Ultrastructure and Maturational Changes in Spermatozoa in the Epididymis of the Pigtailed Monkey, *Macaca nemestrina*

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Ultrastructural characteristics of the acrosome, postacrosomal region, plasma membrane, and cytoplasmic droplet in spermatozoa taken from the caput, corpus, and cauda epididymidis of the pigtailed macaque are described. The subdivision of the postacrosomal region into an anterior and a posterior segment is demonstrated. Maturational changes manifest in the caudal shift of the cytoplasmic droplet and swelling of the plasma membrane are observed during epididymal transit in this species and are similar to those reported for other monkeys. However, the changes in the rostral segment of the acrosome are more striking than any in other Old World monkeys studied to date. In the caput epididymidis, the acrosome is asymmetric because its apical segment extends well beyond the rostral edge of the nucleus and folds under it, giving the acrosome a small but distinct hook shape in sagittal section. In the corpus and cauda, the acrosome contracts down over the nucleus, resulting in the loss of the asymmetry of the contours of the sperm head, and the distinctive hook-shaped apical segment of the acrosome is no longer seen in sagittal section. On the basis of these findings, the pigtailed macaque appears to be a suitable primate model for morphologic analysis of structural variables during epididymal sperm maturation.

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For the scrotal eutherians, there have been relatively few reports on the progressive morphologic differentiation of spermatozoa during their passage through the epididymis. The most striking morphologic changes have been noted in the shape of the acrosome, and of these, the most conspicuous ones occur in the guinea pig, the chinchilla, and the bushbaby, a lorisoid monkey (Fawcett and Hollenberg, 1963; Fawcett and Phillips, 1969; Bedford, 1974). Lesser acrosomal changes have been observed in rabbits and in two species of Old World monkeys (Bedford, 1965; Bedford and Nicander, 1971). The purpose of this report is to call attention to the continuing morphologic differentiation of spermatozoa in the epididymis of the pigtailed macaque (*Macaca nemestrina*). Epididymal sperm maturation in this species is accompanied by acrosomal changes that are more pronounced than those in the other two Old World monkeys which have been examined to date and by changes in the position of the cytoplasmic droplet and swelling of the cell membrane similar to those which occur in other species.

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Materials and Methods

The epididymides of three healthy, sexually mature pigtailed macaques (*M. nemestrina*) were examined. The monkeys were sedated with an intramuscular injection of 100 mg of ketamine (Ketaset, Bristol). Thirty minutes later, general anesthesia was induced with 20 mg/kg of intravenous pentobarbital (Nembutal®, Abbott). In two of the monkeys, the thoracic cavity was opened carefully by midline incision to expose the heart. A 16-gauge stainless steel needle was introduced into the left ventricle and secured in place with ligatures. The right atrium was cut to permit escape of the blood and perfusate. After flushing with 1.8 liters of saline solution, the monkeys were perfused for approximately 20 minutes with 5% glutaraldehyde in 0.16 M collidine buffer. After perfusion, the epididymis was removed and processed for electron microscopy, as described below. Tissue from the epididymis of the third monkey was preserved for electron microscopy by immersion fixation.

Tissue from the caput, corpus, and cauda epididymidis was carefully diced into small cubes (no more than 2 mm^3) and transferred into 5% glutaraldehyde in 0.16 M collidine buffer (*p*H 7.4) at 4 C for 2 hours, according to methods described previously (Hoffer and Greenberg, 1978). The tissue was then rinsed in three changes of 0.2 M collidine buffer for 1 hour and postfixed in 1.3% OsO_4 buffered with 0.67 M collidine. After dehydration in a series of increasing concentrations of cold acetone, the tissue was embedded in Araldite. Sections showing silver to pale-gold interference colors were cut with a diamond knife on a Porter-Blum MT-2 microtome, stained with saturated aqueous uranyl acetate (Watson, 1958) and lead citrate (Venable and Coggleshall, 1965), and examined in a Phillips 200 electron microscope.

Results

Spermatozoa from the caput, corpus, and cauda epididymidis were examined with the electron microscope. No differences were observed between spermatozoa obtained from epididymides fixed by immersion and those fixed by perfusion. Maturational changes were not visible in the nucleus or in components of the flagellum in the three regions of the epididymis examined. In the observations which follow, attention is focused on changes in the shape of the acrosome, the plasma membrane of the sperm head, and the location of the cytoplasmic droplet during epididymal transit.

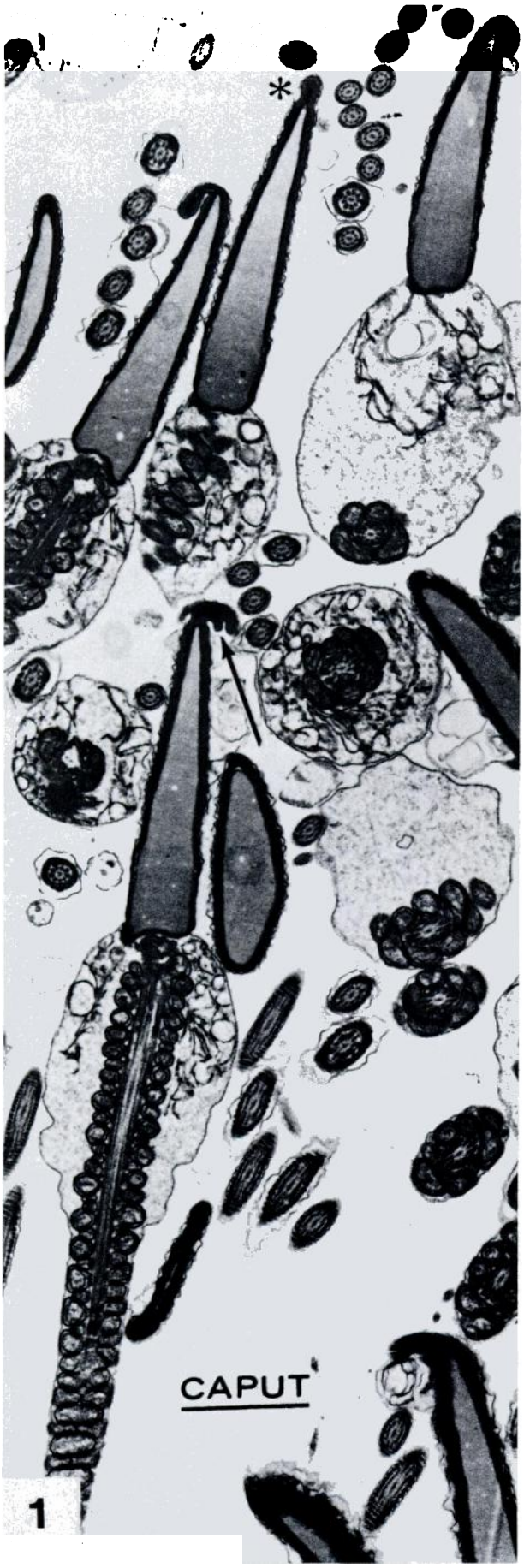
Acrosome

For the purpose of morphologic analysis, it is useful to divide the acrosome or acrosomal cap into three regions: an apical segment that projects beyond the anterior margin of the nucleus, a main or principal segment extending back over the anterior half of the nucleus, and a differentiated equatorial segment of varying length comprising the caudal portion of the cap. The following description is concerned only with the apical segment of the acrosome since neither the principal nor the equatorial segments were observed to undergo maturational changes in the present study.

In the caput epididymis of the pigtailed macaque, the head of the spermatozoon is asymmetric owing to the fact that the apical segment of the acrosome extends well beyond the rostral edge of the nucleus and folds under it.* The ventral groove so formed appears to be deepest anteromedially and to become more shallow as it follows the nuclear periphery in the posterolateral direction. In sagittal section (Figs. 1, 2), the acrosome exhibits a small but distinctly hook-shaped apical segment. In addition, sections passing through the midsagittal plane of the sperm head almost always show a small, characteristic protrusion on the ventral surface of the folded-over apical segment (Fig. 1) or on the surface of the principal segment in the region immediately subjacent to the apical segment (Fig. 2); in the infrequent transverse sections that intersect its narrow base, the protrusion is ridge-like in shape. This protrusion is not seen in transverse sections through more posterior regions of the principal segment or through the equatorial segment of the acrosome, a finding confirming that it is limited to the anteromedial region of the apical segment. There is no accumulation of flocculent material on the concave surface of the hooked apical segment, such as that in the sperm of the lorisoids (Bedford, 1974).

As in the spermatozoa of other species exhibiting rostral elongation of the acrosomal cap, the acrosome contracts down over the nucleus during epididymal sperm maturation, resulting in the loss of the asymmetric contours of the sperm head in the distal epididymal duct. In the corpus, the distinctive hook-shaped apical segment of the acrosome can no longer be observed in sagittal sections. The rostral extension of the acrosome is significantly reduced in size and here projects only slightly beyond the leading edge of the nucleus. There is an accompanying reduction in the volume of the subacrosomal space. In the cauda (Figs. 3, 4), no further changes in acrosomal shape can be detected, and the appearance of caudal sperm in

* For descriptive purposes, the ventral surface of the sperm head is here designated as the one that faces the folded-over apical segment.



this species is very similar to that of caudal sperm in other macaques (Bedford and Nicander, 1971; Fawcett and Phillips, 1969; Fawcett, 1970; Zamboni et al, 1971).

Plasma Membrane and Postacrosomal Region

As described for several other species (Bedford, 1965; Fawcett and Phillips, 1969; Fawcett, 1970), the closeness of fit of the cell membrane over the anterior portion of the sperm head changes as spermatozoa pass through the epididymis. In the caput epididymidis (Figs. 1, 2), the cell membrane adheres relatively closely to the underlying structures of the acrosomal cap, except over the apical segment of the acrosome, where it is often separated from the region surrounding the hooked tip of the acrosome. By contrast, in the corpus and cauda epididymidis (Fig. 3), the plasmalemma is more loosely applied over the entire area of the acrosomal cap, and the membrane in this region appears ruffled, highly irregular in outline, and separated from the acrosome by a clear area of variable width. However, the membrane in the postacrosomal region remains smoothly contoured and tightly applied to the underlying dense lamina.

The postacrosomal region is the same in sperm from the caput and cauda epididymidis. It is divided into anterior and posterior segments, as in man (Zamboni et al, 1971; Pedersen, 1972; Bedford, 1974). In its posterior segment, the postacrosomal dense lamina of sperm of the pigtailed macaque is homogeneous and completely fills the narrow space between the cell membrane and the nuclear envelope, whereas, in the longer anterior segment, the space between the plasma membrane and the nuclear envelope varies in width and is only partially filled with material of the dense lamina (Figs. 2, 4).

Fig. 1. Electron micrograph of spermatozoa from the caput epididymidis of the pigtailed macaque, showing several sagittal sections through the cytoplasmic droplet, nucleus, and hook-shaped apical segment of the acrosome. The small protrusion on the ventral surface of the folded-over apical segment (→) is readily observed in sections through the midsagittal plane of the sperm head. In one spermatozoon (*), the acrosome has already begun to contract down over the nucleus, suggesting that acrosomal maturation of this gamete is more advanced than that in most of the other sperm in this region of the epididymis. The cytoplasmic droplet, which contains numerous vesicular and membranous elements, extends caudad from the level of the posterior ring to a point located between one-third and one-half of the way along the length of the midpiece (×10,225).

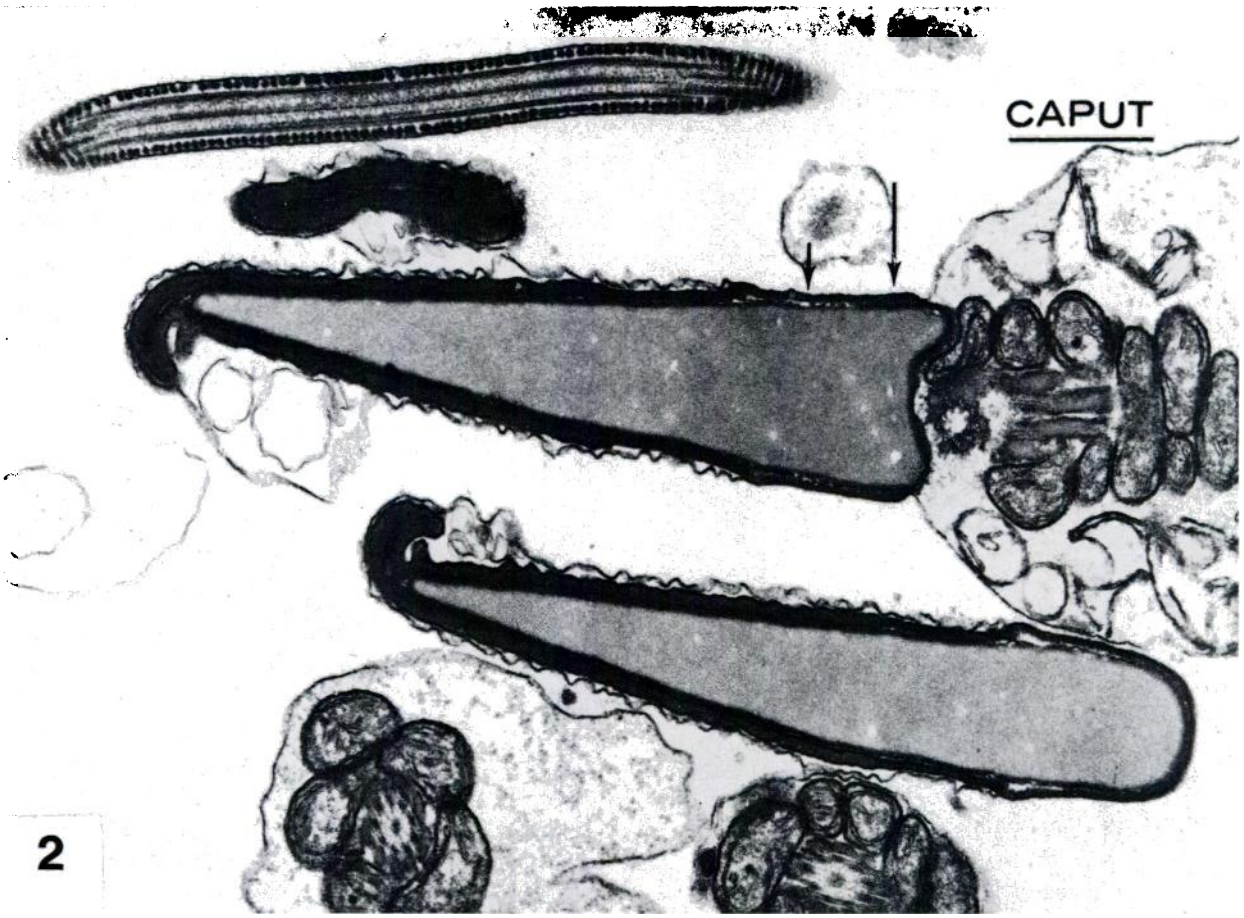


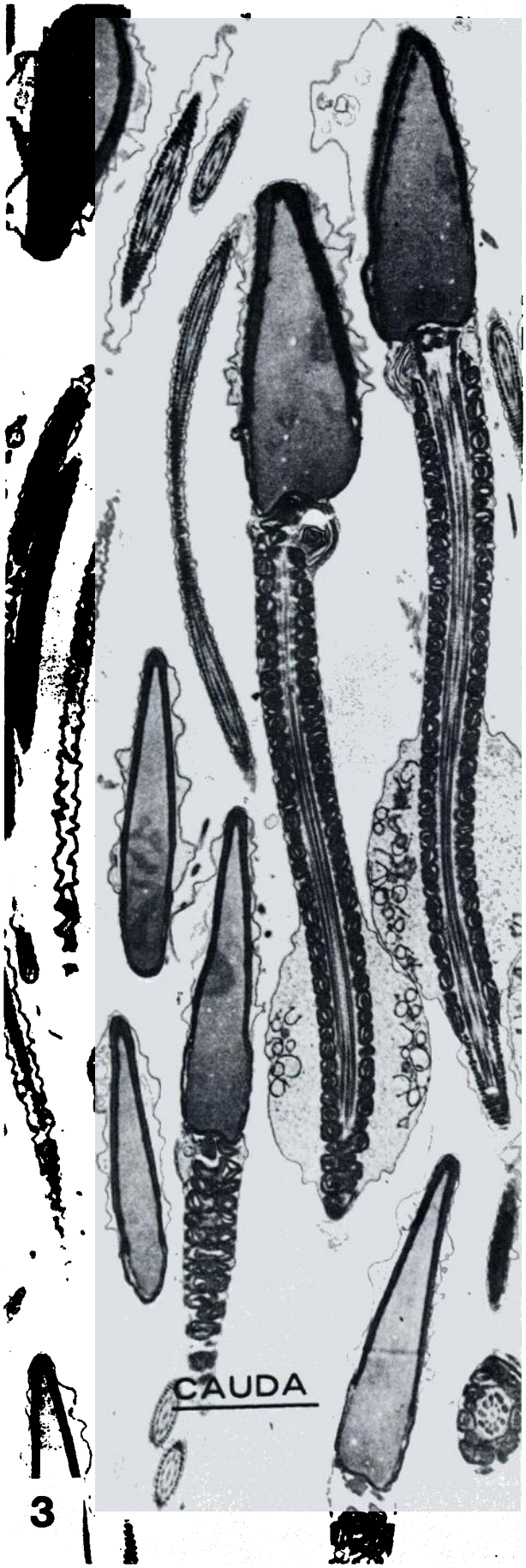
Fig. 2. Sagittal sections of the heads of two spermatozoa from the caput epididymidis showing the hook-shaped apical segment of the acrosome. Notice the small protrusion on the ventral surface of the principal segment immediately subjacent to the folded-over apical segment. The cell membrane adheres relatively closely to the underlying structures of the acrosomal cap, except over the apical segment of the acrosome, where it is separated from the region surrounding the hooked tip of the acrosome. In the posterior segment of the postacrosomal region of the nucleus (long arrow), the postacrosomal dense lamina is homogeneous and fills the narrow space between the cell membrane and the nuclear envelope, whereas in the longer anterior segment (short arrow), the space between the plasma membrane varies in width and is only partially filled with material of the dense lamina (×24,750).

Cytoplasmic Droplet

Unlike the cytoplasmic droplet of human spermatozoa (Zamboni et al, 1971), the cytoplasmic droplet in *M. nemestrina* has a relatively simple structural organization characterized by the presence of flattened vesicles, a few vacuoles with or without a pale flocculent content, occasional fragments of redundant nuclear membrane, and cytoplasmic ground substance (Figs. 1, 2, 3, 4).

In spermatozoa of the caput epididymidis, the cytoplasmic droplet extends caudad from the level of the posterior ring to a point located between one-third and one-half of the way along the length of the midpiece (Figs. 1, 2). Cytoplasmic droplets are seen in somewhat less than half of all transverse sections through the midpiece but are seen in every sagittal section that includes the entire length of the midpiece, a finding which suggests that all spermatozoa in the caput epididymidis of the pigtailed macaque possess a cytoplasmic droplet.

In the corpus and the cauda (the latter in Fig. 3), migration of the cytoplasmic droplet has already occurred, and the droplet is now situated at the caudal end of the midpiece, just anterior to the annulus. No other changes in the cytoplasmic droplet can be observed in this region, and here, too, every sperm seen in a sagittal section that includes the entire midpiece exhibits a cytoplasmic droplet.



Discussion

That the epididymis plays a significant role in sperm maturation is no longer disputed. Most of the maturational changes which sperm undergo in the epididymis are biochemical or physiologic in nature (Bedford, 1975). Attempts at correlating these changes with histologic regions or specific activities of the epididymal epithelium in scrotal eutherians have been hindered, in part, by the difficulty of recognizing maturational changes in spermatozoa at successive levels of the epididymal duct by direct morphologic examination. Thus, no morphologic changes in structures within the sperm tail have been recognized to date, despite the well-documented changes in motility patterns which are associated with sperm maturation and are dependent on these same organelles. With respect to the maturation of the acrosome, however, striking changes have been noted, but only in the epididymides of hystricomorph rodents (Fawcett and Hollenberg, 1963; Fawcett and Phillips, 1969) and of a lorisoid monkey (Bedford, 1974). Among families of Old World or anthropoid monkeys, the ultrastructural changes in the acrosome that have been reported in the epididymides of two haplorhine species, *Cebus apella* and *Macaca fascicularis* (Bedford and Nicander, 1971), are minor. In the present study, we describe ultrastructural changes of sperm in the caput, the corpus, and the cauda of the pigtailed macaque, *M. nemestrina*. While the changes in the position of the cytoplasmic droplet and in the swelling of the cell membrane reported here are not unique (see Fawcett, 1970; Bedford, 1975, 1979), the changes in the rostral segment of the acrosome are much more striking than those in the two other Old World monkeys studied to date (Bedford and Nicander, 1971; Bedford, 1974). This information is relevant to physiologic and biochemical studies of sperm maturation in the primate epididymis. It invites consideration of *M. nemestrina*, a heretofore little-studied species, as a primate model for morphologic analysis of structural variables in the maturation of epididymal spermatozoa.

Fig. 3. During epididymal sperm maturation in the pigtailed macaque, the acrosome contracts down over the nucleus. In the cauda epididymidis, as shown here, the head of the spermatozoon is no longer asymmetric in sagittal sections. The rostral elongation of the acrosome is significantly smaller than in the caput, projecting only slightly beyond the leading edge of the nucleus. The cytoplasmic droplet is displaced to the caudal end of the midpiece, just anterior to the annulus; its ultrastructural characteristics are otherwise unchanged (×9167).

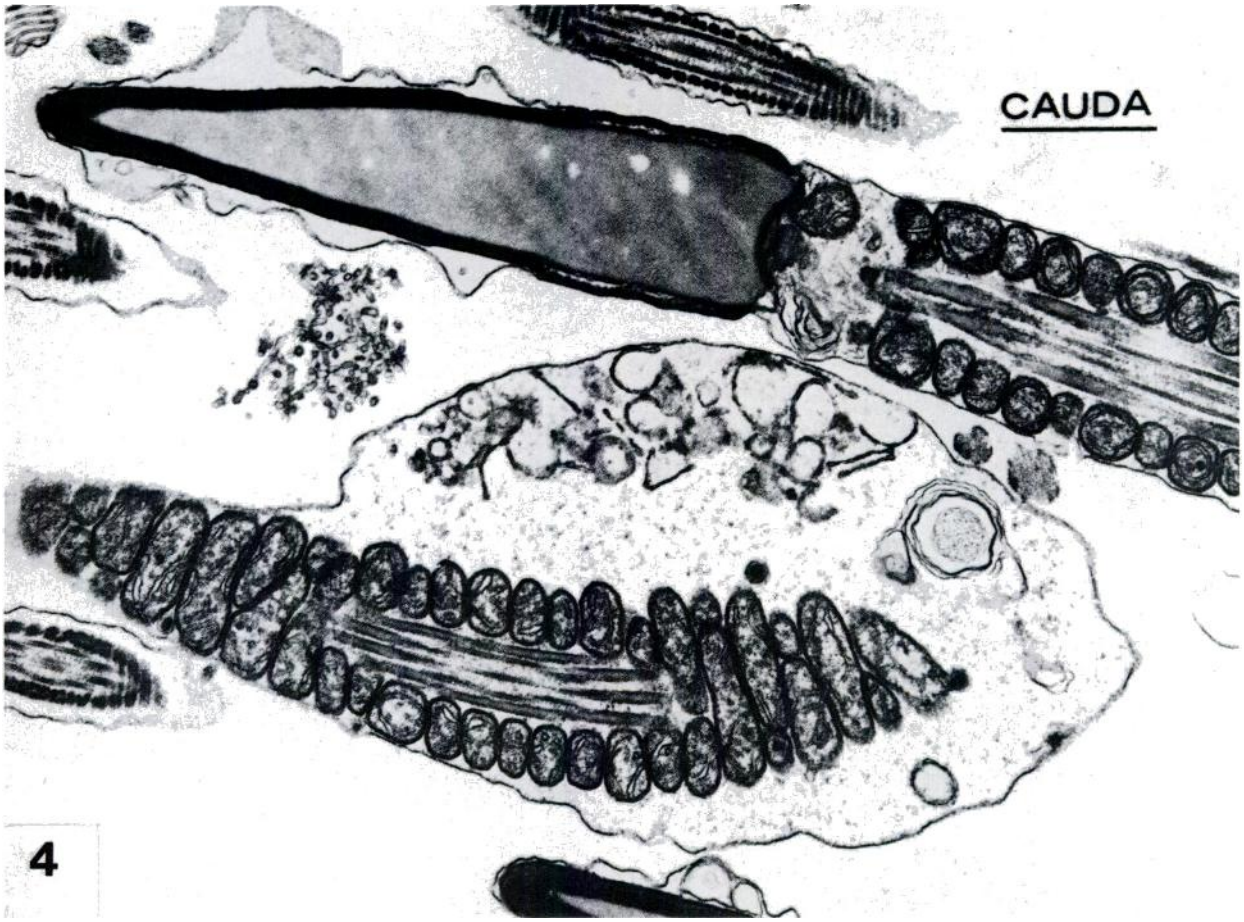


Fig. 4. Sagittal section of the head of one and the cytoplasmic droplet of another spermatozoon, from the cauda epididymidis. The acrosome is symmetric in this region of the epididymis, and the rostral extension of its apical segment projects only slightly beyond the leading edge of the nucleus. The volume of the subacrosomal space is significantly reduced (compare with Fig. 2). The plasma membrane is more loosely applied over the entire area of the acrosomal cap, and the membrane in this region appears ruffled, highly irregular in outline, and separated from the acrosome by a clear area of variable width. The cytoplasmic droplet has shifted caudad and is no longer situated at the level of the posterior ring; its ultrastructure is the same as in caput spermatozoa (×24,400).

The functional significance of the changes observed in this study have not been elucidated. It is not known whether the continuing morphologic differentiation of the acrosome during epididymal transit is essential to the acquisition of full fertilizing capacity in those species in which the final form of the acrosome is incomplete at spermiation. In guinea pigs, acrosomal maturation seems to require the influence of some androgen-dependent activity of the epididymal epithelium (Blaquier et al, 1972), but whether this is also true in primates remains to be established. It is logical to assume that the swelling of the plasma membrane observed in this study and in the spermatozoa of many other species during epididymal transit (Fawcett, 1970) is an osmotic phenomenon related to demonstrable changes in permeability and surface charge (Hamilton, 1975), but the exact mechanism whereby the development of these characteristics enhances fertilizing ability is also unclear.

Migration of the cytoplasmic droplet from the neck to the end of the midpiece and its subsequent shedding occur to various extents within the epididymides of almost all animals studied to date (Bedford, 1975). In the present study, the droplet was retained in the epididymal regions examined. Ultrastructural studies of vasal and ejaculated spermatozoa are in progress to determine whether the cytoplasmic droplet is indeed shed in this species.

Although differentiation of the postacrosomal region into anterior and posterior segments has been noted in human spermatozoa (Zamboni et al,

1971; Pedersen, 1972; Bedford, 1974), it is reported here in the monkey for the first time. In the posterior segment of the postacrosomal region, the space between the cell membrane and the nuclear envelope is relatively narrow and almost entirely occupied by material of the postacrosomal dense lamina, while in the longer anterior segment, the lamina is separated from the nuclear envelope by a clear space of varying width. The ultrastructural characteristics of these segments do not appear to change during epididymal transit either in man or in the pigtailed macaque, but the presence of these segments in the postacrosomal region of the epididymal spermatozoa in the present study is noteworthy because of the important role which this region of the sperm head is believed to play in fertilization.

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