

Age-related Variation in Seminiferous Tubules in Men

A Stereologic Evaluation

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Tubular boundary tissue and seminiferous epithelia were evaluated stereologically in testes from 28 men aged 20 to 48 years and 28 men aged 50 to 90 years. Testes obtained at autopsy within 15 hours of death were perfused with glutaraldehyde, embedded in Epon (Ladd Research Industries, Inc., Burlington, VT), sectioned at 0.5 μm , and stained with toluidine blue. Volume densities (percentage of the testicular parenchyma) of various parameters determined by point counting and diameter measurements were used to calculate total volumes, length of tubules, and number of cells. Electron microscopy was used to determine the volume density of myoid cells in the boundary tissue. Significant ($P < 0.01$) age-related reductions occurred in paired testicular weights, paired parenchymal weights, total volume of seminiferous tubules and of seminiferous epithelium, and length of tubules. The volume density and thickness of boundary tissue increased ($P < 0.01$) with age. The volume of boundary tissue per man and the volume density of myoid cells in the boundary tissue did not vary with age. Although the number of myoid cells per man tended to be lower in the older group, the number of myoid cells per cross section of seminiferous tubule was increased ($P < 0.01$) in older men. The age-related thickening of the boundary tissue was not due to an increase in boundary tissue but resulted from a reduction in the length of the seminiferous tubules.

Key words: aging, testis, seminiferous tubules, myoid cells, Sertoli cells, daily sperm production.

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The boundary tissue that surrounds the seminiferous epithelium of seminiferous tubules (also called limiting membrane, peritubular layer, and lamina propria; Bustos-Obregon and Holstein, 1973) is

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composed of two to six layers of myoid cells that are separated from each other by collagen fibrils, elastin fibrils, and microfibrils (Herms et al, 1977). Modification of the boundary tissue has been associated with infertility or azoospermia in men (de Kretser et al, 1975; Salomon and Hedinger, 1982). Also, the tubule boundary tissue changes with advancing age in humans (Bishop, 1970). Age-related changes include the thickening of the boundary tissue, progressive intertubular fibrosis, decreased tubule diameter, thinning of the spermatogenic epithelium and eventual obliteration of the seminiferous tubule (Bishop, 1970).

Thickening of the tubule boundary tissue has been associated with an increase in extracellular components (collagen fibers, microfibrils, and incomplete basement membrane-like coating of the myoid cells; Bustos-Obregon and Holstein, 1973). De Kretser et al (1975) also found that this thickening resulted from changes in existing structures rather than from the formation of new components. These studies, however, were qualitative in nature, and did not address possible contributing factors, such as changes in tubule length, tubule volume/man, and number and size of myoid cells. In addition to thickening of tubule boundary tissue, daily sperm production (Amann, 1970) was shown to be reduced with advancing age in a large series of men (Johnson et al, 1984a). In an attempt to understand age-related changes in human seminiferous tubules that might

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be associated with reduced daily sperm production, a stereologic evaluation of human seminiferous tubules was conducted.

Materials and Methods

Specimens

Testes from a group of 28 men aged 20 to 48 years and a group of 28 men aged 50 to 90 years were obtained at autopsy within 15 hours of death. The average postmortem time was 8.6 ± 0.6 hours for the younger men and 8.1 ± 0.6 hours for the older men. The distribution of subjects according to race or ethnic origin was similar in both groups. Heart attacks accounted for 18% of deaths in the younger and 50% in the older groups of men. Traumatic injury accounted for 78% of deaths in the younger and 46% in the older groups. Subjects in both age groups were selected on the basis of apparent good health prior to death and no history of recent hospitalization or serious illness.

Body weight, height, and the ratio of weight (kg) to height (cm) were similar in the two groups. Younger men had body weights averaging 77.6 ± 3.3 kg, and older men had body weights averaging 79.2 ± 3.7 kg. Height averaged 174 ± 14 cm for younger men and 175 ± 15 cm for older men. Weight-to-height ratios averaged 0.44 ± 0.02 for the younger group and 0.45 ± 0.02 for the older group.

Testes were fixed by vascular perfusion with 2% glutaraldehyde in 0.1 M cacodylate buffer. Five pieces of fixed testis were further fixed in 1% OsO_4 and embedded in Epon. Epon-embedded tissue was sectioned at $0.5 \mu\text{m}$, stained with toluidine blue, and examined by bright-field microscopy. During evaluation, tissue specimens were coded so that the investigators did not know the identity of the subjects or the age group to which they belonged.

Measurements on Seminiferous Tubules

Volume densities, expressed as the percent of testicular parenchyma (testis less tunica albuginea) occupied by various components, were determined by point counting (Elias et al, 1978). Randomness and sufficient sampling were ensured by the use of large Epon sections (two for each of two randomly selected tissue blocks) averaging over 10 mm^2 and by predetermined movement of slides during the evaluation at 400 or $1000 \times$ magnification. Point counting sufficient to achieve a coefficient of variation of approximately 10% or less was conducted for each tissue component of interest. The total number of points scored for each component per man was 2000 for seminiferous tubules, boundary tissue, and seminiferous epithelium and 10,000 for myoid cell nuclei. Volume density of various components multiplied by total parenchymal volume (parenchymal weight/1.05 g/ml) yielded the total volume of that component per man. Once the tubule diameter (100 measurements/man) and tubule volume were determined, the seminiferous tubule length was calculated using the formula for a cylinder (Johnson and Neaves, 1981). Precisions of point counting and tubule diameter measurements of repeated estimates are indicated by coefficients of variation of 9.3, 10.8, 6.6, 11.6, and 4.4 percent, respectively, for seminiferous tubules, bound-

ary tissue, seminiferous epithelium, myoid cell nuclei, and tubule diameters.

Calculation of the Number of Myoid Cells and Daily Sperm Production

The volume of individual myoid cell nuclei was determined by area measurements using a computerized digitizing unit in each of several $0.5\text{-}\mu\text{m}$ serial sections through the entire nucleus (Johnson et al, 1984b; Neaves et al, 1985). Ten nuclei were measured at $1000 \times$ magnification on three randomly selected men in each age group. The number of myoid cells per man was calculated by dividing the product of the parenchymal volume and the volume density of myoid cell nuclei by the average volume of individual nuclei. The relative section thickness (section thickness divided by the average diameter measured by one half the number of sections in the reconstruction or by direct measurements) was much less than the cutoff value of 0.1, below which no correction of these structures is considered necessary (Bolender, 1978). While the absence of this correction might slightly overestimate the absolute number of myoid cells, the relative comparisons between age groups would not be affected. The average volume of individual myoid cells was calculated for each man by dividing the volume of myoid cells per man (volume density of boundary tissue \times percentage of myoid cells in boundary tissue \times parenchymal weight/1.05 specific gravity) by the number of myoid cells per man (Johnson and Neaves, 1981). The percentage of myoid cells in boundary tissue was determined on electron micrographs of boundary tissue from 10 randomly selected men from each age group. A total of 182 micrographs observed at $16,500 \times$ magnification and taken at predetermined locations on 200-mesh grids were scored by a point-counting overlay (Elias et al, 1978). The percentage of myoid cells was calculated as the number of hits over myoid cells divided by the number of total hits over boundary tissue. A total of 31,970 to 41,756 hits over boundary tissue were scored per age group. The boundary tissue thickness was based on 50 tubules measured per man and equaled the sum of the basement membrane and the myoid cellular component.

The number of myoid cells per testis also was calculated for each man by dividing the product of the length of tubules and the number of myoid cell nuclei in tubular cross sections by the diameter of myoid cell nuclei measured at the maximum nuclear length along the length of the tubules in serial sections. Cross sections of 849 to 911 tubules were evaluated per group with an average coefficient of variation of 5.2%. The diameters of nine myoid cell nuclei per man were measured for three randomly selected men per group, with a coefficient of variation of 4.9%.

The numbers of Sertoli cells and spermatids with round nuclei per man were determined by direct counts from testicular homogenates (Johnson et al, 1981, 1984b). From the number of spermatids with round nuclei (Johnson et al, 1981), daily sperm production was calculated by dividing the product of the number of these spermatids in the homogenate and parenchymal weight by the product of the weight of the tissue homogenized and the 8.9-day lifespan of these spermatids.

Statistical Analysis

Differences between age groups were tested by the Student's *t* test (Sokal and Rohlf, 1969). Correlation coefficients were tested for significance (Sokal and Rohlf, 1969).

Results

Both paired testicular and parenchymal weights were reduced ($P < 0.01$) in the men from the older group (Table 1). Both tubular diameter and volume density of the parenchyma occupied by seminiferous tubules were similar ($P > 0.05$) for both groups. Due to differences in parenchymal weights, however, the total volume of the seminiferous tubules per man and the length of the tubules were less ($P < 0.01$) in the older group of men. A larger ($P < 0.01$) percentage of the parenchyma in older adult men was occupied by tubular boundary tissue (Table 1). As a result of the opposing influences of reduced ($P < 0.01$) total parenchymal weight and increased ($P < 0.01$) volume density of boundary tissue in the older men, the total volume of the boundary tissue was similar ($P > 0.05$) in the two age groups. The larger volume density of

boundary tissue with advancing age was associated with a thickening of the boundary tissue (Fig. 1). When evaluated quantitatively, the thickness of myoid cellular and extracellular components or of all components combined was found to be larger ($P < 0.01$) in the older men (Table 1). The basement membrane region (the area between the seminiferous epithelium and the first layer of myoid cell nuclei observed by light microscopy) was similar ($P > 0.05$) in thickness in both groups. The boundary tissue thickness was positively correlated ($r = 0.64$; $P < 0.01$) with the age of the men (Fig. 2).

The volume density of myoid cell nuclei and the volume of individual nuclei were similar in the two age groups (Table 1). The diameters of myoid cell nuclei measured along the length of the tubules were similar ($16.6 \pm 0.7 \mu\text{m}$ for younger and $16.8 \pm 0.9 \mu\text{m}$ for older men, $P > 0.05$). Due to reduced parenchymal weight in the older men, the total volume of the myoid cell nuclei was lower ($P > 0.05$). The number of myoid cells per gram of parenchyma was similar ($P > 0.05$) in both age groups, but the total number of myoid cells per man was reduced ($P <$

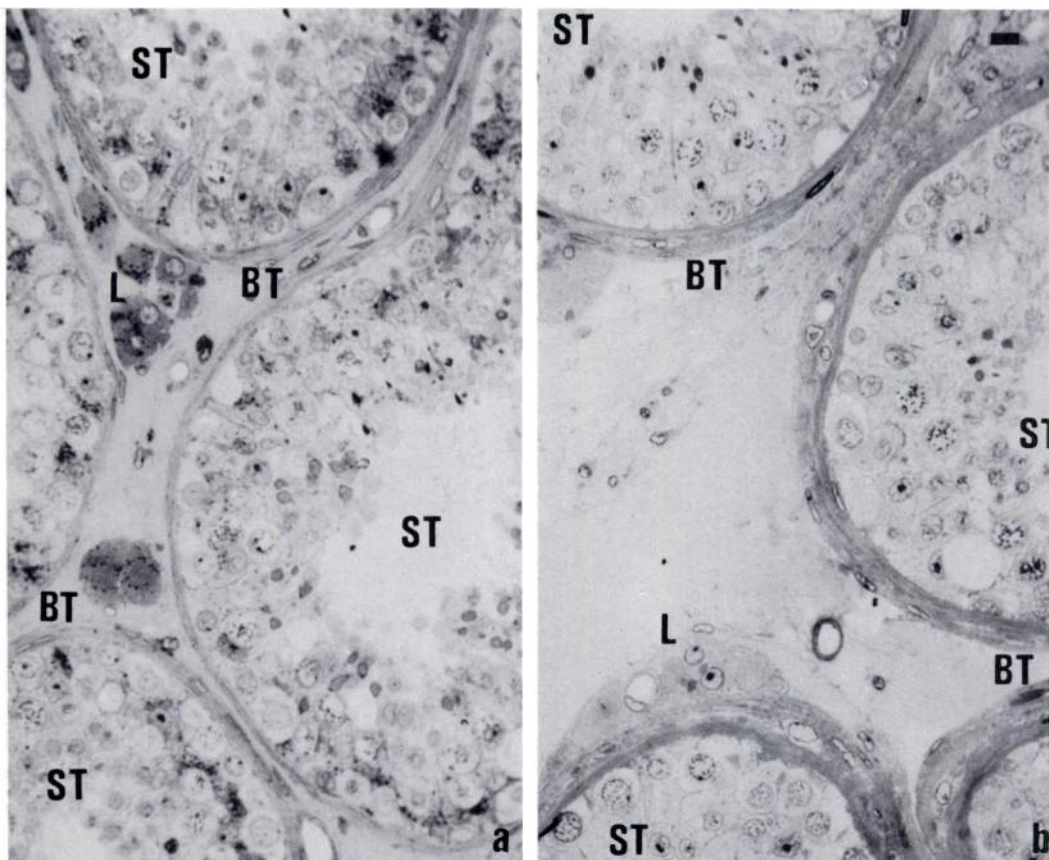


Fig. 1. Comparison of portions of seminiferous tubules (ST) in men from the a. younger and b. older age groups. The boundary tissue (BT) is thicker in the older group of men. Leydig cells (L) are present in the interstitium. Bar length equals $10 \mu\text{m}$ (toluidine blue stain; $\times 63$).

TABLE 1. Age-related Variation in Seminiferous Tubules and Daily Sperm Production in Men

Testicular Component	Age Group*		Significance
	20-48 Yr†	50-90 Yr†	
Weight			
Body (kg)	77.6 ± 3.3	79.2 ± 3.7	NS
Paired testes (g)	46.8 ± 1.8	37.8 ± 2.0	P < 0.01
Paired testicular parenchyma (g)	40.7 ± 1.7	31.4 ± 1.8	P < 0.01
Volume density in parenchyma (%)			
Seminiferous tubule	62 ± 1	59 ± 2	NS
Boundary tissue	9.0 ± 0.3	11.3 ± 0.7	P < 0.01
Lumen	8.8 ± 0.9	10.6 ± 1.0	NS
Seminiferous epithelium	44.1 ± 1.2	37.4 ± 1.8	P < 0.01
Volume per man (ml)			
Seminiferous tubule	23.8 ± 1.0	18.0 ± 1.2	P < 0.01
Boundary tissue	3.5 ± 0.2	3.4 ± 0.2	NS
Lumen	3.5 ± 0.4	3.2 ± 0.4	NS
Seminiferous epithelium	16.9 ± 0.7	11.4 ± 0.9	P < 0.01
Seminiferous tubule			
Diameter (μm)	226 ± 3	224 ± 4	NS
Length/g (m)	14.9 ± 0.5	14.5 ± 0.5	NS
Length/man (m)	598 ± 25	454 ± 28	P < 0.01
Boundary tissue thickness (μm)			
Basement membrane	8.3 ± 0.3	10.9 ± 0.4	P < 0.01
Cellular and extracellular components	1.8 ± 0.1	1.8 ± 0.1	NS
	6.5 ± 0.3	9.1 ± 0.4	P < 0.01
Myoid cell nuclei			
Volume density (%)	0.52 ± 0.02	0.52 ± 0.03	NS
Volume/man (μl)	196 ± 9	158 ± 13	P < 0.05
Average volume of individual nucleus (fl)‡	207 ± 6	208 ± 4	NS
Number			
Per g parenchyma (10 ⁶)	23.9 ± 1.1	24.0 ± 1.3	NS
Per man (10 ⁶)	947 ± 42	760 ± 60	P < 0.05
Number of myoid cell nuclei			
Per tubular cross section	21.2 ± 0.8	25.1 ± 1.0	P < 0.01
Per man based on tubular length (10 ⁶)	743.5 ± 30	665.9 ± 46	NS
Myoid cell			
Volume density of boundary tissue (%)§	32.1 ± 2.2	28.9 ± 2.3	NS
Total volume per man (μl)	1109 ± 51	970 ± 51	NS
Volume of average individual cell (fl)	1197 ± 54	1322 ± 66	NS
Number of cells (10⁶)			
Sertoli cells			
Per g parenchyma	23.9 ± 1.1	16.9 ± 1.0	P < 0.01
Per man	977 ± 61	551 ± 51	P < 0.01
Spermatids with round nuclei			
Per g parenchyma	53.5 ± 3.4	34.1 ± 2.9	P < 0.01
Per man	2226 ± 194	1075 ± 104	P < 0.01
Daily sperm production (10⁶)			
Per g parenchyma	6.0 ± 0.4	3.8 ± 0.3	P < 0.01
Per man	250 ±	121 ± 12	P < 0.01

*Mean ± SEM.

†n = 28.

‡Based on three randomly selected men per age group.

§Based on 10 randomly selected men per age group.

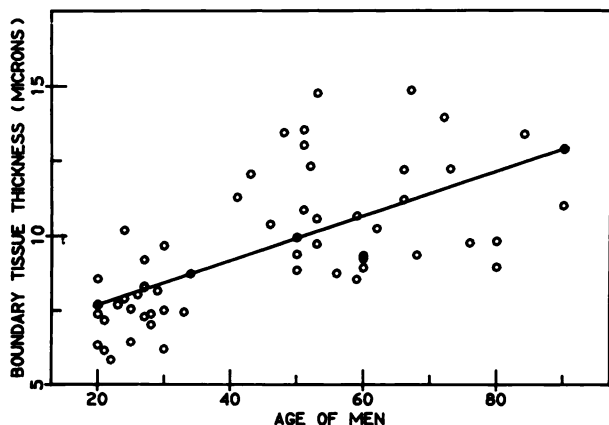


Fig. 2. The relationship between age and the boundary tissue thickness of seminiferous tubules. There was a significant ($r = 0.64$; $P < 0.01$) age-related increase in the thickness of the boundary tissue.

0.05) by about 20%. Given a 20% reduction in the number of myoid cells and a 24% reduction ($P < 0.01$) in the length of the tubules, the number of myoid cell nuclei per tubular cross section was greater ($P < 0.01$) in the older men. When numbers of myoid cells were calculated from tubular length, diameter of myoid cell nuclei along the length of the tubule, and the number of myoid cell nuclei per tubular cross section, the reduction in the number of myoid cells was only 10% ($P > 0.05$).

While the percentage of the boundary tissue occupied by myoid cells (Fig. 3) was less in older men, the difference was not significant in 10 men randomly selected from each group (Table 1). Similarly, the volume of the average individual myoid cell from the two groups did not differ significantly (Table 1).

The volume density of the seminiferous epithelium was reduced ($P < 0.01$) in the older group of men (Table 1). Coupled with reduced parenchymal weight in the older men, the total volume of the seminiferous epithelium per man was significantly reduced. Associated with the age-related reduction in seminiferous epithelial volume, the numbers of spermatids with round nuclei and Sertoli cells per gram of parenchyma and per testis also were reduced ($P < 0.01$). Daily sperm production per gram of parenchyma and per testis was significantly ($P < 0.01$) lower in the group of older men with reduced seminiferous epithelial volume.

Discussion

Our finding of increased boundary tissue thickness with advancing age (Fig. 1; Table 1) confirms previous observations (Bishop, 1970). Likewise,

the thinning of the spermatogenic epithelium reported by Bishop (1970) was comparable to the reduced ($P < 0.01$) seminiferous epithelial volume density and volume per man seen in our study. Our study, however, did not reveal an age-related reduction in the diameter of the seminiferous tubules (Table 1) or progressive intertubular fibrosis. Age was found to be significantly correlated with tubular boundary tissue thickness (Fig. 2) and the volume density of boundary tissue ($r = 0.47$; $P < 0.01$). In addition, boundary tissue thickness was correlated negatively with daily sperm production ($r = -0.53$, $P < 0.01$).

The increased thickness of tubular boundary tissue in older men could result from: 1) increased thickness of the basement membrane, 2) increased number of myoid cells, 3) increased size of individual myoid cells, 4) increased extracellular components between myoid cells, 5) a greater reduction in tubule length than in the number of myoid cells, or 6) a combination of these factors. The failure to detect differences in the basement membrane thickness (Table 1) obviated the first possibility. Likewise, the fact that the number of myoid cells tended to decline with age eliminated the second possibility. While the size of individual myoid cells was slightly greater in the older men, the difference was not statistically significant, which eliminated the third possibility. Although the extracellular component of the boundary tissue (Fig. 3) was slightly greater in older men (Table 1), this difference also was not statistically significant, negating the fourth possibility.

Our findings, which are based on a quantitative analysis, differ from what might be inferred from qualitative studies of human seminiferous tubules (Bustos-Obregon and Holstein, 1973; de Kretser et al, 1975). In these earlier studies, collagen fibers and other extracellular components of the boundary tissue were found to be increased in tubules from normal or abnormal testes with thickened boundary tissue. Likewise, our finding of no age-related increase in total boundary tissue volume per man was based on whole testes and differs from earlier studies of testicular biopsies concluding that thicker boundary tissue in older men resulted from deposition of new layers of connective tissue (Engle, 1942; Molnar, 1965).

The single factor that contributed most toward increased boundary tissue thickness was the disproportionate reduction (24%) in seminiferous tubular length compared to the reduction (10 to 20%) in the number of myoid cells (Table 1). This increased the

number of myoid cells around the remaining seminiferous epithelium. While the resulting increased stacking of myoid cells may not be immediately evident in a given view of seminiferous tubules (Figs. 1 and 3), it was detected as a larger number of myoid cells per cross section of tubule (Table 1). The increased number of myoid cells per cross section was not due to differences in size (diameter or volume) in the average individual myoid cell nucleus.

Using the method based on the percentage myoid cell nuclei and the parenchymal volume, values for the number of myoid cells per man were higher when compared with values from the method based on the number of myoid cells per tubule cross section and the tubule diameter and length. As suggested in Materials and Methods, the absence of a stereologic correction factor for the percentage of myoid cell nuclei could cause a slight overestimation of the values obtained by the former method. The use of histologic sections thinner than 1μ would tend to underestimate the values obtained by the latter method.

The volume of boundary tissue per man and the volume of the average individual myoid cell were similar ($P > 0.05$) in the two age groups (Table 1). While the volume of myoid cells per man tended to be

reduced in the older group of men, the difference was not significant ($P > 0.05$). Although the two methods

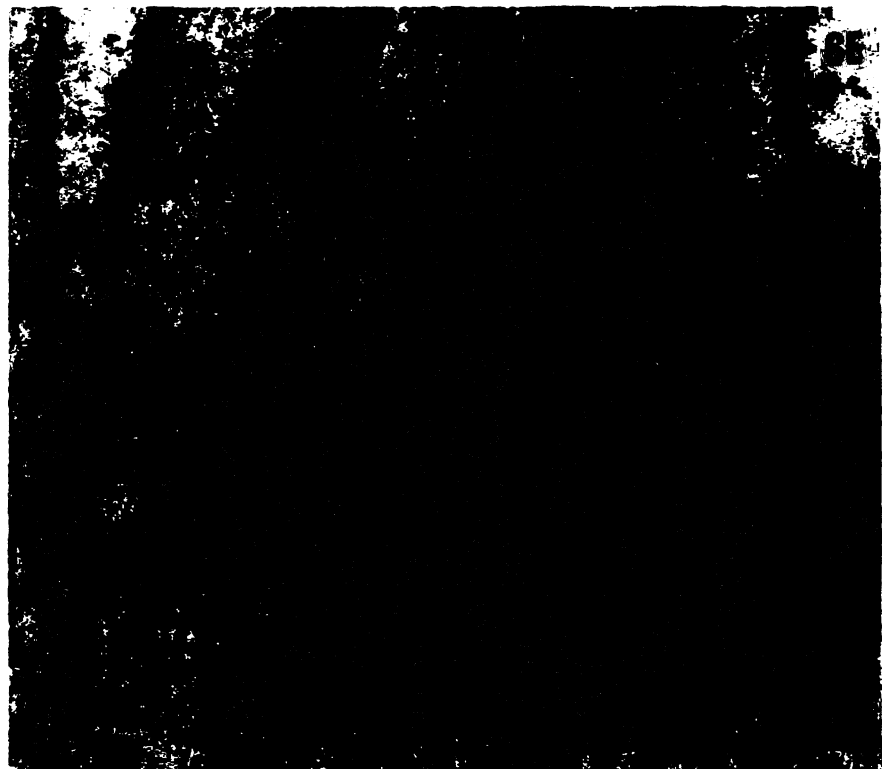
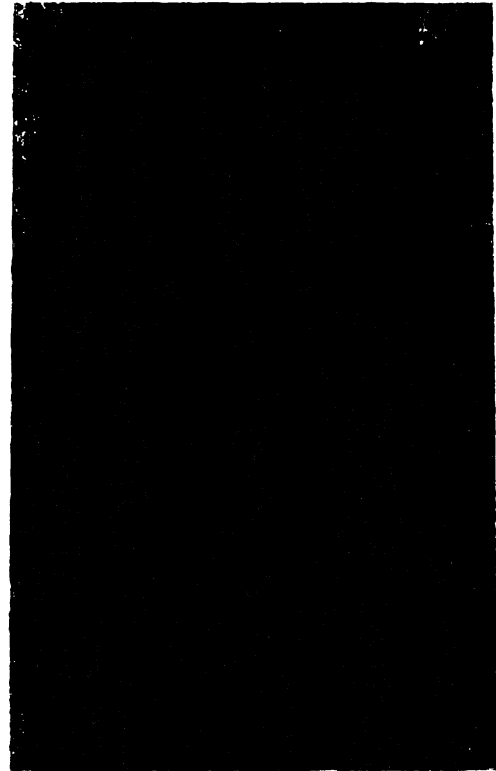


Fig. 3. Electron micrograph of boundary tissue from a man in the a. young group and b. older group. The boundary tissue is composed of a non-cellular (NC) component and a cellular component of myoid cells. The nucleus (N) of one myoid cell is labeled in each specimen. The basal lamina (BL), the innermost part of the basement membrane adjacent to the seminiferous epithelium (SE), is similar in thickness in both groups. While the boundary tissue is thicker in older men, the composition of the boundary tissue is similar. Bar length equals $1 \mu\text{m}$ (uranyl acetate + lead citrate stain; $\times 4700$).

used in this study showed smaller numbers of myoid cells in older men (reductions of 20% and 10%, see Table 1), only the volume density method revealed a statistically significant change. Hence, numbers of myoid cells in older men do not decrease enough to offset the thickening of the boundary tissue caused by age-related reductions in the length of the seminiferous tubule.

Reduction in volume density and volume per man of the seminiferous epithelium with increasing age corresponds to the reduction in the numbers of both Sertoli cells and the germ cells composing the seminiferous epithelium (Table 1). The volume of seminiferous epithelium per man was correlated to daily sperm production per man ($r = 0.66$; $P < 0.01$) and the number of Sertoli cells per man ($r = 0.67$; $P < 0.01$). Presumably, the loss of these two cell populations brings about the reduction in seminiferous epithelial volume. Furthermore, thickening of the boundary tissue conceivably could reduce the flow of metabolites to the seminiferous epithelium and further reduce its sperm production potential.

This study shows that age-related thickening of the boundary tissue of seminiferous tubules in the apparently normal testis results from a reduction in the length of seminiferous tubules instead of an increase in the boundary tissue itself. Indeed, the volume of boundary tissue per man was almost identical in the two age groups (Table 1).

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