Studies on the Interstitial Cells of the Testis

II. The Ultrastructure in the Adult Guinea Pig and the Effect of Stimulation with Human Chorionic Gonadotropin

Leonard Merkow, M.D., Ph.D.,* Hernan F. Acevedo, Ph.D.,* Malcolm Slifkin, Ph.D.,* and Matias Pardo

A STUDY OF THE ULTRASTRUCTURE of the testicular tissue from normal adult guinea pigs, with and without human chorionic gonadotropin (HCG; Ayerst) stimulation, was undertaken. This study is an outgrowth of investigations designed to correlate the fine structure of the Leydig cell, as seen by the electron microscope, with its androgen biosynthetic capabilities as observed at different ages and as influenced by exogenous HCG stimulation.

Previous studies [1] revealed the existence of a striking difference between the fine structure of the Leydig cells of immature animals (3 weeks old) and that of the exogenously HCG-stimulated animal of the same age group. This communication describes the ultrastructural morphology of endogenously stimulated (normal adult) Leydig cells and the effect of in-vivo administration of HCG upon these cells.

Materials and Methods

A total of 10 adult guinea pigs (cavys, obtained from Alden H. Forbes Laboratories), were utilized in these experiments. Their ages ranged from 8 to 9 months, and their weight was 723–1140 gm. Of these animals, 5 were given intraperitoneal injections of 100 international units (I.U.) of HCG daily for 15 days; the other 5 (controls) were inoculated with normal saline. At 24 hr. after the last injection, all animals were sacrificed by decapitation. Cubes (1 mm.) of testis were sectioned and immediately fixed in 1% phosphate-buffered osmium tetroxide at pH 7.4. The cubes of tissue were kept in fixative and were refrigerated for 1–2 hr. Rapid dehydration through a series of cold ethanols was followed by treatment with propylene oxide. The tissue was then embedded in Epon 812. Sections 1 μ thick were prepared and stained with methylene blue. With the light microscope large clusters of Leydig cells were easily identified between seminiferous tubules in these sections. Ultrathin sections displaying silver or gold interference colors were ob-

From the Departments of Experimental Pathology and of Research in Endocrinology and Metabolism, William H. Singer Memorial Research Institute of the Allegheny General Hospital, Pittsburgh, Pa. 15212.

Presented in part at the Sixty-fourth Annual Meeting of The American Association of Pathologists and Bacteriologists, Washington, D. C., Mar. 10–12, 1967.

Accepted for publication Aug. 19, 1968.

Address for reprint requests: William H. Singer Memorial Research Institute, Allegheny General Hospital, East North Ave., Pittsburgh, Pa. 15212.

* Adjunct Assistant Professor at Pennsylvania State University.

tained with a Porter-Blum MT-1 or MT-2 ultramicrotome using a glass knife. These ultrathin sections were stained with a 5% solution of uranyl acetate followed by lead citrate. All ultrathin sections were then examined with a Philips 100B or EM300 electron microscope.

Samples of remaining testicular tissue were simultaneously fixed in buffered formalin, embedded in paraffin, sectioned at 5 μ, and stained with hematoxylin and eosin.

Results

Normal Adult Leydig Cells

Light Microscopy. Interstitial cells of Leydig from the normal adult (control) animals were easily found in small clusters between seminiferous tubules (Fig. 1). Most seminiferous tubules contained immature and mature spermatozoa. The fine structure of the mature spermatozoa was similar to other species. The Sertoli cells contained many vesicles, a paucity of small mitochondria, and scant rough endoplasmic reticulum.

Electron Microscopy. Identification of the interstitial cells by electron microscopy was facilitated by their abundant smooth endoplasmic reticulum (Fig. 2 and 3) and relation to the lamina propria consisting of collagen and a basement membrane adjacent to seminiferous tubules (Fig. 2). The cytoplasm of these cells usually contained an abundance of this type of endoplasmic reticulum. At higher magnification, adjacent parallel cisternae or tubules appeared to have anastomotic cross connections. Lipid vacuoles and membrane-bound lipochrome (lipofuchsin) pigment bodies were frequently noted interspersed between the smooth endoplasmic reticulum (Fig. 2–6).

An occasional interstitial cell contained large, less-dense osmiophilic lipid vacuoles associated with smooth endoplasmic reticulum and a few elements of nondilated rough endoplasmic reticulum (Fig. 2 and 4). The Golgi complex was usually well developed, but centrioles were not observed in these Leydig cells.

Lipochrome pigment bodies exhibited both more and less dense osmiophilic regions (Fig. 2–6). The less dense areas appeared granular (Fig. 4 and 6) and occasionally displayed a structural crystalline appearance (Fig. 5). Either one or more dense components existed in these pigment bodies (Fig. 3–6). These aggregates of pigment were always bound by an outer limiting membrane (Fig. 3–6). At low magnification some of the pigment bodies contained a dense structure consistent with a myelin figure (Fig. 4 and 6). Similar membrane-bound bodies contained multiple laminated whorled concentric narrow membranes resembling an onion skin (Fig. 6). True crystalloids of Reinke were not observed.

In these Leydig cells, mitochondria adjacent to lipid and pigment bodies displayed the usual lamelliform cristae extending toward the center (Fig. 2, 4, and 5). Some mitochondria assumed a different morphologic appearance from the classic form. Adjacent cristae were widened and appear to have undergone partial fusion with each other. They formed a central star-like configuration in any plane of section (Fig. 7). Other cells contained very elongated, large, rod-shaped mitochondria with short parallel lamelliform cristae (Fig. 8). This type of mitochondria was usually found in several parallel groups.

Interstitial cells of Leydig exhibited a variation in morphology from one cell to another. In some, there were numerous stacks of parallel smooth endoplasmic reticulum. Frequently, these stacks displayed bulbous dilatation at each end (Fig. 7). In others, one or more double-membrane-limited pigment bodies contained one or more variably stained vacuole(s); a number of parallel smooth-surfaced membranes; and smaller, round, dense granular elements within the background (Fig. 8). Within these pigment bodies the smooth-surfaced parallel membranes also displayed dilated bulbous ends (Fig. 8).

Annuli of the nuclear membrane in various planes of section were readily discernible (Fig. 2).

HCG-Stimulated Adult Leydig Cells

Light Microscopy. Epon-embedded thick sections of HCG-stimulated testis showed an increase in both number and size of Leydig cells (Fig. 9A and 9B).

Electron Microscopy. Low-power electron microscopy of such a field reveals the overall appearance of cellular organelles (Fig. 10). Even at this magnification one could easily discern that both smooth and rough endoplasmic reticulum and the Gogli complex were increased in number. As in control Leydig cells (Fig. 2 and 3), the smooth endoplasmic reticulum was the most prevalent organelle. In addition, the lipid vacuoles and pigment bodies tended to be located toward the periphery of these cells, and mitochondria, though smaller than those observed in control Leydig cells, appeared increased in number (Fig. 10).

At higher magnification, the smooth endoplasmic reticulum was increased in density, and the number of parallel arrays of rough endoplasmic reticulum were more notable (Fig. 11). The Golgi complex was more numerous within a given cell (Fig. 10–13). The increased parallel arrays of rough endoplasmic reticulum, as well as smooth endoplasmic reticulum, were frequently dilated (Fig. 12–14).

Lipid vacuoles were variable in size, nearly always circular, and peripheral in location (Fig. 10–18). They varied considerably with respect to affinity for osmium tetroxide from one cell to another and within the same cell. Within their limiting membrane these lipid vacuoles displayed continuity with peripheral densely osmiophilic material resembling lipochrome pigment and myelin figure material (Fig. 15). In numerous HCG-stimulated cells, lightly staining circular lipid vacuoles were circumscribed by numerous concentric cisternae of smooth endoplasmic reticulum and few cisternae of rough endoplasmic reticulum, both arranged in a whorl-like fashion (Fig. 16 and 17). This cytoplasmic arrangement of lipid vacuoles and endoplasmic reticulum was noted only infrequently in control animals.

Pigment bodies in HCG-stimulated Leydig cells varied in size, shape, and density (Fig. 18–21). Frequently, the limiting membrane of several pigment bodies of different size were in contact with one another (Fig. 19 and 20). In addition, some pigment bodies made contact with the outer membranes of lipid vacuoles (Fig. 11 and 18). The granular matrix and/or onion-skin-like appearance in the pigment bodies (Fig. 20 and 21) was similar in morphologic appearance to myelin and pigment material associated with lipid vacuoles (Fig. 15). The smooth endoplasmic reticulum, displaying dilated bulbous ends, was not seen in stacks in the cytoplasm or in pigment bodies of HCG-stimulated adult Leydig cells. Ribosomes were more frequently encountered in clusters (Fig. 11, 17, and 19). Nuclei displayed peripheral areas of increased density consistent with nucleolar changes (Fig. 18). Centrioles were occasionally observed adjacent to increased numbers of Golgi elements (Fig. 12). Desmosomes previously observed only rarely[2] (Fig. 10, 15, and 16), as well as other organelles or structures not previously observed in the control adult or the unstimulated or HCG-stimulated Leydig cell from immature guinea pigs,[1] were also encountered. These will be described in a subsequent publication.

Discussion

The ultrastructure of the adult Leydig cell with respect to the nucleus and cytoplasmic organelles was similar to that described by others in several species.[2–7] Comparing the ultrastructure of the mature (adult) and the immature guinea pig Leydig cells described in a previous publication,[1] one finds quite notable differences. Such differences in the ultrastructure, therefore, would represent in the mature animal a primary stimulation of the Leydig cell by the animal's endogenous hy-

pophyseal gonadotropins, a secondary response of the Leydig cell to its endogenous androgen production, or both.

In the present experiments, one must keep in mind that in HCG-stimulated animals the resulting ultrastructure of Leydig cells represents a combined effect of both endogenous and exogenous hormones. Since HCG stimulation in the adult also produces ultrastructural changes, this would indicate that normally endogenously stimulated Leydig cells are capable of further responding to hyperstimulation beyond the pharmacologic level. This evidence suggests that some morphologic alterations in the adult HCG-treated animals are due probably to the exogenous stimulation.

The smooth endoplasmic reticulum was increased in density in the control animals, similar to that in the HCG-stimulated immature guinea pigs.[1] The HCG-stimulated adult Leydig cell showed an even greater proliferation of smooth endoplasmic reticulum, an organelle that has been correlated to steroid production by different investigators.[2,4,7-12] Human gonadotropins have also been shown to produce proliferation of Leydig cells and their smooth endoplasmic reticulum in the human.[13,14] The presence of increased and dilated rough endoplasmic reticulum, as well as more abundant polyribosomes, suggests an increase in metabolic activities of hyperstimulated Leydig cells. These observations are consistent with the increment in protein synthesis [15] and increase in ribonucleic acid content of rat interstitial cell as reported by Jarlstedt and Steward.[8] The presence of multiple Golgi elements also is consistent with this hypothesis. Conversely, the elongated giant mitochondria and enlarged mitochondria with star-shaped cristae are probably under control of endogenous hormones, since they were not observed in HCG-stimulated animals, either immature [1] or mature. In all HCG-treated animals, the mitochondria were small but more abundant. Since centrioles were noted in some HCG-stimulated Leydig cells, cellular division probably can be increased by hyperstimulation in the adult. This suggests that Leydig cells per se retain the capability for mitosis. This phenomenon was not observed in the immature HCG-treated guinea pigs [1] and is quite rare in mature guinea pig Leydig cells.[2] Mitotic activity becomes manifest at puberty [16] in the rat Leydig cell, when secretory activity is induced by the animal's own pituitary gonadotropins. Therefore, one can speculate that in the adult this capacity remains dormant and can be stimulated by HCG.

Whorls of myelin figure material and the signet-ring appearance of lipid vacuoles have been noted by others,[2,7,18,19] as well as ourselves.[1] One might suggest that lipid vacuoles associated with myelin figure

material and/or the presence of material similar morphologically to pigment, might be one of several processes whereby pigment bodies are formed. Increase in pigment body size also may be due to the addition of precursor pigment material. The presence of variable-sized —i.e., both small and large—pigment bodies in contact with one another, suggests that coalescence may be a third process of pigment body formation. Therefore, pigment body formation may occur by (1) formation with a lipid vacuole, (2) enlargement in size, and (3) coalescence of several different-sized pigment bodies. The limiting membrane of pigment bodies was double and therefore similar to that of lipid vacuoles. The presence of alkaline phosphatase [14,17] within these increased numbers of pigment bodies supports the view that these organelles may represent residual bodies [17] at some stage of development associated with the increased metabolic activity of HCG-stimulated Leydig cells.

Localization of both lipid vacuoles and pigment bodies frequently in peripheral cytoplasmic areas, as well as the disappearance of the smooth-surfaced parallel membranes displaying dilated bulbous ends within the cytoplasm and pigment bodies, suggest that these organelles may play a role in steroid metabolism. The peripheral location would enable lipid vacuoles and pigment bodies to be involved in the regulatory and secretory processes of steroid release from the cell cytoplasm. Variation in lipid vacuole content, as well as circumscription by cisternae of endoplasmic reticulum, may morphologically represent consumption and/or production of osmiophilic materials within these vacuoles.[1] Similar concentric membranes of endoplasmic reticulum arranged around a central lipid vacuole or inclusion have been described in the adult mouse and guinea pig.[2,7] A role of depot storage of cholesterol has been suggested for this morphologic phenomenon.[7]

Summary

Testicular tissue was obtained from 10 adult guinea pigs (8–9 months old). Five of the animals were given 100 I.U. of HCG daily for 15 days; the other 5 were given injections of normal saline and were used as controls. Light microscopy revealed an increase in size and number of Leydig cells following hyperstimulation with HCG. Electron microscopy showed the presence of increased smooth endoplasmic reticulum, rough endoplasmic reticulum, and Golgi elements. A relationship between pigment bodies and lipid vacuoles is described and discussed. The ultrastructural observations in exogenously stimulated Leydig cells indicate a potential for a considerable increase in metabolic activity.

References

1. MERKOW, L., ACEVEDO, H. F., SLIFKIN, M., and CAITO, B. J. Studies on the interstitial cells of the testis. I. The ultrastructure in the immature guinea pig and the effect of stimulation with human chorionic gonadotropin. *Amer J Path* 53:47–61, 1968.
2. CHRISTENSEN, A. K. The fine structure of testicular interstitial cells in guinea pigs. *J Cell Biol* 26:911–935, 1965.
3. CHRISTENSEN, A. K. The fine structure of interstitial tissue of the rat testis at various ages and after experimental treatment. (Abst.). *Anat Rec* 133:367–368, 1959.
4. CHRISTENSEN, A. K., and FAWCETT, D. W. The normal fine structure of opossum testicular interstitial cells. *J Biophys Biochem Cytol* 9:653–670, 1961.
5. NISHIMURA, R., and KONDO, I. Morphological study on the interstitial tissue of the testicles. *Urol Int* 18:1–24, 1964.
6. YAMADA, E. Some observations on the fine structure of the interstitial cell in the human testis as revealed by electron microscopy. *Gunma Symp Endocr* 2:1–17, 1965.
7. CHRISTENSEN, A. K., and FAWCETT, D. W. The fine structure of testicular interstitial cells in mice. *Amer J Anat* 118:551–571, 1966.
8. JARLSTEDT, J., and STEWARD, V. W. Content of ribonucleic acid in rat interstitial cells at different ages. *Endocrinology* 82:1063–1065, 1968.
9. CHRISTENSEN, A. K., and MASON, N. R. Comparative ability of seminiferous tubules and interstitial tissue of rat testes to synthesize androgens from progesterone-4-^{14}C in vitro. *Endocrinology* 76:646–656, 1965.
10. FAWCETT, D. W., and BURGOS, M. H. Studies on the fine structure of the mammalian testis. II. The human interstitial tissue. *Amer J Anat* 107:245–269, 1960.
11. CONNELL, G. M., CONNELL, C. J., and EIK-NES, K. B. Testosterone synthesis by the two-day-old chick testis in vitro. *Gen Comp Endocr* 7:158–165, 1966.
12. CHRISTENSEN, A. K. Fine structure of steroid-secreting cells. Second International Congress on Hormonal Steroids, Milan, 1966, pp. 179–180.
13. SMITH, B. D., LEESON, C. R., and BUNGE, R. G. Microscopic appearance of the testis in Klinefelter's syndrome before and after suppression of gonadotrophin production with testosterone. *Invest Urol* 5:58–72, 1967.
14. DE KRETSER, D. M. Changes in the fine structure of the human testicular interstitial cells after treatment with human gonadotropins. *Z Zellforsch* 83: 344–358, 1967.
15. STAEHELIN, T., BRINTON, C. C., JR., WETTSTEIN, F. O., and NOLL, H. Structure and function of E. coli ergosomes. *Nature (London)* 199:865–870, 1963.
16. NIEMI, M., and IKONEN, M. Histochemistry of the Leydig cells in the postnatal prepubertal testis of the rat. *Endocrinology* 72:443–448, 1963.
17. FRANK, A. L., and CHRISTENSEN, A. K. Localization of acid phosphatase in lipofuscin granules and possible autophagic vacuoles in interstitial cells of the guinea pig testis. *J Cell Biol* 36:1–13, 1968.
18. CARR, I., and CARR, J. Membranous whorls in the testicular interstitial cell. *Anat Rec* 144:143–147, 1962.

19. CRABO, B. Fine structure of the interstitial cells of the rabbit testes. Z *Zellforsch* 61:587–604, 1963.

The authors are deeply indebted to Dr. Robert C. Grauer for his constructive criticism in the preparation of the manuscript. We also wish to thank Miss Rachel Trivuncich, Miss Margaret Lischner, and Mr. Thomas Snell for the photographic reproductions, and Mrs. Joan Weissman and Miss Stella Marie Panza for secretarial assistance.

Legends for Figures

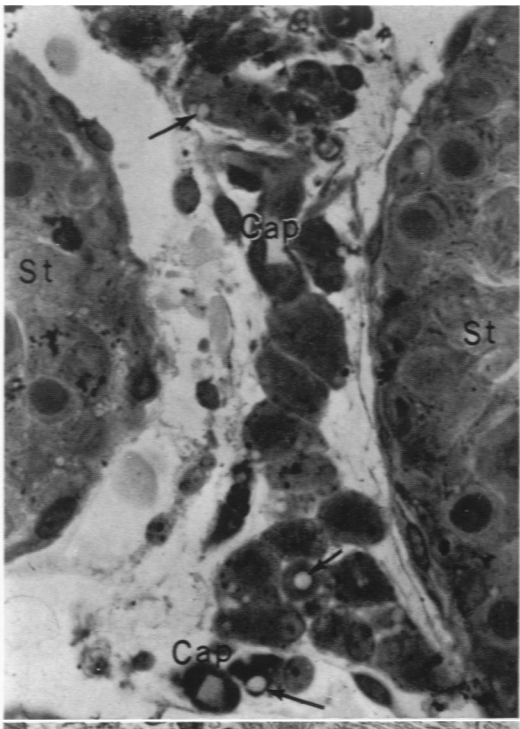
Figures 1 and 9 represent tissue embedded in Epon and stained with methylene blue. Figures 2–8 and 10–21 represent material embedded in Epon and stained with uranyl acetate and lead citrate.

Fig. 1. A 1-μ thick section of a cluster of interstitial cells of Leydig situated between two seminiferous tubules *(St)*. Several interstitial cells contained multiple intracytoplasmic vacuoles *(arrows)*. Small capillaries *(Cap)* are associated with the Leydig cells. × 400.

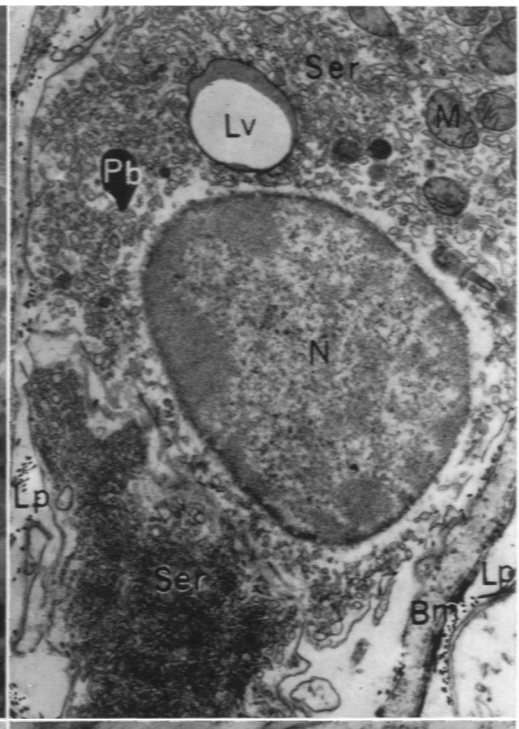
Fig. 2. Interstitial cell from adult guinea pig. Low magnification reveals abundant smooth endoplasmic reticulum *(Ser)* occupying most areas of cell cytoplasm. Cisternae are quite dense in lower portion of figure. Lipid vacuole *(Lv)*, mitochondria *(M)*, nucleus *(N)*, and pigment body *(Pb)* are present. Basement membrane *(Bm)* and collagen of lamina propria *(Lp)* can be seen along periphery of this cell. × 8000.

Fig. 3. Interstitial cell from adult guinea pig. Abundant smooth endoplasmic reticulum *(Ser)* is arranged in parallel elongated cisternae. Pigment body *(Pb)* at center displays lightly osmiophilic material representing a lipid vacuole *(Lv)*. At upper left, another pigment body displays a granular matrix and several more dense regions. A small pigment body is also noted at upper right *(arrow)*. Mitochondria *(M)*. × 10,500.

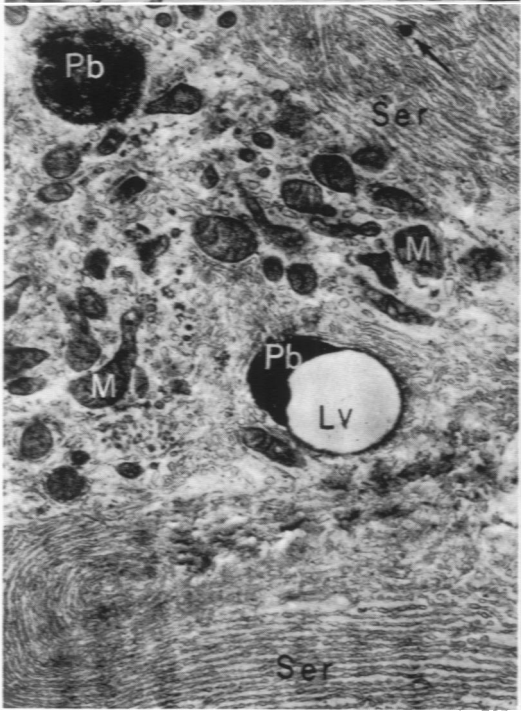
Fig. 4. Interstitial cell from adult guinea pig containing numerous pigment bodies *(Pb)*. These organelles display a dense region, granular area, and suggestion of a whorled structure *(arrow)*. Mitochondria *(M)* are adjacent to large lipid vacuole *(Lv)*. Solitary cisternae of rough endoplasmic reticulum *(Rer)* is at bottom. Nuclear envelope *(Ne)* shows characteristic "pores" *(arrow)* in transverse plane of section. Nucleus *(N)*. × 18,000.


St
Cap
St
Cap

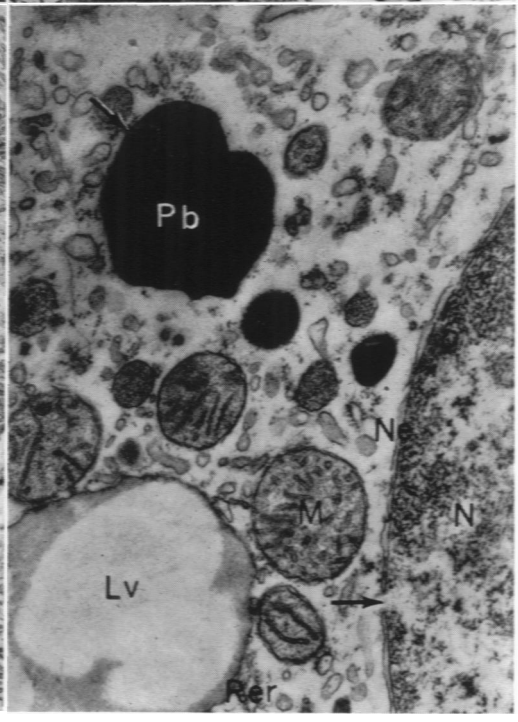
1


Ser
Lv
M
Pb
N
Lp
Ser
Lp
Bm

2


Pb
Ser
M
Pb
M
Lv
Ser

3


Pb
Ne
M
N
Lv
Rer

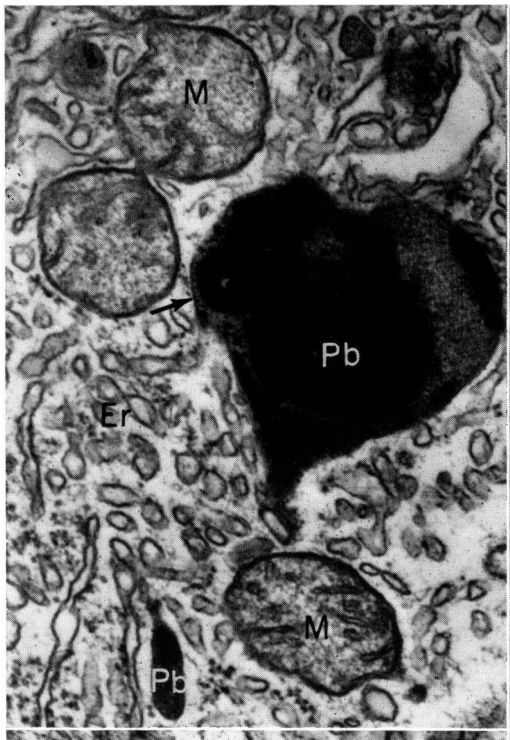
4

Fig. 5. Interstitial cell containing two pigment bodies *(Pb)* between cisternae of endoplasmic reticulum *(Er)*. Note that the larger pigment body contains a less dense region which displays a structured or crystalline pattern. One of the more dense regions *(arrow)* is circular and is suggestive of a myelin figure. Mitochondria *(M)*. × 32,800.

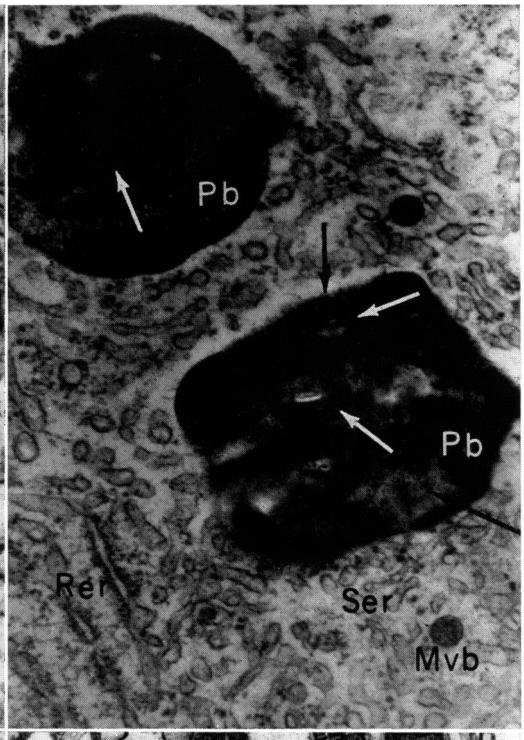
Fig. 6. Interstitial cell containing two pigment bodies *(Pb)*. Higher magnification shows these organelles to contain well-oriented delicate membranes in whorl formation resembling myelin figures *(arrows)*, granular background, and limiting membrane. Surrounding cytoplasm contains smooth endoplasmic reticulum *(Ser)*, rough endoplasmic reticulum *(Rer)*, and a multivesicular body *(Mvb)*. × 32,000.

Fig. 7. Several mitochondria *(M)* in adult interstitial cell. Cristae appear swollen and fused, displaying a star-like configuration regardless of the plane of section. At lower left, several cisternae of smooth endoplasmic reticulum *(SER)* are arranged in parallel and display bulbous dilatation of each end *(arrows)*. × 22,800.

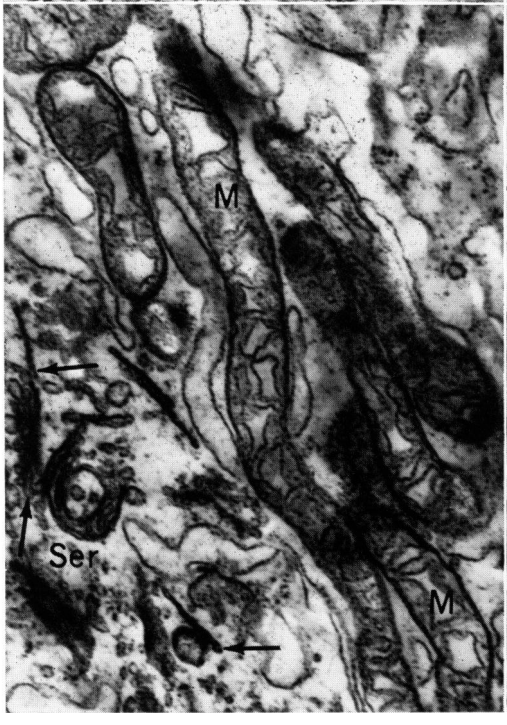
Fig. 8. Giant, elongated, rod-shaped mitochondria *(M)* within interstitial cell from adult guinea pig exhibit short lamelliform cristae. Adjacent pigment body *(Pb)* contains a lighter osmiophilic vacuole *(V)*, several dense circular regions, numerous delicate membranes or fibrils displaying bulbous dilatation of their distal ends *(arrows)*, and a granular background. × 34,000.



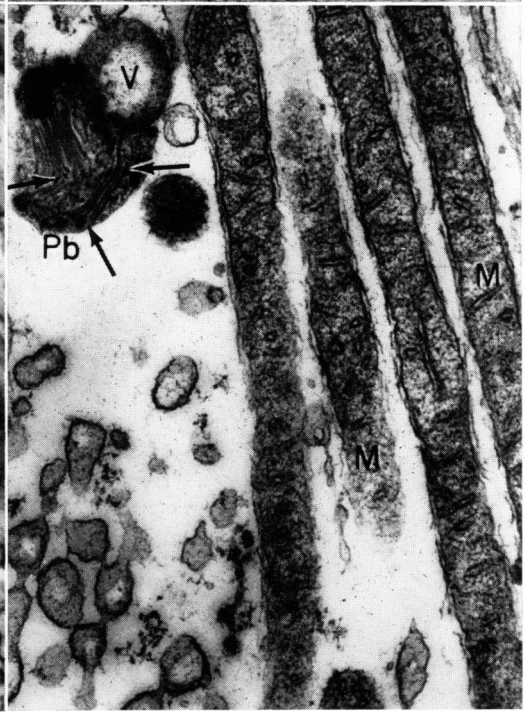
5



6



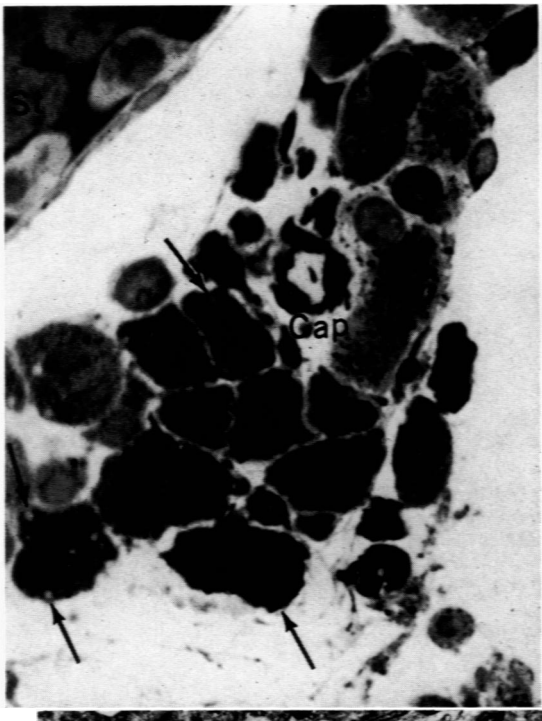
7



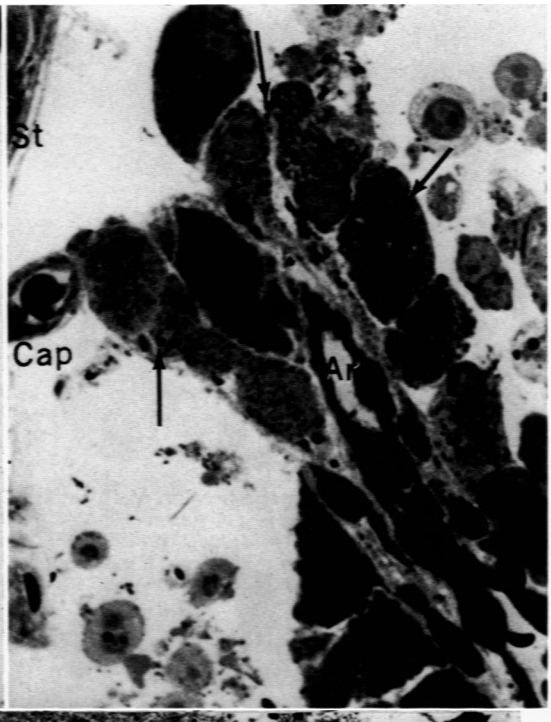
8

Fig. 9. A 1-μ thick section of clusters of interstitial cells of Leydig situated between seminiferous tubules *(St)* from an HCG-stimulated adult testis. **A.** Numerous darker staining Leydig cells contain small vacuoles *(arrows)*. A capillary *(Cap)* is evident. Nucleoli are prominent in several nuclei. × 800. **B.** An arteriole *(Ar)* and a capillary are interspersed or adjacent, respectively, to a cluster of Leydig cells. The small, dark, round cytoplasmic bodies *(arrows)* near periphery of these cells probably represent pigment bodies. × 800.

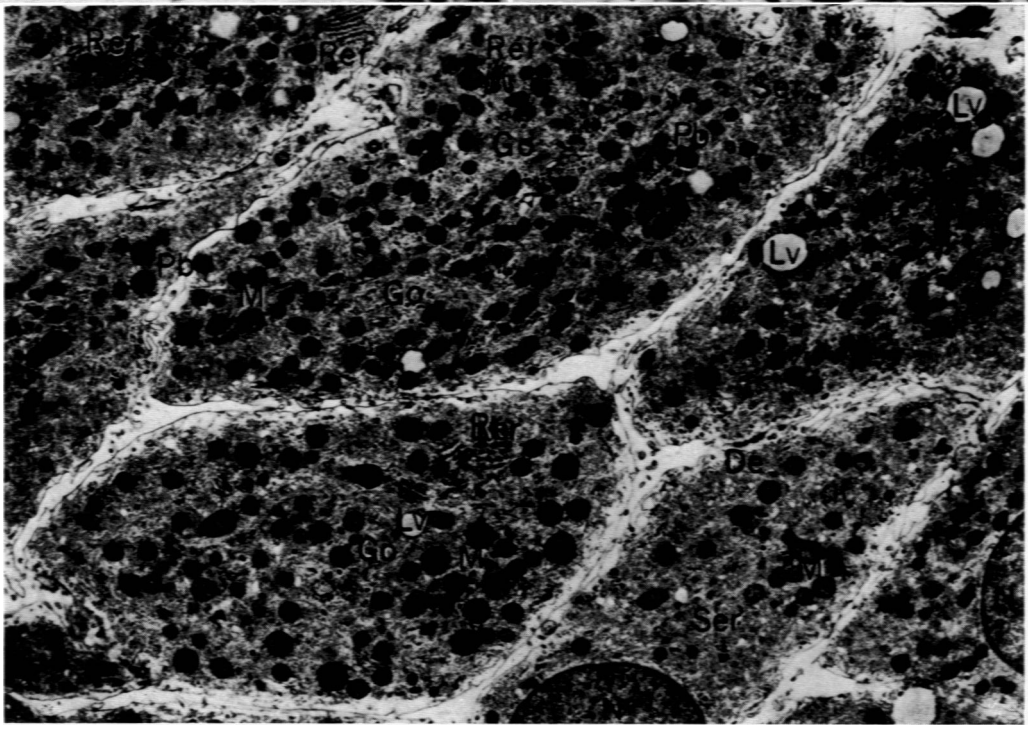
Fig. 10. HCG-stimulated interstitial cells in adult guinea pig. Hyperplastic interstitial cells contain increased rough endoplasmic reticulum *(Rer)*. Golgi complex *(Go)* is prominent. Note that cytoplasmic lipid vacuoles *(Lv)* and pigment bodies *(Pb)* are nearly always arranged in a peripheral location. Smooth endoplasmic reticulum *(Ser)* is also increased. Mitochondria *(M)* are small and uniform. A desmosome *(De)* and two nuclei *(N)* are at lower right. × 3200.



9A



9B

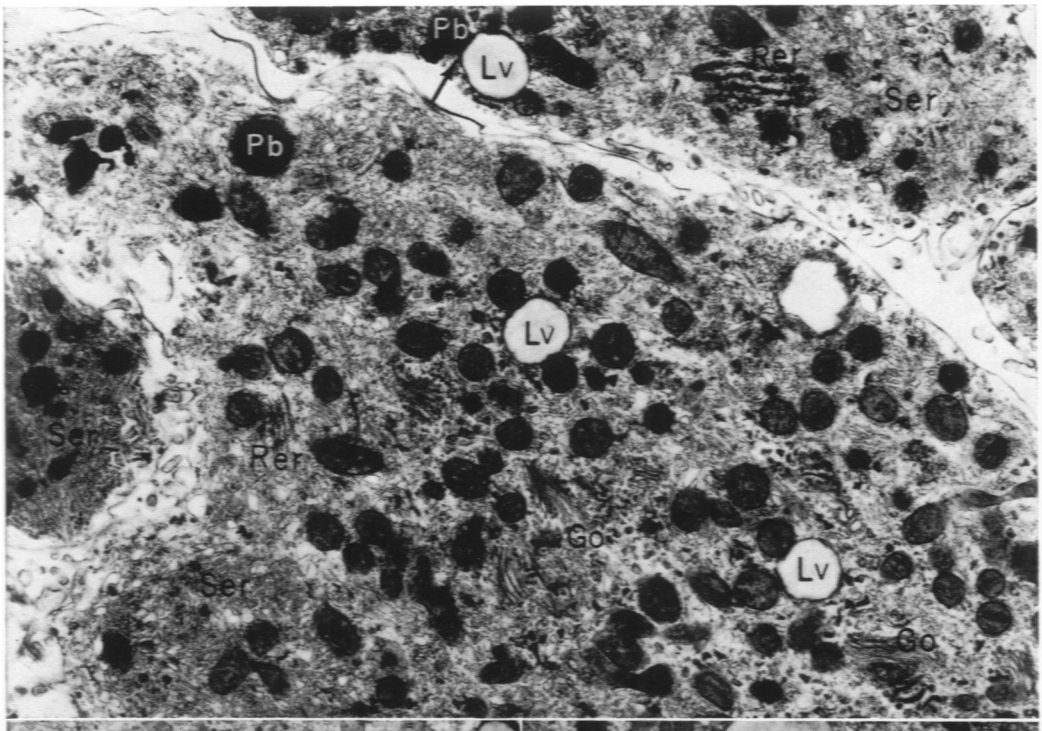


10

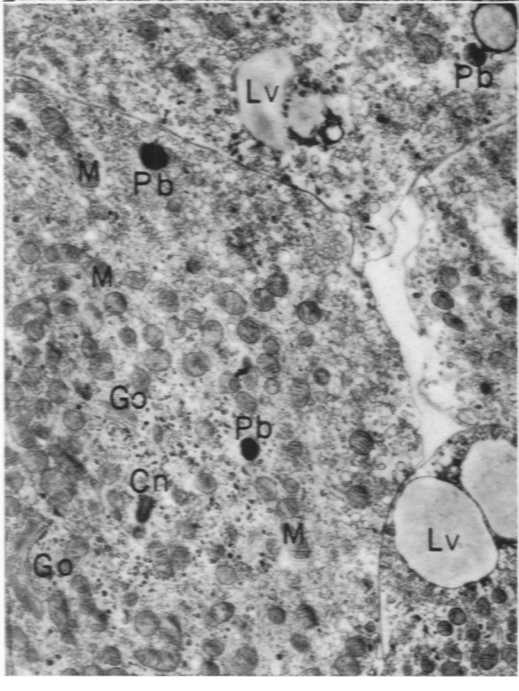
Fig. 11. Higher magnification of HCG-stimulated interstitial cell from adult guinea pig. Rough endoplasmic reticulum *(Rer)*, lipid vacuoles *(Lv)*, and prominent Golgi complexes *(Go)* are evident. Smooth endoplasmic reticulum *(Ser)* and pigment bodies *(Pb)* also appear increased. Lipid vacuoles and pigment bodies tend to be situated in a peripheral location; at top of figure a pigment body is in contact with a lipid vacuole *(arrow)*. × 6600.

Fig. 12. Portions of four interstitial cells of Leydig from HCG-stimulated adult guinea pig. Lipid vacuoles *(Lv)* and pigment bodies *(Pb)* are arranged in the peripheral cytoplasm. Golgi complex *(Go)* is prominent and associated with a centriole *(Cn)*. Mitochondria *(M)* are small and uniform. × 5000.

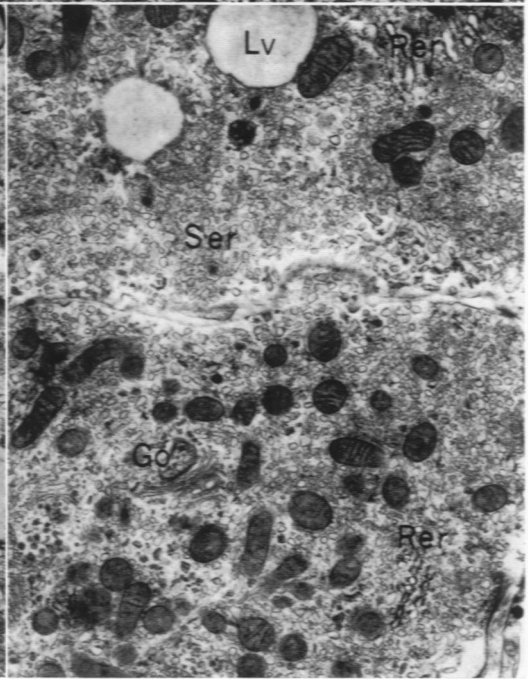
Fig. 13. Two Leydig cells from HCG-stimulated adult guinea pig. Rough endoplasmic reticulum *(Rer)* is increased and slightly dilated. Golgi complex *(Go)*, lipid vacuoles *(Lv)*, and smooth endoplasmic reticulum *(Ser)* are evident. × 7500.


Pb
Lv
Rer
Ser
Pb
Lv
Ser
Rer
Go
Lv
Ser
Go

11


Lv
Pb
M
Pb
M
Go
Pb
Cn
M
Lv
Go

12


Lv
Rer
Ser
Go
Rer

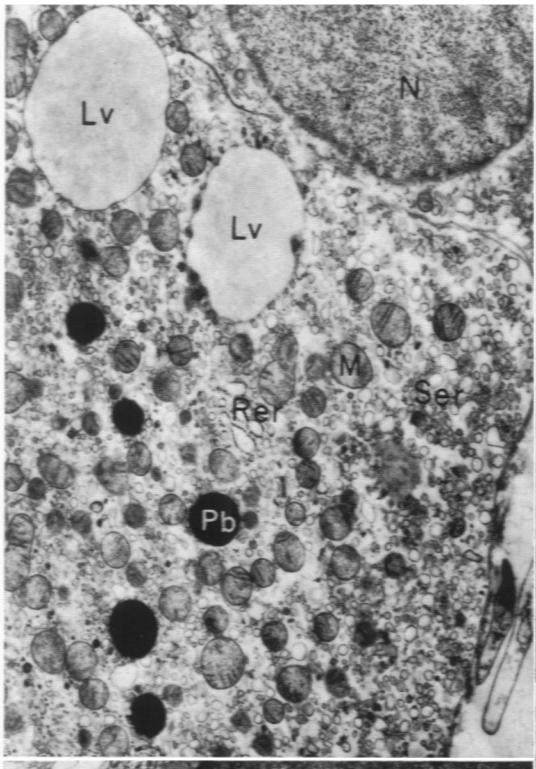
13

Fig. 14. Two Leydig cells from HCG-stimulated adult guinea pig. Two lipid vacuoles *(Lv)* and several pigment bodies *(Pb)* are situated near periphery of lower cell. Note that the rough endoplasmic reticulum *(Rer)* is slightly dilated. Mitochondria *(M)* are small, and smooth endoplasmic reticulum *(Ser)* is vesicular. Nucleus *(N)* × 6000.

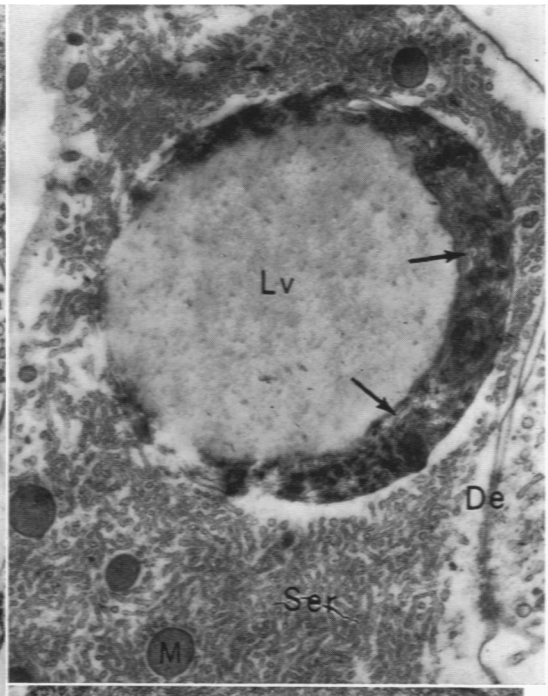
Fig. 15. Two Leydig cells from HCG-stimulated adult guinea pig. The large lipid vacuole *(Lv)* is moderately osmiophilic and contains a peripheral region of delicate fibrils *(arrows)* within its limiting membrane. The filamentous structures resemble myelin and the more dense osmiophilia is consistent with pigment. Adjacent smooth endoplasmic reticulum *(Ser)* is dense. A desmosome *(De)* forms an attachment between these two Leydig cells. Mitochondria *(M)*. × 13,000.

Fig. 16. Three Leydig cells of HCG-stimulated adult guinea pig. Several lipid vacuoles *(Lv)* are circumscribed by whorls of endoplasmic reticulum *(Er)*. Adjacent pigment bodies *(Pb)*, mitochondria *(M)*, and Golgi complex *(Go)* are evident. Nucleus *(N)* and desmosome *(De)* are also present. × 7000.

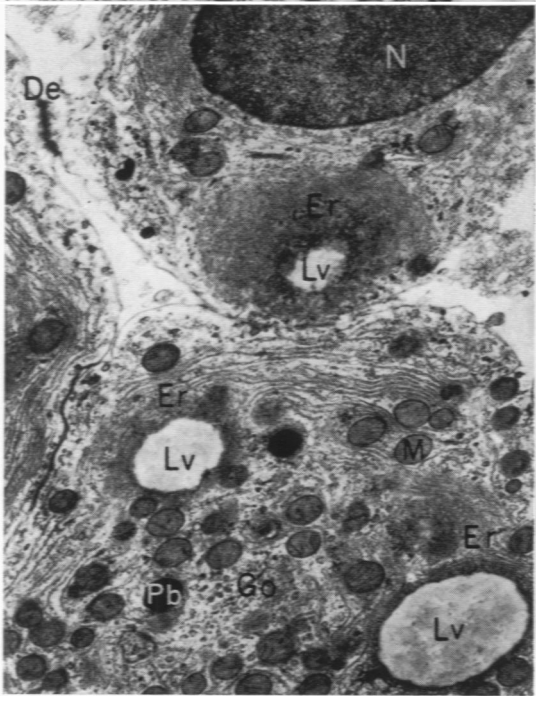
Fig. 17. Higher magnification of a field similar to that in Fig. 16. Two lipid vacuoles *(Lv)* are circumscribed by many cisternae of smooth endoplasmic reticulum *(Ser)* and several cisternae of rough endoplasmic reticulum *(Rer)* in a whorl-like fashion. Pigment body *(Pb)*, mitochondria *(M)*, cluster of ribosomes *(Rnp)*, and nucleus *(N)* are present. × 13,000.


Lv
N
Lv
M
Rer
Ser
Pb

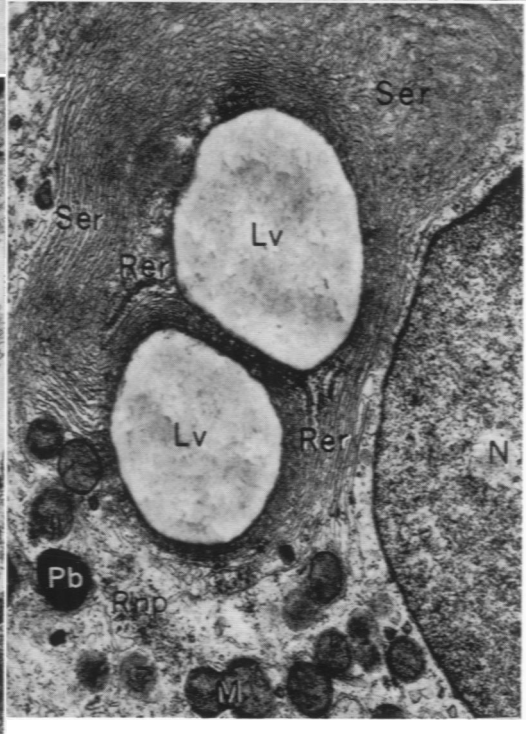
14


Lv
De
Ser
M

15


N
De
Er
Lv
Er
Lv
M
Er
Pb
Go
Lv

16


Ser
Ser
Lv
Rer
Lv
Rer
N
Pb
Rnp
M

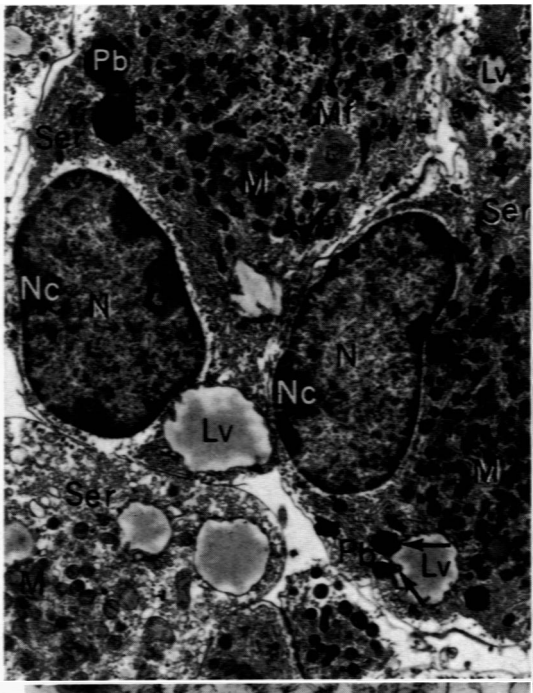
17

Fig. 18. Several HCG-stimulated Leydig cells in adult guinea pig. Lipid vacuoles *(Lv)* and several pigment bodies *(Pb)* are arranged in peripheral location. Abundant smooth endoplasmic reticulum *(Ser)* is dense. A myelin figure *(Mf)* is noted. Several pigment bodies are in contact with limiting membrane of lipid vacuole *(arrow)*. Increased density of nucleoli *(Nc)* is evident in the nuclei *(N)*. Mitochondria *(M)*. × 3200.

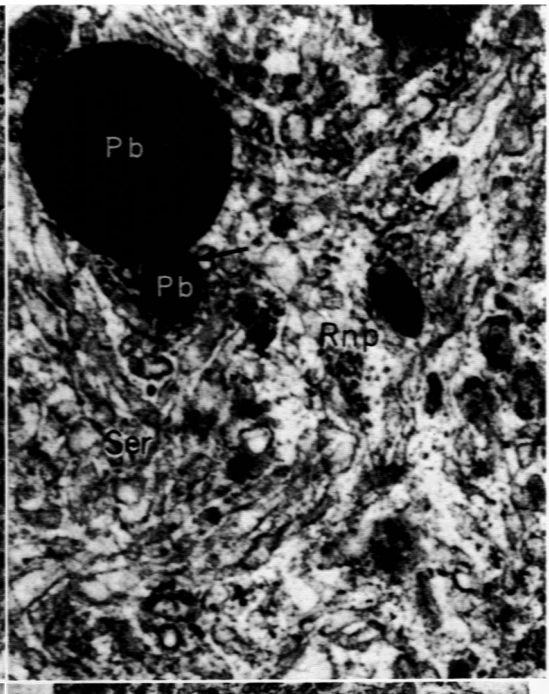
Fig. 19. HCG-stimulated adult guinea pig. Two variable-sized circular pigment bodies *(Pb)* display contact of their limiting membranes *(arrow)*. Several clusters of ribosomes *(Rnp)* and cisternae of smooth endoplasmic reticulum *(Ser)* are evident. × 41,000.

Fig. 20. Large pigment body *(Pb)* displays contact with smaller pigment body *(arrow)*. Larger pigment body shows a double limiting membrane, granular matrix, and a more osmiophilic myelin figure (on the right half). The pigment body on the left also displays myelin figure material. Ribosomes *(Rnp)* and vesicles of smooth endoplasmic reticulum *(Ser)* are also evident. × 18,000.

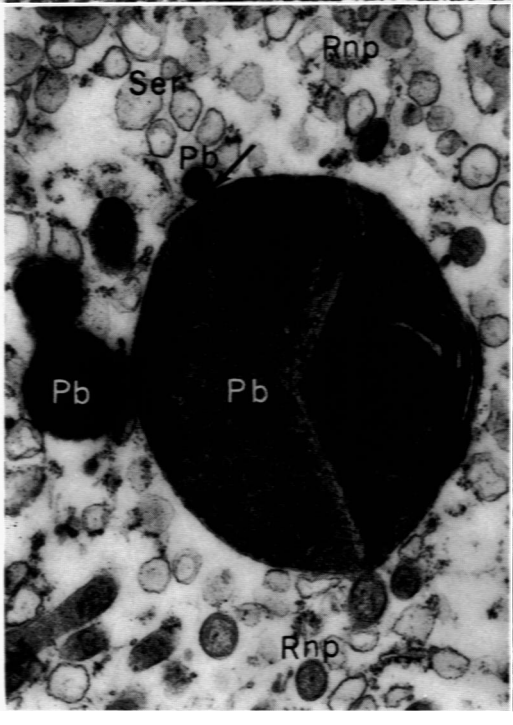
Fig. 21. Pigment body *(Pb)* displays myelin figure material and dense osmiophilia. Lipid vacuole *(Lv)* and rough endoplasmic reticulum *(Rer)* are present. Mitochondria *(M)* are quite small and smooth endoplasmic reticulum *(Ser)* abundant. × 16,000.


Pb
Lv
Mi
Ser
M
Ser
Nc
Nl
N
Nc
Lv
M
Ser
Lv
M

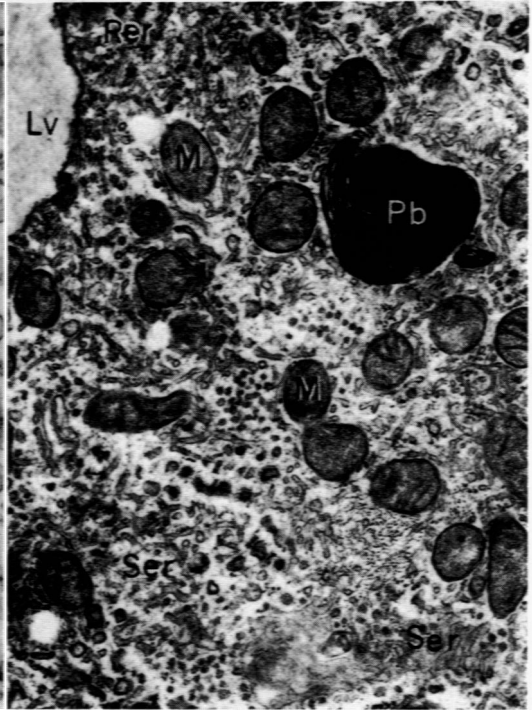
18


Pb
Pb
Rnp
Ser

19


Rnp
Ser
Pb
Pb
Pb
Rnp

20


Rer
Lv
M
Pb
M
Ser
Ser

21