Amphibia, however, the gills do not seem to be essential for ion regulation as neither ligation nor amputation of gills had much effect on transport[17,18].

In spite of the morphological differences mentioned, CAH activity is a common denominator of fish 'chloride-cell'[19,20] and salamander gill MRC. Based on these observations we feel that the amphibian gill MRC could be involved to a large extent in gas exchange and acid-base regulation, thereby providing a possible link between the fish gill 'chloride-cell', the amphibian larval epidermal MRC, and hence the adult amphibian epidermal flask-cell[5,6].

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Scanning and transmission electron microscopic evidence of epithelial phagocytosis of spermatozoa in the terminal region of the vas deferens of the cat

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Summary. Scanning and transmission electron microscopic observations have been made in the terminal region of the vas deferens of the cat, with emphasis on the occurrence of spermiophagy. The present study has revealed that epithelial cells as well as luminal macrophages are extensively and actively involved in phagocytosis of spermatozoa. The mechanism of the spermiophagy is discussed, in relation to a possible role of the epithelial cells, as one function of the vas deferens.
Key words. Cat, vas deferens; vas deferens, cat; spermatozoa; phagocytosis, epithelial; spermiophagy.

Phagocytosis of spermatozoa by the epithelial cells was first demonstrated extensively in the rat vas deferens, and especially in its terminal portion, by Cooper and Hamilton[1]. Phagocytotic activity of the epithelial cells in the vas deferens has since been confirmed in its ampullary region in monkeys and humans[2–5]. Sporadic occurrence of spermiophagy by the epithelial cells in male reproductive tracts other than the vas deferens, such as seminiferous tubule, rete testis and efferent ductule has recently been documented in some mammals[6–8]. However, at present, it is not known whether spermiophagy by the epithelial cells in the vas deferens is a common event in mammalian species or is a phenomenon peculiar to certain species. In this report, the terminal region of the cat vas deferens was studied by SEM and TEM with special attention to the spermiophagic ability of its epithelial cells.

Materials and methods. Four adult male domestic cats were used in this study, and the experiments were carried out in March and April. The animals were anesthetized with Nembutal injected intramuscularly and perfused vascularly through the ascending aorta first with physiological saline solution and next with 2.5% paraformaldehyde and 2% glutaraldehyde in cacodylate buffer (pH 7.2). After perfusion, the whole reproductive tract was removed rapidly. The vas deferens was dissected out and subdivided grossly into proximal, distal and terminal regions. The proximal region was located within the scrotum and the latter two were in the pelvis. A short segment of each region was cut into small tissue blocks. They were immersed in the same paraformaldehyde-glutaraldehyde fixative for another 1–2 and postfixed in 2% phosphate buffered OsO_4 (pH 7.2) for 1 h. Following dehydration by ascending acetone, the tissue was embedded in Epon 812. Thin sections were cut with diamond knives, stained with lead citrate and either uranyl acetate or tannic acid, and viewed in a H-500 or JEM-100S transmission electron microscope (TEM). Thick sections of 1 μ were cut with glass knives and stained with 1% toluidine blue in phosphate buffer. Tissues for scanning electron microscopy (SEM) were prepared and processed as for TEM examination but after dehydration they were dried in a critical point dryer using liquid CO_2, coated with gold-palladium in an Eiko sputter coater, and examined with an HFS-2 field emission scanning electron microscope.

Results and discussion. The terminal vas deferens of the adult cat was less than 10 mm in the region just before it entered the prostate to form the ejaculatory duct (prostatic urethra) (fig. 1). The lumen of the terminal portion was characterized by a rather distended and circular profile. The epithelium lining the lumen was uniform in height and consisted of 1 or 2 layers of low cuboidal cells (fig. 2).

In thin sections, the epithelial cells were somewhat smooth in outline and possessed slender microvilli and an occasional single cilium projecting into the lumen. In the cytoplasm of the cells, there were a few organelles, many lysosomal dense bodies and abundant fine filaments oriented randomly (fig. 3).

When viewed by SEM, the luminal surface of the epithelial cells of the terminal vas was flattened, with a hexagonal cell boundary, and was covered by stubby microvilli which varied slightly in length from cell to cell. A large number of spermatozoa and a few macrophages were located on the epithelial sur-

face throughout the whole length of the terminal vas deferens. Many of the spermatozoa appeared to be intact in structure, but others had degenerated. Usually, varying numbers of spermatozoa were attached to the surface of the macrophages. Under higher magnification most macrophages could be recognized as the cells engulfing the attached spermatozoa by their cytoplasmic processes (fig. 4). Similar spermiophagy by the luminal macrophages has recently been reported not only in the vas deferens but in the entire male reproductive tract of some mammals including human[1,7–11]. A particularly interesting fact is that the epithelial cells of the terminal vas deferens are capable of performing active spermiophagy, which is carried out in various ways (fig. 5a–c). The spermatozoa are trapped by a pseudopod-like flap; in many cases they are incorporated vertically, with either head or tail first, into the cytoplasm, but in rare cases they are taken up by the middle portion between head and tail. The single epithelial cell in figure 5a can be seen engulfing 2 or more spermatozoa simultaneously.

The thin sections through the epithelium illustrated that spermatozoa taken into the epithelial cells were in various stages of digestion within the cytoplasm. Phagocytosis may begin with the formation of a pseudopod-like projection of the apical cell membrane and with invagination of the associated cell membrane to embrace the spermatozoa (fig. 6a). Then the spermatozoa incorporated into the cytoplasm are disintegrated within

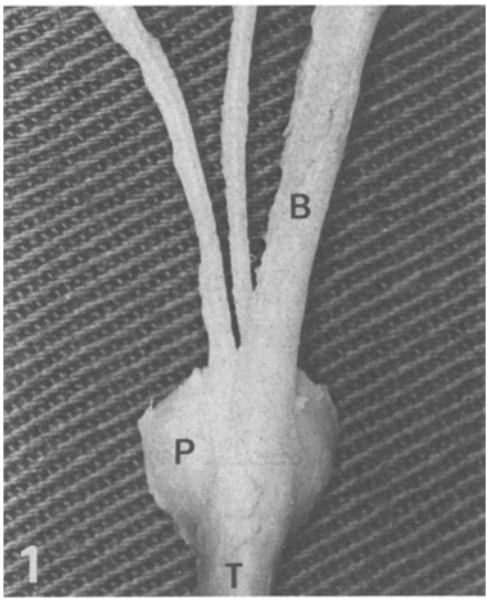

Figure 1. Photograph of the terminal vas deferens of the cat showing its gross anatomical relationship with the neck of the bladder (B) and the prostate (P). the connective tissue sheath enclosing right and left terminal vasa together was removed in order to delineate them. T, membranous urethra.

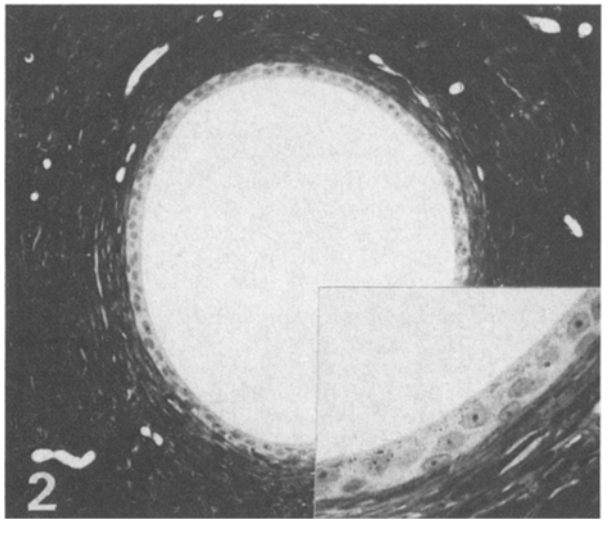

Figure 2. Light micrograph showing the terminal vas deferens in cross section. Note the distended circular profile of the lumen. Insert: Higher magnification of the epithelium bordering the lumen. It consists of 1–2 layers of low cuboidal cells. Epon section, Toluidine blue-stain.

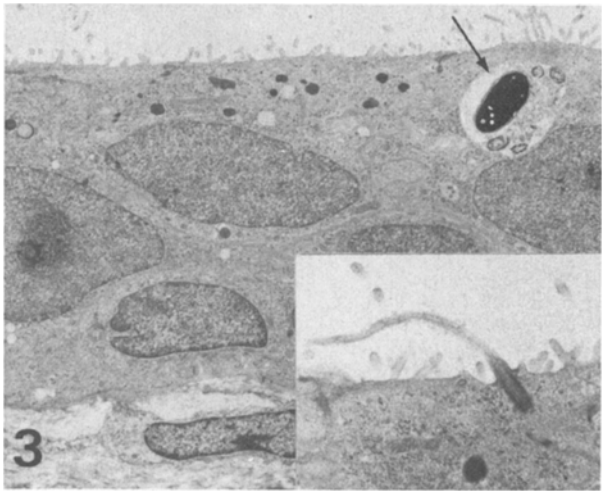

Figure 3. Survey transmission electron micrograph of the epithelium from basal lamina to the lumen in the terminal vas deferens. The epithelial cells are provided with slender microvilli and contain a number of dense bodies presumably of lysosomal nature. Arrow indicates spermatozoon fragments incorporated into the cytoplasm. × 4000. Insert: A single cilium projecting from an epithelial cell. × 9300.

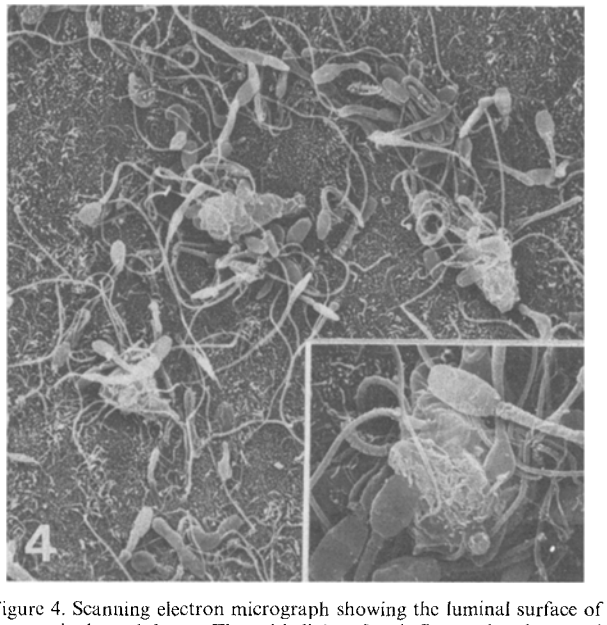

Figure 4. Scanning electron micrograph showing the luminal surface of the terminal vas deferens. The epithelial surface is flattened and covered by stubby microvilli on which a number of spermatozoa and a few macrophages are located. × 930. Insert: Higher magnification of a macrophage engulfing the attached spermatozoa. × 2400.

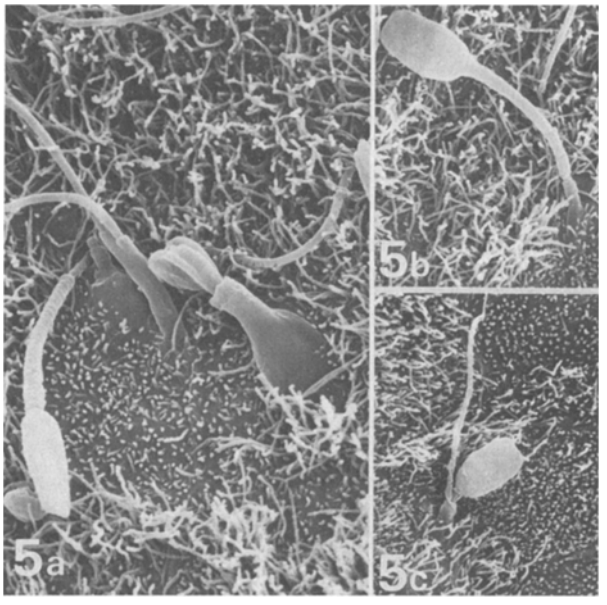

Figure 5. Scanning electron micrographs indicating various modes of spermiophagy by the epithelial cells. Many of the spermatozoa are taken up head-first(a), but others tail-first (b). Rarely they are taken up by the middle portion between head and tail (c). a, × 3600; b, × 3000; c, × 2800.

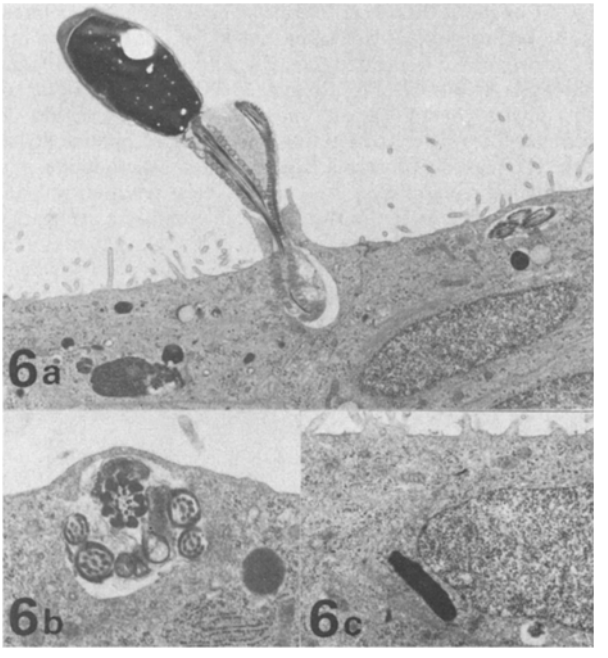

Figure 6. Transmission electron micrographs showing that spermatozoa taken up by phagocytosis are in various stages of digestion in the cytoplasm of the epithelial cells (for detailed explanation see text). a, × 4900; b, × 11,300; c, × 8200.

phagocytotic vacuoles probably by the action of lytic enzymes (fig. 6b) to become condensed masses finally (fig. 6c). In the cat, phagocytosis of spermatozoa by the epithelial cells is observed only in the terminal region of the vas deferens, while spermiophagy by luminal macrophages occurs more or less throughout the length of the vas deferens.

From the present study, the reason why there is epithelial spermiophagy in the terminal vas deferens is not clear, and the problem as to whether the epithelial cells ingest only damaged or abnormal spermatozoa or whether they can ingest even living ones also remaines unsolved. But it seems unlikely that the epithelial cells are merely engaged in selective removal of damaged or surplus spermatozoa[1], because our unpublished observations indicate that the epithelial cells, like luminal macrophages, are also capable of taking up latex beads injected into the cat vas deferens.

The epithelial cells may act to remove several kinds of foreign matter including degenerative spermatozoa in order to scavenge the lumen, though it is not obvious in what way they can discriminate foreign matter from non-foreign matter.

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Observations on the dynamics of argyrophilic nucleolar material in the nuclei of mice spermatids[1]

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Summary. Applying a new silver staining technique it could be shown that in very early spermatids strong argyrophilia in nucleoli is confined to their granular and fibrillar components; fibrillar centers are devoid of silver. During subsequent developmental stages remnants of these nucleolar components are present in the form of intensively silver stained clusters of coiled fibers. As chromatin condensation proceeds, these fibrous structures decrease in size and density and are finally completely absent. The nucleus of the mature sperm contains only the space in which they formerly existed, now silver negative, as the so-called 'nuclear vacuole'.

Key words. Mouse spermatids; nucleoli; silver staining technique.