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Seminiferous Tubule Involution in Elderly Men[1]

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ABSTRACT

The observation of different types of seminiferous tubules (from tubules with normal spermatogenesis to sclerosed tubules) in aging human testes points to the progressive stages of tubular involution in elderly men. The tubules with hypospermatogonesis (reduced number of elongated spermatids) show numerous morphological anomalies in the germ cells, including multinucleated cells. Abnormal germ cells degenerate, causing Steroli cell vacuolation. These vacuoles correspond to dilations of the extracellular spaces resulting from the premature exfoliation of germ cells. Degenerating cells that are phagocytized by Sertoli cells lead to an accumulation of lipid droplets in the Sertoli cell cytoplasm. The loss of germ cells begins with spermatids, but progressively affects the preceding germ cell types, and tubules with maturation arrested at the level of spermatocytes or spermatogonia are observed. Simultaneously, an enlargement of the tunica propria occurs. This leads to the formation of sclerosed tubules, some of which display a low seminiferous epithelium consisting of a few cells—including lipid-loaded Sertoli cells and both Ap and Ad spermatogonia—and others, showing complete sclerosis, are devoid of seminiferous epithelium. The development of tubular involution is similar to that reported after experimental ischemia, which also seems to cause nonspecific effects on the testis such as multinucleate cells, vacuoles, and increased lipids in Sertoli cells.

INTRODUCTION

An involution of testicular function takes place with advancing age (Stearns et al., 1974; Baker et al., 1976; Nieschlag et al., 1983). This hypofunction has its counterpart in morphologic alterations including a variable degree of hypospermatogenesis with fibrosis of the peritubular and interstitial tissues (Kothari and Gupta, 1974; Honore, 1978) and a decrease in the number of Leydig cells (Kaler and Neaves, 1978).

Ultrastructural studies on aging human testes are infrequent and most have principally examined the alterations in germ cells (Holstein et al., 1984; Holstein and Eckmann, 1986), Sertoli cells (Schulze and Schulze, 1981; Johnson et al., 1984; Paniagua et al., 1985), or Leydig cells (Neaves et al., 1985; Paniagua et al., 1986). The objective of the present study is to report on the progressive stages of seminiferous tubule involution leading to complete tubular sclerosis in elderly men.

MATERIALS AND METHODS

Testicular biopsies were obtained approximately 2 h after death from 42 elderly men (from 65 to 89 yr of age) and 10 young adults (27 to 42 yr of age)

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who had not suffered from testicular, endocrine, or related pathology including varicocele. In addition, orchidectomy specimens were obtained from 26 patients (from 66 to 82 yr of age) suffering from prostatic carcinoma and who had received no previous hormonal or drug treatments and who were not suffering from testicular pathology (see Table 1). All of the subjects had fathered at least one child. Workers dealing with toxic agents were excluded.

Testicular specimens were fixed in Bouin's solution and embedded in paraffin. Fifty nonconsecutive 6-μm-thick sections from each testis were stained with hematoxylin-eosin. Five small blocks (1 mm^3) of each testicular specimen were fixed in 4% glutaraldehyde buffered at pH 7.2 with 0.1 M sodium cacodylate for 4 h, and postfixed in 1% osmium tetroxide buffered at pH 7.2 with veronal for 2 h. Afterwards, the blocks were dehydrated in graded ethanol and embedded in araldite. Five nonconsecutive 1-μm-thick sections from each block were stained with toluidine blue. Ultrathin sections were double-stained with uranyl acetate and lead citrate and examined in a Philips-300 electron microscope.

After verification that the proportion of testicular parenchyma occupied by seminiferous tubules (69% tubules, 31% interstitium) was similar in all testes, the different types of testicular parenchyma areas in each testis were classified as 1) areas of tubules with normal spermatogenesis, 2) areas of tubules with slight hypospermatogenesis, 3) areas of tubules with marked hypospermatogenesis, 4) areas of tubules with arrested maturation of spermatogenesis at the level of round spermatids, 5) primary spermatocytes and 6) spermatogonia, 7) areas with Sertoli-cell-only tubules, and 8) areas with fully sclerosed tubules. Two or more different areas could be found within a histologic section measuring less than 5 mm^2. Areas of tubules with normal spermatogenesis were those in which the numbers of each germ cell type per mm^2 of testicular parenchyma were similar to those found in young adult control testes (approximately 250 Sertoli cells, 425 spermatogonia, 625 primary spermatocytes, 775 round spermatids, and 525 elonated spermatids). Areas of tubules with slight hypospermatogenesis were those showing 25% to 50% reduction in the number of elongated spermatids; the numbers of round spermatids, spermatocytes, and spermatogonia were similar to those found in normal young adult testes. Areas of tubules with marked hypospermatogenesis were those showing a decrease from 50% to 90% in the number of elongated spermatids; the numbers of round spermatids, spermatocytes, and spermatogonia showed a reduction between 10% and 30%.

Since the extension of the different areas varied widely among testes and to quantify differences between testes, the numbers of seminiferous tubule profiles corresponding to each tubule type (classified according to their type of area) were counted and expressed as percentages on 5 nonconsecutive paraffin-embedded histological sections (about 4.5 mm^2) from each testis. The number of tubules counted per section varied from 80 to 119. From the values for each testis, the mean ± standard deviation for each age group was calculated (see Table 1). Differences between sections of the same testis were lower than differences between testes. The men were distributed into 4 groups according to age, and after microscopic examination of the testes, each of these groups was divided into two subgroups: A) men with complete spermatogenesis (normal spermatogenesis, slight or marked hypospermatogenesis) in at least 50% of tubules; and B) men with arrested maturation of spermatogenesis in at least 50% of tubules (see Table 1).

RESULTS

Most of the seminiferous tubules in young adult men showed normal spermatogenesis, though some areas of tubules with hypospermatogenesis could be observed; three testes also presented a few isolated sclerosed tubules. The tubules with hypospermatogenesis presented the cytological alterations reported below in elderly men.

The seminiferous tubules in elderly men varied widely among testes, and even within the same testis. In Group A testes (39 elderly men: 20 biopsy specimens and 19 orchidectomy specimens), the testicular parenchyma consisted of areas showing tubules with normal spermatogenesis (Fig. 1) intermingled with areas of hypospermatogenesis. Areas of tubules with arrested maturation of spermatogenesis and sclerosed tubules were occasionally found. In the remaining 29 elderly men (Group B testes), no normal spermatogenesis was observed, but areas of arrested maturation of spermatogenesis at spermatocyte or spermatogonium level, Sertoli-cell-only tubules, and fully sclerosed tubules (Fig. 2) were observed. The percentage distribution of seminiferous tubule types for each age group is shown in Table 1.

TABLE 1. Distribution of seminiferous tubule alterations related to age* in human testes.

Age (yr)	Number of men	Average age (yr)	Percentage[a] of seminiferous tubules showing arrested maturation at the level of						Fully sclerotic
			Normal spermat.	Light hyposp.	Marked hyposp.	Round spermatids	Primary spermato-cytes	Spermato-gonia	Sertoli cell-only	
27–42										
Group A	10	33.2 ± 1	85 ± 3	9 ± 0.5	4 ± 0.3	—	—	—	—	1 ± 1
Group B	0	—	—	—	—	—	—	—	—	—
60–69										
Group A	19	68 ± 2	59 ± 5	18 ± 2	16 ± 2	6 ± 1	—	—	—	1 ± 1
Group B	7	67 ± 2	—	8 ± 2	21 ± 3	34 ± 4	19 ± 2	9 ± 2	—	6 ± 1
70–79										
Group A	15	76 ± 2	31 ± 4	30 ± 3	31 ± 3	7 ± 1	1 ± 1	—	—	—
Group B	13	78 ± 3	—	—	25 ± 3	27 ± 3	21 ± 2	13 ± 2	8 ± 1	6 ± 1
80–89										
Group A	5	85 ± 3	16 ± 2	25 ± 3	20 ± 3	25 ± 3	12 ± 2	—	—	2 ± 1
Group B	9	83 ± 3	—	—	10 ± 2	23 ± 2	26 ± 3	24 ± 3	9 ± 2	8 ± 2

[a]Values are expressed as mean ± standard deviations.

*Group A: men with complete spermatogenesis in a minimum of 50% of tubules; Group B: men with arrested maturation of spermatogenesis in a minimum of 50% of tubules.

The study of araldite-fixed material by light and electron microscopy revealed morphological alterations in both germ cells and Sertoli cells in the different types of altered seminiferous tubules.

Tubules with light or marked hypospermatogenesis. These tubules showed few elongated spermatids and numerous round spermatids, many of them exhibiting anomalies in spermiogenesis. By light microscopy, the most conspicuous anomaly was the occurrence of spermatids showing two or more nuclei (Fig. 3); examination of cross-sections of three tubules from each aging testis (each tubule showing at least 35 spermatids) revealed 9.1 ± 1 percent of bi- or trinucleated spermatids, in contrast with the 1.4 ± 0.2 percent observed in young adults. With electron microscopy, ultrastructural anomalies such as acrosome malformations, redundant nuclear membranes, intranuclear inclusions, excessive cytoplasmic droplets, and irregular configuration of the nucleus (Figs. 4–6) were seen in 35.9 ± 4 percent of the spermatids counted on three 10,000 μm^2 areas (each area showing at least 100 spermatids) from each aging testis and in 18 ± 7 percent of the spermatids present in young adult testes. Spermatocytes and spermatogonia showing ultrastructural anomalies, such as intranuclear inclusions (Figs. 7, 8) and "whorls" of endoplasmic reticulum in the cytoplasm (Fig. 9), were often seen. Some of these cells also showed two or more nuclei (Fig. 10). The Sertoli cells showed abundant secondary lysosomes (Fig. 11), excessive accumulations of lipid droplets (Figs. 3, 12), and vacuoles (Figs. 3, 10) that, by electron microscopy, exhibited an amorphous content similar to that of the tubule lumen. These vacuoles were surrounded by cisternae of smooth endoplasmic reticulum similar to those found in the inter-Sertoli junctional specializations (Fig. 13). Occasionally the vacuoles communicated with the tubular lumen.

Many tubules contained numerous degenerating cells, mainly spermatocytes and spermatids (Fig. 14). These tubules also showed ultrastructural anomalies in the germ cells including bi- and trinucleated cells, and increased amounts of lipids. Apparent sloughing of immature germ cells, mainly multinucleated and degenerated germ cells, was observed in some tubules (Fig. 15). The tunica propria was normal (Figs. 3, 10, 15) or slightly thickened (Fig. 14).

Tubules with arrested maturation at spermatocyte level. These tubules exhibited abundant lipids and vacuoles in the Sertoli cell cytoplasm. Whereas in

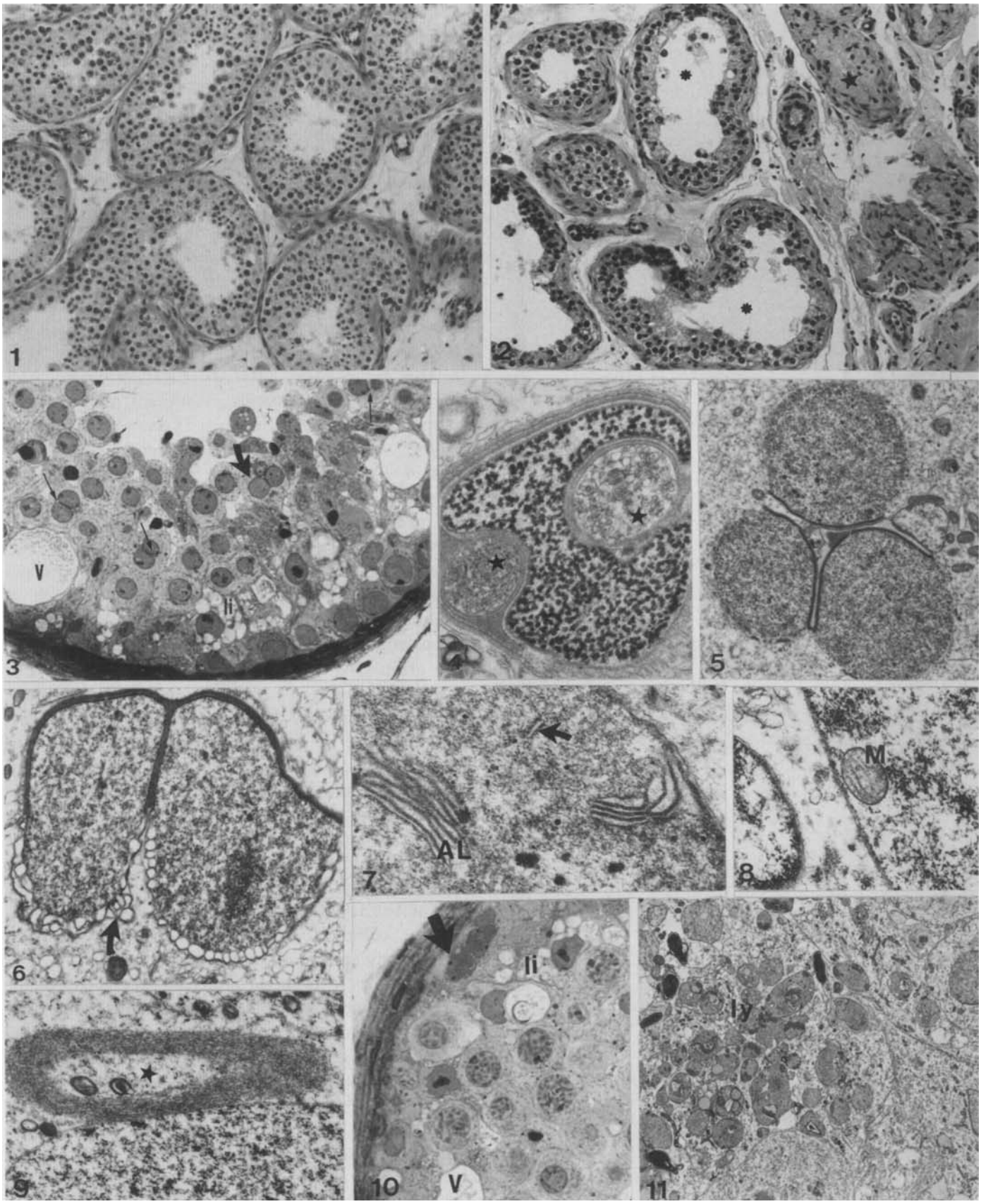

PLATE I.

FIG. 1. Seminiferous tubules showing normal spermatogenesis in an 89-yr-old man. Hematoxylin and eosin. ×125.

FIG. 2. Seminiferous tubules showing maturation arrested at spermatocyte level (*asterisks*) as well as sclerosed tubules (*star*) in a 64-yr-old man. Hematoxylin and eosin. ×125.

FIG. 3. Bi- (*arrows*) and trinucleated (*large arrow*) spermatids, abundant lipid droplets (*li*), and vacuoles (*V*) in a tubule with slight hypospermatogenesis from a 72-yr-old man. Toluidine blue. ×450.

FIG. 4. Irregularly outlined acrosome protruding into the nucleus together with a cytoplasmic mass (*stars*) in a 73-yr-old man. ×9000.

FIG. 5. Trinucleated spermatid showing a single acrosome shared by the three nuclei. 68-yr-old man. ×4500.

FIG. 6. Redundant nuclear membranes (*arrow*) in a binucleated spermatid. 62-yr-old man. ×5500.

FIG. 7. Intranuclear annulate lamellae (*AL*) in a spermatocyte from a 77-yr-old man. *Arrow*: synaptonemal complex. ×9000.

FIG. 8. Intranuclear mitochondrion (*M*) in a binucleated spermatocyte. 69-yr-old man. ×9000.

FIG. 9. Cytplasmic "whorls" of endoplasmic reticulum (*star*) in a spermatocyte from a 72-yr-old man. ×6000.

FIG. 10. Multinucleated spermatogonium (*arrow*) in the testis from a 76-yr-old man. Lipid droplets (*li*); vacuoles (*V*). Toluidine blue. ×600.

FIG. 11. Numerous secondary lysosomes (*ly*) in the cytoplasm of Sertoli cells. 84-yr-old man. ×2350.

some tubules the lipids were more conspicuous than the vacuoles (Fig. 16), in others the vacuoles were predominant. Degenerating cells were also seen. The tunica propria appeared enlarged (Fig. 16).

Tubules with arrested maturation at spermatogonium levels. These tubules were similar to those with spermatocytes except for a more pronounced thickening of the tunica propria and more abundant vacuoles (Fig. 17) or lipids (Fig. 18). Both Ap and Ad spermatogonia were present (Fig. 17).

Sclerosed tubules. Some tubules showed a markedly thickened tunica propria in which abundant collagen fibers and a few peritubular cells could be recognized (Fig. 19). The seminiferous epithelium was reduced to a cuboidal epithelium consisting of a low number of lipid-loaded Sertoli cells and spermatogonia (Figs. 19, 20). The Sertoli cells (with deeply infolded nuclei) and the basal lamina (displaying a multilayered configuration) exhibited the characteristic appearance of the adult, mature Sertoli cells (Fig. 20). The basal lamina extended from the seminiferous epithelium past the boundary of the first layer of peritubular cells (Fig. 20). Other tubules appeared completely sclerosed and were lacking in seminiferous epithelium.

DISCUSSION

A few sclerosed tubules are found frequently in normal adult testes, and their presence has been interpreted as evidence of dysgenetic tubules that never matured (Hatakeyama et al., 1979). The occurrence of a diffuse or focal tubular sclerosis is more significant. Diffuse tubular sclerosis is usually present in both hypogonadotropic and hypergonadotropic hypogonadisms. The finding of focal tubular sclerosis suggests a variety of etiologies including mumps orchitis, arterial lesions, obstruction of the initial portion of the excretory ducts, and dysgenetic lesions (Hatakeyama et al., 1979). The focal tubular sclerosis in elderly men who were fertile does not seem to represent the evolution of dysgenetic tubules; rather, it seems to be the final step of an involutive process which may be deduced from the observation of other tubular alterations that suggest progressive stages of such involution.

The first sign of alteration is the numerous ultrastructural anomalies in germ cell development, principally in spermatids but also in the preceding cell types. Among these anomalies, the formation of multinucleated germ cells is characteristic (Holstein and Eckmann, 1986). The presence of multinucleated cells in the aging human testis is not exclusive of germ cells, since multinucleated Leydig cells (Paniagua et al., 1986) have been reported in old age. The occurrence of several nuclei in a single cytoplasm may be due to karyokinesis without cytokinesis or to cell fusion. Recent evidence supports the idea that multinucleated spermatids in aging human testes are due to cell fusion (Nistal et al., 1986). Although these ultrastructural anomalies are nonspecific and may be seen even in normal testes (Nistal and Paniagua, 1984), their frequency was much higher in the testes of aging men than in young adult testes.

It is not unlikely that abnormally configurated germ cells progress to spermatozoa, and it is to be expected that these cells either degenerate or become sloughed (Holstein and Eckmann, 1986). This agrees with the frequent observation of degeneration and apparent sloughing of germ cells in the seminiferous tubules of aging human testes. The occurrence of germ cells in the lumen of the seminiferous tubules may simply reflect tissue preparation; and, to definitively evaluate sloughing, it would be necessary to locate sloughed germ cells in the epididymis or the

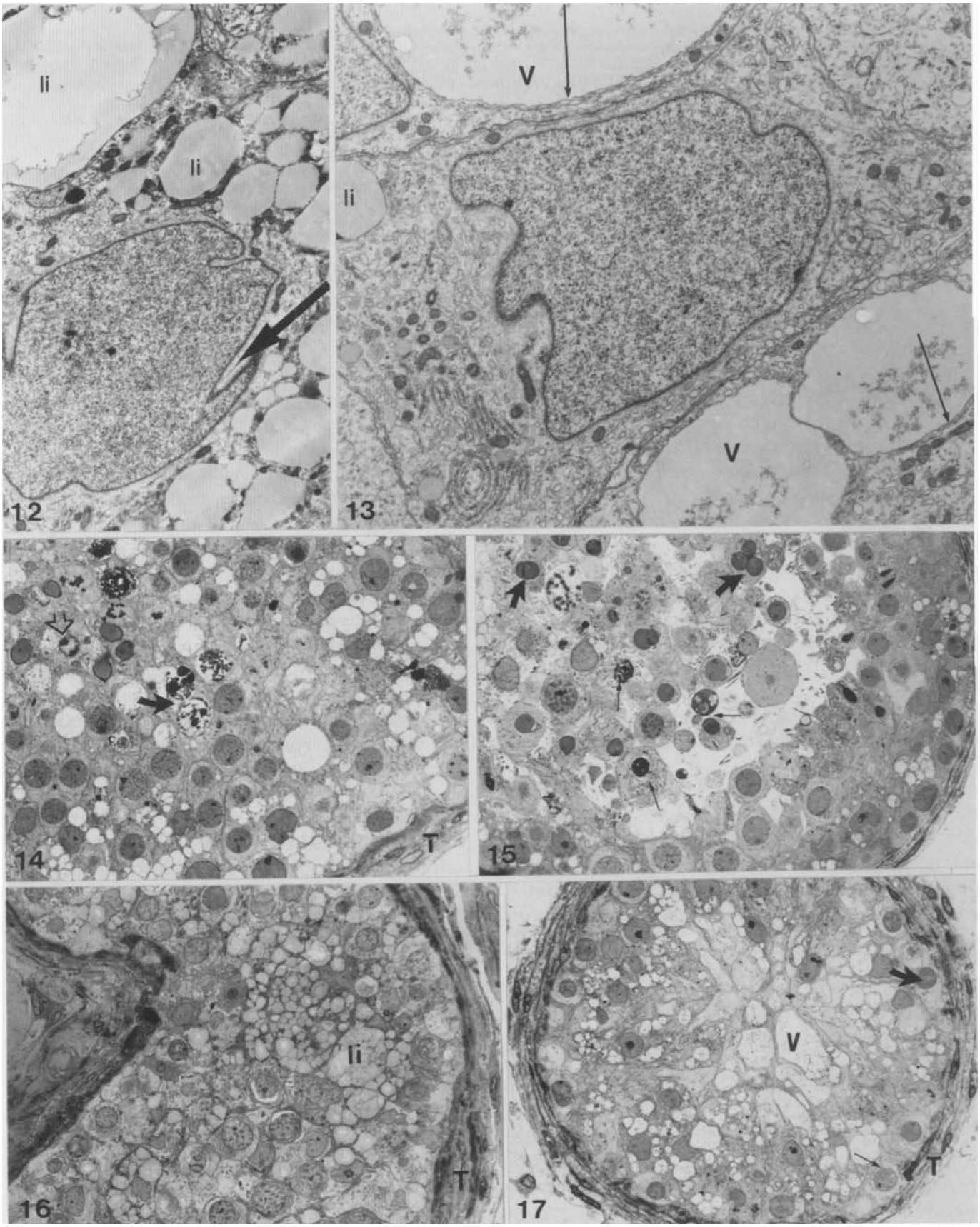

PLATE II.

FIG. 12. Abundant lipid droplets (*li*) in the cytoplasm of a Sertoli cell. The nucleus shows its characteristic irregular outline (*arrow*). 74-yr-old man. ×4500.

FIG. 13. Vacuoles (*V*) partially surrounded by cisternae of smooth endoplasmic reticulum (*arrows*) in the Sertoli cells. Lipid droplets = *li*. 69-yr-old man. ×5000.

FIG. 14. Degenerating spermatids (*open arrow*) and spermatocytes (*arrow*) in a tubule with slight hypospermatogenesis from a 72-yr-old man. The tunica propria (*T*) is slightly thickened. Toluidine blue. ×500.

FIG. 15. Apparent sloughing of immature germ cells, mainly bi- and trinucleated spermatids (*large arrows*), and of degenerating germ cells (*small arrows*) in the testis from a 77-yr-old man. Toluidine blue. ×450.

FIG. 16. Maturation arrested at spermatocyte level in a tubule showing abundant lipid droplets (*li*). The tunica propria (*T*) is thickened. 79-yr-old man. Toluidine blue. ×450.

FIG. 17. Maturation arrested at spermatogonium level in a tubule showing abundant vacuoles (*V*). The tunica propria (*T*) is thickened. Ap (*small arrows*) and Ad (*large arrow*) spermatogonia can be seen. 84-yr-old man. Toluidine blue. ×450.

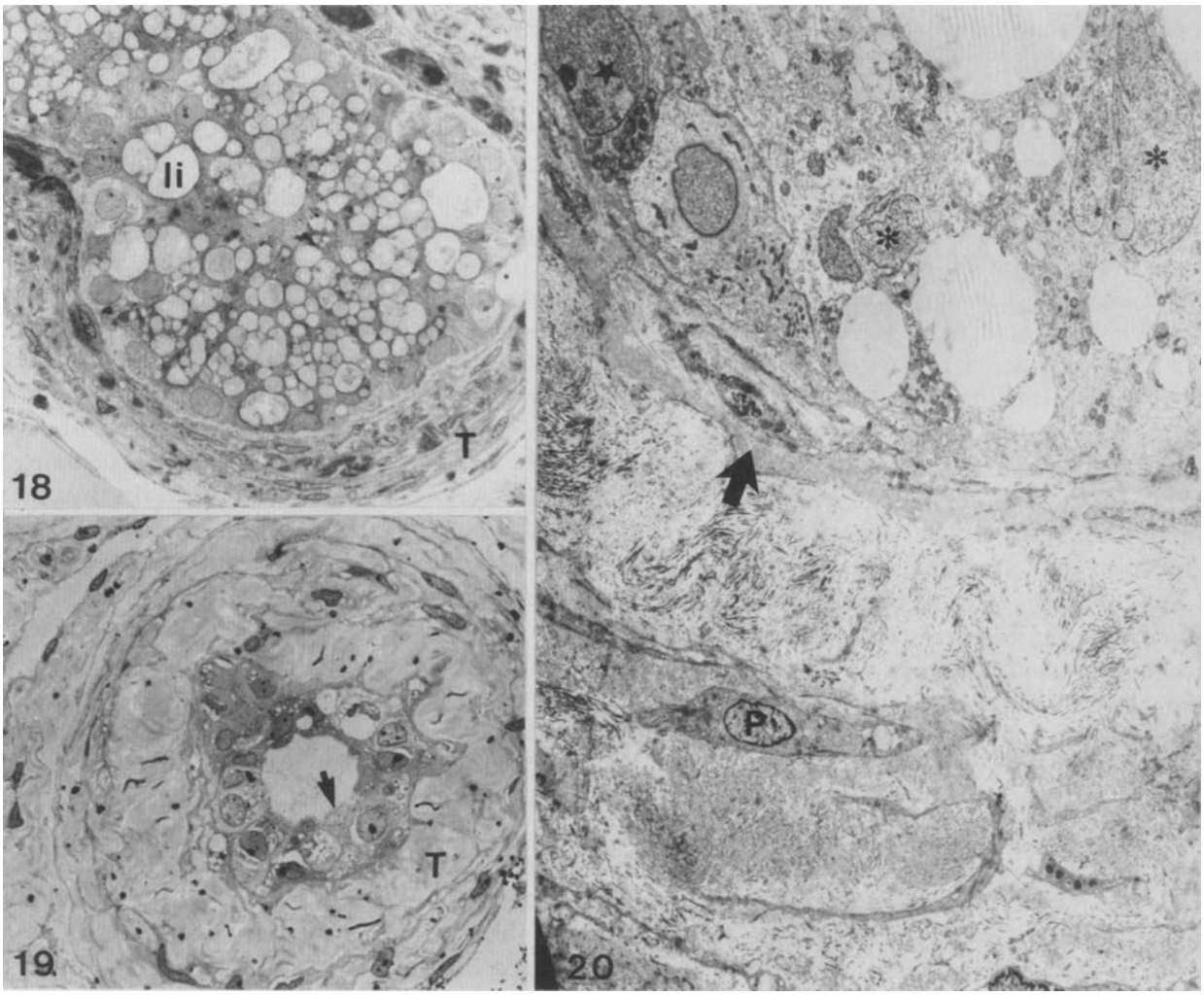

PLATE III.

FIG. 18. Maturation arrested at spermatogonium level in a tubule showing abundant lipid droplets (*li*). The tunica propria (*T*) is thickened. 88-yr-old man. Toluidine blue. ×450.

FIG. 19. Almost completely sclerosed tubule. The tunica propria (*T*) is markedly thickened. The seminiferous epithelium (*arrow*) is low and vacuolated. 71-yr-old man. Toluidine blue. ×450.

FIG. 20. Part of the seminiferous tubule of *Figure 19* showing lipid-loaded Sertoli cells (*asterisks*) and spermatogonia (*star*). The multilayered basal lamina (*arrow*) exceeds the inner layer of peritubular cells (*P*). 81-yr-old man. ×2600.

ejaculate. However, both degenerated and apparently sloughed germ cells were frequent in aging testes and were only observed occasionally in young adult testes. Germ cell loss probably determines the development of vacuoles in the Sertoli cells. Similar vacuoles have been reported by Kerr et al. (1979) in rat testes after surgical induction of cryptorchidism, and by Flickinger (1981) in vasectomized hamsters. This author assumed that the vacuoles are dilations of the extracellular space developed from premature exfoliation of germ cells. Although the vacuoles observed in aging testes appear to lie within the cytoplasm of Sertoli cells rather than between adjacent cells, the occasional observation of vacuoles in continuity with the tubular lumen and the occurrence of smooth endoplasmic reticulum cisternae around the vacuoles suggest that many vacuoles are continuous with the extracellular space. These smooth endoplasmic reticulum cisternae are a characteristic component of the inter-Sertoli junctional specializations commonly found at the Sertoli cell-spermatocyte junctions (Russell and Peterson, 1985).

The accumulation of lipid droplets in the Sertoli cells seems to be a gradual process, starting at 15 yr of age and increasing as time progresses (Lynch and Scott, 1950). The increased lipid content in Sertoli cells has been considered to be derived from phagocytized germ cells (Schulze, 1984). It is probable that not all the degenerating germ cells observed in aging testes are sloughed and that some of them are phagocytized by the Sertoli cells, contributing to the increase in the abundance of secondary lysosomes that finally form the lipid accumulations.

The loss of germ cells progresses giving rise to tubules with maturation arrested at spermatocyte or spermatogonium level. Vacuoles and lipid accumulations are more abundant in these types of tubules. However, in some tubules, lipids are more abundant than the vacuoles; the contrary occurs in other tubules. This feature might be related to the predominant mechanism leading to germ cell loss: germ cell degeneration followed by phagocytosis or germ cell sloughing. The tubules with a few spermatogonia and lipid-loaded Sertoli cells suggests that even the most resistant cell types are also lost. A decrease in the number of Sertoli cells in the aging testis has been reported (Harbitz, 1973). Although it has been reported that Ad spermatogonia are more sensitive to noxious agents than Ap spermatogonia (Schulze, 1979), both A spermatogonium variants are found in these later stages of the seminiferous epithelium. Both finally disappear and leave behind fully sclerosed tubules.

The loss of germ cells might be related to the enlargement of the tunica propria. Both processes occur simultaneously in the aging testis and, therefore, it is not possible to infer which is the cause and which is the effect. It has been assumed that the enlargement of the tunica propria impedes the metabolic exchange of the seminiferous epithelium, thus leading to tubular atrophy (Hadziselimovic, 1977; Prijono and Schirren, 1985). However, it has also been suggested that tubular hyalinization is secondary to the destruction of germ cells and that the fibroblasts of the tubular wall synthesize collagen fibers that fill the tubules (Söderström, 1986).

The causes of tubular sclerosis are possibly multiple and related to the aging of somatic tissues. Among these causes, vascular alterations, such as arteriosclerosis and arteriolar hyalinosis, might play an important role. A correlation between tubular atrophy and testicular arteriosclerosis has recently been demonstrated in the human testis (Regadera et al., 1985). Within the same testis, the well-vascularized areas showed tubules with complete spermatogenesis, whereas the areas showing progressive degrees of arteriosclerosis also presented progressive degrees of tubular atrophy. Testicular arteriolar hyalinosis has been reported to be present in many adult normal testes and to increase with age (Hatakeyama et al., 1966). Associated with this arteriolar hyalinosis are focal tubular lesions, varying from hypospermatogenesis to tubular sclerosis. The involvement of vascular lesions in tubular involution with age agrees with the results of this study, since the tubular involution is similar to that observed after experimental occlusion of the testicular artery (Carmignani et al., 1983; Kaya, 1986), including the occurrence of unspecific morphological alterations such as multinucleated germ cells (Carmignani et al., 1983), the loss of seminiferous epithelium (beginning at spermatid level and following up to the Sertoli cells), the abundance of lysosomes and lipids in the Sertoli cells, and the thickening of the tunica propria. The mosaic pattern distribution of tubular lesions in aging testes agrees with the irregular distribution of vascular lesions in these testes (Regadera et al., 1985).

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