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Ultrastructural studies on the epididymal spermatozoa in the rhesus monkey

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Abstract. Ultrastructural studies on the spermatozoa in different regions of the epididymis of the rhesus monkey have shown that the process of sperm maturation is associated with the caudad migration of the cytoplastmic droplet, a reduction in the volume of the cytoplasmic droplet, and an obvious wrinkling of the plasma membrane surrounding the head of the spermatozoa. These changes are completed by the time the spermatozoa reach the distal-middle segment of the epididymis. The present studies also indicate that spermatozoa are incorporated into the intraepithelial cells in the epidymis. This finding suggests that spermiophagy is a normal occurrence in the epidymis of rhesus monkey.

Keywords. Ultrastructure; epididymis; spermatozoa; rhesus monkey.

Introduction

The epididymis has been recognized as a potential site for the extraneous intervention for purpose of regulating fertility. This conclusion is based on the knowledge that spermatozoa undergo a process of maturation during their passage through the epididymis.

The process of sperm-maturation can be microscopically visualized by the location of the cytoplasmic droplet on the spermatozoa. The cytoplasmic droplet is located in the neck region in such of the spermatozoa which have not yet undergone maturation, whereas in the matured spermatozoa cytoplastmic droplet is either located at the distal end of the midpiece (Bloom and Nicander, 1961) or it is shed-off (Bedford *et al*., 1973) depending on the species.

A detailed light microscopic study of the epididymis of the rhesus monkey has revealed that marked histological differences occur in the histological features of the epithelium in the different regions of the epididymis (Prakash *et al*., 1979). These structural differences have been assumed to reflect functional differences between the epididymal segments and appear to be related to the maturation of spermatozoa. In the studies reported here, we have sought to determine the topographic region of the epididymis where spermatozoa mature, as evidenced by

the location of the cytoplasmic droplet on the spermatozoa. This information is of relevance to the evaluation of anti-fertility agents affecting sperm-maturation in the epididymis of non-human primate species.

In the course of these studies we have also observed, for the first time, that spermatozoa are resorbed in the epididymis of the rhesus monkey under normal physiological conditions; since the process of sperm-resorption may be related to the production of sperm-antibody, this interesting feature is also reported.

Materials and methods

Tissues were obtained from the different epididymal segments (figure 1) of eight, healthy adult rhesus monkeys. The demarcation of the epididymis into initial, middle and terminal segments respectively, was based on histological criteria (Prakash *et al*., 1979). The tissues were fixed by vascular perfusion (Anand Kumar *et al*., 1980a) and processed for electron microscopy (Anand Kumar *et al*., 1980b).

Results

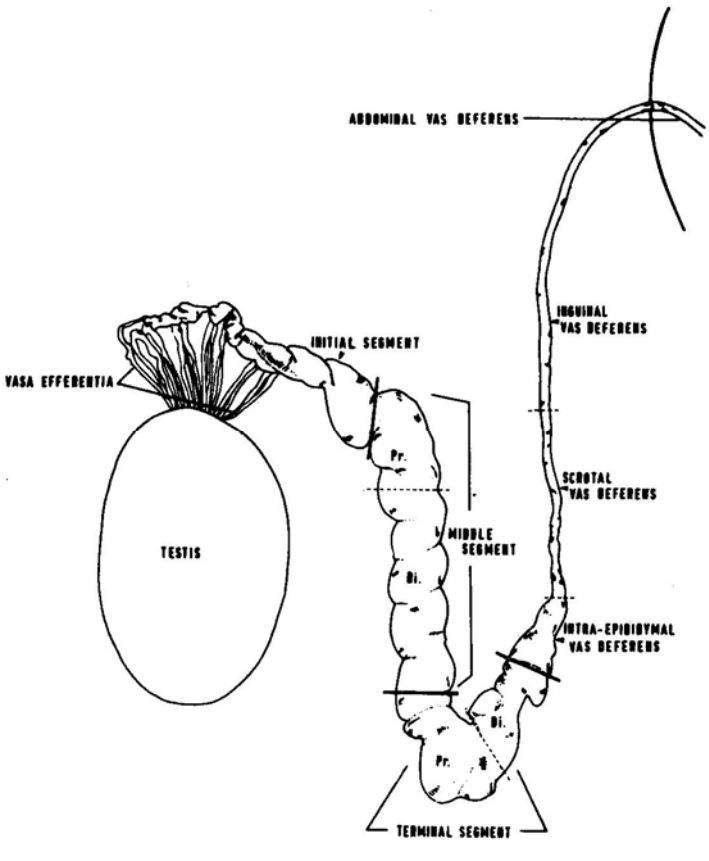
Sperm maturation

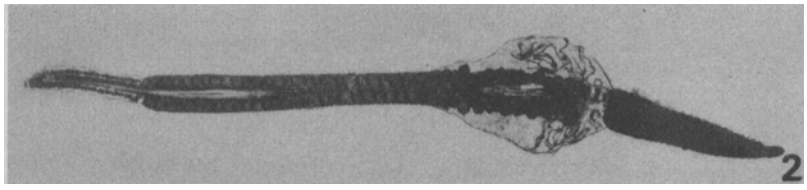
The cytoplasmic droplet occurs as a cuff of residual cytoplasm surrounding the post-acrosomal portion of the spermatozoa. In spermatozoa present in the intial segment of the epidymis, the cytoplasmic droplet is attached to the proximal portion of the midpiece of the spermatozoa (figure 2). The plasma memrane in most of the spermatozoa in the initial segment lies in close apposition to the acrosomal membrane.

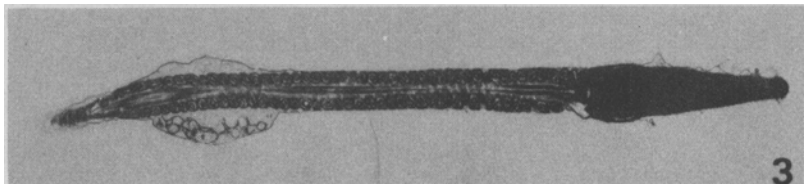
Some of the spermatozoa from the proximal-middle segment (figure 1) of the epididymis show a translocation of the cytoplasmic droplet to the distal end of the midpice of the spermatozoa. In the distal-middle (figure 1) as well as in the terminal segments of the epididymis, the cytoplasmic droplet, in most of the spermatozoa, is located in the distal part of the midpiece (figure 3). Coincident with this translocation, the volume of the cytoplasmic droplet decreases and the plasma membrane of the spermatozoa in the head region is wrinkled and separated off from the acrosomal membrane.

Spermiophagy

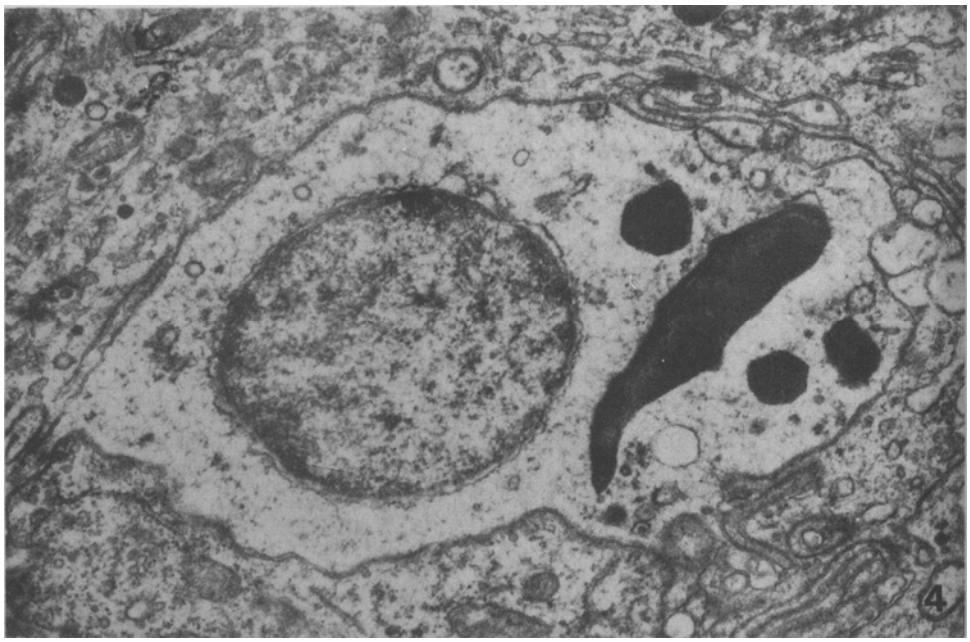
Remnants of spermatozoa occur within the cytoplasm of intraepithelial cells. These cells are pleomorphic with an euchromatic nucleus and they constitute a wandering population of cells. The other cytoplasmic inclusions of these cells comprise lysosomes, lipofuscin granules and lipid droplets (figures 4 and 5) which suggests that they may be similar to the ‘macrophage-like’ basal cells described in previous studies (Ramos and Dym, 1977; Prakash *et al*., 1979).

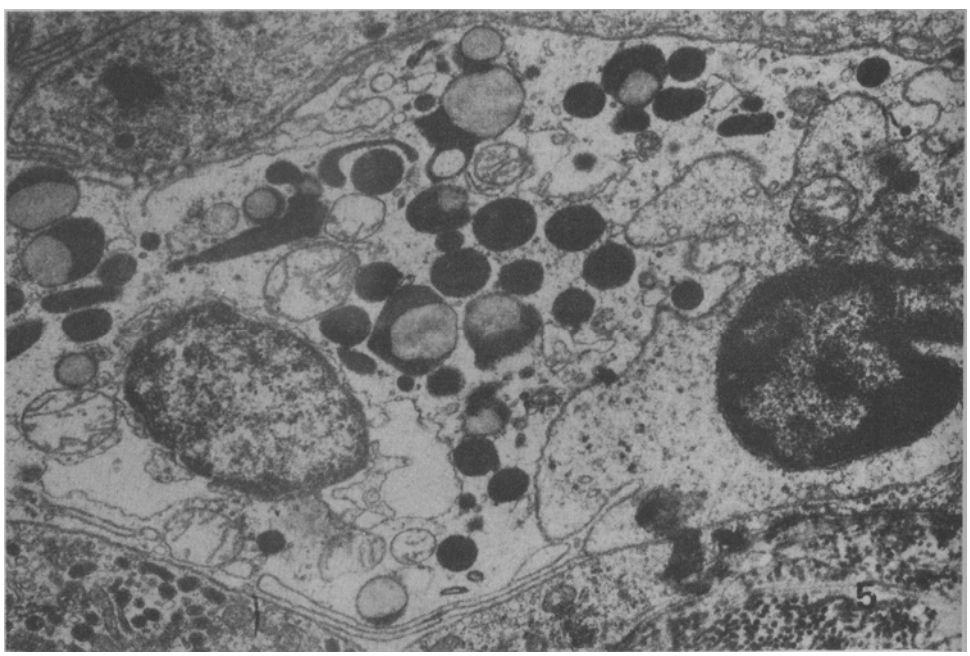






Figures 1-3. 1. Diagrammatic representation of the testis, the vasa efferentia, the epididymis and the vas deferens. The different regions of the epididymis taken for ultrastructural studies are indicated in the diagram. Pr: Proximal. Di: Distal. **2.** Ultrastructure of a spermatozoon from the initial segment of the epididymis. Note that the cytoplasmic droplet surrounds the neck of the spermatozoon. X9000. **3.** Ultrastructure of a spermatozoon from the terminal segment of the epididymis. The cytoplasmic droplet is translocated to the caudal part of the spermatozoon. X9000.





Figures 4-5. A part of a spermatozoon's head can be seen within an intraepithelial cell in the epididymal epithelium. X 10,000. **5.** Electron micrograph illustrating two adjacently lying intra-epithelial cells in the epididymal epithelium. A part of a spermatozoon and cytoplasmic inclusions comprising lipofuscin granules and lysosomes can be seen in one of the intraepithelial cells. X 10,000.

Discussion

A number of criteria such as, acrosomal changes, motility pattern of spermatozoa and changes in the biochemical contitutents of spermatozoa have been used by different investigators to identify events related to the maturation of spermatozoa (Orgebin-Crist *et al*., 1975; Bedford 1975). Microscopic changes reported in relation to the maturation of spermatozoa are the caudad migration of the cytoplasmic droplet and, coincident with this migration, there is a reduction in the volume of the cytoplasmic droplet and a wrinkling of the plasma membrane surrounding the head of the spermatozoa (Bloom and Nicander, 1961; Fawcett and Philips, 1969; Zamboni *et al*., 1971).

In rodents (Bedford 1975), the fully matured spermatozoa in the terminal segment of the epididymis lack cytoplasmic droplets since these are sloughed off. Alsum and Hunter (1978) came to a similar conclusion in the rhesus monkey, on the basis of their optical microscopic studies on spermatozoa in the cauda epididymidis. The present ultrastructural studies, however, indicate that the vast majority of spermatozoa in the terminal segment of the epididymis do not entirely shed off their cytoplasmic droplets–a situation that is similar to that described in the human epididymis (Bedford *et al*., 1973). The reasons for the difference between our results and those of Alsum and Hunter (1978) are not clear but may be due to differences in the microscopic methods used for observation.

The present studies have also shown that the ultrastructural changes attributed to the maturation of spermatozoa are first noticed in the proximal-middle segment of the epididymis where maximal fluid-resorption occurs (unpublished observations). It could well be that ionic shifts in the luminal fluid, resulting from fluid resorption (Crabo, 1965; Levin and Marsh, 1971) contribute to the caudad migration of the cytoplasmic droplet as has been suggested previously (Bedford, 1975). If this is so, then one can conclude that the maturation of spermatozoa in the rhesus monkey is almost completed by the time spermatozoa reach the distal middle segment of the epididymis.

The present observations have revealed that parts of spermatozoa occasionally occur within the cytoplasm of the wandering population of intraepithelial cells in the epididymis of intact, untreated adult male rhesus monkeys. The intraepithelial cells have features characteristic of a phagocytic function, *viz*., presence of lysosomes and lipofuscin granules which have been shown to stain meta-chromatically with toluidine blue (Prakash *et al*., 1979). The presence of spermatozoa within these cells suggests that these cells are capable of spermiophagy, in keeping with an earlier suggestion (Prakash *et al*., 1980), The question as to how the spermatozoa traverse the apically situated tight occluding junctions between the adjoining epithelial cells (Anand Kumar *et al*., 1980a) needs to be elucidated as also the functional significance of spermiophagy under normal physiological conditions.

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