The occurrence of spermiphagy under natural conditions in the cauda epididymidis of the Cape horseshoe bat (*Rhinolophus capensis*)

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Summary. In Cape horseshoe bats large numbers of spermatozoa remain in the cauda epididymidis after copulations have ceased in September. Light microscopy has shown that for 5 months after September phagocytes are present in the lumen of the cauda epididymidis and electron microscopy has shown these cells to be active spermiphages. The large numbers of spermiphages present suggests that these cells could be responsible for removal of most of the excess spermatozoa. Spermiphagy by the epididymal epithelial cells occurs rarely and is not significant in the treatment of excess spermatozoa.

Introduction

In many species of hibernating vespertilionid and rhinolophid bats the cauda epididymidis acts as a site of sperm storage (Strelkov, 1962; Racey, 1979). However, in the seasonally breeding Cape horseshoe bat (*Rhinolophus capensis*), large numbers of spermatozoa remain in the cauda epididymidis after copulations have ceased and a second function associated with the epididymis may then be the removal of excess spermatozoa.

The fate of excess or non-ejaculated spermatozoa in mammals has been investigated and there appear to be four possible ways in which these spermatozoa may be removed. (1) Under natural conditions luminal macrophages may remove decapitate spermatozoa from the epididymis of the bull, monkey and rabbit (Bedford, 1965; Roussel, Stallcup & Austin, 1967). Under the abnormal conditions of vasoligation, vasectomy and azoospermia, spermiphagy by macrophages occurs in man (Phadke & Phadke, 1961; Phadke, 1964), rhesus monkey (Alexander, 1972; Bedford, 1976), mouse (Neaves, 1975) and hamster (Bedford, 1976). (2) It has been reported that spermatozoa undergo dissolution or degradation in the lumen of the epididymis of the guinea-pig (Simeone & Young, 1931), rabbit (Orgebin-Crist, 1969), rat (Cooper & Hamilton, 1977), hamster (Flickinger, Howards & English, 1978), rock hyrax (Millar, 1972) and European mole (Suzuki & Racey, 1976). The fate of the products of sperm dissolution is not known. (3) The ability of the epididymal epithelium to absorb colloidal material and vital dyes by micropinocytosis has been widely reported (Shaver, 1954; Burgos, 1964; Nicander, 1965) but only in very few cases has it been shown that these cells are capable of absorbing portions of spermatozoa (rabbit: Nicander, 1965; Glover, 1969; rat: Flickinger, 1972; mouse: Neaves, 1975). (4) In several species it has been shown that spermatozoa may be voided by spontaneous ejaculation, in seminal discharges or in the urine and in such species these may be the most important means of removing excess spermatozoa (Orbach, 1961; Martan & Risley, 1963; Lino, Braden & Turnbull, 1967; Martan, 1969).

For two species of microchiropteran bat (*Myotis lucifugus* and *M. velifer*) it has been suggested that, in the absence of macrophages and occurrence of only limited sperm dissolution, the excess

spermatozoa in the cauda epididymidis are probably voided during urination (Krutzsch, Crichton & Nagle, 1982). Males of the genus *Rhinolophus* from subtropical and temperate zone latitudes store spermatozoa in the cauda epididymidis during winter hibernation (Gustafson, 1979), and in the Cape horseshoe bat (*R. capensis*) large numbers of spermatozoa remain in the cauda epididymidis after the spring copulations. The aim of this study was to investigate the fate of these non-ejaculated spermatozoa.

Materials and Methods

Specimens of the Cape horseshoe bat (*Rhinolophus capensis*) were collected every month from Table Farm (33°17'S, 26°25'E) in the Cape Province of South Africa, brought back to the laboratory and killed by asphyxiation with CO₂.

Light microscopy. Epididymides were removed from the specimens, fixed in Bouin's fluid for about 2 weeks and thereafter stored in 70% alcohol. Tissues were embedded in Paraplast, sectioned at 5 µm and stained with Ehrlich's haematoxylin and eosin.

Electron microscopy. Pieces of cauda epididymidis (4 mm³) were fixed in cold (4°C) 5% glutaraldehyde in 0.1 M-phosphate buffer (pH 7.3) for 3 days. After primary fixation the tissues were washed in 0.1 M-phosphate buffer, secondarily fixed in 1% osmium tetroxide for 90–100 min and washed again. After rapid dehydration through an alcohol sequence the tissues were embedded in Taab 812. Thick sections were cut with a glass knife on an LKB UM III ultramicrotome, stained with toluidine blue and examined with a light microscope. Ultrathin sections were cut with a glass knife, picked up on copper grids, stained with aqueous uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) and examined under a Jeol JEM-100 II transmission electron microscope.

The density of macrophages per unit area in the lumen of the cauda epididymidis was calculated from light microscope sections. The numbers of macrophages in 20 transverse sections through the lumen of the epididymis of each specimen were counted and the area of the lumen was calculated from the radius. With thickness of the sections known (5 µm) the number of macrophages per unit volume was calculated.

Results

In Cape horseshoe bats spermatogenesis occurred in summer (September to April) and spermatozoa were first released to the epididymides in April. Between May and August these animals hibernated and during this period spermatozoa were stored in the cauda epididymidis (Pl. 1, Fig. 1). Although copulation, which was asynchronous within the population, occurred in August

PLATE 1

Fig. 1. Section through the cauda epididymidis of a Cape horseshoe bat showing the typical appearance of this structure during the period of sperm storage before fertilization. × 106.

Fig. 2. Section through the cauda epididymidis in December, showing the numerous phagocytes (arrows) amongst the stored spermatozoa. × 394.

Fig. 3. Section through the cauda epididymidis in March, showing the reduced numbers of phagocytes (arrows) and spermatozoa. × 450.

Fig. 4. Light micrograph of a section through the cauda epididymidis, showing the typical appearance of the phagocytes (arrows). × 680.

(Facing p. 540)

and September, large numbers of spermatozoa remained in the cauda epididymidis until February so that sperm storage effectively occurred between April and February.

Luminal phagocytes were present in very small numbers (1.8 ± 1.1 macrophages per $10 \times 10^3 \mu\text{m}^3$; mean ± 2 s.d.) in the cauda epididymidis during the period of sperm storage before copulation and first became abundant in October. Between October and February the lumen of the cauda epididymidis contained large numbers of phagocytes (25 ± 10.5 per $10 \times 10^3 \mu\text{m}^3$) (Pl. 1, Fig. 2) but by March the numbers of spermatozoa and phagocytes were reduced (6.6 ± 2.71 per $10 \times 10^3 \mu\text{m}^3$) (Pl. 1, Fig. 3).

With the light microscope, the phagocytes appeared as round or ovoid cells of $5\text{--}20 \mu\text{m}$ in diameter with lightly staining eosinophilic cytoplasm and a large, central nucleus (Pl. 1, Fig. 4).

Electron microscopy showed that two different types of membrane-bound body appeared to be involved in the removal of excess spermatozoa. The first was uncommon and appeared more as an aggregate of several spermatozoa within a common cell membrane than a typical phagocytic cell (Pl. 2, Fig. 5). This membrane-bound body was characterized by a granular cytoplasm, in which the sperm portions were lying free, and an apparent lack of nucleus and other organelles. Although these structures always contained portions of spermatozoa, they were never seen actively engulfing spermatozoa. The second type of membrane-bound body was a typical macrophage, characterized by a varied shape and numerous pseudopodial projections (Pl. 2, Figs 6 & 7). Cellular organelles included a large nucleus of varied shape, mitochondria, primary and secondary lysosomes, various vacuoles, Golgi apparatus and some endoplasmic reticulum. These macrophages were active spermiphages, engulfing numerous spermatozoa (Pl. 2, Figs 6 & 7). The engulfed sperm portions always appeared in membrane-bound vacuoles within the macrophage cytoplasm.

The epithelium of the cauda epididymidis showed considerable micropinocytotic activity but there was little evidence of spermiphagy by the epithelial cells. On three occasions only, sections of sperm tail were seen in the cytoplasm of the principal epididymal cells.

Discussion

From the available literature it is apparent that the fate of excess spermatozoa may vary both from species to species and within a single species. In the seasonally breeding rock hyrax, *Procavia capensis*, spermatozoa that remain in the cauda epididymidis after copulations have ended are degraded (Millar, 1972), while in Cape horseshoe bats (present study) such spermatozoa are destroyed by macrophages. In the mouse, sperm dissolution and spermiphagy by macrophages occur after vasoligation (Neaves, 1975). The destruction of spermatozoa by macrophages, as described in the present study, occurs widely in mammals under unnatural conditions in which the normal expulsion of spermatozoa is prevented. However, under normal conditions macrophages rarely occur in the lumen of the epididymis (Bedford, 1965; Roussel *et al.*, 1967; Krutzsch *et al.*, 1982). The Cape horseshoe bat seems to be an exception to this observation with macrophages being at least partly responsible for the removal of excess spermatozoa. Phagocytes, identified from

PLATE 2

Fig. 5. A cellular aggregate, containing portions of spermatozoa within a common cell membrane. Note that the sperm portions (ax = axoneme complex; m = mitochondria; a = acrosome) are free in the cytoplasm of the aggregate. $\times 15\,225$.

Fig. 6. Part of a typical macrophage from the cauda epididymidis, showing the pseudopodial processes (ps) and disruption of ingested portions of spermatozoa (arrows). $\times 9310$.

Fig. 7. Electron micrograph of a typical macrophage, showing the large central nucleus (n) and ingested portions of spermatozoa (arrows). $\times 9750$.

light microscope sections, are believed to serve a similar function in Geoffroy's horseshoe bat (*Rhinolophus clivosus*) (Bernard, 1984) and spermiphagy by macrophages may be a characteristic of this genus.

The first type of membrane-bound body described in the present study is similar in appearance to a membrane-bound structure that occurs in the epididymis of the bats *Myotis lucifugus* and *M. velifer* and which was seen to contain various sperm parts (Kruttsch *et al.*, 1982). These membrane-limited bodies appear to be associated with treatment of excess spermatozoa although their mode of function and origin are unknown.

The origin of the luminal macrophages in Cape horseshoe bats is unknown. Reid & Cleland (1957) first described the halo cells in the epididymal epithelium and suggested that they may be phagocytes, and ultrastructural studies have since shown the halo cells to be morphologically similar to leucocytes (Bedford, 1965; Dym & Romrell, 1975; Hamilton, 1975). Although it is not possible to identify the halo cells as leucocytes on the basis of morphological evidence alone, it has been suggested that they may be lymphocytes moving from the blood stream through the epididymal epithelium into the lumen (Hoffer, Hamilton & Fawcett, 1973). The halo cells of Cape horseshoe bats were similar in appearance to those illustrated by Hoffer *et al.* (1973) but were at no time sufficiently numerous to account for the large number of luminal macrophages.

Bedford (1976) and Temple-Smith & Bedford (1978) have suggested that spermiphagy only occurs under the unnatural conditions of vasectomy wherein the epididymal lumen is exposed to the immune system following rupture of the epithelium. However, it is unlikely that this is the case in Cape horseshoe bats in which the luminal macrophages occur under natural conditions and histological examination has revealed no breaks in the epithelium. Spermiphagy by epididymal epithelial cells has been reported as a rare occurrence under natural conditions (Glover, 1969; Flickinger, 1972; Neaves, 1975) and is probably not significant in the removal of excess spermatozoa. In Cape horseshoe bats the epididymal epithelial cells showed active micropinocytosis and it is possible that the ingestion of sperm fragments was fortuitous.

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