Age-Related Histologic Changes in the Adult Mouse Testis

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Summary. Age-related changes in the testis were studied histologically in dd-mice from 2 months to 2 years of age. After 6 months of age, vacuoles appeared first singly and later became clustered in the seminiferous epithelium. With the appearance of the vacuoles, the epithelium started to release spermatids and spermatocytes into the lumen. Multinucleated giant cells occasionally appeared in the epithelium and were also released to the lumen. The epithelium, when markedly depleted of germ cells, was composed mainly of Sertoli cells. The lumen of the atrophied tubules occasionally contained accumulations of macrophages, sperm aggregations, and nodules of a homogeneous material covered with flattened epithelial cells. The basement membrane surrounding the atrophied tubules thickened corresponding to the degree of atrophy. The atrophied tubules initially appeared in patches and then spread throughout the testis. Leydig cells increased in amounts with age. The increase of Leydig cells was distinct around severely atrophied tubules with a thickened basement membrane. These changes are discussed in comparison with autoimmunized testes which show similar histologic changes.

Regressive histologic changes in seminiferous tubules occur spontaneously, and develop with aging. Such changes have been reported in men, rats, oxen, mice, and cats, but their causes remain unclear (LÜTZEN and UEBERBERG, 1973; HUMPHREY and LADDS, 1975; HATAKEYAMA et al., 1979; GOSDEN et al., 1982; ELCOCK and SCHONING, 1984). We have noticed that aged mice show testicular degeneration similar to that induced by irradiation, artificial cryptorchidism, and experimental autoimmunization in laboratory animals (NEBEL and MURPHY, 1960; SATO et al., 1981; JÉGOU et al., 1983). Regressive testicular changes, such as those in aged men and animals, also occur spontaneously in young men and can result in male infertility, although the cause remains unknown (WONG and STRAUS II, 1973). Though it would seem that examination of age-related testicular regression may throw light on our understanding of how aging effects the testes and male infertility, no detailed report on histologic changes in the testes in aged animals has been available. In the present study we observe the testes from young to old mice by light microscopy, and describe the onset and development of age-related regressive changes.
MATERIALS AND METHODS

The testis and epididymis were removed from 48 male dd-mice at 2 months to 2 years of age; the testis was weighed. The organs were fixed in Bouin's solution for 3-4 hrs, dehydrated, embedded in paraffin, longitudinally cut into serial sections of 6 μm in thickness, stained with periodic acid-Schiff (PAS) and hematoxylin, and observed by light microscopy. Some sections were stained with von Kossa's silver to detect calcium deposits.

Quantitative measurements

Frequencies of the atrophied tubules and the tubules showing the mature spermatogenetic phase: In all profiles of the tubules in one section of each testis, a count was made of the frequencies (%) of the tubular profiles with vacuoles occupying about one third or more of the epithelium and the tubular profiles lined by spermatozoa along the surface of the epithelium.

Thickness of the basement membrane around the seminiferous tubules: The sections were enlarged to 2,500 times using a light microscopic projector. The thickness of the basement membrane surrounding the seminiferous tubules was measured on the screen.

Amount of Leydig cells: The amount of Leydig cells was calculated from the total weight of the testis and the volume proportion of Leydig cells in the testis. The volume proportion of Leydig cells was obtained by a point-counting method, as follows. With a light microscopic projector, sections of the testis were enlarged to 50 times on a screen with a regular point-lattice at 200 μm intervals. The numbers of points included in the testis and Leydig cells were counted. The volume proportion of Leydig cells occupying the testis was then calculated.

Fig. 1. Testicular weight at various ages. Bars represent means.
RESULTS

Testicular weight ranged from 70 to 120 mg in young adult mice of 2 to 6 months of age, and was from 30 to 95 mg in aged mice of 17 months to 2 years (Fig. 1). The average weight was about 100 mg in the young adult mice, decreasing gradually after 6 months to about half in the fully matured mice of 17 months to 2 years (Fig. 1).

Fig. 2. Mouse testes showing age-related regressive changes in the seminiferous tubules. PAS-hematoxylin. ×50. a. Testis at 4 months of age. All seminiferous tubules appear normal. b. Testis at 9.5 months of age. Small numbers of seminiferous tubules show some atrophy (asterisks). c. Testis at 17 months of age. All tubules are extensively atrophied. The interstitium between the tubules is wide and filled with Leydig cells.
Seminiferous tubules

In the young adult mice, the testis was filled with seminiferous tubules lined with Sertoli cells and 5 or 6 layers of germ cells consisting of spermatogonia, spermatocytes, spermatids, and spermatozoa (Fig. 2a, 4a).

Histologic changes in the testis appeared after 6 months and developed with age (Fig. 2-5). Initially, the appearance of single vacuoles was noticed in the middle layer of the seminiferous epithelium (Fig. 4a). The vacuoles were 5-30 \( \mu \)m in diameter. They increased in number and became clustered in the epithelium with age (Fig. 2–4).

With the appearance of the vacuoles, regressive changes in the germ cells began to occur (Fig. 4). The epithelium released spermatids and spermatocytes in the pachytene phase into the lumen. Multinucleated giant cells containing several round nuclei of spermatids occasionally appeared in the epithelium and were also released to the lumen (Fig. 4b). Those tubules with marked depletion of the germ cells displayed an epithelium composed almost only of Sertoli cells (Fig. 4d).

Tubules showing the regressive changes initially appeared in patches among the tubules of normal appearance (Fig. 2b), and gradually spread throughout the testis (Fig. 2c). A testis containing a large number of atrophied tubules with distinctive regressive changes was frequent in the aged mice (Fig. 6). The testicular weight was less in organs with more frequent atrophic tubules (Fig. 7). The tubules showing the mature spermatogenetic phase were generally 15–40% in the testis in young mice, while in the

Fig. 3. Testis at 2 years of age. PAS-hematoxylin. \( \times \)100. The tubules on the right side appear normal, but those on the left side are severely atrophied. The basement membrane around the atrophied tubules (double arrows) is thicker than that around the normal tubules (single arrows). The interstitium around the atrophied tubules is wide and filled with Leydig cells.
Age-Related Changes in the Adult Mouse Testis

Organ after 6 months of age, the frequency was occasionally under 15%.

The tubules decreased in outer diameter as the atrophy became prominent (Fig. 2-4). The diameter was about 200 μm in the normal tubules packed densely with germ cells and about 120 μm in the most severely atrophied tubules. The thickness of the basement membrane surrounding the atrophied tubules remained unchanged before 1 year of age but thickened according to the degree of the atrophy thereafter (Fig. 3, 4, 8).

The atrophied tubules often included irregularly shaped nodules of a homogeneous material containing several macrophages with PAS-positive cytoplasm (Fig. 5a). The nodules bulged from the basement membrane into the lumen, obstructing it; they were covered by flattened epithelial cells (Fig. 5a). Similar nodules also appeared in the rete testis and efferent duct; these contained a few cells with flattened nuclei (Fig. 5b). The nodules appeared in about 15% of the testes in mice at 6 months to 1 year of age and about 40% of the testes after 1 year of age.

The testis with these nodules generally revealed intratubular sperm aggregations (Fig. 5c). The aggregates were stained strongly with PAS (Fig. 5c) and with von Kossa’s silver to prove calcification. The epithelium of the tubules containing the sperm aggregates was flattened or fragmented (Fig. 5c). These aggregates appeared in about 20% of the testes of mice between 6 months and 1 year of age and about 55% of the testes after 1 year of age.

Fig. 4. Seminiferous tubules showing various degrees of atrophy. PAS-hematoxylin. ×260. [Images a-d]

a. Normal tubule at 6.5 months of age. A single vacuole is seen in the epithelium (arrow).
b. Atrophied tubule at 9.5 months of age. Multinucleated giant cells are seen in the seminiferous tubules (arrows). Many vacuoles are visible in the epithelium.
c. Atrophied tubule at 15 months of age. The appearance of vacuoles and a decrease of germ cells are noticed in the epithelium.
d. Severely atrophied seminiferous tubule at 17 months of age. The tubule is lined only by the vacuolated Sertoli cells.
The lumen of the atrophied tubules occasionally contained macrophages, 10–30 μm in diameter, ingesting spermatozoa and degenerating spermatids (Fig. 5d). The lumen of the rete testis also contained accumulations of such macrophages (Fig. 5e). The macrophages appeared in about 35% of the testes at 6 months to 1 year of age and about 65% of the testes after 1 year of age.

Fig. 5. Abnormal seminiferous tubules and excurrent ducts in aged mice. PAS-hematoxylin. 

a. Seminiferous tubule with a nodule of homogeneous material containing several macrophages. The nodule is covered with flattened epithelial cells. The epithelium possesses no germ cells. ×300. 
b. Efferent ducts with nodules, bulging into the lumen from the basement membrane and being covered with flattened epithelial cells. ×300. 
c. Seminiferous tubules filled with sperm aggregations. ×150. 
d. Atrophied tubules filled with macrophages containing spermatozoa. ×300. 
e. Duct of the rete testis containing an accumulation of macrophages ingesting spermatozoa. ×300. 
f. Arteriole (center) showing an abnormally thick wall due to a deposit of PAS-positive and fibrous material under the endothelium. ×250.
Fig. 6. Frequency of the atrophied tubules in each testis at various ages. The testes containing no atrophied tubules are shown in a belt under the horizontal 0 line.

Fig. 7. Correlations between the frequency of the atrophied tubules and testicular weight.

Fig. 8. Thickness of the basement membrane around the seminiferous tubules at 2 months (2M) and 17 months (17Y5M) of age. The numbers, 1, 2 and 3 on the horizontal axis express normal tubules, moderately atrophied tubules with moderately decreased germ cells, and severely atrophied tubules consisting mainly of Sertoli cells. Circles represent the data from the testes consisting of similar tubules, and dots, from the testes containing various tubules. The dots for the values from each testis are connected by a line.
In mice 2–4 months old, the testes filled with tubules of normal appearance left a narrow interstitium, containing Leydig cells in small groups (Fig. 2a, 4a). The interstitial space with Leydig cells tended to widen with age (Fig. 2, 4). Though the amount of Leydig cells increased, their average size did not change with aging (Fig. 9). The Leydig cells in particular showed accumulations in the widened interstitium around atrophied tubules with a thickened basement membrane after 1 year of age (Fig. 3).

A few arterioles showing abnormal walls were found in the testis with severely atrophied seminiferous tubules after 1 year of age (Fig. 5f). The walls of these arterioles were thickened by a strongly PAS-positive and fibrous material deposited between the endothelium and the smooth muscle layer (Fig. 5f).

**Epididymis in aged mice**

Spermatozoa in the epididymal duct disappeared in mice with testes showing remarkable atrophy. Few or no spermatozoa were seen in the epididymal duct in mice with testes in which the atrophied tubules comprised 35 to 90% of all tubular profiles and the tubules showing the mature spermatogenetic phase made up 15% or less. The principal cells in the body of the epididymis lacking in spermatozoa had PAS-positive inclusions as described in our previous papers (ABE et al., 1982, 1983).

**DISCUSSION**

The mice used in this study are sexually matured at 1.5 months of age (TAKANO, 1980). Testicular regression was spontaneously produced after 6 months and became prominent about 1.5 years after birth. The changes appeared to occur and develop in relation to aging.
The appearance of vacuoles in the seminiferous epithelium was the first notable change. A similar change could be induced by irradiation, artificial cryptorchidism, and experimental autoimmunization in animals (Nebel and Murphy, 1960; Sato et al., 1981; Jegou et al., 1983). Although we could not determine under the light microscope whether or not the vacuoles were located in the Sertoli cells, electron microscopy of the autoimmunized testis has demonstrated that the vacuoles originate from the endoplasmic reticulum (Kierszenbaum and Mancini, 1973). With the appearance of the vacuoles, the seminiferous epithelium undergoes a disappearance of germ cells. The order of this germ cell disappearance is different according to the causes of the testicular regression; germ cells disappeared from spermatozoa in the testes in aged, immunized, and cryptorchid mice (Smith, 1919; Freund et al., 1953; Payne, 1956), and from spermatogonia and early spermatocytes in irradiated testes (Craig et al., 1961; Abe et al., 1983); however the vacuoles are similarly produced in these testes (Freund et al., 1953; Nebel and Murphy, 1960). Vacuolization in the aged mice is considered to be a common change in the seminiferous epithelium with the damaging of germ cells.

Atrophied seminiferous tubules showing profiles with a depletion of germ cells and vacuolization appeared in patches in the testis. The uneven distribution of the atrophied tubules in the testis suggests that the cause of the atrophy might have acted on the testis locally but not generally. Sasano and Ichijo (1969) gave especial attention to the changes in the blood supply as the cause of senile changes in the human testis; they noticed that the atrophy of the seminiferous tubules was more advanced around the distal part of the arterial supply, demonstrated microangiographically. In the present study we found abnormal arterioles which may cause focal reduction of the blood supply to promote the aging process of the testis (Suoranta, 1971), but they were too rare to be taken as the cause for seminiferous tubule degeneration.

We observed that, with advancing age, spermatids and spermatocytes were released to the lumen. The germ cells have been believed to be able to differentiate through the support of the Sertoli cells (see review by Fawcett, 1986). The Sertoli cells never show cell-division after puberty (see review by Fawcett, 1986), and the aged Sertoli cells may be functionally depleted enough (see review by Bishop, 1970) to allow the release of developing germ cells. In addition, the Sertoli cells form the blood-testis barrier which isolates the germ cells with an antigenicity from the environment around the seminiferous tubules (Dym and Fawcett, 1970). The age-related deterioration of the Sertoli cells may cause a breakage in the blood-testis barrier, resulting in the production of antibodies to the germ cells and induction of an autoimmune reaction. In fact, Gosden et al. (1982) observed the penetration of lanthanum into seminiferous tubules in mice aged 20–23 months. This indicates such a breakage in the blood-testis barrier.

The breakage of the blood-testis barrier has been suggested also in the experimental allergic orchitis formed by injections of the testis homogenate with adjuvants (Johnson, 1970). Johnson (1970) found that acriflavine permeability into the tubules increased in immunized testes, and postulated that the testicular damage was associated with an injury of the barrier which then induced the autoimmune response for germ cells. Histologic changes in the testes in aged mice developed similarly to those in the testes with allergic orchitis (Brown et al., 1963; Yantorno et al., 1971). It is possible that an autoimmune reaction develops in the testes of aged mice.

The experimental allergic orchitis showed infiltrations of lymphocytes, monocytes, and polymorphs in the testis (Sato et al., 1981). In the present study, though the
infiltrations of lymphocytes and plasma cells were not clear, macrophages ingesting dead spermatozoa appeared in the damaged tubules and in the rete testis. The rete testis is known to be the weakest portion of the blood-testis barrier; macrophages were frequent in this portion (JOHNSON, 1972; KORMANO and REIJONEN, 1976). This suggests that the macrophages arose in reaction to the breakage of the blood-testis barrier.

The rete testis and atrophied tubules occasionally possessed nodules of homogeneous material, and the markedly atrophied seminiferous tubules in aged mice were accompanied by a thickened basement membrane. The tubules of the Sertoli cell-only syndrome, i.e., those composed mainly of the Sertoli cells as seen in markedly atrophied tubules (WONG and STRAUS II, 1973; SHERINS and HOWARDS, 1978), are also surrounded by a thick basement membrane (BUSTOS-OBREGÓN and HOLSTEIN, 1973). The thickening of the basement membrane is known in renal glomerulonephritis to be induced by an autoimmune mechanism, showing deposits of antigen-antibody complexes (MARTINEZ-HERNANDEZ and AMENTA, 1983). Whether the thickening of the basement membrane and the nodules of the homogeneous material at the basement membrane in the testes of aged mice occur as deposits of antigen-antibody complexes remains a question.

Along with the above mentioned changes in seminiferous tubules, Leydig cells changed. The total volume of the cells increased with aging, but their average size showed no alteration. This indicates that the number of Leydig cells increased with aging. The increase of Leydig cells has been observed in the testes of aged men, rats, cats, and oxen, and has been considered to be a compensation for the low productivity of testosterone in each Leydig cell in the aged testis (LÜTZEN and UEBERBERG, 1973; KOTHARI and GUPTA, 1974; HUMPHREY and LADDS, 1975; HONORÉ, 1978; PIRKE et al., 1978; ELCOCK and SCHONING, 1984). However, Leydig cells exhibit hyperplasia, particularly around the atrophied seminiferous tubules in the testes in aged men (HONORÉ, 1978) and in experimentally damaged testes (WAKSMAN, 1959; KERR et al., 1979; SATO et al., 1981). AOKI and FAWCETT (1978) believed that the atrophied seminiferous tubules radiate some diffusable agents which influence Leydig cells to proliferate. We noticed that Leydig cells became numerous only around the atrophied tubules in mice after 1 year of age. The aged Sertoli cells seem to effect proliferation of the Leydig cells.

In the present study, we examined age-related histologic changes in the testes of adult mice. It has been implied that an autoimmune reaction plays a primary role in age-related changes in the testis, though immunological examinations are needed to conclude this. Further studies of the testicular regression which spontaneously appeared in the dd-mice may also serve for the understanding of the Sertoli cell-only syndrome, one of the causes of male infertility.

REFERENCES


