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Ultrastructure and light microscopical study of a Leydig cell tumor of the testis associated with bilateral gynaecomastia

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Summary. Light and electronmicroscopic study of a Leydig cell testicular tumor in an 18-year-old male is presented. Bilateral gynaecomastia and normal hormonal blood levels were found. Emphasis on the diagnostic value of electronmicroscopy is remarked upon, based on the following ultrastructural characteristics of the cells; 1) Ovoid shaped nuclei with ondulating contours and dispersed and homogeneous chromatin, 2) Rich agranular endoplasmic reticulum with frequent special modifications, such as membranous whorls with a central cytoplasmic mass or lipid droplets, 3) Numerous mitochondria with occasional tubular cristae, 4) Numerous lipid vacuoles. Other structures also identified in this tumor are Reinke crystalloids, cytoplasmic microbodies, myelin figures, gap-type junctional complexes and paracrystalline inclusions of Payer type E, which are less common.

Key words: Gynaecomastia, Leydig, Tumor, Ultrastructure

Introduction

Interstitial cells of the testis, which have undeniable endocrine activity, give rise to tumors which show unclear hormonal behaviours and uncertain prognoses. The early gonadal stroma can become Leydig cells. The neoplasms from this stroma are often a mixture of Leydig and Sertoli cells with other less differentiated elements. Thus, tumors which have only one type of these cells, such as Leydig cell tumors, are infrequent (Collins and Pugh, 1964; Mostofi and Price, 1973).

In the present paper, we show the light and electron microscopy of a histologically benign Leydig cell tumor in an 18-year-old patient, who suffered bilateral gyneacomastia. We emphasize the ultrastructural pattern, making particular reference to the occurrence of Reinke crystalloids, which are an essential element to typify the origin of the tumor cells and are an uncommon finding in these cells.

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Materials and methods

Case Report

An 18-year-old male, with normal morphometric constitution, came in for a consultation for a painful testicular tumor and mild swelling of both breasts. Upon exploration, a painful right testicular hydrocele stood out, and a small mass with slightly greater consistency than the rest of the parenchima was noted. The sexual characteristics were normal for his age, with the exception of a bilateral gynaecomastia. Blood hormonal levels showed no significant alterations. Consequently, right orchidectomy and subcutaneous mastectomy were performed. The macroscopic study of the testis showed a round tumoral mass of two centimeters diameter, encapsulated, with a homogeneous yellow surface, close to the albuginea.

The whole testicle was removed, fixed with 10% formaldehyde, where it remained for 5 hours. Afterwards, a sample from the tumor was postfixed in 2% glutaraldehyde in Milloning buffer for 4 hours. The rest of the specimen was submitted to paraffin wax embedding and sectioned in 5 μ thickness for light microscopy. The samples for electron microscopy were submitted to 1% Osmium tetroxide in phosphate buffer at pH 7.3 for 2 hours. Afterwards, the tissue was dehydrated and embedded in Vestopal. Ultrathin sections were cut on a LKB ultratome microtome and stained with uranyl acetate and lead citrate. The micrographs were taken by an EM Zeiss M 10 B at 60 kv.

The breast sample for the light microscopy were taken from subdermic condensations of tissue showing white aspect with irregular borders among the adipose tissue.

Results

Light microscopy

The histological study of the specimen revealed a clear delineation between the tumor and the adjacent testicular parenchyma by means of a fine connective capsule. The neoplastic cells showed an epithelial appearance, well defined limits and eosinophylic cytoplasms, adapting a homogeneous trabecular growth pattern. Their nuclei were round, conspicuous nucleoli, scarce pleomorphism, and very occasional mitotic figures were also seen. Cytoplasms showed fine sudanophilic lipid droplets in a high percentage of them. In spite of being numerous, we could not find any Reinke crystalloids in the numerous tissue sections which were studied. A fine net of capillaries was observed among cells, giving a significant endocrine-like appearance to the tumor. Vascular or capsular invasions were not found. Samples corresponding to the mastectomies showed a typical pattern of gynaecomastia.

Electron microscopy

The nuclei of tumoral cells showed ovoidal or slightly ondulating contours (Fig. 1). Their chromatin were mostly dispersed and homogeneous, with small condensation points which are more evident close to the nuclear membrane. Nucleoli, generally alone, were conspicuous. Numerous mitochondria, displaying lamellar cristae rather than tubular, stood out in the cytoplasm of the cells (Fig. 2A). The endoplasmic reticulum was abundant. The smooth one prevailed, which often formed special spiral-like structures enclosing either portions of cytoplasm with ribosomes and mitochondrias, lipid droplets (Fig. 2B) or conformed structures which resembled myelin figures (Fig. 2A).

Lipid droplets, similar to the morphologically neutral fats, were frequently present in the cytoplasm (Fig. 1). These droplets may be related to endoplasmic reticulum or not. Many cells contained round membrane-bounded dark bodies (Fig. 2C), which ranged from 80 to 240 nm in diameter, and were often located near the cell membrane or the Golgi cisternae. Filament structures, composed of sets of parallel microfilaments, were observed in some cells; these structures being similar to the Payer type E paracrystalline inclusions (Payer, 1980) (Fig, 2A).

The most noteworthy fact was the occurrence of isolated neoplastic elements with a crystalloid polygonal structure, mostly hexagonal, in their cytoplasms. Those

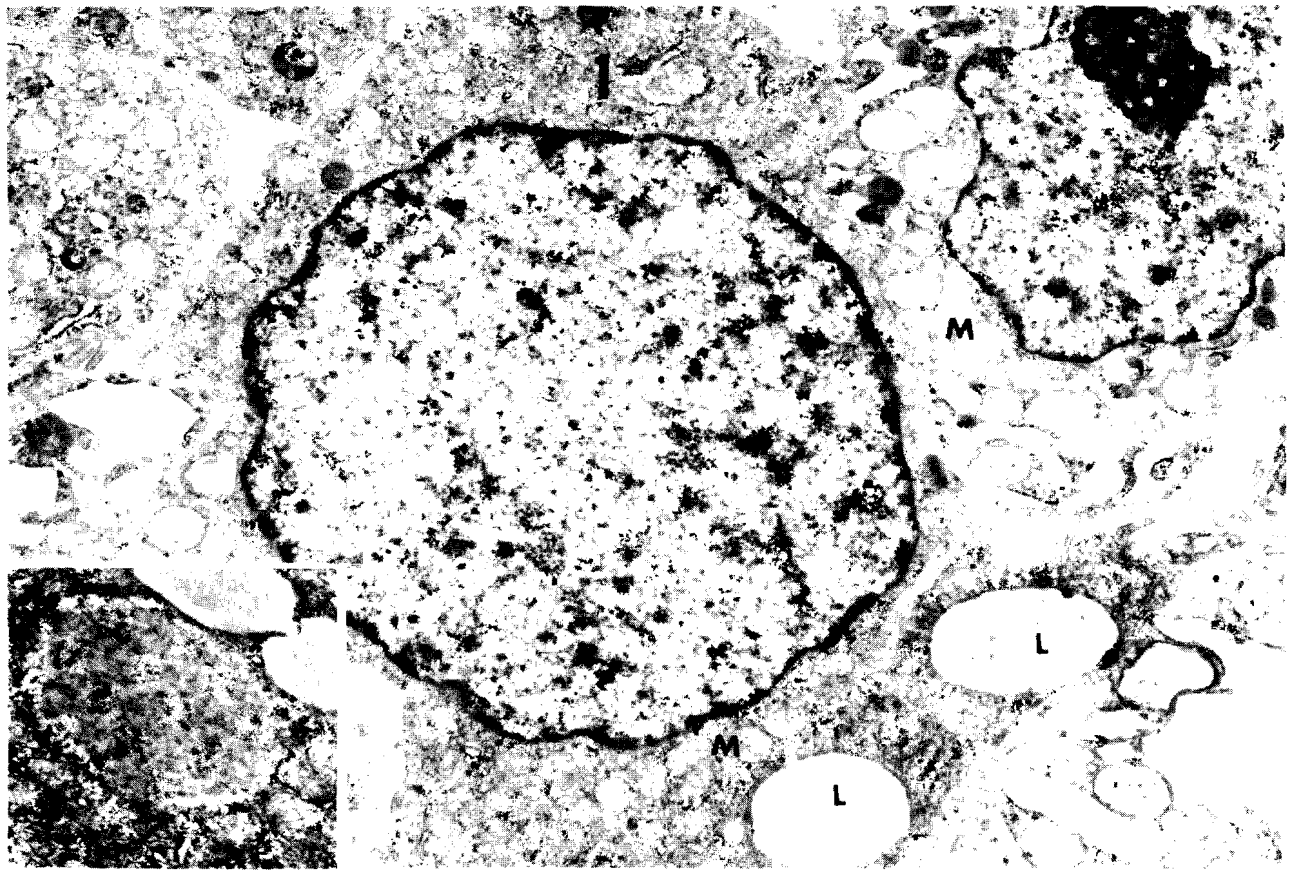

Fig. 1. Electron micrograph of neoplastic Leydig cells showing large sized nuclei with slightly ondulated nuclear membrane dispersed and homogeneous chromatin with small condensation points, numerous mitochondria (M) and lipid droplets (L) × 10,000. (Inside) Reinke crystalloid with a typical reticular structure × 18,900

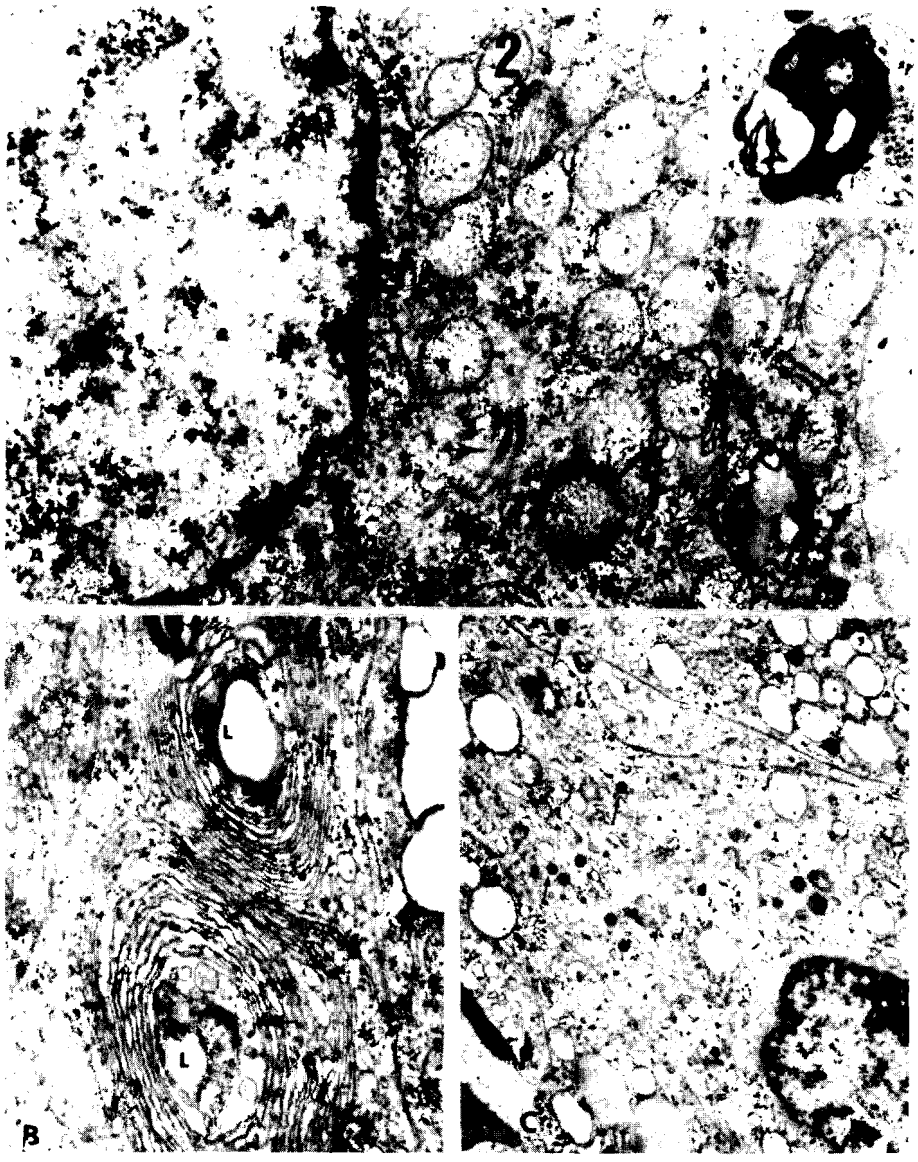

Fig. 2. A) Electron micrograph of tumoral cells showing numerous mitochondrias with lamellar and tubular cristae, and paracrystalline inclusions (arrow) × 25,000. (Inside) Lamellar structure which resembles myelin figures. × 12,000. B) Cytoplasmic membranous whorls with a central endoplasmic mass (arrow) and lipid droplets (L) × 24,000. C) Tumorous Leydig cells with round, membrane-bounded dark bodies (arrow) × 12,500

structures, were composed of filamentous units which were arranged as a fine reticular net, showing the ultrastructural morphology of Reinke crystalloids (Fig. 1). Finally, frequent junctional complexes of the desmosome type were noted between the membranes of the adjacent neoplastic cells.

Discussion

The present study reports a gonadal stroma tumor composed exclusively of Leydig cells showing a typical morphological pattern and associated with bilateral gynaecomastia. The latter occurs in adults at a frequency ranging from 12% (Chen et al., 1982) to 15% (Tackray, 1978). Several explanations have been proposed for this phaenomenon, all of them related to an overproduction of androgens, or in some cases even related to an overproduction of strogens (Mostofi and Price, 1973). But none of these are totally convincing nor applicable to our case, in which the blood hormone levels were normal. The histological pattern was of a benign tumor and 5 years later there were no metastasis nor alterations of the hormonal figures.

Another aspect that we think is interesting, is the ultrastructural morphological pattern of these tumors, since we consider the morphological features of the neoplastic cells, which are present both in our case and in those of other authors, very characteristic. The following morphological characteristics are sufficient to establish a diagnosis of origin of the tumoral cells, even without identifying the pathognomonic Reinke crystalloids: 1) A round nuclear contour whose membrane often appeared ondulated, the chromatin was homogenously dense, dispersed and with peripherical condensations and clumps of granular aggregates (Sohoval et al., 1977; Sworn and Buchanan, 1981). However, some more pleomorphic tumors, e.g. those described by Feldman et al. (1982) show greater folding in the nuclear membrane, and give a more irregular nuclear contour. 2) In the cytoplasm, an abundant smooth endoplasmic reticulum stood out. It formed spiral-like structures composed of concentric cisternae (Cervós-Navarro et al., 1964; Beals et al., 1965; Sotodate et al., 1970; Carr, 1972; Sworn and Buchanan, 1981; Feldman et al., 1982), including occasionally a portion of cytoplasm (Sworn and Buchanan, 1981; Feldman et al., 1982) with mitochondria in its center (Sohoval et al., 1977) or lipid droplets. The highly numerous mitochondrias were seen with occasional tubule cristae (Sohoval et al., 1977; Sworn and Buchanan, 1981; Feldman et al., 1982). 3) Other structures we observed were less constant in the literature, such as junctional complexes of the gap type (Carr, 1972; Sohoval et al., 1977; Sworn and Buchanan, 1981) and myelin figures (Cervós-Navarro et al., 1964; Sohoval et al., 1977; Feldman et al., 1982). In the same way, we have found, with a certain frequency, membrane-bounded dark granules in the tumorous cells,

similar to the normal human testis (Fawcet and Burgos, 1960), or those described by Reddy and Shovoda (1972) in Leydig cells of the testis of rats, mice or guinea pigs, or the microbodies described in tumoral cells by Beals et al. (1965) and Sohoval et al. (1977). Our finding of paracrystalline inclusions in the same cells of the tumor, is an interesting addition to the aforementioned parameters. The significance of these inclusions is still unknown, being related to the formation of Reinke crystalloids by some authors (Fawcet and Burgos, 1960; Sohoval et al., 1973). These inclusions have been described both in normal testis (Fawcet and Burgos, 1960; Yasazumy et al., 1967; Sohoval et al., 1973; Payer, 1980), and in displastic ones (Livni et al., 1977; Nistal; 1978; Paniagua et al., 1984), but we have not found them described in neoplastic cells by other authors.

Lastly, according to the descriptions in the literature, we also include Reinke crystalloids within the group of less constant features. They were found in our material by electron microscopy, but not by the light microscopy, after having sectioned many blocks of tumoral tissue. These cyrstalloids have also been described by some authors (Beals et al., 1965; Leung et al., 1971), whereas others do not mention them (Sohoval et al., 1977; Sworn and Buchanan, 1981; Chen et al., 1982; Feldman et al., 1982). Mostofi and Price (1973) show only 3 cases containing crystalloids out of 12 registered ones. On the other hand, Sotodate et al. (1970) observed them by light microscopy and not by electron microscopy, which is in sharp contrast with our results. For these reasons, the identification of Reinke crystalloids is considered by us to be of value, since the nature of the tumoral cells is fully established with this feature but according to our experience it is necessary to perform numerous sections of tumoral tissue, in order to obtain it. But at the same time, we think that the absence of these crystalloids does not impede the ultrastructural diagnosis of Leydig cell tumors, on the basis of other differential characteristics more commonly presented.

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