Electron Microscopic Studies on Dog Testicular Interstitial Cells

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Received June 15, 1974

Summary. The ultrastructure of the dog testicular interstitial cells is described. The cells are characterized by numerous tubules of agranular endoplasmic reticulum, many large lipid droplets, different types of dense bodies including heterogeneously dense bodies, giant mitochondria containing lipid droplets, a few microtubules, and well-developed cytoplasmic filaments and their specific organization. Acid phosphatase reaction at ultrastructural level reveals that some of the lipid droplets change into the heterogeneously dense bodies, lysosomes. The cytoplasmic filaments are occasionally arranged in large bundles piled closely in an extensive area adjacent to the Golgi region in the cytoplasm. Some of these large bundles show conspicuous circular or spiral configurations which are composed of elaborate arrangements of both circular and longitudinal filaments and accompany tubules of agranular endoplasmic reticulum running parallel to the longitudinal filaments.


The present study deals with the cytology of the dog testicular interstitial cells with special reference to the acid phosphatase activity in their dense bodies and the specific organization of their intracytoplasmic filaments.

Materials and Methods

A total of 10 adult mongrel dogs aged 2 to 5 years, were used from January to February. Both testes were removed from each animal under thiamylal sodium anesthesia. These testes were perfused with 2.5% glutaraldehyde in either phosphate or cacodylate buffer, pH 7.4, following the technique recommended by Christensen (1965) and then cut into small pieces and placed in the same freshly prepared fixative for 3 hrs. The tissue was washed overnight in the buffer containing sucrose and
then postfixed in 2% osmium tetroxide in Millonig's buffer, pH 7.4, for 1.5 hrs. After dehydration in a graded series of acetone, the tissue was embedded in epoxy resin. Thin sections were cut with glass knives on a Porter-Blum ultramicrotome, mounted on copper grids, and doubly stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963). Electron micrographs were taken with a JEM T8 microscope. For correlated light microscopic studies thick sections were cut and stained with 1% toluidine blue.

For demonstration of acid phosphatase the testes perfused with 2.5% glutaraldehyde in cacodylate buffer were used. The tissue washed overnight in the buffer was cut by a vibratome (Oxford Laboratory) into sections 40 μ in thickness. The sections were incubated in either the medium of Gomori (1952) or the medium modified by Barka and Anderson (1962) at 37°C for 20 to 30 min. Control sections were incubated in the above medium either lacking substrate or containing 0.01 M sodium fluoride, inhibitor of acid phosphatase.

After these experimental and control sections were washed in the buffer, they were postfixed in 2% osmium tetroxide in Millonig's buffer for 1 hr, and then dehydrated. In some of the sections incubated for acid phosphatase, postosmification was omitted in order to distinguish reaction product from osmiophilia (Brunk and Ericsson, 1972). The procedures after dehydration were the same as those mentioned for the conventional technique.

Results

In thick Epon sections stained with toluidine blue, the dog testicular interstitial cells are distributed singly or in groups in the loose connective tissue with blood capillaries among seminiferous tubules and are easily detectable as round or polygonal cells with many lipid vacuoles (Fig. 1). In the electron microscopy, however, one layer of collagen fibrils, one to three layers of contractile cells and several layers of fibroblasts or their transitional cells (Fig. 2) with lipid droplets to interstitial cells accompanying macrophages and mast cells are distinguishable between the seminiferous tubules and the interstitial cells, though the fibroblast or transitional cell layer is sometimes absent. The contractile cells also often contain large lipid droplets (Fig. 3), some of which are surrounded even by a thin layer of flat cisternae of agranular endoplasmic reticulum.

The interstitial cells, when they are in groups, are firmly attached by a gap junction in a straight or wavy line of different length and occasional small desmosome-like junctions (Fig. 5). In some of the areas where a projection from one cell reaches deep into an adjacent cell, one can see either an arciform gap junction longitudinally cut (Fig. 5) or
a ring-shaped one transversely cut (Fig. 5, Inset). The cytoplasm is characterized by the presence of numerous cisternae of agranular endoplasmic reticulum and of many large lipid droplets. These cisternae are mainly tubular, but around the lipid droplets and mitochondria they are frequently closely piled to form lamellar arrays of flattened cisternae with fenestration. In addition, small numbers of elements of granular endoplasmic reticulum and occasional glycogen particles are also scattered in the cytoplasm. The Golgi apparatus is relatively well developed and mainly located perinuclearly. Centrioles are seen surrounded by the Golgi apparatus in the juxtanuclear region. An occasional cilium can be observed protruding from one of these centrioles (Fig. 13). The mitochondria usually are round, oval, or rod-shaped and have tubular or vesicular cristae (Fig. 4, 5), but elongate mitochondria up to 2.5 μ in length, those with lamellar cristae, cup-shaped mitochondria (Christensen and Chapman, 1959), or giant mitochondria up to 3 μ in diameter are occasionally encountered. Some mitochondria, irrespective of their size and form, contain one or more lipid droplets in their matrix (Fig. 4, Inset).

Besides lipid droplets, there are membrane-bound, varying sized, dense bodies of different types measuring up to 3 μ in diameter; heterogeneously dense bodies frequently containing internal structures such as parallel tubules or tortuous membranes (Fig. 4, 5); smaller and homogeneously dense bodies with no internal structures;
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Fig. 4. Part of a testicular interstitial cell from adult dog, showing closely packed filaments. Er granular endoplasmic reticulum, H heterogeneously dense bodies containing tubular structures, L lipid droplets, M mitochondria. ×18,000. Inset shows a giant mitochondrion containing lipid droplets. ×10,000

dense bodies having myelin figures in their periphery (Fig. 5). It is of interest that gradual transitional forms from lipid droplets to such heterogeneously dense bodies are followed (Fig. 6–8). When the testicular interstitial cells are tested for acid phosphatase activity, reaction product is found to be present in most of these dense bodies (Fig. 9–12). Furthermore, in the histochemical preparations omitting post-osmification, lipid droplets usually appear as a clear space, but some of them indicate strong activity localized in the marginal area surrounding the clear space (Fig. 9). Such bodies are thought to correspond morphologically to the transitional form shown in Figure 7. Some of the smaller and homogeneously dense bodies, 0.1 to 0.4 µ in diameter, show no reaction product and may possibly correspond to peroxisomes (Reedy and Svoboda, 1972a, b) (Fig. 12). In the Golgi area, heavy deposition of reaction product is frequently discernible in the innermost membranous cisterna and its several associated vesicles (Fig. 11).

In the dog testicular interstitial cells there are frequently observed numerous cytoplasmic filaments. In some sections, bundles of parallel filaments are closely packed in an extensive area adjacent to the Golgi region in the cytoplasm opposite to the eccentrically located nucleus. In this area, which may reach 4 µ in diameter,
Fig. 5. Part of testicular interstitial cells from adult dog, showing two gap junctions of different kinds (arrows) and one desmosome-like junction (arrowhead). The gap junction in the bottom right corner of this micrograph is cut longitudinally and suggests that it is located at the apex of a projection extending from one cell to another. C collagen fibrils, D dense body with myelin figures, L lipid droplets, H heterogeneously dense bodies containing tortuous membranous structures, g glycogen particles, M mitochondria. ×22,800. Inset shows a cross section of the same kind of gap junction as mentioned above. ×22,800

Fig. 6-8. Part of a testicular interstitial cell from adult dog, showing different stages of transformation from lipid droplets into heterogeneously dense bodies. L lipid droplet. ×22,000. Figure 6 shows a lipid droplet containing transversely sectioned, parallel tubules in its narrow peripheral region (arrows). This type of lipid droplet seems to be in the early stage of transformation to heterogeneously dense bodies. Figure 7 shows a lipid droplet with a heterogeneously dense, cap-like area containing longitudinally sectioned, parallel tubules (arrow). This type body is thought to be in the more progressive stage of transformation to heterogeneously dense bodies and corresponds to a typical transitional type from lipid droplet to heterogeneously dense body. Figure 8 shows a typical heterogeneously dense body containing transversely sectioned, parallel tubules (arrow).
other cell organelles and inclusions are few and large bundles of filaments are arranged in a form of one or more whorls (Fig. 4, 14). Some of such large bundles show, when cut transversely, circular, or spiral configurations of 0.6 to 1 µ in diameter

Fig. 9-11. Part of a testicular interstitial cell from adult dog, showing acid phosphatase reaction product in the cytoplasm. Figure 9 shows a preparation omitting postosmification after acid phosphatase reaction, and reaction product surrounds a lipid droplet (L) which appears as a clear space. This type body is thought to correspond to the transitional type in Figure 7. ×22,000. Figure 10 shows reaction product on a typical heterogeneously dense body containing transversely sectioned, parallel tubules (arrow). L lipid droplet. ×24,400. Figure 11 shows the Golgi area where heavy reaction product is visible in the innermost membranous cisterna of the Golgi apparatus and some of its associated vesicles. G Golgi membranes, Ly lysosome, M mitochondrion. ×24,400

Fig. 12. Part of a testicular interstitial cell from adult dog, showing acid phosphatase-reactive bodies and non-reactive smaller bodies with a moderately dense matrix (arrows) in groups. The latter bodies are thought to be microbodies (peroxisomes). M mitochondrion. ×22,800

Fig. 13. The peripheral cytoplasm of a testicular interstitial cell from adult dog, showing a cilium and a pair of centrioles surrounded by the Golgi complex (G). ×22,700

Fig. 15. Part of a testicular interstitial cell, showing a large spiral configuration composed of doubly or triply coiled spirals. This configuration is a transverse section through the specific portion of a bundle of longitudinal filaments. Each spiral consists of both a longitudinally sectioned, spiral microfilament and numerous transversely sectioned, longitudinal filaments which are disposed at uniform intervals in a row along the spiral microfilament and fused with the latter. Note that in this case tubules of agranular endoplasmic reticulum (arrows) are present not only in the central area surrounded by the spiral but also in the outer zone between circles consisting of the spiral. ×31,500

Fig. 16. Part of a testicular interstitial cell, showing a pair of parallel-running straight lamellae. These lamellae are a longitudinal section through the specific portion of a bundle of longitudinal filaments. Each lamella consists of both a longitudinally sectioned longitudinal filament and of numerous transversely sectioned, spiral microfilaments which are thought to be lined along the longitudinal filament in a row. In the center of this bundle occur crossly cut agranular tubules (arrows) and glycogen particles. ×31,500
Fig. 14. Part of a testicular interstitial cell from adult dog, showing circular or spiral configurations, dense band-like structures of different shapes (arrowheads) and paired parallel-running straight lamellae (asterisk) in the area closely packed with bundles of longitudinal filaments. Note that the circular or spiral configurations and the dense band-like structures or the paired straight lamellae correspond to the cross sections and the oblique or longitudinal sections through the specific portions of the bundles of longitudinal filaments respectively, and the tubules of agranular endoplasmic reticulum (arrows) are situated within and between these bundles of filaments. G the Golgi complex. ×31,500
in which crossly sectioned coarse filaments, about 80 Å in diameter, are arranged at uniform intervals in a row along circular or spiral microfilaments, about 20 Å in diameter (Fig. 15). On the other hand, they appear, when cut longitudinally or obliquely, either merely as dense bands of 0.2 to 0.35 μ in width crossing the bundles of filaments (Fig. 14) or as paired parallel and straight lamellae, up to 1.7 μ in length, which are thought to consist of longitudinally sectioned coarse filaments and crossly sectioned microfilaments (Fig. 16). Furthermore, the coarse filaments continue with neighboring common filaments (Fig. 16).

On the basis of these findings of cross and longitudinal sections through the same structures, it can be suggested that the specific portions of the bundles of filaments are composed of the usual longitudinal filaments and circular or spiral microfilaments and that the former filaments are supported in their inside by the latter filaments and both filaments are fused with each other. Such specific portions of the bundles of filaments show different configurations according to the section (Fig. 14). The areas filled with filaments are seen in all the testes examined, but it is not certain whether they appear in every interstitial cell. The grade of development of these intracytoplasmic filaments seems to be different in the individual animals used.

The lumen surrounded by the specific portions of the bundles of filaments may be filled with randomly distributed other longitudinal filaments and occasional glycogen particles (Fig. 14, 16) and ordinarily penetrated by one to five tubules of agranular endoplasmic reticulum running parallel to the longitudinal filaments. In some cases a lipid mass is seen bounded by a limiting membrane in the lumen. In addition, the spaces among these specific bundles also are penetrated by similar longitudinal tubules of agranular endoplasmic reticulum accompanying longitudinal filaments (Fig. 4, 14). Some of these tubules seem to be connected anywhere with those from the surrounding agranular reticulum (Fig. 4).

A few microtubules are randomly dispersed among agranular cisternae or filaments, but double-walled tubules (CHRISTENSEN and FAWCETT, 1961) are not recognized. So-called dark and light cells, which are due to the difference in electron density of the cytoplasmic matrix, are not distinguishable in this study using perfusion fixation.

Discussion

The interstitial cells contained many large lipid droplets in their cytoplasm. The abundance of lipid droplets is comparable with that described in guinea pig interstitial cells (CHRISTENSEN, 1965; FRANK and CHRISTENSEN, 1968). The dog may thus belong to one of the species having the most abundant lipid droplets in interstitial cells. In addition, there were observed different types of dense bodies besides the lipid droplets, especially heterogeneously dense bodies and transitional type bodies from the lipid droplets to the heterogeneously dense bodies. On the other hand, the acid phosphatase test revealed that most of the dense bodies had enzyme activity and that the transitional type bodies also showed a more or less strong activity in their marginal, heterogeneously dense area surrounding the lipid material. Thus, it may be suggested that most of the dense bodies, including heterogeneously dense bodies, are lysosomal in nature and that these heterogeneously dense bodies are derived from the lipid droplets. Such an alteration of lipid droplets into lysosomes has not previously been cytochemically demonstrated in the testicular interstitial cells and
supports the findings described by Aoki and Massa (1972) who showed a transformation of lipid droplets into lipofuscin in the testicular interstitial cells of mice stimulated by interstitial-cell-stimulating hormone.

Lipofuscin granules composed of dense pigment, granular matrix, and lipid droplets have been reported in the testicular interstitial cells of the guinea pig (Christensen, 1965; Frank and Christensen, 1968), and some components of these granules have shown a positive acid phosphatase activity (Frank and Christensen, 1968). Somewhat similar granules have been described as lipochrom pigments in man (Fawcett and Burgos, 1960; Yamada, 1965; Kretser, 1967) and in the rat (Leeson, 1963). In the present study, however, such typical lipofuscin granules were not detected, but in an old dog kept more than 10 years we found many typical lipofuscin granules (unpublished data). Therefore, the present authors distinguished the large heterogeneously dense bodies observed mingled with lipid droplets from typical lipofuscin pigment. The former bodies resembled granules termed as lipofuscin in the mouse (Aoki and Massa, 1972) and boar (Belt and Cavazos, 1967) and as lipochrome pigment bodies in immature and mature guinea pigs (Merkow et al., 1968a, b).

In this study, giant mitochondria and/or mitochondria containing lipid droplets in their matrix were sometimes encountered. Since the dogs used in this work were sacrificed in their natural breeding season from January to February, the presence of such unusual mitochondria is thought to be related to hyperfunction of testicular interstitial cells because of the increase of similar mitochondria in the same organ stimulated by gonadotropins (Kretser, 1967; Aoki and Massa, 1972), and the lipid droplets-containing mitochondria may be the resulting exhausted ones, as suggested by Dahl (1971) in the ovarian thecal glands stimulated by gonadotropins.

The most striking characteristic in the fine structure of the dog testicular interstitial cells, when compared with that of other animals reported up to now, is the occurrence of abundant filaments and their specific arrangements in the cytoplasm. Cytoplasmic filaments of similar nature have been found in the testicular interstitial cells of the boar (Belt and Cavazos, 1967) and squirrel monkey (Belt and Cavazos, 1971) and in the undifferentiated testicular interstitial cells of man (Fawcett and Burgos, 1960) and rat (Leeson, 1963), but such a peculiar organization of filaments as reported in this paper has not yet been noticed.

Concerning the functional significance of specific bundles composed of elaborate arrangements of circular and longitudinal cytoplasmic filaments in the dog testicular interstitial cells, it is suggested that the specific bundles may play a role in supporting the connecting tubules between adjacent large stacks of agranular cisternae including the Golgi complex and that the places where many circular or spiral configurations of the specific bundles are observed may correspond to a main point of intersection of these connecting tubules running in various directions. This interpretation is based on the following findings: (1) the specific bundles are observable only in the places where a large number of filaments are concentrated, but not in the areas where loosely distributed filaments are found mingled with cisternae of agranular endoplasmic reticulum; (2) the former places are closely surrounded by large stacks of agranular cisternae intimately connected with each other; (3) within or among these specific bundles, tubules of agranular endoplasmic reticulum run along longitudinal filaments composing the bundle and seem to be continuous with tubules
from the surrounding large stacks of agranular cisternae including the Golgi complex.

It is well known that agranular endoplasmic reticulum in steroid-producing cells may be an important organelle associated with steroid biosynthesis. Therefore, in the dog testicular interstitial cells containing well-developed cytoplasmic filaments, such elaborate organization of filaments may be necessary to secure the pathway for tubules connecting each stack of agranular cisternae which is separated from each other by the invasion of compact masses of filaments.

Acknowledgments. The authors are thankful for the technical assistance of Mr. M. Mieno and Mr. K. Kawao in the Department of Pathophysiology.

References


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