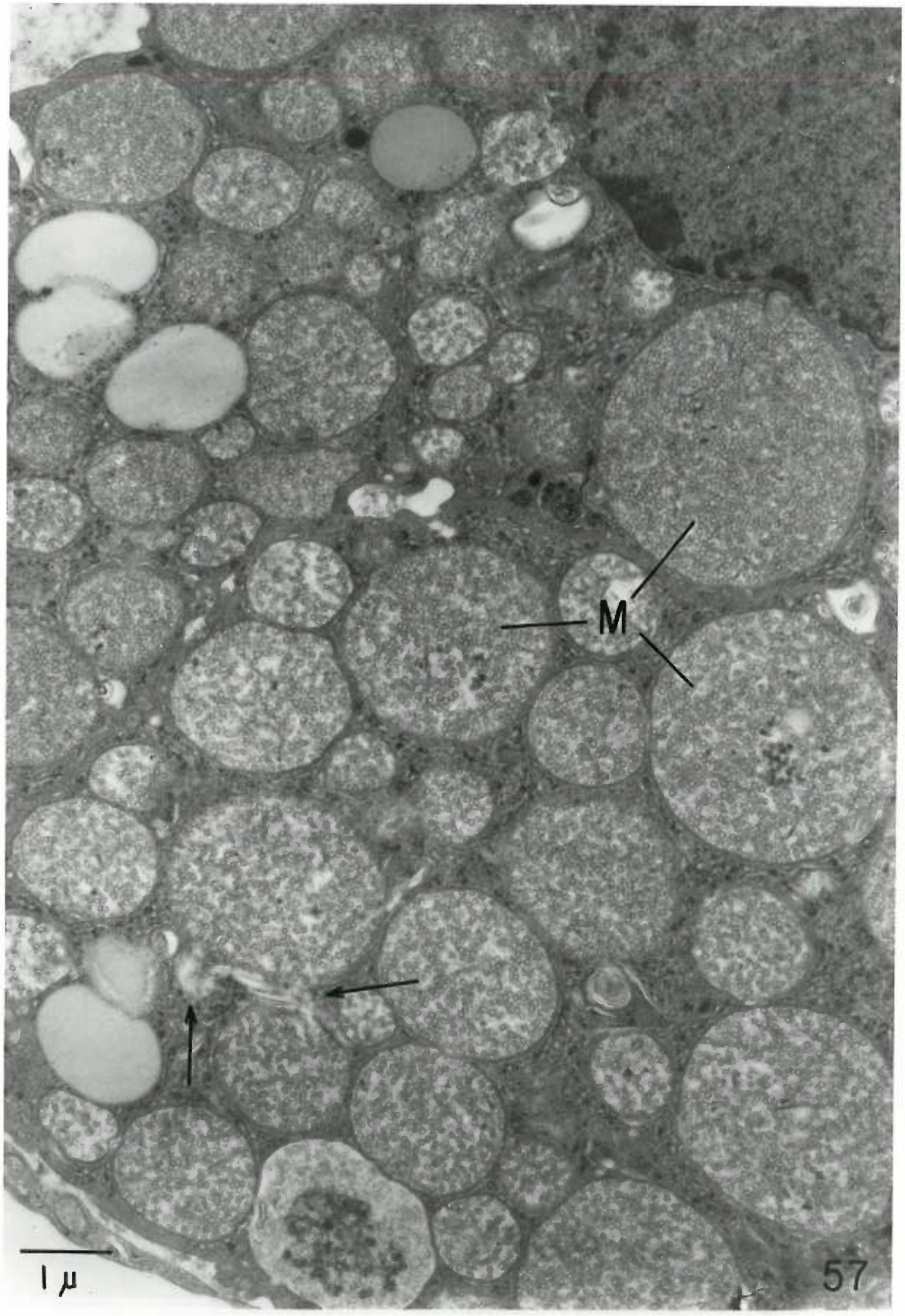

M
1 μ
57

FIGURE 58

Morphologically altered light (LC) medullary cells of the zona reticulo-medullaris at day 25 of gestation. Magnification 10, 560 X. These cells exhibit swollen and rarefied mitochondria (M) and a paucity of membrane-limited catecholamine storage granules. Note the cytoplasmic fragment in the sinusoidal lumen (arrow).

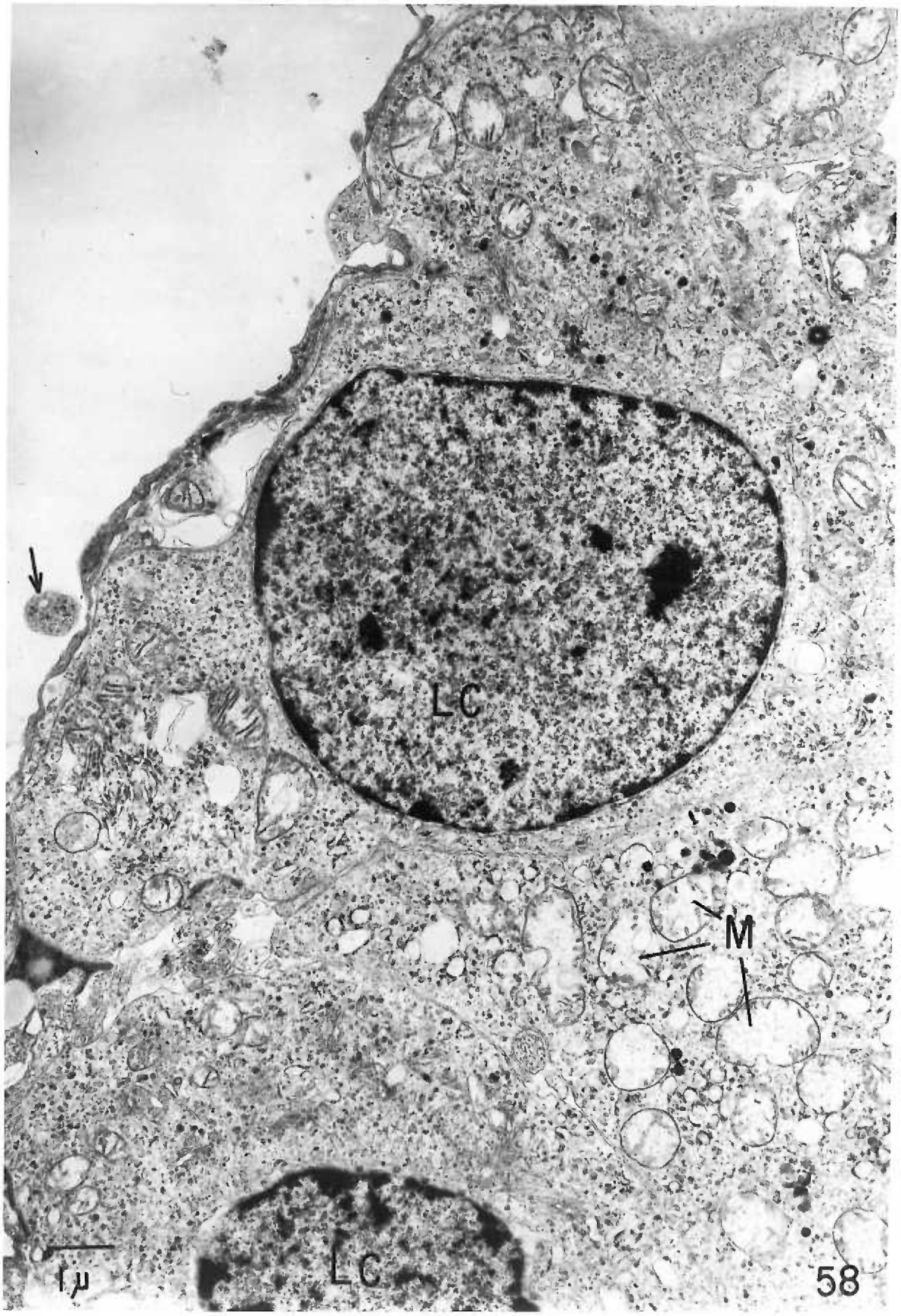

LC
M
LC
1μ
58

FIGURE 59

Sinusoid of the zona reticulo-medullaris at day 25 of gestation. Magnification 13, 640 X. Note that the sinusoidal lumen (SL) contains a variety of cortical cell elements such as mitochondria (M), polyribosomes (Ps), smooth endoplasmic reticulum (SER) and lipid droplets (L).

End = endothelium

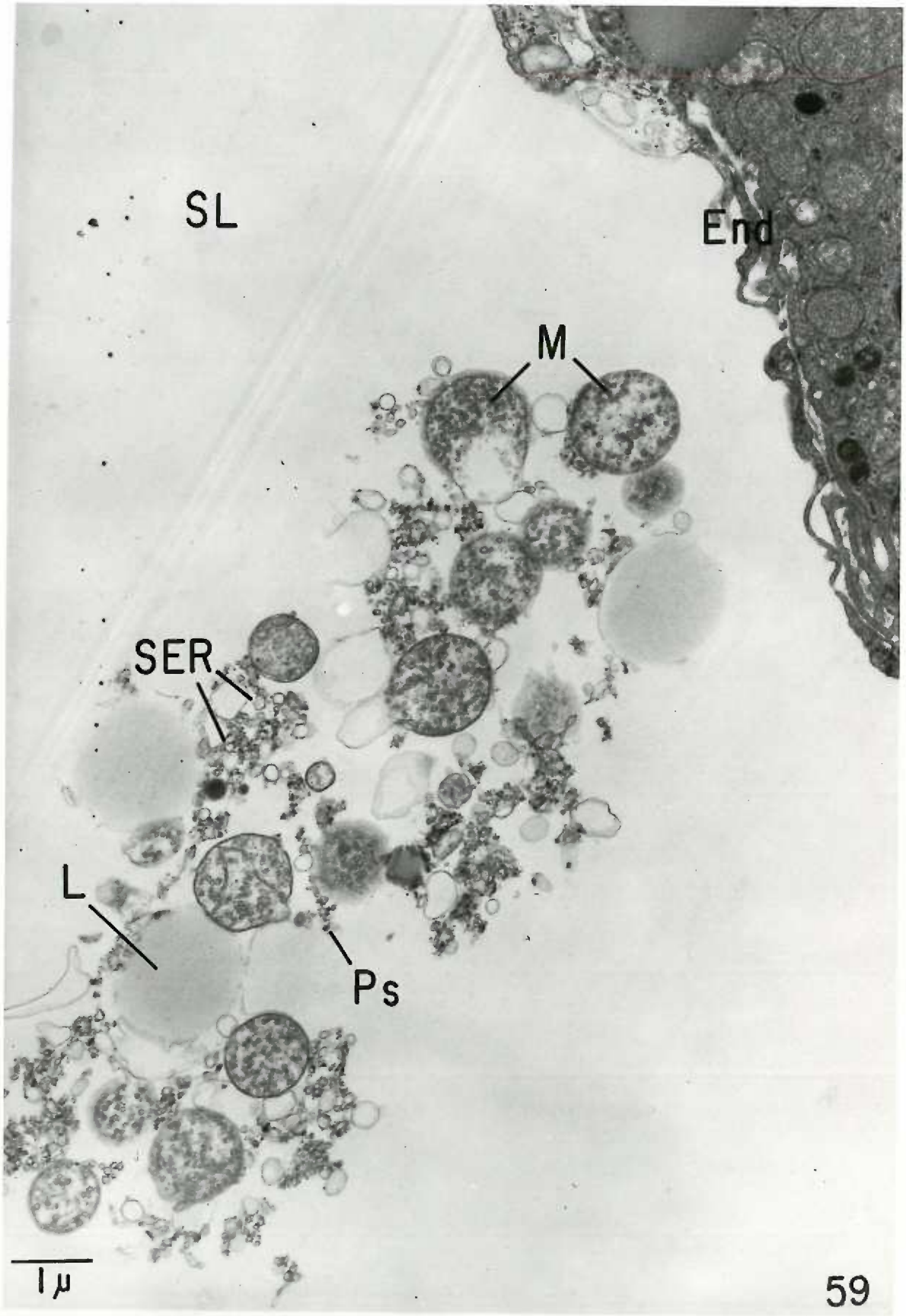

SL
End
M
SER
L
Ps
1 μ

FIGURE 60

Light (LC) medullary cells of the zona reticulo-medullaris at day 25 of gestation. Magnification 13, 640 X. Note that cytoplasmic portions of these cells are evaginating through the sinusoidal endothelium (End). The medullary cell at the left exhibits an area of cytoplasmic concentration, which morphologically appears to be in the process of flowing (arrows) towards the evaginated process.

Sinusoidal luman (SL)

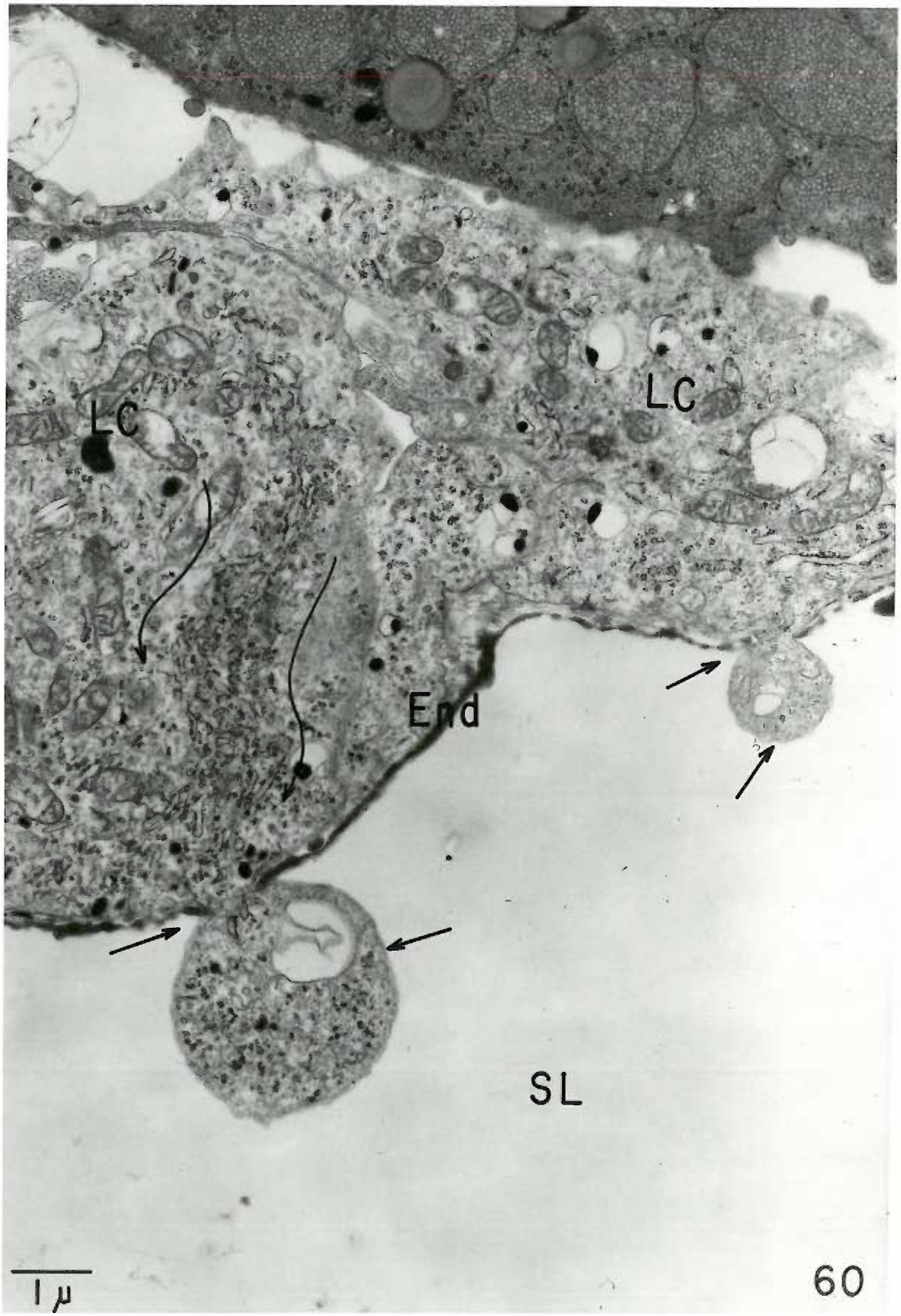

LC
LC
End
SL
1 μ
60

FIGURE 61

Dark cortical (DC) and light medullary (LC) cells of the zona reticulo-medullaris at day 25 of gestation. Magnification 15, 840 X. Note the evagination of a cytoplasmic process (arrow) through the sinusoidal endothelium (End).

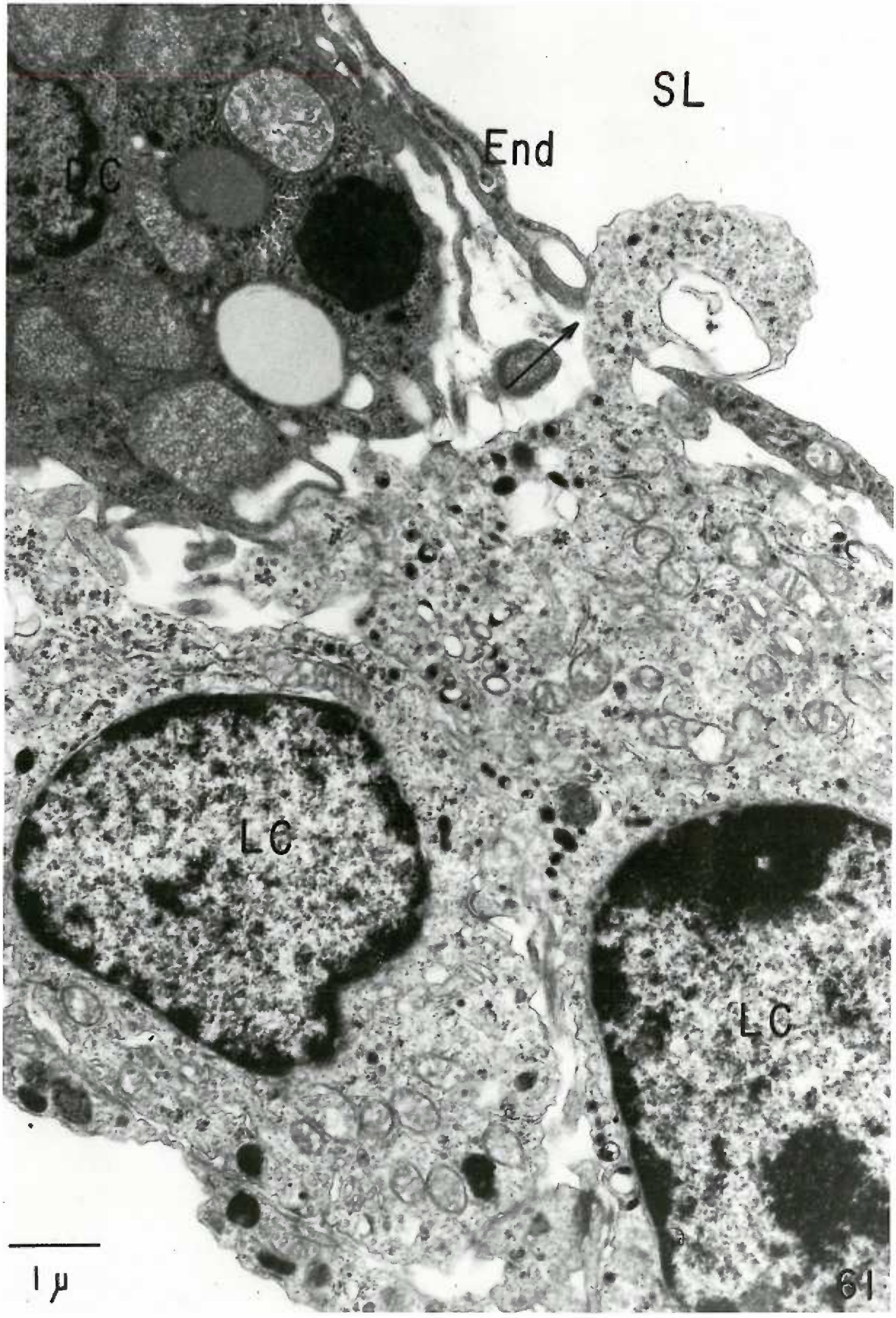

SL
End
DC
LC
LC
1 μ
61

FIGURE 62

Dark cortical cell (DC) of the zona reticulo-medullaris at day 25 of gestation. Magnification 22, 440 X. Note the close association of lipid, mitochondria and membranous structures i. e. LERM complex (arrows).

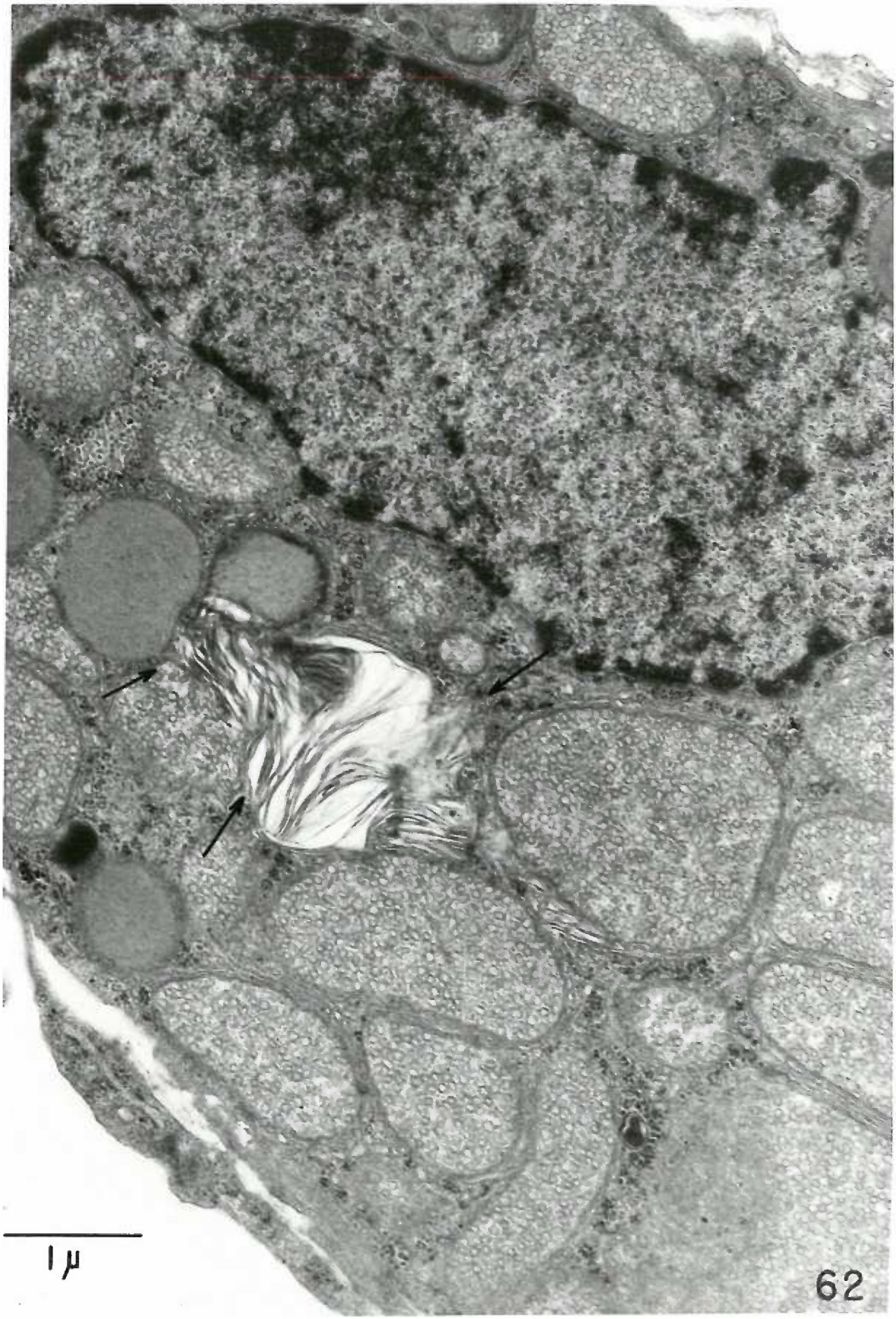
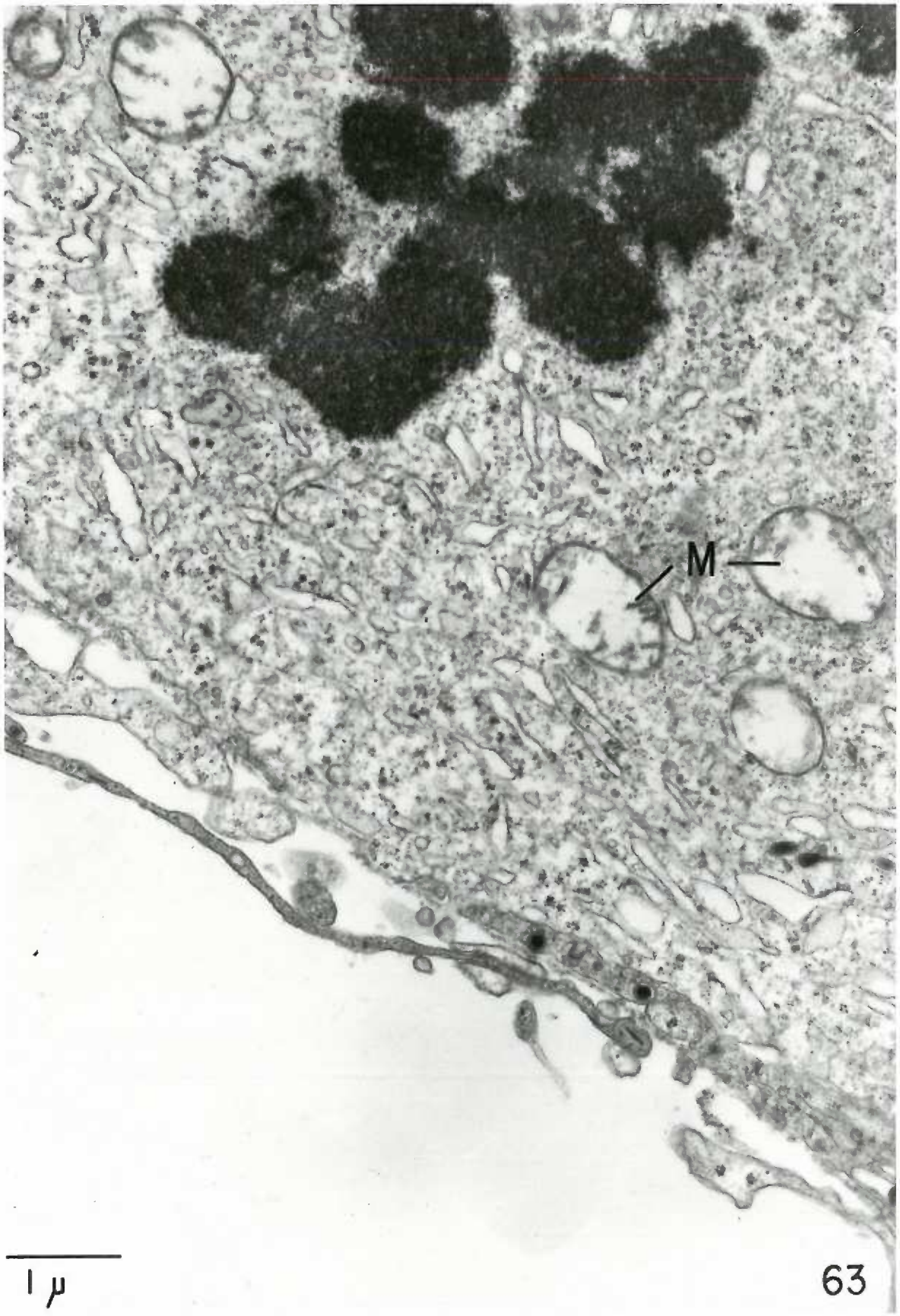

1μ
62

FIGURE 63

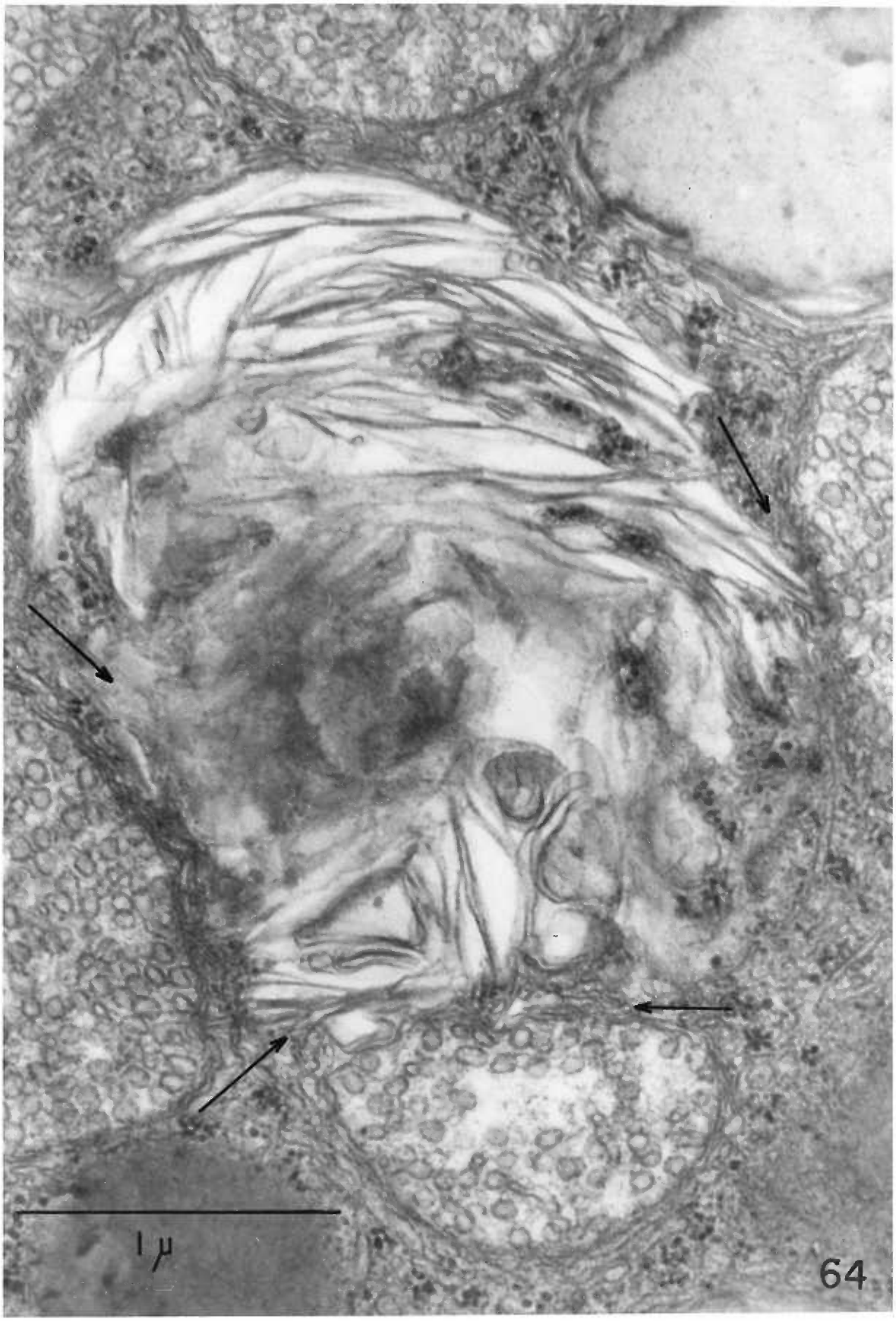
Morphologically altered light medullary cell of the zona reticulo-medullaris at day 25 of gestation. Magnification 21, 000 X. Note the mitotic figure, swollen and rarefied mitochondria (M), and the paucity of membrane-limited catecholamine storage granules.



63

FIGURE 64

Dark cortical cell of the zona reticulo-medullaris at day 25 of gestation. Magnification 54, 638 X. Note the close association of lipid, membranous structures and mitochondria i. e. LERM complex (arrows).


1 μ
64