Nagoya J. med. Sci. 30: 225-251, 1967.

AGE AND SEX DIFFERENCES IN THE FINE STRUCTURE OF THE MOUSE ADRENAL CORTEX

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ABSTRACT

Sex and age differences were examined in the adrenal cortices of thirty five mice with special reference to their zonation.

The zonal variety was found in the shape and inner structure of the mitochondria. Elongated form of mitochondria was characteristic in the glomerulosa cells, and frequent in young animals. The internal structure of the mitochondria was almost tubular or rectimembranous. In general the former was predominant in the deeper zone of the cortex, and the latter in the outer zone. In old animals, however, most mitochondria of all zones possessed tubular internal structures.

In old males, lipid droplets were localized at the upper part of the cortex, particularly in the zona glomerulosa. Golgi apparatus was, in general, more developed in young animals, mainly in the zona glomerulosa.

Vacuoles bounded by a smooth membrane were abundant in the deeper cortical cells. Rough surfaced endoplasmic reticulum was characteristic in the glomerulosa cells. Both the light and the dark cells were recognized in all zones of the cortex, though the latter were very frequently observed in the deeper zone.

Pigment bodies were noticed in the zona reticularis, more frequently in males than females.

X zone was well preserved only in young females.

INTRODUCTION

Electron microscopic studies of the adrenal cortex have been made by many authors during the past ten years[1)-33)]. Zelander[17)] described in detail the fine structures of the adrenal cortex of the normal and hydrocortisone-treated adult male mice.

There has been, however, no description of sex and age difference in the ultrastructure of the mouse adrenal cortex. The structure and the function of the adrenal cortex should be studied with consideration to sex and age of animals.

In this study, many striking differences by sex and age were observed in the structure of the mouse adrenal cortex under light and electron microscopes. Furthermore, the X zone firstly described by Masui and Tamura[34)], and Howard-Miller[35)], was studied electron microscopically.

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Received for publication May 29, 1967.

MATERIALS AND METHODS

Animals: In the present investigation, 6 groups of normal SMA mice[36] were used, of both sexes and of the young, adult and old. They were an inbred strain obtained from the center supplying laboratory animals in this medical school. They are shown in Table 1.

TABLE 1

sex→ / age↓	female		male	
	days of age	No. of animals	days of age	No. of animals
young	67～ 70	7	67	6
adult	418～460	5	390～408	6
old	586～646	5	618	6

These mice lived in groups of 5 or 6 in alminium jars at an ambient temperature of 25 ± 1°C and fed with NMF mouse bread (Oriental Yeast Co. Ltd.) and water. General appearances were normal in all cases.

Fixation and Embedding: After decapitation, both adrenal glands were immersed in the fixation solution for less than a minute, rid of the surrounding fat without injuring the capsule, and cut in half with a razor blade. This and continued fixation were made at an ambient temperature of 2°C.

The left adrenal gland was fixed in blood isotonic 1% OsO_4 solution containing 0.045 g sucrose/ml of fixative[37] buffered with acetate-veronal of pH 7.4 for 2 hours, and dehydrated in graded aceton after staining with uranyl acetate saturated in 60% aceton.

The right one was fixed in 2.5% or 6% phosphate-buffered (pH 7.4) glutaraldehyde for 3 or 4 hours, rinsed in phosphate buffer (pH 7.4) for 30 minutes, and then fixed in 1% OsO_4 solution mentioned above for 2 hours, and dehydrated in graded aceton.

All specimens were infiltrated in a 1∶1 mixture of aceton and Epon, and then embedded in Epon (Luft)[33].

Sectioning: The specimens embedded in gelatine capsules were trimmed with a razor blade under a stereomicroscope. The specimen was trimmed in order to show the capsule, all cortical zones and medulla in the top surface.

Sections of 1 to 2 μ in thickness were cut with a Porter-Blum microtome, stained with Giemsa solution for 12 hours, and examined light microscopically.

Thin sections for electron microscopy were carefully cut to keep the continuity from the capsule to the medulla, placed on slit meshes so that all cortical zones were continuously visible in a slit of the mesh, stained with 1% uranyl acetate for $1\frac{1}{2}$ hours, rinsed in 0.005 M citrate buffer (pH 5.5) several times, stained with 20% Raynold's solution[39] with 0.01 N NaOH added, and then rinsed in 0.02 N NaOH and distilled water.

Some of the thin sections were stained with 2% uranyl acetate and the specific solution, the composition of which was: 1.5 g of lead nitrate, 1.5 g of lead acetate, 1.5 g of lead citrate, 3 g of sodium citrate, 24 ml of 4% sodium hydroxide solution, and 130 ml of distilled water.

Electron microscopy: The observations and electron micrographs were made with a Hitachi HU-11 A-type electron microscope manipulated at 50 kV or 75 kV.

Initial magnifications of the electron micrographs were from 430 to 35,000 times.

RESULTS

1. Light microscopic observation

In all specimens, the capsule, cortex and medulla were observed to be apparently normal. Some light microscopic findings of each group are shown in Table 2.

TABLE 2. Light microscopic observation

	capsular cell layers	cell layers			amount of lipid droplet	amount of pigment	thickness of medullary capsule	X zone
		Z. glo.	Z. fas.	Z. ret.				
young females	3~ 4	+~++	++++	—	++++	+	—	++++
young males	4~ 6	+~++	++++	+~++	+++~++++	++	1~ 2	—
adult females	5~ 6	+	++++	++~+++	+++~++++	++~+++	2~ 4	—
adult males	5~ 8	+	++~+++	+	+~++	+	1~ 2	—
old females	5~ 6	+~++	+++	++~+++	++~+++	++~+++	3~10	—
old males	6~10	+~++	+~++	+~++	+~++	+~++	0~ 3	—

cell layers: nothing —, 1~4 +, 5~7 ++, 8~10 +++, 11~ ++++
amount of lipid droplet and amount of pigment: scarce +, moderate ++, abundant +++, quite abundant ++++.

Capsular cell layers in old males were largest in number, consisting of 6 to 12 cell layers, and least in young females. The capsules were generally greater in thickness in the old and in males. This finding is concomitant with the description of Dribben and Wolfe[40].

No noticeable difference was found in the thickness of the glomerular cell layers among all groups.

In contrast to the capsules, the fascicular cell layers in young animals were quite larger in number of cells than in the old, and the age difference was particularly prominent in males. In old males, the fascicular zone was very thin and the fascicular structure could not be distinctly observed, and in addition most of the lipid droplets were visible in the outer part of the cortex, mostly in the glomerular zone.

Lipid droplets in the adrenal cortex of males were observed more abundantly in the young than in the old, although no significant age difference was noticed in females.

Large pigment bodies stained greenish-yellow with Giemsa were observed in the reticular zone, most frequently in old animals.

Only in young females the special fetal zone, the so-called X zone, was well preserved, measuring about one half of the whole cortex at the maximum (Fig. 2). These cells were very similar to those described by Jones[41)-44)] and others[45)46)].

Cortico-medullary connective tissues were especially well developed in the adult and old females compared with any other groups.

2. Electron microscopic observation

(*1*) *Capsules* (Figs. 9–14)

The capsules were composed of flat capsular cells, collagen fibrils, nerve cells and blood vessels (Figs. 9-14). Capsular cell layers varied in the number of cells according to sex and age, as noted above. The fine structure of the capsules examined here was almost conformable to Zelander's description[17)].

Various numbers of "Capsular membrane "(Figs. 9, 11 a, 14 a), termed by Zelander, were seen running almost parallel to the adrenal surface, with occasional connection of two membranes into one in all cases.

The collagenous fibril bundles appeared as weaving very close to the capsular cells or between the capsular membranes (Figs. 9, 10, 11 a, 12, 13). The amount of collagen fibrils was apparently scarce in females, and most abundant in old males. The amount, in general, seemed to be proportional to the thickness of the capsules.

Cytoplasmic organelles in the capsular cells were generally small in amount, especially in the old and in females.

Occasionally adjacent capsular cells were longitudinally attached closely to each other, and there could be often observed desmosome-like structures (Figs. 9, 11 a, 14 a, 14 b). The capsular membrane surrounding a capsular cell group appeared like a basement membrane.

Mitochondria were polygonal in shape and had the typical cristae (Figs. 9, 10, 13, 14 b).

Occasionally a few oval, dense bodies with a double smooth bounding membrane were visible, and they seemed to be the so-called "globules", termed by Zelander, by their size and shape (Figs. 10, 14 b, 36, 42).

A few capsular cells possessed a polygonal shaped osmiophilic homogeneous substance that seemed to be a lipid droplet (Figs. 9, 12).

Small numbers of so-called "pale round bodies", described by Zelander, averaging 410 mμ in diameter, with a dense unit membrane and 10 to 20 regularly ordered annular profiles within it, could be noticed in occasional capsular cells of all cases (Fig. 11 a). Their appearances were of the same frequency as those in the capillary endothelial cells (Fig. 11 b), and fewer in females.

TABLE 3. Electron microscopic observation

zone	organelles ↓	animals→	young males	young females	adult males	adult females	old males	old females
Z. glo. cells	mito-chondria	shape	elongated round	elongated round	round	round	round elongated	elongated round
		size (μ)	0.4~1.5	0.4~1.3	0.4~1.5	0.4~1.5	0.3~1.3	0.3~1.9
		inner struct.	recti. tubular	recti. tubular	tubular recti.	recti. tubular	tubular	recti. tubular
	lipid	size (μ)	0.3~1.6	0.6~1.8	0.7~2.2	0.7~2.1	0.4~2.2	0.7~2.4
	Golgi	develop.	well	well	moderate	moderate	well	moderate
	globule	aver. size (μ)	0.36	0.41	0.62	0.54	0.51	0.50
	vacuole	amount	moderate	a few	moderate	a few	a few	a few
	endopl. ret.	surface	granular agranular	granular agranular	granular agranular	granular agranular	granular agranular	granular agranular
Z. fas. cells	mito-chondria	shape	round	round	round	round	round	round
		size (μ)	0.4~1.9	0.5~1.3	0.4~1.9	0.4~1.7	0.4~1.3	0.4~1.4
		inner struct.	recti. tub. cyclo.	tubular recti.	recti. tubular	tubular	tubular	tubular
	lipid	size (μ)	0.5~2.4	0.7~2.3	0.5~2.4	0.7~2.5	0.3~1.8	0.7~1.9
	Golgi	develop.	moderate	moderate	poor	poor	well	moderate
	globule	aver. size (μ)	0.54	0.43	0.60	0.51	0.44	0.44
	vacuole	amount	moderate	moderate	abundant	moderate	abundant	moderate
	endopl. ret.	surface	agranular	agranular	agranular	agranular	agranular	agranular
Z. ret. cells	mito-chondria	shape	round	(X zone) various	round	round	round	round
		size (μ)	0.3~1.6	0.4~2.6	0.4~1.9	0.4~1.3	0.3~1.4	0.4~1.2
		inner struct.	tubular recti.	lamelli-form cristae	tubular recti.	tubular	tubular	tubular
	lipid	size (μ)	0.4~2.0	few	0.7~2.1	0.7~2.1	0.5~2.1	0.5~1.7
	Golgi	develop.	poor	poor	poor	poor	moderate	moderate
	globule	aver. size (μ)	0.53	0.50	0.54	0.47	0.44	0.55
	vacuole	amount	abundant	abundant	abundant	abundant	moderate	a few
	endopl. ret.	surface	agranular	granular agranular	agranular	agranular	agranular	agranular

(2) *Zona glomerulosa* (Figs. 15-20, 38, 44)

In all cases, the glomerular cells were disposed in clumps, with the capsular ends arched (Fig. 17). They were cuboidal in shape and normally adapted to compose a large round or oval cell-island, surrounded by an epithelial basement membrane (Figs. 17, 18). The glomerular cell was separated from the adjacent epithelial cells by a very narrow space without collagen fibrils, and a few occasional desmosomes could be encountered (Fig. 17). Between the adjacent glomerular cells, no basement membrane could be observed. A few microvilli extended into the periendothelial space (See (6)).

Two kinds of epithelial cells could be distinguished, *i.e.* the light and dark cells (Figs. 16, 17, 19, 25). In the dark cells, large numbers of lipid droplets and polygonal shaped mitochondria were observed, while in the light ones cytoplasmic organelles were poorly developed and the cytoplasmic vacuoles were abundant. The dark cells were generally less frequently seen in this zone than the deeper cortical zones.

From the viewpoint of sex and age, the dark cells were generally more often observed in the old and in males.

Occasionally some flattened cells, which possessed several specific granules the same as those seen in the perivascular cells (See (6)), merged into the glomerular cell columns, and often closely situated at the juxtaposition of the glomerular cells as if they, structurally, composed a part of the column (Figs. 38 a, 38 c). In addition, a few adjacent glomerular cells often contained similar granules (Fig. 38 c). These granules appeared round or oval, occasionally dumb-bell-like in shape, and had a double unit membrane, ranging in minor axis from 0.1 to 0.3 μ (Fig. 38 b).

No noticeable difference was seen in the architecture of the glomerular zone in all groups.

The nuclei with double membranes of the glomerular cells were generally irregular in shape, and located near the center of the cells. Chromatin were spaced close to the marginal zone, and some nuclear pores were visible (Fig. 43). One or two nucleoles could be seen near the nuclear center.

Mitochondria were abundant and varied in shape and size; some were round or oval, while others were elongated (Fig. 44). The elongated forms of mitochondria were characteristic in this zone in young animals, but not in other groups. They were most frequently noticed in the upper portion of the outer glomerular "dome" in young females. Very small mitochondria with dense matrix were often observed in an uppermost cell of the glomerular zone in females (Fig. 16). Two kinds of mitochondria were observed according to their internal structures. One was the rectimembranous type, described by Zelander[17], in which the inner membranes along two adjacent light regions were approximately parallel (Fig. 39 a), and the other the tubular type, described by Sabatini *et al.*[6] and others[5,8,9,11,14,29], in which the reflections of the inner mitochondrial membrane protruded inward (Fig. 39 c). Most mitochondria found in one cell usually had a similar internal structure. Mitochondria of larger size were generally of the rectimembranous type, and the smaller ones the tubular type.

Lipid droplets in this zone showed no essential difference in amount in all groups. Some lipid droplets in adult and old animals were slightly larger in size and sometimes lost their osmiophilic material in a part of the periphery (Fig. 27).

The globules, noted in capsules, were composed of a part of very dense regions and most part of the numerous fine and homogeneous granules (Figs. 36, 42). They were less in number in this zone than in the deeper zones of the cortex, and 2 to 3 in number per cell. Occasionally a few globules had internal vesicular structures (Fig. 40). The globules were various in size, ranging from 0.3 to 0.7 μ in diameter, and often irregular in shape. They were less in number in this zone of the old than in the young.

The Golgi apparatus was generally well-developed in this zone of all animals, particularly in the young. It was situated close to the nuclear membrane, showing the typical appearance of a system of 6 to 9 layered membranes, plentiful associated vesicles and a few vacuoles (Figs. 16, 17).

The cytoplasmic vesicles and vacuoles were less numerously scattered in this zone than in the deeper zones of the cortex. The cytoplasmic vesicles seem to be the vesicular form of the smooth-surfaced (agranular) endoplasmic reticulum.

Considerable amount of rough-surfaced (granular) endoplasmic reticulum was characteristically present at random as the filamentous profiles. Occasionally in all groups, agranular endoplasmic reticulum appeared as the multi lamellar profiles of the small stacks of them (Fig. 22). They appeared almost freely in the cytoplasm, although sometimes mitochondria and lipid droplets were closely bounded by their curved cisternal profiles (Fig. 45).

The cytoplasmic vacuoles, surrounded by a thin membrane, were somewhat more frequently observed in males, and seemingly similar to the Golgi vacuoles, as Lever had suggested[1].

Numerous ribosomes were diffusely dispersed throughout the cytoplasm in the form of polysomes.

The composite osmiophilic formation assumed to be pigment bodies was rarely present in this zone in old animals.

(3) *Zona fasciculata* (Figs. 7, 21-28, 40, 41)

In the fascicular zone, the epithelial cells had abundant microvilli (Figs. 21-23, 25-28).

The nuclei were almost regularly round in shape and their fine structures were similar to those of the preceding zone.

Extremely abundant lipid droplets and mitochondria, in general, were dispersed throughout the cytoplasm, although small amounts of tiny lipid droplets were scattered in old males (Fig. 27). The lipid droplets varied in size, particularly in old animals.

Mitochondria were almost round or oval in shape and varied in size in all groups, and the elongated form was scarce. In young males, occasional mitochondria had the cyclomembranous internal structures (Fig. 39 b), described by Zelander[17] in detail, while most of the mitochondria took the appearance of tubular or rectimembranous form. The rectimembranous type predominated in young animals, while the tubular type was predominant in old ones. In adult animals, the internal structures of the mitochondria were almost rectimembranous in males (Figs. 24, 25), while the tubular types were predominant in females (Fig. 26). The mitochondria of all cells in the deeper fascicular zone were almost of the tubular type.

Lipid droplets were almost uniform in size, and numerously found in young animals and adult females, while those in the other groups varied markedly in size and were less numerous. Irregularly layered membranous structures occasionally appeared in the periphery of lipid droplets (Fig. 27), but they seemed to be artefacts due to glutaraldehyde fixation.

Golgi apparatus, in the fascicular zone, was moderately developed. Compared with those of the glomerular zone, their structures were somewhat incomplete, in which the lack of Golgi vacuoles or decrease in number of Golgi vesicles were seen (Figs. 21, 24, 25, 41). Occasionally Golgi apparatus with complete lack of Golgi vacuoles and vesicles were hardly distinguishable from multi lamellar layered agranular endoplasmic reticulum (Fig. 22), although the multi lamellar profiles were more regularly arranged in the latter.

There were observed some globules, which were in general very similar to those of the preceding zone in their profiles and frequency. No sex and age differences in frequency of the globules were noticed. In adult and old animals, they varied markedly in size, and the large ones were sometimes indistinguishable from the pigment bodies in their appearance (Figs. 27, 40).

Large numbers of cytoplasmic vacuoles were diffusely dispersed throughout the cytoplasm. They were more numerous than those in the glomerular zone, and more abundant in males than females. Occasionally cytoplasmic vacuoles were observed at the juxtaposition of the lipid droplets and mitochondria (Fig. 24).

Extremely numerous agranular endoplasmic reticulum were diffusely observed in the cytoplasm as the vesicular or tubular form (Fig. 24). Occasionally some of them appeared to be like the same multi lamellar profiles noted in the preceding zone. These features, however, were more frequent in this zone. Very rarely in old females there could be observed a strange structure, in which a few mitochondria were closely bounded by concentric multi lamellated agranular endoplasmic reticulum (Fig. 46), very similar to the so-called "large lamellated chondriosphere" described by DeRobertis and Sabatini[5].

Very few granular endoplasmic reticulum were present in this zone.

Quite a number of the ribosomes appeared as polysomes.

Particularly in old males, small numbers of pigment bodies were observed in this zone, which were supposed to belong mostly to the so-called "iron-containing type"[17] (See (4)).

(4) *Zona reticularis* (Figs. 29-33)

The reticular cells were gradually continuous with the deeper fascicular cells, and situated in clusters between the network of blood vessels. The microvilli were somewhat fewer in this zone. The epithelial cellular contact was sometimes intimate, and a few small desmosomes were found.

Particularly in adult and old females, thick medullary capsules were frequently observed in the space between the cortex and the medulla. They seemed to be composed of fibroblasts and collagen fibrils. In some cases of males, the reticularis cells contacted directly with the medullary cells (Fig. 30). Large vessels were sometimes seen running in the space parallel to the medullary surface.

In contrast to the upper zones of the cortex, a single basement membrane was usually visible between the epithelial cells and the capillary endothelium in this zone (Fig. 31). The dark cells were more frequently observed than in the outer zones.

In old males, the total width of the fascicular and reticular zones was extremely small, and furthermore the two zones were almost indistinguishable.

The nuclei were similar to those of the fascicular cells.

Mitochondria were markedly fewer in this zone, particularly in old animals. They were almost round, rarely elongated in shape and quite varied in size. The internal structures of mitochondria were rectimembranous or tubular in males, and almost tubular in females. An open form of mitochondrion[3] was occasionally observed in older animals (Fig. 39c).

Lipid droplets in this zone were generally less numerous and smaller in size, and present near the nuclei.

The frequency of the globules was about two times that in the outer cortical cells, and 5 to 6 per cell. They were markedly varied in size and shape. Occasionally large ones, about 1.2 μ in diameter, were present, and they resembled the pigment bodies mentioned above.

Golgi apparatus was generally ill-developed in this zone, although they were somewhat well-developed in old animals.

The cytoplasmic vacuoles were characteristically numerous in this zone, although they were less numerous in old animals.

Agranular endoplasmic reticulum was quite abundantly dispersed throughout the cytoplasm as the vesicular or tubular form.

Abundant ribosomes were seen as polysomes or free particles.

The most characteristic finding in this zone was the appearance of large amounts of pigment bodies. Two types were encountered as Zelander has described[17]. They were (1) the iron-containing type, composed of extremely dense granules, a composite of intensely osmiophilic regions and light areas (Figs. 48 a, 48 b), and (2) the lipofuscin pigment, composed of arcuate stripes combined with dense granules and light areas (Figs. 49 a, 49 b). Most pigment bodies in females belonged to the former, while the two types were visible in males.

Pigment bodies were generally less in number in females and in the young.

(5) *X zone* (Figs. 8, 34-37)

The X zone abutted directly on the inner fascicular zone. Its inner border met directly the medulla without boundary connective tissues, and, occasionally, irregularly extended into the medulla. A few small desmosomes were sometimes encountered at the portion of the cellular attachment (Fig. 35). In contrast to the fascicular cells, the X zone cells were smaller in size (Figs. 7, 8). A small amount of collagen fibrils was seen in the intercellular space.

Round or oval nuclei were present near the center of the cells.

Large numbers of bizarre shaped mitochondria were observed throughout the cytoplasm. They were round, oval, elongated, club-shaped, ring-shaped or dumb-bell like in shape and of various sizes, ranging from 0.4 to 2.6 μ in diameter (Figs. 34, 35). Many mitochondria were bounded partly by multi lamellated membranes. Most of the mitochondria had lamelliform cristae. It is of great interest that some of the mitochondria had one or more dense homogeneous round bodies in their matrices (Fig. 37). These intramitochondrial bodies were very similar to the cytoplasmic lipid droplets in the fascicular cells, although the former were conspicuously smaller in size and less in density.

Several globules were also visible, and seemed to be the same as those in other cortical epithelial cells (Fig. 36).

It was characteristic that a large amount of granular endoplasmic reticulum could be observed as filamentous or vesicular profiles (Figs. 34, 36).

Numerous agranular vesicles and vacuoles were also visible.

(6) *Periendothelial space*

The vascular channels of the adrenal cortex in mice were covered by a thin walled sheet arranged discontinuously with the endothelial cell. In this discontinuity, approximately 500 Å in width, the outer and inner endothelial plasma membranes appeared to have merged into only a single membrane, in place where the vascular lumen connected with the periendothelial space (Figs. 9, 11 b).

The endothelial cell had a small amount of cytoplasmic organelles near the nucleus; a few polygonal shaped mitochondria with typical cristae, the pale round bodies noted above, globules, Golgi apparatus, centrioles, a few filamentous granular endoplasmic reticulum and considerable amount of ribosomes (Figs. 9, 11 b, 17, 18). The endothelial cells in the glomerular and the fascicular zone had a basement membrane, which was situated closely to the epithelial basement membrane (Figs. 17, 18). Usually a single basement membrane was visible between the endothelial and the epithelial cells in the reticular and X zone (Figs. 31, 34). A widespread space, called here "periendothelial space", similar to Disse's space was formed between the endothelial and the epithelial cells. There were observed abundant microvilli of the epithelial plasma membrane, small amount of collagen fibrils and the so-called perivascular cells, termed by Sheridan and Belt[39], in the periendothelial space.

The perivascular cells possessed small numbers of mitochondria with typical cristae, a few globules, moderate amount of vesicles, filamentous granular endoplasmic reticulum, occasional pigment bodies and considerable amount of specific granules (Fig. 38 b).

These granules were also observed in the upper portion of the glomerular domes as noted in (2) (Figs. 38 a, 38 c). The perivascular cells in the deeper portion of the cortex scarcely possessed these granules. Usually the perivascular cells had no basement membrane. Most pigment bodies observed in the outer zones of the cortex were present in the perivascular cells. Hence, the perivascular cells are presumably macrophages.

Some granules with a limiting membrane, ranging from 0.1 to 0.3 μ, could be seen in the periendothelial space particularly in the reticular zone (Figs. 47 a, 47 b).

Sometimes nerve cells and arterioles were observed in the periendothelial space.

DISCUSSION

Capsule

The interesting sex and age difference was noticed in the thickness of the capsule. The thickening was more prominent in males in each age group.

Dribben and Wolfe[40] reported a proliferation of connective tissue with age in the rat, and Meyers and Charipper[47] also noticed a general thickening of the capsule with age in the hamster.

The thickening of the capsule depends on increase both in amount of collagen fibrils and in number of capsular cell layers with advancing age.

Desmosome like structures were observed in the capsular cells in this study, though Zelander[17] observed no transitional cells between the capsular and the glomerular cells. Yamori *et al.*[21] also noted that no special cells were found excepting fibroblasts in the capsular tissue. Although it is doubtful whether the specific structure observed at the capsular cellular attachment is a true desmosome, it cannot be assumed to be an artefact, since it was visible in most capsules. If it is a desmosome itself, a possible speculation that reserve cells of the glomerular epithelia exist in the capsules may be made. In addition some of the capsular cells possessed occasional lipid droplets, though the internal structures of mitochondria differed from the general appearance of those in the adrenocortical parenchymal cells.

The thickening of the capsule in older animals presumably suggests that the reserve cells in the capsule can not maturate into the glomerular cells and new reserve cells are continuously formed in older animals.

There could also be evidently noticed a thickening of the medullary capsules in old animals, especially in females as Whitehead reported[48]. It may be the result of involution of the X zone cell[49] which is more characteristic and preserved for a longer time in females. The medullary capsule seemed to be composed of fibroblast-like cells and collagen fibrils.

Periendothelial space

The space between the adrenocortical epithelia and the vascular endothelium has been variously termed: "Subendothelial space"[1][2][15][29], "Perisinusoidal space"[50], "Periendothelial space"[17], "Extravascular space"[14], or "Perivascular space"[30].

Many authors reported cells in this space as fibroblasts[17][30], macrophages[29][30] or numbers of the reticuloendothelial system[50]. In the present observation they seemed to be macrophages rather than fibroblasts, because they often apparently possessed secretory granules and pigment bodies.

Numerous microvilli extended into the periendothelial space communicating with the interparenchymal space, as described by many authors. Carr[14] suggested that these filamentous prolongations of the plasma membranes may provide additional surface area through which transport could occur, and that they were more prominent in humans than rats[1].

Small amount of fibrils in this space seemed to be almost collagenous, although Sheridan and Belt[3] interpreted them as reticular or collagen fibrils.

Two basement membranes were noticed in the glomerular and fascicular zones; one on the vascular endothelium and the other on the epithelial cells. A single basement membrane observed in the reticular and X zones seems to belong to the vascular endothelium rather than the epithelial cell.

Rinehart and Farquhar[50] described two basement membranes in the anterior pituitary lobe, and Ekholm *et al.*[51][52] reported two basement membranes in the thyroid, although Lever[1][2] observed merely a single basement membrane in the rat adrenal cortex.

Green and Van Breemen[53] reported the presence of secretory granules in the periendothelial space of the anterior pituitary lobe. In addition, Lever[1][54] observed semiopaque materials filling this space in the hyperactive states of the adrenal cortex and medulla of rats. Zelander[17], however, observed nc material in this space in the mouse adrenal cortex. In the present observation, a few granules that seemed to be secretory granules could be observed in the periendothelial space.

Yeakel[55] noted an enormous vascular dilatation in albino rats of old age.

Meyers and Charipper[47] on the other hand reported the development of areas of hemorrhage in the zona reticularis with increased age in the golden hamster, and Loeb[56] also did so. In the present observation, however, no sex and age difference was noticed both in the vascular lumen and the periendothelial space.

Zonation

Some trials at subdivision of the zona fasciculata have been done. Zelander[17] divided the adrenal cortex of mice into the glomerular, the outer and inner fascicular, and the reticular zones, and suggested that the division of zones is by no means sharply demarcated and the transition between the outer and inner fascicular zone particularly diffuse. Brenner[29] made a similar trial in rhesus monkeys, and Sheridan and Belt[30] in guinea pigs. Sabatini and DeRobertis[6] tried to differentiate the rat adrenal cortex into four zones, the glomerular, the intermediary, the fascicular and the reticular according to the mitochondrial structure, the lipid droplets and the structure of the ground cytoplasm, as Lever[1] did.

Since the changing process from the deeper glomerulosa to the outer fasciculata is very gradual and the transition between the outer and inner fascicular zones is more gradual, subdivision of the fascicular zone seems to be almost impossible under the electron microscope.

Pilgrim[57] noted the occurrence of nodular hyperplasia in the adrenals of senile mice of the C3H strain, as Yeakel[55] reported in albino rats.

In the present observation, the cortical nodule was observed only in a case of adult female.

Blumenthal[58] found a progressive increase in diameter of the gland with age, and noted that the peak actually appeared later in females, and that the adrenals were in general larger in females than males. The present observation supports his description. A decrease in thickness of the cortex in the old was particularly prominent in males, although not so apparent in females.

From the viewpoint of the cell migration theory of Gottschau[59], it may be speculated that the thickening of the adrenal capsule and the thinning of the cortex in old animals result from the maturation disturbance of the reserve cells in the adrenal capsule.

Epithelial cells

Since Hoerr[60] described the "light" and the "dark" cells in the guinea pig adrenal cortex, many workers have discussed on these cells of some species[1)3) 15)17)28)].

In the present study, the dark cells were more frequently observed in the deeper zone of the cortex, and their grade of appearance was higher in the old and in males. The dark cell may probably indicate the charged form of the cortical cell, and, on the contrary, the light one may represent the discharging or discharged form as Lever suggested[3]. But some of the dark cells seemed to be distinctly degenerative cells.

The significance of the proportion of these two kinds of cells among all groups is unknown.

Mitochondria

Since Palade[61] found the special "filamentous" internal structures of mitochondria of the adrenal cortex, mitochondrial internal structures have been observed in various species by many workers: as "tubulo-saccular", "rectimembranous" and "cyclomembranous" in the mouse adrenal cortex (Zelander[17]): as "tubular", "vesicular" and "tubulo-vesicular" (Sabatini and DeRobertis[6]), "tubular (Belt[9]; Belt and Pease[8]) and "saccular" and "filamentous" (Lever[1)3)]) in the rat: as "tubular" (Belt[11]; DeRobertis and Sabatini[5]) and "saccular" and "filamentous" (Lever[1]) in the hamster: as "cristae-form" and "tubular" in the human (Carr[14]): and as "lamelliform", "tubular" and "lamelliform cristae" in the rhesus monkey (Brenner[29]).

In the present study on mice, mitochondrial internal structures were found as almost rectimembranous or tubular in all groups, and occasionally as cyclomembranous in young males. The tubulo vesicular profile of the internal structure is considered to be a profile of the inner mitochondrial tubules. Sabatini and DeRobertis[6], however, suggested that the tubular structure of the glomerulosa underwent a process of disorientation and dilatation of the tubules to form the tubulo-vesicular elements of the intermediary zone, and that in a second stage of differentiation, by fragmentation of the tubules, the vesicular structure of fasciculata was formed. In the kidney of a grasshopper Beams *et al.*[62] reported a tubular component in the mitochondria, and Fawcett[63] also described the same profile in the mitochondria of hepatic cells.

On the other hand Luft and Hechter[12] examined the cow adrenal cortex under hypoxia, and observed the mitochondria showing many vesicles attached to their inner bounding membranes, but after perfusion with oxygenated blood, he found most of the vesicles had disappeared and the mitochondria had displayed the usual form, with cristae.

Belt and Pease[8] reported that in most sites of steroid secretion (adrenal cortex, corpus luteum, theca interna and granulosa of the graafian follicles, interstitial cells of testis and placenta of the rat), the internal structure of the mitochondria was in the form of tubular reflections of the internal mitochondrial membrane, rather than plate-like cristae, and suggested that tubules would furnish a considerably greater surface area per unit volume than would cristae. This interpretation seems interesting and very reasonable.

Luse[15] speculated that the appearance of various type of mitochondria suggested a turnover of them, with constant formation and degeneration of mitochondria independent of aging of the cell.

The elongated forms of mitochondria were observed mainly in the glomerular zone in the present investigation, as reported by many workers[6][15][17][30].

Many observations have been made on the accumulation of dense material within mitochondria[1][3][4][6][13][15][25][28][64][65], and some workers have regarded these materials as intramitochondrial lipid accumulation[1][3][13][15][65]. Lever[1] suggested that an osmiophilic substance was either elaborated by or accumulated within the mitochondria, and the intermediary forms between mitochondria and lipid droplets were discernible. Zelander[17], however, denied any association of mitochondria with lipid droplet. It has not been still resolved whether or not lipid droplets are formed within the mitochondria. On the other hand, Giacomelli *et al.*[28] suggested that amorphous intramitochondrial deposits were possibly aldosterone precursors in the zona glomerulosa of the rat adrenal gland stimulated by sodium restriction.

In the mouse adrenal cortex examined here, such intramitochondrial bodies were frequently visible merely in the X zone of young females. They probably seem to be lipid materials in their density and homogeneous profiles, although they were quite smaller than the cytoplasmic lipid droplets, as many authors suggested.

Openings of mitochondrial unit membranes, described by Lever[3], were extremely rarely observed here particularly in the old.

Mitochondrial shape and internal structure seemed to be considerably varied with sex and age. The sex and age difference was, however, not so apparent, since the variability was remarkable among animals even in the same group and among the cells of an animal.

Jayne[66][67] found light microscopically quite a number of substantial changes including fragmentation and vesiculation in the mitochondria of older rats, but the present light microscopical study revealed no age changes.

Lipid droplets

Whitehead[65] reported that lipid in the adrenal cortex of a female mouse had remained a little longer than that of males of comparable age, and that in both males and females, the lipid became variable in amount and distribution with increasing age, and the peak was at the first five months of life, but from six months onwards the average amount of lipid became less and in some cases almost disappeared. Jayne[66][67] also noted the reduction in amount of cytoplasmic lipids in older rats.

In the present observation, lipid droplets became reduced in amount with age, and were obviously less numerous in males than females.

Variation in the size of lipid droplets in old animals was not so prominent as the features reported in the cortisone treated animals[17], but their variation and decrease in number may show the lower secretory activity with increasing age.

In the mouse adrenal cortex, lipid droplets were generally more abundant in the fascicular zone than the glomerular and reticular zones, except in old males.

The amounts of lipid droplets in the adrenal cortex vary by species, and they are in general more numerous in the mouse[17] and the rat[6] than the guinea pig[30] and the hamster[5].

Some workers tried to classify the lipid droplets according to their shape and structure. However, from the viewpoint of the functional appearance of lipid droplets, the amount and size of them seem to be rather important. Lipid droplets have been often reported as large vacuoles by many workers[15][17][21][28][30]. But they seem to be artefacts. In the preparation dehydrated in ethanol series, lipid droplets are often present as large vacuoles, but they result from the extraction of osmiophilic lipid material. In the fixation of adrenal glands, dehydration should be made as rapidly as possible in aceton series.

Brenner[29] noted that during periods of maximal secretory activity of the adrenocortical cells lipid droplets diminished in volume and often disappeared completely. Deane[69] reported that the waxing and waning of the lipid droplets may be presumed to correspond to the storage and release of steroid precursor molecules.

Sabatini and DeRobertis[6] described in the adult rat that the lipid droplets were larger and irregular in the glomerulosa and smaller in the intermedia. The present author observed the same finding only in old males.

Brenner[29] suggested the intimate relationship between lipid droplets and the tubules of the agranular endoplasmic reticulum might be junctions across which steroid precursor transport could occur. Certainly these appearances were occasionally noticeable in the present investigation.

Golgi apparatus

The ultrastructural finding of the Golgi apparatus observed here almost corresponds to the criteria of Dalton and Felix[70]. They were composed of a system of smooth membranes, several small vesicles and a few large vacuoles, and more developed in the glomerular zone than the deeper zones. These findings are almost identical to the description of some workers[29][30].

Golgi apparatus was more developed in young animals than in the old, while no sex difference of development was observed in the present study.

Lever's speculation[1] that the intracellular sacs (vacuoles) may be Golgi vacuoles can never be supported under electron microscopy, although both look very similar.

Globules

The "globules" appeared as small dense bodies very similar to the "microbodies" described by Rhodin[71], and Rouiller and Bernhard[72]. They have been observed in the adrenocortical cells of various species by many workers under different names, as a "microbody" in the rat[10], as a "dense body" in the guinea pig[30] and the rhesus monkey[29].

Zelander[17] suggested that these bodies were deemed desirable to distinguish from Rhodin's "microbodies" which were provided with a simple boundary membrane and contained finer granules more evenly dispersed in a ground substance of the same density as mitochondrial ground substance. In addition, Brenner[29] speculated that they were probably lysosomes, although their content of acid hydrolase has not yet been measured. On the other hand, Novikoff and Essner[73] suggested that the acid phosphatase positive peribiliary granules might correspond to be Duve's lysosomes. Whether or not the globules are acid phosphatase positive is currently being investigated. Under the process of this study, the globules are probably deemed to be lysosomes.

Belt[10] suggested that in the adrenal cortex the microbody (called here as globule) was a common precursor of lipid droplet and mitochondria.

In the present observation, the transitional form between the globule and lipid droplet could not be noticed, although occasional globules with the internal vesicular structure were considered to be transitional forms of the mitochondria with dense matrix and vesicular internal structure. On the other hand, the large globules were very similar to pigment bodies. Hence, it may be speculated that a small globule becomes larger and transforms into a pigment body.

The globules were more in number in the deeper zones of the cortex than the glomerular zone, and they were generally larger and more variable in size in old animals than in young ones.

Cytoplasmic vacuoles

A large number of cytoplasmic vacuoles were present in the mouse adrenal cortex as reported by many workers, and more numerously in the fascicular and reticular zones than in the glomerular zone.

DeRobertis and Sabatini[5] reported that in some cases of hamsters, the vacuoles could be interpreted as canaliculi communicating with the surface membrane of mitochondria, and that in the deeper cortex of the rat[6], large vesicles and more clear vacuoles were seen near the edge as if their content was diluted, and some of these vacuoles protruded on the surface. Analogous findings were often visible in the present observation, although the significance of these findings is doubtful.

Yamori *et al.*[21] reported an increase of the agranular endoplasmic reticulum and various vacuoles after ACTH administration or formalin stress. Hence, abundant cytoplasmic vacuoles in a cell may represent a cellular discharge after the highest secretory activity of the cortical cells.

Cytoplasmic vacuoles were slightly more numerous in males than females, and most abundant in adult animals in which the secretory activity was supposed to be greatest.

Since they are possibly variable due to the physiological condition of the animals, sex and age difference of cytoplasmic vacuoles under the same functional condition of animals can be hardly obtained.

Endoplasmic reticulum

The frequent appearance of a granular endoplasmic reticulum in the glomerular zone of young animals may represent protein synthesis of a high degree as Palade suggested[74][75].

Christensen and Fawcett[64], and Enders[76] suggested that the cells associated with steroid synthesis had an agranular endoplasmic reticulum. The present author also observed the agranular endoplasmic reticulum abundantly in the deeper zone cells supposed to be associated with steroid synthesis. The agranular endoplasmic reticulum was most abundant in adult animals. It may also be related with the secretory cycle.

Ross *et al.*[31] described an extensive tubular anastomotic network of agranular endoplasmic reticulum in the foetal zone of the human adrenal cortex, and Brenner[29] more recently reported on a similar feature in the adrenal cortex of the rhesus monkey. The agranular endoplasmic reticulum, however, has been generally observed in the form of isolated vesicles in most other species[6][17][30]. Brenner[29] suggested that immersion in glutaraldehyde or glutaraldehyde-formaldehyde mixtures containing 4.5% sucrose always preserved the tubular form of agranular endoplasmic reticulum.

A few stacks of agranular endoplasmic reticulum were observed freely or surrounding lipid droplets or mitochondria in this study, although many authors[29)32)77)] reported similar structures of the granular endoplasmic reticulum. These small stacks of agranular endoplasmic reticulum may become larger and include a few mitochondria or lipid droplets within them. Thus the large lamellated chondriospheres may be formed. The mitochondria in the chondriospheres may degenerate and gradually disappear.

Pigment bodies

At least two types of pigment have been reported in the adrenals light microscopically; a black one believed to be melanin and a yellow one considered to be a lipochrome.

Melanin pigment, as reviewed by Bourne[78)], has been found in the adrenals of various mammalian species. On the other hand Findley[79)] reported that the lipochrome pigment was present in largest amount in the zona reticularis cells and the amount increased with age in humans. Dribben and Wolfe[40)] also found the pigments more abundantly in older animals.

The pigment bodies observed here were also classified into two types electron microscopically.

One belonged to the so-called lipofuscin type and the other the iron-containing type. They seemed to completely correspond to Zelander's criteria[17)]. The iron-containing type of pigment seems to be identical with the yellow lipochrome, however the lipofuscin type of pigment distinctly differs from the melanin pigment in structure.

Also in the present observation the pigment bodies were more abundant in males and in the old. The majority of the pigments in females was of the iron-containing type, while in males the lipofuscin type was predominantly noticed. The significance of the distribution of the pigments is unknown. Probably they may be related with the secretory function of sexual hormones.

SUMMARY

Sex and age differences of the mouse adrenal cortex were examined, light and electron microscopically.

1. *Capsules*: Capsular cell layers were largest in number in old males, and least in young females. In general, the capsules were greater in thickness in the old and in males. The amount of collagen fibrils was almost proportional to the thickness of the capsules. Cytoplasmic organelles in the capsular cells were smaller in amount in the old and in females.

2. *Z. glomerulosa*: The glomerular cell layers showed no noticeable sex and age difference in thickness. The elongated mitochondria were more frequently

observed in young animals than in the old. The very small mitochondria with dense matrix were mainly observed in females. The Golgi apparatus was particularly well-developed in young animals. The globules were larger in size in the old than in the young. The cytoplasmic vacuoles were slightly more frequently seen in males.

3. *Z. fasciculata*: The fascicular cell layers in young animals were definitely larger in number than in the old, and the age difference was particularly prominent in males. Lipid droplets were more abundantly observed in the young than in the old in males, although, in females, no significant age difference was noticed. Lipid droplets, in general, varied in size in old animals. Occasional mitochondria in young males had cyclomembranous internal structures. Rectimembranous mitochondria predominated in the young. Generally in females the tubular type was predominant. The Golgi apparatus in this zone was well-developed in old males only. The cytoplasmic vacuoles were more abundant in males than in females.

4. *Z. reticularis*: Considerable layers of medullary capsules were observed particularly in adult and old females, while, in males, the reticular cells almost directly contacted with the medulla. X zone was visible merely in young females. In old males, the total width of the fascicular and reticular zones was extremely narrow. The internal structures of mitochondria were the cyclomembranous or the rectimembranous profiles in young males, the rectimembranous or the tubular in adult males, and almost tubular in the other groups. The Golgi apparatus in this zone was more developed in the old than in the young. The pigment bodies were more abundant in males than in females. The majority of the pigment bodies in females was of the iron-containing type, while in males the lipofuscin type was predominantly noticed.

5. Two kinds of epithelial cells were observed in all cortical zones, *i.e.* the light and the dark cells. The dark cells were more frequently observed in males and in older animals.

ACKNOWLEDGEMENT

The author wishes to sincerely thank to Prof. H. Tauchi for his kind guidance and advice, to Dr. S. Kozuka and Dr. M. Hoshino for their helpful suggestions, and to Mr. J. Aoki and Mr. T. Nakashima for their excellent technical assistance.

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Abbreviations

aER. agranular endoplasmic reticulum
B. basement membrane
CC. capsular cell
CE. capillary endothelial cell
CF. collagen fibril
CL. capillary lumen
CM. capsular membrane
D. desmosome
enB. endothelial basement membrane
epB. epithelial basement membrane
G. Golgi apparatus
gER. granular endoplasmic reticulum
Gl. globule
L. lipid droplet
M. mitochondria
Med. medulla
mv. microvilli
N. nucleus
ner. nerve
NP. nuclear pore
P. polysome
PB. pigment body
PES. periendothelial space
PM. plasma membrane
PRB. pale round body
PVC. perivascular cell
R. red blood cell
Va. vacuole
Ve. vesicle

EXPLANATION OF FIGURES

FIG. 1. Young male (67 days of age). Light microscopic picture of the whole cortex. The medulla can be seen in upper right. Note a large amount of lipids in the fascicular zone. ×130.

FIG. 2. Young female (67 days of age). X zone occupy almost half of the cortex in the right. No zona reticularis can be seen. ×130.

FIG. 3. Adult male (403 days of age). Note the thickening of the capsule and thinning of the cortex. ×130.

FIG. 4. Adult female (418 day of age). A large amount of lipids can be seen. ×130.

FIG. 5. Old male (618 days of age). The thickening of the capsule and thinning of the cortex are quite prominent. Most lipids can be seen in the uppermost portion of the cortex. ×130.

FIG. 6. Old female (627 days of age). Note the medullary capsule in upper right. ×130.

FIG. 7. Young female (70 days of age). Survey view of zona fasciculata. Cytoplasm is characterized by numerous lipid droplets and mitochondria. ×2,100.

FIG. 8. Young female (70 days of age). Survey view of X zone. Note bizarre shaped mitochondria and some intramitochondrial bodies. The medulla can be seen in upper right. ×2,100.

FIG. 9. Young female (67 days of age). Capsule (radially sectioned). A few capsular membranes are present. Arrows indicate desmosome-like structures. Some mitochondria and collagen fibril bundles (transversely sectioned) are also shown. ×9,000.

FIG. 10. Old male (618 days of age). Capsule. Collagen fibrils show the striation with typical periodicity. Golgi apparatus can be seen in upper right. ×12,400.

FIG. 11 a. Young male (67 days of age). Capsule. A pale round body (PRB) in the capsular cell. ×18,000.

FIG. 11 b. Young male (67 days of age). Capsule. A pale round body in the endothelial cell. Note the discontinuity of the endothelial cell (annular pore) in upper left (arrow). ×12,400.

FIG. 12. Adult male (403 days of age). Capsule. Three lipid droplets are present. ×36,000.

FIG. 13. Old male (586 days of age). Capsule. Pigment bodies in the cytoplasm. ×18,000.

FIG. 14 a. Young female (67 days of age). Capsule. Note a desmosome-like structure (arrow). ×18,000.

FIG. 14 b. Young male (67 days of age). Capsule. A double plasma membrane and a desmosome-like structure can be seen. ×36,000.

FIG. 15. Young male (67 days of age). Glomerulosa cell. Numerous lipid droplets and mitochondria are seen. Mitochondria have the tubular internal structures. ×9,000.

FIG. 16. Young female (67 days of age). Glomerulosa cell. Elongated mitochondria are characteristic. Golgi apparatus is well developed. ×9,000.

FIG. 17. Adult male (403 days of age). Glomerulosa cell. The outermost part of glomerular cell column is arched. Two basement membranes can be seen in the periendothelial space. Note a few granular endoplasmic reticulum. ×9,000.

FIG. 18. Adult female (418 days of age). Glomerulosa cell. Numerous rectimembranous mitochondria in the upper cell. ×9,000.

FIG. 19. Old male (618 days of age). Glomerulosa cell. The dark cell (in the left) possesses numerous mitochondria and some lipid droplets. The light cell (in the right) has fewer mitochondria and no lipid droplets. ×9,000.

FIG. 20. Old female (627 days of age). Capsule and glomerulosa cell. Large lipid droplets and various sized mitochondria are present in a glomerulosa cell (right). A few globules also can be seen. ×9,000.

FIG. 21. Young male (67 days of age). Outer fasciculata cell. A few microvilli can be seen in the upper part. Mitochondria have tubular internal structures. ×9,000.

FIG. 22. Young male (67 days of age). Inner fasciculata cell. Lipid droplets are somewhat fewer than in the outer fasciculata cell (Fig. 21). Several stacks of agranular endoplasmic reticulum can be seen at the center. Numerous microvilli are noticed in lower right. ×9,000.

FIG. 23. Young female (70 days of age). Inner fasciculata cell. Varied size of lipid droplets are seen. Note numerous agranular vesicles. ×9,000.

FIG. 24. Adult male (403 days of age). Outer fasciculata cell. Tubular or rectimembranous mitochondria are abundantly seen. Note lack of Golgi vacuoles. Tubular form of agranular endoplasmic reticulum can be seen in upper left. Numerous cytoplasmic vacuoles are present close to lipid droplets in the right. ×9,000.

FIG. 25. Adult male (403 days of age). Inner fasciculata cell. The light (left) and the dark (lower right) cells are distinguishable Numerous vesicles and vacuoles can be seen in the light cell, but not in the dark one. Numerous microvilli extend into the periendothelial space (upper right). ×9,000.

FIG. 26. Adult female (418 days of age). Fasciculata cell. Tubular mitochondria and large lipid droplets are abundantly seen. Polysomes are dispersed throughout the cytoplasm. ×13,500.

FIG. 27. Old male (618 days of age). Fasciculata cell. Note tiny lipid droplets and large globules resembling pigment bodies. Numerous vesicles and incomplete Golgi apparatus also can be seen. ×9,000.

FIG. 28. Old female (627 days of age). Fasciculata cell. Note numerous microvilli. A perivascular cell is present in lower center. Numerous vesicles and vacuoles can be seen. ×9,000.

FIG. 29. Young male (67 days of age). Reticularis cell. Note tubular mitochondria, two globules and agranular vesicles. Golgi apparatus is small and somewhat incomplete. A lipofuscin type of pigment (upper right) and collagen fibrils (lower right) are visible. A perivascular cell (center) has mitochondria with typical cristae and filamentous granular endoplasmic reticulum. ×9,000.

FIG. 30. Adult male (403 days of age). Reticularis-Medulla junction. Large rectimembranous mitochondria and small lipid droplets are present. Numerous small vesicles and large vacuoles can be seen. In lower left, a medullary cell is characterized by some cathecolamine granules. ×13,500.

FIG. 31. Adult female (418 days of age). Reticularis cell. Note only a single basement membrane in the periendothelial space (upper right). A few tubular mitochondria, no lipid droplets, many large globules, small vesicles and incomplete Golgi apparatus are characteristic. ×9,000.

FIG. 32. Old male (618 days of age). Reticularis-medulla junction. Note quite well developed Golgi apparatus. A tangentially sectioned nucleus (left) has several pores. ×9,000.

FIG. 33. Old female (646 days of age). Reticularis cell. Some elongated mitochondria and large globules are seen. Microvilli are somewhat fewer than in the fascicular zone. Golgi apparatus (lower left) is small in size. ×9,000.

FIG. 34. Young female (67 days of age). X zone cell. A single basement membrane in the periendothelial space, many bizarre shaped mitochondria, filamentous granular endoplasmic reticulum and numerous agranular vesicles and vacuoles are characteristic. Cellular contact is sometimes very intimate (arrow). ×9,000.

FIG. 35. Young female (67 days of age). X zone cell. Mitochondrion shows a round, elongated, ring-shaped or dumb-bell-like form. Cellular contact is very intimate (right). ×27,000.

FIG. 36. Young female (67 days of age). X zone cell. A globule bounded by a double unit membrane can be seen in lower left. Filamentous granular endoplasmic

reticulum is characteristic. ×36,000.

FIG. 37. Young female (67 days of age). X zone cell. Intramitochondrial bodies can be seen in three mitochondria. ×40,000.

FIG. 38 a. Young female (67 days of age). Capsule-glomerulosa junction. Small dense granules can be seen both in a perivascular cell and a glomerulosa cell. ×9,000.

FIG. 38 b. Young female (67 days of age). Zona glomerulosa. High magnification of a perivascular cell. Specific dense granules are bounded by double membranes. ×63,000.

FIG. 38 c. Young female (67 days of age). Glomerulosa cell. An uppermost glomerulosa cell has small dense granules. ×9,000.

FIG. 39 a. Young male (67 days of age). Fasciculata cell. Rectimembranous mitochondria. ×18,000.

FIG. 39 b. Young male (67 days of age). Fasciculata cell. Cyclomembranous mitochondria. ×18,000.

FIG. 39 c. Old female (627 days of age). Fasciculata cell. Tubular or open form of mitochondria can be seen. ×17,600.

FIG. 40. Adult male (403 days of age). Fasciculata cell. A globule with internal vesicular structure can be seen (left). The other globules (right) are very similar to pigment bodies. ×9,000.

FIG. 41. Adult male (403 days of age).. Fasciculata cell. Some incomplete Golgi apparatus, small stacks of agranular endoplasmic reticulum (upper right) and tubular agranular endoplasmic reticulum can be seen. ×13,500.

FIG. 42. Adult female (418 days of age). Fasciculata cell. Globules are bounded by double membranes, and composed of some homogeneous dense regions and many fine granules. ×36,000.

FIG. 43. Old female (627 days of age). Glomerulosa cell. Many nuclear pores can be noticed. ×16,000.

FIG. 44. Young male (67 days of age). Glomerulosa cell. Some elongated mitochondria and granular endoplasmic reticulum are characteristic. ×13,500.

FIG. 45. Old female (627 days of age). Fasciculata cell. A lipid droplet (center) is partly bounded by highly ordered stacks of agranular endoplasmic reticulum which show junctions between the cisternae of the stacks and the mitochondrial unit membrane (arrow). ×44,300.

FIG. 46. Old female (627 days of age). Reticularis cell. The so-called "large lamellated chondriosphere". Two mitochondria, bounded by multi concentric agranular endoplasmic reticulum, are shown. ×17,600.

FIG. 47 a. Young male (67 days of age). Zona reticularis. Several granules (left) can be seen in the periendothelial space. One is in the capillary lumen (arrow). ×9,000.

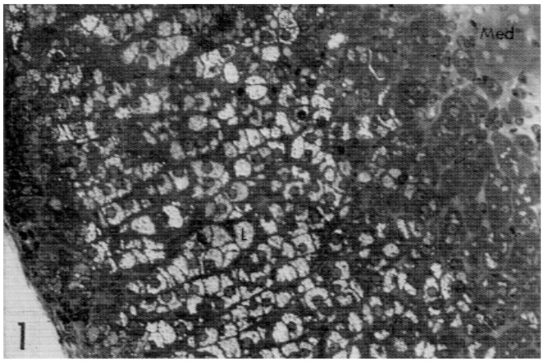
FIG. 47 b. Young female (67 days of age). X zone. Several granules same as in Fig. 47 a can be seen in the periendothelial space. ×9,000.

FIG. 48 a. Old female (627 days of age). Reticularis cell. The so-called "iron-containing type" of pigment. ×9,000.

FIG. 48 b. Adult female (418 days of age). Reticularis cell. High magnification of the "iron containing type" of pigment. ×38,700.

FIG. 49 a. Adult male (403 days of age). Reticularis cell. The so-called "lipofuscin type" of pigment. ×9,000.

FIG. 49 b. Adult male (403 days of age). Reticularis cell. High magnification of the "lipofuscin type" of pigment. ×36,000.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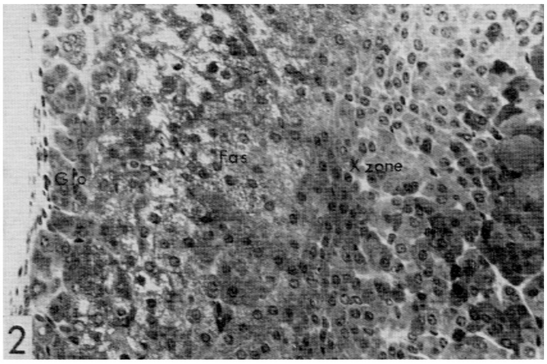
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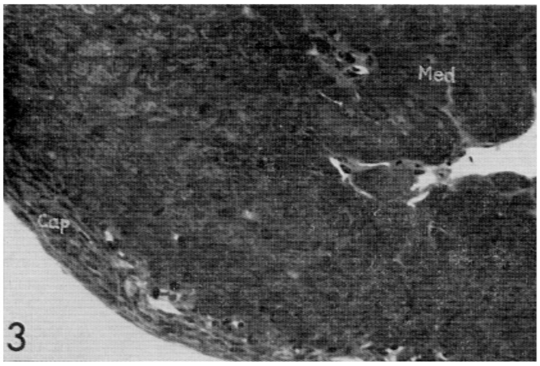
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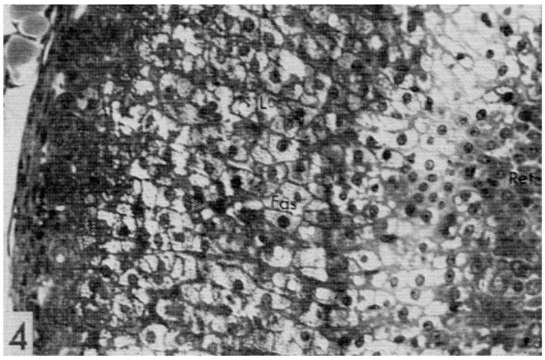
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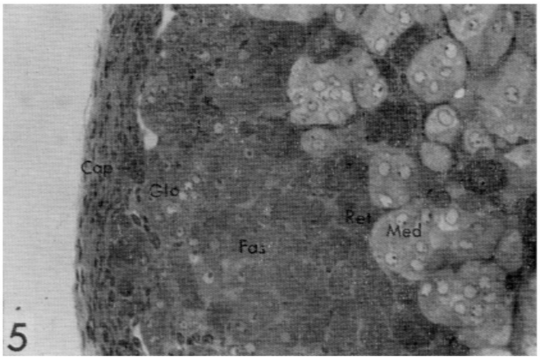
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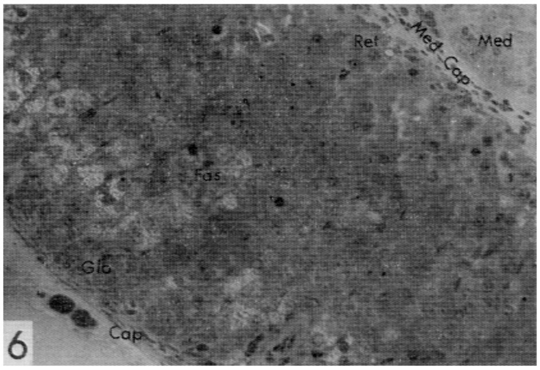
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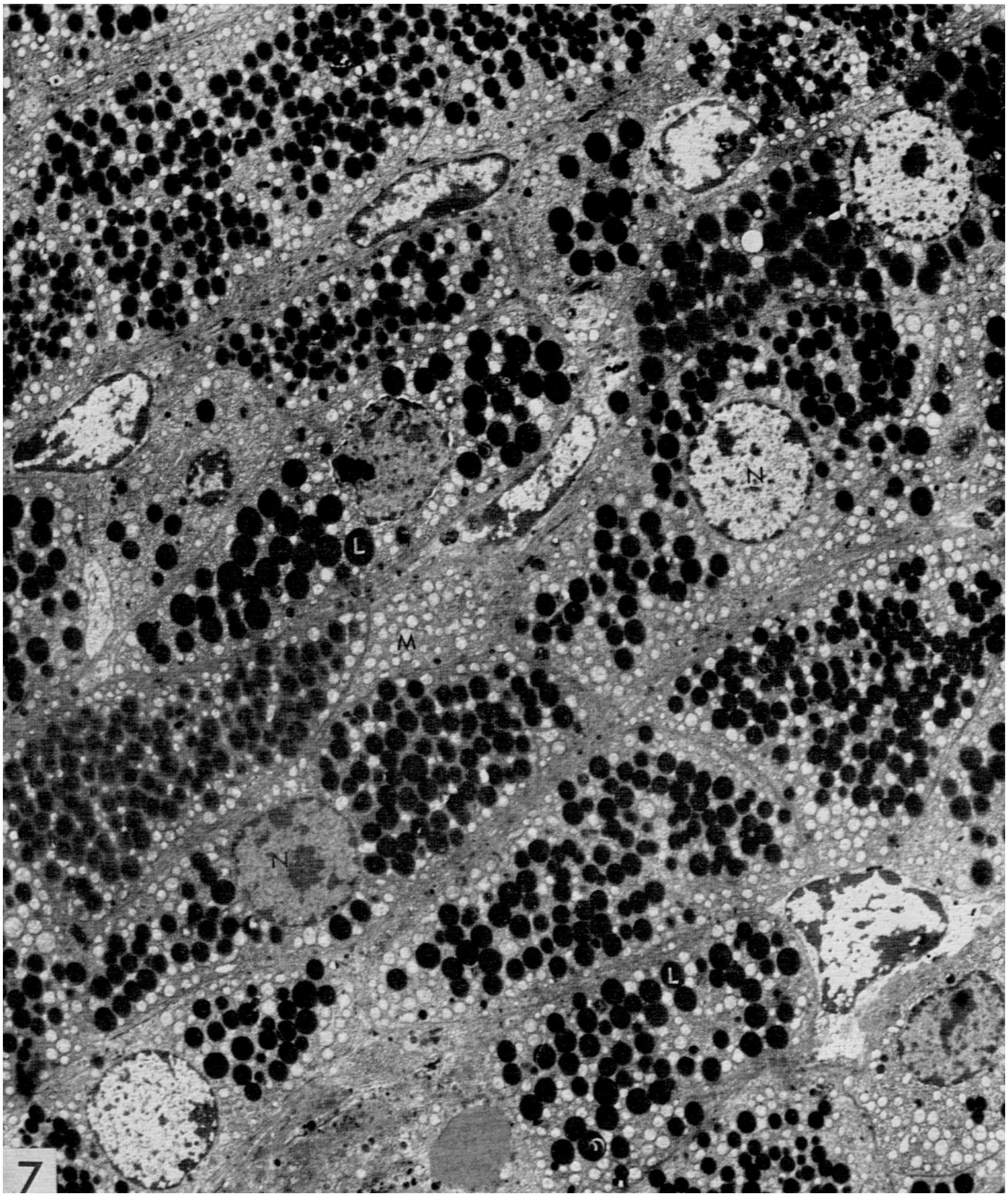
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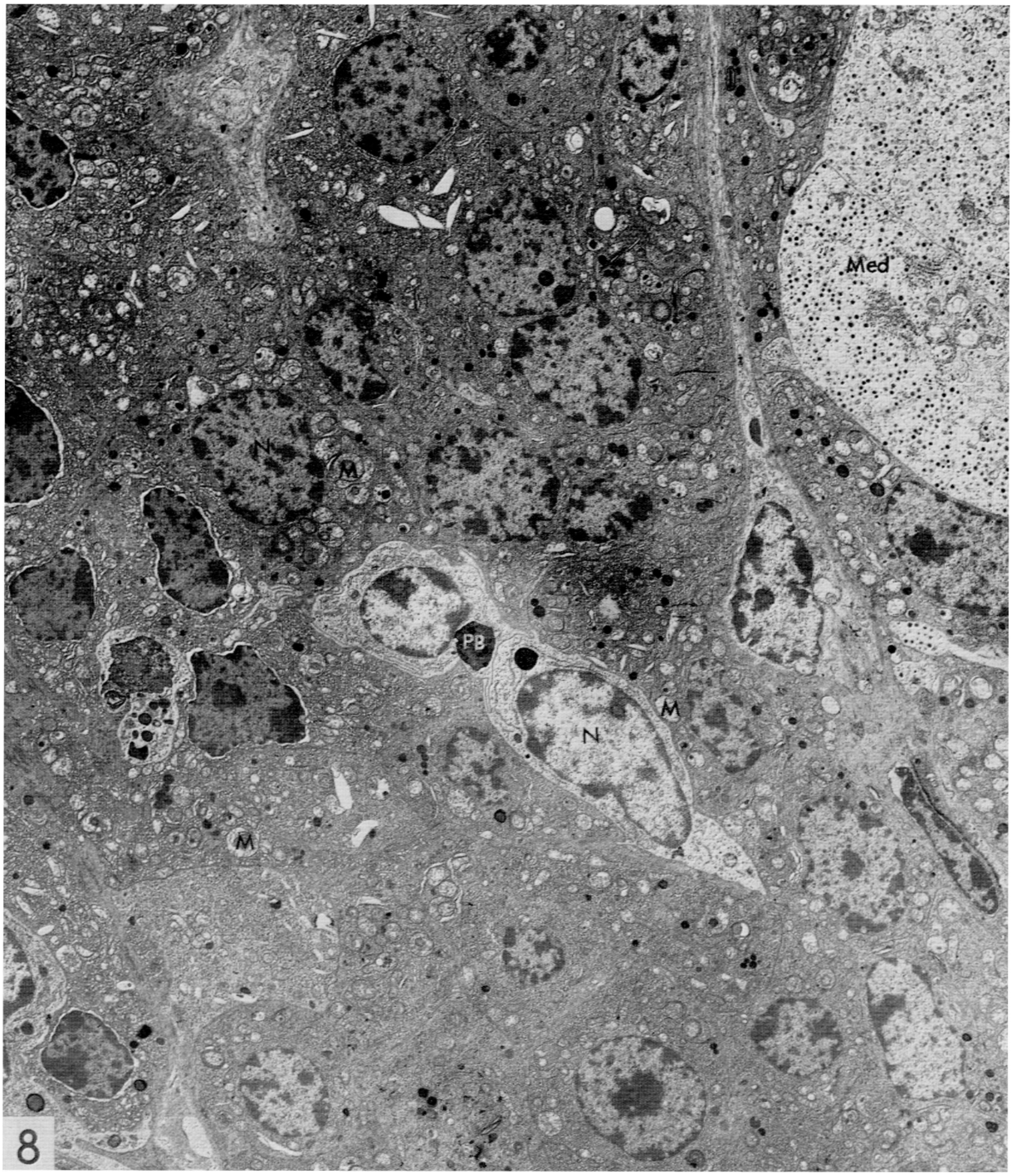
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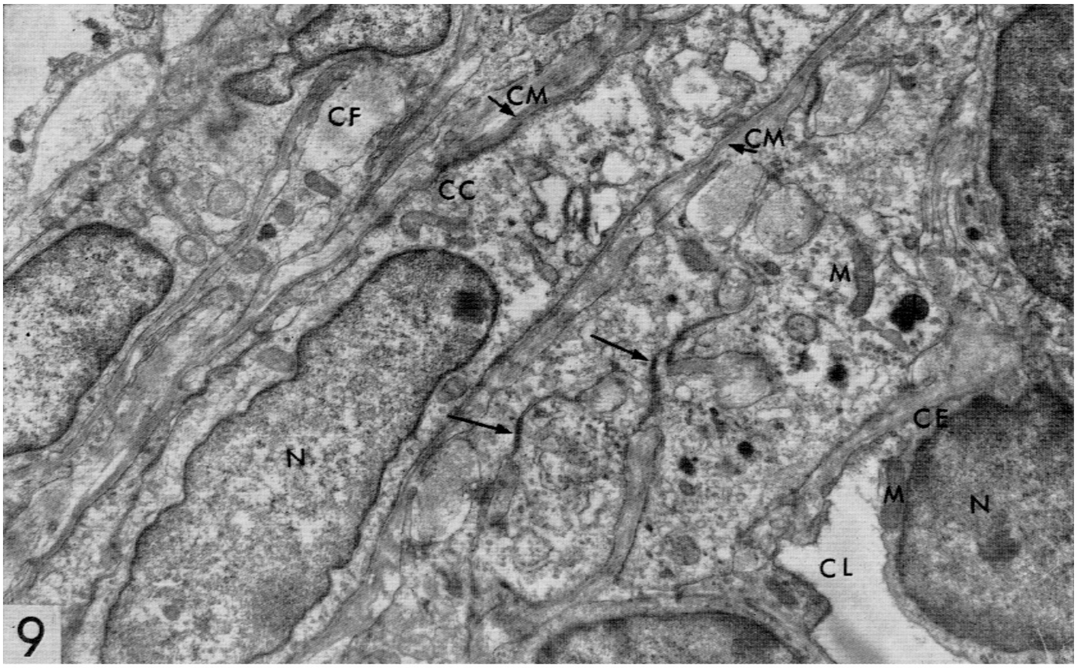

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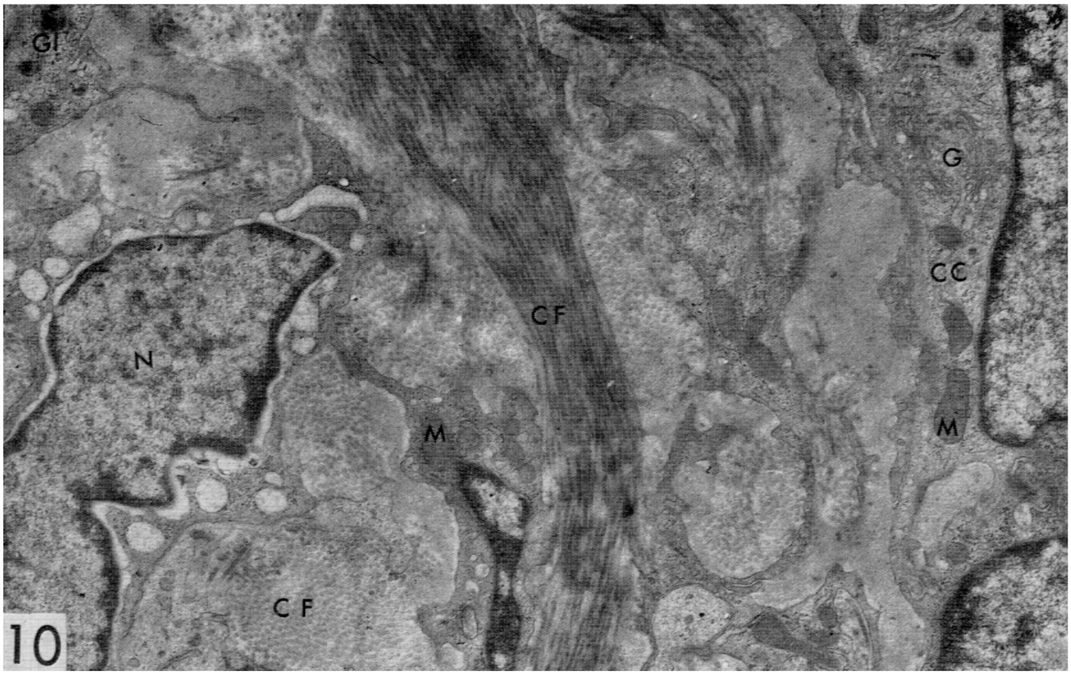

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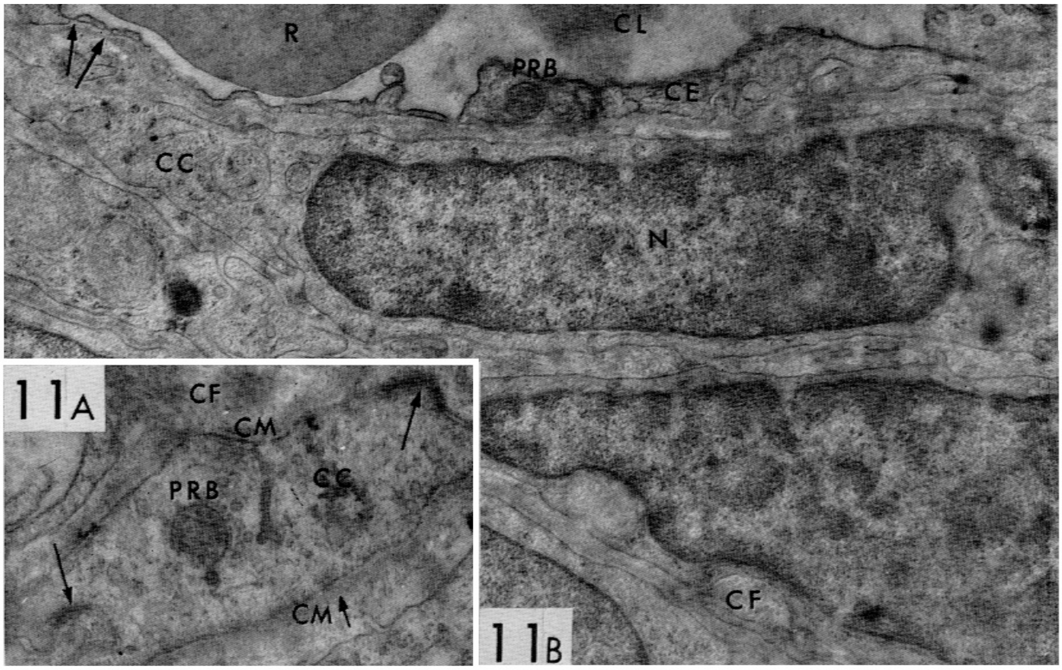
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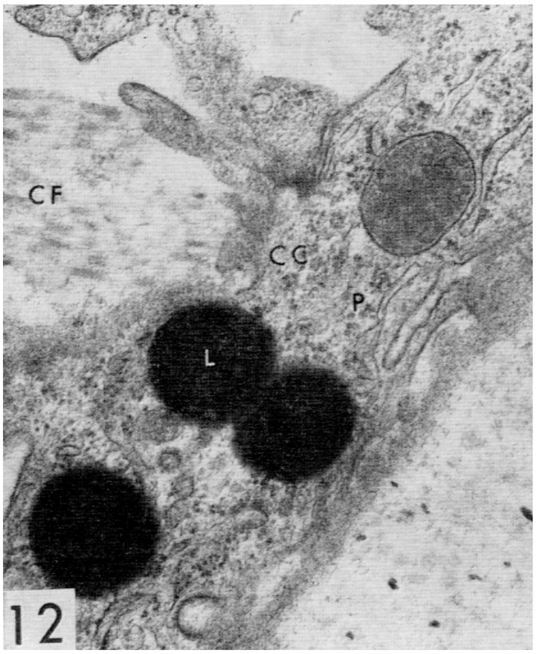
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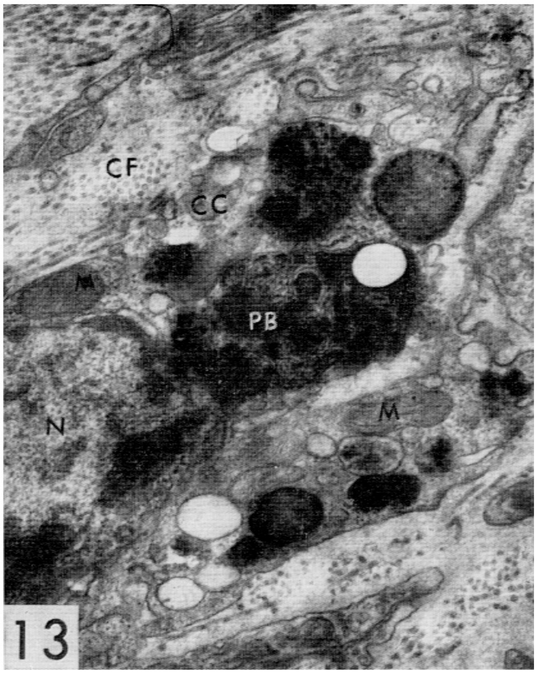
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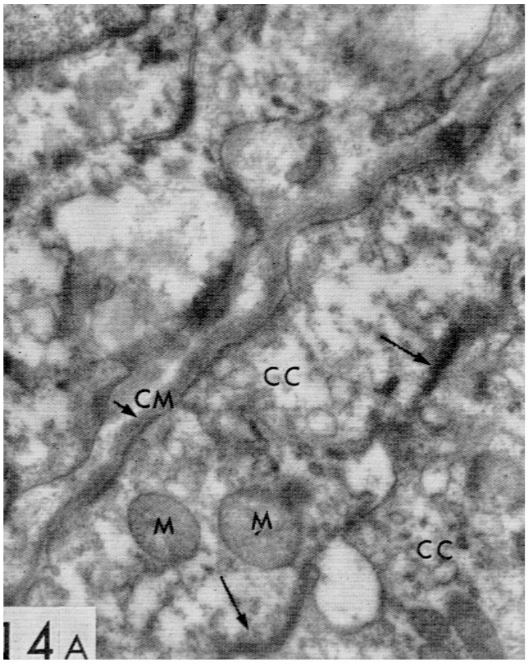
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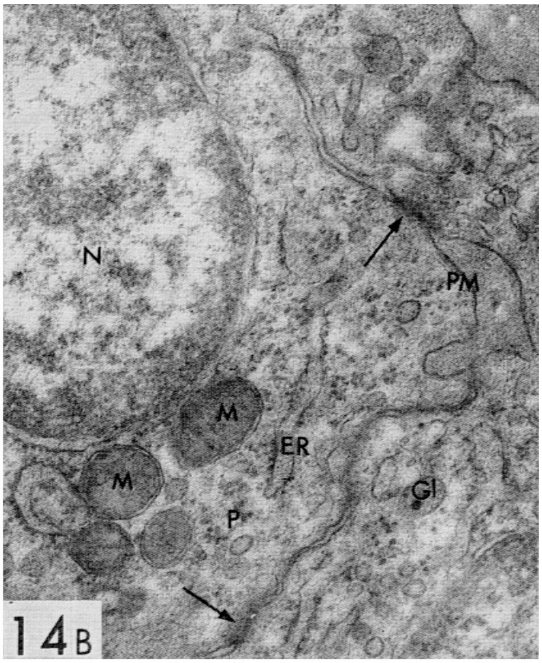
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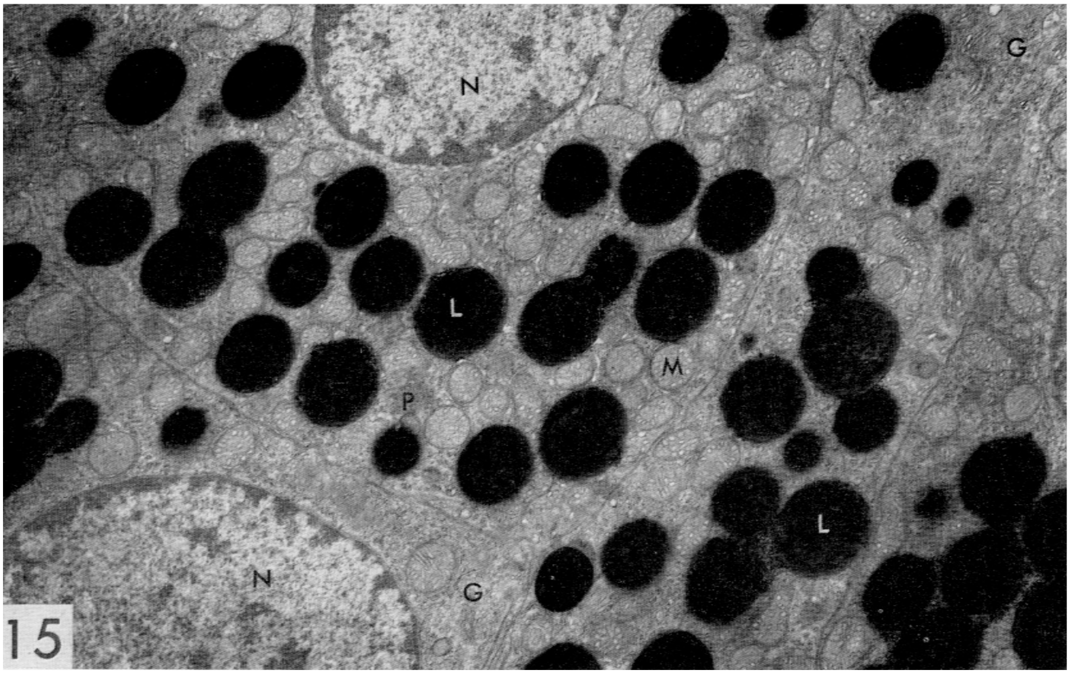
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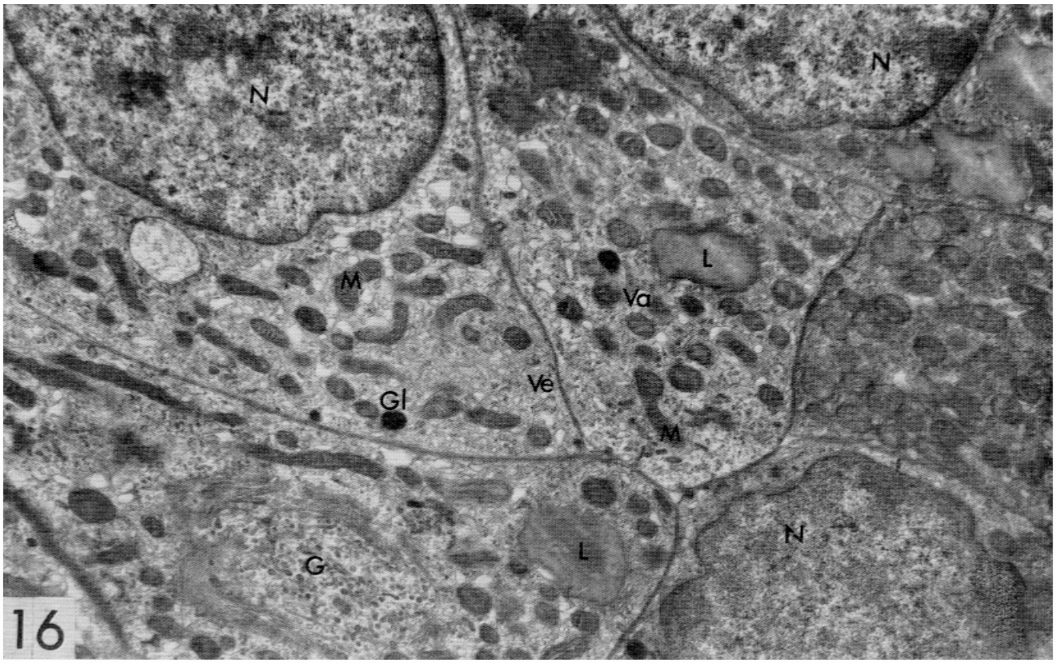
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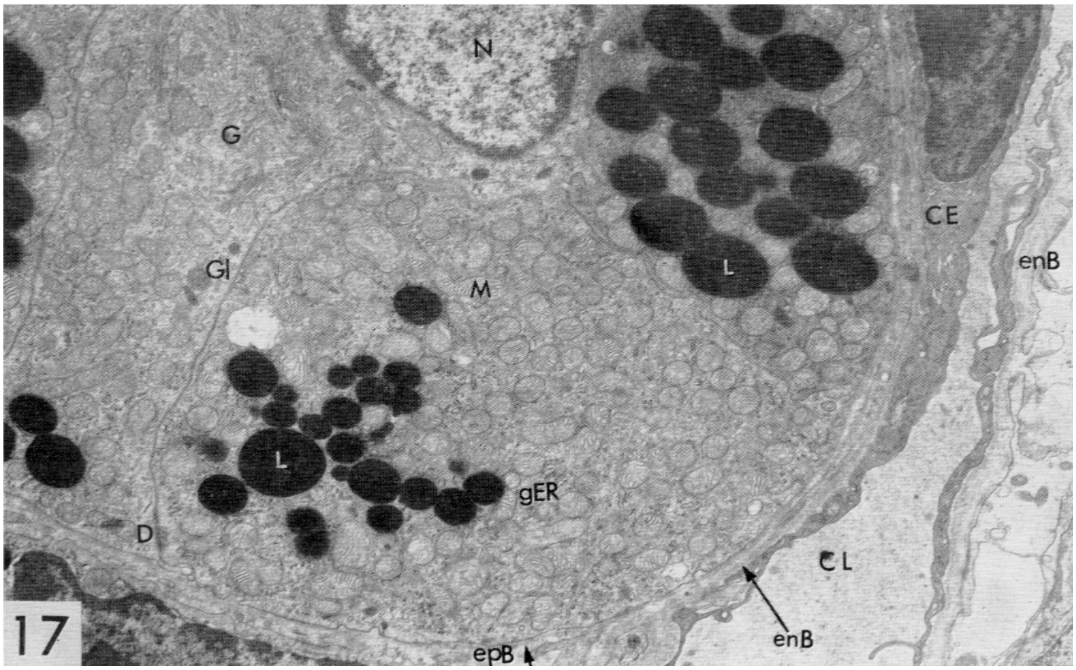
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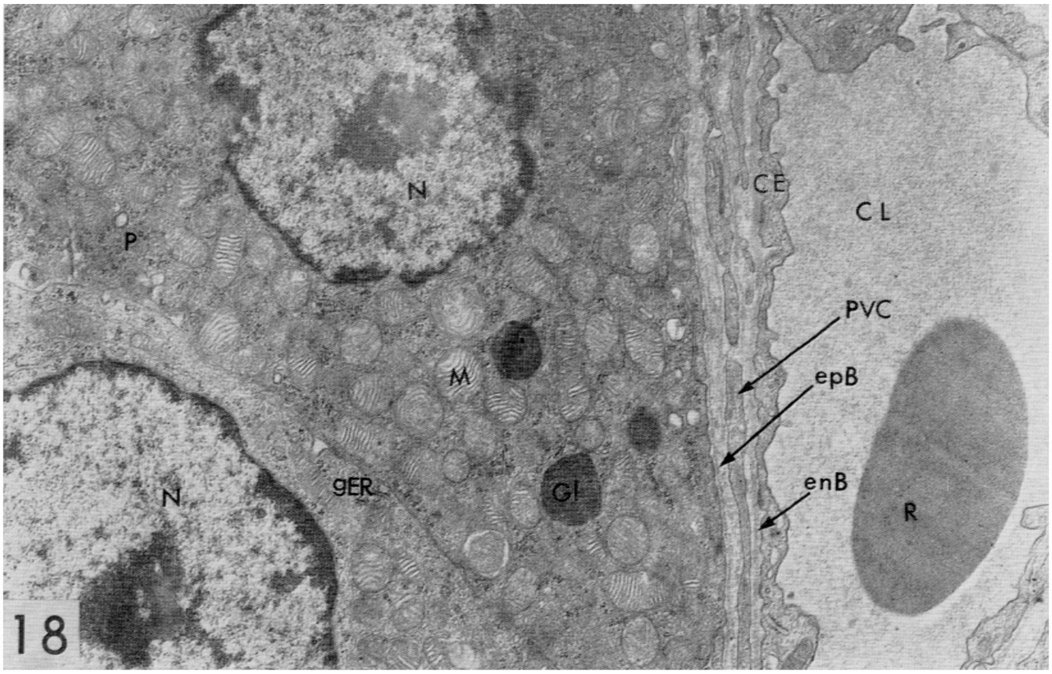

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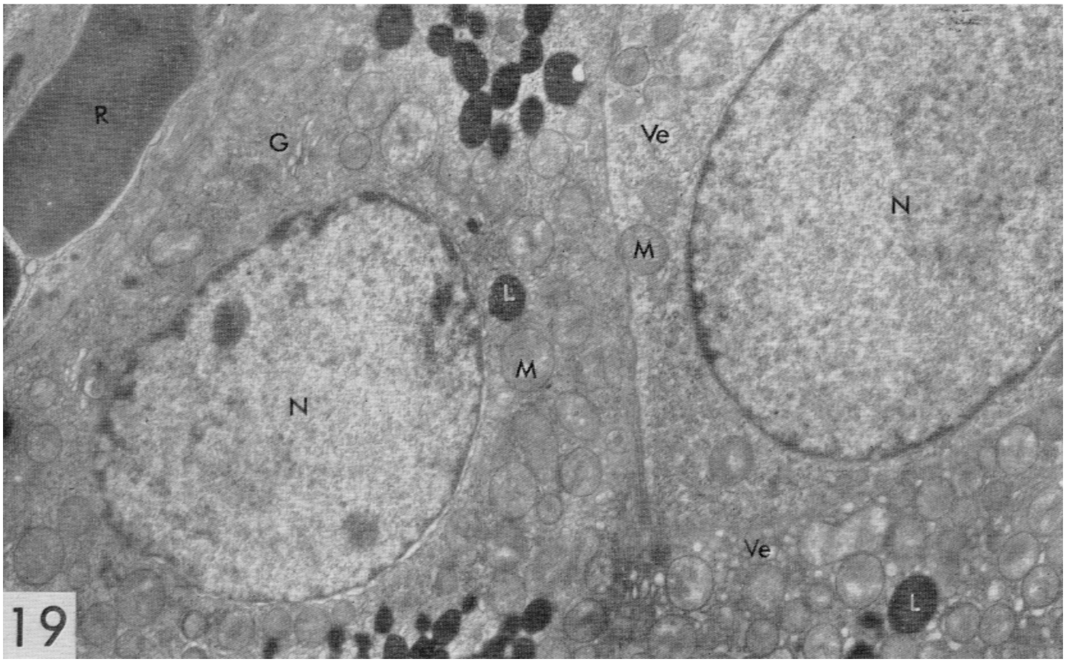

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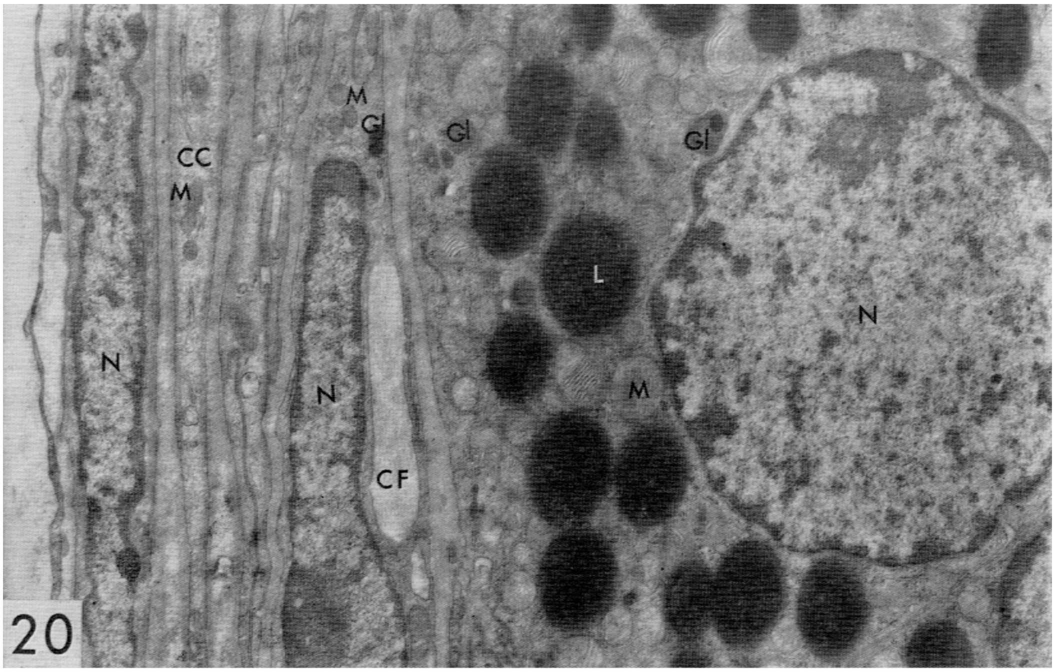
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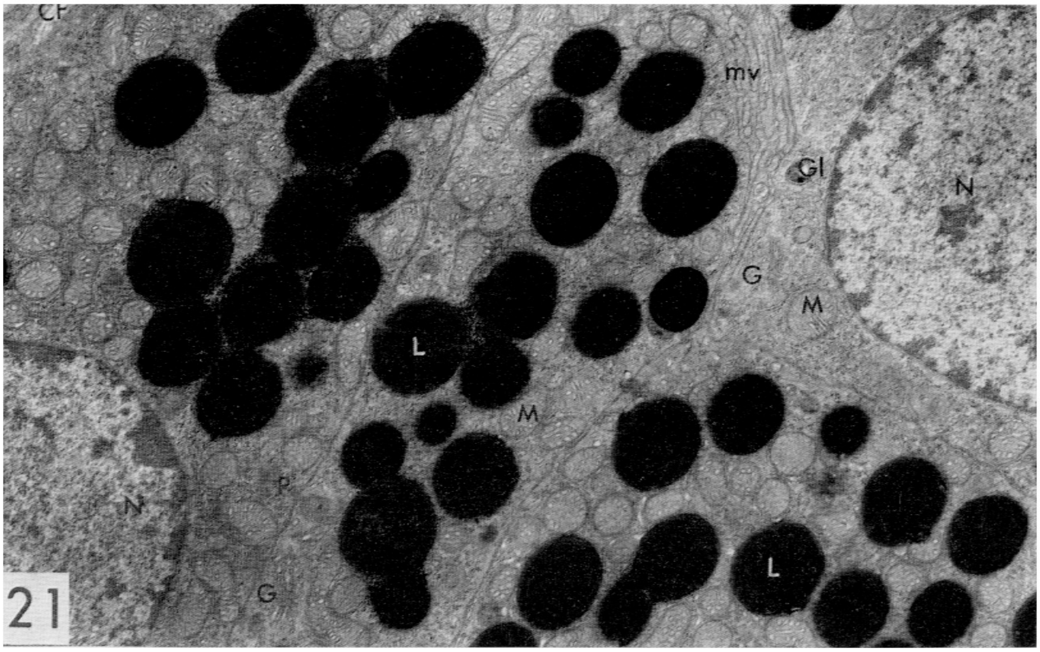
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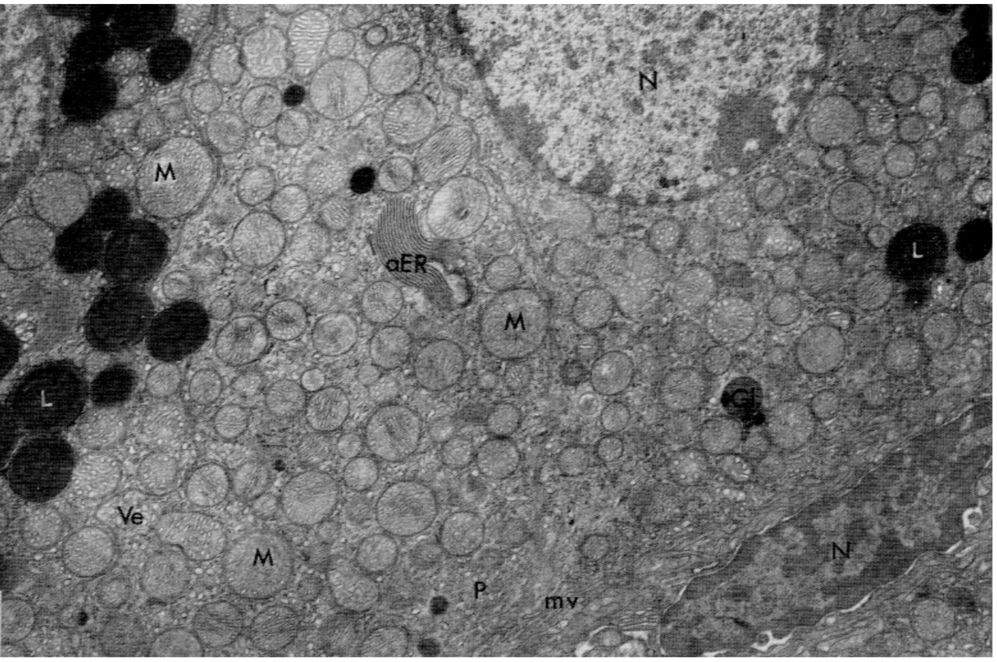

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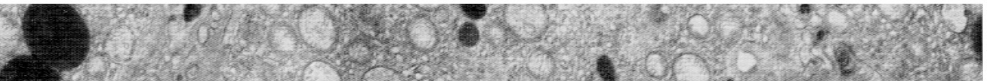

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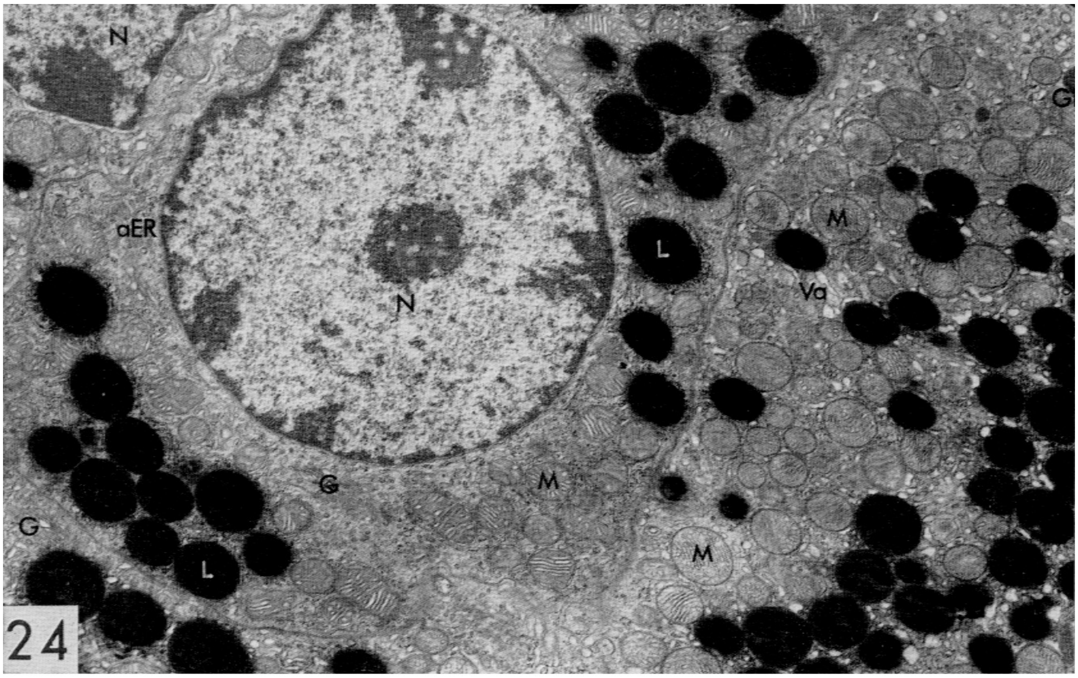

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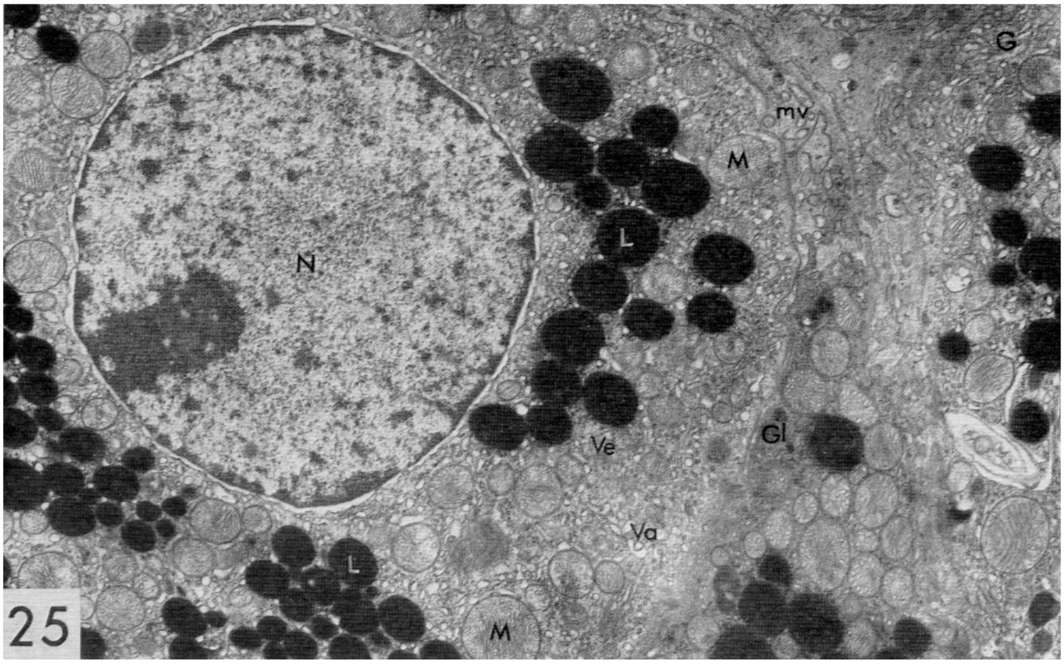

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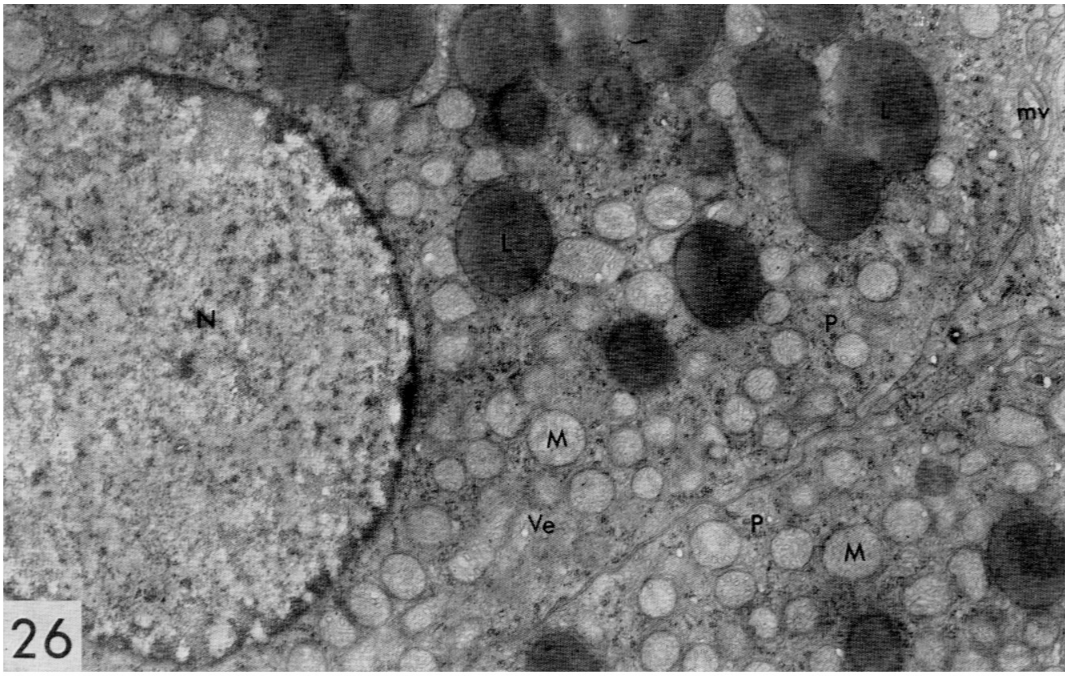
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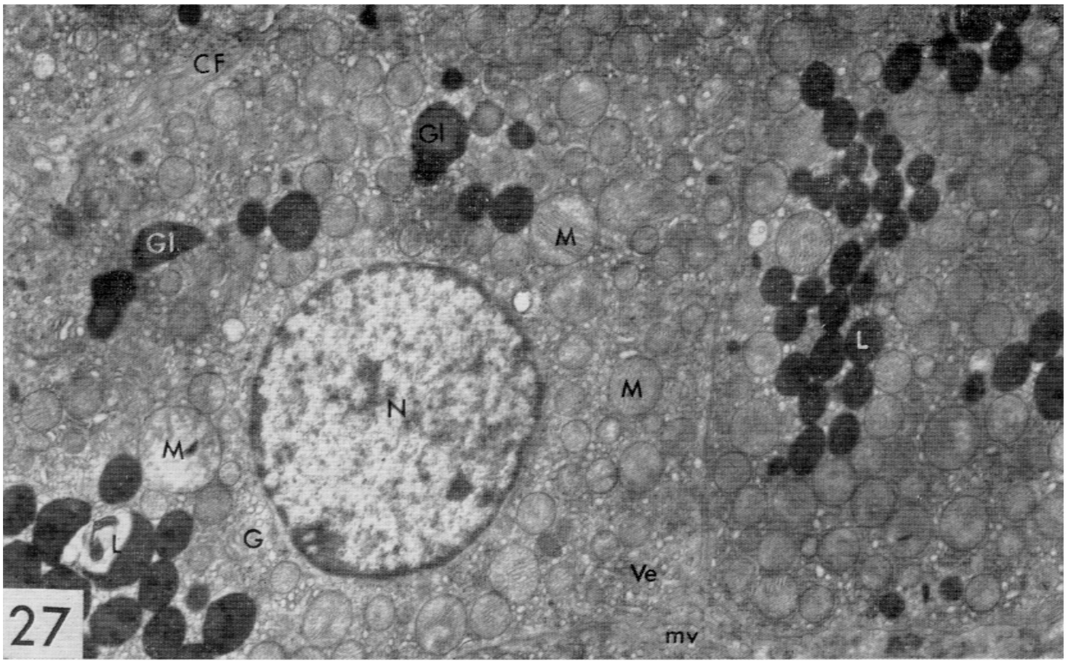
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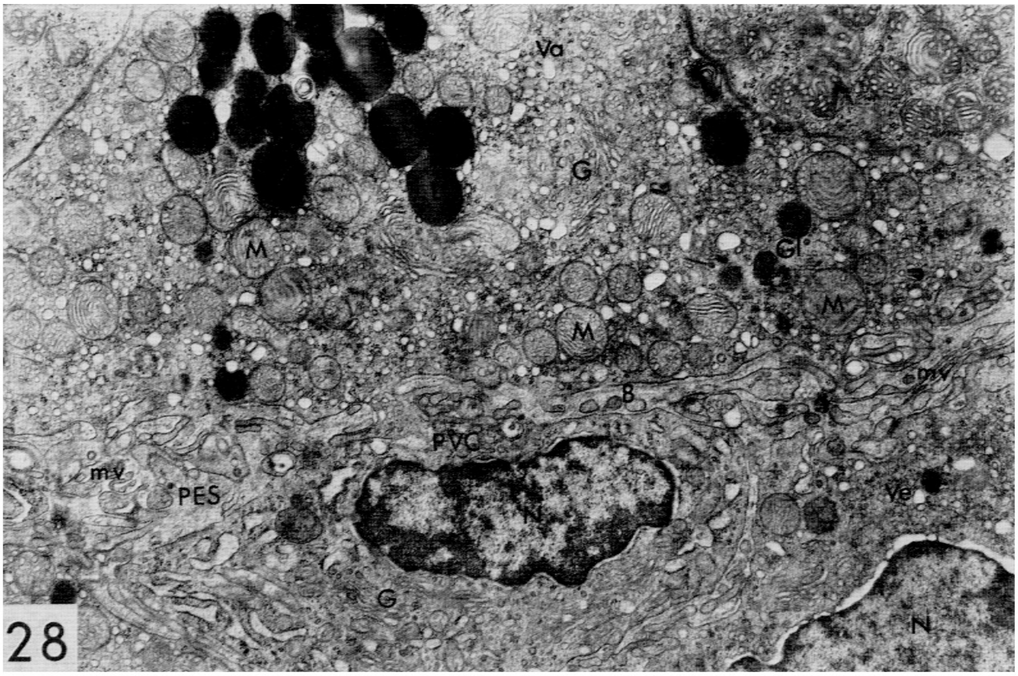

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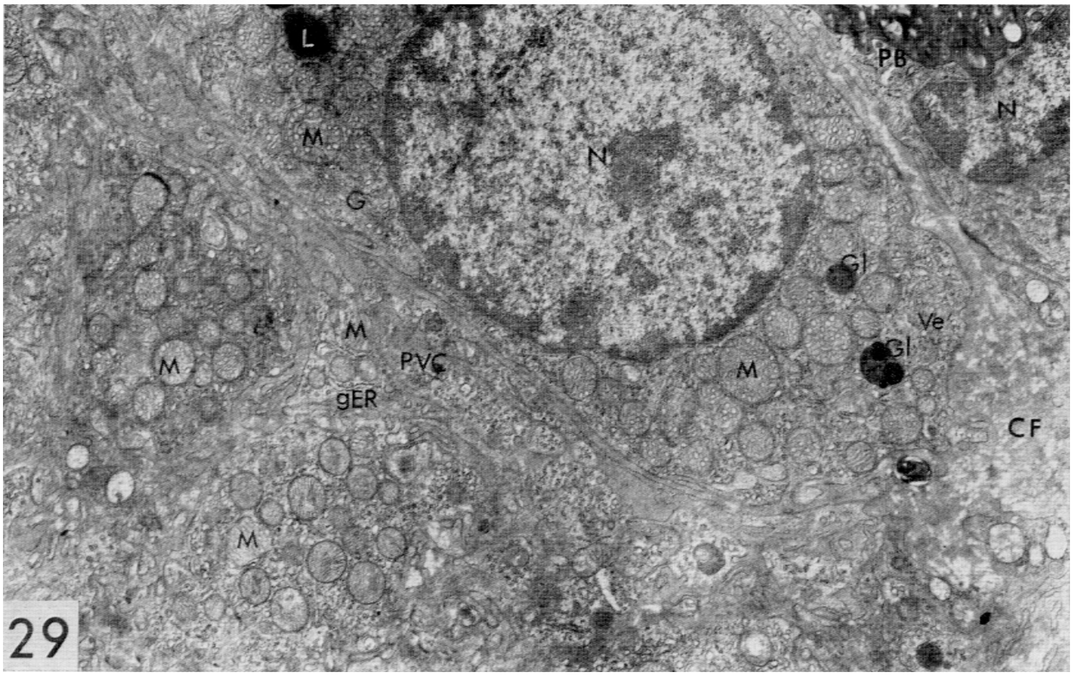

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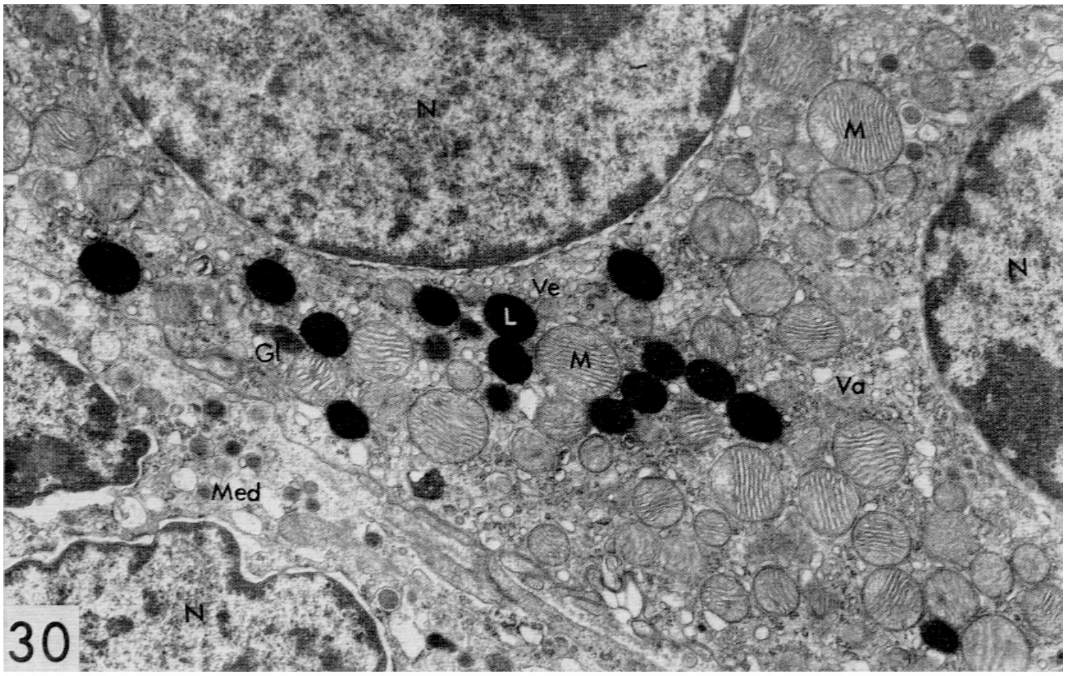

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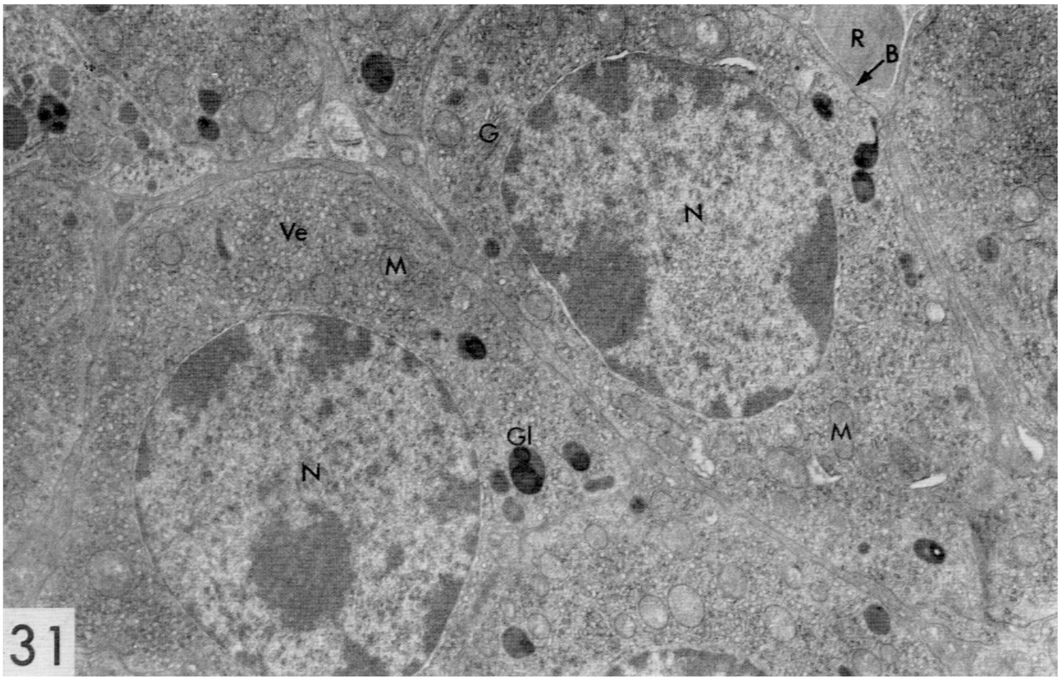

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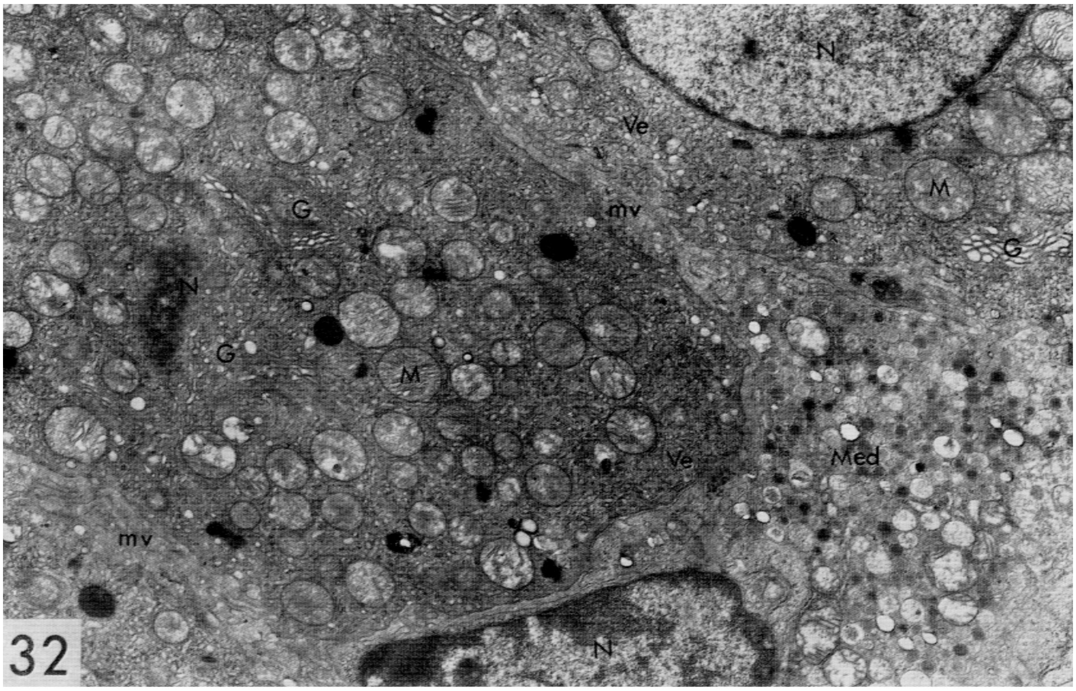
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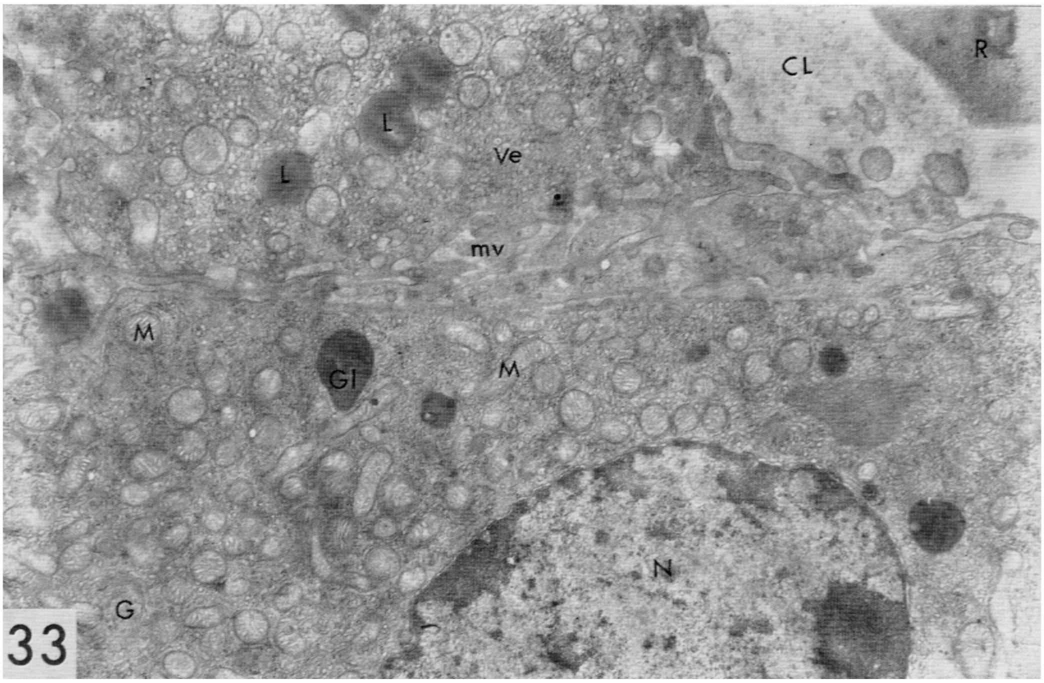
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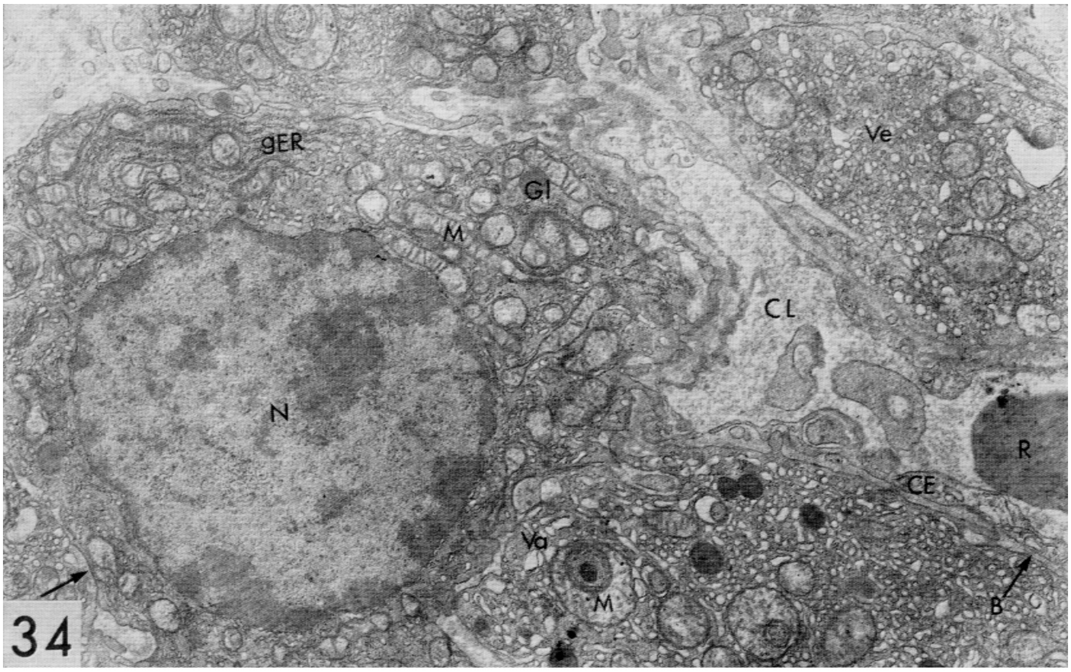
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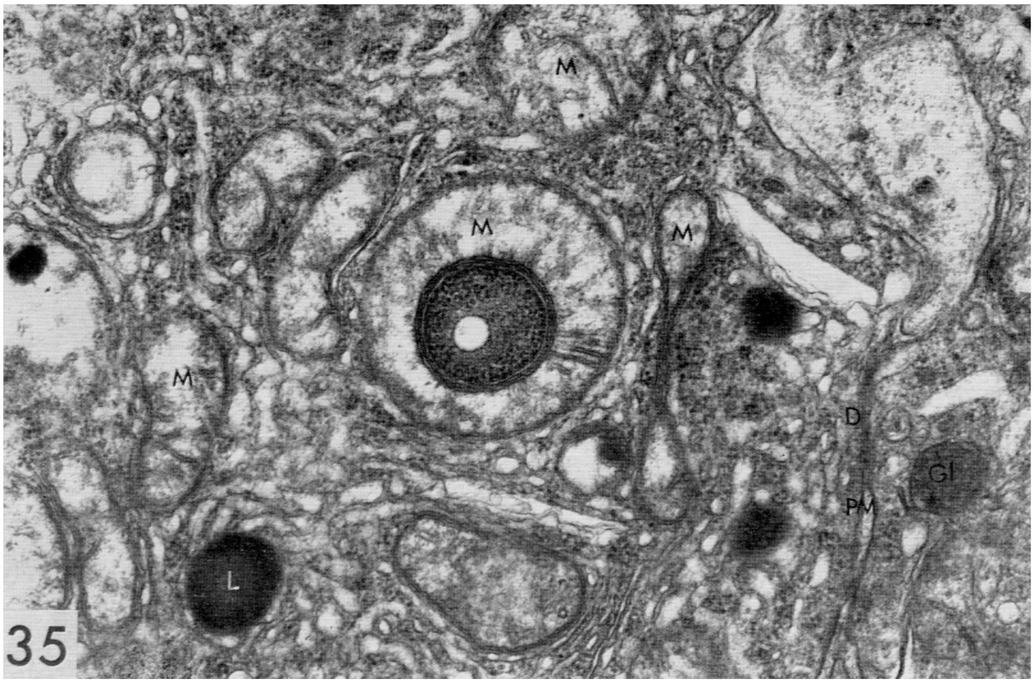
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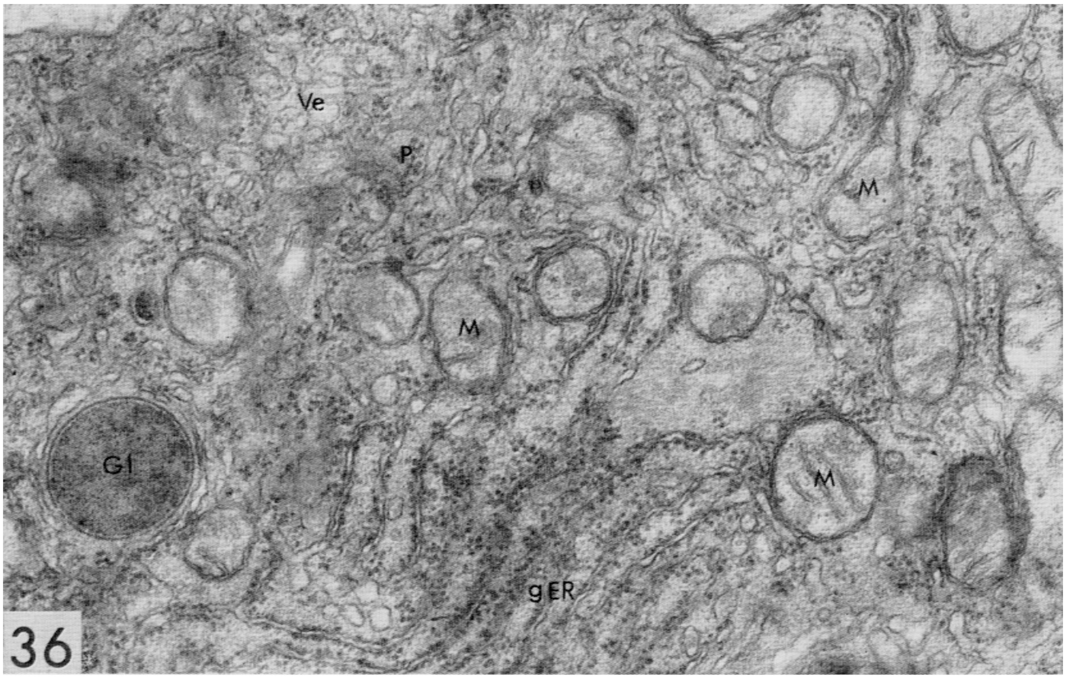
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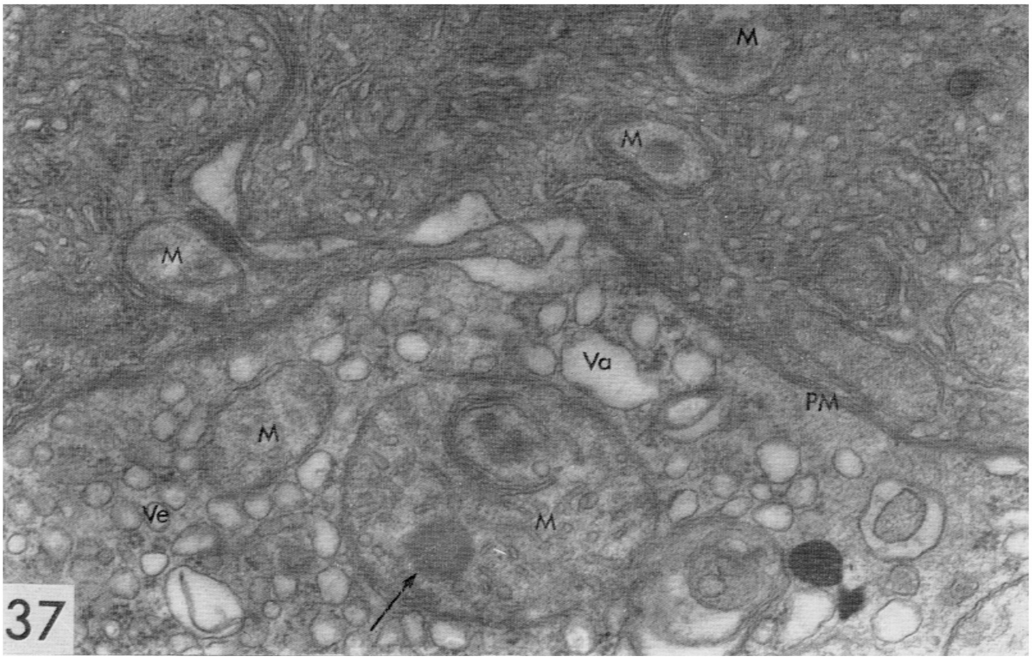
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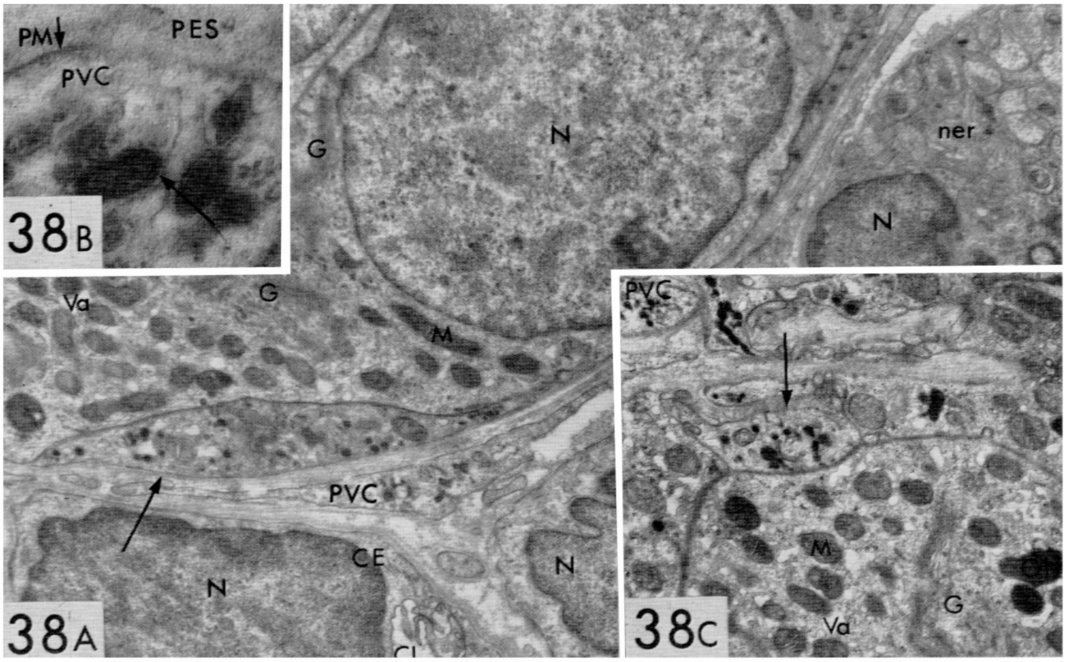
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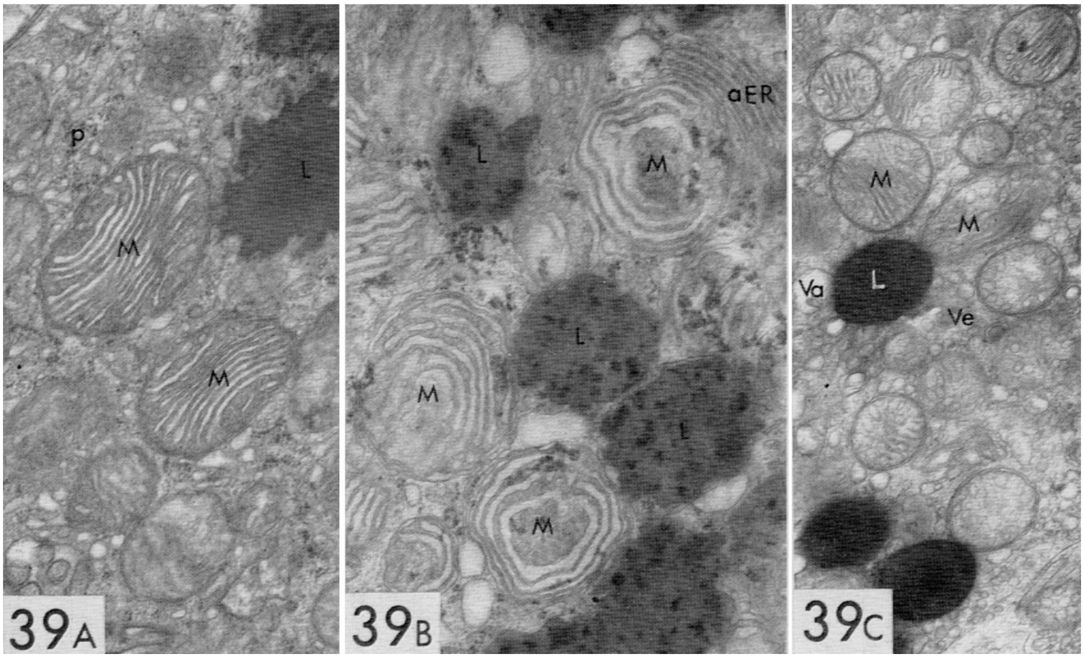
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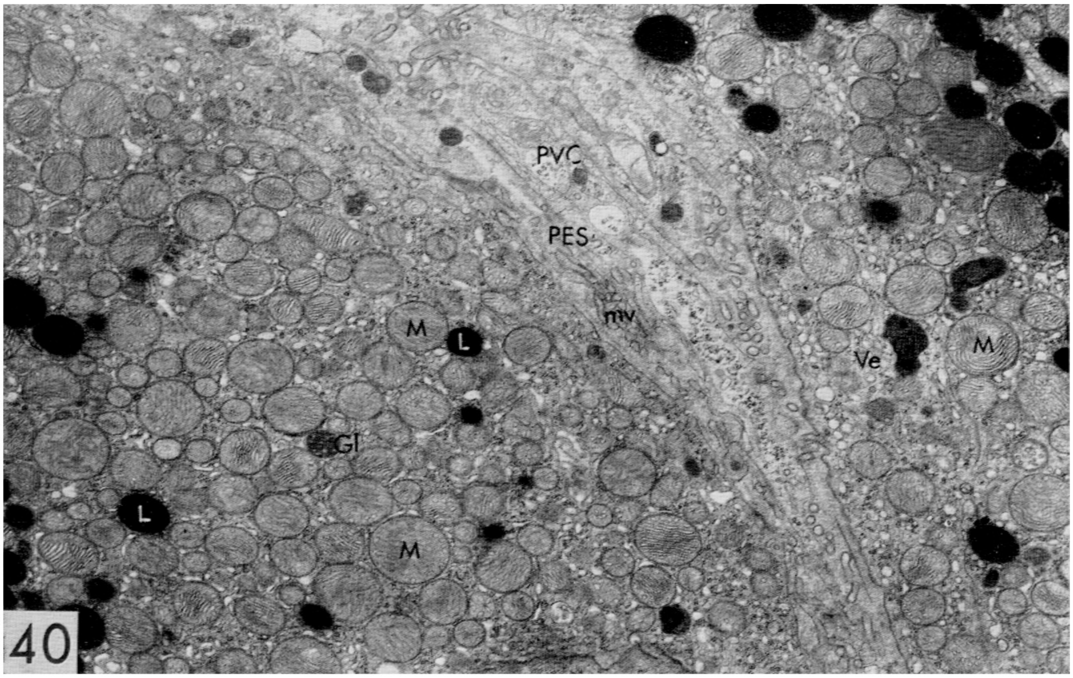
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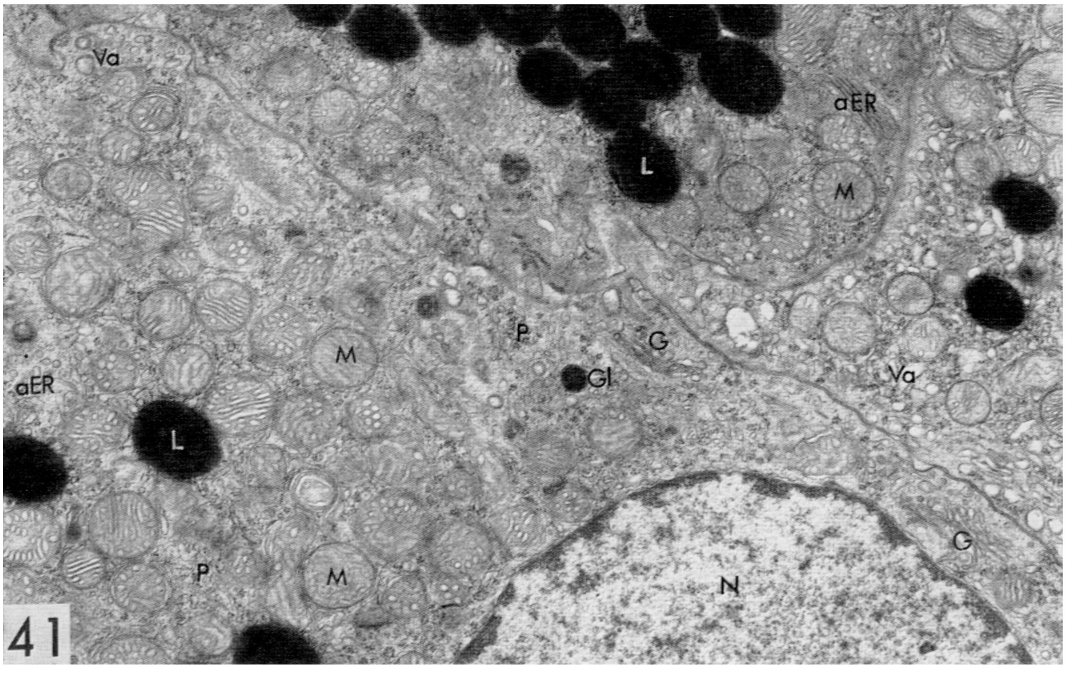
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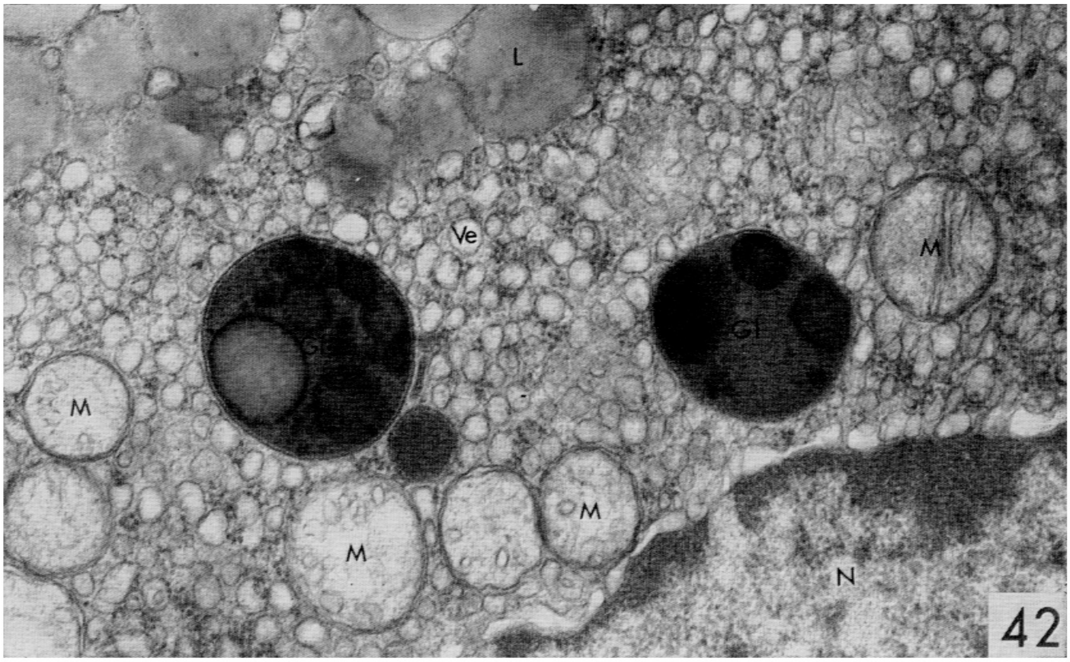

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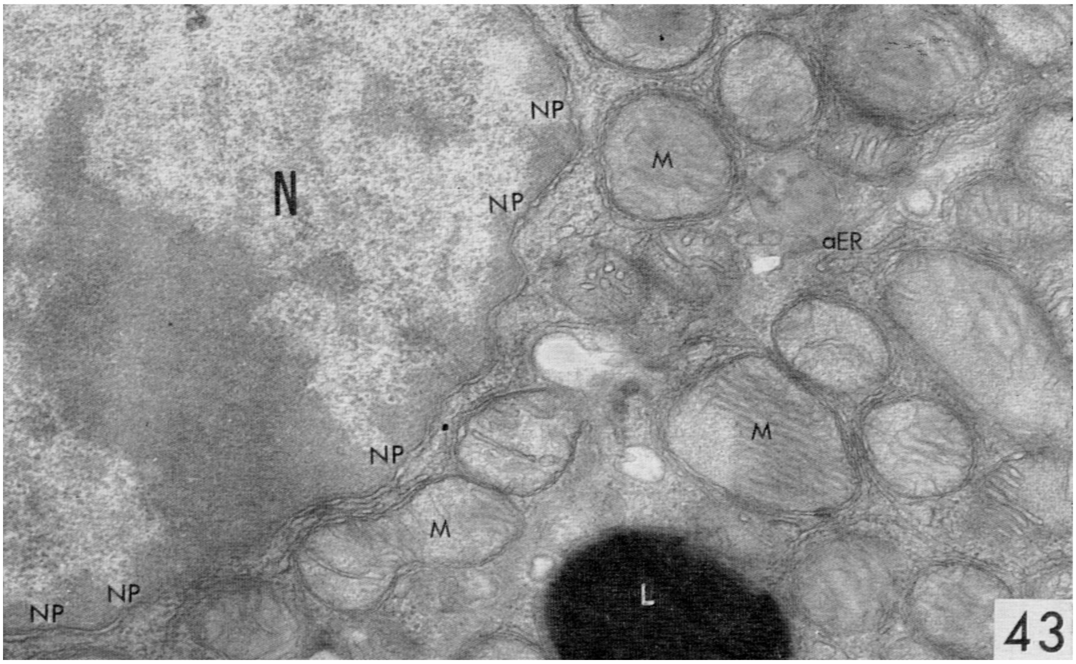

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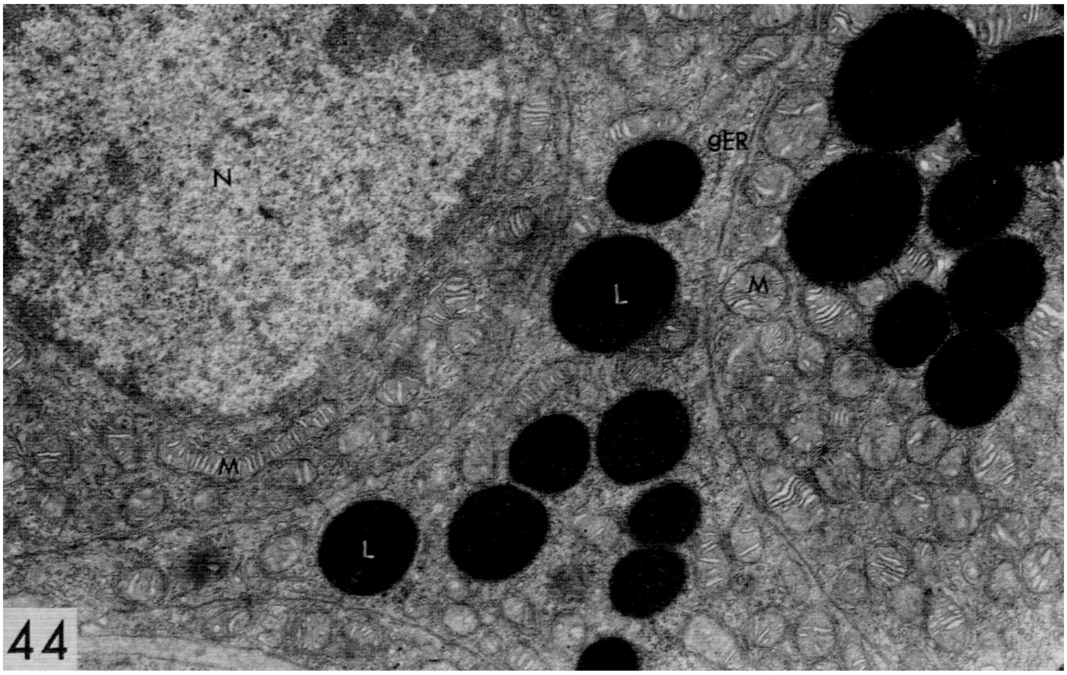
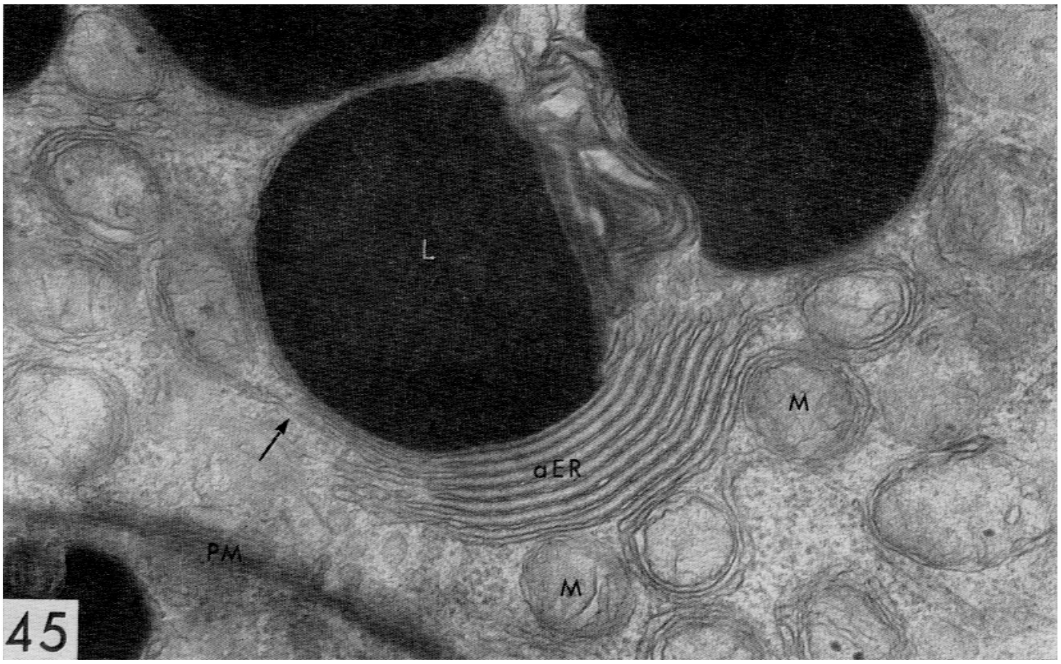
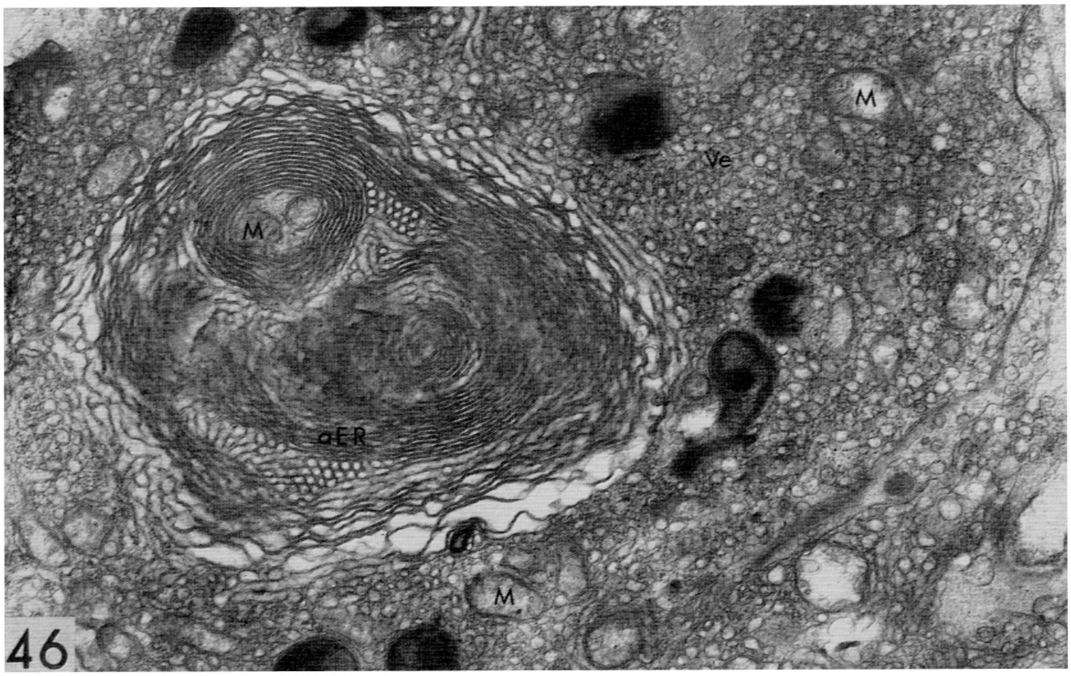
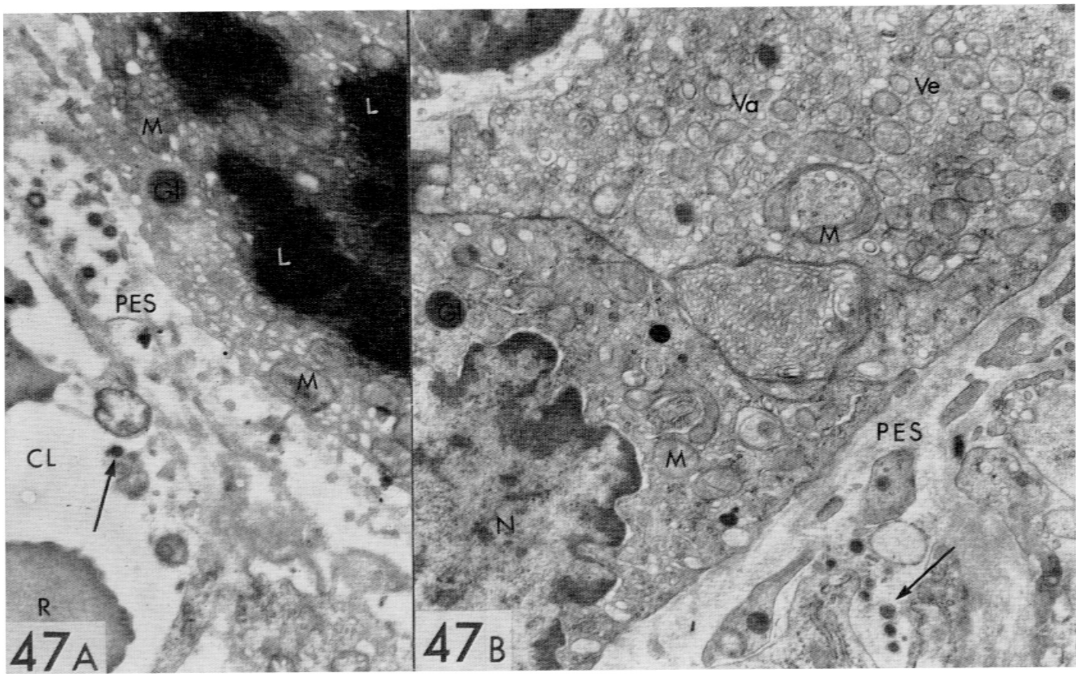
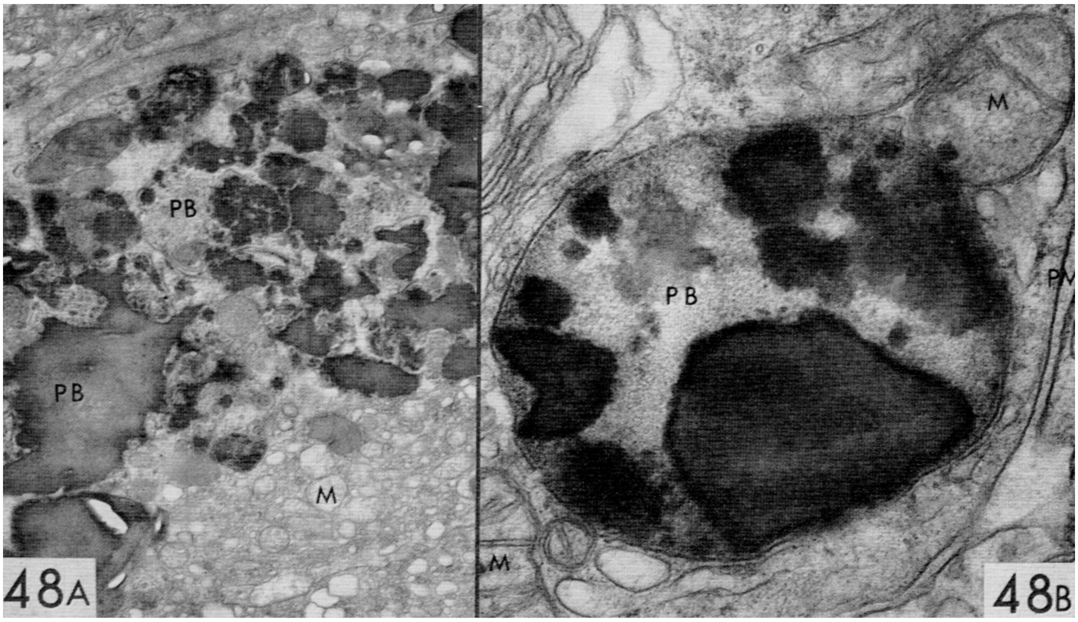
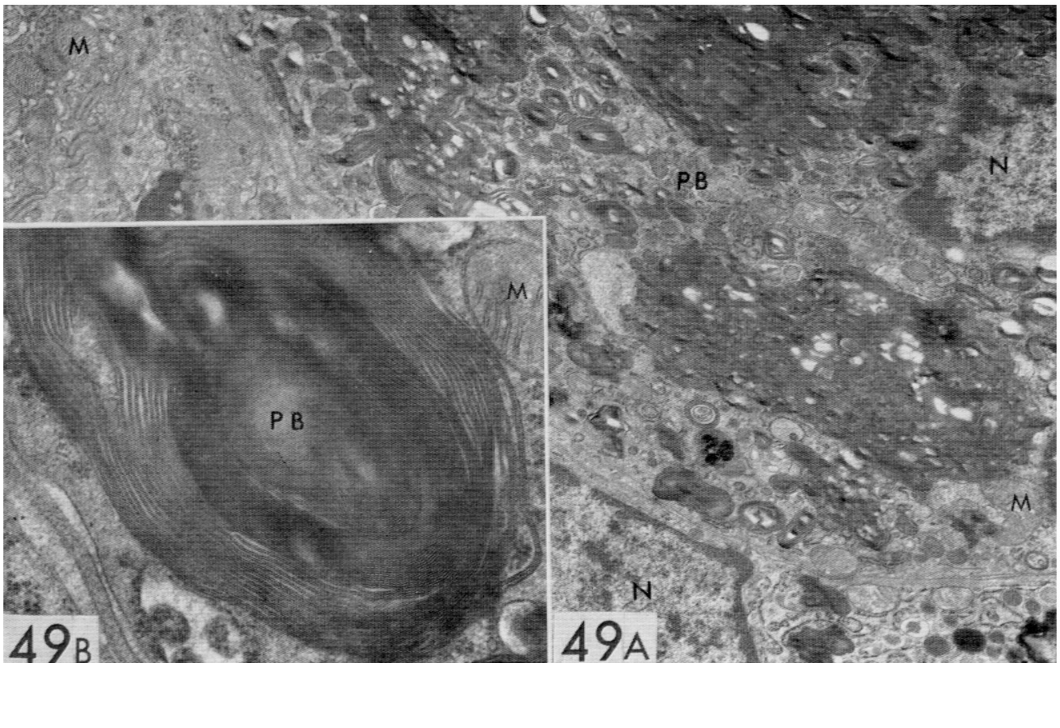

N
gER
L
M
M
L
44


L
M
aER
PM
M
45


M
Ve
M
aER
M
46


L
M
L
Gl
PES
M
CL
R
47A
Va
Ve
M
Gl
M
PES
N
47B


PB
PB
M
48A
M
PB
PM
M
48B


M
PB
N
M
PB
M
N
49B
49A