Scanning and Transmission Electron Microscope Observations of the Terminal Segment of the Cat Seminiferous Tubule: Epithelial Phagocytosis of Spermatozoa and Latex Beads*

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Summary. The terminal segment of the seminiferous tubule in the testis of the adult domestic cat was investigated using scanning and transmission electron microscopy. The terminal segment gradually tapers towards the tubulus rectus, while transforming into tall columnar supporting cells termed modified Sertoli cells which protrude into the lumen of the tubulus rectus in the form of a plug. There is no distinct central lumen in the plug, rather, it is a narrow slit-like cleft surrounded by the modified Sertoli cells.

The modified Sertoli cells of the plug as well as the cuboidal cells lining the tubulus rectus revealed an active phagocytosis of spermatozoa and even injected inert latex beads. These epithelial cells are thought to act as a protective filtration line for foreign bodies including unwanted spermatozoa.

The terminal segment of the seminiferous tubule which connects the ordinary seminiferous tubule to the rete testis is a short tapering segment of about 1 mm or less in length; in addition, in many mammalian species, its apical portion protrudes into the lumen of the tubulus rectus—which is a part of the rete testis—in the form of a plug.

The epithelium of the terminal segment, as it approaches the tubulus rectus, gradually loses spermatogenic cells such as spermatids and spermatogonia, coming to consist at the plug solely of tall, columnar supporting cells called modified Sertoli cells. It has generally been accepted that the plug-like protrusion of modified Sertoli cells may function as a valve preventing the reflux of spermatozoa and testicular fluids from the rete testis.

The terminal segment has recently attracted the attention of some investigators who have suggested that the lining epithelium, especially the plug-forming epithelium, plays a significant role in the removal of spermatozoa; ultrastructural evidence in support of this phenomenon has been shown in various mammalian species including the monkey (DYM, 1974), boar (OSMAN, 1978), rat (NYKÄNEN, 1979), rabbit (OSMAN, 1979) and in cattle (WROBEL et al. 1982).

We have previously observed that the epithelial cells of the terminal region of the vas deferens of the cat reveal the active phagocytosis of spermatozoa as well as inert particles such as latex beads experimentally injected into the lumen (MURAKAMI et al., 1984 a, b). The present investigation by scanning (SEM) and transmission electron microscopy (TEM) aims to clarify the fine structure of the terminal segment of the seminiferous tubule of the cat. Special attention has been given to the question of whether or not the modified Sertoli cells of this portion are responsible for the phagocytosis of spermatozoa and latex beads as is the case in the epithelium of the terminal vas deferens of the same animal.

MATERIALS AND METHODS

Four adult male cats were used in this study. The animals were anesthetized with sodium pentobarbital (Nembutal) injected intramuscularly and fixed by vascular perfusion through the abdominal aorta using

*This paper is dedicated to the memory of the late Professor Emil TONUTTI (1909-1987) of Ulm University in Germany.
Fig. 1. Longitudinal section of the terminal segment of the cat seminiferous tubule revealed under the light microscope. At the tip of the terminal segment, the epithelial lining consists solely of tall, columnar supporting cells called modified Sertoli cells which protrude, in the form of a plug, into the lumen of the tubulus rectus (TR).

Fig. 2. SEM image of a longitudinally cut terminal segment of the seminiferous tubule. The epithelial lining forms a plug-like protrusion in the tubulus rectus (TR) as shown in Figure 1. The approximate course of a channel continuous to the central lumen of the ordinary seminiferous tubule is indicated by the broken line. The area surrounded by the rectangle is enlarged as in Figure 3. ×430
a mixture containing 2.5% glutaraldehyde and 2% formaldehyde in cacodylate buffer (pH 7.2). In two of the cats, the unilateral testis was exposed through an incision to the scrotum and 0.2 ml of physiological saline containing polyvinyl toluene latex beads of 1.0 \( \mu \text{m} \) in diameter was injected into the lumen of the rete testis near its vascular pole for 30 min before fixation by perfusion. The testes of all the experimental animals were then removed. One to three mm thick sections, including the proximal portions of the lobuli testis, were cut with razor blade on an autoclopper (Sorval type 2), then immersed in the same fixative for an additional 1 h, and finally placed in 2% osmium tetroxide in phosphate buffer (pH 7.2) after a brief washing in cacodylate buffer solution.

For TEM, the tissues were dehydrated through a series of graded alcohol and embedded in an eponaraldite mixture. Thin sections were made with a diamond knife on a Reichert-Jung ultratome, stained with uranyl acetate and lead citrate, and examined with a JEM 2000EX TEM. Semithin sections of 1 \( \mu \text{m} \) were also prepared for light microscopic examination and stained with 1% buffered toluidine blue.

For SEM, the specimens were critical-point dried in liquid carbon dioxide after dehydration in graded alcohol, coated with gold-palladium in an Eiko ion sputter coater, and viewed with an HFS-2 SEM. Organic reagents such as acetone, propylene oxide and isoamyl acetate were not used during the procedure for SEM because they solved latex beads completely or partially.

RESULTS

The rete testis of the cat is located in the mediastinum running along the long axis of the testis and belongs to the axial rete type according to the classification by Benoit (1926), while the rete of the rat and man lies on the surface of the testis and beneath the tunica albuginea, and is categorized as a type of superficial rete.

The terminal segment of the seminiferous tubule tapers down, the diameter of the segment being reduced to approximately half or less when compared with the diameter of the ordinary seminiferous tubule. The epithelial lining loses spermatogenic germinal cells, including spermatids and spermatagonia, as it approaches the tubulus rectus. It finally comes to consist solely of tall, columnar supporting cells termed modified Sertoli cells, some of which are deeply stained with toluidine blue. At the tip of the terminal segment the modified Sertoli cells protrude, in the form of a plug or lip, into the lumen of the tubulus rectus, which is slightly expanded and is a part of the rete testis. In the cat the formation of the plug is not uniform through the circumference of the free margin of the terminal segment. Figure 1 shows a longitudinal semithin section in the terminal segment of the seminiferous tubule. On one side of the tubular wall the epithelium bulges considerably into the tubulus rectus to form a plug, whereas on its opposite side there is no plug formation of the epithelium. The modified Sertoli cells, at the junction with the tubulus rectus, abruptly alter into simple cuboidal cells characteristic of the rete testis. In semithin sections, the modified Sertoli cells forming a plug and

Fig. 3. Enlargement of the area marked with the rectangle in Figure 2, showing an irregular cleft bordered by closely adjoining modified Sertoli cells. The cleft contains spermatozoan tails which are being phagocytosed by modified Sertoli cells. Asterisk indicates the narrow lumen between the plug and the wall of the tubulus rectus. \( \times 1,300 \)
the simple cuboidal cells of the tubulus rectus seem to be in contact with each other or are separated by only a narrow lumen.

The SEM appearance of the terminal segment in longitudinal section is illustrated in Figure 2. The central lumen continuous with the lumen of the ordinary seminiferous tubule gradually narrows while running along the long axis of the terminal segment and opens into the narrow lumen formed between the epithelial lining of the tubulus rectus and the modified Sertoli cells of the plug. In a portion of the plug, the distinct central lumen disappears and is replaced with an irregular slit-like cleft bordered by the apices of closely adjoining modified Sertoli cells, through which spermatozoa and testicular fluid pass from the seminiferous tubule to the rete testis (Fig. 3). The free surfaces of individual modified Sertoli cells comprising the plug are more or less protruded into the lumen of the tubulus rectus and provided with short stubby microvilli. Large numbers of spermatozoa are seen attached to the free surfaces of the modified Sertoli cells. Some of the spermatozoa seem to be undergoing phagocytosis by the modified Sertoli cells (Fig. 4).

When viewed by TEM, the modified Sertoli cells of the plug display highly lobulated nuclei which are situated at variable levels in the cytoplasm. The cells also contain a moderate number of rod-shaped mitochondria, tubules of rough surfaced endoplasmic reticulum randomly scattered with infrequent concentric arrays, sparse smooth surfaced endoplasmic reticulum, a poorly developed Golgi apparatus, abundant microtubules and microfilaments oriented parallel to each other, and occasional dense bodies of lysosomal nature. Vacuoles of various sizes are frequently visible in the apical region of the cytoplasm of some modified Sertoli cells. The lateral border of the modified Sertoli cells is relatively straight and attached at variable levels to adjacent modified Sertoli cells by

Fig. 4. SEM image showing the tip of a plug. Modified Sertoli cells are protruded into the lumen of the tubulus rectus and many spermatozoa are attached to the surfaces of these cells. ×1,800
such devices as tight junctions and desmosomes; however, a junctional specialization characteristic of the typical Sertoli cells in the ordinary seminiferous tubule is not detected. The intercellular space between adjoining modified Sertoli cells is generally narrow except for an occasional dilation. Among the ordinary modified Sertoli cells are a few modified Sertoli cells with a cytoplasm of higher electron density. However, no distinct ultrastructural difference can be found between these two types of cells. The cells of the latter type may correspond to those stained intensively with toluidine blue in light microscopy.

The modified Sertoli cells, especially those of the plug, are actively phagocytic, and phagocytosed spermatozoa at various stages of degeneration are often encountered in the apical region of the cytoplasm near the free surfaces (Figs. 5, 6). In addition to spermatozoan fragments such as heads and tails, whole bodies of spermatozoa are also not infrequently found which are being internalized into the cytoplasm. Both spermatozoa and their phagocytosed fragments are enclosed within phagocytic vacuoles or associated with lysosomal dense bodies. They may be disintegrated by lytic enzymes to finally become a part of the cytoplasm. Varying numbers of degenerating spermatozoa are also found within the cuboidal epithelial cells of the tubuli recti, being located opposite to the modified Sertoli cells forming the plug (Fig. 6). Latex beads injected into the lumen of the rete testis were observed to be extensively phagocytosed both by modified Sertoli cells forming the plug and by epithelial cells lining the tubulus rectus (Fig. 7).
DISCUSSION

The epithelial lining of the terminal segment of the seminiferous tubule of the cat consists mainly of tall columnar cells termed modified Sertoli cells, as has previously been reported in some other species. At the tip of the terminal segment, these modified Sertoli cells project into the lumen of the tubulus rectus in a manner of plug formation. There may possibly be a species difference concerning the plug because no plug-like structure could be detected in the rabbit (OSMAN, 1979). Some ultrastructural differences have been shown between the modified Sertoli cells constructing the plug and the typical Sertoli cells of the ordinary seminiferous tubule, one of these being that the former cell lacks the junctional specialization characteristic of the latter cell. The absence of a typical Sertoli-Sertoli junction in the plug region has been already documented in man (LINDNER, 1982), the monkey (DYM, 1974), boar (OSMAN, 1979) and in cattle (WROBEL et al., 1982); this fact is thought to be attributable to the lack of spermatogenic cells in this region (OSMAN, 1978).

There have been different arguments on the presence of a central lumen in the terminal segment. In the plug of the cat, a distinct lumen disappeared, being replaced with a slit-like cleft which was bordered by closely arranged modified Sertoli cells. In many

![TEM image showing spermatozoa phagocytosed within the cytoplasm of a modified Sertoli cell. Spermatozoan heads with eroded peripheral chromatin appear to be in a stage of degeneration more advanced than those with acrosomes. TRC: a part of the cytoplasm of an epithelial cell lining the tubulus rectus. ×11,300. Insert: A phagocytosed spermatozoan within the cytoplasm of an epithelial cell of the tubulus rectus. ×8,500](image)
species the plug region of the terminal segment also exhibits no patent lumen under normal conditions (OSMAN, 1978; WROBEL et al., 1982), and it has been reported that the central lumen becomes visible only under experimentally increased testicular pressure either following ligation of the ductuli efferentes (OSMAN, 1979) or after injection of corrosion compounds for casting into the rete testis (HEES et al., 1987).

The presence of phagocytosed spermatozoa within the modified Sertoli cells of the terminal segment has been noted in various species (DYM, 1974; OSMAN, 1978, 1979; NYKÄNEN, 1979; WROBEL et al., 1982). Similar spermiophagy by the epithelium of the tubulus rectus has also been documented in some species (DYM, 1974; OSMAN and PLOEN, 1978; SINOWATZ et al., 1979). In the cat, the modified Sertoli cells forming the plug and the epithelial cells lining the tubulus rectus revealed the active phagocytosis of spermatozoa and even injected inert latex beads. The ingestion of latex beads by spermiophagic epithelial cells has recently been reported in the terminal region of the vas deferens of a number of species including the cat (MURAKAMI et al., 1984 b, 1985, 1986). The uptake of latex beads is thought to offer circumstantial evidence for the phagocytic property of the epithelial cells in the male reproductive tract, though the immunological mechanism involved may differ between the phagocytosis of spermatozoa and that of latex beads. From the present data it is obvious that, in the cat, the modified Sertoli cells in the terminal segment of the seminiferous tubule and the epithelial cells of the tubulus rectus behave physiologically in an attempt to remove foreign bodies, probably including

![Fig. 7. TEM view of a section through a plug and a tubulus rectus. Large numbers of latex beads are seen in the lumen between modified Sertoli cells forming the plug (upper half of the picture) and lining epithelial cells of the tubulus rectus (lower half of the picture). They are also observed within the cytoplasm of both the modified Sertoli cells and the epithelial cells of the tubulus rectus (arrows). ×4,200](image-url)
damaged spermatozoa.

This function would be facilitated by the narrowness of the cleft in the plug region, as this ensures close contact between the passing spermatozoa and the modified Sertoli cells. Besides the epithelial cells involved in spermiophagy, a few macrophages loaded with degenerating spermatozoa were occasionally seen in the lumen of the tubulus rectus, but were never found in the lumen of the terminal segment of the seminiferous tubule.

REFERENCES


