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Light and Electron Microscopical Analysis of Cell Types in Human Seminoma

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Summary. Samples of solid tumours were obtained from six patients with classical seminoma, either from the center or the periphery of the tumour as well as from macroscopically normal-looking testicular tissue. The tissues were examined by light and electron microscopy.

In different places of the affected testis, three different tumour cell types were found:

1. Intratubular, basal tumoral cells showing abundant glycogen, a peripheral web of microfilaments and thin cell expansions.

2. In the periphery of the tumour, cells are found towards the seminiferous tubular lumen, characterized by having less glycogen, many endoplasmic reticulum membranes and free ribosomes and fewer cytoplasmic evaginations.

3. In the center of solid tumours, rounded cells exhibiting neither glycogen nor cell expansions and few figured elements in the cytoplasm are found.

The tumour cells of type 1 show characteristics of human embryonic germinal cells, those of type 3 being the more differentiated and forming most of the tumoral mass.

Hence classical seminoma is formed not only by large clear cells as stated in the literature, but instead by more cell types, as described in the present work.

Introduction

In the literature, the histological picture of classical seminoma is described as related to an homogenous population of pale, large cells, resembling spermatogonia (Dixon and Moore, 1952; Pierce, 1966; Blandy, Hope-Stone, Dayan, 1970; Gallager, 1972; Mostofi, 1973). Connective tissue strands, groups of free connective tissue cells or lymphatic infiltration can be found surrounding irregular or rounded tumour cell formations. Anaplastic seminomas are clearly different from the above referred description and are identifiable by their varied nuclear forms and abundant tumor cell mitoses. The so-called spermatocytic seminoma, whose tumour cells exhibit cytological characteristics of primary spermatocytes, are relatively rare (Rosai, Khodadoust and Silber, 1969). Mixed tumours are more frequent. They are formed by different tumoral tissues, thus offering an heterogeneous aspect (Hedinger, 1973).

After the comprehensive investigations of Dixon and Moore (1952) it is accepted that seminomas are germinal cell tumours. Stevens (1964) was able to demonstrate experimentally that in the mouse, testicular tumours can originate from primordial germ cells. Furthermore Pierce (1966) found cytological characteristics of germinal cells in cells of human seminoma. He described the ultrastructural aspect of clear tumour cells that are found in solid seminoma.

Examination of testicular tumours, either on semi-thin sections or under the electron microscope allow us to conclude that in classical seminoma as well as those of seminiferous tubules or in solid tumours, more characteristic cell types are found.

Material and Methods

In 1973, 21 patients (aged 27 to 41), bearing different types of testicular tumours, were operated at the Urology Service of the Armed Forces Hospital Hamburg-Wandsbek. From these patients, six had a classical seminoma. (The ages of the patients were: 1 = 24, 2 = 25, 3 = 27, 4 = 31, 5 = 34, 6 = 35 years old).

Immediately after excision of the testis, pieces of tissue were taken from the center or from the periphery of the tumour and also from different places of the rest of the testis. Tissues were fixed for one hour in phosphate buffered 6% glutaraldehyde (820 mOsm) and then post-fixed in 1% OsO_4 ($2^1/_2$ hours). After alcohol dehydration, the samples were embedded in Epon 812 (Luft, 1961). Semi-thin and ultrathin sections were made in a Reichert Ultramicrotome (OmU2) using glass or diamond knives. For light microscopy the sections were stained with toluidine blue-pyronin (for details see Holstein and Wulfhekel, 1971). For identification of polysaccharides, some sections were stained with PAS following the indications of Nevalainen, Laitio and Lindgren (1972). For the electron microscopy (Philips EM 300) the sections were contrasted with lead citrate (Reynolds, 1963).

Observations

Some of the testes from our six patients were evidently enlarged. They contain in the hiliary region, solid, whitish tumour tissue about 2 cm in diameter, occasionally not clearly delimited from the brownish, normal looking seminiferous tubules of the surrounding testicular parenchyme. In the relatively small tumours, no necrotic areas were found. Light microscopical observation of each of the testicular components revealed that tumour cells can occur in many places: in the seminiferous tubules, where the lamina propria still appears to be intact, in the interstitium between normal seminiferous tubules or as cellular groups in solid tumours.

Seminiferous Tubules

The seminiferous tubules in the macroscopically non tumoral tissue of the compromised testis showed a normal diameter and contained all types of germ cells, from spermatogonia to mature spermatids, thus exhibiting a normal aspect of intact spermatogenesis. The tubular wall was formed of four to five layers of thinned myofibroblasts, the thickness of the wall being normal.

However, in the testes of three patients (2, 4, 5) it was noted that in some places, in apparently normal seminiferous tubules, large isolated cells were found at a basal position, between the spermatogonia (Fig. 1). These cells have a large nucleus with translucid caryoplasm. The cytoplasm contains a material strongly stained by toluidin-blue, which is found mostly to one side of the nucleus but that can occasionally fill the whole cellular space up. This material corresponds to polysaccharide, as shown by a positive PAS reaction. A thin peripheral zone does not contain polysaccharide and thus appears like a clear rim. This unusual cell type is not normally found in the germinal epithelium. They are pathological cells which correspond to tumour cells of the seminoma.

In the neighbourhood of seminiferous tubules with apparently intact spermatogenesis sections of relatively smaller tubules are frequently found. They contain isolated germ cells (Fig. 1), like for instance, A pale spermatogonia (Clermont, 1966), primary spermatocytes or spermatids, predominant Sertoli cells and closely applied to the basal membrane, large, polysaccharide containing cells of

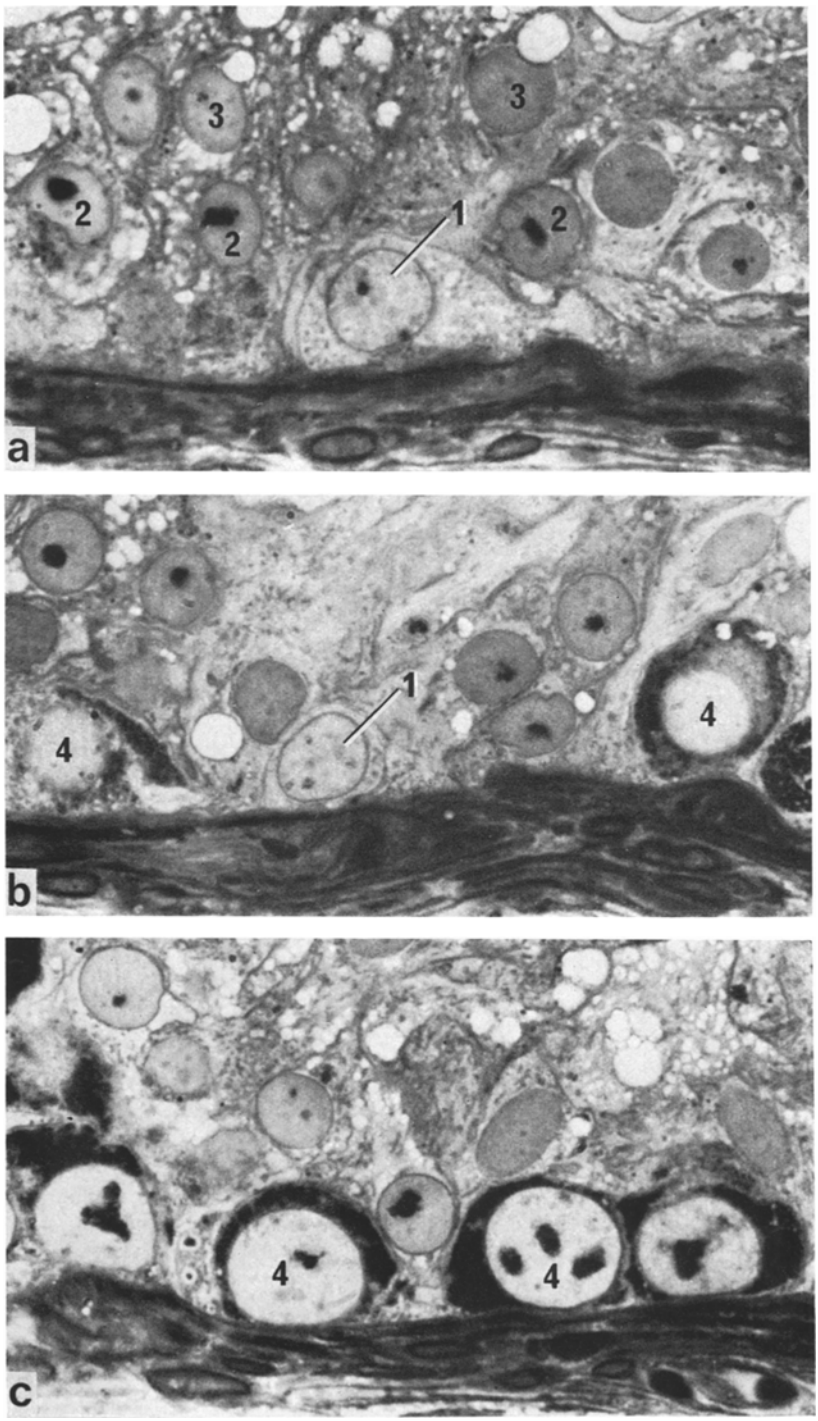

Fig. 1 a—c. Different regions of seminiferous tubules from a 31 years old patient. a A pale spermatogonium (*1*), Sertoli cells (*2*) and spermatids (*3*) are seen in the section. b Isolated tumour cells (*4*) next to a pale spermatogonium (*1*). c Tumour cells closely packed on the basal membrane in tubules otherwise showing only Sertoli cells. Semi-thin section, Toluidin blue. × 800

the type described above. No A dark spermatogonia are found in these tubules. The tubules that contain a more conspicuous basal layer of these atypical cells exhibit also a thickening of the lamina propria.

Interstitium

Occasionally large cells, which do not correspond to Leydig cells, are found in normal looking testicular tissue, far removed from solid tumours located between normal seminiferous tubules with intact spermatogenesis. These large cells have also a large nucleus and a conspicuous nucleolus, the cytoplasm being occupied by large aggregations of PAS positive material which leave a clear rim underneath the cell membrane. The aspect of these cells is identical to that of the polysaccharide-containing cells previously described within the seminiferous tubules.

Solid Tumours

In solid tumours different cell types can be found (Fig. 2). In the periphery the already described, polysaccharide-rich cells, are found nearly exclusively. The PAS positive cytoplasmic material is polarized, disposed as a half-moon near the nucleus, thus displacing it to an eccentric position in the cell. The closest to the center of the tumour that one inspects, the poorer is the deposition of PAS positive material as found in semi-thin sections, but the clear ectoplasmic rim can anyway distinctly be seen. Most of the tumour mass is formed by large, clear cells. In their cytoplasm fewer particles are seen, just some PAS positive granules and a clear rim which is not particularly visible. In addition, there are other cell types in solid tumours. Rounded small cells that can be identified as lymphocytes on account of their small, compact nuclei. Other larger cells can also be observed, displaying bizarre shapes, probably of connective tissue origin. This "accompanying" cell types will not be further described here.

Electron Microsopy

Three types of tumour cells can be recognized by electron microscopy in the different tissular components of a testis bearing a seminoma.

1. Large cells (Fig. 3) that are found at the basal part of the seminiferous tubules, free in the interstitium or in the periphery of solid tumours. Their large nuclei exhibit a flocculent and granular caryoplasm and usually sections of one to two large nucleoli with a honey-comb aspect. The cell organelles are mostly clustered near the nucleus. The mitochondria are rounded and display cristae with vacuolar dilatations and a clear matrix. In spite of the fact that the mitochondria are sometimes close to one another, no electron dense cementing substance can be found between them, as it is usually the case in normal spermatogonia. The Golgi zone is scarce and only a few endoplasmic reticulum vesicles or cisternae, lined by ribosomes, are observed. Abundant free ribosomes are found

Fig. 2a—c. Semi-thin sections of a solid seminoma from a 31 years old patient. a Tumour cells (*1*) from the periphery of the solid tumour with glycogen accumulation in a halfmoon zone and a clear peripheral rim in the cytoplasm. b Tumour cells more centrally located in solid tumours exhibit few stained granules but the clear peripheral rim of the cell is well defined. c Tumour cells in the center of solid tumours are clear and have no stained granules. Only a few cells show a sharply outlined clear peripheral rim. Toluidin blue. × 800

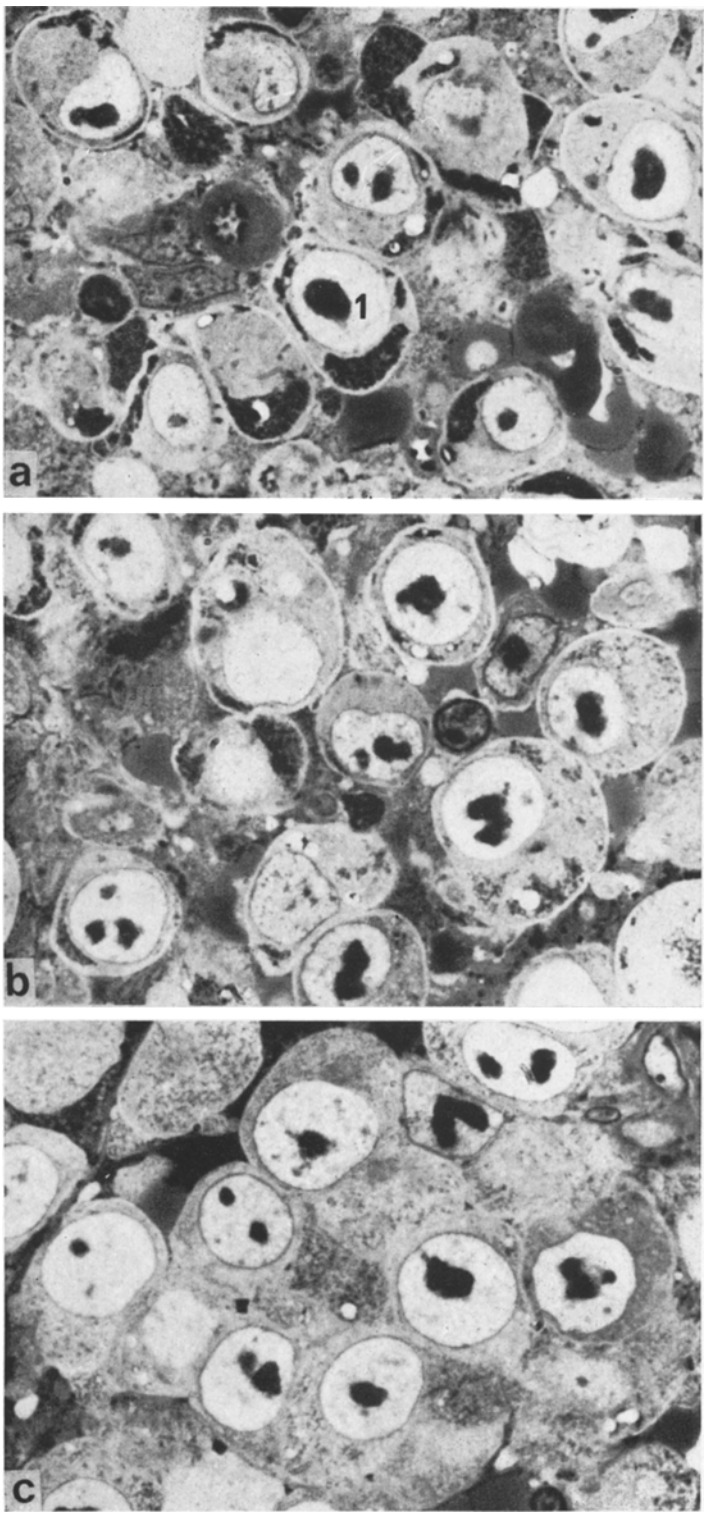

Fig. 2

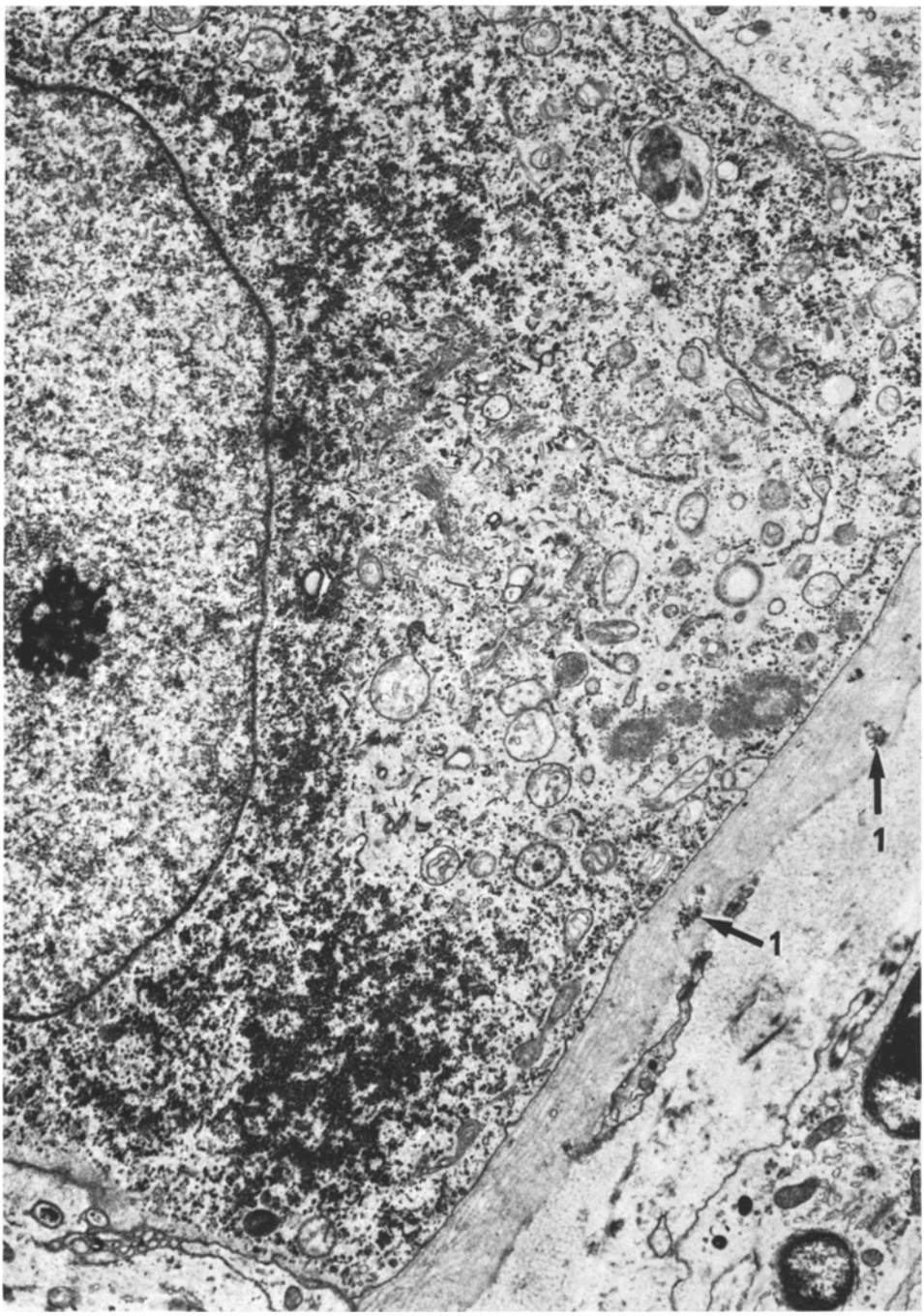

Fig. 3. Section of an intratubular basal tumour cell in the testis of a 25 years old patient. The cytoplasm shows numerous glycogen granules and an area free of them where cell organelles and endoplasmic reticulum are found. The basal membrane is thickened and presents many layers. At these places, accumulation of glycogen granules (*1*) are seen. $\times 10000$

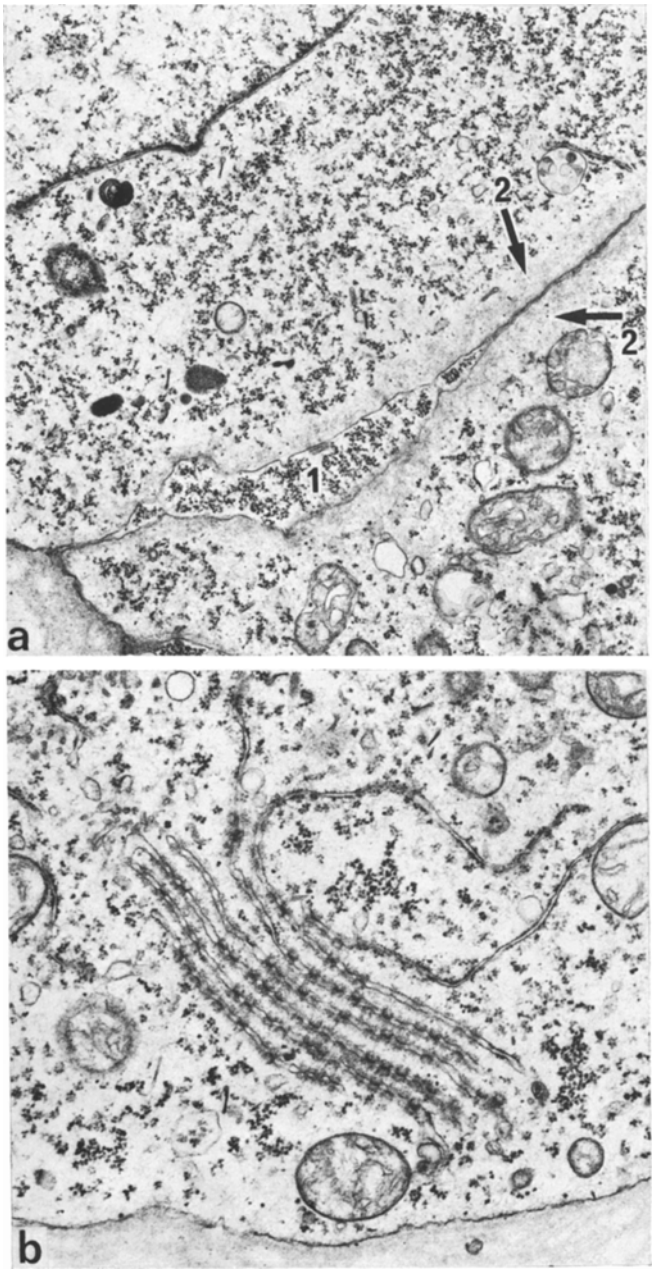

Fig. 4. a Glycogen deposition (*1*) is frequently found in the intercellular spaces between two intratubular basal tumour cells. The arrows (*2*) point to the peripheral microfilamentous web of the tumour cells. × 11500. b Packed annulated lamellae in the cytoplasm of an intratubular basal tumour cell. × 17000

all over the cytoplasm. In many cells, packed annulate lamellae are seen connected to the endoplasmic reticulum cisternae (Fig. 4 b).

The unique aspect of these cells is due to the large amount of glycogen granules that are densely packed in the cell periphery leaving an empty space for the cell organelles. These granules have an average diameter of approximately 300 Å and form mostly rosettes, so that they can be clearly differentiated from the free ribosomes (average diameter approximately 150 Å) that are found dispersed in the whole cytoplasm. Furthermore, the entire cell is crossed by fine microfilaments (average diameter approximately 60 Å) running in different directions. Immediately underneath the cell membrane they build in most cells a thick network that leaves no free space between the figured elements of the cytoplasm. This network corresponds to the clear rim seen at the cell periphery in light microscopy. This microfilamentous web surrounds the whole cell but is rather scarce in the basal aspect of the cell. The outer cell membrane presents in many points expansions (Fig. 5) similar to microvilli, with a core of densely packed microfilaments which are connected to the microfilamentous web at the cell periphery. The cellular expansions are either isolated or packed in groups, projecting themselves in the intercellular space or in the cellular space of nearby Sertoli cells, running under foldings of the cell membrane of the latter. Penetration in neighbouring germ or tumour cells was rarely seen.

It is to be remarked that these glycogen-rich cells, when found isolated at the basal membrane of the seminiferous tubule, are directly apposed to apparently normal type A dark spermatogonia. If these glycogen-rich cells form, on the contrary, a dense layer over the basal membrane, only type A pale spermatogonia (Clermont, 1966) can be found between them. It was not possible to correlate the types A L and B spermatogonia (Rowley, Berlin and Heller, 1971) with any of the tumoral cell types to be described in the present work.

In some places it is difficult to separate normal from pathological cells since type A dark spermatogonia also contain clusters of glycogen granules in their cytoplasm. The peripheral web of microfilaments and the cell expansions and the lack of electron dense cementing substance between the mitochondria are the characteristics pathognomonic to identify a pathological basal tumour cell. Desmosomes are found both between spermatogonia and spermatogonia and basal tumour cells microfilaments radiate from these areas into the cytoplasm. Moreover, there are desmosomes in invaginations of facing membranes from the one and same cell. Intercellular bridges are never found between the glycogen-rich tumour cells, but they are common between normally looking spermatogonia. In this cases, though rarely, some curious pictures are seen (Fig. 6). Within a wide cytoplasmic bridge between two spermatogonia the sections of two adjacent rounded profiles are seen, occasionally covered by a thickened cell membrane, thus closely resembling a typic intercellular bridge between germinal cells. Supposedly, this corresponds to the section of a ringshaped tubular structure which has no obvious connections to the outer cell membrane. A few microtubules are seen running through this region as it is normally found in the intercellular bridges connecting germinal cells.

In the seminiferous tubules which contain a great number of the tumour cells described, the basal membrane is obviously thickened (Fig. 7) and splitted

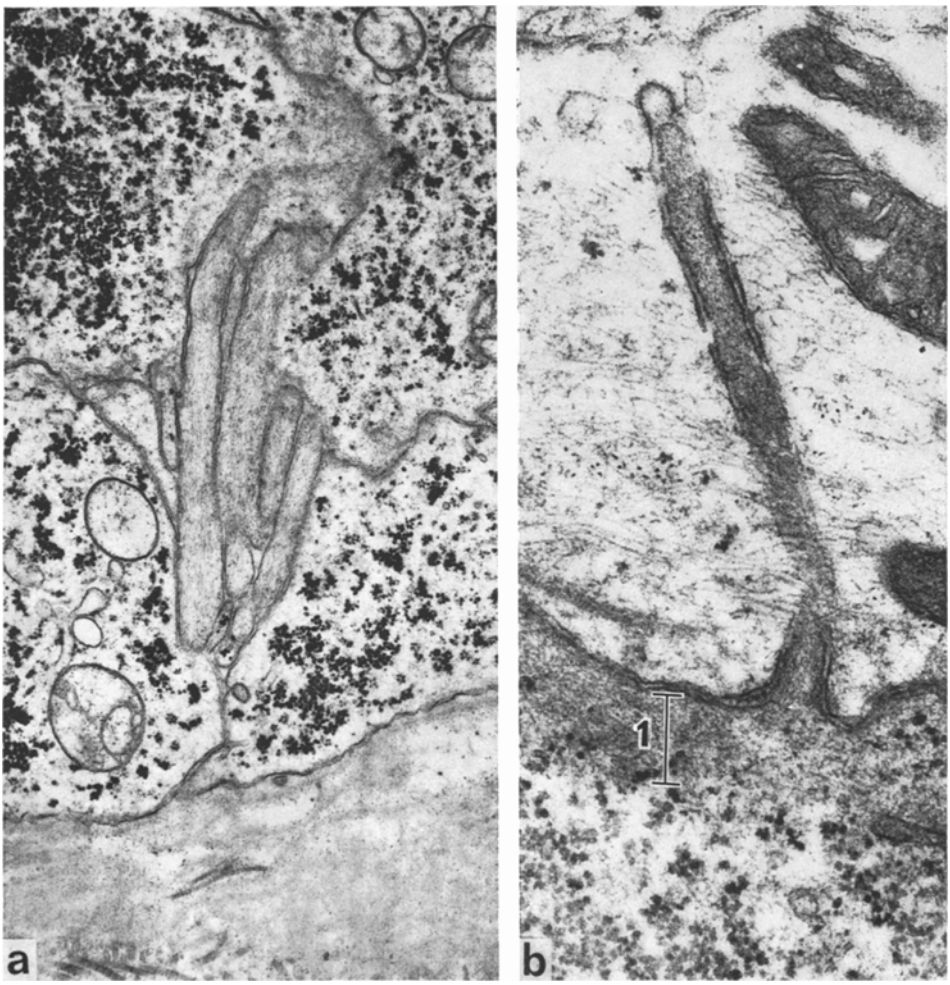

Fig. 5a and b. Cell expansions of intratubular basal tumour cells (34 years old patient). a The cell expansions, which contain microfilaments, extend themselves as bundles in the intercellular space. × 17000. b Cytoplasmic expansion penetrating into a Sertoli cell. The former starts from the peripheral microfilamentous web (*1*). × 40000

in various layers. The other components of the lamina propria, i.e. contractile cells and intercellular connective tissue fibrillar elements, seem to be normal in appearance.

In a good number of tubular sections glycogen deposition is found expanding the intercellular space between the tumour cells in the neighbourhood from the basement membrane (Fig. 4a). In these places, discrete glycogen accumulations can be also seen between the layers of the greatly thickened non cellular component of the lamina propria (Figs. 3a and 7b).

2. Another cell type is found in the tubular lumen, in the interstitium and at the periphery of solid tumours. It can be separated from cell type 1 due to its

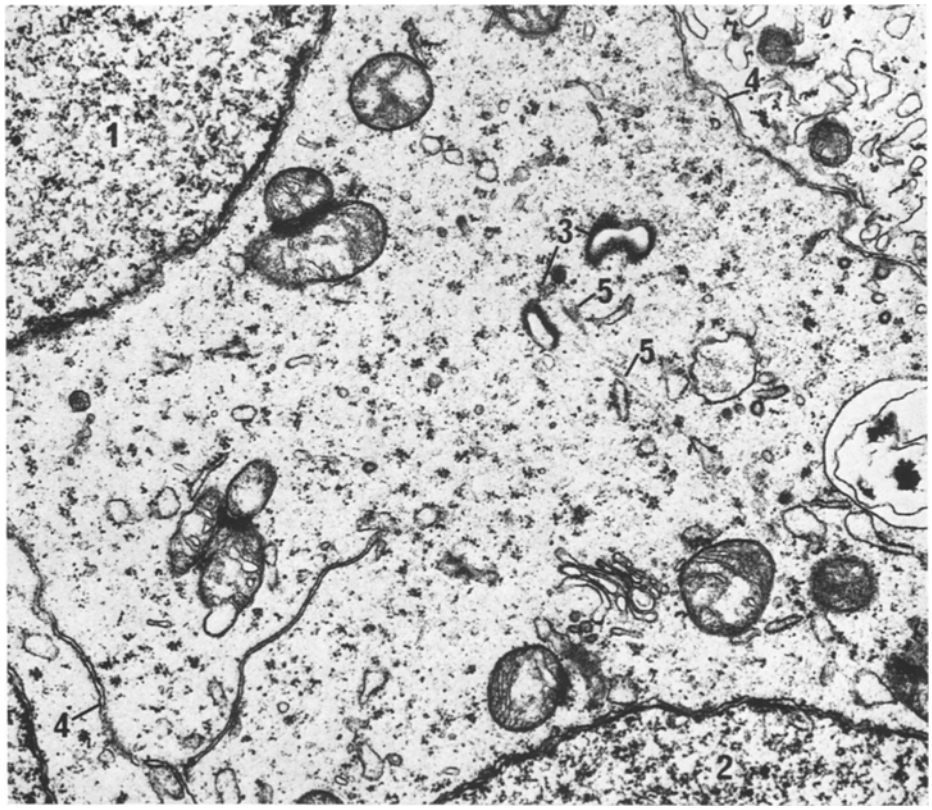

Fig. 6. Wide cytoplasmic bridge between two type A pale spermatogonia (*1*, *2*) showing two dark profiles (*3*), similar to an intercellular bridge but lacking a connection to the cell membrane. Between these dark profiles microtubuli are seen (*5*). × 20300

rounder form and because it shows less cell expansions. Moreover, these cells contain relatively less glycogen and hence, more cytoplasmic membranes, in the form of vesicles, short tubules and small electron dense cytoplasmic bodies. Other cell characteristics are similar to those of cell type 1: large, translucid nucleus with a prominent nucleolus, pale mitochondria, few Golgi elements and a microfilamentous cell web peripherally located.

3. Large tumour cells, with a translucid cytoplasm (Fig. 8) were found exclusively in solid tumours. They differ from the two preceding types in the paucity of figured elements of the cytoplasm. There are only a few mitochondria with fewer, thin cristae and a very light matrix. Very few elements of the Golgi zone or endoplasmic reticulum are found. In a few places, flattened endoplasmic reticulum cisternae, lined by ribosomes, can be seen at the cell periphery. Occasionally, packed annulate lamellae can be discerned. Glycogen granules are very scarce, and in addition free ribosomes and a finely granular material are seen in the cytoplasm. The rim of microfilaments is thin, the cell expansions are missing and desmosome-like structures between tumour cells are not at all observed.

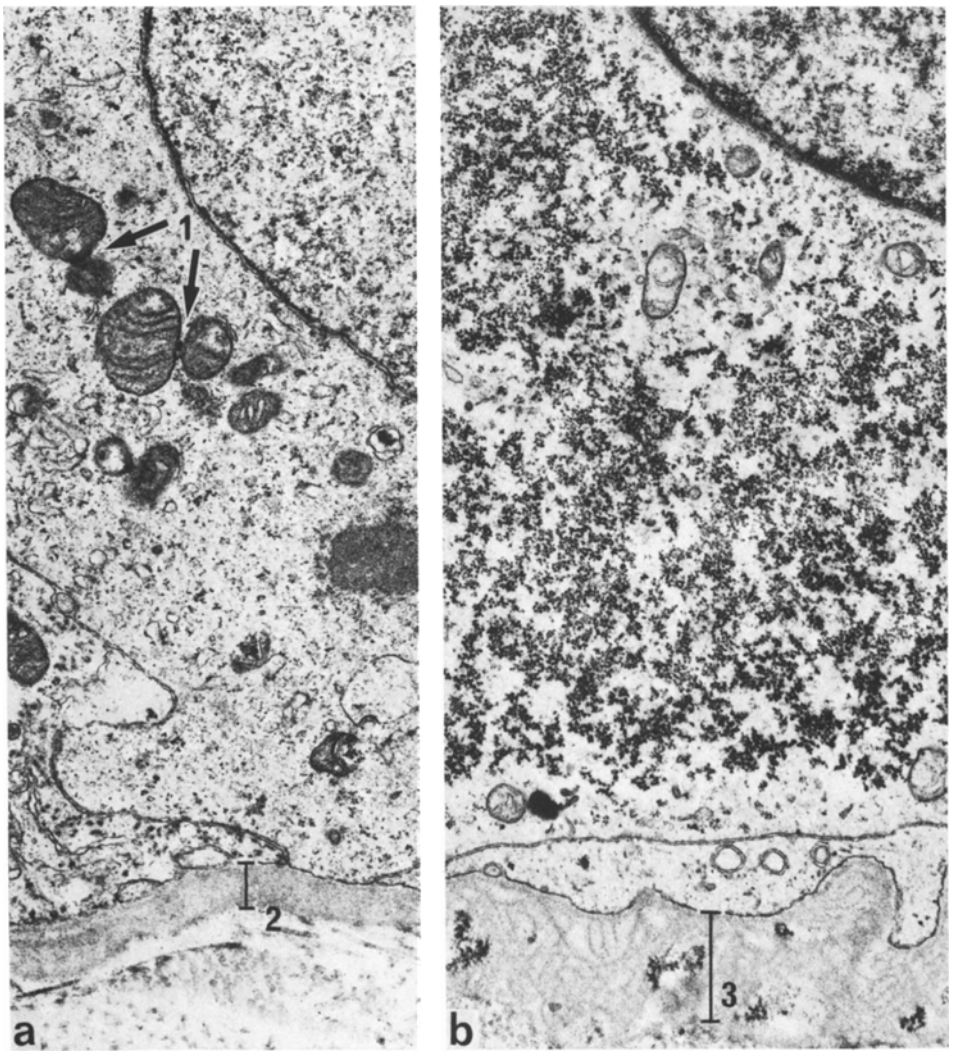

Fig. 7. a Normal spermatogonium in a seminiferous tubule with intact spermatogenesis (from a seminoma in a 25 years old patient) Note the electron dense cementing inter-mitochondrial substance (*1*). The thickness of the basal membrane is normal (*2*). b Basal tumour cells in a neighbour tubule (from the testis of the same patient). The basal membrane is noticeably thickened, and splitted in various lamellae. Glycogen accumulation is also observed. × 14000

Discussion

Contrary to the opinion predominant in the literature that classical seminomas are composed of a uniform cell type, the clear cells (Teppo, 1973; Mostofi, 1973), the present observations demonstrate that at least three different tumoral cell types can be found. They do not differ too much in cell or nuclear size but they can be differentiated on account of cytoplasmic characteristics: the cell type 1

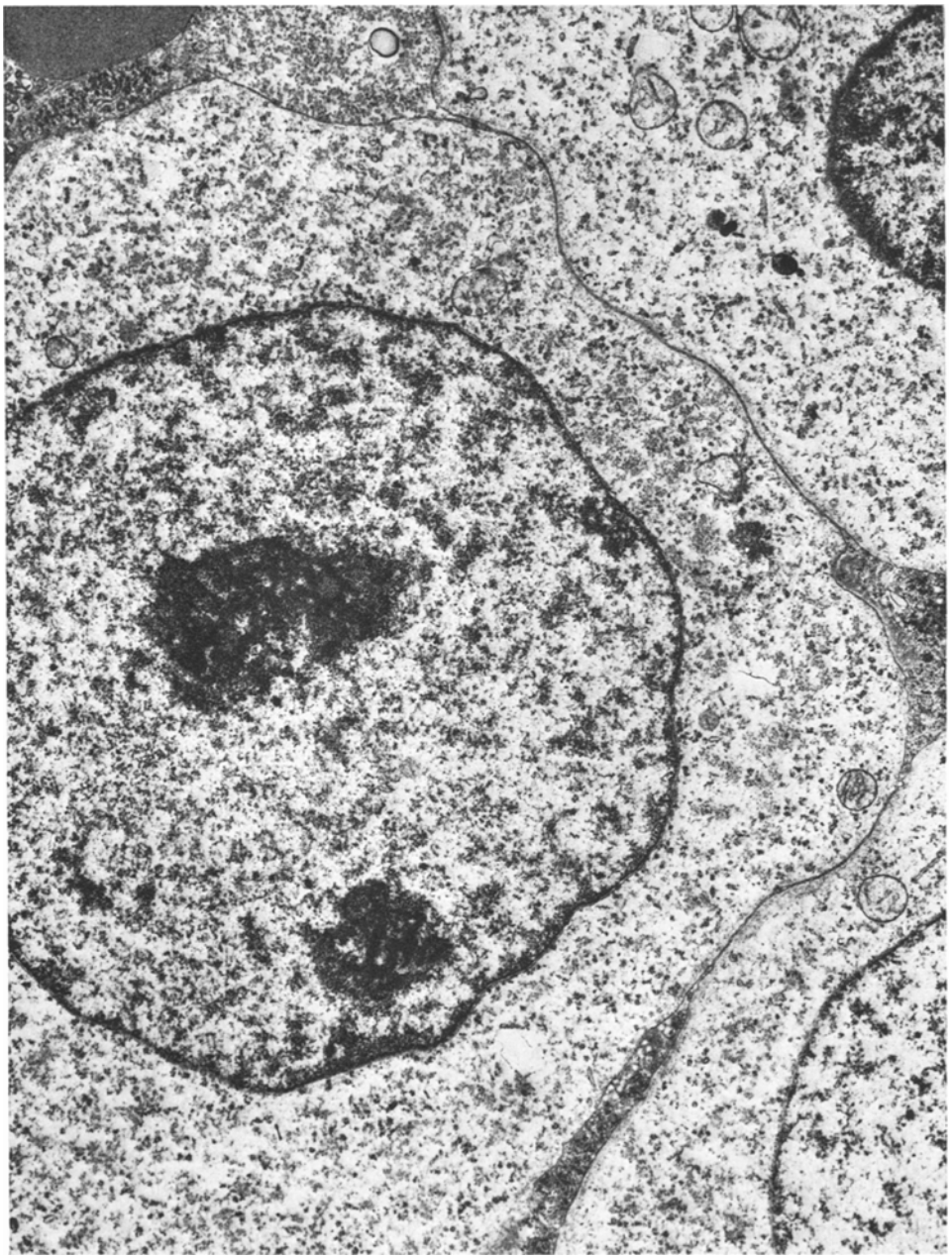

Fig. 8. Section of a cell in a solid tumour from the testis of a 24 years old patient. The cytoplasm contains abundant free ribosomes and a finely granulated material but only few other figured elements. × 10000

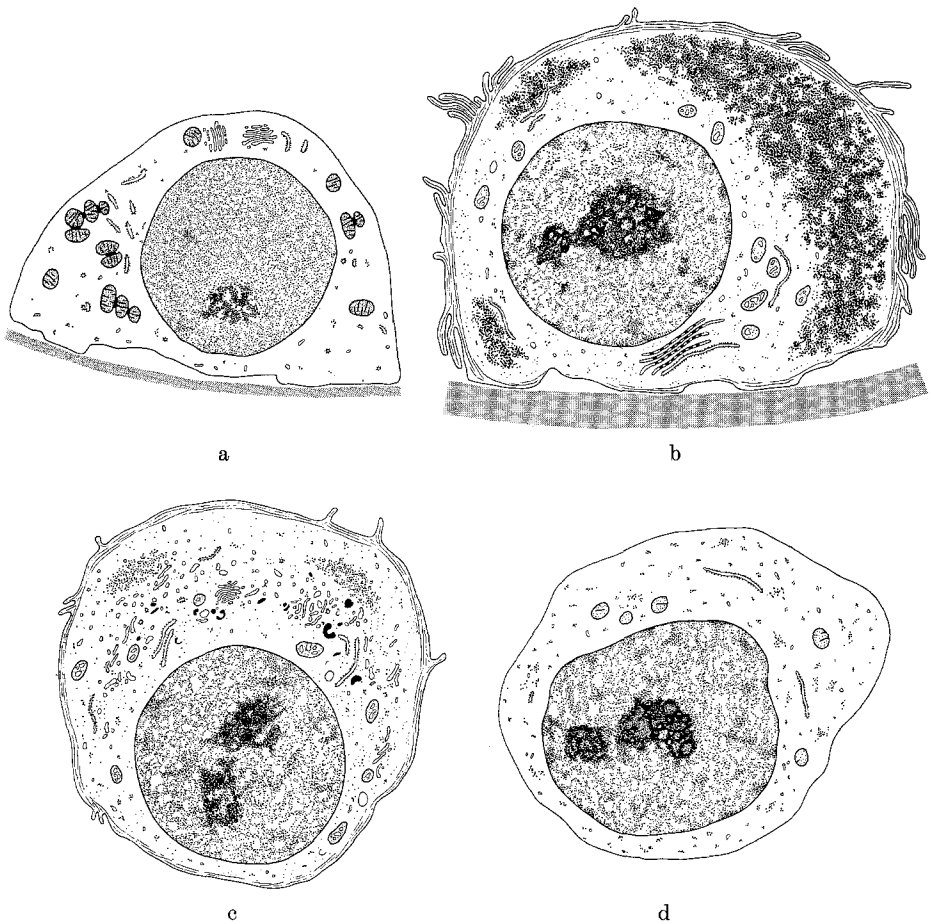

Fig. 9a—d. Semi-schematic drawing of a normal spermatogonium and of the three tumour cell types described here in the testis of a patient bearing a seminoma. a Normal spermatogonium. b Intratubular tumoral cell. c Tumoral cell found in the interstitium and at the periphery of the solid seminoma. d Tumoral cell found in the center of solid tumours. The drawings are based on electronmicrophotographies

has masses of glycogen granules, a rim of microfilaments under the cell membrane and numerous thin cytoplasmic evaginations. The cell type 2 contains less glycogen but more endoplasmic reticulum membranes and free ribosomes. There is also a rim of microfilaments but the cytoplasmic evaginations are few. The cell type 3 is strikingly translucid, with scarce figured elements in the cytoplasm and lacks both glycogen granules and cell prolongations. These three cell types are not normally found in the seminiferous epithelium and should be considered as seminoma tumoral cells.

In fact, these three cell types do not appear too often in solid tumours. The bulk of them corresponds to the clear cell type 3, upon which the diagnose of classical seminoma is based.

However, in the cases we analyzed, it could be regularly established that in the compromised testis, pathological cells (considered to be tumoral cells), were found not only in solid tumours but also in their periphery as well as isolated in the interstitial tissue between the seminferous tubules and even within the tubules themselves.

From the extensive works of Dixon and Moore (1952) and Mostofi (1973), it is known that seminoma can also grow intratubularly. Isolated tumoral cells in otherwise apparently normal seminiferous tubules are difficult to identify in paraffin sections since common fixatives will dissolve the glycogen away. On the other hand, seminoma cells are easy to recognize in semi-thin sections, where glycogen is very well preserved.

Based on the electron microscopical findings, it may be assumed that the tumoral cell types described correspond to steps in the development of the seminoma. Tentatively, the cells type 1 can be considered as immature tumour cells that are found in the seminiferous tubules and at the periphery of solid tumours. They exhibit clear characteristics of embryonic human germinal cells i.e. the early gonocytes, like abundance of glycogen granules, a peripheral microfilamentous web and thin cellular expansions (Wartenberg, Holstein, Vossmeyer, 1971). The microfilaments have a diameter of approximately 60 Å, thus resembling the contractile elements in the myofibroblasts of the lamina propria (Bustos-Obregón and Holstein, 1973) or in the contractile cells of the subepithelial wall of the epididymal duct (Baumgarten, Holstein and Rosengren, 1971). Microfilaments and cell expansions are structures associated to cellular ameboid motion. This type of motion has been claimed to occur in the gonocytes (Witschi, 1948) and cinematographically demonstrated by Blandau, White and Rumery (1963). In the case of tumoral cells it may be related to the invasiveness of the tumour (David and Mangakis, 1963; Ambrose, 1967). In the so-called embryonary carcinoma of the testis in the mouse Pierce and Beals (1964) found similar tumour cells, also rich in glycogen and exhibiting a peripheral microfilamentous web.

The fact that in the testis of the same patient tumoral cells having the characteristics of embryonic germinal cells are found intratubularly or at the periphery of solid tumours whereas in the center the cells lack these characteristics, supports the idea of a morphological differentiation of the tumour cells. Both embryonic and fully developed tumour cells were found to constitute the classical seminoma in the cases we examined.

In the seminiferous tubules with apparently intact spermatogenesis, where basal, isolated tumour cells are found, two interesting cytological details were observed:

1. Desmosome-like structures are often seen both between normal looking spermatogonia as well as between basal tumour cells. Normally, in human material, desmosomes or desmosome-like attachments are very seldom found between type A spermatogonia (unpublished observations). Bustos and Clermont (1968) have described similar attachments between intermediate type of spermatogonia and Sertoli cells in the rat.

2. Type A pale spermatogonia in the affected testis are occassionally connected by wide intercellular bridges. A medial ring-shaped element is found sectioned, that evokes a typical intercellular bridge (Dym and Fawcett, 1971) but is not

related to the cell membrane. This connection may supposedly be broken under pathological conditions. Alternatively it may also be assumed that this bridge-like structure has an identity of its own, not necessarily related to cell division.

The cell type 2 is more frequently found in the lumen of the seminiferous tubules or near the center of solid tumours. Because of its paucity in glycogen and cell expansions, it may be considered as a more mature tumoral cell type. Pierce (1966) has described the ultrastructure of this cell as typical of seminoma cells.

The clear cell of type 3 is the more developed. It constitutes the bulk of the tumour and is remarkably poor in figured elements of the cytoplasm. The lack of cytoplasmic expansions may point out to the inability of this cell to migrate away from its place.

It is generally assumed that the seminoma cells derive from germinal cellls. However, it is not known whether the tumour cells are abnormal germ cells that under unknown circumstances can, after puberty, undergo pathological proliferation or whether they derive from adult spermatogonia that are affected by an unknown noxa.

In the case of extragonadal teratocarcinoma the tumour cells are supposed to originate from misplaced primordial germ cells (Pierce and Beals, 1964). In classical seminomas there are still few observations to support our findings that "embryonic" as well as "mature" tumour cells may be found in the same tumour. Considering the topographical arrangement of the embryonic-like seminoma cells in the seminiferous tubules, the following points should be emphasized:

1. In seminiferous tubules with apparently normal spermatogenesis, isolated tumour cells are located mainly in a basal position close to type A dark spermatogonia, although type A pale spermatogonia are also present.

2. In seminiferous tubules with spermatogenesis greatly disturbed, numerous tumour cells are found basally located, close to type A pale spermatogonia, type A dark spermatogonia being very rare.

3. In seminiferous tubules showing no spermatogenesis, the tumour cells are densely packed at the base and also in luminal clusters of cells. There are no spermatogonia.

The more tumour cells are found, the lesser are the germinal cells. These observations may be taken to mean that the intratubular seminoma cells are not of embryonic origin but derived from spermatogonia (probably from type A dark). Like in other rapid growing tumours they may adquire the cell characteristics of their embryonic cell ancestor.

Concerning the problem of the origin of the cells in classic seminoma, more refined investigations are needed for a detailed understanding of the cytological characteristics of testicular tumours.

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