ACROSOMAL DISRUPTION IN SPERM

Freeze-Fracture of Altered Membranes

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ABSTRACT

"Capacitation" is a physiological event which alters sperm to permit rapid penetration through oocyte investments and fusion between gametes. Acrosomal "reaction," the physiological release of acrosomal contents, occurs after this facilitating process. In this study, acrosomal "disruption" of guinea pig and rat sperm was achieved in vitro by incubating sperm together with the follicular contents of superovulated mice. The samples contained both "reacted" and "disrupted" sperm. Thin sections of affected sperm revealed rupture and vesiculation of the plasma membrane overlying the acrosome, as well as loss of both the outer acrosomal membrane and the acrosomal content. Freeze-fracture revealed disintegration of the characteristic geometric patterns in regions of the acrosomal and plasma membranes thus disrupted and major modifications in particle distribution in the sperm tail. In the guinea pig, strands of 6-8-nm particles, usually confined to the plasma membrane of the midpiece, which overlies mitochondria, also appeared in the principal piece. Likewise, in rat sperm, bands of similarly small particles formed acute angles throughout the membrane of the principal piece. Compared with the membranes of control preparations, these membrane alterations are apparently a direct consequence of incubation with ovarian follicular contents.

Various regions of mammalian sperm have highly specialized functions (1–3). These areas also reveal distinctive membrane differentiations in freeze-fracture; this is true of sperm in the rat, rabbit, bull, water buffalo, monkey, and man (4–11). It is particularly true in the case of guinea pig sperm, one of the most elaborately differentiated in the species studied (12).

Portions of the plasma membrane of the guinea pig sperm head display a discoid "quilt" of elevations and depressions. That part overlying the equatorial segment of the acrosome has a serrated appearance, while islands of particles in rectilinear array inhabit limited portions of the membrane overlying the postacrosomal dense lamina. Also, large areas of the acrosomal membrane exhibit a cobblestone array of particles. The nuclear envelope composing the anterior limit of the implantation fossa contains unusually large particles. In the tail, the plasma membrane of the midpiece has sheaths made up of small particle strands over the mitochondria. Beyond the roughened anulus, the plasma membrane of the principal piece has a linear “zipper” of large particles opposite fiber 1. In the rat, the particle arrays are similar but less elaborate.

The implications of these focal variations are, first, that they participate in regional functional
specializations, and second, that segregating elements within the membrane are responsible for their organization.

Functional alterations would be expected to result in changes in the particle patterns. A major functional modification, prerequisite for the fusion of gametes, is capacitation (1, 13, 14), whereby sperm attain the ability to penetrate ova rapidly and fertilize them. One morphologic correlate of the reaction is disruption of a part of the plasma membrane of the head and areas of the outer acrosomal membrane (1, 15). This structural manifestation of a physiologic response (capacitation) can be induced in vitro (16), simulating its natural occurrence in the neighborhood of eggs in the fallopian tubes. The present paper is concerned with alterations which occur in the freeze-fracture appearance of the membranes during this event in rat and guinea pig sperm, particularly the demonstration of altered particle distribution in the tail and modifications in the appearance of the plasma and acrosomal membranes of the head. In the Discussion, we comment on the use of this model system for studying the general phenomena of membrane particle organization, mobility, and function.

MATERIALS AND METHODS

Materials

The sperm used in these studies were removed from the epididymides and vasa deferentia of sexually mature Sprague-Dawley rats and guinea pigs. Ovarian follicular contents were obtained from the fallopian tube fimbria of superovulated Swiss albino mice. 4 guinea pigs, 5 rats, and 27 mice, plus the following reagents, were employed: concanavalin A and 3,3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, Mo.); horse-radish peroxidase (Worthington Biochemical Corp., Freehold, N. J.); human chorionic gonadotropin (HCG) (Ayerst Laboratories, New York); gonadotropin from pregnant mare’s serum (PMS) (Sigma); K ferrocyanide (Fisher Scientific Company, Springfield, N. J.); tissue culture medium RPMI 1640 with added glutamine (Grand Island Biological Co., Oakland, Calif.).

Methods

COLLECTION OF FOLLICULAR CONTENTS AND SPERM, AND INCUBATION FOR ACROSOMAL DISRUPTION: Female mice were injected intraperitoneally with 6 IU PMS in 0.5 ml saline, and 48 h later with 6 IU HCG in 0.5 ml H₂O. The mice were killed 15–20 h after the injection of HCG for collection of the oocytes and adherent cumulus oophorus from the fimbriated end of the fallopian tubes. The oviductal contents were placed in CO₂-equilibrated RPMI 1640 tissue culture medium (pH 7.2), and sperm from the caudae epididymides and vasa deferentia of the male rats or guinea pigs was added. The sperm-oocyte mixtures in 50-μl aliquots were then transferred to Falcon plastic tissue culture dishes (Falcon Plastics, Division of BioQuest, Oxnard, Calif.) containing sterile mineral oil; the dishes had been kept in an incubator at 37°C and saturated with 5% CO₂ in air. Subsequently, they were placed on a tilting device for 3–5 h at 37°C before the cells were removed and processed for thin sectioning and freeze-fracturing (17).

Control preparations consisted of sperm incubated in the culture medium without the addition of oviductal contents, and also of sperm incubated for 10–30 min in physiological saline, 50% saline and 50% H₂O, or distilled water alone.

PREPARATION OF SPECIMENS FOR ELECTRON MICROSCOPY: The suspensions of sperm or follicular contents mixed with sperm were gently pelleted and then fixed and processed for sectioning and freeze-fracturing in the manner described previously (12).

OBSERVATIONS

Interaction between Sperm and Follicle Cells

After sperm are incubated in vitro with mouse oocytes for 3–5 h at 37°C in CO₂-equilibrated tissue culture medium, nearly all are motile. Frequently the plasma membrane overlying the acrosome breaks and sometimes vesiculates (Fig. 4). With the rupture of the outer acrosomal membrane (Figs. 6–8), the acrosomal content swells and leaks out. In the sperm head, only the plasma membrane of the postacrosomal segment and the inner acrosomal membrane remain intact (Figs. 1 and 2).

Whether or not individual acrosomes are disrupted, sperm are partially phagocytosed by granulosa cells of the cumulus oophorus and completely by PMN leukocytes and monocytes (if present). The granulosa cells are identifiable as such by their anular nexuses (18) and extensive gap junctions, which distinguish them from monocyte-derived macrophages. Various stages of contact (Figs. 2 and 3) can be detected in both thin-sectioned and freeze-fractured material. A feature both constant and common to all cells which contact and engulf sperm is the frequent contiguity of pseudopodia and ruffles of the phagocyte with the postacrosomal region of the sperm plasma membrane (Figs. 2 and 3), reminiscent of the site...
of contact and fusion between oocyte and sperm (1, 3). This is the area between the serrated elevation and the striated ring where sparse small islands of particles persist in rectilinear geometric array (12).

**Acrosomal Disruption**

After incubation with follicular contents to induce acrosomal disruption, no obvious alterations in freeze-fracture appearance are observed in the postacrosomal plasma membrane, the nuclear envelope, the implantation fossa, or the plasma membrane of the endpiece. However, modifications do occur in the patterned regions of the acrosomal and plasma membranes of the head and the plasma membrane of the mid- and principal pieces of the tail. None of the membrane particle changes observed in freeze-fracture are discernible in thin section.

**HEAD—PLASMA AND ACROSOMAL MEMBRANES:** In sperm removed from the epididymis and vas deferens, regions of the plasma membrane of the head in both guinea pigs and rats reveal highly organized patterns and more subtle rectilinear ones in zones of the acrosomal membrane (12).

After acrosomal disruption, comparable patterns have not been observed in the vesicles or membrane fragments adjacent to areas of plasma and acrosomal membrane rupture (Fig. 5). In the membranes retained by the sperm, and most evident in the rat, patterns of the plasma and acrosomal membranes progressively disappear after acrosomal disruption, so that in the majority of sperm examined at the 5-h interval, geometric patterns were only faintly recognizable (Figs. 10 and 11). Since random particles in these membranes persist, the original arrays were not the product of membrane particles in the usual sense, but somehow reflected soluble, membrane-associated substances or other configurations of the membrane constituents. Although the same phenomenon may take place in guinea pig sperm, intermediate stages in the dissolution of the hexagonal and rectilinear patterns are difficult to visualize: the observer either sees the pattern or he does not.

**TAIL—RAT:** Generally, the plasma membrane of the rat tail is unremarkable save for a diminutive large-particle zipper opposite fiber 1 in the principal piece (12). However, after acrosomal disruption, closely aggregating particles appear in bands of 8–9 nm, 4–12 rows wide (Figs. 12–14). The packing of particles in the bands at first assumes a rectilinear pattern (Figs. 23 and 24), and occasionally they extend across the entire width of the tail (Fig. 13). Commonly, the particulate bands are not oriented perpendicular to the axis of the sperm, but lie in pairs at acute angles (Figs. 13 and 14). No substructure has been identified in thin section which could account for the pattern of the particulate strips. However, when the membrane overlying the interstices between the ribs of the principal piece occasionally retracts during concanavalin A agglutination, the angles of the retracted areas are the same as those of the strips, suggesting that the interstices may be a common factor in the orientation of the particulate strips and sites of membrane infolding.

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**Abbreviations used in legends:**

A, acrosome  
M, mitochondrion  
a, "A" face, freeze-fracture  
N, nucleus  
AM, acrosomal membrane  
PM, plasma membrane  
b, "B" face, freeze-fracture  
V, vesicles

**Figure 1** Rat sperm undergo acrosomal disruption after in vitro incubation with fallopian tubal contents from superovulated female mice. In the head, only the postacrosomal segment of the plasma membrane (PM) and the inner acrosomal membrane (AM) remain intact. The sperm tails stay attached to the heads, but are not contained within the plane of sections shown in Figs. 1-3. × 49,000.

**Figure 2** A sperm partially ingested by a granulosa cell from the cumulus oophorus. Note that the sperm is grasped in the postacrosomal region, a constant feature of sperm-phagocyte and sperm-oocyte interaction. AM, acrosomal membrane. × 36,000.

**Figure 3** Freeze-fractures of rat sperm and a mouse granulosa cell. Numerous sperm (arrows) are in contact with this phagocytic cell. All the sperm heads have lost the major parts of their plasma and acrosomal membranes. × 15,000.

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FIGURE 4 Guinea pig acrosome. The earliest definitive stage of disruption is vesiculation of the plasma membrane (PM) overlying the acrosome (A). V, vesicles. × 26,000.

FIGURE 5 Freeze-etched preparation of a guinea pig sperm head at a stage of disruption comparable to that of the thin-sectioned acrosome in Fig. 4. The plasma membrane (PM), still in continuity with the sperm, retains its quilted hexagonal pattern, while the vesicles (V) (presumably derived from this membrane) appear relatively smooth and patternless. At later stages, no typical quilted plasma membrane pattern is visible at all. The acrosomal membrane (AM) also loses its usual linear-particle identity and may do so earlier than the plasma membrane. N, nucleus. × 41,000.
FIGURES 6–8 Acrosomes of three guinea pig sperm fixed during disruption. After the overlying plasma membrane forms vesicles, the outer acrosomal membrane ruptures, frequently curling (Fig. 6). The rarified portion of the acrosomal content becomes hydrated and seeps out (Figs. 6 and 7), followed by the escape of the denser content (Fig. 8). This low-magnification image suggests that the broken ends of the acrosomal (AM) and plasma membranes (PM) are adhesive (arrows). Fig. 6, x 12,000; Fig. 7, x 16,000; Fig. 8, x 19,000.

FIGURE 9 Portion of acrosomal membrane in a rat sperm displaying the common linear cobblestone pattern in an area adjacent to a region of random particles similar to that shown in the rectangle in Fig. 10. The pattern in this A face is ordinarily equally prominent in the B face. x 78,000.

FIGURES 10 and 11 After 3-h incubation with follicular contents (generally eliciting acrosomal disruption), the pattern in the control preparation (Fig. 9) is now very difficult to detect (Fig. 11), possibly reflecting the loss of a substance formerly intercalated in the lipid matrix of the membrane. Fig. 11 is an enlargement of the area shown in the rectangle in Fig. 10. Fig. 10, x 34,000; Fig. 11, x 100,000.
FIGURES 12-14 Freeze-fractures of the rat sperm tail plasma membrane. After acrosomal disruption, bands of small particles (arrows) occasionally appear in the membrane of the midpiece and commonly in the principal piece as shown here. The bands are often paired, forming acute angles, and sometimes extend across the entire width of the A face exposed by the fracture. Note that the particles of the bands are smaller than those which generally surround them. Fig. 12, × 59,000; Fig. 13, × 70,000; Fig. 14, × 170,000.
Such long, narrow bands are not seen in the mid-
and principal pieces of control sperm incubated in
culture medium without fallopian tubal contents.

TAIL—GUINEA PIG: Ordinarily, the highly
organized strands of small particles in the plasma
membrane overlying mitochondria (Fig. 15) are
sharply delimited near the swelling of the neck and
distally at the anulus (Fig. 18). The principal piece
has a well-developed longitudinal doublet of large
(\(> 9\) nm) particles opposite fiber 1 (Fig. 18, inset)
and a scattering of large particles in a somewhat
circular swirl. Generally, few small (6–8 nm) par-
ticles and certainly no strands are found there.

During incubation with oviductal content, one
among the several sequential changes in the particle
pattern of the tail appears to be correlated with
acrosomal disruption. The strands of particles in
the midpiece of some sperm disassemble (Figs. 16
and 17) and others draw apart, while similar
strands take form in the principal piece, where they

**FIGURE 15** Strands of small particles normally found in the A face of the midpiece plasma membrane in
guinea pig sperm. \(\times 120,000\).

**FIGURE 16** 3 h after incubation with ovarian follicular contents, the formerly orderly midpiece strands are
in total disarray. A few remain intact (arrows), but vary in orientation. \(\times 90,000\).

**FIGURE 17** Almost total dissociation of the strands is evident in this region, even though the plasma
membrane is still in close contact with the underlying mitochondrion (M). Ordinarily, sheaths of particle
strands only overlie the mitochondria (12). \(\times 82,000\).
are usually not observed (Figs. 19 and 20). These beaded strands are often associated with the longitudinal double row of larger particles (Fig. 19). Since scant particles and no particulate strands of that size were noted in the membrane of the principal piece before the acrosome reaction, it is possible that they migrated from the midpiece. In addition, patches of particles in rectilinear array, similar to those observed in the rat, are sprinkled throughout the plasma membrane of the guinea pig tail (Fig. 22).

**TAIL—CONTROLS:** Besides the development of closely gathered bands in the rat principal piece and the emergence of strands there in the guinea pig, other alterations in particle patterns are discernible in the plasma membrane of the tail after incubating the sperm with oocytes. These patterns, however, also exist after incubation at 25° or 35°C in tissue culture medium alone, in physiological saline, or in varying concentrations of saline and distilled water. Since sporadic acrosomal disruption may occur under these conditions (19), we cannot rule out that possibility. The most common pattern in the rat and guinea pig after the incubation of sperm in tissue culture media is illustrated in Figs. 22–24. Another pattern in the guinea pig principal piece, not customarily seen in sperm removed from the epididymis or vas deferens, but present in occasional sperm during all the control procedures employed, is comprised of the multiple, longitudinal, rectilinear arrays of large particles as depicted in Fig. 21.

As we indicated earlier, no striking alterations were observed in the equatorial or the important postacrosomal regions of the plasma membrane, nor in the implantation fossa.

**DISCUSSION**

**Acrosomal Disruption**

When sperm draw near to recently ovulated eggs, the outer acrosomal membrane and overlying plasma membrane fragment, creating openings to release enzymes of the acrosome. "Acrosomal reaction" (1, 15) is the term describing this process which serves to facilitate the penetration of sperm through the cumulus oophorus and the zona pellucida. In this paper, however, the term acrosomal disruption rather than reaction is used, since the morphologic (not the physiologic) consequences of plasma and acrosomal membrane rupture were the subject of analysis and mixtures of reacted and pathologically disrupted sperm were used. Seen in thin section, the major membrane alterations after incubating sperm together with follicular contents are found in the sperm head, whereas in freeze-fracture the most notable changes occur in the tail.

**HEAD:** In sperm removed shortly after incubation for acrosomal disruption, the plasma and acrosomal membranes they retain keep their distinctive appearance, i.e., an hexagonal, quilted pattern in the plasma membrane and a fine linear pattern in the acrosomal membrane. However, vesicles associated with the areas of disruption and presumably derived from fragments of the two membranes no longer have distinguishing features: they are relatively smooth, without pattern, and they have few particles. Later, after 3–5 h of incubation, intact sperm of both the guinea pig and rat still have distinctly identifiable plasma and acrosomal membranes, while the membranes of disrupted sperm have totally, or almost totally, lost their patterns. In the head, the loss of architec-

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**Figure 18** Juncture of the midpiece (right half of micrograph) and principal piece (left half) at the anulus in a control guinea pig sperm removed from the cauda epididymis. Observe particularly the sharp demarcation between the strands of 6–8-nm particles of the midpiece and the large 10–15-nm particles of the principal piece. The *inset* is a comparable A face through the plasma membrane opposite fiber 1, with the linear doublet of large particles which constitute the zipper (12). × 190,000; *inset*, × 150,000.

**Figure 19** After incubation of sperm for acrosomal disruption, the small particle strands are found in the principal piece and often abut against the zipper. The plasma membrane of the principal piece has far fewer large particles than that of the control sperm (Fig. 18). × 115,000.

**Figure 20** Incubated in the same manner as in the preceding figure, strands of small particles are randomly distributed in the A face (a) of the plasma membrane on the side opposite the zipper as well. The zipper is visible here in the B face (b). We interpret the emergence of these strands in the principal piece as a consequence of migration from the midpiece after acrosomal disruption. This pattern is not present in control sperm incubated in tissue culture medium or saline without follicular contents. × 76,000.

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FIGURES 21–24 Patterns of particles which are seen in freeze-fracture preparations of epididymal sperm incubated in tissue culture medium or saline, with or without follicular contents. These have no specific correlation to acrosomal disruption. Fig. 21: Guinea pig sperm principal piece. Linear arrays of large particles on the B face (b) were constant features in a small proportion of all preparations examined. a, A face. Fig. 22: Small aggregates of particles in rectilinear array can be found throughout the plasma membrane of the guinea pig sperm tail after incubation in tissue culture medium (even for 1 h) before fixation. Figs. 23 and 24: Rectilinear “waffle-iron” arrays of particles appear in the plasma membrane of the mid- and principal pieces of the rat sperm tail as well. In the rat, the arrays are often composed of small particles, identical to those which comprise the angulated bands in the principal piece after incubation with follicular contents. These two arrays are indistinguishable from those which we interpret as early stages in the formation of angulated bands and may indeed represent incomplete phases of this phenomenon. Fig. 21, × 51,000; Fig. 22, × 220,000; Fig. 23, × 120,000; Fig. 24, × 140,000.

The structural pattern is restricted, as far as we have observed, to portions of the plasma membrane overlying the acrosome and to the acrosome itself.

As previously discussed (12), loss of the patterned arrays may reflect the solubilization of enzymes intercalated within the hydrophobic matrix of the membranes or a rearrangement of membranous elements for expansion of the membrane. After acrosomal disruption, the membranes may stretch, but this has not yet been established.
Incisive interpretation of these observations demands correlative biochemical experimentation to resolve several questions: Are the enzymes associated with these membranes soluble under the experimental conditions employed? If so, is there a quantitative relationship between the amount of membrane incubated and the enzyme(s) recovered? Can the recovered enzyme(s) form patterns in artificial membranes similar to those of the native plasma and acrosomal membranes? Or do other membranes with like patterns simply lose their geometric configurations after expansion?

Significantly, the smooth areas and the rectilinear arrays in the plasma membrane where it overlies the postacrosomal dense lamina persist. Perhaps they do so in order to subserve the sperm's penultimate function, fusion with the oocyte plasma membrane (1, 3, 19).

TAIL: After acrosomal disruption in guinea pig sperm, the strands of particles in the plasma membrane of the midpiece disperse, apparently released from their position overlying mitochondria, and migrate to the principal piece. Mere induction of motility does not produce the same pattern of particulate strand disassemblage, nor has it been observed in populations of intact motile or quiescent sperm incubated under the same conditions of time, temperature, pH, oxygen tension, and suspension medium. Apparently, then, alteration in the particles of the tail is a consequence of acrosomal disruption. The precise event which initiates particle movement and reaggregation in guinea pig spermatozoa is not known, primarily because of acrosome and plasma membrane breakage and leaking of acrosomal enzyme content, both occurring during incubation procedures. Separation of the two phenomena of membrane disruption and enzyme leakage would require bathing intact spermatozoa with the content of acrosomes collected from other sperm. This has not yet been attempted. As a third potentially triggering factor, exposure of the sperm to enzymes or other substances present in the oviductal fluid of superovulated females could elicit the movement.

After acrosomal disruption, the strands of small particles which appear in the plasma membrane of the principal piece may not be those which were formerly observed in the midpiece. Other particles present in the membrane of the principal piece could potentially be organized into strands. We do favor the assumption, however, that the nascent strands in the principal piece derive from those in the midpiece. (Without cognizance of the midpiece strands' function [12], we are hard pressed to explain why they do disband and migrate.) Nevertheless, this assumption affords us a means of calculating the rate of particle movement within the plasma membrane, a potentially useful statistic for those interested in the biology of membranes. The parameters of distance and time are measurable. Distance can be determined by using the anulus as the nearest starting point of strands drifting tailward (the remote part of the neck representing the farthest point) and by measuring the length of membrane from the anulus to the farthest strand discernible in freeze-fracture after acrosomal disruption. The time of acrosomal disruption can be established by phase microscope observation of a small number of sperm mixed with excess fallopian tubal content. Directly after acrosomal disruption, the sperm can be rapidly fixed in an acrolein-glutaraldehyde mixture. With fixation at various intervals after disruption, and with enough fractures through the plasma membrane to expose the anulus and most of the principal piece, the rate of movement could be fairly well calculated.

The fact that the strands of small particles in the midpiece often abut against the longitudinal array of large particles in the principal piece after acrosomal disruption may be merely fortuitous; the strands may simply eddy at the least flexible portion of the membrane.

After acrosomal disruption in rat sperm, the function of the bands in the principal piece is likewise enigmatic. Their angulation and the spacing between fibrous ribs appear to match flat areas of plasma membrane retraction after agglutination of sperm tails with concanavalin A. The similarity in the bands' spacing and widths suggests that the particles give rigidity to focal areas of the membrane. Their significance in a broader sense, however, is unknown. Nonetheless, the generation of well-delineated aggregates of small particles in the principal piece in both species studied intimates that this phenomenon may be widespread in mammalian sperm and warrants further study.

Perhaps the particle migration observed in the guinea pig as well as the particle aggregation noted in the rat are the natural sequelae of degenerative changes. Interruption of the plasma membrane of the head may alter the state of the entire membrane matrix, modifying its fluidity and signaling.
the imminent death of the cell. The particles, possibly instrumental in maintaining the mobility of sperm until the ovum is contacted, are no longer needed. Released from their sites of activity, they now either flow about aimlessly or attain a physically stable position which has been determined by other factors. The motility of the guinea pig sperm tail could account for their drifting into the principal piece. The casual abutment of particles along the zipper supports this possibility.

In the rat, the paracrystalline arrays may be the most stable configuration of the particles, which may have become dispersed in their active state. The emergence of rectilinear arrays in culture media in a matter of time could support the latter view. Certainly, in both guinea pig and rat, an alteration in the restraining elements which formerly held the particles firmly in another domain is evident.

CONTROLS: Rectilinear arrays of particles aggregate both under experimental conditions and in the tissue culture medium containing control preparations. The formation of such aggregates during incubation is likely to occur in many membranes. In size and aggregate configuration, these clusters closely resemble previously described structures such as type III gap junctions (20), a similarity which emphasizes the need for careful examination of control material when such membrane modifications are being interpreted. The straight-line pattern could reflect degeneration or it could simply be a common configuration difficult to categorize in terms of a specific function on the basis of architecture alone.

At present, these assemblies of rectilinear arrays in both the rat and guinea pig do not lend themselves to measurement of the rate of particle movement. Their size (sometimes similar to that of other, surrounding particles), their unknown origin (anywhere in the plasma membrane), and their lack of distinguishing formation en route while they are migrating as individual particles preclude any advantages over red blood cell or lymphocyte models of particulate movement. If, however, the particles are antigen-specific or can be tagged in other manners, they too could become a useful model of membrane particle flow. The simple attributes that sperm are elongate, highly polarized cells, millimeters in length, make them auspicious for such studies. Granted enough hallmarks for precise localization both in fractures and in thin-sectioned preparations, plus sufficient lengths of membranes for facilitating accurate measurements, sperm have the potential for yielding more information than can be gained from studies limited to spherical cells.

The multiple linear arrays of particles in the principal piece of some guinea pig sperm imply that a subpopulation of sperm exists. Our impression is that this subpopulation is best represented in groups of motile rather than quiescent sperm, and is independent of the type of medium containing sperm suspensions. Suspended in physiological saline for 2 h, many sperm from the vas deferens become motile, and often some exhibit this pattern. Currently, we know too little about this sort of array to speculate on its functional significance.

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