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Scanning Electron Microscope Observation of the Seminal Vesicle in the Japanese Monkey with Special Reference to Intraluminal Spermiophagy by Macrophages

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Summary. The seminal vesicle of the adult Japanese monkey was observed by scanning electron microscopy. A number of spermatozoa are found scattered on the luminal surface. Some of them are normal in appearance, while others disintegrated. The epithelial cells bulging slightly into the lumen are devoid of cilia, but provided with diffusely distributed microvilli. The fracture surface reveals that the epithelium of the seminal vesicle consists of columnar epithelial cells with secretory activity and occasional basal cells, in accordance with the finding obtained by transmission electron microscope in other mammals.

The most striking observation of this study is the occurrence of a number of macrophages lying on the epithelium of the seminal vesicle. They also are seen to engulf the disintegrated spermatozoa in bulk. The intraluminal macrophages observed may serve to dispose of spermatozoa which have migrated into the seminal vesicle for some unknown reason.

In recent years, light and electron microscopic studies of the seminal vesicle under normal and experimental conditions have been extensively made in numerous mammalian species (DEANE and her co-workers, 1960, 1965; RIVA, 1967; TONER and BAILLI, 1969; GOHARA, 1971; DAHL et al., 1973a, b, c; BEIER and KÜHNEL, 1974; FLICKINGER, 1974). It has been demonstrated that the columnar epithelial cells bordering the lumen of the seminal vesicle are, like other protein-secretory cells, characterized by the presence of secretory vacuoles with dense granules derived from the Golgi apparatus and by well developed rough endoplasmic reticulum. It has also been revealed in the same epithelium that castration causes reduction in cell size, loss of rough endoplasmic reticulum and absence of secretory vacuoles, while testosterone administration induces the reverse alterations. It is currently believed that the seminal vesicle is not a site of storage of spermatozoa as believed previously, but a secretory gland whose activity level is controlled to a great degree by androgens.

To our knowledge, neither the occurrence of macrophages nor spermiophagy by them in the lumen of the seminal vesicle has been depicted in literature. However, some authors (STIEVE, 1930; MATHIS, 1943) have pointed out occasional migration of spermatozoa into the lumen of the seminal vesicle. Scanning electron microscopic study affords visualization of a selected area larger than that attainable by transmission electron microscopy and hence is particularly useful in the study of the mucous

surface of reproductive organs. We applied the technique to the seminal vesicles of monkeys and frequently observed a number of macrophages which were engulfing spermatozoa on the seminal epithelial surface. HÜCKER and AUMÜLLER (1976) first observed the rat seminal vesicle with a scanning electron microscope, but they did not mention either the occurrence intraluminally of spermatozoa or phagocytosis of them by macrophages.

In the present investigation we have attempted to provide a scanning electron microscope survey of the seminal vesicle of the Japanese monkey paying particular attention to macrophages and their intraluminal spermiphagy.

Materials and Methods

After separate breeding for at least 3 months, three healthy adult Japanese monkeys (*Macacus fuscatus*), 4–6 years and weighing 8–15 kg, were anesthetized with nembutal and perfused through the ascending aorta first with physiological saline and then with Karnovsky's aldehyde fixative for half an hour. The seminal vesicle was quickly removed and immersed for an additional 3 hrs in the same fixative. With the aid of a dissecting microscope the seminal vesicle was cut into small pieces. Tissue blocks were gently washed several times with physiological saline to rinse away the mucus from the lumen, postfixated for 2 hrs with 2% OsO_4 in Millonig's buffer, and dehydrated in an ascending acetone series. Most specimens were then

Fig. 1. Survey micrograph of the luminal surface of the seminal vesicle. In the lumen, tortuous ridges of the mucosa are apparent. Note a few spermatozoa attached to the epithelial surface. $\times 880$

dried by the critical point method using CO₂, coated with gold by the ion sputter method, and viewed in a field emission scanning electron microscope (HFS-2). Some specimens were fractured by the freeze resin cracking method (SHIMADA et al., 1976) prior to the critical point drying.

Results

A low magnified view of the epithelial surface of the seminal vesicle revealed intricately anastomosing mucosal ridges by which the lumen can be subdivided into several irregular compartments. It was of interest that spermatozoa were seen singly or in a cluster on the epithelial surface. Some of the spermatozoa were deteriorated or disintegrated, but others appeared to still retain normal structure (Fig. 1). The individual epithelial cells covering the lumen protruded slightly into the lumen, so that each cell could be defined from its neighbors by relatively deep furrows. The epithelial cells were devoid of cilia, but diffusely provided with stubby microvilli varying in population from cell to cell. Occasionally, there were epithelial cells whose microvilli adhered to each other presumably by coagulation of a secretory substance (Fig. 2), but extrusion of secretory granules from the epithelial surface could not be detected in our materials. Indented apical surface of the epithelial cells probably resulting from granule discharge was occasionally found.

In vertical fracture of the seminal vesicle, the epithelium was entirely uniform

Fig. 2. Free surface of the epithelial cells lining the lumen. The epithelial cells protrude slightly into the lumen and are provided with stubby microvilli. Occasionally there are epithelial cells whose microvilli are intermingled with a coagulation of a presumable secretory substance. $\times 7,800$

and consisted of columnar cells intermingled with occasional low basal cells. The nucleus of the epithelial cell was spherical to ovoid and exhibited clumps of chromatin and conspicuous nucleoli. Parallel rows of the cisterns of rough endoplasmic reticulum were usually extensive in the basal half of the cells. A detailed image of the fractured epithelial cell is shown in Figure 3. In this figure, concentrically arranged, a well-developed Golgi apparatus and a large number of round pits containing the marginally located dense granules due to conglomeration of fine spherical materials can be seen. These scanning electron microscopic findings of the fractured epithelium of the seminal vesicle of the Japanese monkey are well correlated with the previous descriptions of the same tissue in other mammals obtained by transmission electron microscopy.

The most striking finding in the survey of the luminal surface of the seminal vesicle of the Japanese monkey was the occurrence of numerous macrophages on the epithelial surface, and their phagocytotic process for spermatozoa could be observed extensively. The macrophages appeared usually singly or in pairs but sometimes

Fig. 3. Fractured surface of the apical portion of an epithelial cell showing many secretory vacuoles containing dense granules and a concentrically arranged Golgi apparatus. $\times 25,000$

Fig. 4. Macrophage in the lumen of the seminal vesicle. Thin, pseudopodial processes extend radially from the cell body. $\times 3,900$

in clusters especially in indented portions where highly condensed aggregations of spermatozoa were often found. The macrophages varied in size from 15 to 35 μm . In general, they were spherical or elongated in shape and possessed short, ridge-like, densely distributed microvilli. From the macrophage surface, a number of pseudopodial processes radiated and tended to interdigitate with microvilli of the underlying epithelial cells (Fig. 4). Fragments of disrupted spermatozoa were often seen attached to the surface of a macrophage (Fig. 5). This may be the early stage in spermiophagy. Some later stages of the spermiophagic process could be recognized frequently. Some macrophages were relatively irregular and extended thin, veil-like flaps of pseudopodial processes to engulf the sperm head. Figure 6 demonstrates a more advanced stage in the fractured specimen. This macrophage exhibits a rather round contour and loses pseudopodial processes on its surface presumably as a result of satiation with spermatozoa. In the cytoplasm there are fusiform inclusions suggesting ingested sperm heads and tails in bulk. In this study there was no evidence of any endocytotic uptake of spermatozoon by the epithelial cells of the seminal vesicle.

Fig. 5. Intraluminal macrophage adhering to fragments of disrupted spermatozoa. $\times 2,700$

Fig. 6. Fractured surface of an intraluminal macrophage. It contains disrupted, ingested portions of many spermatozoa. $\times 2,600$

Discussion

The scanning electron microscope observation of the seminal vesicle of the Japanese monkey confirmed and extended the knowledge by previous transmission electron microscope studies in other mammals.

The epithelial cells of the seminal vesicle revealed fine structures in common with those of protein producing cells or cells in an actively secretory state, although the image of secretion release of the cells could not be obtained in the present study. Occurrence of spermatozoa in the lumen of the seminal vesicle has been long known. This fact was explained previously as evidence for storage of spermatozoa in the seminal vesicle. However, as this view is no longer tenable, the occurrence of spermatozoa should be accounted for by their aberrant migration into the vesicle. Whether or not spermatozoa always exist in the seminal vesicle if the animal is sexually active remains unclear. As to this question, a previous description by STIEVE in 1930 is noteworthy to mention: He described that the seminal vesicle of sexually active men who had been pressed to lead an ascetic life for a long time contained a number of spermatozoa, whereas the seminal vesicle of those who had sexual intercourse just prior to the examination contained no spermatozoa. It is not surprising that there were abundant spermatozoa in the seminal vesicle of the Japanese monkey used in the present study, since the individual animal was housed in a separate cage for some months before the experiment.

Occurrence of macrophages and the intraluminal spermiophagy by them have been recently demonstrated in the epididymal duct in several species under normal and experimental conditions by light and electron microscopy (PHADKE, 1964; HOLSTEIN, 1969; ALEXANDER, 1972; FLICKINGER, 1975; MURAKAMI et al., 1977) but not in the seminal vesicle. In the present study the monkey seminal vesicle revealed a number of macrophages under spermiophagic activity. The main function of these macrophages apparently is to dispose of spermatozoa migrating into the seminal vesicle, although it could not be decided whether the macrophages ingest only selectively deteriorated or dead spermatozoa or living ones too. The question whether these macrophages are mesenchymal inhabitants of the mucous membrane or are derived from monocytes in the blood remains to be solved.

It was once believed that lipofuscin pigment found in the epithelial cells of the seminal vesicle was derived from the ingested spermatozoa (STIEVE, 1930; MATHIS, 1943; WATZKA, 1943). In our study, however, there was no figure indicating any stage of phagocytosis of spermatozoa by the epithelial cells of the seminal vesicle.

ニホンザル精囊の走査電子顕微鏡的観察、とくに大食細胞による 囊腔内精子たべこみについて

村 上 正 浩, 杉 田 新
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成熟ニホンザルの精囊を走査電子顕微鏡で観察した。多数の精子が囊腔表面に散在し、そのあるものは正常の形態を保っているが、あるものは崩壊している。精囊の内腔面を縁

どる上皮細胞は線毛を欠き、軽く内腔に向け突出しており、均等に分布した微絨毛を備えている。切断面で見ると、上皮は分泌性の円柱細胞とときにその間に介在する基底細胞ら構成されており、ほかの動物でこれまでに報告された透過電子顕微鏡による所見とよく一致する。

この研究で特記すべき所見は、上皮表面に多数の大食細胞が在存することであり、またこれら大食細胞が崩壊した精子を大量にたべこんでいることである。大食細胞は何らかのきっかけで精嚢腔にはいり込んだ精子の除去を行なうのであろう。

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