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Response of the human testis to long-term estrogen treatment: Morphology of Sertoli cells, Leydig cells and spermatogonial stem cells

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Summary. The present investigation is concerned with the morphological changes observed in human testicular tissue following prolonged estrogen administration. Testicular material obtained from 11 transsexual patients who had been submitted to long-term estrogen treatment prior to sex-reversal surgery was studied by means of light- and electron microscopy.

The testes of all patients examined present a more or less uniform appearance: There are narrow seminiferous cords surrounded by an extensively thickened lamina propria. They contain Sertoli cells and spermatogonia exclusively. There is no evidence of typical Leydig cells.

The persisting spermatogonia show the characteristic features of pale type-A spermatogonia, whereas dark type-A spermatogonia are almost completely eliminated from the epithelium. In view of the fact that spermatogonia that survived radiotherapy and treatment with various noxious agents have recently been regarded as the stem cells of the human testis, it is suggested that also the majority of those spermatogonial types that are less sensitive to disturbances of the endocrine balance may consist of stem cells. The present results, therefore, corroborate the concept that the stem cells of the human testis may be derived from pale type-A spermatogonia or the variants of this cell type.

Sertoli cells display two types of ovoid nuclei. In contrast to untreated material the nuclei lie adjacent to the basal lamina, and organelles and telolysosomes are confined to the apical cytoplasm. The apico-basal differentiation of mature cells, therefore, is not observed. Moreover, typical organelles and inclusions of mature cells are absent, as are the junctional specializations. Thus, Sertoli cells have transformed into immature cells, resembling precursors prior to puberty.

Fibroblast-like cells in the interstitial tissue, which display strongly lobulated nuclei, a well-developed smooth endoplasmic reticulum, lipid droplets, and numerous inclusions are assumed to represent dedifferentiated Leydig cells.

Since after estrogen treatment serum testosterone and gonadotropin levels are known to be reduced, it appears that the morphological changes correlate well with the endocrine status.

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Because of obvious ethical constraints few experimental data are available relating the effects of hormones to the morphology of human testicular cells. As substitutes for experiments not possible in man, more or less well-defined disturbances of spermatogenesis have been used (Schulze et al. 1976; Chemes et al. 1977). Such a disturbance of spermatogenesis is found in transsexual males as a result of long-term estrogen treatment. While the endocrinological changes following estrogen therapy have been clearly established in the past (Slaunwhite et al. 1962; Yanaihara and Troen 1972; Oshima et al. 1974; Rodriguez-Rigau et al. 1977; Leinonen et al. 1981), morphological studies are far more limited and the status of the overall testicular structure is yet to be defined. Early light-microscopic investigations already indicated a deleterious effect of exogenous estrogens on the human testis (Dunn 1941; Howard et al. 1950; de la Balze et al. 1954, 1962). Two recently published electron-microscopic studies confirmed these results but each of them was confined to special components of the testicular tissue (Lu and Steinberger 1978; Payer et al. 1979).

The first attempt at a more comprehensive investigation was performed recently by Schulze (1984). In extending this examination to a greater number of patients it was possible in the meantime to substantiate and validate these initial observations. The present paper, therefore, contains comprehensive data on the morphology of testicular tissue from eleven transsexual patients who had been treated with estrogens for several years prior to sex-reversal surgery. It provides information on the unique alterations of Sertoli and Leydig cells and on the functional significance of the few persisting germ cells.

Materials and methods

Testicular material was obtained from 11 transsexual patients (aged 22–48 years) who had been treated 1–12 years with various amounts of estrogens, mostly estradiol, prior to sex-reversal surgery (see Table 1 for specific details). Tissues were initially fixed in phosphate-buffered 6% glutaraldehyde (820 mOsm) for 1 h and then postfixed in 1% OsO_4

Table 1. Type and dosage of hormone and duration of treatment

Patient	Age at surgery (yr)	Hormone treatment	Duration of treatment (yr)
1	22	Estrogens	1
2	34	Ethinyl estradiol	12
3	48	Estradiol undecylate 4 × 100 mg/year	10
4	33	Estrogens 100 mg/month	3
5	29	Estradiol valeriate 40 mg/2 weeks	1
6	39	Estradiol undecylate 100 mg/3 weeks	4
7	31	Estrogens	7
8	23	Estradiol undecylate 100 mg/4 weeks	1
9	27	Estradiol undecylate 100 mg/4 weeks	3
10	40	Estrogens	3
11	31	Estradiol undecylate 100 mg/4 weeks	3

(2.5 h). After alcohol dehydration the samples were embedded in Epon 812. Semithin sections were stained with toluidine blue/pyronine, while ultrathin sections were contrasted according to Reynolds (1963). Ultrathin sections were examined in a Philips EM 300 electron microscope.

Results

Histology

After prolonged estrogen treatment the testes of all patients present a more or less uniform appearance (Fig. 1a). Instead of tubules there are seminiferous cords with conspicuously small diameters (70–90 μm). They contain only two cell types: spermatogonia and Sertoli cells (Fig. 1b). Occasionally, mitoses are found within the cords. The lamina propria is extensively thickened due mainly to an accumulation of collagen material adjacent to the basal lamina. The basal lamina itself shows a wavy pattern consisting of a reticulum of anastomosing layers.

In comparison to normal conditions the area occupied by interstitial tissue has markedly increased. Apart from fibroblasts there are a few clusters of small cells containing dark granules (Fig. 1c). Typical Leydig cells do not occur. Cell clusters and blood vessels are often enveloped by thick lamellae of collagen fibrils. Likewise, peripheral nerves show an increased amount of endoneurium and the perineural cells are separated by unusually thick layers of collagen fibrils. Four cases show hyalinosis of small arterioles, a deposition of hyaline material subjacent to the endothelium, which concentrically or excentrically compresses the lumen. With continuing estrogen therapy the number of seminiferous cords declines, the spermatogonia disappear and the cords become smaller. Numerous macrophages occur in the surrounding tissue and among the Sertoli cells inside the cords. After 10 years of treatment the testicular tissue mainly consists of completely hyalinized seminiferous cords.

Cytology of spermatogonia

In all cases examined the germ cell population consists of spermatogonia exclusively. These spermatogonia are not only found adjacent to the basal lamina but also in the middle of the cords (Figs. 1b, 3). Practically all of them belong to the pale type-A spermatogonia (Ap), whereas dark type-A spermatogonia (Ad) are absent from the epithelium. Pale and dark type-A spermatogonia are originally distinguished by the different staining pattern of their nuclei in light-microscopic preparations.

The persisting spermatogonia often lie isolated and show round or oval nuclei with homogeneously distributed chromatin and up to four nucleoli, which contact the nuclear membrane or occur in more central portions of the nucleus (Figs. 3, 4a, b). Occasionally, there are round condensations of a granular material, which is interpreted as heterochromatin. Mitochondria are usually connected by an electron-dense substance and often are associated with membranes of the rough endoplasmic reticulum. Golgi complexes are moderately developed (Fig. 4b). Glycogen is either found scattered throughout the cytoplasm or accumulated in membrane-bound areas. Frequently, the organelles are concentrated around the nucleus so that large areas of the cytoplasm are devoid of cell organelles (Figs. 1b, 4a).

Apart from these characteristic Ap spermatogonia, numerous variants occur. They differ from those described above mainly in the heterogeneous distribution of their chromatin. Such an irregular nuclear staining pattern is apparent even at the light-microscopic level. Fig. 4a demonstrates another variant with a nucleolus surrounded by a clear halo. A striking feature is the occurrence of numerous multinucleate spermatogonia (Fig. 3). Polyploid nuclei and a large number of cells with conspicuous blebs of their nuclear membranes are regularly found.

Cytology of Sertoli cells

Sertoli cells in seminiferous cords of treated patients are tall slender cells, which adhere to one another by their lateral and apical surfaces (Figs. 3, 5a). Except for a few lamellar processes, which extend into the cytoplasm of neighboring cells, they have smooth contours. They possess conspicuously round or oval nuclei, which lie in the basal cytoplasm (Figs. 1b, 2, 3, 5a). Binucleated cells were observed in two incidences.

The majority of the nuclei shows dense, homogeneously distributed chromatin and small nucleoli attached to the nuclear membrane (Figs. 2, 3, 5a, b). In addition, less dense and comparatively larger nuclei occur. Their chromatin appears to be in a less condensed state, the translucent interchromatin space has enlarged. Furthermore, these nuclei contain numerous irregular clumps of a heterogeneous population of particles consisting of fibrils and granules (Figs. 2, 4a, 5b). In large aggregates the granules measure ~20 nm in diameter, in smaller clusters they have diameters of ~40 nm. This cluster formation of differently sized granules throughout the nucleus corresponds closely to the dense spots seen in the light microscope.

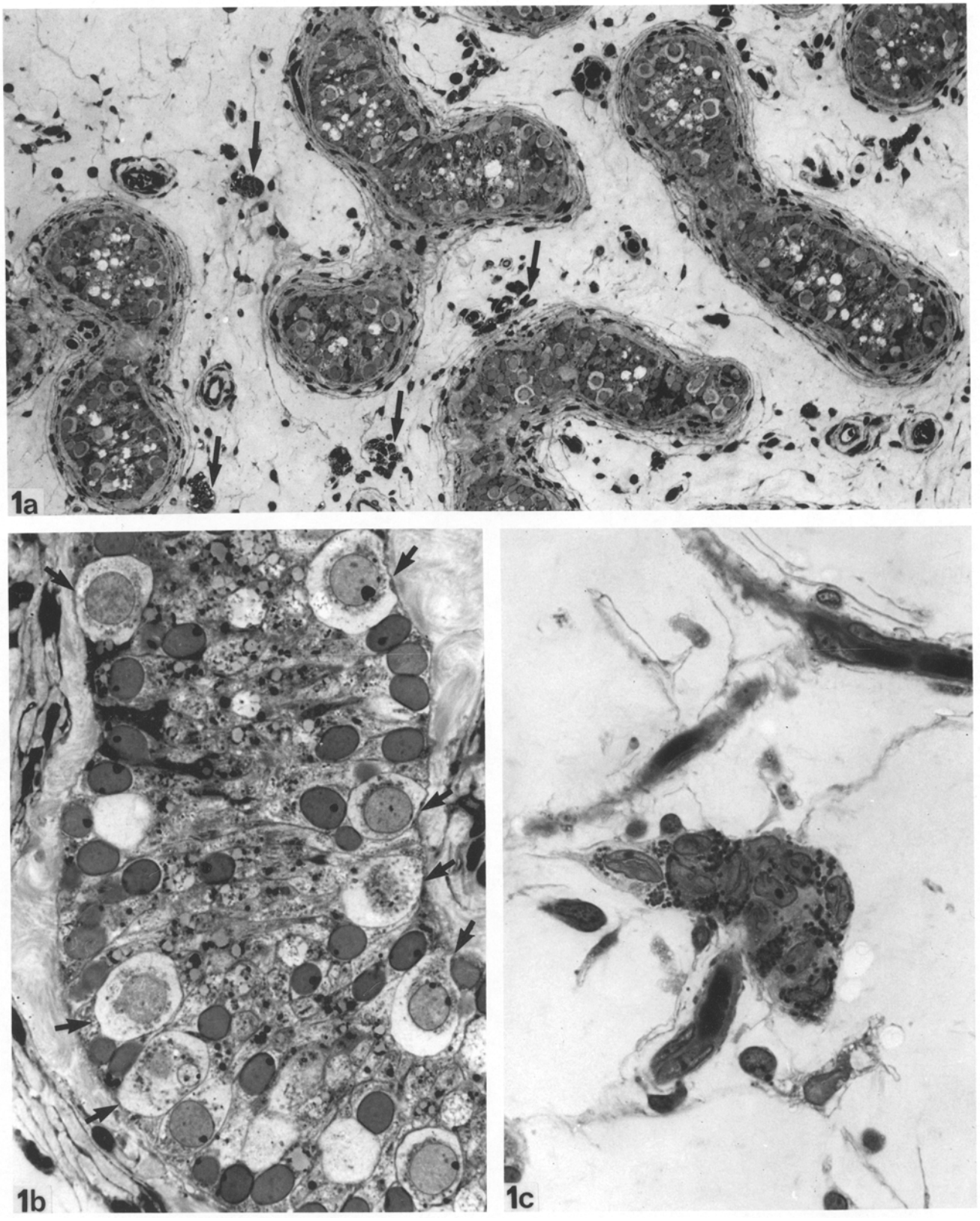


Fig. 1a–c. Light micrographs illustrating the typical morphology of estrogen-treated testes. **a** Narrow seminiferous cords containing only two cell types are surrounded by a thickened lamina propria. The interstitial tissue occupies a large area and apart from blood vessels exhibits a few clusters of small cells (*arrows*). Typical Leydig cells are absent. ×195. **b** Part of a seminiferous cord displaying two cell types: pale type-A spermatogonia (*arrows*) and Sertoli cells with ovoid nuclei. ×920. **c** Interstitial tissue. A cluster of fibroblast-like cells with lobulated nuclei and dark granules in the vicinity of capillaries. ×920

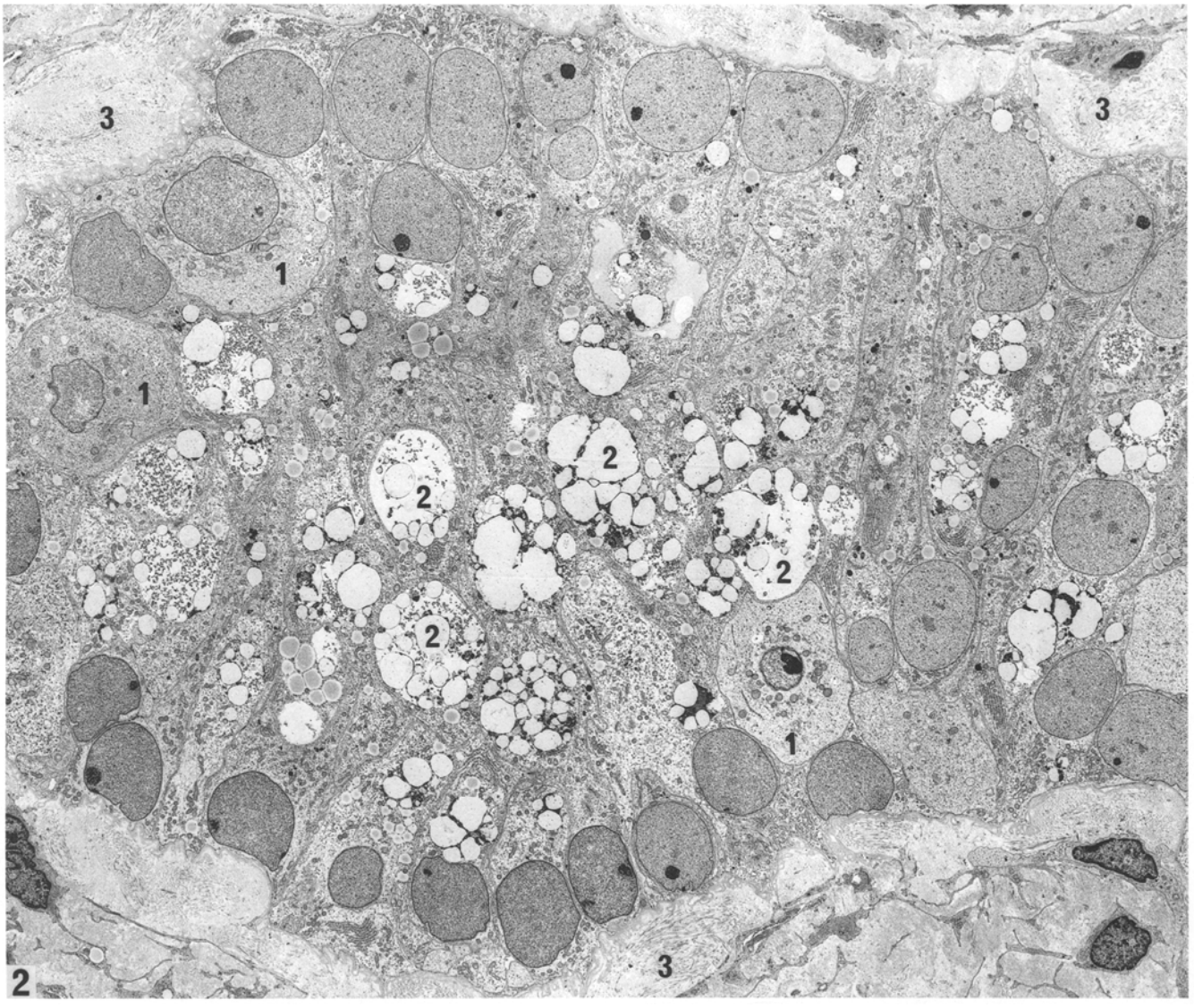


Fig. 2. Low-magnification electron micrograph of a cross-sectioned seminiferous cord demonstrating two aspects of Sertoli cell nuclei. The nuclei have smooth contours and are located adjacent to the basal lamina in a palisade-like manner. Some nuclei (*at bottom*) are small and dark, other nuclei (*at top*) are larger and their pale chromatin exhibits dense spots. Note that the cytoplasm of the respective cells does not show any morphological differences. *1* spermatogonia; *2* lipid inclusions in the apical cytoplasm of Sertoli cells; *3* collagen fibrils of the lamina propria. × 2000

The apical cytoplasm of the cells displays numerous lipid droplets and telolysosomes, which in turn consist of lipid droplets, osmiophilic material and vacuoles rich in glycogen (Figs. 2, 3, 5a). There are also many small electron-dense vesicles, i.e., primary lysosomes. A striking feature is the abundance of rough endoplasmic reticulum (Figs. 3, 5a). It occurs mainly in the form of fenestrated cisternae arranged in conspicuous parallel arrays, but there are also sections of what appears to be bowl-like structures. A pale flocculent material is preserved in the lumina of the cisternae. Often, the latter are continuous at their margins with narrow tubular elements of the smooth endoplasmic reticulum. In contrast to the normal situation, membranes of the smooth endoplasmic reticulum, however, are scanty. Annulate lamellae, microbodies and clusters of granules are not observed. Likewise, crystalloids of Charcot-Böttcher do not occur.

Furthermore, there are no junctional specializations, especially tight junctions between Sertoli cells in estrogen-treated material. Only occasionally do cisternae run parallel to the cell membrane (Fig. 5a). Regularly, intermediate junctions are apparent between the apical and lateral surfaces of neighboring Sertoli cells (Fig. 4b) and between Sertoli cells and spermatogonia. There are numerous degenerating Sertoli cells, which are identified by their dark cytoplasm and irregularly shaped nuclei.

Cytology of Leydig cells

Well-developed Leydig cells do not occur in any of the patients treated with estrogens. Instead, the interstitial tissue displays small clusters of cells with dark granules and lobulated nuclei. In the light microscope these cells strongly

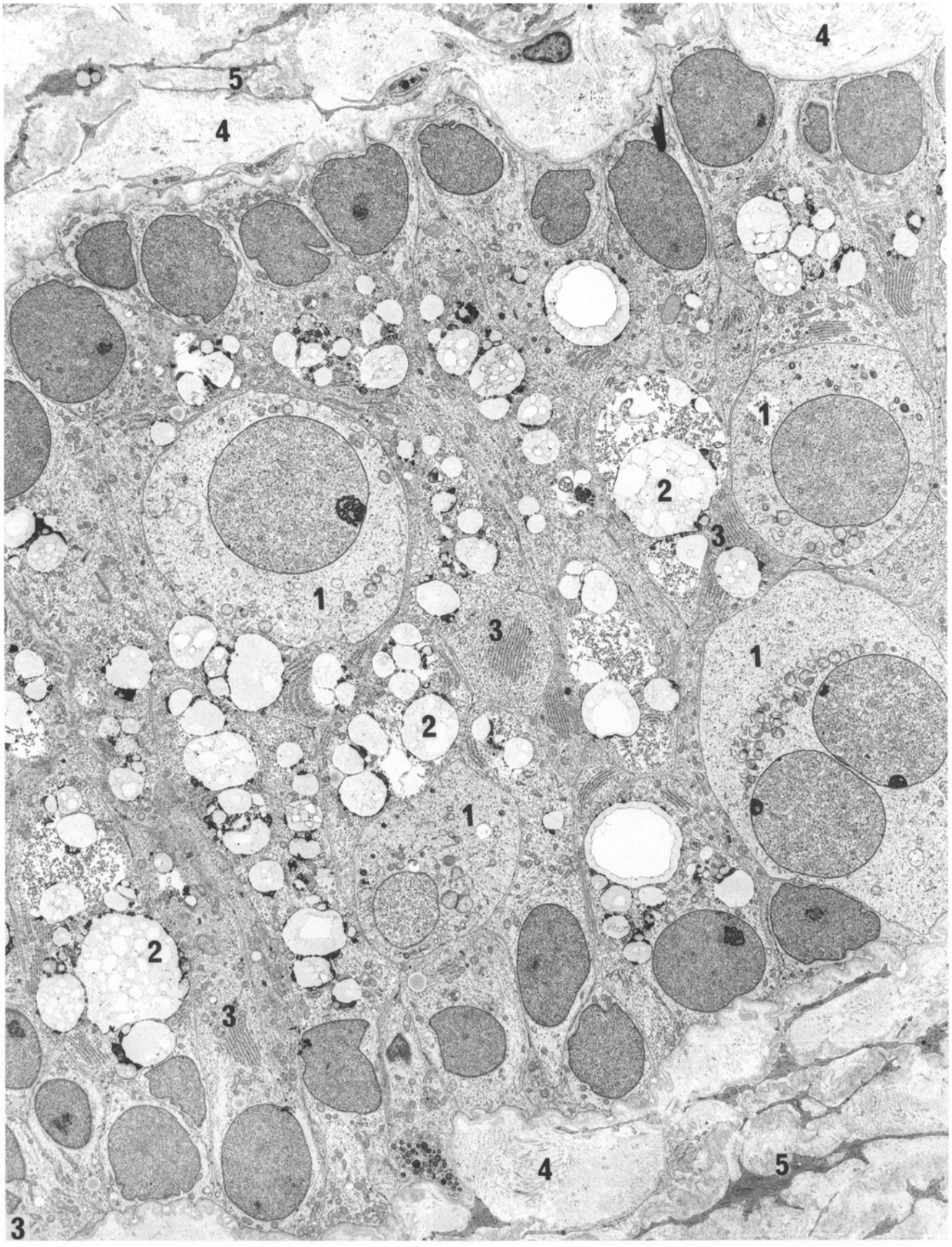
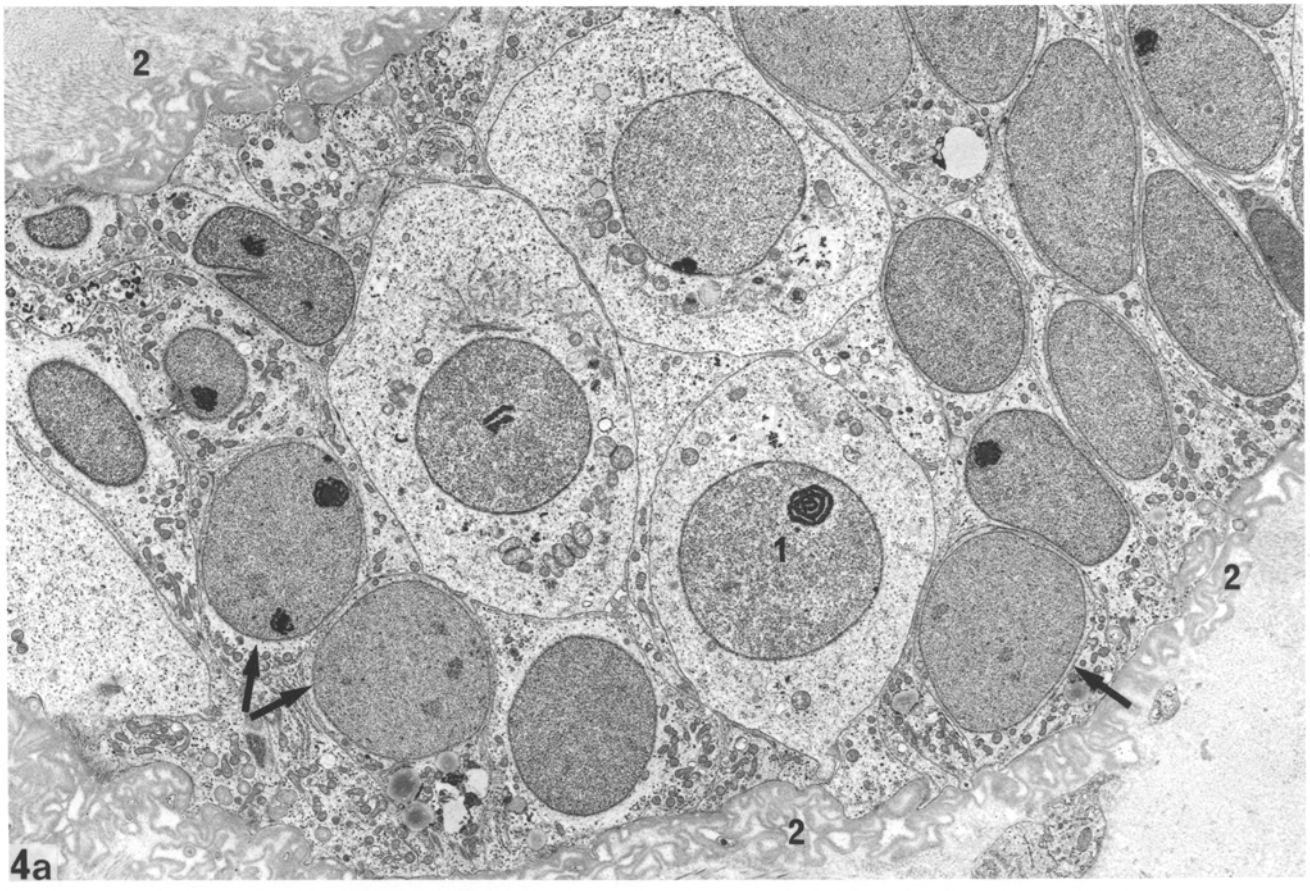


Fig. 3. Cross section of a seminiferous cord. In addition to Sertoli cells there are isolated spermatogonia (*1*), which also occur in the middle of the cord. On the right, a spermatogonium with two nuclei. Sertoli cells are narrow and display smooth contours except for a few thin processes. The polar differentiation resembles that of immature cells: Nuclei lie in the basal cytoplasm, whereas the apical cytoplasm is filled with lipid droplets, telolysosomes (*2*), and abundant rough endoplasmic reticulum (*3*). The lamina propria is extensively thickened displaying a broad band of collagen fibrils (*4*) subjacent to the basal lamina; *5* myofibroblasts of the lamina propria. × 3000



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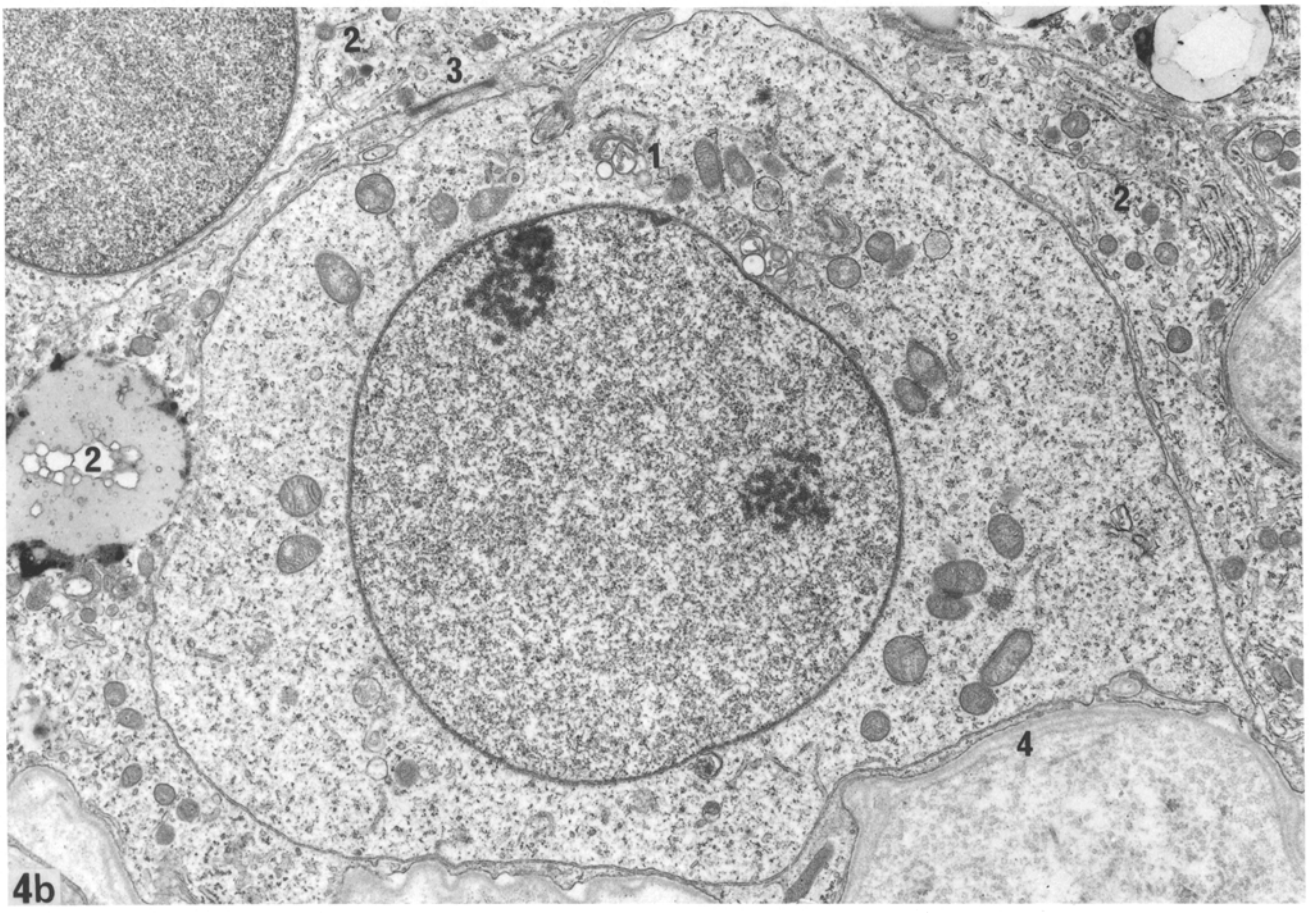


4b

resemble fibroblasts (Fig. 1c). However, electron micrographs reveal an abundance of smooth endoplasmic reticulum in the form of anastomosing tubules, numerous lipid droplets and telolysosomes and rare Reinke crystals (Fig. 6a, b). The cells display submembranous dense areas and are partially covered by a basal lamina (Fig. 6b). They are connected by gap junctions. Occasionally, individual cells with condensed cytoplasm and signs of cellular disintegration are found within clusters of well-preserved cells. As under normal conditions, autonomic nerves occur in the neighborhood of Leydig cells displaying "synapses par distance".

With increasing duration of estrogen therapy the cells described above become less frequent. Instead, cells increase in number with little or no smooth endoplasmic reticulum, a few telolysosomes, lipid droplets and bundles of 10-nm filaments (Fig. 7). Small surface areas are covered by a basal lamina. After ten years of treatment only small cells with long processes and sparse cytoplasm around the irregularly shaped nuclei are found.

Discussion

The inhibitory effect of estrogens on testosterone production has been unequivocally demonstrated in the past, based mainly on studies performed on testes of aged men with carcinoma of the prostate (Slaunwhite et al. 1962; Yanaihara and Troen 1972; Oshima et al. 1974; Leinonen et al. 1981). In the meantime the effects of prolonged estrogen treatment on the circulating levels of hormones in young male transsexuals have been reported by Rodriguez-Rigau et al. (1977). These authors found suppressed concentrations of gonadotropins and testosterone, while concentrations of estradiol were elevated. They suggested that estrogens may alter androgen production via two different mechanisms, i.e., (i) an immediate direct effect on the Leydig cells, and (ii) an indirect effect via suppression of gonadotropins, which requires additional time and higher doses. The endocrinological data mentioned above are in agreement with those reported by Payer et al. (1979) of patients treated with ethinyl estradiol. They are also confirmed by the endocrinological data from the only patient of this study who was selected for hormonal measurement.

The testicular morphology of the eleven estrogen-treated patients of the present investigation has been shown to be remarkably uniform. None of the hitherto published studies on the effects of exogenous estrogens describes comparable uniform and advanced alterations. These alterations are related to the well-documented morphology of normal human testicular tissue (for references, see Holstein and Roosen-Runge 1981; Schulze 1984) and discussed below with regard to the endocrinological findings mentioned above. As will be shown in the following sections the morphology appears to correlate well with the endocrine status.

Spermatogonia

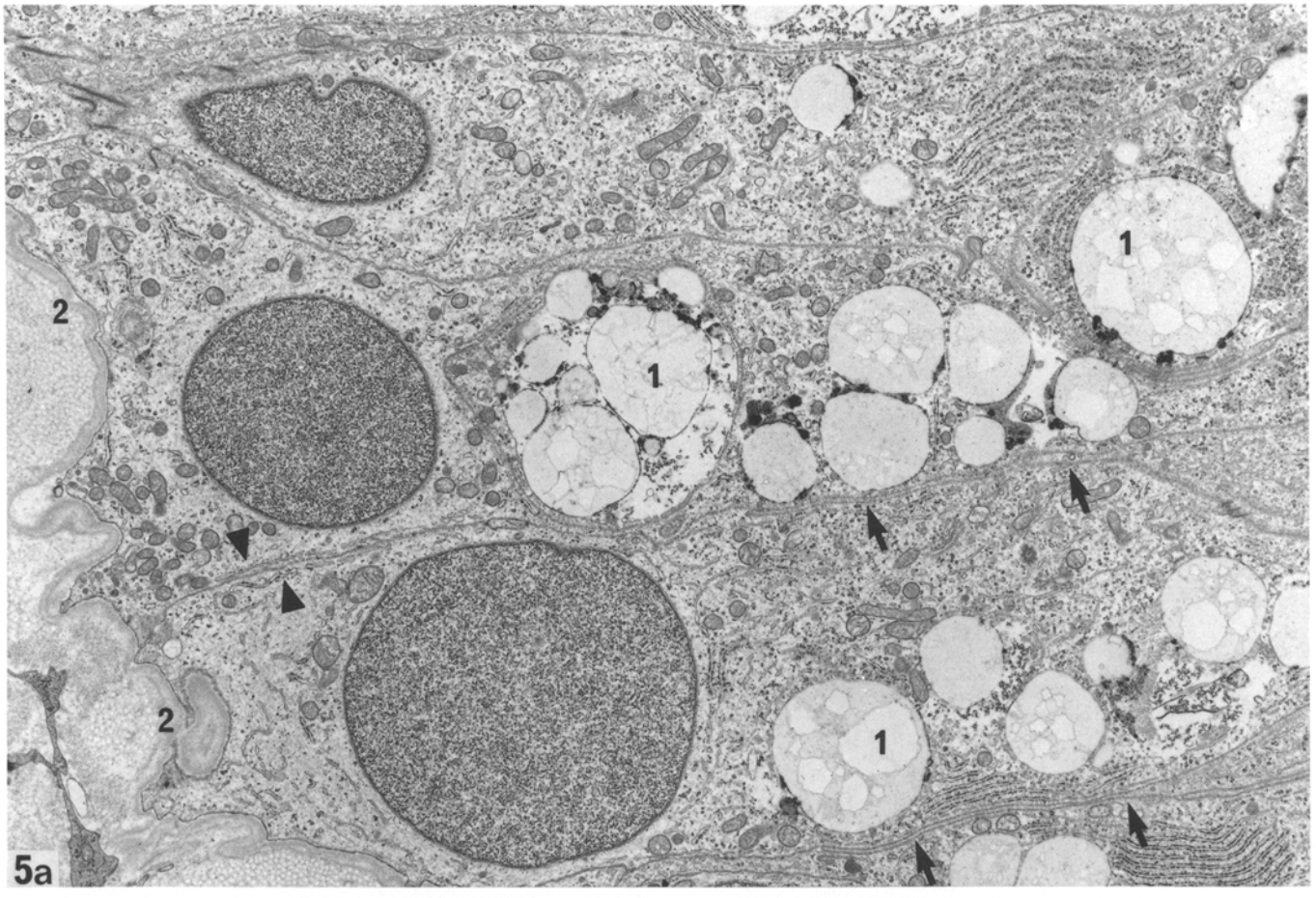
The only germ cells to survive estrogen treatment are spermatogonia. The majority belongs to the pale type-A spermatogonia (Ap), while dark type-A spermatogonia (Ad) are almost completely absent from the epithelium. Apart from the typical pale staining of their chromatin, the persisting spermatogonia often display a concentration of organelles around the nucleus. These cell variants, in particular, resemble the immature spermatogonia of the fetus (Wartenberg et al. 1971; Fukuda et al. 1975) and the so-called "fetal spermatogonia" described by de Kretser (1968) in hypogonadotropic hypogonadism.

The survival of Ap spermatogonia and the elimination of Ad spermatogonia are findings that closely resemble those following radiotherapy and treatment with alkylating agents (Schulze 1979). As mentioned previously (Schulze 1981), they are also found with increasing periods of incubation in organ culture of human testes (Chowdhury et al. 1975) or following treatment with the antiandrogen, cyproterone acetate (Schulze 1978). Likewise, the only germ cells that occur in postpuberal cryptorchidism display the morphological features of intact normal Ap spermatogonia (Schulze 1984).

It has been concluded, therefore, that various noxious agents uniformly influence the seminiferous epithelium in that they reduce the germ cell population to one and the same cell type: Ap spermatogonia. However, there is no evidence of any morphological criteria that could explain the resistance of Ap spermatogonia. Instead, experimental studies in the rat and mouse showed that the radioresistance of a small population of spermatogonia could be attributed to their long cell cycle (Oakberg 1971; Huckins 1978; Huckins and Oakberg 1978). In extrapolating these data from animals to man, it is conceivable that Ap spermatogonia persist after radio- and chemotherapy because – due to their low mitotic rate – they are in a stage of relative resistance at the time of treatment. The same may apply to Ap spermatogonia that survive other unfavorable conditions.

Furthermore, it has already been firmly established in the rat and mouse that radioresistance is a characteristic feature of spermatogonial stem cells (Withers et al. 1974; Oakberg 1975). In recent studies on human testicular tissue, therefore, spermatogonia that survive the various clinical conditions mentioned above, have been regarded as the stem cells of the human testis (Schulze 1979, 1981). Likewise, it is reasonable to suggest that the majority of those spermatogonial types surviving long-term estrogen treatment may consist of stem cells since they are less sensitive to disturbances of endocrine balance. The present findings, therefore, corroborate the concept that – in contrast to the hitherto prevailing view that favored Ad spermatogonia (Clermont 1966) – the stem cells of the human testis may be derived from Ap spermatogonia or the variants of this cell type.

Fig. 4. Spermatogonia. **a** Tangential section of a seminiferous cord representing three of the persisting pale type-A spermatogonia surrounded by smooth-contoured Sertoli cell nuclei (among them three large, pale nuclei, *arrows*). Nuclei of spermatogonia are round and reveal the characteristic homogeneously distributed chromatin. Nucleoli are either attached to the nuclear membrane or positioned more centrally. One of the nucleoli displays a clear halo (*1*). The organelles of two spermatogonia are concentrated around the nucleus so that large areas of the cytoplasm are devoid of organelles; *2* undulating multilayered basal lamina. ×3700. **b** Typical isolated, pale type-A spermatogonium adjacent to the basal lamina. The nucleus contains two nucleoli. Mitochondria are connected by an electron-dense substance. *1* Golgi complex; *2* Sertoli cells; *3* desmosome-like junction connecting two Sertoli cells; *4* multilayered basal lamina. ×8600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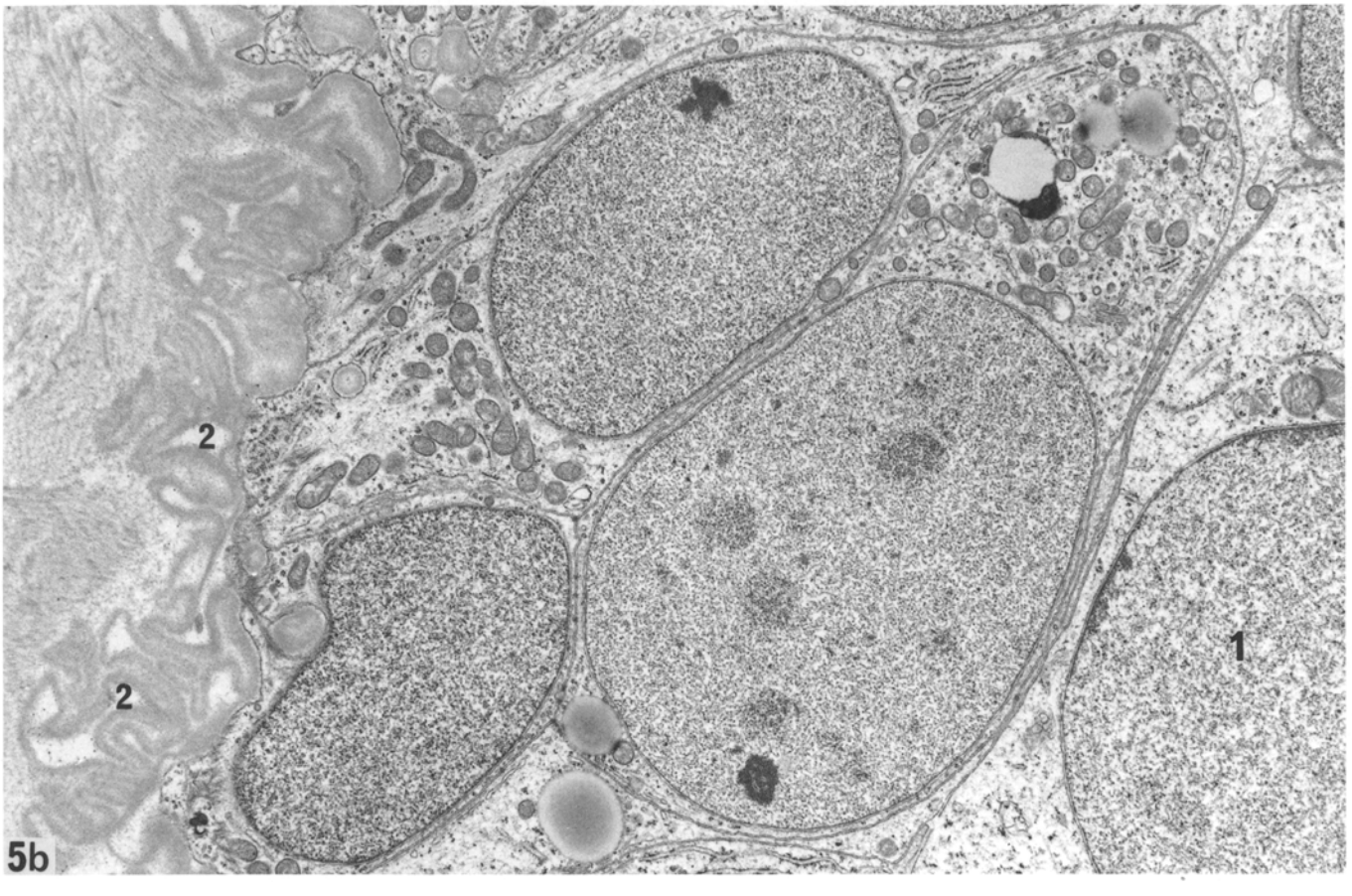
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Sertoli cells

Estrogen therapy not only has a profound effect on germ cells and the composition of spermatogonia but also on Sertoli cells, which are transformed into immature cells. With their unusual smooth contours Sertoli cells resemble columnar epithelial cells, the more so since their nuclei are located adjacent to the basal lamina and organelles and inclusions are confined to the apical cytoplasm. The characteristic apico-basal differentiation of mature cells where the nuclei lie in the middle of the cytoplasm and organelles and inclusions are present only in the basal cytoplasm (Schulze 1984), is not observed.

Moreover, in contrast to untreated material the nuclei are round or ovoid in shape. There are no annulate lamellae, microbodies, clusters of minute granules and Charcot-Böttcher crystalloids, special organelles and inclusions that under normal conditions facilitate the identification of Sertoli cell cytoplasm within the seminiferous epithelium. Likewise, the usual abundance of smooth endoplasmic reticulum is missing, while the granular endoplasmic reticulum is remarkably well-developed so that the cells appear to be well equipped for protein synthesis. Of particular interest is the lack of junctional specializations, a finding that will be thoroughly discussed below.

The features of Sertoli cells in estrogen-treated patients mentioned so far are characteristic of undifferentiated cells. There are, however, two differences: the cells display an enormous amount of inclusions, and the population of nuclei is not homogeneous. From studies on the effects of cyproterone acetate and radio- and chemotherapy it has become apparent that the various lipid inclusions in Sertoli cells are derived from phagocytized germ cells (Schulze 1984). Most likely, the lipid inclusions after estrogen therapy are also due to phagocytosis of the numerous germ cells, which are supposed to degenerate as a result of treatment.

Concerning the different karyological aspects, the predominating nuclei are small and ovoid and display dense, relatively homogeneous chromatin. These nuclei, therefore, resemble those found in immature cells. Other nuclei, however, appear strikingly different. They are larger and their chromatin is less dense; the latter appears to be swollen and unraveled, displaying numerous electron-dense clusters of different-sized granules, one category of which may correspond to perichromatin granules. Within this context it is worthwhile mentioning that similar inhomogeneous chromatin structures are discernible as a stress response in nuclei of fibroblasts after heat-shock treatment (Welch and Suhan 1985). The functional significance of the two nuclear aspects after estrogen therapy is not clear. It is difficult to explain why adjacent Sertoli cells display different appearances of their nuclei without any other morphological indications as to a difference in the functional state of these cells.

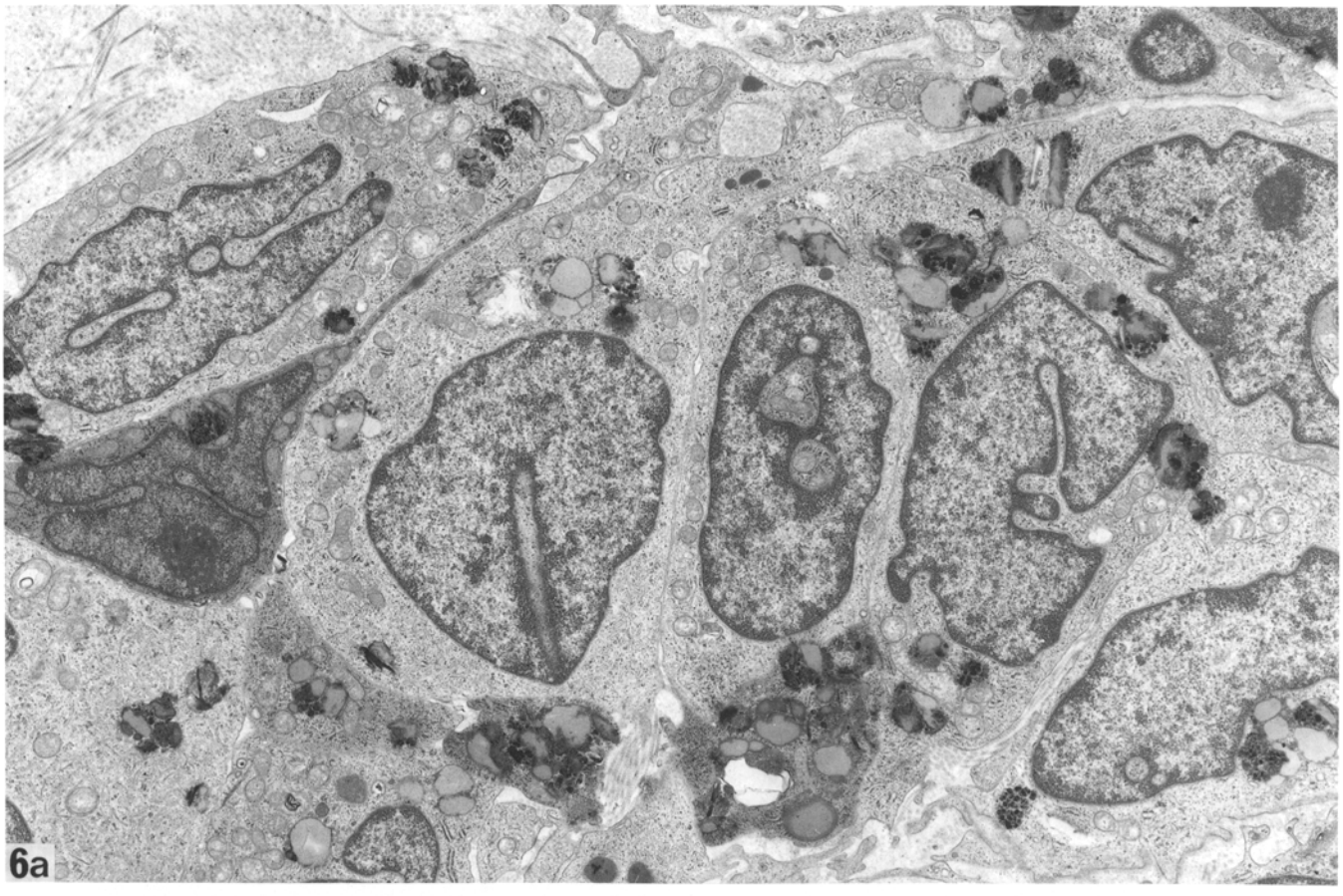
Except for these two features, i.e., the occurrence of inclusions and two different nuclear aspects, estrogen-treated Sertoli cells resemble the immature cells prior to puberty (Mancini et al. 1960; Leeson 1966; Vilar 1970; Hadžiselimović and Seguchi 1974), in hypogonadotropic hypogonadism (de Kretser 1968) and in so-called hypoplastic zones (Sohval 1956; Plattner 1962; Huber et al. 1968).

These Sertoli cells have one feature in common; they are located in seminiferous cords instead of tubules, and they are associated only with a few spermatogonia. Prepuberal Sertoli cells begin to differentiate only at the time of puberty with rising levels of gonadotropins. The differentiation leads to an increase in volume and the development of cytoplasmic processes. Simultaneously, the nuclei become lobulated, while the nucleoli occupy a more central position. Charcot-Böttcher crystalloids appear for the first time, and membranes of the smooth endoplasmic reticulum proliferate (Hadžiselimović and Seguchi 1974). Undifferentiated Sertoli cells in hypogonadotropic hypogonadism are subject to morphological alterations comparable to those during puberty after treatment with gonadotropins. The changes even include the appearance of junctional specializations and the development of a lumen (de Kretser and Burger 1971). The third case of immature Sertoli cells occurs in the convoluted cords of hypoplastic zones. Since these cords occupy only circumscribed areas of normal and pathologically altered testes, the reason for the persistence of their prepuberal features is still a matter of discussion. Based on the observation that Leydig cells are not encountered within the circumscribed areas but in the surrounding tissue, a locally insufficient hormonal stimulation has been implicated (Halley 1963; Schulze 1984). According to Halley (1963) migration of Leydig cells into these undifferentiated areas may lead to maturation of the cords.

Reference to the above-mentioned immature Sertoli cells and their differentiation into mature cells following hormonal stimulation is of special interest. The Sertoli cells described here, being deprived of any hormonal stimulation after estrogen treatment, appear to have passed through the opposite development. Presumably mature cells have transformed into immature cells, and seminiferous tubules that formerly must have contained a normal germinal epithelium (e.g., patient No. 3, father of four children) have transformed into cords without lumina.

Sertoli cells described in the present study – like all other immature Sertoli cells – neither form tight junctions nor any other component of the junctional specializations. Since tight junctions have been shown to be the morphological basis of the blood-testis barrier (Dym and Fawcett 1970), this response of Sertoli cells to long-term estrogen treatment again raises the question as to whether the development and maintenance of the blood-testis barrier are really independent of hormonal influences as hitherto assumed (Vitale

Fig. 5. Sertoli cells. **a** Portion of a seminiferous cord with radially arranged columnar Sertoli cells, which – with the aid of long, thin processes – interdigitate with Sertoli cells of the opposite side of the cord (*arrows*). Sertoli cells display the typical polar differentiation following estrogen treatment. Apart from various inclusions, the apical cytoplasm contains an unusual abundance of parallel-arranged cisternae of the rough endoplasmic reticulum. There are no junctional specializations; only occasionally membranes of the endoplasmic reticulum run parallel to the cell membrane (*arrowheads*). *1* Lipid droplets within telolysosomes; *2* basal lamina. ×6400. **b** Portion of a seminiferous cord with three Sertoli cell nuclei. The two nuclei on the left belong to the small, dark type with dense, homogeneously distributed chromatin. The nucleus on the right is larger, the chromatin appears less condensed, the interchromatin space wide. There are numerous irregular clumps of a heterogeneous population of particles consisting of fibrils and granules. *1* Spermatogonium; *2* multilayered basal lamina forming a reticulum. ×8800



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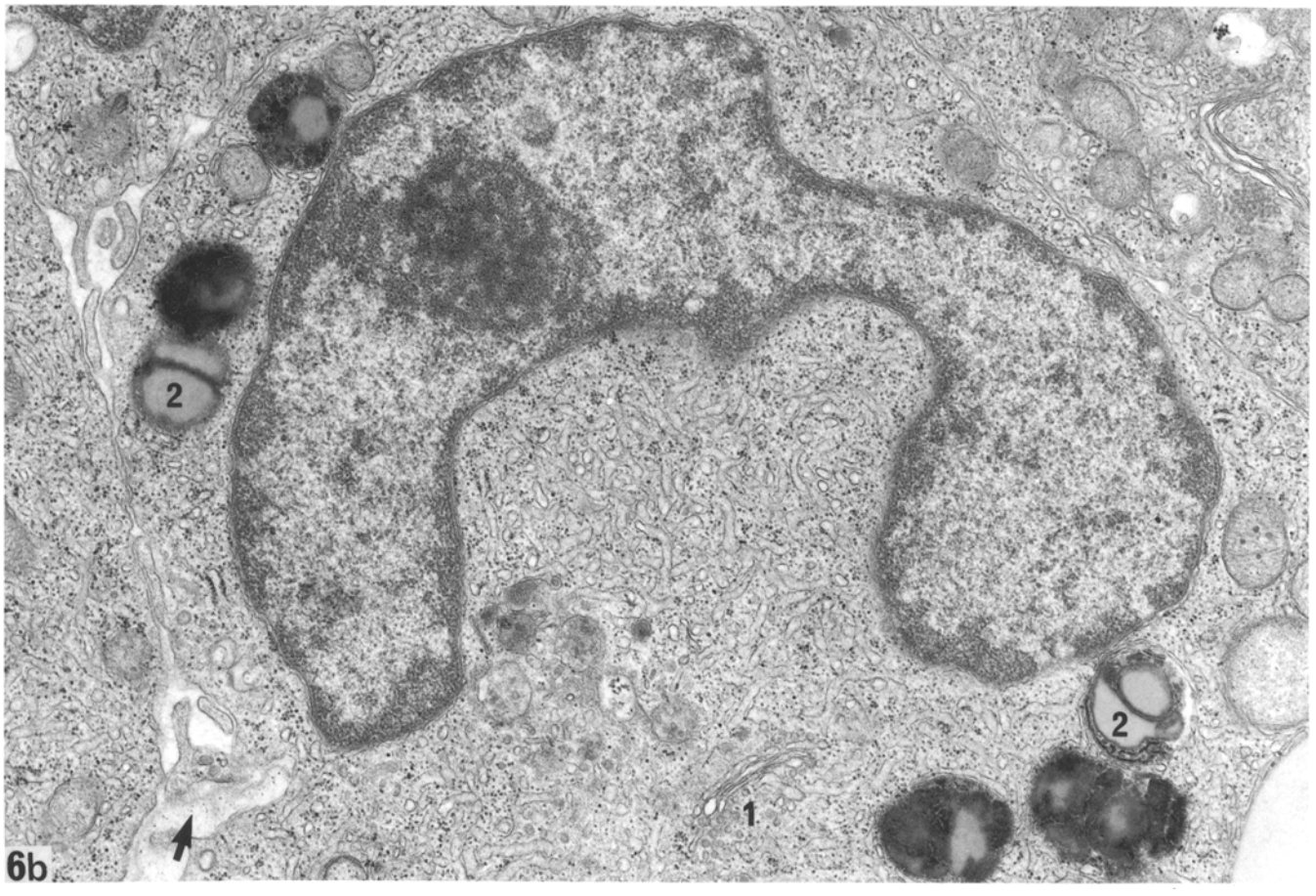

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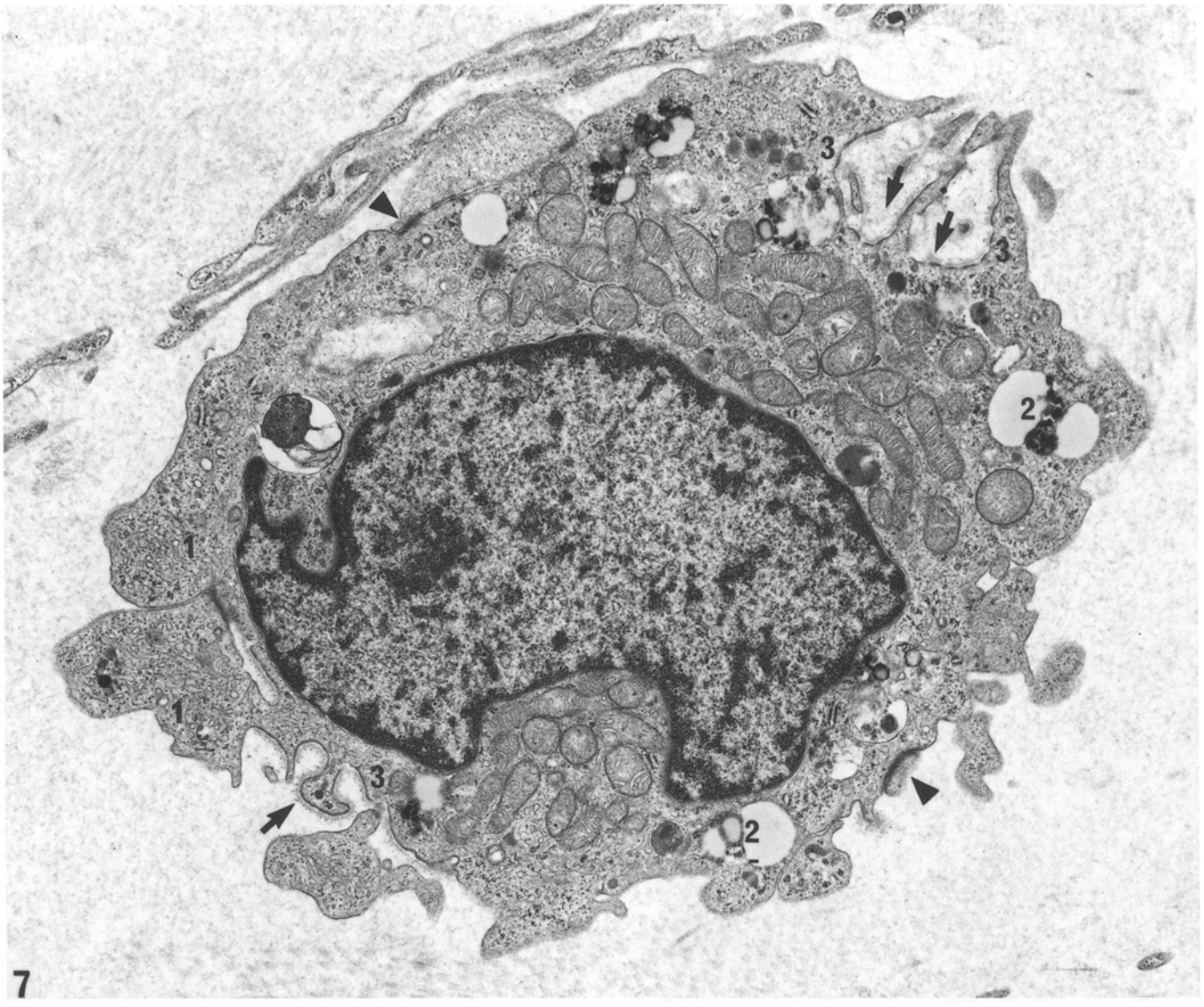

Fig. 7. Electron micrograph illustrating a cell type found frequently in the interstitial tissue. It exhibits many features of Leydig cells: areas filled with smooth endoplasmic reticulum (*1*), electron-dense inclusions (*2*), and submembranous dense areas (*3*). It is partly covered by a basal lamina (*arrows*), and via desmosome-like junctions (*arrowheads*) it is connected with cytoplasmic processes of neighboring cells. × 12900

et al. 1973). This assumption was based on the observation that in rats a suppression of circulating gonadotropins by daily injections of clomiphene and estrogens from birth only delayed but did not prevent the appearance of the blood-testis barrier. Moreover, the blood-testis barrier and, hence, the occluding junctions of adult rats have been demonstrated to remain intact following hypophysectomy (Aumüller et al. 1978; Hagenäs et al. 1978).

The evident inconsistency of these results with the results of the present paper may indicate the difficulty of making valid comparisons between the data in man and rat. On the other hand, it could presumably be attributed to the different duration of gonadotropin suppression. The patients presented here have been submitted to an exceptionally long duration of hormone suppression, which by far exceeds that realized in experimental animals. Furthermore, it is conceivable that also the permanently high level of circulating estrogen may play a role in the regression of tight junctions.

The long duration of treatment may also be one reason why the present findings are at variance with those of Lu and Steinberger (1978). While in the present study major

Fig. 6. Dedifferentiated Leydig cells. **a** Cluster of fibroblast-like cells in the interstitial tissue. The nuclei are conspicuously lobulated, the cytoplasm contains prominent smooth endoplasmic reticulum and electron-dense inclusions. These cells are considered dedifferentiated Leydig cells. × 6400. **b** Comparable dedifferentiated Leydig cell with lobulated nucleus at higher magnification revealing the abundance of smooth endoplasmic reticulum. It consists of a labyrinth of anastomosing tubules. The cell displays submembranous dense areas and is in part covered by a basal lamina (*arrow*). *1* Golgi complex; *2* inclusions. × 20600

alterations of Sertoli cell morphology have been shown to be readily apparent, these authors described Sertoli cells after estrogen therapy as resembling normal cells except for an accumulation of lipid droplets. Apart from differences in the duration of treatment, this discrepancy may conceivably be explained by differences in the doses administered.

Leydig cells

A remarkable feature of the testis after administration of estrogens is the absence of typical Leydig cells. Like Sertoli cells, Leydig cells transform into immature cells.

In all patients, Leydig cells could only be differentiated with certainty by use of electron-microscopic analysis. In the present paper only the abundance of smooth endoplasmic reticulum and electron-dense inclusions are reminiscent of Leydig cells. Furthermore, the ability of the cells to form clusters and to establish contacts in the form of gap junctions is an indication that they may be derived from Leydig cells. The conspicuous lobulations of the nuclei, however, suggest their classification as altered Leydig cells.

The fact that in some patients increased numbers of intermediate stages between these altered Leydig cells and fibroblast-like cells with little or no smooth endoplasmic reticulum and numerous intermediate filaments occur, supports the assumption that Leydig cells after estrogen treatment dedifferentiate into immature cells.

These results are in striking accordance with the only previous data on the ultrastructure of estrogen-treated Leydig cells published by Payer et al. (1979). The latter authors studied the effect of long-term treatment with estrogens alone or in combination with medroxyprogesterone acetate on the morphology of Leydig cells from transsexual males and found a similar spectrum of interstitial cell appearances ranging from normal Leydig cells to fibroblast-like cells. Consequently, these findings prompted them to suggest that a dedifferentiation of Leydig cells may take place. Likewise, earlier workers in the field basing their conclusions on light-microscopic studies of human testes following administration of stilbestrol, a non-steroid estrogen, described a gradual transformation of Leydig cells into fibroblast-like cells (de la Balze et al. 1954, 1962). In addition, they demonstrated that these fibroblast-like cells not only differed from normal fibroblasts in their morphology but also in their histochemical reaction.

However, Leydig cells not only disappear via dedifferentiation into fibroblast-like cells. The presence of occasional disintegrated elements within clusters of well-preserved cells indicates that degeneration and dissolution possibly contribute to the loss of Leydig cells.

The assumption pointed out previously that Leydig cells dedifferentiate into fibroblast-like cells is especially noteworthy in the light of the widely accepted concept on the origin of Leydig cells. According to this concept the differentiation of Leydig cells in human embryos occurs via mesenchymal cell maturation, while at the time of puberty and in the adult testis they may arise from primitive fibroblast-like "stem cells" (Sniffen 1950; Mancini et al. 1952, 1963; de la Balze et al. 1960; Pelliniemi and Niemi 1969; Hooker 1970; Holstein et al. 1971; Christensen 1975). Support for this theory has very recently been provided by investigations in the rat in which the regeneration of numerous Leydig cells after their experimental destruction and depletion is explained by their differentiation from mesenchymal precursor cells (Kerr et al. 1985; Jackson et al. 1986). Evaluation of published data as well as own findings suggest that in man interstitial cells with a morphology intermediate between fibroblasts and myofibroblasts may correspond to the immature stem cells (Fawcett and Burgos 1960; de Kretser 1967; Christensen 1975; Schulze 1984). These cells, in turn, cannot be distinguished from fibroblast-like cells found in estrogen-treated testis. The present data suggest that following estrogen treatment mature Leydig cells dedifferentiate into immature cells, which closely resemble the putative precursor cells.

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