

## HISTOLOGICAL DIVISION OF MOUSE EPIDIDYMIS BASED ON REGIONAL DIFFERENCES

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**Abstract** We divided the mouse epididymis into 5 segments (Segments I-V) based on regional differences in histology to obtain a fundamental division that reflects the functions of this organ. Segments I-III are contained in the caput epididymidis, Segment IV in the corpus epididymidis, and Segment V in the cauda epididymidis. For this classification, we observed morphological features of the epididymal duct in adult mice by light and electron microscopy, and examined postnatal development of the epididymal duct qualitatively and quantitatively. We also performed experimental studies to examine the effects of ligation/cutting of the efferent ducts, epididymal duct ligation at the border between Segments III-IV, testis irradiation, cryptorchidism, and estrogen administration on epididymis histology. In these studies, we found that the epididymal duct in each segment responds as a functional unit when the epididymis is subjected to pathological conditions. In conclusion, the present histological division is useful as a basic division for studies on the mouse epididymis.

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**Key words:** epididymis; mouse; histology; division; regional difference

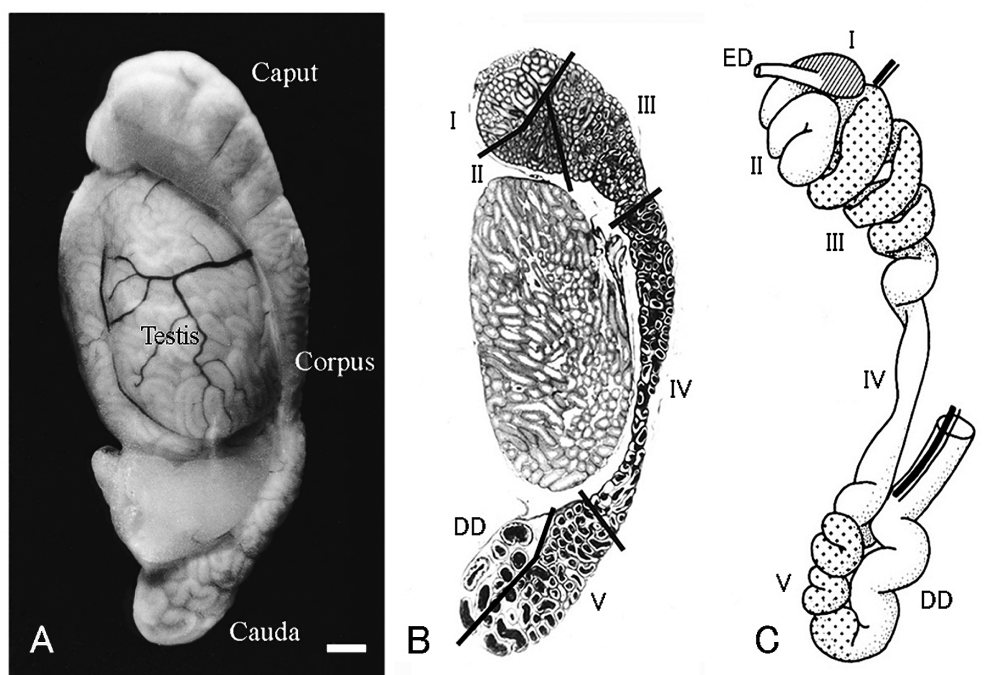
### Introduction

The epididymis is involved in sperm maturation and storage. The functions of this organ are regulated by sex hormones<sup>1-4</sup>. Schleicher et al. examined autoradiography in mice injected with <sup>3</sup>H-DHT/<sup>3</sup>H-estradiol, and found that distribution of these isotopes shows regional differences in the epididymis<sup>5</sup>. Spermatozoa undergo molecular modification while passing through the duct, acquiring fusibility with oolemma and motility<sup>6</sup>. Protein molecules involved in sperm maturation are secreted by epithelial cells, and thus the morphological features of these epithelial cells are important for elucidating the mechanisms of sperm maturation. We divided the mouse epididymis into 5 segments (Segments I-V) based on regional differences in histology<sup>7</sup>. Recently, numerous papers dealing with localization of gene-expressing proteins in the mouse epididymis have been published<sup>8</sup>. In these papers, most

authors use general terms when describing the localization of proteins; the initial segment, caput epididymidis, corpus epididymidis, and cauda epididymidis. However, these terms have created misunderstanding, as cross sections of the epididymis show various profiles depending on the cut position.

Johnston et al. investigated more than 2000 genes that are selectively and specifically expressed in the mouse epididymis and determined 6 different transcriptional units<sup>9</sup>. These units correspond to our Segments I-V, thus suggesting the validity of our division. As our early papers about the histological division of the mouse epididymis were written in Japanese, in this review, we will introduce our early studies, showing the evidence for our division, and describe our experimental studies.

This review will deal with the following subjects: 1) Histological division of the mouse epididymis and characteristic features of each



**Figure 1** Mouse testis and epididymis A, Lateral view of the left testis and epididymis of an adult mouse. Bar is 1 mm. Caput, caput epididymidis; Corpus, corpus epididymidis; Cauda, cauda epididymidis. B, Longitudinal section of the testis and epididymis stained with PAS. The borders between Segments I-V (I-V) are indicated by lines. Bouin fixation, Paraffin section, PAS-hematoxylin. C, Schematic diagram of the lateral view of the epididymis without capsule. ED, efferent ducts; I-V, Segments I-V; DD, deferent duct.

segment; 2) Postnatal development of the epididymis; 3) Epididymis after efferent duct ligation/cutting; 4) Effects of testis irradiation or cryptorchidism, 5) Epididymis after epididymal duct ligation at the border of Segments III and IV; and 6) Effects of estrogen administration on epididymis histology.

### **Histological division of mouse epididymis and characteristic features of each segment**

The epididymis is macroscopically divided into the caput epididymidis, corpus epididymidis and cauda epididymidis (Figure 1A).

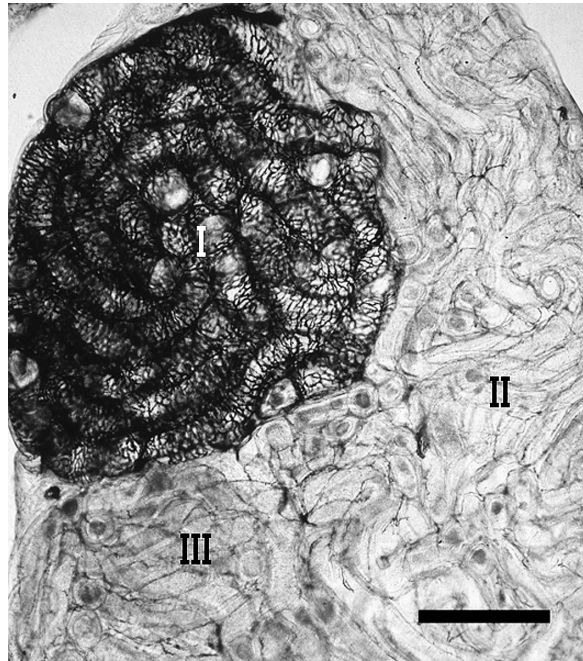
However, we divided the mouse epididymis into 5 segments (Segments I-V) based on regional differences in duct diameter, luminal diameter, epithelial height, and PAS-stainability of the epithelial principal cells (Figure 1B)<sup>7</sup>. Segments

I-III are contained in the caput epididymidis, Segment IV in the corpus epididymidis, and Segment V in the cauda epididymidis. The epididymis appears to be partitioned by connective tissue septa; however, Segments I-V are continuously settled in a connective tissue cylinder that spirals and forms the caput, corpus, and cauda epididymidis (Figure 1C). Segment I appears as a reddened area because a dense network of capillaries surrounds the duct (Figure 2)

The light microscopic features of the epididymal duct and ultrastructure of the principal cells in Segments I-V are described below (Figures. 3, 4)<sup>7,10</sup>.

### **Segment I**

This segment is characterized by a high and pale epithelium. The epididymal lumen is



**Figure 2** Thick section of caput epididymidis after India ink perfusion. Epididymal duct in Segment I (I) is surrounded by a dense capillary network. Capillaries in Segments II and III (II and III) form coarse networks. Epon embedded. Bar is 500  $\mu\text{m}$ .

empty, except in the distal part where it contains spermatozoa and PAS-positive materials<sup>11)</sup>. Long stereocilia are seen on the luminal surface of the principal cells. The apical cytoplasm of the cells is stained with PAS to detect carbohydrates in the distal part but not in the proximal part of this segment. The ellipsoidal nucleus is situated basally. Large Golgi apparatus is present above the nucleus. Small PAS-positive granules are scattered in the supranuclear cytoplasm. Infranuclear cytoplasm is relatively wide and stained with hematoxylin. Ultrastructurally, the principal cells have small vesicular smooth endoplasmic reticulum distributed densely throughout the cytoplasm. Dense bodies are scattered in the supranuclear cytoplasm. Lamellar rough endoplasmic reticulum forms a fingerprint pattern occupying a large area of the infranuclear cytoplasm.

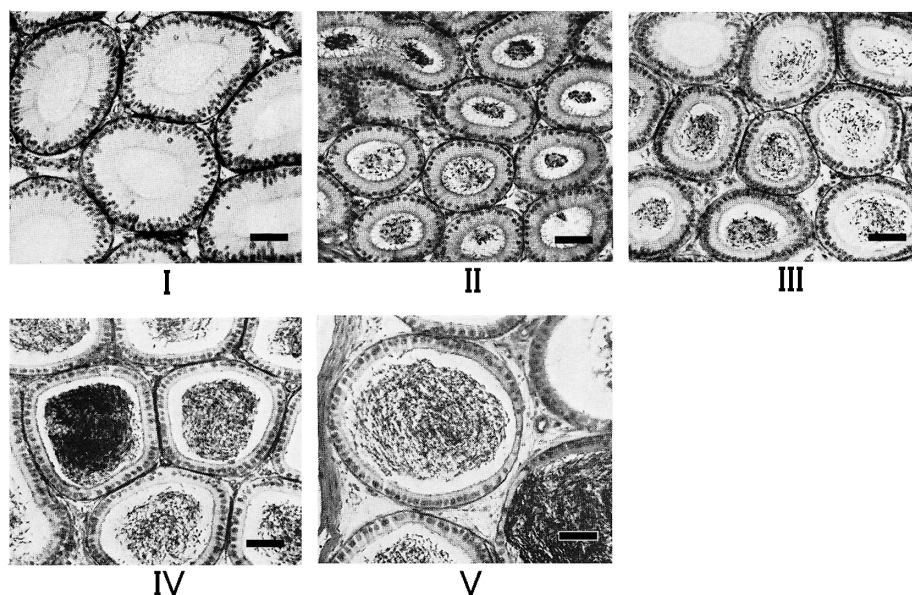
Noteworthy is the presence of a dense network of fenestrated capillaries situated just

under the basal membrane<sup>12)</sup>.

Segment I has long been described as the initial segment<sup>13)</sup>. The main function of the initial segment is testicular fluid absorption<sup>14)</sup>. The presence of fenestrated capillaries suggests highly active epithelium in this segment.

### Segment II

The duct is thinner and the epithelium is lower than in Segment I. A few spermatozoa and PAS-positive materials are typically seen in the epididymal lumen. Stereocilia and apical cytoplasm in the principal cells are strongly stained with PAS. Ellipsoidal nuclei are situated basally in columnar cells. Supranuclear cytoplasm containing large Golgi apparatus is moderately stained with PAS. Prominent vacuoles are observed in the supranuclear cytoplasm. Ultrastructurally, the principal cells contain large multivesicular bodies scattered in the supranuclear cytoplasm. Vesicular



**Figure 3** Photomicrographs of epididymis. I-V, Segments I-V. Bar is 50  $\mu$ m. Bouin fixation, paraffin section, PAS-hematoxylin.

rough endoplasmic reticulum studded with a few ribosomes is distributed throughout the cytoplasm.

This combination of findings, that is, strong PAS-stainability in stereocilia and apical cytoplasm of the principal cells, and luminal PAS-positive materials, suggests that PAS-positive materials are secreted by the principal cells into the lumen in Segment II and attach to the spermatozoa. These PAS-positive materials were found to be glycoprotein, as they were stained with bromphenol blue<sup>11</sup>.

### Segment III

The epididymal lumen is wider and contains more spermatozoa with PAS-positive materials than in Segment II. The principal cells lack PAS-stainability in stereocilia and vacuoles typically seen in Segment II. The Golgi apparatus is only weakly stained with PAS. Ultrastructurally, the principal cells contain tubular rough endoplasmic reticulum densely distributed throughout the cytoplasm. Small vesicular granules are seen in the infranuclear cytoplasm.

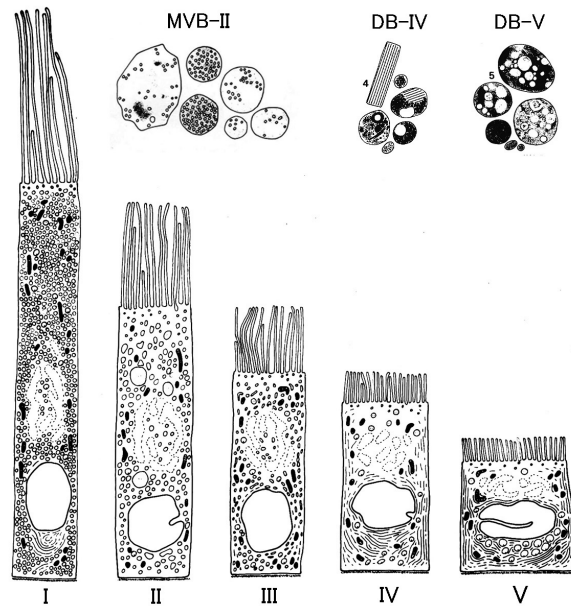
As the principal cells in Segment III show different PAS-stainability from those in Segment II, they are considered to secrete different types of glycoprotein into the lumen from those in Segment II. For this reason, Segment III should be distinguished from Segment II.

### Segment IV

The epididymal lumen increases in size and contains numerous spermatozoa with strongly PAS-positive materials. The principal cells are low columnar, having brush border-like microvilli. Round or slightly flat nuclei are situated basally. Two nuclei are frequently overlapped vertically in cells<sup>15</sup>. Large Golgi apparatus is present in the supranuclear cytoplasm. PAS-positive granules are scattered in the infranuclear cytoplasm. Ultrastructurally, the principal cells have tubule-containing inclusions<sup>16</sup> and lamellar rough endoplasmic reticulum in the middle and basal cytoplasm.

Ratkin et al. reported that AEG homologs are expressed in the Golgi apparatus of the principal cells and in the lumen in the corpus





**Figure 4** Diagram showing ultrastructure of principal cells of epididymal duct. Golgi apparatus is enclosed by dotted lines. The Golgi area is located above the nucleus, and the cytoplasm is filled with well developed organelles. I-V, Segments I-V. MVB-II, Large multivesicular bodies (left) are seen in Segment II. DB-IV, Tubule-containing dense bodies are seen in Segment IV. DB-V, Large dense bodies with foamy appearance are seen around nuclei in Segment V.

epididymidis in the mouse<sup>17</sup>). AEG is an acidic epididymal glycoprotein involved in fusibility with oolemma<sup>18,19</sup>).

### Segment V

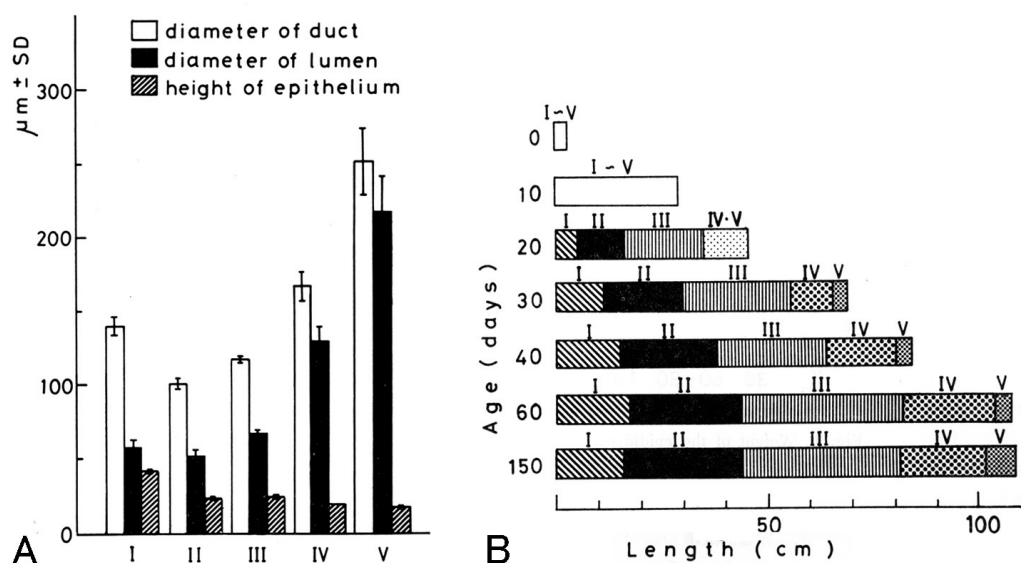
The epididymal lumen is extremely wide containing numerous spermatozoa and strongly PAS-positive materials. The principal cells are low columnar or cuboidal, having brush border-like microvilli. Slightly flat nuclei are situated basally. Large nuclei with a deep invagination of the nuclear membrane are frequently observed after 120 days of age<sup>15</sup>). Large PAS-positive granules surround the nuclei. A Golgi apparatus is situated in the supranuclear cytoplasm. Ultrastructurally, large dense bodies with foamy appearance are seen around nuclei. Lamellar rough endoplasmic reticulum is distributed in the middle and basal cytoplasm. In addition to

the features described above, clusters of clear cells are seen in the epithelium in the proximal part of Segment V and compact layers of smooth muscles surround the duct in the distal part of Segment V.

As the principal cells in Segment V contain numerous large dense bodies (lysosomes), one of their functions is to absorb and degrade luminal materials. It is also known that clear cells absorb luminal materials<sup>20</sup>). Thick layers of smooth muscle surrounding the epididymal duct function during ejaculation.

### Quantitative measurement of the duct

We performed quantitative studies of the epididymal duct in adult mice<sup>7</sup>). Ductal and luminal diameters, and epithelial height in each segment are shown in Figure 5A. Marked regional differences were seen.



**Figure 5** Measurement of epididymal duct. A, Diameters of the duct and lumen, and height of the epithelium of the epididymal duct in each segment. I-V, Segments I-V. Bars represent standard deviations. B, Total and regional lengths of the epididymal duct at each age.

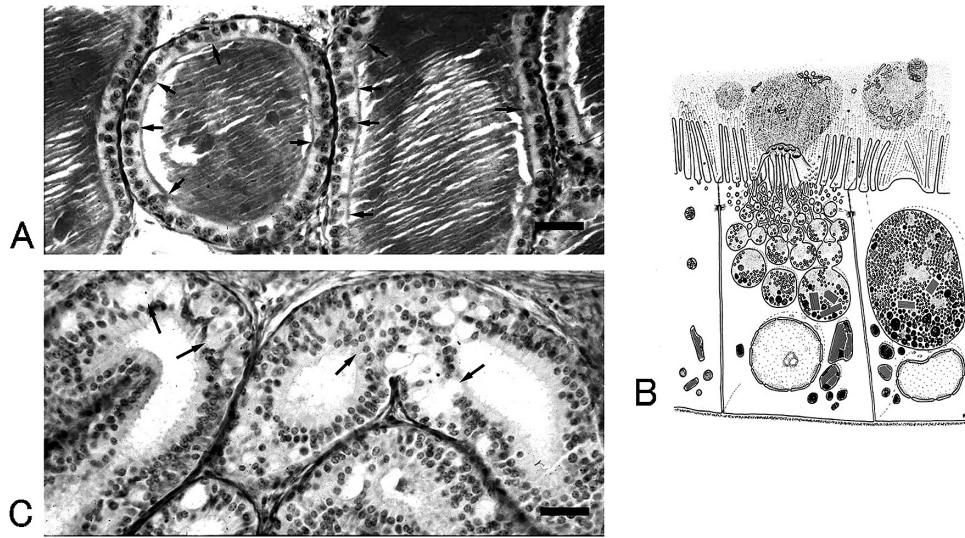
Luminal volume was obtained by the point-counting methods. The length of the duct was calculated from the ductal volume and the average value of the ductal diameter in each segment. The proportions of ductal length were 13% in Segment I, 28% in Segment II, 36% in Segment III, 18% in Segment IV, and 4% in Segment V. The proportions of luminal volume were 7% in Segment I, 10% in Segment II, 22% in Segment III, 38% in Segment IV, and 24% in Segment V.

As Segment II has a long duct and small luminal volume, spermatozoa readily interact with secretory products from the epithelial cells. Segment V has a short duct and large luminal volume, which allows spermatozoa to remain in the lumen until ejaculation.

### Postnatal development of mouse epididymis

We qualitatively and quantitatively examined the postnatal development of the epididymis at 10-day intervals after birth<sup>7</sup>. The principal cells are undifferentiated at 10 days of age. They begin to differentiate in Segments I-III at 20 days of age and are completely differentiated in Segments I-V at 30 days of age. Spermatozoa appear in the lumen at 40 days of age. Total length of the duct increases from birth to 60 days of age, after which it remains constant (Figure 5B). Total volumes of the duct and lumen increase rapidly from 20 days and 30 days of age, respectively, until 60 days of age, after which they remain constant.

Differentiation of the principal cells in Segments I-III is followed by that in Segments IV and V. This suggests that the secretory products from Segments I-III induce differentiation of the principal cells in Segments IV-V. The results of this study indicate that the mouse epididymis is fully mature at 60 days of age.



**Figure 6** Epididymis in Segment IV. A, Photomicrograph of the epididymis after efferent duct ligation. PAS-positive inclusions appear in the principal cells (arrows). The lumen contains sufficient PAS-positive materials, but no spermatozoa. B, Schematic diagram of the principal cells containing PAS-positive inclusions. C, Photomicrograph of the epididymis after epididymal duct ligation at the border of Segments III-IV in juveniles. Peculiar pale, vacuolated cells are clustered in the epithelium (arrows). The lumen is narrow and contains no spermatozoa or PAS-positive materials. A, C, Bar is 50  $\mu$ m. Bouin-fixation, paraffin section, PAS-hematoxylin.

### Epididymis after efferent duct ligation/cutting

We examined the histological changes in the mouse epididymis after efferent duct ligation/cutting to determine the effects of spermatozoa and testicular fluids on epididymis histology<sup>21-24</sup>. Surgery was performed in adults (90 days of age) or juveniles (20-30 days of age). PAS stained longitudinal sections were prepared and observed under a light microscope at 4 weeks after surgery or at 60 days of age, respectively. The epididymis showed prominent changes in Segments I and IV. **Segment I:** The principal cells showed similar characteristics to those in Segment II after surgery in adults and did not differentiate after surgery in juveniles. Pores in fenestrated capillaries decreased in number after surgery in adults<sup>12</sup>.

These results suggest that differentiation and function of Segment I depends on the materials present in the testicular fluids, and that the principal cells in Segment I have the potential to function as those in Segment II; however this ability is suppressed by testicular fluids. Avram et al. demonstrated that a combination of dihydrotestosterone and testicular exocrine secretions are both related to the maintenance of the initial segment<sup>25</sup>.

**Segment IV:** PAS-positive inclusions appeared in the principal cells after surgery in adults or juveniles (Figure 6A).

The lumen contained sufficient PAS-positive materials, but no spermatozoa. Ultrastructure of the principal cells containing PAS-positive inclusions was examined at 1 week after surgery in adults. Accumulated multivesicular bodies were seen in the supranuclear cytoplasm, and

nipple-like protrusions were seen on the luminal surface with ductules opening to the lumen and multivesicular bodies (Figure 6B). The multivesicular bodies frequently formed giant multivesicular bodies up to  $10\mu\text{m}$  in diameter. The giant multivesicular bodies often contained bundles of fine cylindrical tubules (Figure 6B). Dense bodies containing the same bundles of cylindrical tubules as seen in the multivesicular bodies were also seen in the basal cytoplasm (Figure 6B)<sup>26)</sup>.

It is believed that the multivesicular bodies were formed as a result of luminal material absorption, and that they become fused and enlarged. It is probable that fine tubules may be formed by condensation or digestion of the ingested luminal material, as inclusions containing bundles of tubules are also found in the principal cells in Segment IV in normal mice<sup>10)</sup>.

### **Effects of testis irradiation or cryptorchidism on Segment IV**

We examined the chronological histological changes in mouse epididymis after testis irradiation or cryptorchidism to determine the response of the epididymal duct to the disappearance and reappearance of spermatozoa<sup>27,28)</sup>.

In Segment IV, PAS-positive inclusions appeared at 1 week after the disappearance of spermatozoa in the epididymal duct, and disappeared soon after the reappearance of spermatozoa.

The findings suggest that some PAS-positive material is bound to spermatozoa and, if not bound, is reabsorbed by the principal cells in Segment IV and deposited as intracellular inclusions. The principal cells in Segment IV are apparently capable of physiologically processing of the accumulated PAS-positive material.

### **Epididymis after epididymal duct ligation at the border of Segments III and IV**

We examined histological changes in the mouse epididymis after epididymal duct ligation at the border of Segments III-IV to determine the effects of secretory products from the proximal segments on Segments IV-V<sup>21,22,29,30)</sup>. The procedure was performed in adults (90 days of age) or juveniles (20-30 days of age). PAS-stained longitudinal sections were observed with a light microscope at 4 weeks after surgery or at 60 days of age, respectively.

Although the lumen dilates with PAS-positive materials in Segment III, the epithelial cells in Segments I-III showed their original characteristics. However, in Segment IV in treated in juveniles, the lumen was narrow and contained no spermatozoa or PAS-positive materials, and peculiar pale, vacuolated cells were clustered in the epithelium (Figure 6C). On the other hand, in treated in adults, few or no pale cells were seen in the epithelium in Segment IV.

These findings suggest that the differentiation of epithelial cells in Segment IV is dependent on the luminal contents entering from the proximal duct.

### **Effects of estrogen administration on epididymis histology**

We examined adult mice subcutaneously administered an emulsion of 1 mg estradiol benzoate in 0.1 ml olive oil twice at 1-week intervals. At 1 week after the 2<sup>nd</sup> injection, the epithelium in Segments I-III showed marked regression; however, that of Segments IV-V did not. According to Schleicher et al., nuclear labeling of <sup>3</sup>H-estradiol in the principal cells was present in the caput epididymidis, but not in the corpus and cauda epididymidis<sup>5)</sup>.

Exogenous estrogen binds to the estrogen receptors present in the principal cells in



Segments I-III, thus suppressing the function of these cells.

### Conclusions

We first divided the mouse epididymis histologically into these 5 segments (Segments I-V) in 1980. We have studied the morphological features of in Segments I-V by light microscopy and electron microscopy, and have qualitatively and quantitatively documented the postnatal development of the epididymal duct in each segment. We have performed experimental studies on the mouse epididymis, in which Segments I-V individually respond as functional units. We strongly recommend that other researchers use this division in their studies on changes in knockout mice or localization of gene-expressing proteins in the mouse epididymis, thereby facilitating the complete elucidation of epididymal function.

### References

- 1) Escheverria FMG, Cuasnicu PS, Blaquier JA. Identification of androgen-dependent glycoproteins in the hamster epididymis and their association with spermatozoa. *J Reprod Fertil* 1982;64:1-7.
- 2) Eberspaecher U, Roosterman D, Kratzschmar J et al. Mouse androgen-dependent epididymal glycoprotein CRISP-1 (DE/AEG) : isolation, biochemical characterization, and expression in recombinant form. *Mol Reprod Dev* 1995;42:157-72.
- 3) Hess RA, Zhou Q, Nie R et al. Estrogen and epididymal function. *Reprod Fert Dev* 2001;13: 273-83.
- 4) Tong MH, Song WC. Estrogen sulfotransferase: discrete and androgen-dependent expression in the male reproductive tract and demonstration of an in vivo function in the mouse epididymis. *Endocrinology*. 2002;143:3144-151.
- 5) Schleicher G, Drews U, Stumpf WE, Sar M. Differential distribution of dihydrotestosterone and estradiol binding sites in the epididymis of the mouse. An autoradiographic study. *Histochemistry* 1984;81:139-47.
- 6) Yanagimachi R. Mammalian Fertilization. In: Knobil E, Neill JD, eds. *The Physiology of Reproduction*, 2<sup>nd</sup> Edition. New York: Raven Press, 1994;189-317.
- 7) Takano H. [Qualitative and quantitative histology and histogenesis of the mouse epididymis, with special emphasis on the regional difference (author's transl)] *Kaibogaku Zasshi* 1980;55:573-87. Japanese
- 8) Cornwall GA, Lareyre J-J, Matusik RJ, Hinton BT, Orgebin-Crist M-C. Gene expression and epididymal function. In: Robaire B, Hinton BT, eds. *The Epididymis from Molecules to Clinical Practice*. New York: Kluwer Academic/Plenum Publishers, 2002;169-99.
- 9) Johnston DS, Jelinsky SA, Bang HJ et al. The mouse epididymal transcriptome: transcriptional profiling of segmental gene expression in the epididymis. *Biol Reprod* 2005;73:404-13.
- 10) Abe K, Takano H, Ito T. Ultrastructure of the mouse epididymal duct with special reference to the regional differences of the principal cells. *Arch histol jpn* 1983;46:51-68.
- 11) Abe K, Takano H. Changes in distribution and staining reactivity of PAS-positive material in the mouse epididymal duct after efferent duct ligation. *Arch Histol Cytol* 1988;51:433-41.
- 12) Abe K, Takano H, Ito T. Microvasculature of the mouse epididymis, with special reference to fenestrated capillaries localized in the initial segment. *Anat Rec* 1984;209:209-18.
- 13) Benoit J. Recherches anatomiques, cytologiques et histophysiologiques, sur les voies excrétrices du testicule chez les mammifères. *Arch Anat Histol Embryol* 1926;5:173-412.
- 14) Levine N, Marsch DJ. Micropuncture studies of the electrochemical aspects of fluid and electrolyte transport in individual seminiferous tubules, the epididymis and vas deferens in rats. *J Physiol* 1971; 213:557-70.
- 15) Takano H, Abe K, Ito T. [Morphological features of the nuclei of the principal cells in the epididymal duct in mice, with special emphasis on the regional differences in the duct and age-related changes (author's transl)] *Hokkaido Igaku Zasshi*

- 1985;60:848-55. Japanese
- 16) Abe K, Takano H, Ito T. Tubule-containing inclusions in the epithelial cells of the mouse epididymal duct. *Arch histol jpn* 1983;46:69-77.
- 17) Lea OA, French FS. Characterizaion of an acidic glycoprotein secreted by principal cells of the rat epididymis. *Biochim Biophys Acta* 1981;668:370-6.
- 18) Rankin TL, Tsuruta KJ, Holland MK, Griswold MD, Orgebin-Crist MC. Isolation, immunolocalization, and sperm-association of three proteins of 18, 25, and 29 kilodaltons secreted by the mouse epididymis. *Biol Reprod* 1992;46:747-66.
- 19) Cuasnicu PS, Ellerman DA, Cohen Dj, Busso D, Morgenfeld MM, DaRos VG. Molecular mechanisms involved in mammalian gamete fusion. *Arch Med Res* 2001;32:614-8.
- 20) Vanha-Perttula T, Arya M. Lectin staining of rat testis and epididymis after ligation of excurrent ducts at different levels. *Biol Reprod* 1985;33:477-85.
- 21) Takano H, Abe K, Ito T. [Effects on the mouse epididymis of juvenile ligation of the ductuli efferentes or proximal epididymal duct: qualitative and quantitative histological studies (author's transl)] *Hokkaido Igaku Zasshi* 1981;56:133-41. Japanese
- 22) Takano H, Abe K, Ito T. [Changes in the mouse epididymis after ligation of the ductuli efferentes or proximal epididymal duct: qualitative and quantitative histological studies. (author's transl)] *Kaibogaku Zasshi* 1981;56:79-90. Japanese
- 23) Abe K, Takano H. Cytological response of the principal cells in the initial segment of the epididymal duct to efferent duct cutting in mice. *Arch Histol Cytol* 1989;52:321-6.
- 24) Abe K, Takano H, Ito T. Response of the epididymal duct in the corpus epididymidis to efferent or epididymal duct ligation in the mouse. *J Reprod Fertil* 1982;64:69-72.
- 25) Avram C, Yeung CH, Nieschlag E, Cooper TG. Regulation of the initial segment of the murine epididymis by dihydrotestosterone and testicular exocrine secretions studied by expression of specific proteins and gene expression. *Cell Tissue Res* 2004;317:13-22.
- 26) Abe K, Takano H, Ito T. Appearance of peculiar multivesicular bodies in the principal cells of the epididymal duct after efferent duct cutting in the mouse. *Arch histol jpn* 1984;47:121-35.
- 27) Abe K, Takano H, Ito T. Response of epididymal duct to the temporary depletion of spermatozoa induced by testicular irradiation in mice. *Anat Rec* 1983;207:17-24.
- 28) Abe K, Takano H. Response of the mouse epididymal duct to the disappearance and reappearance of spermatozoa induced by temporal cryptorchidism. *Arch histol jpn* 1987;50:315-24.
- 29) Abe K, Takano H, Ito T. Appearance of peculiar epithelial cells in the epididymal duct of the mouse ligated epididymis. *Biol Reprod* 1982;26:501-9.
- 30) Abe K, Takano H, Ito T. Interruption of the luminal flow in the epididymal duct of the corpus epididymidis in the mouse, with special reference to differentiation of the epididymal epithelium. *Arch histol jpn* 1984;47:137-47.