Studies on the Interstitial Cells of the Testis

III. The Ultrastructure in the Immature Mongolian Gerbil and the Effect of Stimulation with Human Chorionic Gonadotropin

Leonard Merkow, MD, PhD, Hernan F. Acevedo, PhD, Malcolm Slifkin, PhD, and Matias Pardo

In recent years, the Mongolian gerbil has become a useful experimental animal.[1,2] This desert rat is a unique animal since, unlike other rodents and man, its main adrenal steroid is 19-hydroxy-11-deoxycortisol.[3] Because of this species difference, and as an outgrowth of previous studies on the guinea pig,[4,5] we initiated an investigation on the ultrastructure of the interstitial cells of Leydig and alterations produced following administration of human chorionic gonadotropin (HCG). This communication describes the morphology of the immature gerbil Leydig cell and also compares its ultrastructure to that of the immature guinea pig in both stimulated and unstimulated animals.

Materials and Methods

Testicular tissue was obtained from 16 5-week-old Mongolian gerbils (*Meriones unguiculatus*) sacrificed by decapitation.* One half of the animals were injected intraperitoneally with 100 IU of HCG daily for 15 days. Control animals were inoculated with normal saline. Their average weight was 20 g at the beginning of the experiment (3 weeks old) and 35 g at the end. One-mm cubes 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 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 Resin 812. One-μ thick sections were prepared and stained with methylene blue. Clusters of Leydig cells were identified between seminiferous tubules in these sections with the light microscope. Ultrathin sections displaying silver or gold interference colors were obtained with a Porter-Blum MT-1 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.

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

Presented in part at the Sixty-sixth Annual Meeting of the American Association of Pathologists and Bacteriologists, San Francisco, Cal, March 9–11, 1969.

Accepted for publication August 25, 1969.

Address for reprint requests: Dr. Robert C. Grauer, Director, Singer Memorial Research Institute, Allegheny General Hospital, East North Ave, Pittsburgh, Pa 15212.

* Obtained from Dr. M. C. Chang, Worcester Foundation, Shrewsbury, Massachusetts, and the Colonial Pet Shop, Waukesha, Wisconsin.

Results

Light Microscopy

In all control (unstimulated) animals small clusters of Leydig cells within a relatively large interstitial space were associated with capillaries as seen in paraffin-embedded sections (Fig 1). Seminiferous tubules either lacked or displayed a small lumen and did not contain spermatozoa in control animals (Fig 1). Within animals stimulated with HCG, large clusters of Leydig cells filled and obliterated the potential interstitial spaces (Fig 2). Both hyperplasia and hypertrophy of interstitial cells of Leydig were evident in all stimulated animals (Fig 2). Seminiferous tubules were larger than in control animals and contained spermatozoa (Fig 2).

In unstimulated animals, 1-μ sections of Epon-embedded testis showed that Leydig cells were mainly of a "light" cell type, and infrequently displayed many vacuoles (Fig 3). Following stimulation, both "light" and "dark" Leydig cells were evident, the latter predominating (Fig 4). In addition, most of these dark Leydig cells displayed small, dark-staining cytoplasmic bodies (Fig 4).

Electron Microscopy

The most striking feature of unstimulated Leydig cells within the gerbil was the presence of numerous lightly osmiophilic staining scalloped or irregularly shaped lipid vacuoles occupying a considerable area of the cytoplasm (Fig 5). Usually, a well developed perinuclear Golgi complex was in close proximity to the multitude of lipid vacuoles (Fig 5). Vesicular or tubular smooth endoplasmic reticulum (Fig 5) and rare cisternae of nondilated rough endoplasmic reticulum were noted. Small mitochondria with lamelliform cristae were evident at the periphery of a cluster of lipid vacuoles and between vesicles of smooth endoplasmic reticulum (Fig 5). Annulate lamellae were very rarely observed within the cytoplasm of unstimulated Leydig cells.

Most unstimulated Leydig cells showed infrequent small or medium-sized pigment bodies consisting of a homogeneously dense matrix surrounded by a double limiting membrane and often associated with a small lipid vacuole (Fig 6). Interdigitations of the plasmalemma were observed occasionally between adjacent Leydig cells (Fig 6). Rarely, a vacuole containing solid rod-shaped structures in a transverse plane of section was noted, usually in proximity to a lipid vacuole (Fig 7).

The most outstanding cytostructural alteration within stimulated Leydig cells was the prominence of lipid bodies (Fig 8–10). These

membrane-bound organelles assumed different types of surface contour although, for the most part, they displayed an irregular or scalloped border (Fig 9 and 10). Where smooth endoplasmic reticulum circumscribed lipid bodies, a more circular, though still slightly irregular configuration, was noted (Fig 8). These lipid bodies were quite homogeneous in density and displayed a slightly lighter central region (Fig 8 and 9).

Individual Leydig cells displayed an increase in smooth endoplasmic reticulum (Fig 8), clusters of parallel-arrayed rough endoplasmic reticulum (Fig 9), and more numerous Golgi complexes (Fig 12). Dense lipid bodies were associated with the increased parallel-arrayed rough endoplasmic reticulum (Fig 9).

Increased numbers of annulate lamellae were evident within the cytoplasm of stimulated Leydig cells (Fig 10 and 11). These annulate lamellae were larger and individual lamella displayed dilated lateral margins (Fig 10 and 11). Direct continuity between this organelle and both rough and smooth endoplasmic reticulum was evident (Fig 10). Occasionally, the more distal connection with endoplasmic reticulum circumscribed mitochondria (Fig 10).

Centrioles and increased numbers of enlarged pigment bodies were observed within HCG-stimulated Leydig cells (Fig 12). These enlarged pigment bodies displayed a granular matrix associated with focal and mottled dense areas (Fig 12). This organelle assumed an irregular configuration depending upon the plane of section and frequently displayed well-formed myelin figure material replacing most of the matrix in a fingerprint-like pattern (Fig 13). Small vacuoles were evident in some pigment bodies adjacent to myelin figure material (Fig 14). In an occasional stimulated Leydig cell, large foci of nonrosette polysomes of glycogen were observed (Fig 15). The plasmalemma was more frequently noted to interdigitate or form complex folds between adjacent Leydig cells (Fig 16). Large cytoplasmic myelin figures surrounding lipid vacuoles were present within endothelial cells of capillaries adjacent to Leydig cells (Fig 17).

Discussion

Although the guinea pig and gerbil are both rodents and their Leydig cell ultrastructure in the unstimulated animals appears similar, there are prominent differences between these two species following stimulation by HCG.

It is evident that exogenous administration of HCG produces marked ultrastructure alterations within the Leydig cell in both species. These

alterations can be ascribed to a primary effect of HCG or a secondary effect due to biochemical changes within the Leydig cell resulting from exogenous stimulation.[4] In contrast to the guinea pig, HCG causes maturation of the germ cells in the immature gerbil.

Morphologically, by light as well as electron microscopy, the alterations produced by HCG on such organelles as the lipid vacuoles were strikingly different from that of the immature guinea pig.[4] The dark appearance of cells observed by light microscopy in the immature gerbil was probably due to increased numbers of osmiophilic lipid bodies and increased endoplasmic reticulum. Lipid vacuoles in the gerbil became more prominent and dense rather than less osmiophilic as in the guinea pig.[4] In addition, the plasmalemma was found to assume a more complex arrangement between adjacent Leydig cells than in nonstimulated animals. This morphologic arrangement may be of physiologic importance since it could facilitate and/or enhance exchange or transport of intercellular materials.

HCG stimulation increased the number of annulate lamellae. This organelle of an obscure nature and function has been found in normal and abnormal cells of several endocrine glands.[6-12] It has been reported to be more often attached or adjacent to the nuclear envelope in the primordial human follicle when estrogen production was dominant in the normal menstrual cycle.[8] Annulate lamellae have been encountered under certain specific conditions—such as during the period of rapid cell division[7] (proliferative phase) or during the secretory period if there is hyperestrogenism[6] in glandular cells of the endometrium. This suggests that their appearance may be a secondary effect mediated through hormones elicited by gonadotropins. Clusters of glycogen particles also were observed in the stimulated gerbil, but not in the guinea pig. These alterations produced by exogeneous HCG, as well as the production of myelin figures in the capillary endothelial cells adjacent to stimulated Leydig cells, suggest that biochemical as well as morphologic differences within testicular tissue exist between the gerbil and guinea pig.

The nonspecific response of increased smooth endoplasmic reticulum is a prominent alteration noted in HCG-stimulated gerbil Leydig cells. This increase in smooth endoplasmic reticulum, however, is probably not relative since it does not represent a degranulation of rough endoplasmic reticulum. Moreover, we also observed a concomitant increase in the rough endoplasmic reticulum in HCG-stimulated Leydig cells. The increased quantity of smooth endoplasmic reticulum in Leydig cells has also been reported to be most striking following gonadotropin

stimulation in man [13] and in rat.[14] The notable propinquity of endoplasmic reticulum to osmiophilic lipid bodies in the stimulated animal may represent an increased biochemical relationship and function between these organelles.

Recently, Pedersen and Larsen [15] have demonstrated ultrastructural changes in the human luteal cells during the first trimester of pregnancy, a period in which the ovary is under strong stimulation by HCG produced by the trophoblast. Such changes characterized by hypertrophy of luteal cells, proliferation of the smooth endoplasmic reticulum and Golgi, and the formation of myelin figures are similar to our observations in HCG stimulated Leydig cells.

Since the main adrenal steroid produced in the gerbil is 19-hydroxy-11-deoxycortisol, it is possible that the androgenic biosynthetic pathways at the testicular level could also be different in this animal leading to the production of steroids other than testosterone and 4-Androstenedione. Such a difference could account for the morphologic alterations observed in the gerbil but not in the guinea pig.

Summary

Tissue was obtained from the testes of 16 5-week-old Mongolian gerbils. Eight animals were administered 100 IU of HCG daily for 15 days. The other 8 control animals were injected with normal saline.

Both light and electron microscope studies revealed alterations in stimulated animals when compared to controls. A light type of Leydig cell predominated in control animals, whereas a light or dark Leydig cell was manifest in stimulated testis as observed in Epon-embedded sections, with the dark type predominating.

Several striking ultrastructural differences were observed in HCG-stimulated gerbil Leydig cells when compared to corresponding guinea pigs. The most outstanding feature was the presence of densely osmiophilic lipid vacuoles in sufficient quantity to produce a dark type Leydig cell as observed by light microscopy. Annulate lamellae were noted with considerably increased frequency in stimulated gerbils. Differing morphologic alterations in stimulated gerbils when compared to the guinea pig may be accounted for by the possible presence of a different testicular biosynthetic pathway for androgen production.

References

1. Marston, J. H., and Chang, M. C. The breeding, management and reproductive physiology of the Mongolian gerbil (*Meriones unguiculatus*). *Lab Anim Care* 15:34–48, 1965.

2. RICH, S. T. The Mongolian gerbil (*Meriones unguiculatus*) in research. *Lab Anim Care 18*:235–243, 1968.
3. OLIVER, J. T., and PERON, F. G. 19-hydroxy-11-deoxycortisol, a major steroid secreted by the adrenal gland of the Mongolian gerbil. *Steroids 4*:351–363, 1964.
4. 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*:47–62, 1968.
5. MERKOW, L., ACEVEDO, H. F., SLIFKIN, M., and PARDO, M. 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. *Amer J Path 53*:989–1007, 1968.
6. ANCLA, M., and DEBRUX, J. Occurrence of intranuclear tubular structures in the human endometrium during the secretory phase and annulate lamellae in hyperestrogenic states. *Obst Gynec 26*:23–33, 1965.
7. CAVAZOS, F., GREEN, J. A., HALL, D. G., and LUCAS, F. V. Ultrastructure of the human endometrial glandular cell during the menstrual cycle. *Amer J Obst Gynec 99*:833–854, 1967.
8. HERTIG, A. T., and ADAMS, E. C. Studies on the human oocyte and its follicle. I. Ultrastructure and histochemical observations on the primordial follicle stage. *J Cell Biol 34*:647–675, 1967.
9. HERTIG, A. T. The primary human oocyte: Some observations on the fine structure of Balbiani's vitelline body and the origin of annulate lamellae. *Amer J Anat 122*:107–138, 1968.
10. MARSHALL, R. B., ROBERTS, D. K., and TURNER, R. A. Adenomas of the human parathyroid. Light and electron microscopic study following selenium 75 methionine scan. *Cancer 20*:512–524, 1967.
11. ROSS, M. H. Annulate lamellae in the adrenal cortex of the fetal rat. *J Ultrastruct Res 7*:373–382, 1962.
12. HARRISON, G. A. Some observations on the presence of annulate lamellae in alligator and sea gull adrenal cortical cells. *J Ultrastruct Res 14*:158–166, 1966.
13. DEKRETSER, D. M. Changes in the fine structure of the human testicular interstitial cells after treatment with human gonadotropins. *Z Zellforsch 83*: 344–358, 1967.
14. VILAR, O., STEINBERGER, A., and STEINBERGER, E. An electron microscopic study of cultured rat testicular fragments. *Z Zellforsch 78*:221–233, 1967.
15. PEDERSEN, P. H., and LARSEN, J. F. The ultrastructure of the human granulosa lutein cell of the first trimester of gestation. *Acta Endocr 58*:481–496, 1968.

[*Illustrations follow*]

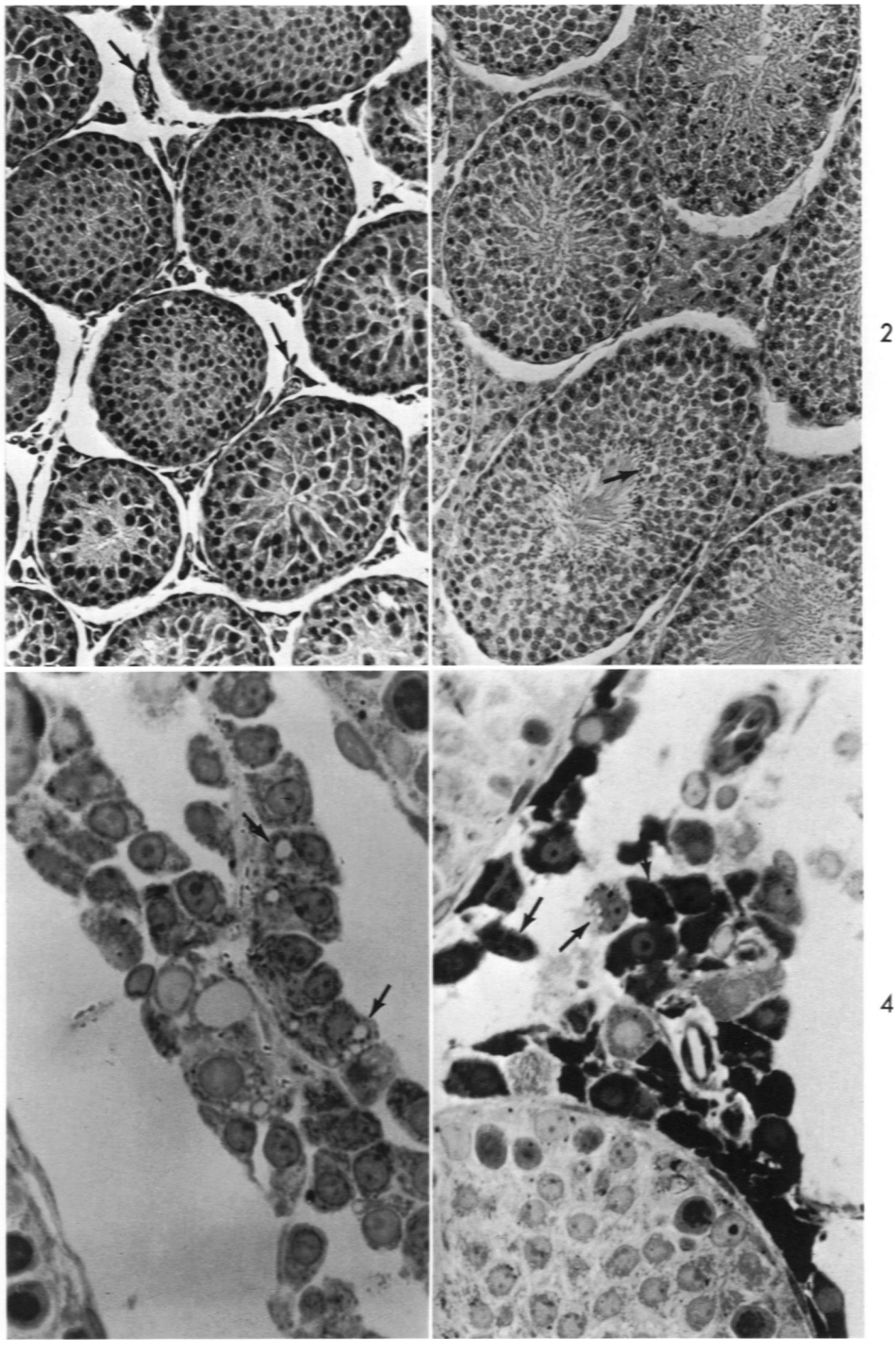
Legends for Figures

Fig 1. Testicular tissue from 5-week-old unstimulated control Mongolian gerbil. Seminiferous tubules are small, usually lack lumen, and do not contain spermatozoa. Small groups of Leydig cells and associated capillary *(arrows)* are in intertubular regions. Leydig cells are usually in close proximity to basement membrane of tubules. H & E. × 168.

Fig 2. Testicular tissue from 5-week-old gerbil stimulated with HCG. Enlarged and increased numbers of interstitial cells of Leydig nearly fill intertubular regions. These uniform cells have eosinophilic granular cytoplasm. Seminiferous tubules are larger and contain spermatozoa *(arrow)*. H & E. × 168.

Fig 3. Immature unstimulated gerbil. Cluster of light staining Leydig cells appears relatively uniform. Vacuoles *(arrows)* are evident in some cells. 1-μ thick Epon-embedded section stained with methylene blue. × 800.

Fig 4. Immature gerbil stimulated with HCG. Light and dark staining Leydig cells are present. Some light cells display cytoplasmic vacuoles *(arrow)*. Many of the predominating dark Leydig cells manifest small cytoplasmic dense bodies *(arrows)*. 1-μ thick Epon-embedded section stained with methylene blue. × 600.



1 2

3 4

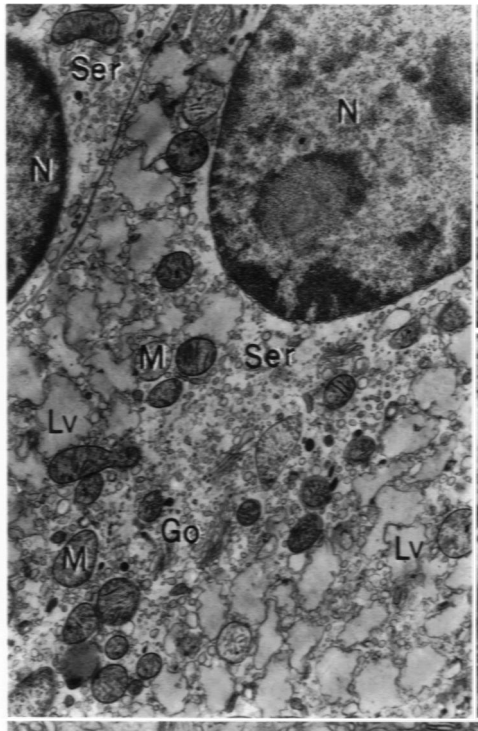
Fig 5. Survey of two Leydig cells from immature unstimulated gerbil. Numerous slightly osmiophilic scalloped or irregularly shaped lipid vacuoles (*Lv*) occupy most of the cytoplasm. Perinuclear Golgi complex (*Go*), vesicular smooth endoplasmic reticulum (*Ser*), and small mitochondria (*M*) are noted. Two nuclei (*N*) show peripheral condensation of chromatin. This and all subsequent figures represent osmium-fixed Epon-embedded material stained with uranyl acetate and lead citrate. × 9000.

Fig 6. Immature unstimulated gerbil. Interdigitations of the plasmalemma (*Pl*) were observed infrequently between adjacent Leydig cells. Membrane bound pigment body (*Pb*) is evident. × 55,000.

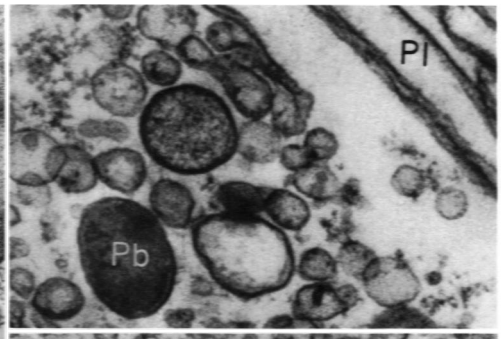
Fig 7. Immature unstimulated gerbil. Vacuole contains many dense rod-shaped structures in transverse plane of section (*arrow*). These structures were rarely observed. Adjacent mitochondria (*M*) and lipid vacuole (*Lv*) are evident. × 32,500.

Fig 8. Immature HCG stimulated gerbil. Densely osmiophilic lipid bodies (*Lb*) assume differing surface contours depending upon amount of circumscribing smooth endoplasmic reticulum (*Ser*). × 21,000.

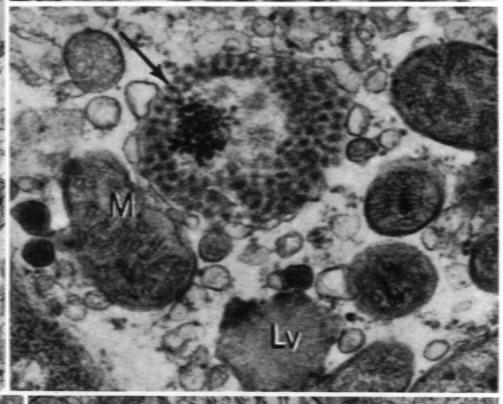
Fig 9. Immature HCG stimulated gerbil. Abundant parallel arrayed rough endoplasmic reticulum (*Rer*) is associated with several densely osmiophilic lipid bodies (*Lb*) and mitochondria (*M*). × 16,000.



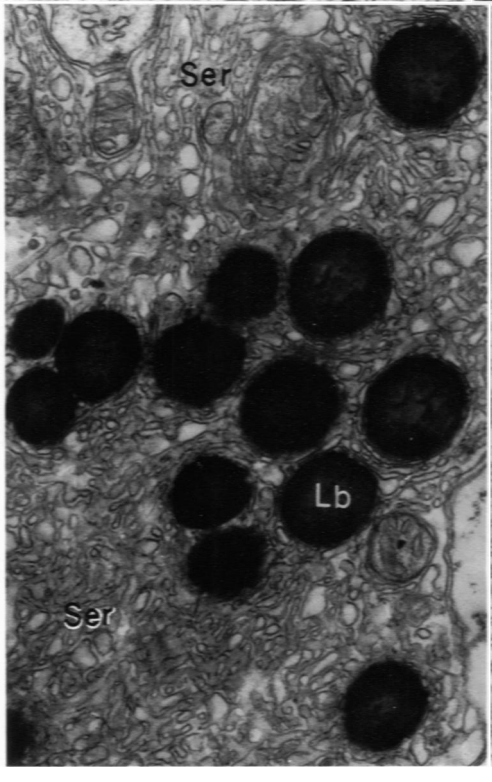
5



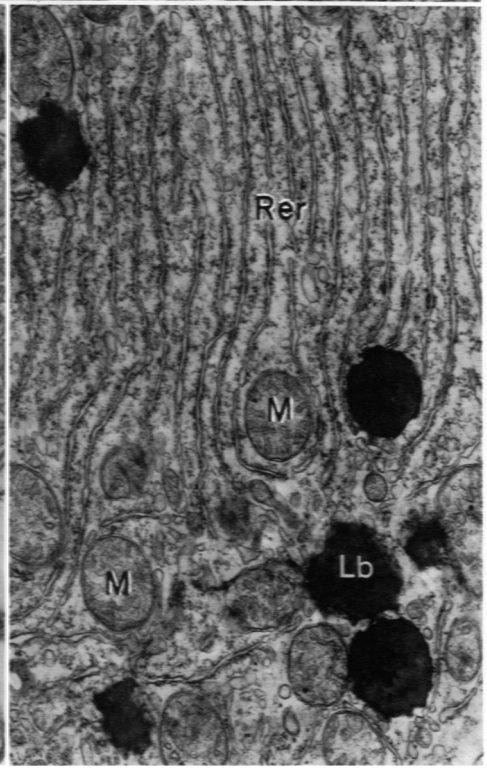
6



7



8

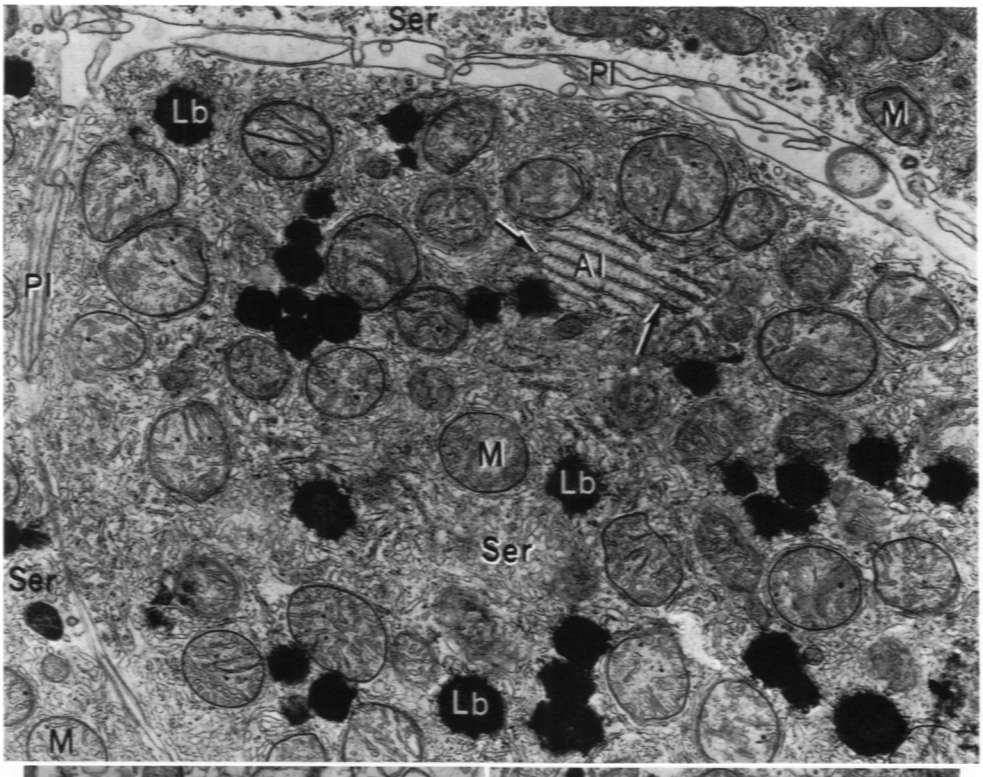


9

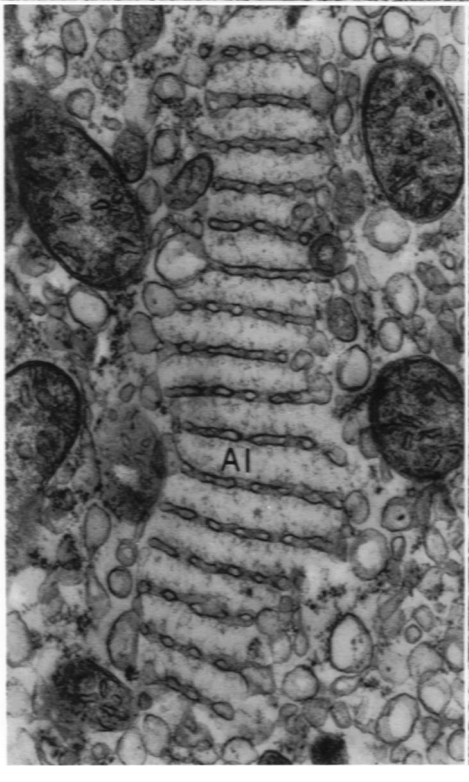
Fig 10. Immature HCG stimulated gerbil. Three Leydig cells display various amounts of smooth endoplasmic reticulum (*Ser*), mitochondria (*M*), and interdigitations of the plasmalemma (*Pl*). The center Leydig cell shows numerous densely osmiophilic lipid bodies (*Lb*) and annulate lamellae (*Al*). Latter organelle is contiguous with both smooth endoplasmic reticulum and rough endoplasmic reticulum (*arrows*). × 16,000.

Fig 11. Immature HCG stimulated gerbil. Enlarged and more frequently observed annulate lamellae (*Al*) display periodic constrictions, interlamellar granular material, and dilatation of lateral margins into vesicle. × 37,000.

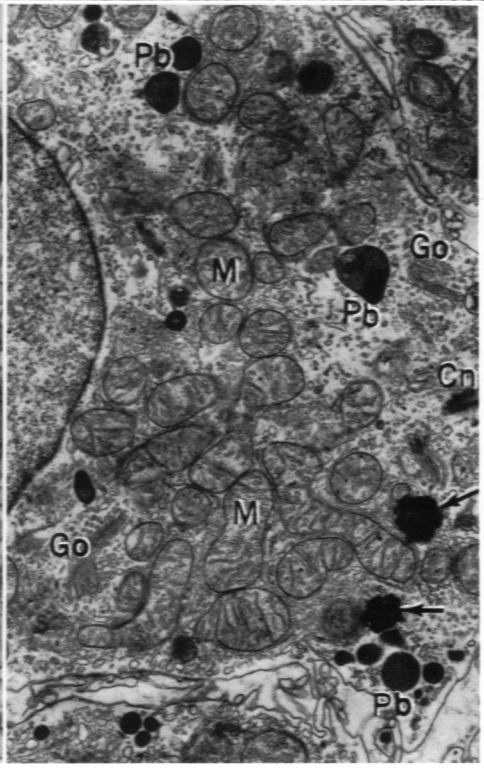
Fig 12. Immature HCG stimulated Leydig cell. Pigment bodies (*Pb*) displaying granular matrix and focal dense regions are at different stages of development. Centriole (*Cn*), Golgi complex (*Go*), several osmiophilic dense bodies (*arrows*), and numerous mitochondria (*M*) are evident. × 10,500.



10



11



12

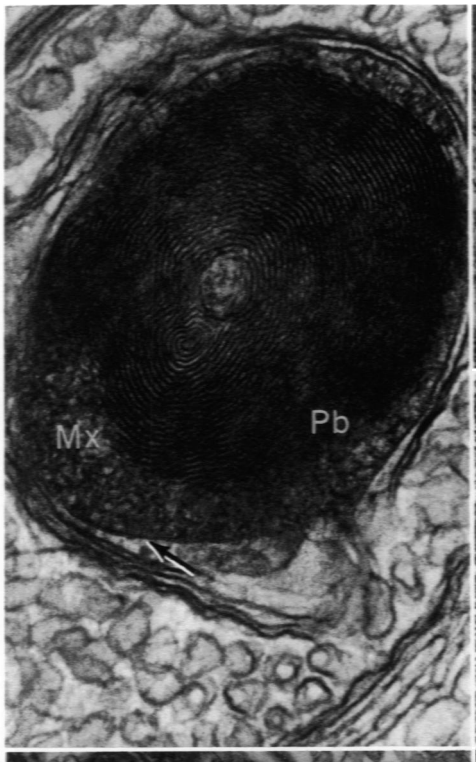
Fig 13. Immature HCG stimulated gerbil. Pigment body (*Pb*) displays well formed myelin figure material in fingerprint-like pattern. Double limiting membrane (*arrow*) and light staining matrix (*Mx*) remain evident. × 80,000.

Fig 14. Pigment body (*Pb*) consists of small vacuole (*V*), granular matrix (*Mx*), and myelin figure material (*Mf*). × 54,000.

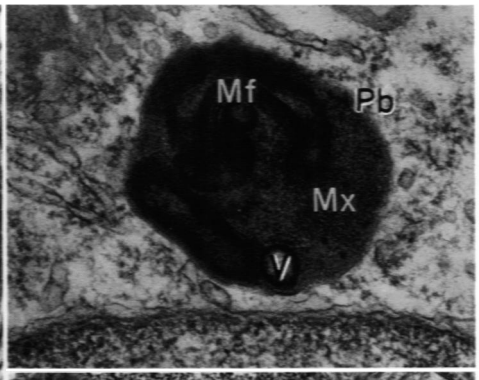
Fig 15. Immature HCG stimulated gerbil. Clusters of nonrosette darkly staining polysomes of glycogen (*Gly*) are adjacent to cluster of smooth endoplasmic reticulum (*Ser*) in one of two Leydig cells. × 15,500.

Fig 16. Immature HCG stimulated gerbil. Plasmalemma (*Pl*) displays complex interdigitations and folds between two adjacent Leydig cells. This was more frequently observed and to an even greater degree in stimulated than nonstimulated Leydig cells of gerbil. × 13,250.

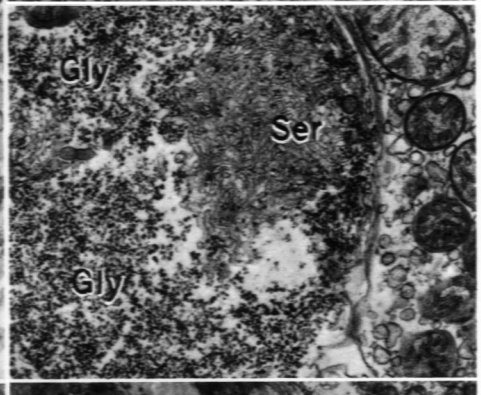
Fig 17. Immature HCG stimulated gerbil. Capillary adjacent to several Leydig cells. One of three cytoplasmic myelin figures (*Mf*) displays central vacuole (*V*) containing lipid. Red blood cell (*Rbc*) within lumen appears adjacent to endothelial cell nuclei (*N*). × 4300.



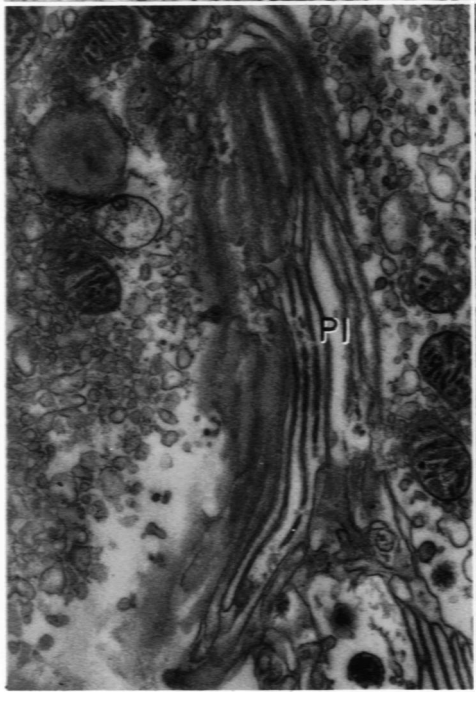
13



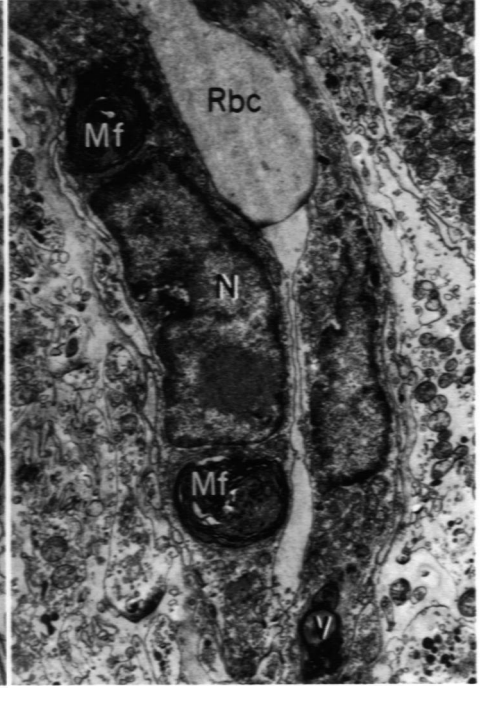
14



15



16



17