# 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*

Leonard Merkow, M.D., Ph.D.,* Hernan F. Acevedo, Ph.D., Malcolm Slifkin, Ph.D.,* and Barbara J. Caito

ANDROGEN PRODUCTION in man, as well as in other species, varies with respect to age.[1-4] Such changes at different ages have not been correlated with a study of the fine structure of the interstitial cells of Leydig. Moreover, there is a paucity of reports attempting to correlate ultrastructure of Leydig cells with androgen production in animals of the same species. We have initiated a study on the fine structure of interstitial cells in guinea pigs at various ages through their life span and of the effect of human chorionic gonadotropin (HCG, obtained as A.P.L. brand from Ayerst Laboratories, Inc., New York, N. Y.) stimulation. This represents the first part of a series of investigations attempting to correlate such fine structure with the biosynthetic capabilities of testicular tissue.

Ultrastructurally, interstitial cells in most species display similar subcellular organelles differing chiefly in number rather than type of organelle.[5-13]

Several studies on the fine structure suggest that steroid biosynthesis may be attributable to the abundant smooth endoplasmic reticulum of interstitial cells.[7,14-16] Recently, Connell *et al.*[17] reported the presence of increased numbers of polyribosomes and osmiophilic bodies concurrent with altered mitochondria in interstitial cells of 2-day-old chicks stimulated with HCG.

This initial communication represents an electron microscopic study of the Leydig cells of immature guinea pig testis and the effects upon it by HCG stimulation.

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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. 15212.

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

* Adjunct Assistant Professor at Pennsylvania State University.

## Materials and Methods

Testicular tissue was obtained from ten 3-week-old guinea pigs (cavys, Alden H. Forbes Laboratories, Pittsburgh, Pa.) sacrificed by decapitation. Their weight ranged from 174 to 209 gm. One-half of the animals were previously given intraperitoneal injections of 100 I.U. of HCG daily for 15 days. Control animals were given inoculations of normal saline. One-millimeter 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 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 $\mu$ thick were prepared and stained with methylene blue. Large clusters of Leydig cells were easily 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 electron microscope.

Samples of remaining testicular tissue were simultaneously fixed in buffered formalin, embedded in paraffin, sectioned at 5 $\mu$, and stained with hematozylin and eosin.

## Results

The light microscopy of testis from all immature control (unstimulated) animals revealed small clusters of Leydig cells within the large interstitial spaces (Fig. 1). Seminiferous tubules were small and did not contain mature spermatozoa in both control and experimental animal testes (Fig. 1 and 2). Conversely, in all the immature animals stimulated with HCG, large clusters of Leydig cells completely filled and obliterated the potential interstitial spaces (Fig. 2). Seminiferous tubules appeared small and surrounded by clusters of Leydig cells (Fig. 2). Both hyperplasia and hypertrophy of interstitial cells of Leydig were evident in all the stimulated animals (Fig. 2).

Identification of interstitial cells in guinea pig testis by electron microscopy was facilitated by their abundant smooth endoplasmic reticulum (Fig. 3–10) and relation to the basement membrane of seminiferous tubules (Fig. 3 and 6). Higher magnification of individual Leydig cells showed a vesicular type of abundant smooth endoplasmic reticulum nearly filling the cytoplasm (Fig. 3–5). Lipid vacuoles stained lightly and displayed a characteristically irregular border of their limiting membrane (Fig. 3–5). Membrane-bound lipochrome (lipofuscin) pigment bodies, frequently noted to be interspersed among the smooth endoplasmic reticulum, were quite diminutive and displayed a homogeneous density (Fig. 3–5). Rough endoplasmic reticulum was rarely observed and polyribosomes were evident in focal areas of the cytoplasm (Fig. 4 and 5).

After 15 days of stimulation with HCG, the Leydig cells were found

with considerable ease in ultrathin sections by low power examination. A survey view showed 5–6 Leydig cells with slightly increased density of smooth endoplasmic reticulum (Fig. 6). The lipid vacuoles were lighter staining than in unstimulated animals, decreased in number, and displayed a circular configuration of their limiting membrane (Fig. 6 and 7). The lipochrome pigment bodies exhibited both more and less dense osmiophilic regions (Fig. 9 and 10). The less dense areas appeared granular and one or more dense components, usually at the periphery, existed in these pigment bodies (Fig. 7–10). The appearance of lipochrome pigment bodies in HCG-stimulated animals was similar to that of the more differentiated lipochrome pigment bodies observed in adult guinea pig Leydig cells (personal observation). Strands of rough endoplasmic reticulum were increased in number and displayed dilatation (Fig. 7 and 9). The prominence and number of Golgi complexes was increased (Fig. 6). Mitochondria in both the unstimulated and stimulated interstitial cells of Leydig appeared similar morphologically (Fig. 3–9). Occasionally, the enlarged pigment bodies displayed an "onion skin" or myelin figure appearance (Fig. 9).

## Discussion

Shoen [18] has demonstrated that steroidogenic function and cellular morphologic alterations could not be correlated by means of light microscopy. It appears more likely that structure could be correlated with function by means of electron microscopy. The ultrastructure of guinea pig Leydig cells has revealed striking differences between HCG-treated and untreated immature animals.

The effects of HCG upon the different enzyme systems of the testosterone biosynthetic pathway in the guinea pig testis as well as in other species has been well documented.[19–21] Since HCG stimulates androgen production in the immature animal, one may interpret the observed ultrastructural differences as due to either primary stimulation of the cell by HCG, or secondary response of the cell to androgen production (testosterone), or both. It is also known that estrogen stimulation of uterine smooth muscle [22] and the effect of monocrotaline upon pulmonary arteriolar smooth muscle [23] results in hyperplasia of many cytoplasmic organelles.

The smooth endoplasmic reticulum is well developed in most interstitial cells, and it has been suggested both morphologically and biochemically that this organelle could be related to steroid production.[7,8,14–17,24] Pituitary gonadotropins,[25] as well as several toxic chemicals, produce proliferation of Leydig cells and their smooth endoplasmic

reticulum.[26,27] Various types of smooth endoplasmic reticulum, such as tubular, cisternal, fenestrated, or intermediate have been described in the guinea pig testis by Christensen.[7] Myelin whorls or figures formed from portions of the smooth endoplasmic reticulum and usually surrounding a lipid body have also been reported in Leydig cells of several species.[5,7,14,28] The myelin figures within the pigment bodies observed in the HCG stimulated animals were similar in appearance to those described by other investigators.[1,7,11,29]

The lipochrome pigment bodies and lipid bodies also appeared similar to that reported in Leydig cells of several species [7,10,11,16,29] and in a hilar-cell tumor of a human ovary.[30] The lipid vacuoles in the stimulated animals were sparse, rounded, and less osmiophilic. This suggests the possibility of increased utilization of the lipids (cholesterol?) for steroid biosynthesis. The enlargement and differentiation of the lipochrome pigment bodies following HCG stimulation may also be related to steroid production. Frank and Christensen have recently shown that acid phosphatase is present within these lipochrome pigment bodies.[31] In addition, the pigment bodies within stimulated immature Leydig cells in our study displayed myelin figure material and a dense osmiophilic matrix, constituents imparting a morphologic similarity to those observed in the adult guinea pig.[32]

The increased prominence of rough endoplasmic reticulum in the HCG-stimulated animal may be related to increased protein synthesis.[33]

## Summary

Testicular tissue was obtained from ten 3-week-old guinea pigs. Half of the animals were administered 100 I.U. of HCG daily for 15 days. The other 5 were given injections of normal saline as controls. Light microscopy revealed hyperplasia of Leydig cells in all stimulated animals. Electron microscopic studies indicated differences between control and treated animals. Leydig cells from all control testis contained a paucity of rough endoplasmic reticulum and polyribosomes, abundant, irregular, and more osmiophilic lipid vacuoles, and diminuitive pigment bodies. Enlarged Leydig cells of all the HCG-treated animals displayed increased foci of dilated rough endoplasmic reticulum, formation of polyribosomes, circular and less osmiophilic lipid vacuoles, larger pigment bodies, and hypertrophy of the Golgi complex. The implications of the organelle changes observed are discussed.

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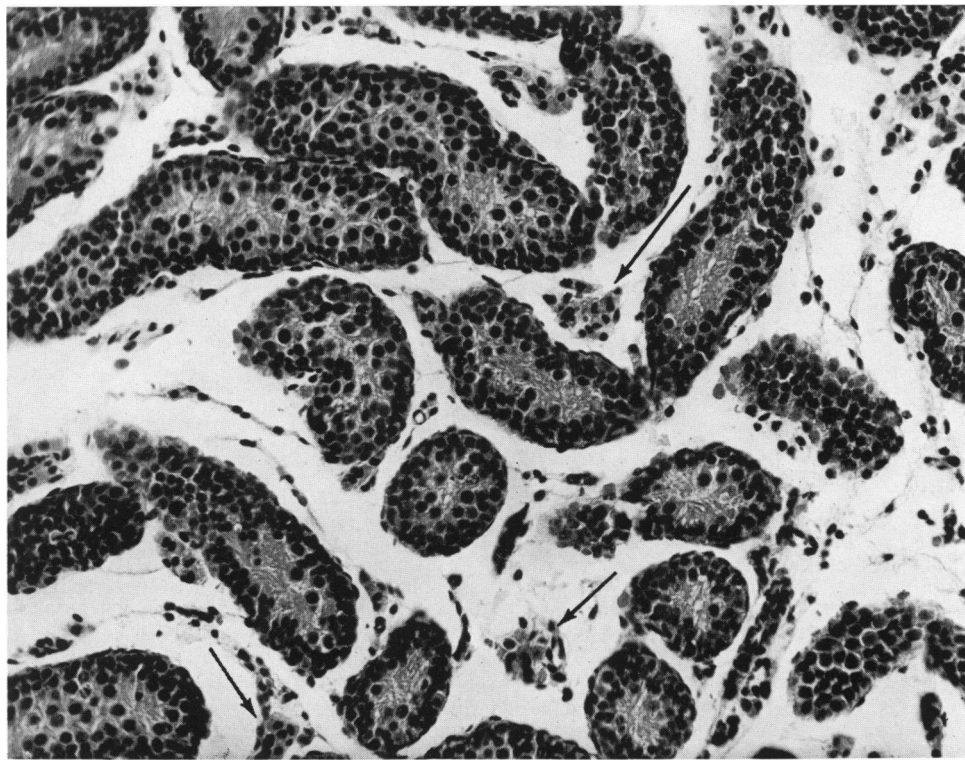
## Legends For Figures

**Fig. 1.** Testicular tissue from a 3-week-old unstimulated control immature guinea pig. Seminiferous tubules do not contain mature spermatozoa. Regions between tubules contain only an occasional small group of Leydig cells (*arrows*). The lack of cells and possibly fixation artifact produce a wide "space" between tubules. Hematoxylin and eosin stain. × 275.

**Fig. 2.** Testicular tissue from a 3-week-old immature guinea pig stimulated with HCG. Increased size and number of interstitial cells of Leydig fill intertubular regions. These cells have eosinophilic granular cytoplasm and are quite uniform. Little space remains between tubules. Seminiferous tubules do not contain mature spermatozoa. Hematoxylin and eosin. × 275.



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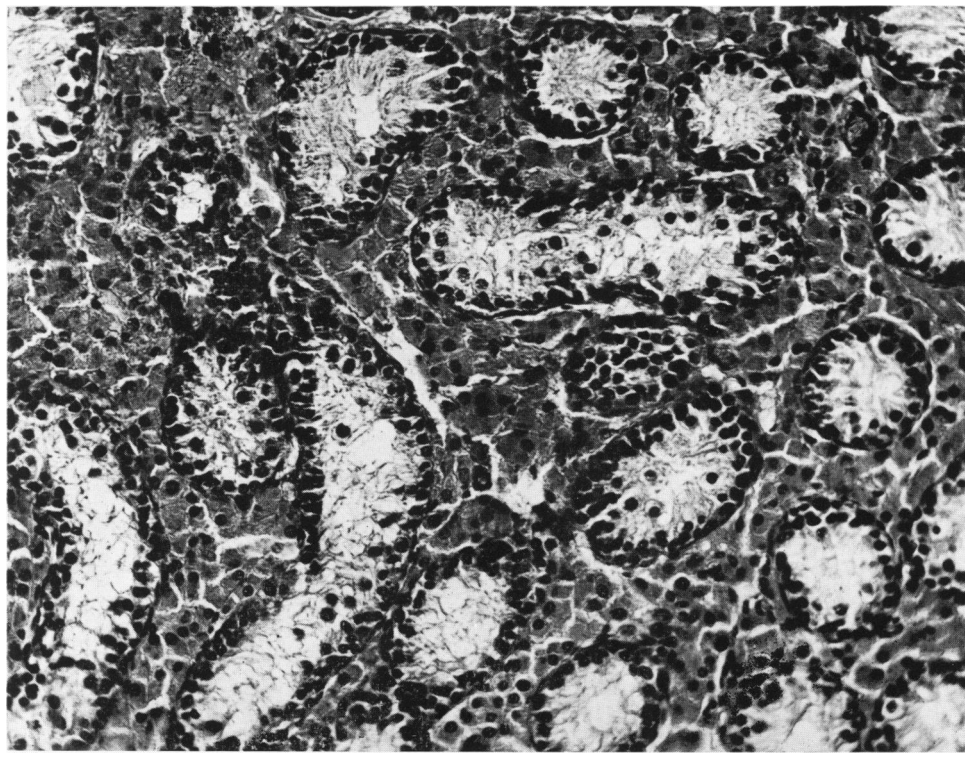

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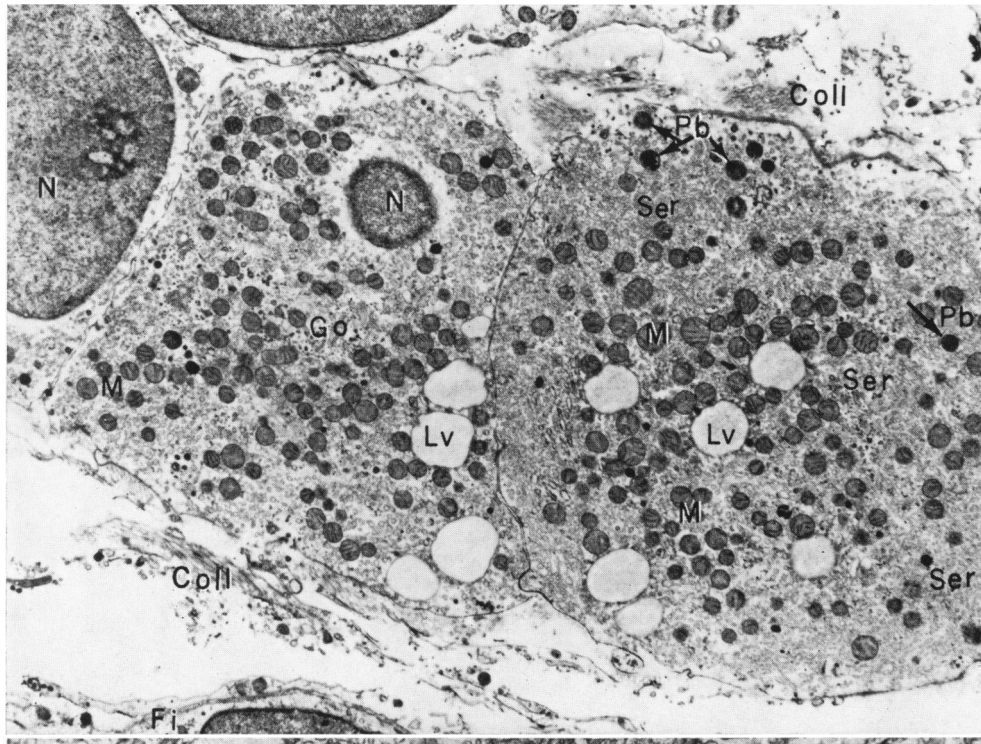
Figures 3–10 represent osmium-fixed Epon-embedded material stained with uranyl acetate and lead citrate.

**Fig. 3.** Portions of 3 Leydig cells from testis of unstimulated 3-week-old immature guinea pig. Cytoplasm is filled with smooth endoplasmic reticulum (*Ser*). Mitochondria (*M*) tend to cluster toward central portion of cell. Pigment bodies (*Pb*) are small (*arrows*). Irregular lipid vacuoles (*Lv*) have an affinity for osmium. The Golgi (*Go*) and nuclei (*N*) are evident. Adjacent to cells there is collagen (*Coll*) and a fibroblast (*Fi*). × 5400.

**Fig. 4.** High magnification of Leydig cell from unstimulated immature guinea pig. Lipid vacuoles (*Lv*) are irregular and contain osmiophilic material. Pigment bodies (*Pb*) are quite small. Smooth endoplasmic reticulum predominates and is associated with many small mitochondria (*M*). × 17,000.

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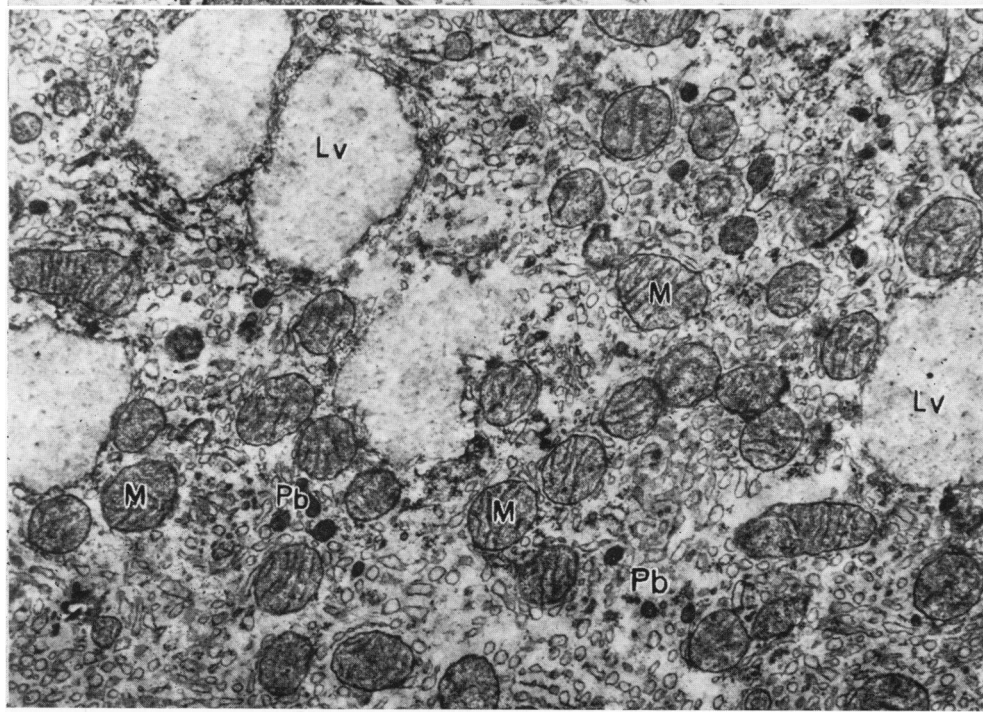


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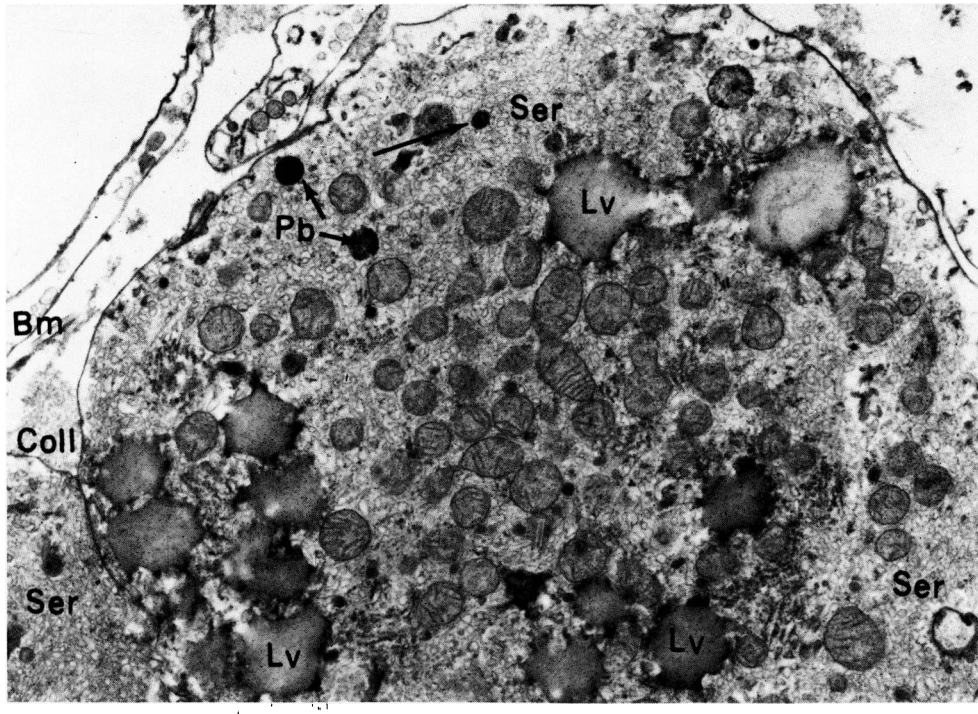
**Fig. 5.** Transverse section from Leydig cell of unstimulated immature guinea pig. Smooth endoplasmic reticulum (*Ser*) is associated with small or medium-sized pigment bodies (*Pb, arrows*) and rather large scalloped or irregular bordered lipid vacuoles (*Lv*). Collagen (*Coll*) and basement membrane (*Bm*) are at left. × 7200.

**Fig. 6.** A survey low-power view of 3 Leydig cells adjacent to tubule in testis from HCG-stimulated 3-week-old immature guinea pig. Number of organelles appears increased. Some mitochondria (*M*) are larger and more round. Pigment bodies (*Pb*) are enlarged. There is an increase in cisternal smooth endoplasmic reticulum (*Ser*). Golgi complex (*Go*) is prominent. Scarce lipid vacuoles (*Lv*) are relatively clear and round. A fibroblast (*Fi*), connective tissue, and basement membrane (*Bm*) are adjacent to cells of seminiferous tubules (*St*) at right. × 5500.

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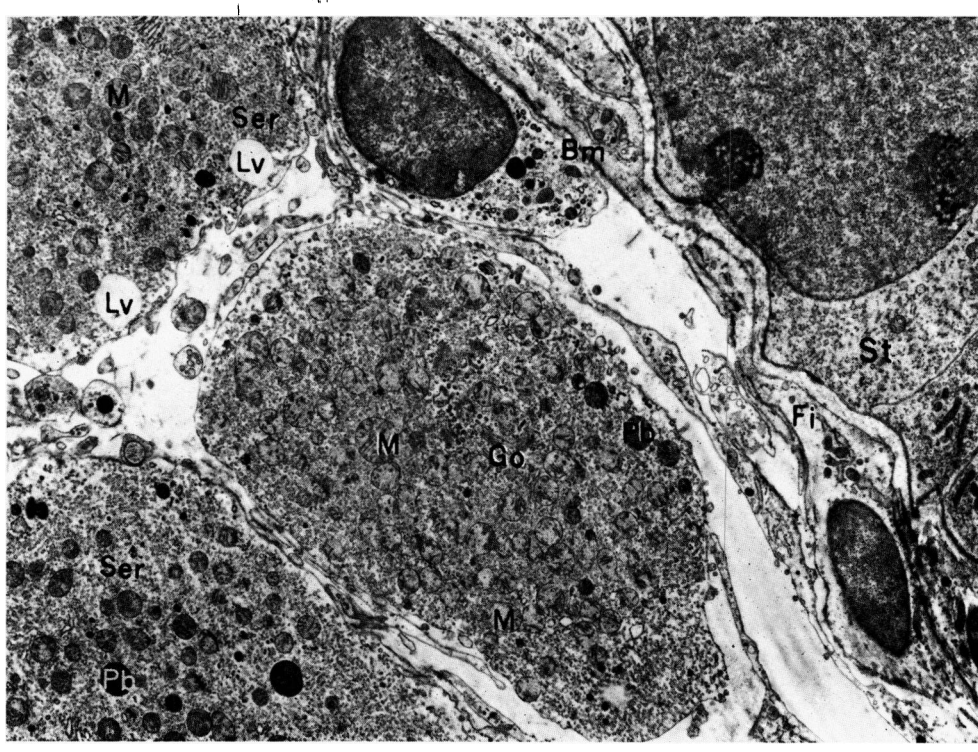


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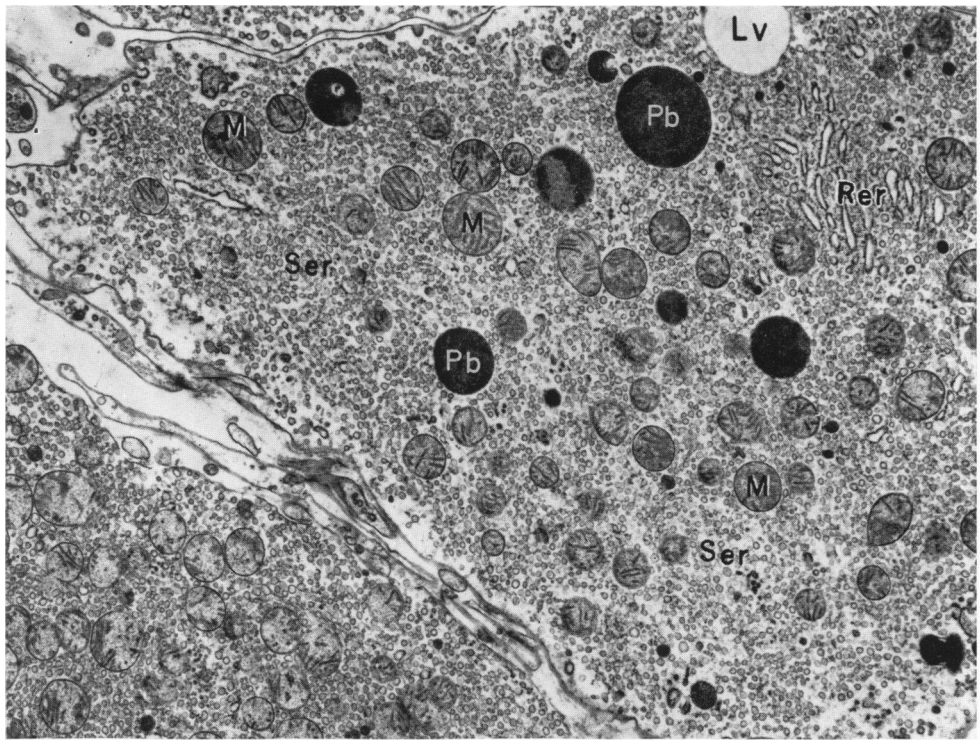
**Fig. 7.** Higher magnification of Fig. 6. Pigment bodies (*Pb*) are enlarged and have dense regions. Rough endoplasmic reticulum (*Rer*) is increased in size and somewhat dilated. Cisternal smooth endoplasmic reticulum (*Ser*) fills area between mitochondria (*M*). Lipid Vacuole (*Lv*). × 10,000.

**Fig. 8.** Portion of a Leydig cell from HCG-stimulated guinea pig. Enlarged pigment body (*Pb*) shows lighter matrix with focal peripheral increase in osmiophilia. Small (*arrows*) and large *Pb* are evident. Both free (*Rnp*) and attached (*Rer*) ribonucleic acid particles are evident (*arrows*). × 17,000.

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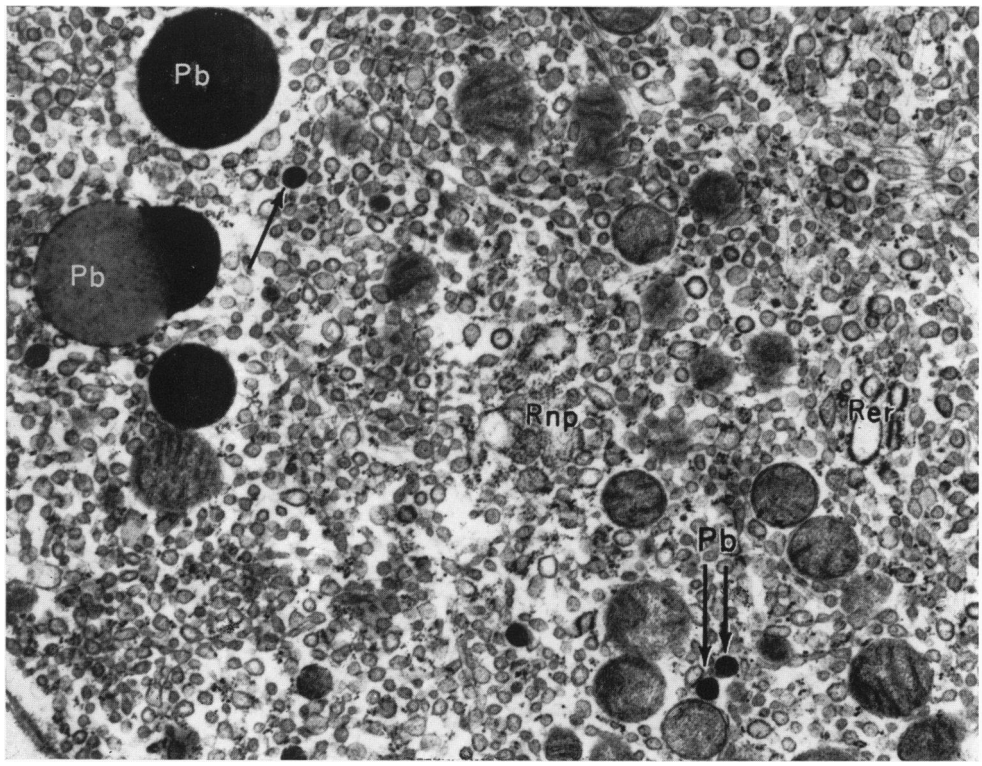


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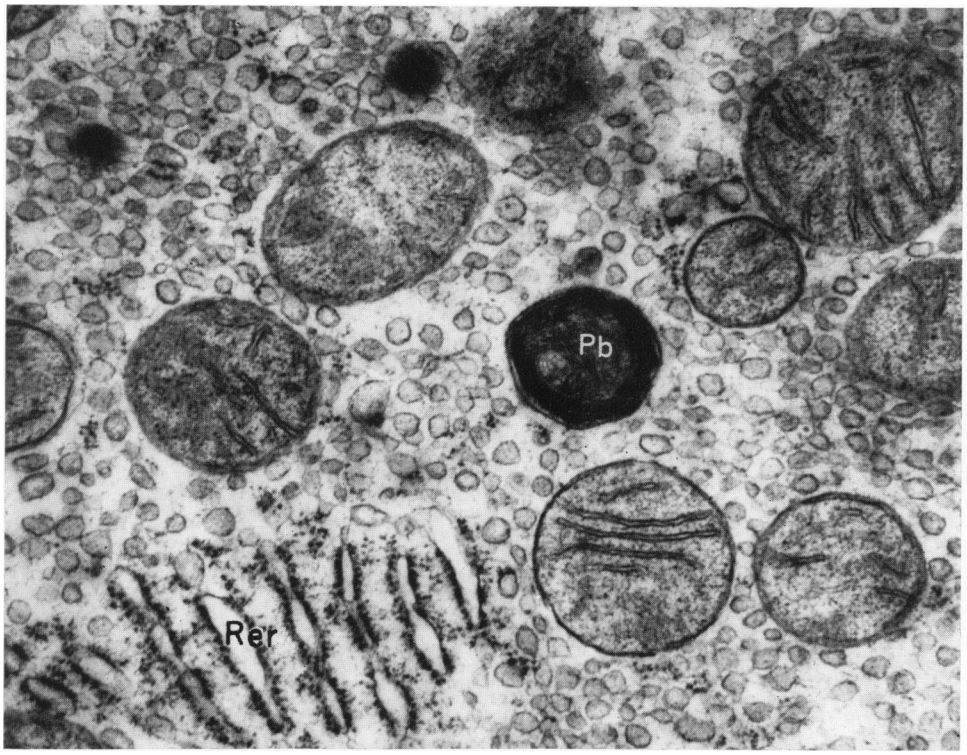
**Fig. 9.** Portion of a Leydig cell from HCG-stimulated guinea pig. Rough endoplasmic reticulum (*Rer*) is increased and displays some dilatation. Adjacent to it is a pigment body (*Pb*) containing myelin figure material. Background of this pigment body is composed of a granular matrix. × 32,000.

**Fig. 10.** High magnification of pigment body (*Pb*) from HCG-stimulated guinea pig. Pigment body consists of a double limiting membrane surrounding a less dense granular matrix and focal areas of more dense osmiophilic material. × 60,000.

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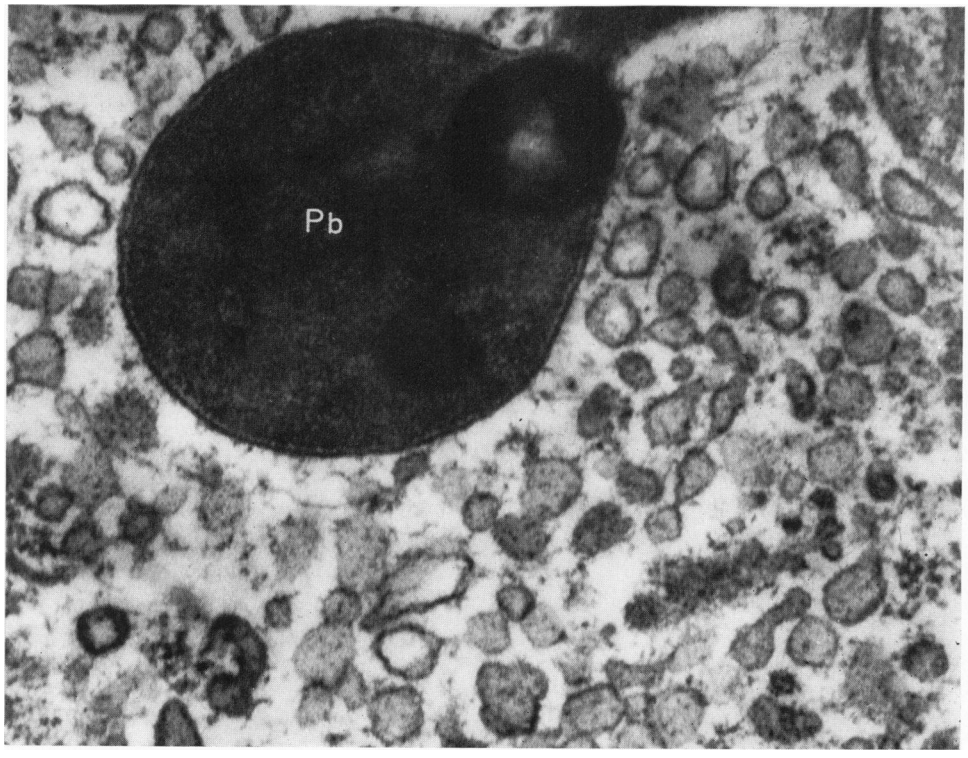


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