ASYMMETRY OF SPERMIATION AND SPERM SURFACE CHARGE PATTERNS OVER THE GIANT ACROSOME IN THE MUSK SHREW SUNCUS MURINUS

G. W. COOPER and J. M. BEDFORD

From the Departments of Obstetrics and Gynecology and Anatomy, Cornell University Medical College, New York 10021

ABSTRACT

Spermatozoa of the shrew Suncus murinus, a mammal with abdominal testes, exhibit four unusual features: a giant acrosome; a dorsoventral asymmetry of their spermiation; a dorsoventral asymmetry of their head surface character; and also apparent surface maturity as they enter the epididymis. A Sertoli cell-periacrosomal cisternal complex envelops the giant acrosome during spermatid maturation. Spermiation is heralded by asymmetrical disorganization of the subplasmalemmal components of this complex and is completed by retraction of the Sertoli cell from the ventral and then the dorsal face of the spermatid head. This sequence of release is correlated with an asynchronous acquisition of negative surface charges on the spermatid head—demonstrable on glutaraldehyde-stabilized cells by the binding at pH 1.8 of positively charged colloidal particles of ferric oxide. Mature epididymal spermatozoa exhibit an asymmetry in the patterns of distribution of bound colloid over the dorsal vs. ventral surfaces of the sperm head, as well as regional differences between the tail midpiece and principal piece. Surface distributions of anionic residues and lectin (Con A)-binding sites characteristic of mature Suncus spermatozoa are demonstrable within the testis, unlike the situation in most mammals where distinct modifications of the sperm surface occur during epididymal passage.

In all mammals studied to date, spermatozoa entering the epididymis from the testis are incompetent to fertilize ova and acquire this ability as they pass through the epididymis. Such functional maturation must involve change in several structural and biochemical characteristics, only some of which are elucidated (3). Chemical modifications of the surface overlying the mammalian sperm head and tail have been demonstrated during epididymal transit. These were first detected at physiological pH as a difference in the electrophoretic mobility and orientation of populations of cooled rabbit spermatozoa recovered from the caput and cauda epididymidis, respectively (2). Subsequently, regional differentiation of the sperm surface during epididymal passage has been confirmed in the rabbit and other scrotal mammals, using positively charged ferric oxide hydrosols at pHs of less than 2.0. Such electron-dense colloids allow one to visualize the surface distribution of fixed negative charges which appear to be acquired in species-specific patterns on different regions of the sperm surface during transit through the epididymis (10, 6, 7, 31, 32). This, and additional
evidence of regionality in the distribution of lectin-(13, 27) and antibody-binding sites (21, 22, 24, 18), indicate that the surface of the mature mammalian sperm also differs abruptly in its properties over several anatomically distinct domains: the acrosome; the equatorial region and postacrosomal region of the head; and the midpiece and principal piece of the tail. Within each of these regions, moreover, the concentration and/or spatial distribution of the intramembranous particles of the plasmalemma can differ from one another in subregions along the sperm axis (15).

When it was considered that differentiation of the sperm surface in scrotal mammals is a post-testicular event, it seemed of interest to investigate the surface properties of spermatozoa in a testicular or nonscrotal mammal. We have approached this question first using Suncus murinus, the Asian musk shrew. This species is a member of one of the several orders of Eutheria whose testes are nonscrotal, being situated at the internal inguinal ring (subtengumental) in Suncus (12).

The first observation of note described herein is the unusually enormous acrosome of the Suncus spermatozoon which, in contrast to the acrosomes of certain other mammals (guinea pig, chinchilla [17] rabbit, monkey [5]), appears not to change its form as the epididymis is traversed. Second, unlike those of other mammals studied, Suncus spermatozoa in the caput epididymidis already have mature surface properties, at least as judged by the similarity of their colloid- and lectin-binding properties to those of sperm in the cauda epididymidis. Our observations on the surface properties of Suncus spermatozoa also provide evidence that differentiation of the molecular topology of the sperm surface can occur not only between subregions along the sperm axis, but also between opposing surfaces of the head, here designated as dorsal and ventral surfaces (see Results). Asymmetry of the properties of the dorsal and ventral surfaces of the mature Suncus spermatozoon head is first indicated in the testis during the process of sperm release from the Sertoli cell. Unlike that in the guinea pig and chinchilla (17) where the Sertoli cell appears to retract uniformly from the acrosomal region during spermiogenesis, spermiogenesis in Suncus begins with a differential dissociation of the Sertoli cell from the ventral surface of the sperm head. The relationship between the acquisition of detectable negative charges at the sperm surface and the process of sperm release from the Sertoli cell is detailed in this report.

MATERIALS AND METHODS

Suncus murinus males were supplied by Dr. G. L. Dryden, Department of Biology, Slippery Rock State College, Slippery Rock, Pa. The testes and epididymides of eight males were excised under ether anesthesia. Samples to be studied by transmission electron microscopy were immersed for 1 h at room temperature in 2.5% glutaraldehyde (Fisher Scientific Co., Pittsburgh, Pa., 50% biological grade) in a 0.1 M phosphate buffer (pH 7.2) containing 0.5% NaCl (23). Tissue slices were rinsed in 0.1 M phosphate buffer (pH 7.2) + 0.5% NaCl and postfixed for 1 h at room temperature in 1% OsO4 in the same buffer.

For studies of the distribution of cell surface negative charges, spermatozoa from three males were liberated by mincing testis, caput, and cauda epididymidis in Tyrode’s solution and washed once by slow-speed centrifugation in Tyrode's before resuspension in 2.5% glutaraldehyde in phosphate-NaCl buffer for 1 h at room temperature. Suspensions of glutaraldehyde-stabilized cells were then washed in phosphate-NaCl buffer and in distilled, deionized water and resuspended for 10 min in a solution of ferric oxide hydroxol (5% acetic acid (pH 1.8)) (20), the preparation of which has been described previously (11). Colloid-exposed cells were washed sequentially with 12% acetic acid solution (pH 2.4) and distilled, deionized water, and were postfixed for 1 h at room temperature in 1% OsO4 in phosphate-NaCl buffer (pH 7.2).

Tissues and cell suspensions processed for electron microscopy were dehydrated through a graded series of ethanol and propylene oxide and embedded in Epon 812. Thin sections were examined with a Philips EM 200 at 60 kV or a JEOL 100B at 80 kV. Sections of cells exposed to ferric oxide hydroxols were usually viewed and photographed without contrast staining, whereas those processed for conventional electron microscopy were stained for 10 min with lead citrate (28) and 3% uranyl acetate in 50% ethanol. All measurements of ultrastructural dimensions are based on calibrations made with carbon diffraction gratings of 463 nm spacing (Ladd Research Inc., Burlington, Vt.).

Estimates of the percent area of four different regions of the Suncus spermatozoon surface occupied by bound ferric oxide colloidal particles were made by the method of Loud (25), and by direct estimation on electron micrographs of sections tangential to sperm plasma membranes. For a direct estimate, a grid of 1 mm2 squares was drawn on photographs enlarged x 100,000. At this magnification, individual ferric oxide particles measured about 0.2-1 mm in dimension, corresponding to 2-10 nm in absolute dimensions (Figs. 6-8). The percent of each 1 mm2 on the grid occupied by an image or images of colloidal particles was estimated and summed for the total number of squares to give a direct estimate of the surface area occupied by colloidal particles. Loud’s method and direct estimates gave similar values for the mean percent of area occupied by bound colloidal particles over different regions of the
TABLE 1

Mean Percent Area ± SE of the Plasma Membrane over the Head and Tail Regions of Suncus Spermatozoa Occupied by Bound Ferric Oxide Particles

<table>
<thead>
<tr>
<th></th>
<th>Head-acrosome</th>
<th>Tail regions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean % of 10 μm²</td>
<td>Mean % of 1.0 μm²</td>
</tr>
<tr>
<td>Dorsal surface</td>
<td>27.4 ± 1.0</td>
<td>Midpiece</td>
</tr>
<tr>
<td>Ventral surface</td>
<td>26.3 ± 1.0</td>
<td>Principal</td>
</tr>
</tbody>
</table>

Data based on direct measurements on electron micrographs of the distributions of ferric oxide particles bound at pH 1.8 to spermatozoa isolated from the cauda epididymidis of three Suncus males.

Sperm morphology. Data were obtained by direct estimation of the mean percent particle distribution over 10 μm² of the dorsal and ventral surfaces of the Suncus spermatozoon head and over 1 μm² of the midpiece and principal piece of the tail (Table 1).

Concanavalin A (Con A) and wheat germ agglutinin (WGA) conjugated with fluorescein isothiocyanate (fl-Con A and fl-WGA, Miles Laboratories, Inc., Elkhart, Ind.) were used to detect lectin-binding sites on Suncus spermatozoa. Spermatozoa from the testis and successive regions of the epididymis of two males were fixed for 30 min in 2% formaldehyde (generated from paraformaldehyde) in 0.1 M phosphate buffer (pH 7.2) + 0.9% NaCl (PBS). Fixed cells were washed in PBS containing 1% bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, Mo.) and exposed for 30 min to solutions of either lectin in 1% BSA in PBS at concentrations of 13-25 μg/ml. Sperm were washed by microfuge centrifugation in three changes of 1% BSA in PBS and populations from respective epididymal regions and testis were examined with a Leitz incident light fluorescence and phase-contrast microscope, using selection excitation filters for FITC (2 × KP490) and suppression filters TK510/515. The specificity of binding of fl-Con A to epididymal and testicular spermatozoa was checked by the inclusion of 0.1 M α-methyl-D-mannoside (Sigma) in the fl-Con A incubation and in the washing solutions.

RESULTS

Sperm Morphology

The form of the Suncus spermatozoon is illustrated in Fig. 1. The content of the acrosome, translucent under phase-contrast optics, appears separated from the dense nucleus by a refractile margin corresponding to the subacrosomal space (Fig. 2). The asymmetry of the rostral projection of the subacrosomal space has been used as one of three facets defining the dorsoventral axis of the flattened sperm head. The nucleus of the Suncus spermatozoon occupies about 4.5-5 μm of a total length of 21-24 μm for the head, as measured in the midsagittal plane. The inner acrosomal membrane extends over the anterior four-fifths of the nucleus in this plane, and the posterior margin of the acrosome ends at the site of an indentation of the nuclear surface (Fig. 2). The postacrosomal “cap” is located over the remainder of the nucleus, and the posterior limit of distribution of the subplasmalemmal material in this region is correlated with a major discontinuity in colloid-binding patterns between the head and tail. The sperm tail is about 110 μm in length, of which some 65 μm are occupied by the mitochondrial sheath of the tail midpiece.

The dorsoventral axis of the flattened sperm head has three morphological features which permit the arbitrary designation of one face of the head as the ventral surface. These features are: (a) the rostral projection of the subacrosomal space which inclines toward the ventral surface of the acrosome (Fig. 2); (b) the electron-translucent gap separating the plasma membrane from the outer acrosomal membrane over the ventral surface and margin of the acrosome (Figs. 2, 11); and (c) the falciform base of the nucleus which extends farthest posteriorly on the ventral surface in sagittal sections (Fig. 2).

Colloid-Binding Patterns of Epididymal Spermatozoa

Caput and cauda epididymal spermatozoa exposed to ferric oxide hydrosols at pH 1.8 have identical surface properties revealed as distinct distribution patterns of colloidal particles on the dorsal and ventral surfaces of the sperm head, and on the midpiece and principal piece of the tail (Table I; Figs. 3 and 6-8). Colloid-binding properties of the plasma membrane differ, however, over the entire ventral vs. dorsal surfaces of the head. The dorsal surface consistently bound colloid in an aggregated pattern of particle distribution (Figs. 3, 6), in which the dimensions of individual particles...
ranged from about 2 to 10 nm. There were areas of 100 nm or more in width on the dorsal surface that were free of bound colloid. On the ventral surface, by contrast, colloidal particles were bound in a more dispersed pattern and the dimensions of colloid-free areas usually were smaller than on the dorsal surface (Figs. 3, 7). Electron micrographs of sections cut tangential to the sperm surface over the acrosomal region, the mitochondrial sheath of the midpiece, and the principal piece of the tail, respectively, were enlarged to ×100,000 and the mean percent of surface area occupied by bound colloidal particles was estimated (Table 1). Although the particle density and their pattern of distribution were clearly different on the ventral and dorsal surfaces of the acrosome (Figs. 3, 6, 7), there was no significant difference in the mean percent of area occupied by colloidal particles on either surface.

In agreement with results obtained with tangential sections, cross sections of the sperm head showed colloidal particles with different distributions on the ventral vs. dorsal surfaces (Fig. 4) as evidenced by frequent 100-nm gaps in the binding profile on the dorsal surface. These gaps correspond in dimension to the colloid-free areas seen on the dorsal face of the head in tangential sections. Occasionally, similar gaps appeared in cross sections of the ventral surface, but, as a reflection of the different charge distributions seen in planar views, these were not found with the same regularity. Colloidal particles were bound characteristically in clumps which extended up to 40 nm beyond the outer limits of the plasma membrane of the dorsal surface (Fig. 4). Fig. 3 demonstrates that the dispersed pattern of colloid binding characteristic of the ventral surface extends around the curvature of the acrosomal margin to the dorsal surface where there is an abrupt transition to an aggregated pattern of colloid binding. Because of the small radius of curvature over the postacrosomal region, it was not possible to obtain frontal

![Figure 1](suncus_spermatozoon_from_cauda_epididymidis.png)

**Figure 1** Suncus spermatozoon from cauda epididymidis. The disproportionately large, heart-shaped acrosome is separated from the nucleus by a phase-translucent subacrosomal space. Arrow indicates the posterior limit of the midpiece mitochondrial sheath. Phase contrast after glutaraldehyde fixation. Bar = 10 μm. × 1,350.

![Figure 2](sagittal_section_through_head_of_suncus_spermatozoon_fixed_in_situ_in_the_cauda_epididymidis.png)

**Figure 2** Sagittal section through the head of a Suncus spermatozoon fixed in situ in the cauda epididymidis. The ventral (V) surface of the sperm head is distinguishable from the dorsal (D) by three criteria described in the text. The point of discontinuity between colloid-binding properties characteristic of the midpiece and head surfaces, respectively, occurs at the posterior limits (arrows) of the electron-dense subplasmalemmal material in the postacrosomal region. Uranyl acetate and lead citrate. Bar = 0.5 μm. × 30,000.
views of extensive areas of the postacrosomal membrane. In sagittal sections, however, there was no obvious difference in the pattern of binding at pH 1.8 over the postacrosomal region and the contiguous acrosomal surface on each face of the sperm head.

The mean percent of surface area occupied by colloidal particles was lower over the midpiece than the principal piece of the tail, although the general distribution patterns of particles bound on each region were similar (Fig. 8). The midpiece binding pattern extended from the posterior limits of the electron-dense perinuclear material lying beneath the plasma membrane in the postacrosomal region to the distal end of the mitochondrial sheath. Counts on sections tangential to the tail plasma membrane showed that about one-third of the principal piece surface was occupied by colloidal particles. This value, obtained for this region in all other mammalian sperm examined by us, appears to be the only constant feature of sperm surface colloid binding among different eutherian species. Ferric oxide binding characteristic of the midpiece, acrosomal, and postacrosomal regions.

**Figure 3** Planar view of ferric oxide colloid binding on the dorsal (D) and ventral (V) surfaces of the *Suncus* acrosome, presented in oblique section. Colloidal particles are bound in an aggregated pattern on the dorsal surface and in a more dispersed pattern on the ventral surface. The site of transition from the D to V patterns of binding occurs at the acrosome margin (arrow). Unstained. Bar = 500 nm. x 28,130.

**Figure 4** Transverse section through the head of a *Suncus* spermatozoon. Spaces between clusters of colloidal particles bound on the dorsal (D) surface of the acrosome correspond in width to the dimensions of the colloid-free areas seen on this surface in Fig. 3. Nucleus (N). Unstained. Bar = 500 nm. x 28,050.

**Figure 5** Spermatid isolated from the testis showing ferric oxide colloid binding at pH 1.8 exclusively over the ventral (V) surface of the acrosome before spermiation. Unstained. x 28,150.
Figure 6. Planar view of the pattern of colloid binding on the dorsal head surface in a section tangential to the acrosome plasma membrane. Ferric oxide colloidal particles are bound at pH 1.8 in elongated clusters. Bar = 100 nm. × 122,500.

Figure 7. Planar view of the pattern of colloid binding on the ventral surface of the Suncus acrosome showing the dispersed distribution of colloidal particle binding, as compared to that on the dorsal surface shown in Fig. 6. Bar 100 nm. × 122,500.

Figure 8. Tail pattern of binding at pH 1.8 of ferric oxide colloidal particles. Midpiece of tail, section tangential to the plasma membrane. Bar = 100 nm. × 121,200.
of the sperm head at pH 1.8 appears to be species specific and may differ radically among closely related groups of mammals.1

Testicular Spermatozoa and Spermatids

Suncus spermatozoa taken at successive levels of the epididymis have the same surface properties with respect to colloid binding. By contrast, testicular spermatozoa had head surface properties similar to those of epididymal spermatozoa. The principal and midpiece regions of all spermatid tails have colloid-binding distributions similar to those of epididymal spermatozoa, while both surfaces of the postacrosomal region bound colloid when spermatids remained associated with Sertoli cells after fixation, provided the Sertoli cell plasma membrane was not in close contact with this region. Over the acrosome, however, only the ventral surface bound colloidal particles before spermiation was completed. Such binding was seen frequently in cases where intact Sertoli cells were partially separated from the ventral face of spermatid heads. In contrast, colloid binding did not occur on the dorsal surface before completion of Sertoli cell dissociation at this interface. After physical separation of the spermatid from the Sertoli cell during isolation from the testis, the most common colloid-binding profile over the spermatid acrosomal region is one in which ferric oxide particles are exclusively bound on the ventral surface of the head (Fig. 5). The sequential acquisition of these negative charges, first over the ventral and then over the dorsal surface of the acrosome, could possibly reflect molecular events determining the asymmetrical dissociation of the Sertoli cell from the spermatid acrosome.

Lectin Binding

The binding affinity for fl-Con A or fl-WGA did not change in Suncus sperm taken from successive regions of the epididymis. As judged by their fluorescence, Suncus spermatozoa from the testis or caput or cauda epididymidis all bound Con A weakly but uniformly over the head and tail. Spermatozoa incubated with fl-Con A and α-methyl-d-mannoside were completely nonfluorescent. There was essentially no binding of fl-WGA to Suncus spermatozoa from any region of the male tract, with the exception of those sperm whose acrosome was obviously damaged or had detached from the head, in which cases the acrosome displayed a bright green fluorescence. Suncus spermatozoa exhibited no autofluorescence in the system used.

Spermatid-Sertoli Cell Periacrosomal Complex

Subsurface specializations appear within the Sertoli cell cytoplasm opposite the developing acrosome during the cap phase of spermatid maturation. These appear as parallel bundles of microfilaments beneath the Sertoli cell plasma membrane, and as continuous cisternae which appear to partition the subsurface microfilaments from the remainder of the Sertoli cell cytoplasm (Figs. 9, 10). The zone of contact between the Sertoli cell and regions of the spermatid plasma membrane posterior to the acrosome never exhibits these subsurface specializations. Before spermatid release, Sertoli cell processes holding three to five spermatids are divided into projections, each of which envelopes one or at most two spermatids (Figs. 12–15). Both the microfilament bands and subsurface cisternae persist around the acrosome up to the onset of spermiation.

Spermiation

Spermiation is signalled by visible changes over that part of the Sertoli cell in contact with the ventral surface of the spermatid acrosome. Sertoli cell dissociation at this interface begins coincidently with the appearance of negative charges demonstrable at pH 1.8 on the ventral surface of the sperm head. Localized blebbing of the Sertoli cell surface at sites where the subplasmalemmal microfilaments have either disappeared or have been displaced (Figs. 12–14) constitutes the first morphological indication of reduced adhesion between the spermatid and Sertoli cell. These blebs interpose themselves between the ventral surface of the head and the remainder of the differentiated Sertoli cell periacrosomal complex (Figs. 12, 14). Subsequent displacement and/or disorganization of the microfilaments and subsurface cisternae appears to destabilize extensive areas of the Sertoli cell surface, as indicated by multiple sites of blebbing. The ventral face of the acrosomal region

1Cooper, G. W., and J. M. Bedford. Unpublished observations.
remains in interrupted contact with these blebs at a
time when the acrosomal region on the dorsal
surface of the head is in contact with a largely
intact Sertoli cell periacrosomal complex (Figs. 12,
14). Negative charges ionized at pH 1.8 are not
demonstrable on the dorsal surface before retrac-
tion of the Sertoli cell. In most cases, subsurface
components of the periacrosomal complex associ-
ated with the dorsal surface do not break down to
the extent seen at the ventral surface (Figs. 14, 15).

**FIGURE 9** A segment of a *Suncus* spermatid acrosome (*Ac*) enveloped by a Sertoli cell periacrosomal
complex. Bands of microfilaments (arrowheads) constitute the subplasmalemmal elements of the
periacrosomal complex and are separated from the Sertoli cell cytoplasm (*Ser*) by a largely continuous
endoplasmic reticulum (open squares in cisternae). The ventral surface of the acrosome (*V*) is partially
dissociated from the Sertoli cell, while the dorsal (*D*) surface remains closely apposed to the Sertoli cell
plasma membrane. Bar = 200 nm. x 64,230.

**FIGURE 10** Transverse cut through a Sertoli cell process enclosing two spermatid acrosomes (*Ac*) by
individual periacrosomal complexes (*Pc*). The cytoplasm of the Sertoli cell is studded with cross sections of
microtubules arranged parallel to the long axis of the spermatids. Bar = 0.5 μm. x 15,850.

**FIGURE 11** Sagittal section through the acrosomes of two spermatozoa lying in register in the lumen of the
cauda epididymidis. The plasma membrane over the ventral (*V*) surface of the sperm head is separated from
the outer acrosomal membrane (arrows) by a distinct electron-translucent gap. This "gap" is one of three
morphological features used to establish the dorsoventral axis of the sperm head. Bar = 200 nm. x 56,250.
Different stages of impending spermiation of sister spermatids, with associated Sertoli cell processes, residual bodies (R), and two cap-phase spermatids (Spm). Spermatids 1 and 2 are segregated into individual cytoplasmic projections of the Sertoli cell. Dissociation of the Sertoli cell from the heads has occurred to varying degrees around spermatids 1, 2, and 4 at sites where blebs of the Sertoli cell cytoplasm (open triangles) are now interposed between the surface of the Sertoli cell and displaced components of the periacrosomal complex. Bar = 1 μm. × 9,470.
Spermiation is completed by sequential withdrawal of the Sertoli cell process, first from the ventral and subsequently from the dorsal surface of the spermatid head.

DISCUSSION

The spermatozoa of *Suncus murinus* display four unusual features. First, they have an unusually large acrosome whose proportions dwarf the sperm nucleus. Second, the surface of the mature head shows a dorsoventral asymmetry, first evidenced during spermiation in the preferential acquisition of negative charges on its ventral surface. Third, as a probable reflection of this asymmetry, spermatid release from the Sertoli cell first occurs over the ventral and then later along the dorsal surface of the head. Fourth, in contrast to previous observations on the epididymal sperm of other mammals, *Suncus* spermatozoa entering the epididymis subsequently show no change in affinity of the sperm surface for positively charged colloidal particles or for lectins (Con A or WGA) during epididymal passage.

The dorsoventrally asymmetrical character of the surface overlying the acrosomal and postacrosomal regions of *Suncus* spermatozoa indicates that, in this species, regional differentiation over the sperm head is even more complex than might have been supposed from studies on other mammalian spermatozoa (7, 32, 13, 27). The significance of a dorsoventral difference such as that described here is not known, but this asymmetry may direct the way in which spermatozoa interact with the egg and its vestments at fertilization.

Studies of the structural aspects of mammalian spermiogenesis and spermiation have revealed a periacrosomal complex of smooth endoplasmic reticulum and bands of microfilaments in the Sertoli cell cytoplasm adjacent to the acrosomal region of the spermatid head (26, 19, 14). Other than the absence of parallel lines of membrane fusion, the Sertoli cell-periacrosomal complex closely resembles the occluding junctional complexes at the base of adjacent Sertoli cells (14, 16). The subsurface components of the Sertoli cell-periacrosomal complex disappear before the onset of spermiation in the cat, mouse, guinea pig, and chinchilla (19, 17) and rabbit (26). In *Suncus*, however, the first indication of the onset of spermatid release is asynchronous displacement of these structures from their subplasmalemmal location in the Sertoli cell, along the ventral surface of the acrosome.

Dislocation of the components of the periacrosomal complex from their immediate subsurface position in the Sertoli cell can be considered as an important criterion for the initiation of spermiation in *Suncus*. That this is true for other mammals is indicated by morphological observations on the relationship between the time of disappearance of the periacrosomal complex and the onset of spermiation (19, 17, 26) and by our analysis of micrographs in studies purporting to demonstrate the “induction of spermiation” in the hamster and rat (8, 29, 30). Close inspection with a magnifying glass of the micrographs in the latter reports reveals that these authors have interpreted some of their micrographs incorrectly. First, in sections through the acrosomal region of hamster sperma-

**FIGURE 13** *Suncus* spermatid in early stage of spermiation. Disorganization of the Sertoli cell periacrosomal complex at the level of the spermatid nucleus (D, open triangle) is similar to that over the corresponding region of the head of spermatid 1 in Fig. 12. The components of the complex are beginning to be displaced from the Sertoli cell plasma membrane at the ventral margin of the acrosome (arrow). The large residual body (R) is connected at the neck region of the spermatid. Bar = 1 μm. × 12,000.

**FIGURE 14** A more advanced stage of the asymmetrical spermiation which shows earlier release of the ventral surface of the spermatid head. The components of the periacrosomal complex opposed to the ventral surface of the spermatid are displaced away (arrows) from the Sertoli cell plasma membrane by blebs of cytoplasm, while the periacrosomal complex opposed to the dorsal (D) surface remains intact. Bar = 1 μm. × 12,470.

**FIGURE 15** Cross section of spermatid head showing complete retraction of the Sertoli cell process from the ventral (V) surface of the acrosome. Arrow points to a whirl of endoplasmic reticulum which appears to have continuity with that of the periacrosomal complex opposed to the dorsal surface of the acrosome. Release of the spermatid is accomplished by continued curling up of the Sertoli cell process (at open triangle). Bar = 1 μm. × 12,870.
demonstrable difference in the distributions of dependent structural changes, on ultrastructural the suggestion that modification of the spermatid release in the rabbit has previously prompted toll cell dissociation (5). The Sertoli cell surface Con A or WGA of caput and cauda epididymidis morphology, and the character of the sperm changes in several features of the sperm cell. We arrows), when in fact these "spaces" are located within the Sertoli cells and surround subplasma-membrane microfilaments of persisting junctions between Sertoli cells and spermatid acrosomes. These comments have been included to illustrate how a failure to appreciate the role of the periacrosomal complex in Sertoli cell-spermatid adhesion can bring erroneous interpretations to the sequence of morphological events of spermiation.

The preferential acquisition of negative charges on one face of the spermatid acrosome in *Suncus* occurs in concert with the onset of surface blebbing and asymmetrical disorganization of the periacrosomal complex. An ultrastructurally visible increase in the thickness of the outer lamina of the spermatid plasmalemma coincident with spermatid release in the rabbit has previously prompted the suggestion that modification of the spermatid surface may be directly involved in spermatid-Sertoli cell dissociation (5). The Sertoli cell surface associated with *Suncus* spermatids does not bind significant amounts of ferric oxide colloid at pH 1.8, and thus, unlike several other cell types, appears not to have exposed sialic acid residues ionized at this pH as major terminal constituents of its surface glycoproteins. The focus of future investigations on the regulation of spermatid release should be directed to the existence and nature of changes in the surface chemistry of spermatids, as well as Sertoli cells, during spermiation.

Functional maturation of sperm in the mammalian epididymis has been linked with demonstrable changes in several features of the sperm cell. We have focussed previously on motility, on -S-S- dependent structural changes, on ultrastructural morphology, and the character of the sperm surface (4, 6, 9). The absence in *Suncus* of a demonstrable difference in the distributions of negative surface charge or the binding affinities for Con A or WGA of caput and cauda epididymidis sperm was unexpected in the light of sperm surface changes demonstrated consistently in the epididymis of all the scrotal mammals studied (2, 10, 7, 32). However, *Suncus* spermatozoa do appear to undergo functional maturation in transit through the epididymis. We have observed that an increasing capacity for progressive motility correlated with a change in position of the tail cytoplasmic droplet (from the neck region to the midpiece-principal piece junction) occurs in spermatozoa taken from successive levels of the epididymis. Moreover, with epididymal passage the nuclear chromatin and tail of the *Suncus* spermatozoon become progressively crosslinked by -S-S- bridges (9) as do those of other eutherian spermatozoa. The apparent absence of surface changes in *Suncus* sperm during their movement through the epididymis suggests that post-testicular sperm maturation is somewhat less complex in *Suncus* than in other mammals studied. We initially thought that adoption of a scrotal position by the epididymis might have increased the complexity of maturation in the epididymis, and that molecular changes at the sperm surface might be a sequitur of the evolution of the scrotal condition. That this notion is too simple an interpretation is indicated by the independent finding (J. M. Bedford and R. L. Miller, unpublished observations) of marked sperm surface changes in both the ferric oxide colloid- and lectin-binding affinities as a consequence of epididymal sperm passage in the hyrax *Procavia capensis* which has both testis and epididymis located completely within the abdomen.

The giant proportions of the acrosome seem to be peculiar to *Suncus*. The modest dimensions of the acrosome in elephant, hyrax, and whale, for example, indicate that its disproportionate size in *Suncus* is not related to the testicondid condition per se, and its unremarkable form in the mole and tree shrew make it unlikely that its size in *Suncus* is a common feature of the sperm of other insectivores. The significance of the manifold shapes and sizes of the acrosome in different mammals is not known, but the giant acrosome of *Suncus* spermatozoa again raises a question about the function of the acrosomal content in mammalian fertilization (1, 2).

We wish to thank Mrs. E. Sanidad and Ms. Miu Ying Kiu for their technical assistance and Dr. G. L. Dryden for supplying us with *Suncus* males.

This work was supported in part by National Insti-
tutes of Health grants HD-07257 and HD-09215 and a
grant from the Ford Foundation.

Received for publication 23 September 1975, and in
revised form 16 January 1976.

REFERENCES

1. Allen, G. J., M. W. H. Bishop, and T. E.
Thompson. 1974. Lysis of photographic emulsions by
mammalian and chicken spermatozoa. J. Reprod.
Fertil. 36:249-252.

properties of rabbit spermatozoa during passage
through the epididymis. Nature (Lond.). 200:1178-
1180.

penetration of spermatozoa through the investments
of the mammalian egg. In Physiology and Genetics of
Reproduction. E. M. Coutinho and F. Fuchs, editors.

fate of spermatozoa in the epididymis. In Handbook of
Physiology. Male Reproductive System. Sec. 7,
Vol. V. R. O. Greep and E. B. Astwood, editors.
American Physiological Society, Washington, D.C.
303-318.

structural changes in the acrosome and sperm mem-
branes during maturation of spermatozoa in the
testis and epididymis of the rabbit and monkey. J.
Anat. 100:527-543.

1973. The maturation of spermatozoa in the human

1972. Post-meiotic changes in the nucleus and mem-
branes of mammalian spermatozoa. In Genetics of the
Spermatozoon. R. A. Beatty and S. Gluecksohn-
Waecht, editors. Bogtrykkeriet Forum, Copen-
hagen. 69-89.

1973. Mechanism of sperm release. In Regulation of
Mammalian Reproduction. S. J. Segal, R. Crozier,
P. A. Corfin, and P. G. Condilfe, editors. Charles
C Thomas, Publishers, Springfield, Ill. 166-182.

disulfide bonds in the nucleus and accessory
structures of mammalian spermatozoa during matura-
tion in the epididymis. J. Reprod. Fertil. (Suppl.).
13:65-75.

10. Cooper, G. W., and J. M. Bedford. 1971. Acquisi-
tion of surface charge by the plasma membrane of
mammalian spermatozoa during epididymal matura-

density change in the vitelline surface following
fertilization of the rabbit egg. J. Reprod. Fertil.
25:431-436.

Sustained fertility after CdCl2 injection by a non-
scrotal mammal Sancus murinus (Insectivora, Sori-

Molecular probes of spermatozoan structures. Proc.

cells and germ cells. In Male Fertility and Sterility. R.
E. Mancini and L. Martini, editors. Academic

15. Fawcett, D. W. 1975. The mammalian sper-

16. Fawcett, D. W. 1975. Gametogenesis in the male:
Prospects for its control. In The Developmental
Biology of Reproduction. C. L. Markert and J.
Papaconstantinou, editors. Academic Press, Inc.,
New York. 25-53.

vations on the release of spermatozoa and on
changes in the head during passage through the

18. Fellous, M., G. Gackelin, M.-H. Buc-Caron, P.
Dubois, and F. Jacob. 1974. Similar location of an
early embryonic antigen on mouse and human
spermatozoa. Dev. Biol. 41:331-337.

junctional specialization of Sertoli cells in the semi-

1968. Positive and negative colloidal iron as cell

21. Hjort, T., and K. B. Hansen. 1971. Immuno-
fluorescent studies on human spermatozoa. I. The
detection of different spermatozoal antibodies and
their occurrence in normal and infertile women.

infertile couples. Analysis of sperm agglutinins and
immunofluorescent antibodies in 657 men.

of the central nervous system for electron micros-
12:160-186.

Hammerling, and M. P. Lardis. 1973. Topographi-
cal location of H-Y antigen on mouse spermatozoa

15:481-487.

of cell contacts in the seminiferous tubules of some

Cooper and Bedford Sperm Surface Asymmetry and Spermiation 427


