Adrenal Cortex in Retired Breeder Mongolian Gerbils *(Meriones unguiculatus)* and Golden Hamsters *(Mesocricetus auratus)*

Ultrastructural Alterations in the Zona Reticularis

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Alterations occur predominantly in cells of the zona reticularis in retired breeder Mongolian gerbils and golden hamsters. In gerbils, the size and number of lipid droplets increased and numerous large lipid-lysosomal complexes were observed in zona reticularis parenchymal cells or in macrophages, which were more numerous than in younger animals. Abundant lipofuchsin pigment was observed in zona reticularis parenchymal cells, and mitochondria contained electron-opaque inclusions. In the hamster, lipid droplets were present in inner zona reticularis cells and there was an increased amount of pigment within macrophages. Some of the alterations in gerbils and hamsters may at least in part reflect a stimulation of zona reticularis cells in retired breeders, attributable to increased prolactin secretion. (Am J Pathol 95:347–358, 1979)

THE ZONA RETICULARIS in young, mature Mongolian gerbils is a well-defined zone which is free of lipid droplets, contains few, if any, cisternae of smooth endoplasmic reticulum and a few cisternae of rough endoplasmic reticulum, but possesses numerous lysosomes.[1] The zona reticularis and zona fasciculata are separated by a unique intermediate zone of cells which contains concentric whorls of rough endoplasmic reticulum.[2] These structures respond to stress or ACTH,[3] transforming into focal areas of smooth endoplasmic reticulum. The ultrastructure of the adrenal gland in the golden hamster is of interest inasmuch as there are no lipid droplets in zona-fasciculata–zona-reticularis cells and elongate mitochondria assume a whorl-like appearance (chondriospheres).[4,5]

Most studies of adrenal morphology have concentrated on young mature, fetal, or newborn animals; there have been only a few ultrastructural studies on the adrenal gland in older rats,[6] mice,[7] and humans,[8] but none in the golden hamster or gerbil. By light microscopy, there have been studies of the adrenal gland in a number of animals,[9,10] including the hamster.[11] The purpose of the present report is to compare the adrenal cortex of retired breeder gerbils and hamsters with that of their younger counterparts as described in the literature.[1,4,5]

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Materials and Methods

Six female retired breeders at least 1 year of age were obtained from Tumblebrook Farms (North Brookfield, Mass). Six 1-year-old female golden hamsters were obtained from Ancare Corporation (Manhasset, NY). Six young, mature female gerbils and hamsters were employed for comparison with the older animals.

Animals were killed by decapitation, the adrenal gland removed quickly and trimmed of adherent fat, and slices 1 mm thick were fixed in 3% purified glutaraldehyde (Ladd Research Industries, Burlington, Vt) buffered to pH 7.4 with 0.1 M phosphate. While in buffer, smaller pieces of tissue having the desired zonal orientation were cut with a microscalpel and processed for electron microscopy as described previously.[1] Before thin sections were cut, 1-mm-thick sections were stained with toluidine blue to verify the zonal position of the tissue. Thin sections were cut with glass knives and stained with uranyl acetate [12] and lead citrate [13] before examination with a Siemens 101 electron microscope.

Organs were fixed in 10% formalin buffered to pH 7.0 with phosphate, trimmed of adherent fat and connective tissue, and weighed. All data were expressed as the mean ± SEM and analyzed statistically by the Student t test.

Results

The body weights of retired breeder gerbils and hamsters were significantly greater than those of younger counterparts (Table 1). The kidney, spleen, liver, adrenal, and ovary were significantly heavier in retired breeder gerbils than in younger animals. In the hamster, the liver and ovary in older hamsters were significantly heavier than those of younger animals (Table 1).

Adrenocortical Ultrastructure: Mongolian Gerbil

Alterations in the ultrastructure of the adrenal gland occur principally, although not exclusively, in the zona reticularis in retired breeder Mongolian gerbils; none of these changes in ultrastructure is observed in zona reticularis cells of young female gerbils.[1]

Focal, homogeneously electron-opaque inclusions were observed within the matrix of some zona reticularis mitochondria (Figure 1). In section, the inclusions were predominantly spherical and the number of inclusions varied from one to six in a single mitochondrion. Occasionally, lipid droplets were associated closely with the outer membrane of mitochondria (Figure 1) or, in another section, appeared to be incorporated into the mitochondrial matrix (Figure 1). Mitochondria varied in size and shape; cristae were tubular, and connections with the inner mitochondrial membrane were often demonstrable.

Lipid droplets appeared in parenchymal cells of the zona reticularis (Figure 2), whereas they were absent in zona reticularis cells of younger mature animals. The size of the droplets was variable and some of them were extremely large but not surrounded by a distinct membrane (Figure 2). The contour of the droplets was smooth or irregular at some points,

Table 1—Organ Weights of Young, Mature and Aged Gerbils and Hamsters

Group	Body weight (g)	Organ weights (mg)					
		Heart	Kidney	Spleen	Liver	Adrenal	Ovary
Young gerbils	57 ± 2*	296 ± 27	481 ± 31	52 ± 5	2440 ± 126	30.5 ± 0.6	13.0 ± 1.2
Old gerbils	65 ± 1†	309 ± 13	736 ± 13‡	79 ± 2‡	3365 ± 58‡	53.0 ± 2.1‡	50.7 ± 1.6‡
Young hamsters	106 ± 2	540 ± 18	1136 ± 35	98 ± 4	5090 ± 183	10.1 ± 0.5	25.0 ± 2.0
Old hamsters	132 ± 8‡	647 ± 71	1239 ± 106	109 ± 10	5622 ± 273§	12.6 ± 1.3	11.0 ± 2.6§

* Mean ± SEM
† $P < 0.05$
‡ $P < 0.001$
§ $P < 0.01$

suggesting a site for fusion of smaller droplets with the large structures (Figure 2). In other parenchymal cells, small lipid droplets of light electron opacity or other larger, more electron-opaque droplets were incorporated into structures similar to lipofuchsin pigment (Figure 3). Numerous cisternae of smooth endoplasmic reticulum and a small amount of rough endoplasmic reticulum were observed in some parenchymal cells (Figure 3), whereas rough endoplasmic reticulum predominated in other cells.

The number of macrophages in the zona reticularis was greater than in the other two zones of the cortex. Small mitochondria with simple tubular cristae (Figures 4 and 5) facilitated identification of macrophages from neighboring parenchymal cells in which mitochondria were larger and the cristae were more extensively developed. Macrophages also contained varying amounts of rough endoplasmic reticulum or polyribosomes (Figure 5). Remnants of secondary lysosomal material were frequently found within macrophages (Figure 4), often imparting a considerable size to the macrophages (Figure 5). Discrete areas of incorporated material within the cytoplasm of the cell were consistent with engulfment of material at different times by the macrophage (Figure 5).

Concentric whorls of rough endoplasmic reticulum in cells between the zona fasciculata and zona reticularis (Figure 6) were identical in location and structure to those in younger animals.[2] However, lipid droplets were more numerous in these cells (Figure 6) than in those of younger animals. Endoplasmic reticulum was almost entirely rough-surfaced; most of it was localized to whorls except for a few cisternae which were scattered throughout the cytoplasm (Figure 6).

Zona glomerulosa cells (Figure 7) were identical to those in younger animals [1]; endoplasmic reticulum was predominantly smooth-surfaced, and a prominent Golgi apparatus with numerous associated vesicles was a consistent finding (Figure 7). Mitochondria were smaller than those of cells in the inner zone and could be identified by plate-like cristae with a dense mitochrondrial matrix. Lipid droplets were usually small and infrequently surrounded by lysosomal membranes (Figure 7).

In zona fasciculata cells, lipid droplets varied in size (Figure 8), but none of them equalled those in the zona reticularis. Numerous cisternae of smooth endoplasmic reticulum filled the cytoplasm and were interspersed among other cytoplasmic organelles (Figure 8). Electron-opaque inclusions were sometimes seen in zona fasciculata cells (Figure 8).

Adrenocortical Ultrastructure: Hamster

The ultrastructure of the adrenal gland of young, mature hamsters was identical to that described previously [4,5] and will not be described here.

Alterations in the adrenal gland of retired breeder hamsters occurred predominantly in the zona reticularis and, therefore, only the structure of this zone will be described. Lipid droplets were observed in almost all cells of the inner zona reticularis of retired breeders, whereas no lipid droplets have been reported in these cells from young, mature hamsters.[4,5] Lipid droplets of variable size were partially, or sometimes completely, encircled by elongate mitochondria (Figure 9); connection of the double membrane to the body of the mitochondrion was not always demonstrable (Figure 10).

Lipid droplets and fragments of lipofuchsin-like pigment, undoubtedly originating from adrenocortical cells, were observed in macrophages (Figure 11). Macrophages were particularly numerous in zona reticularis cells and could be identified by mitochondria with simple, plate-like cristae.

Discussion

Our study is the first to show with the electron microscope that lipid droplets are present in the zona reticularis of retired breeder gerbils and hamsters soon after the end of their breeding span (1 year). With the light microscope, Meyers and Charipper [11] reported an increase in sudanophilic and cholesterol-positive vacuoles in aged hamsters. Changes in the present study were less severe than those seen in older animals. In old (21 months) senile hamsters, severe morphologic changes have been observed, which included atrophy of the cortical parenchyma and, in rare instances, its replacement by connective tissue, areas of vascular hemorrhage, and necrosis of cells, resulting in defects of cell cords.[11,14] In various strains of old mice, Jayne [15] observed brown degeneration and multinucleated giant cells filled with pigment in the zona reticularis.

Leishmania infection in hamsters is also followed by an accumulation of lipid droplets and cholesterol, with some cells exhibiting a signet ring appearance.[16] Leathem and Stauber [16] noted that the response was related to the degree of infection and attributed it to stress. This does not appear likely because ACTH release is a major component of the stress response and Yates [5] did not observe similar changes in the adrenal cortex of the hamster after ACTH injection.

We reported that estrogen also induces accumulation of lipid droplets in zona reticularis cells of young, mature, male and female gerbils.[17] By light microscopy, estrogen produces lipid and pigment deposition in the hamster [18] and mouse.[19] Although the mechanism which produces accumulation of lipid droplets in hamsters and gerbils is unknown, it is possible that a common alteration could account for similar ultrastructural appearances in retired breeders and after estrogen treatment: enhancement of prolactin secretion. In retired breeders, increased secretion of prolactin has been reported by Lewis and Wexler,[20] although the ultrastructure of mammotrophs and blood levels of prolactin have not been studied in retired breeder gerbils and hamsters. With estrogen treatment in the gerbil, there is hypertrophy of mammotrophs in the anterior pituitary gland, increased synthesis and secretion of prolactin, and induction of intranuclear inclusions.[21]

Gerbils and hamsters are related taxonomically and belong to the same family. The gerbil and hamster have a remarkably efficient mechanism for water conservation, presumably as an adaptation to an arid environment, their natural habitat.[22,23] Prolactin is important in some animals for regulation of water balance, although this has not been shown for gerbils and hamsters. However, the adrenal gland is essential for survival of gerbils and hamsters inasmuch as adrenalectomized gerbils do not survive despite replacement therapy,[24] whereas with the hamster, a special regimen developed in our laboratory [25] ensures survival of the animals after bilateral adrenalectomy.

Several functions have been suggested for the zona reticularis. Hoerr [26] observed increased numbers of degenerating and dead cells in this zone and postulated that it was a zone of increased cell degeneration and cell death. However, this interpretation has subsequently been questioned because dead and degenerating cells are found in other zones as well [27] and stereologic studies show that it has a reactivity to ACTH similar to that of the zona fasciculata.[28] The zona reticularis has also been postulated to be a source of adrenal sex steroids,[29] and in the gerbil there appears to be an interrelationship between the adrenal cortex and the ovary since ovariectomy stimulated the adrenal gland and there is a marked increase in plasma cortisol.[30] If this is correct, the changes observed in the zona reticularis in the present study may represent compensatory alterations following diminution in ovarian function, which occurs during aging. The lipid droplets in the zona reticularis would provide cholesterol and cholesterol esters, precursors for steroidogenesis in the adrenal gland.[31]

References

1. Nickerson PA: Fine structure of the Mongolian gerbil adrenal cortex. Anat Rec 171:443–456, 1971
2. Nickerson PA, Curtis JC: Concentric whorls of rough endoplasmic reticulum in adrenocortical cells of the Mongolian gerbil. J Cell Biol 40:859–862, 1969
3. Nickerson PA: Effects of ACTH on membranous whorls in the adrenal gland of the Mongolian gerbil. Anat Rec 166:479–490, 1970
4. DeRobertis E, Sabatini D: Mitochondrial changes in the adrenocortex of normal hamsters. J Biophys Biochem Cytol 4:667–670, 1958
5. Yates RD: Fine structural observations on untreated and ACTH treated adrenocor-

tical cells of the zona reticularis of Syrian hamsters. Z Zellforsch Mikrosk Anat 66:384–395, 1965
6. Szabó D, Dzsinich C, Ökrös I, Stark E: The ultrastructure of the aged rat zona fasciculata under various stressing procedures. Exp Gerontol 5:335–337, 1970
7. Sato T: Age and sex differences in the fine structure of the mouse adrenal cortex. Nagoya J Med Sci 30:225–251, 1967
8. Dobbie JW: Adrenocortical nodular hyperplasia: the ageing adrenal. J Pathol 99:1–18, 1969
9. Blumenthal HT: Aging processes in the endocrine glands of various strains of normal mice: Relationship of hypophyseal activity to aging changes in other endocrine glands. J Gerontol 10:253–267, 1955
10. Jayne EP: Histochemical and degenerative changes in the adrenal cortex of the rat with age. J Gerontol 12:2–8, 1957
11. Meyers MW, Charipper HA: A histological and cytological study of the adrenal gland of the golden hamster (*Cricetus auratus*) in relation to age. Anat Rec 124:1–25, 1956
12. Stempak JG, Ward RT: An improved staining method for electron microscopy. J Cell Biol 22:697–701, 1974
13. Reynolds ES: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 17:208–212, 1963
14. Lombard Ch, de Boissezon P, Pierre M: Les signes histologiques de la sécrétion dans le cortex surrénal. Ann Endocrinol (Paris) 7:165–172, 1946
15. Jayne EP: A histo-cytologic study of the adrenal cortex in mice as influenced by strain, sex, and age. J Gerontol 18:227–234, 1963
16. Leathem JH, Stauber LA: Adrenal lipid and alkaline phosphatase in hamsters infected with *Leishmania donovani*. Endocrinology 50:311–173, 1952
17. Nickerson PA: Fat accumulation in the adrenal gland: Effect of oestradiol benzoate on the zona reticularis of the Mongolian gerbil adrenal gland. Pathol Eur 10:287–297, 1975
18. Koneff AA, Simpson ME, Evans HM: Effects of chronic administration of diethylstilbestrol on the pituitary and other endocrine organs of hamsters. Anat Rec 94:169–195, 1946
19. Tobin CE, Birnbaum JP: Some factors influencing brown degeneration of the adrenal gland in the Swiss albino mouse. Arch Pathol 44:269–281, 1947
20. Lewis BK, Wexler BC: Changes in LH and prolactin in arteriosclerotic female breeder rats. Atherosclerosis 21:301–314, 1975
21. Nickerson PA: Induction of intranuclear inclusions by estrogen in mammotrophs of the Mongolian gerbil. J Ultrastruct Res 44:41–48, 1973
22. Bond CR: The golden hamster (*Cricetus auratus*): Care, breeding, and growth. Physiol Zool 18:52–59, 1945
23. Rich ST: The Mongolian gerbil (*Meriones unguiculatus*) in research. Lab Anim Care 18:235–243, 1968
24. Cullen JW, Scarborough DE: Behavioral and hormonal prophylaxis in the adrenalectomized gerbil (*Meriones unguiculatus*). Horm Behav 1:203–210, 1970
25. Nickerson PA, Molteni A: Survival of bilaterally adrenalectomized hamsters drinking saline sucrose. J Appl Physiol 31:675–678, 1971
26. Hoerr N: The cells of the suprarenal cortex in the guinea pig: Their reaction to injury and their replacement. Am J Anat 48:139–197, 1931
27. Greep RO, Deane HW: The cytology and cytochemistry of the adrenal cortex. Ann NY Acad Sci 50:596–615, 1949
28. Mazzocchi G, Robba C, Belloni AS, Rigotti P, Gambino AM, Nussdorfer GG: Investigations on the turnover of adrenocortical mitochondria. IV. A stereological study of the effect of chronic treatment with ACTH on the size and number of rat zona reticularis mitochondria. Cell Tissue Res 168:1–19, 1976

29. Blackman SS: Concerning the function and origin of the reticular zone of the adrenal cortex. Bull Johns Hopkins Hosp 78:180–217, 1946
30. Nickerson PA: Stimulation of the adrenal gland of the Mongolian gerbil after ovariectomy. Cell Tissue Res 165:135–139, 1975
31. Malamed S: Ultrastructure of the mammalian adrenal cortex in relation to secretory function. Handbook of Physiology. Section 7, Vol 6: Adrenal Gland. Edited by RO Greep, EB Astwood. Washington DC, American Physiological Society, 1975, pp 25–39

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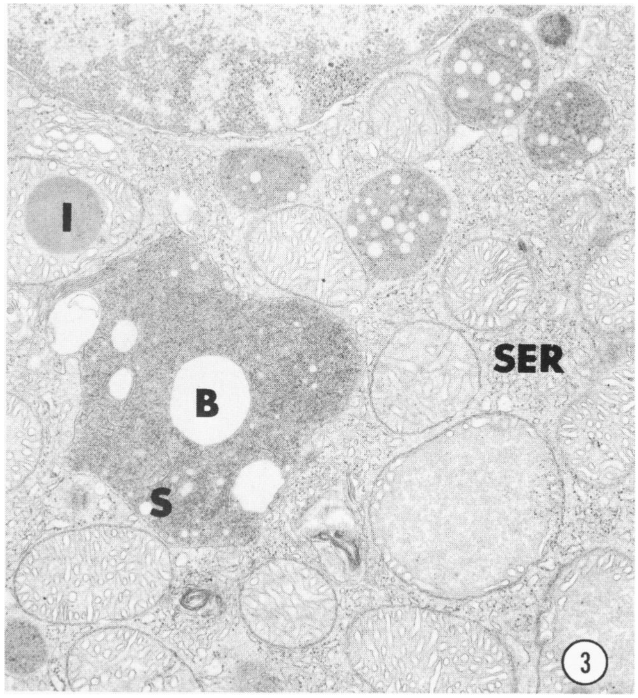


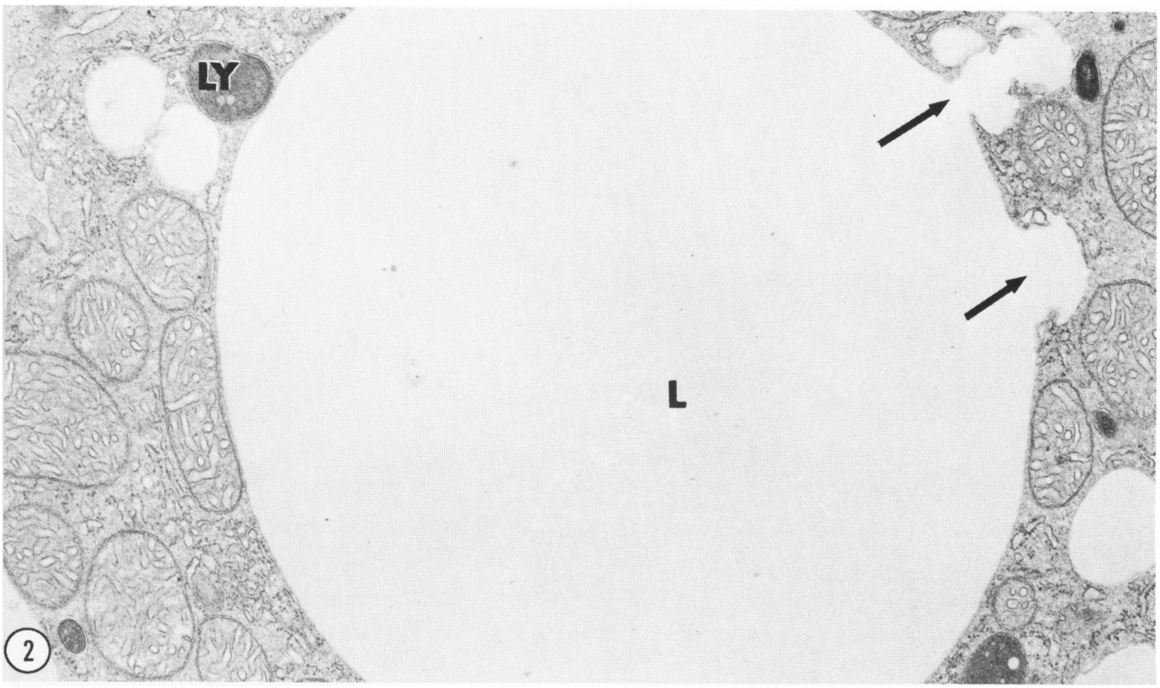
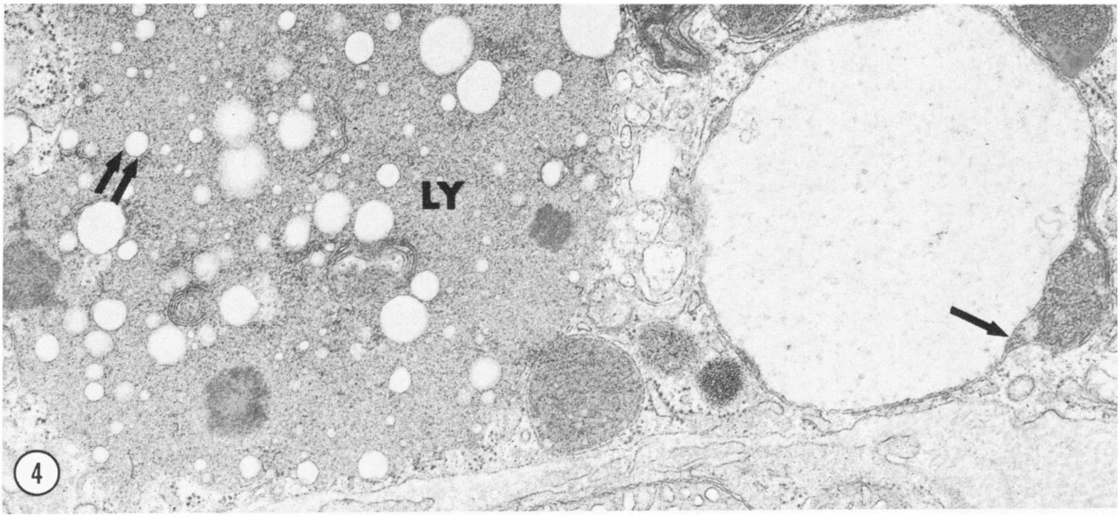


Figure 1—Zona reticularis cell in gerbil. Mitochondria contain electron-opaque inclusions (*I*) of varying size. Cristae are predominantly tubular. Lipid droplets are close to mitochondrial membranes (*L*) or appear to be incorporated (*L2*) into the mitochondrial matrix (× 21,600) (with photographic reduction of 8%) **Figure 2**—Large lipid droplet (*L*) in zona reticularis cell. The lipid droplet is not membrane-bound but shows irregularities along the contour (*arrow*), where smaller droplets appear to fuse with the larger structure. *LY*, secondary lysosome (× 22,400) (with photographic reduction of 8%) **Figure 3**—Zona reticularis cell. Large areas of the cytoplasm contain lipofuchsin pigment which is membrane-bound and contains small (*S*) or large (*B*) lipid droplets incorporated into the structure. Mitochondrial cristae are tubular, and the matrix has an occasional electron-opaque inclusion (*I*). *SER*, smooth endoplasmic reticulum. (× 17,600) (with photographic reduction of 8%)



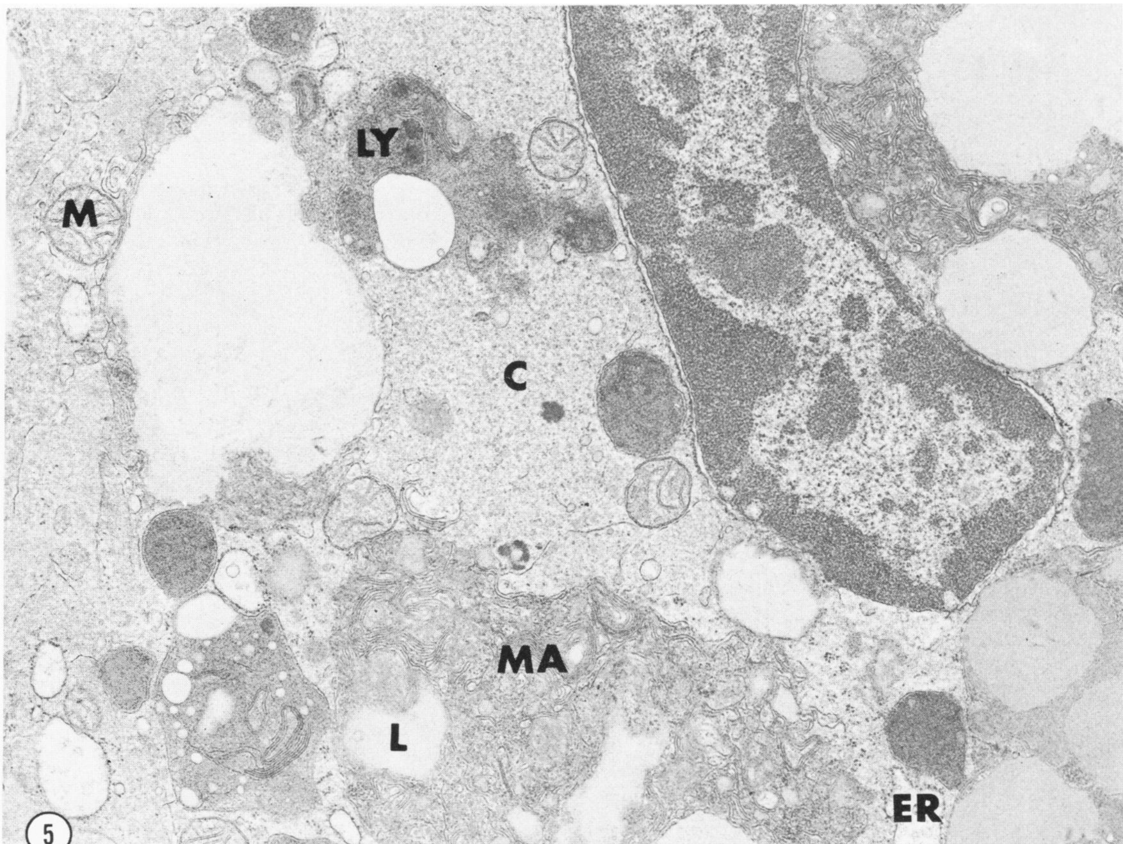
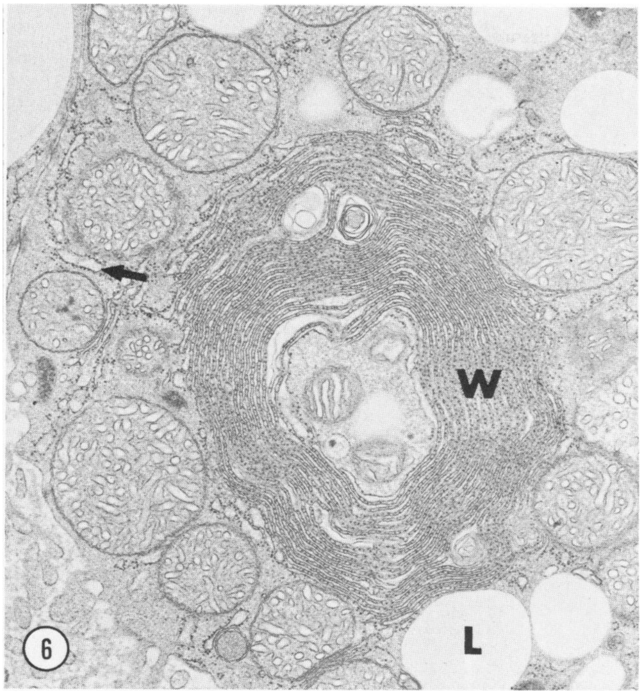
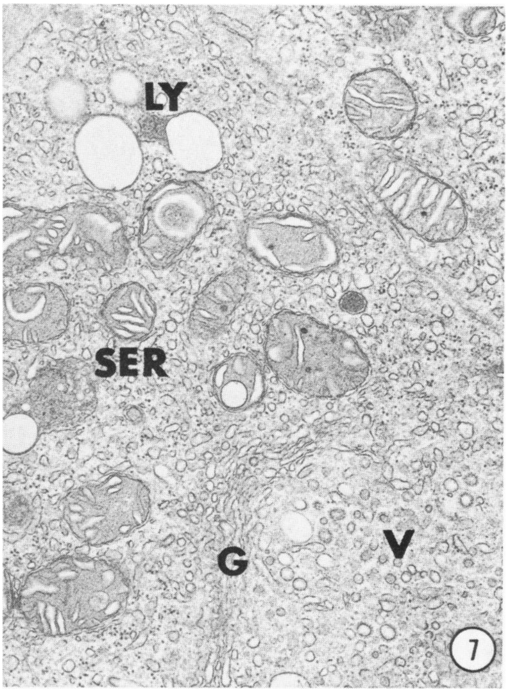


Figure 4—Macrophage within zona reticularis. Discrete areas of secondary lysosomal material is observed in th cytoplasm (*LY*). In 1 case, a large electron-lucid structure with a lightly flocculent matrix is surrounded by a rim c dense material which in turn is connected to a larger electron-opaque lysosome (*arrow*). In other areas, small lipi droplets are embedded in a more electron-opque matrix (*dougle arrows*). (× 20,800) (With photographic reduction c 8%) **Figure 5**—Macrophage in the zona reticularis containing discrete lipid droplets (*L*) embedded in a matri (*MA*) showing remnants of cytoplasmic membranes. Mitochondria (*M*) of the macrophage have simple tubula cristae. Cytoplasm (*C*) of macrophage contains numerous polyribosomes (*P*). *LY*, lysosome; *ER*, rough endoplasmi reticulum. (× 20,800) (with photographic reduction of 8%)





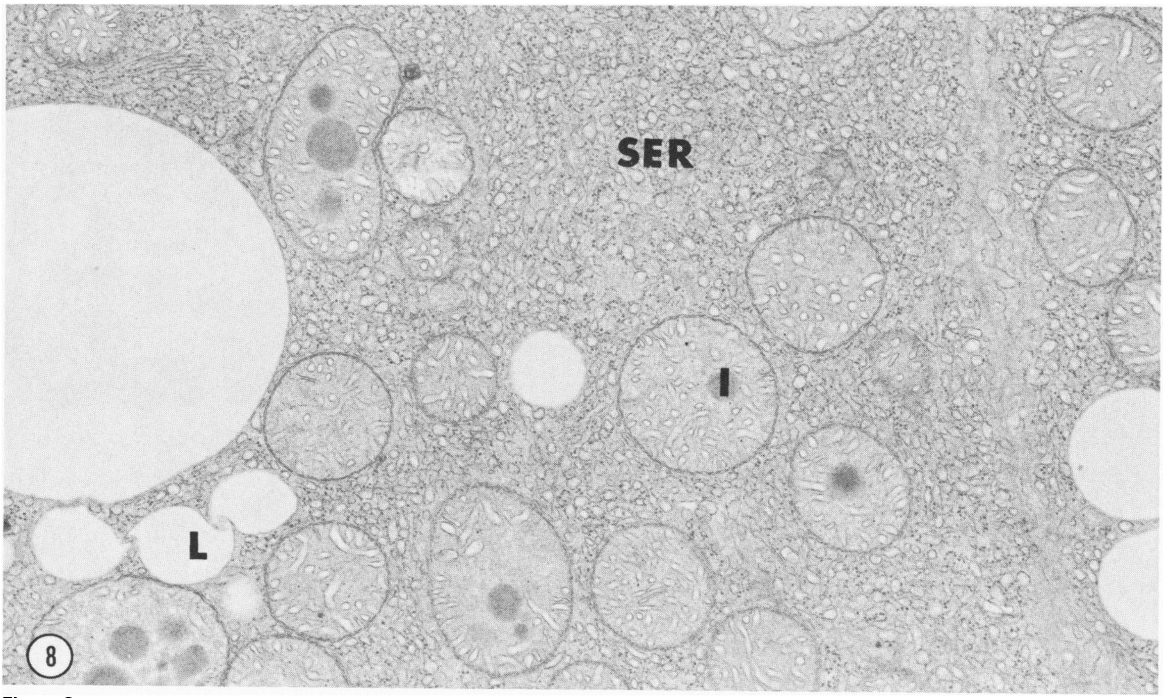
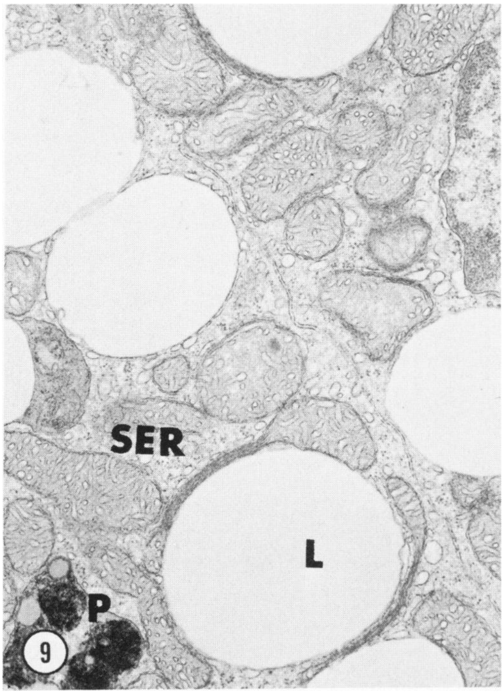
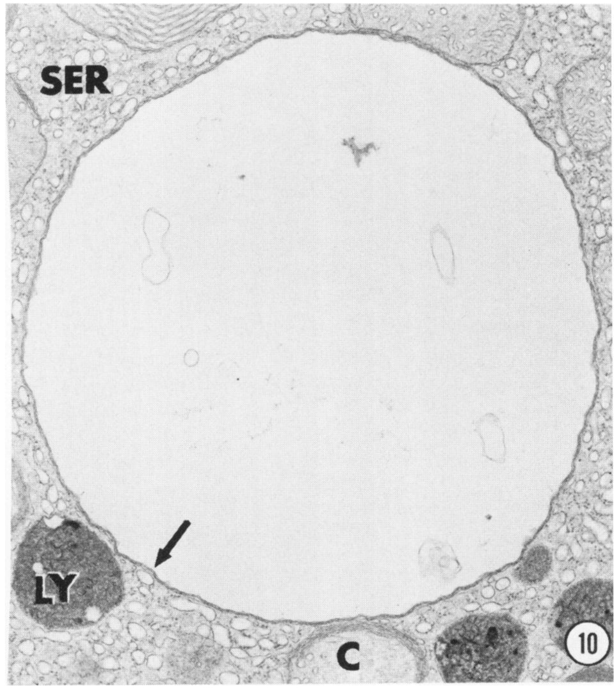


Figure 6—Cell in intermediate zone between the zona fasciculata and zona reticularis. A whorl of concentric membranes of rough endoplasmic reticulum (*W*) occupies a focal area of cytoplasm. A few lipid droplets (*L*) are scattered throughout the cytoplasm. Tubular cristae occupy the matrix of mitochondria. A few isolated cisternae of rough endoplasmic reticulum (*arrow*) are seen in the cytoplasm. (× 17,600) (with photographic reduction of 8%) **Figure 7**—Zona glomerulosa cell. Mitochondria are oval to elongate in contour, showing plate-like cristae and an electron-opaque matrix. A Golgi complex (*G*) has numerous vessicles (*V*) associated with the inner concave face. Endoplasmic reticulum is smooth surfaced (*SER*). *LY* lipid-lysosome complex. (× 21,000) (with photographic reduction of 8%) **Figure 8**—Zona fasciculata cell. The cytoplasm is filled with numerous cisternae of smooth endoplasmic reticulum (*SER*). Mitochondria contain electron-opaque inclusions (*I*). The size of lipid droplets (*L*) is variable. (× 23,200) (with photographic reduction of 8%)





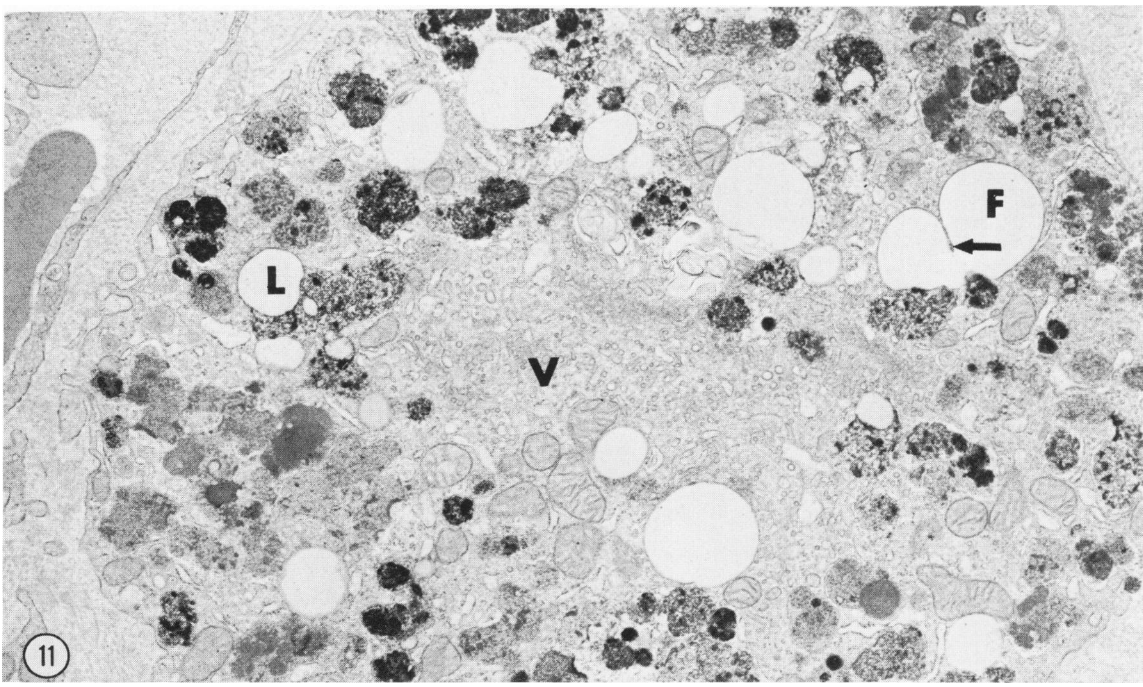


Figure 9—Portions of cells from inner zona reticularis of aged hamster. Some lipid droplets (*L*) are partially encircled b elongate mitochondria. *P*, lipofuchsin pigment; *SER*, smooth endoplasmic reticulum. Mitochondrial cristae are tubular (× 18,400) (with photographic reduction of 8%) **Figure 10**—A large lipid droplet in zona reticularis cell of aged hamster is surrounded by a double membrane (*arrow*). *SER*, somewhat dilated smooth endoplasmic reticulum; *LY* lysosome. Some mitochondrial cristae (*C*) appear in circles beneath the inner mitochondrial membrane. (× 17,600 (with photographic reduction of 8%) **Figure 11**—Macrophage in extrasinusoidal space within the inner zona reticularis of an aged hamster. Many discrete areas of secondary lysosomal material are present in the cytoplasm o the macrophage. Lipid droplets appear associated with the pigment (*L*) or largely free (*F*) but possessing a limiting membrane (*arrow*). *V*, Golgi apparatus with numerous vesicles. (× 14,400) (with photographic reduction of 8%)