Virchows Arch [Pathol Anat] (1982) 397:17–27

Virchows Archiv A

Ultrastructure of a Virilizing Ovarian Leydig-Cell-Tumor

Hilar Cell Tumor

N. Schnoy
Klinikum Charlottenburg der Freien Universität Berlin, Pathologisches Institut (Direktor: Prof. S. Blümcke), Spandauer Damm 130, D-1000 Berlin 19

Summary. A case of virilizing ovarian hilus cell tumor (Leydig-cell tumor) in a 37 year old female was studied by light and electron microscopy. The ultrastructural features of this rare and almost allways benign tumor are compared with those reported in the literature and with findings in normal and neoplastic interstitial cells of the testis. Tubulovesicular hyperplasia and formation of whorl structures of the endoplasmatic reticulum together with the presence of exocytosis vesicles on the cell surface may be the morphological manifestation of endocrine activity of the tumor. The identity of ultrastructural and optical diffraction characteristics of the crystal inclusions in both cells (hilar and testicular interstitial) favours the assumption of an homology of both cells and their neoplasms.

Key words: Ovarian tumor – Hilus cell tumor – Leydig cell tumor – Virilism – Electron microscopy

Differentiated androblastomas of the ovary may present as tumors of the Sertoli cells or Leydig cells, most often, however, they occur as a combination of both cell types. Leydig cell tumors almost always show secretion of male sex hormones [7, 20]. The cell matrix of the rare pure Leydig cell tumor (about 40 recorded cases in the literature [7]) consists of the hilar cell of the ovary which was described by Berger [2]. It is assumed to be identical with the interstitial cell of the testis. Electron microscopic studies of normal interstitial testicular cells [5, 6], Leydig cell tumors [4, 20] and the Leydig cell component of arrhenoblastomas [1, 14, 18] can be found in the literature. However, we found only 2 studies on the electron microscopic structure of ovarian tumors consisting only of Leydig cells [10, 16].

Offprint requests to: N. Schnoy at the above address

0340-1227/82/0397/0017/$02.20

Clinical Data

One year after hormone contraceptive therapy of 10 years duration was discontinued in a 37 year old female, she presented with amenorrhea and progressive virilisation. In addition she complained of baldness, male physical features, beard growth, seborrhoea, weight gain, increased perspiration, increased libido and hypertension which was difficult to control with medication (190/120). Except for a hypertrophied clitoris, the pelvic examination revealed no pathological results. Serum testosterone levels were elevated (10.5 ng/ml) and remained so in the HCG test. Production of cortisone was normal and could be suppressed in the dexamethasone test. Laparoscopically the left ovary was seen to be somewhat enlarged but there were no signs of an ovarian tumor. Selective catheterisation of the adrenal and ovarian veins showed that the left ovary was producing the increased testosterone levels [11]. The specimen obtained during the left-side adnexectomy showed an ovary $4 \times 3 \times 1{,}5$ cm in size with a smooth surface and a flat elevation of 0,5 cm in diameter in the hilar region. When this region was cut a round, soft light brown colored tumor with sharply defined borders and a diameter of 1,5 cm was seen. The surface of the tumor was covered with a very narrow capsule.

Material and Methods

Directly after removal of the ovary tumor tissue was conserved in a 5% glutaraldehyde solution (kept at a pH of 7,3 by means of a 1/15 molar phosphate buffer) for electron microscopic study. Thereafter block contrasting with a 2% osmiumtetroxyde solution was performed, the specimens were then embedded in araldite. The ultrathin sections were then contrasted in a 5% uranylacetate solution in a 50% ethyl alcohol and lead citrate solution.

Light Microscopic Findings

Light microscopically the tumor is seen to consist of large isomorphic closely packed cells. Most cannot be distinguished from normal hilar cells. The cytoplasm is weakly acidophile, finely granulated and shows perinuclear condensation in paraffin sections. The nuclei are nearly round and show little variation in size. Most contain a prominent, peripherally located nucleolus, or several nucleoli which vary in size. Scattered cells are greatly enlarged and the cytoplasm as well as the nuclei show a greater variety of shapes. In semi-thin sections one often recognizes interdigitations of the cytoplasm and deep indentations of individual nuclei. On the whole, however, distinct polymorphism cannot be observed. Lipopigment and small fat droplets can be seen only in very small quantities. In contrast Reinke crystals are numerous in paraffin sections and can be seen even better in semi-thin sections in the cytoplasm as well as in scattered nuclei. The border between the tumor and ovarian stroma is always clearly defined.

Electron Microscopic Studies

The ultrastructure of the tumor cells consists of interstitial spaces containing several collageneous fibres and blood capillaries. The polyhedral cells often form short branches of cytoplasm; in addition interdigitations of neighbouring cells often exist (Figs. 2a and 3d). Once every so often the cell membrane discontinues and the cytoplasmatic structures extend to neighbouring cells, giving the impression of cytoplasmatic continuity. One often sees "secretion" as electron-negative pinocytotic vesicles with a diameter of 0,1 µm in the intercellular space which is 0,02 µm wide (Fig. 1). This phenomen, seen in cells with and without Reinke crystals, is accompanied by a widening of the cisternae of the endoplasmatic reticulum, a sign of increased synthetic activity.

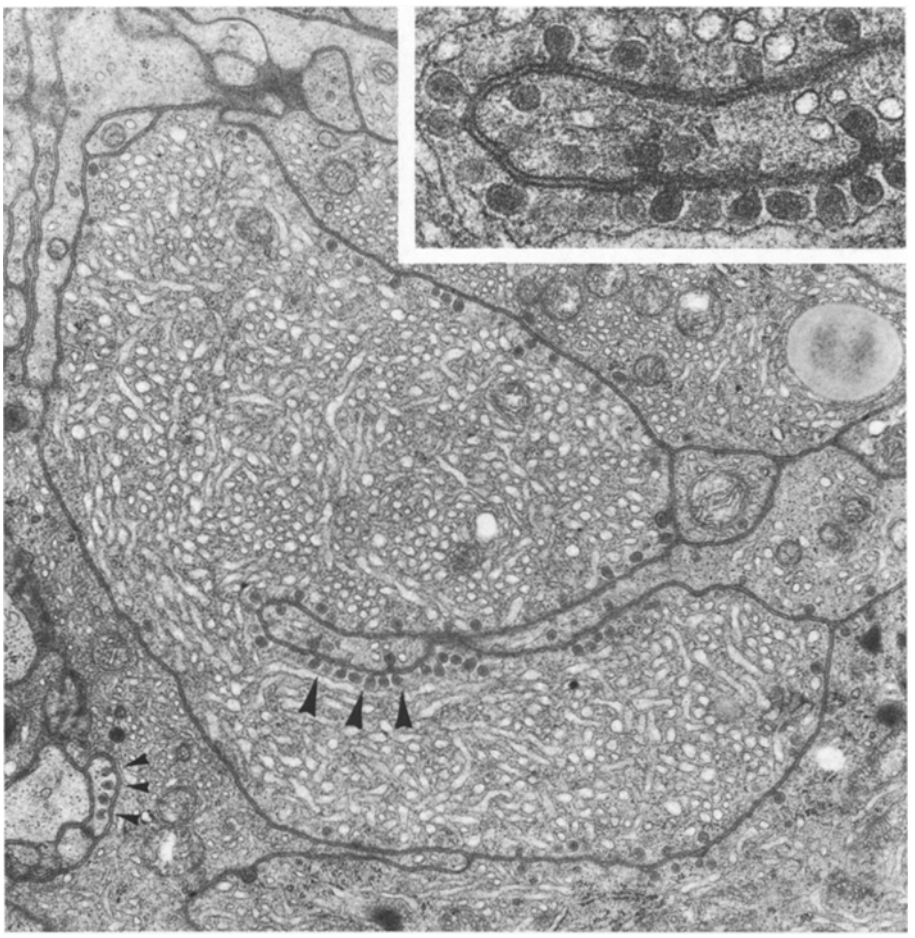


Fig. 1. Exocytosis of the synthesized products on the cell membrane (*arrowheads*). Vesicle diameter = 0,1 µm. × 1,700 (*inset* × 53,500)

The nucleolus of the Leydig tumor cell is round or oval shaped. The nuclear membrane shows pores, contains folds and the chromatin is more dense toward the periphery. The nucleolus is usually large, sometimes contains irregular convolutions and contains inclusions of lipid droplets (Fig. 3d). Nuclei containing two nucleoli are not rare.

The cytoplasm contains many organelles. The mitochondria are positioned at irregular intervals and are usually round, sometimes oval-shaped. They sometimes contain cristae but usually have, however, an irregular tubular internal structure. Often the mitochondria and endoplasmatic reticulum are located near one another (Fig. 3a). Now and then giant mitochondria can be found.

The endoplasmatic reticulum is present in large amounts (Figs. 1 and 2a). It shows a generally smooth surface which extends focally into small

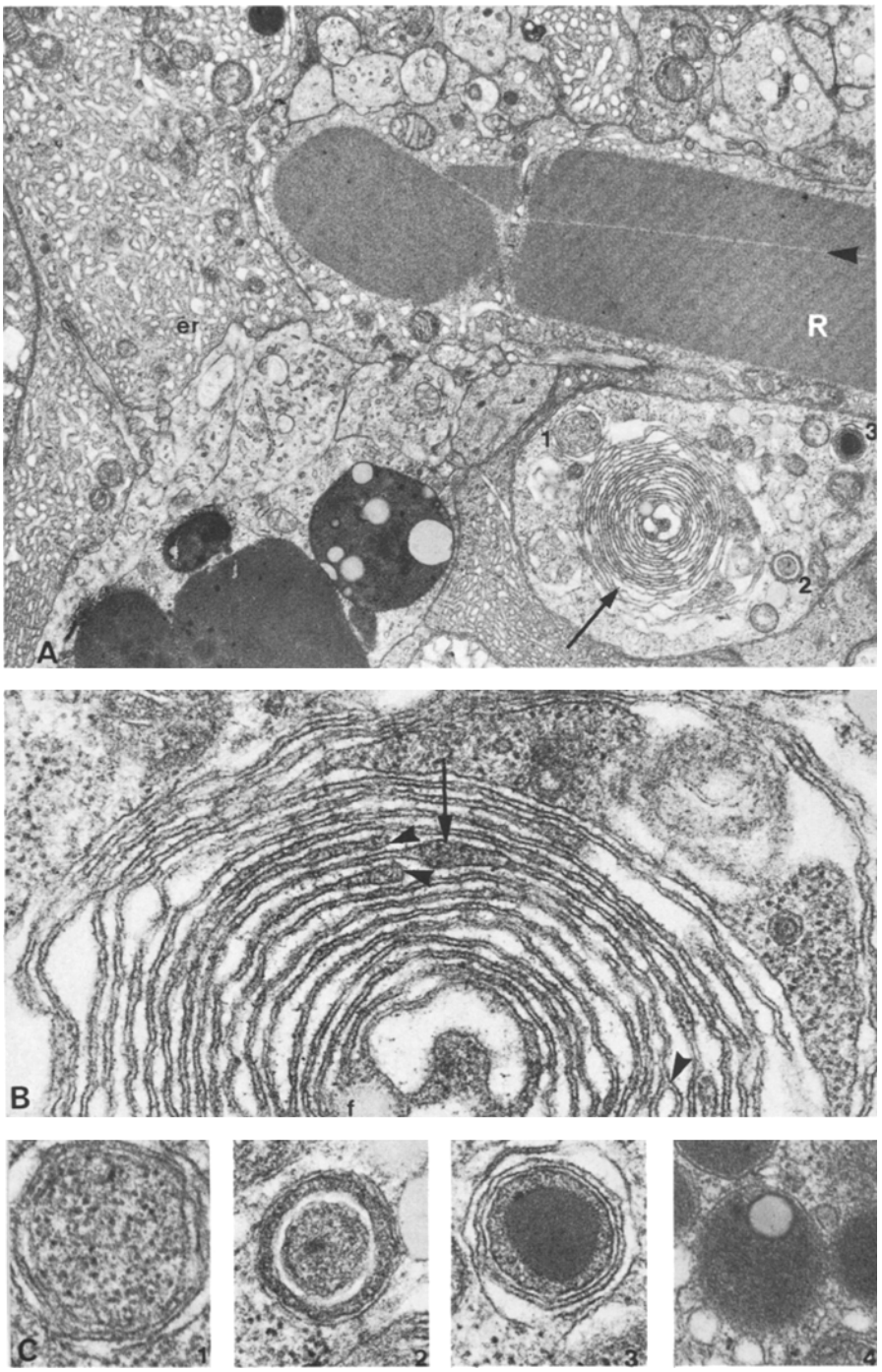


Fig. 2A–C. Ultrastructural characteristics of cytoplasm of tumor cells: **A** Tubulovesicular hyperplasia (*er*) and whorls (*arrow*) of the mostly smooth endoplasmatic reticulum. Focal cytoplasmic degeneration (the numbers correspond to the enlargements in c). Reinke crystal (*R*) with dislocation (*arrowhead*). ×10,500. **B** Whorls of smooth endoplasmatic reticulum with branches and piston-like distension (*arrows*) as well as inclusion of fat droplets (*f*). Part of a. ×52,000. **C** Hypothetical steps in the formation of focal cytoplasmic segregation beginning with segmentation of ribosome-filled cytoplasm compartments in slings of endoplasmatic reticulum (*1*), with increasing density (*2*), to a complete homogenisation (*3, 4*), finally with inclusions of fat droplets (*4*). ×52,000

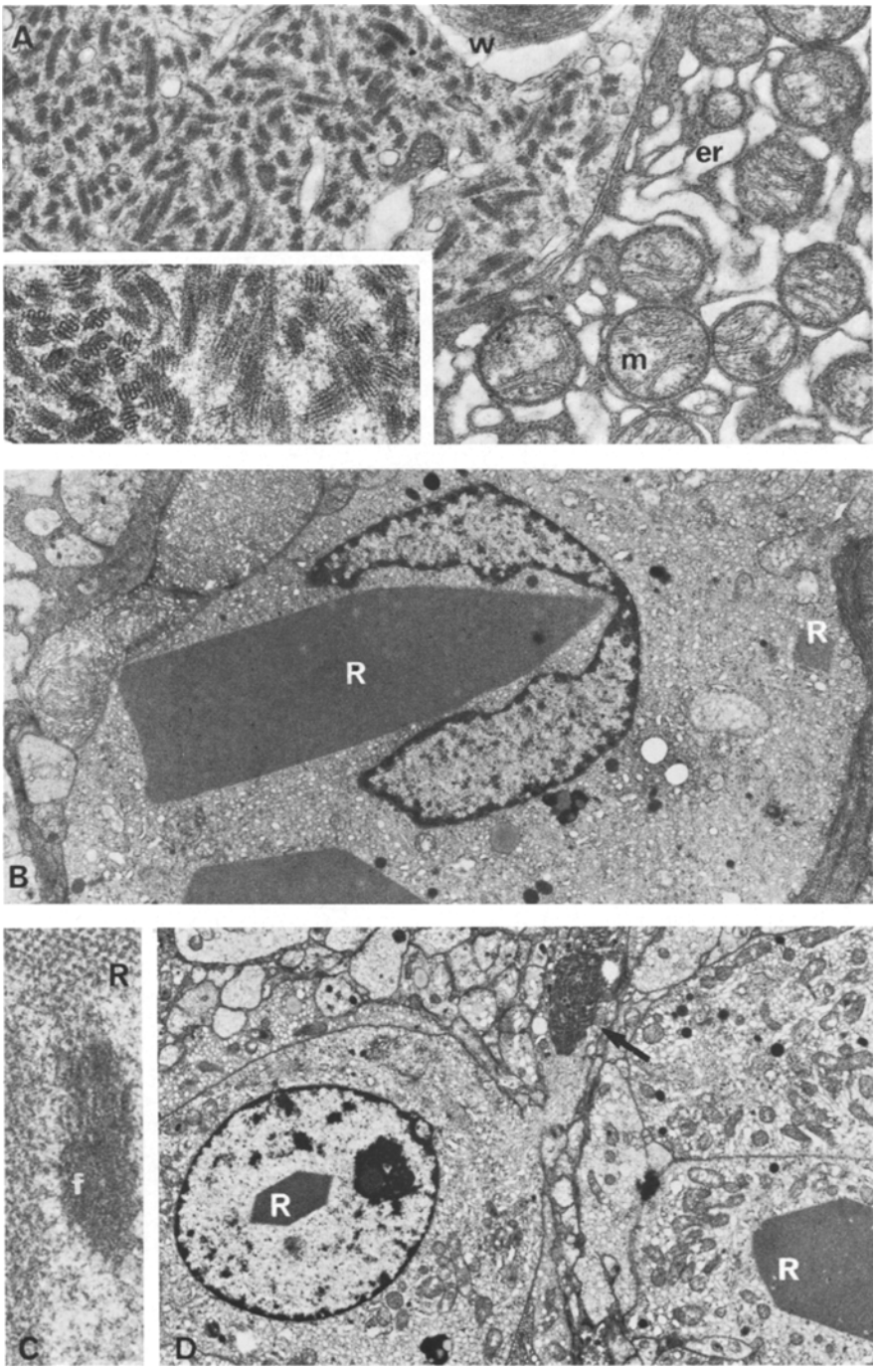


Fig. 3A. Topographically close relationship of tubular endoplasmatic reticulum structures (*er*) to the mitochondria (*m*). Spiral- shaped hyperplasia of endoplasmatic reticulum (*w*). Cristalloid precursors (*f*). × 20,000. *Inset:* Precrystalloid structures in high magnification, consisting of meandering lines (sheets) with 150 Å intervals and fine granular substructure (× 42,000) **B** Typical Reinke-crystal (*R*) in the longitudinal axis with deformation of the nucleus by the needle-shaped tip of crystal. × 6,000. **C** Filamentous cristalloid precursors (*f*) directly beside an intranuclear Reinke crystal (*R*). × 65,000. **D** Intranuclear inclusion in crystal (*R*) and intracytoplasmatic crystalloid precursors (as in a) in the same tumor cell. × 5,000

areas with few ribosomes. Tubules, which are very close to one another and irregularly branched and anastomosed are present, together with vesicles with small lumens and large cisterns. These various forms are all seen in the same tumor cell, however, usually one form is dominant.

Often all of the endoplasmatic reticulum of 1 cell is in the form of spirals (Figs. 2a, b and 3a). They often contain ribosome-rich cytoplasm components or fat droplets. These spirals show branching and a piston-like dilatation especially in the blindending final segment (Fig. 2b). Sometimes a transition to layered osmiophilic lamellar formed bodies is found in the middle of these spirals. The centers of the above mentioned bodies become unstructured or plaquelike granulated electron dense zones (Fig. 3a).

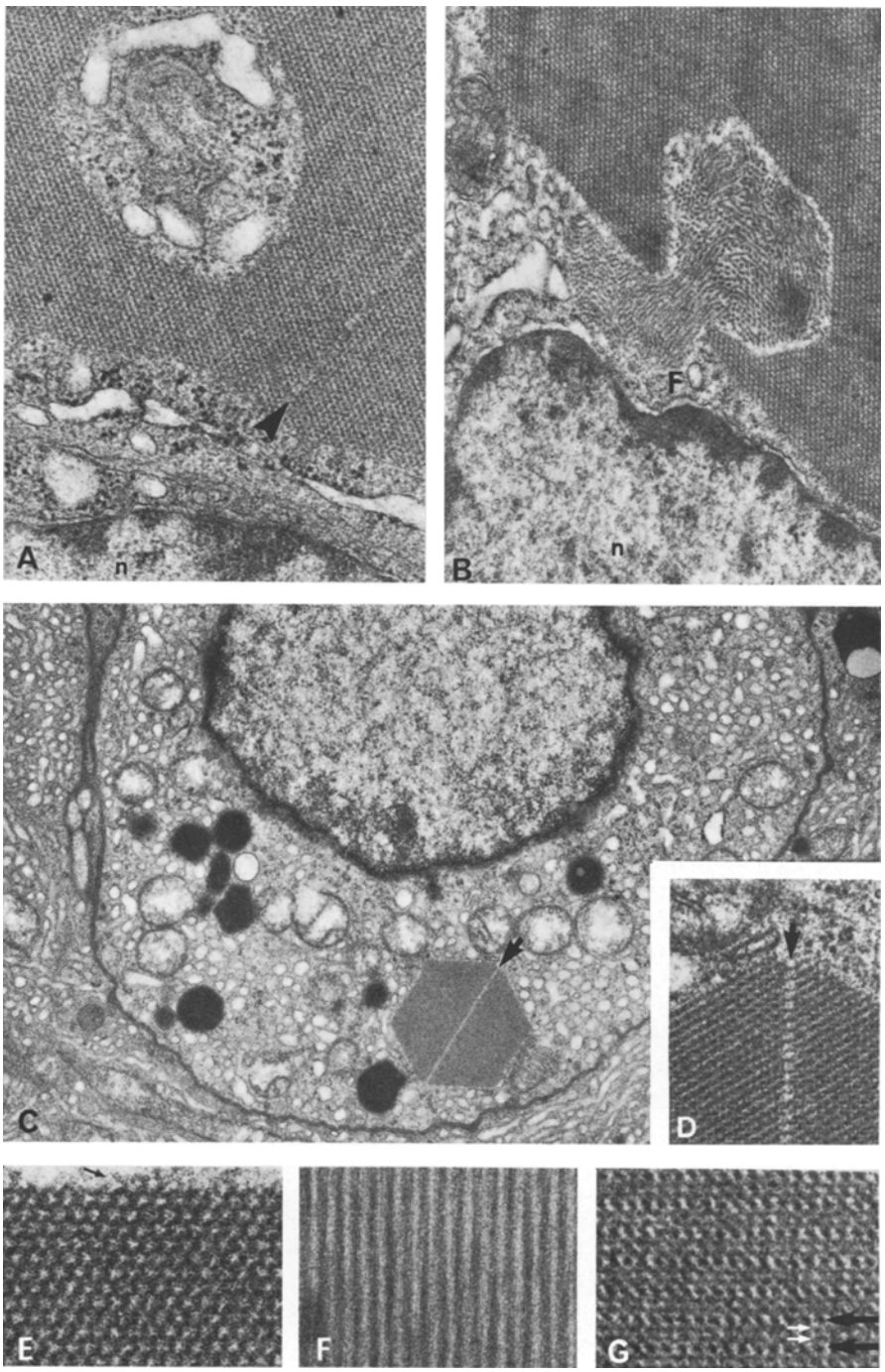
Outside of these spirally formed hyperplastic regions, local cytoplasm segregation involving the endoplasmatic reticulum can be seen (Fig. 2c). Small round areas of cytoplasm are contained in one ore more convolutions of the endoplasmatic reticulum. In part, they contain isolated ribosomes; in other areas they show a density which may border on full homogenity. In addition, fat droplets are present in these areas and form lysosome-like bodies by their confluence which are assumed to be the substrate of the pigment. They show morphological similarity to lipofuscin granules.

The most obvious and numerous are the crystalloid inclusions. The Leydig crystals are not membrane bound. They border on the endoplasmatic reticulum or other cytoplasmatic elements. Occasionally, the nuclear and cytoplasmatic membranes are deformend (turned inwards or outwards) (Fig. 3b). Peripherally they show "chips of crystalline fragments" which have clearly defined congruent lines bordering the corresponding side of the next crystal.

The crystals are rectangular or have a needle-like shape. In cross seftion they are often hexagonal (Fig. 4c). Within the crystals one finds varying substructures, denpending on the section (Fig. 4e–g). In a section cut transversly to the longitudinal axis the crystals show a hexagonal prismal structure (honeycomb) in which 1 microcylinder is surrounded by 6 others. In sections along the crystals longitudinal axis two other main planes with various structural patterns can be recognized. One of these consists of identical parallel lines at intervalls of 150 Å (Fig. 4f); the other consists of dots arranged in parallel lines (Fig. 4g), in which two lines are made up of tiny dots and the third of thick dots. The intervalls between the dot lines are 50 Å. The sectional phases which lie between the described main planes show many variations in the electron microscope, often reminding the observer of a textile-like structure.

Characteristically "lines of dislocation" can often be found within the crystal; these usually extends outside of the median plane, longitudinally or horizontally through the crystals (Figs. 2 and 4a, c, d). Nagano [19] studied these lines in the interstitial cells of the testis. In the filamentous pattern within these fracture lines one of the three parallel lines is missing. More-

Fig. 4A. Reinke crystal near nucleus (*n*) with inclusion of cytoplasmatic elements. Dislocation in crystal reticulum (*arrowhead*). ×32,500. **B** Hexagonal recess in Reinke crystal with filamentous precursors which extend into the cytoplasm (*F*), *n*, nucleus. ×36,000. **C** Tumor cell with hexagonal transverse incision of a Reinke crystal with typical line of dislocation (*arrow*). ×9,700. **D** Line of dislocation with formation of periodical connecting links (*arrow*) between the crystal parts. Part of Fig. 4c, ×26,200. **E** Periodic hexagonal honeycomb pattern consisting of filaments 50 Å thick, cut transversly to the longitudinal axis of the crystal. The hexagonal edge of the crystals is 200 Å long. Isolated globular substructures (*arrow*) hint at subunits in the filamentous walls. ×39,600. **F** Parallel lines at intervals of 150 Å in the longitudinal axis of the crystal ×92,500. **G** Pattern of 3 periodically recurring parallel lines, consisting of 1 row of thick dots (*large arrows*) and 2 of thin dots (*small arrows*). Distance between each row of dots is 150 Å. ×90,000


A
n
B
F
n
C
D
E
F
G

over, sometimes pocketlike inclusions of cytoplasmic components can be found within the crystals (Fig. 4a, b). On the edge of the crystal we found a rhomboid shaped recess which communicates with the surrounding cytoplasm. Within this inclusion there are dense filament structures, which we assume to be precursors of the crystals (Fig. 4b).

In addition to the large crystals which can be seen in the light microscope, there are small more irregularly located precrystalline structures, which in their density sometimes take up all of the cytoplasm. When magnified they are seen to consist of a bundle of six parallel lines or, as in the orthograd section, of a twisted band with a hint of granulated substructure (Fig. 3a, c, d). Within these crystals we found a periodicity of 150 Å, similar to that of large Leydig crystals. Within the nucleus crystalloid inclusions can be found either alone or combined with crystals or their precursors in the cytoplasm (Fig. 3c–d). The third form of these precursors is detectable in a few cells as groups of irregularly positioned long filaments (Fig. 4b). There is no correlation between the crystalloid inclusion or their precursors and the frequency or variety of shapes of other cytoplasmatic structures.

In summary our results indicate that the tumor described is defined by the following structural characteristics:

1. Unusually strong development and pleomorphism of the endoplasmatic reticulum with tubulovesicular and spiral formations.
2. Exocytosis on the surface of the cells.
3. Crystalloid cell inclusions:

a) Crystalloid inclusions can be found within the nucleolus and cytoplasm in the same cell.

b) Precursors of the crystals are found in the same cell as are formed crystals; the precursors are seen as bundles of filaments or twisted bands.

c) In the 3 main planes, the substructure of the crystals is identical to the inclusions, which are seen in the Leydig cells of the testis.

d) The diffraction of the crystalline structure of Leydig cells is identical to that of the crystals of the tumor.

Discussion

Hilar cell tumors have been described in association with Stein-Leventhal-Syndrom [10, 16], in familial incidence [18], combined with endometrial hyperplasia and endometrial polyps as well as with endometrial carcinomas [7]. The majority of the pure hilar cell tumors show androgenic hormonal activity [7, 20]. Jeffcoate and Prunty [13] were able to prove the existence of steroid synthesis of a hilar cell tumor in vitro. In contrast, in tumors with a hilar cell component (arrhenoblastoma, Sertoli-Leydig-cell-tumor), oestrogen activity is seen [22]. Völpel [27] reported a case of hilar cell hyperplasia in adrenogenital syndrome. Obviously the majority of pure Leydig cell tumors are benign. However 3 cases of malignancy with metastases are described in the literature [8, 25].

When examined the tumor cells show the same structural elements as normal Leydig cells: a large amount of smooth endoplasmatic reticulum

with twisted tubules and wide cisterns and formation of whorls with inclusions of fat droplets and free ribosomes.

One of the characteristic structures and a morphologic equivalent of a high rate of synthesis in tumor cells is the presence of abundant endoplasmatic reticulum, which is also seen in endocrinologically active adrenal tumors [26]. It is within these structures that numerous steps in the synthesis of steroids are assumed to take place [6]. The increased presence of these structures as well as the formation of whorls of the endoplasmatic reticulum was experimentally proven by Merkow and co-workers on embryonic and mature Leydig cells of guinea pigs after stimulation with human chorionic gonadotropin [15].

The many whorls which can be seen in the endoplasmatic reticulum are obviously a characteristic finding in the Leydig cell tumors and were also seen in the two comparable studies of Merkow [16] and Berendsen [1]. The polymorphism in the appearance of the endoplasmatic reticulum and the observation of varying shapes in the neighbouring cells is not an artefact in the fixation process; rather both phenomena speak for the fact that these are morphological equivalents of varying activity of the endoplasmatic reticulum.

In as much as ultrastructural pictures allow conclusions about dynamic processes, degenerative cytoplasmatic products seem to be part of a process which is initiated by localized incorporation of cytoplasm into convolutions of the endoplasmatic reticulum. This process ends in total, central condensation of osmiophilic material. Possibly this is a step in the formation of the pigment seen in Leydig cells [1].

The numerous negative pinocytotic vesicles, which can be assumed to represent a channeling process of the synthesized products have, as yet, not been mentioned in the literature.

The tumor contained an unusually large number of Reinke crystals. Since they were first described by Reinke in 1896 [21] these crystals are used to identify Leydig cells and tumors arising from these cells.

A stereological analysis of Reinke crystals was done by Mori and co-workers [17]. The optical diffraction pattern of the main planes was demonstrated by Nagano in 1976 [19]. We examined these findings in the crystals of ovarian tumor and were able to certify the identity of the structures: the diffraction patterns of the main planes of the crystals in interstitial testicular cells are identical to those of the crystals in the tumor.

Morphologically similar crystals have been identified in virally infected cells as well as in cells treated with Vincristin [3] and in human tumor cells. The possibility of virus induced crystal formation has been discussed by Fawcett and Burgos [9]. Viral inclusions have been described in estrogen induced Leydig cell tumors in mice [23]. "Virus-like" particles have been described in a human Sertoli-Leydig cell tumor by Murad and co-workers [18]. We found no viral inclusions in our material. The widespread presence of Reinke crystals in normal testicular cells after puberty lessens the probability of viral induction as an aetiologic factor.

The intranuclear position of the cristalline material and its precursors has been observed in human interstitial testicular cells [6]. These crystals may be formed in the nucleus by the same stimulus which initiates the formation in the cytoplasm after transport of smaller structural elements through nuclear pores. The observation of precursors of crystals in mitochondria seems to speak for this interpretation [16]. We were able to demonstrate these as short fibrous bundles and twisted structures. As yet they have not been described in this form. Previous studies [16] described tubular forms of the precursors. In contrast to previous studies [1, 16] we found precursors and complete crystals, in the cytoplasm of the same tumor cell as a hexagonal pocket shaped "surface lesion".

Reinke postulated the protein nature of these crystals in 1896 [21]; this was certified by histochemical studies done by Janko and Sandberg [12]. The detailed chemical nature of these crystals and their relationship to the endocrine activity of the cells has not yet been explained. The fact that, except in cases of precocious puberty, these crystals do not appear until puberty [24] speaks for a close relationship to the endocrine activity of the cells, as does the presence of large amounts of crystalloid inclusions in the cells of the androgen-producing tumor described here.

References

1. Berendsen PB, Smith EB, Abell MR, Jaffe RB (1969) Fine structure of Leydig cells from an arrhenoblastoma of the ovary. Am J Obstet Gynecol 103:192–199
2. Berger L (1922) Sur l'existence de glandes sympaticotropes dans l'ovaire et le testicule humains; leurs rapports avec la glande interstitielle du testicule. CR Acad Sci (Paris) 175:907–909
3. Boulanger PA, Torpier G, Biserte G (1970) Investigations on intranuclear paracrystalline inclusions induced by adenovirus 5 in KB cells. J Gen Virol 6:329–332
4. Cervos-Navarro J, Tonulli E, Bayer JM (1964) Elektronenmikroskopische Untersuchungg eines androgenbildenden Leydigzelltumors. Endokrinologie 47:23–51
5. De Kretser DM (1967) The fine structure of testicular interstitial cells in men of normal androgenic status. Z Zellforsch 80:594–609
6. De Kretser DM (1967) Changes in the fine structure of the human testicular interstitial cells after treatment with human gonadotrophins. Z Zellforsch 83:344–358
7. Dunniho DR, Grieme DL, Wolfe RJ (1966) Hilus cell tumors of the ovary. Obstet Gynecol 27:703–713
8. Echt CR, Hadd HE (1968) Androgen excretion patterns in a patient with metastatic hilus cell tumor of the ovary. Am J Obstet 100:1055–1061
9. Fawcett DW, Burgos MH (1960) Studies on the fine structure of the mammalian testis. II. The human interstitial tissue. Am J Anat 107:245–269
10. Green JA, Maqueo M (1966) Histopathology and ultrastructure of on ovarian hilus cell tumor. Am J Obst Gynecol 96:478–485
11. Hardt W, Sörensen R, Schnoy N, Gräf KJ, Moltz L, Schmidt-Gollwitzer M (1980) Zur Diagnostik androgenproduzierender Tumoren bei der Frau. Geburtshilfe Frauenheilkd 40:373–380
12. Janko AB, Sandberg EC (1970) Histochemical evidence for the protein nature of the Reinke crystalloid. Obstet Gynecol 35:493–503
13. Jeffcoate SL, Prunty FTG (1968) Steroid synthesis in vitro by a hilar cell tumor. Am J Obstet Gynecol 101:684–688
14. Jenson AB, Fechner RE (1969) Ultrastructure of an intermediate sertoli leydig cell tumour. A histogenetic misnomer. Lab Invest 21:527–535

15. Merkow L, Acevedo HF, Slifkin M, Caito BJ (1968) 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. Am J Pathol 53:47–61
16. Merkow LP, Slifkin M, Acevedo HF, Pardo M, Greenberg, WV (1971). Ultrastructure of an interstitial (hilar) cell tumor of the ovary. Obstet Gynecol 37:845–859
17. Mori H, Fukonishi R, Fjii M, Hataji K, Shirais T, Matsumoto K (1978) Stereological analysis of Reinke's crystals in human leydig cells. Virchows Arch [Pathol Anat] 380:1–9
18. Murad TM, Mancini R, George J (1973) Ultrastructure of a virilizing ovarian Sertoli-Leydig cell tumor with familial incidence. Cancer 31:1440–1450
19. Nagano T (1976) Crystalloid inclusions in human testicular cells. In: Yamada E, Mizuhira V, Kurosumi K, Nagano T (eds) Recent progress in electron microscopy of cells and tissues. Georg Thieme, Stuttgart pp 82–94
20. Novak ER, Woodruff JD, Linthicum JM (1963) Evaluation of the unclassified tumors of the Ovarian Tumor Registry. Am J Obstet Gynecol 87:999–1007
21. Reinke F (1896) Beiträge zur Histologie des Menschen. I. Über Kristalloidbildungen in den interstitiellen Zellen des menschlichen Hodens. Arch Mikrobiol Anat 47:34–44
22. Scully RE (1953) An unusual ovarian tumor containing Leydig cells but associated with endometrial hyperplasia in a port-menopausal woman. J Clin Endocrinol 13:1254–1263
23. Stephens RJ, Pourreau-Schneider N, Gardner WU (1968) Cytological morphology of a virus-containing mouse testicular interstitial cell tumor. J Ultrastruct Res 22:494–507
24. Sternberg WH (1949) The morphology, androgenic function, hyperplasia, and tumors of the human ovarian hilus cells. Am J Pathol 25:493–521
25. Stewart RS, Woodard DE (1962) Malignant ovarian hilus cell tumor. AMA Arch Pathol 73:91–99
26. Valente M, Penelli N, Segato P, Bevilacqua L, Thiene G (1978) Androgen producing adrenocortical Carcinoma. A histological and ultrastructural study of two cases. Virchows Arch [Pathol Anat] 378:91–103
27. Völpel M (1971) Hiluszellwucherungen im Ovar bei connatalem adrenogenitalem Syndrom. Virchows Arch [Pathol Anat] 352:43–49

Accepted May 24, 1982