Plasma-membrane Diversity in a Highly Polarized Cell

DANIEL S. FRIEND
Department of Pathology, University of California School of Medicine, San Francisco, California 94143

INTRODUCTION

Plasma-membrane Diversity

In their classical experiments on membrane fluidity, Frye and Edidin (1) triggered further research on the fluid mosaic model of membrane structure (2) in numerous other laboratories. Since then, investigators in this field have also emphasized the planar, functionally specialized mosaics within the generally fluid bilayer of the membrane. These mosaics comprise areas where specific groups of lipids, proteins, glycoconjugates, and sterols aggregate to mediate specific functions. To cite several different facets of the membrane-domain concept, we can recall the now familiar work of Goldstein et al. (3) on the distinctive participation of coated pits in receptor-mediated endocytosis. Other thought-provoking membrane regionalities are the rosette-particle arrays instrumental in mucocyst secretion in *Tetrahymena* (4), the need for specific lipids for the complete activity of cytochrome *c* oxidase and other membrane-related enzymes (5), and the variations of phospholipid content in continuous membrane systems. (Consider the nuclear envelope and the rough-surfaced endoplasmic reticulum [6].)

The Symposium on Plasma-Membrane Diversity (see footnote) involved the existence and topography of such polysaccharide, lipid, and protein mosaics in the plasma membrane. We addressed ourselves to the broad questions: Do our methods and techniques verify the presence of domains in living cells? Where these membrane domains apparently exist, are their structure and composition pertinent to specific membrane-functions? How are domains sustained in a supposedly fluid environment? And when they reside in the depth as well as the plane of the bilayer, can we manipulate them to modify plasma-membrane and cell function?

Plasma-membrane Diversity in a Highly Polarized Cell

Since motile sperm were first observed by Leowenhok via light microscopy more than 300 years ago, we have been aware that the spermatozoon is a highly polarized cell. Austin (7) and Bedford and Cooper (8), among others, authoritatively demonstrated that the various regions of this cell served different functions. For instance, on the anterior part of the head, the plasma membrane fuses with the underlying acrosome, thereby freeing enzymes for sperm penetration to the surface of the egg. And in the equatorial and post-acrosomal segments, the membrane adheres to and merges with the egg's plasma membrane. Housing mitochondria, the mid-piece provides the cell with energy, while the principal-piece propagates the flagellar wave. Fawcett (9), by electron microscopy of thin sections, described the major architectural features of each functional area in guinea-pig sperm. More recent observations, obtained by freeze-fracture, have revealed the complexity of this structural mosaicism of the plasma membrane itself in the many and diverse "working" regions of the cell (10–13).

The central concerns of my paper are: (*a*) numerous domains of the guinea-pig sperm plasma membrane—lipid as well as protein—are morphologically detectable; (*b*) contemporary cytochemical techniques permit us to identify high concentrations of several lipid-classes in this particular plasma membrane; and (*c*) correlating the specific structural patterns with composition (where it is known) and function (when it is known) may warrant generalization about the plasma membranes of many secretory cell-types.

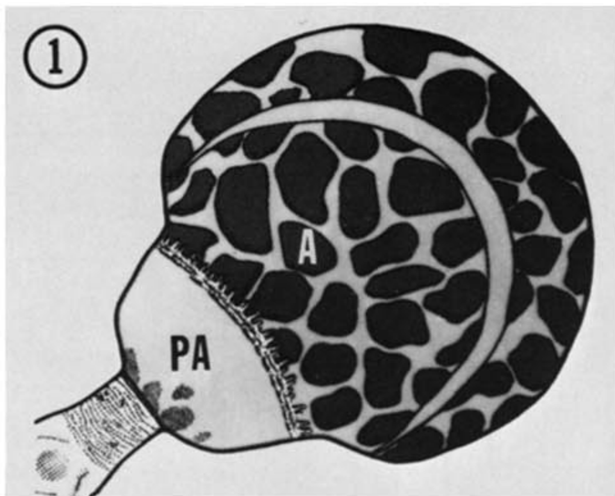
RESULTS AND DISCUSSION

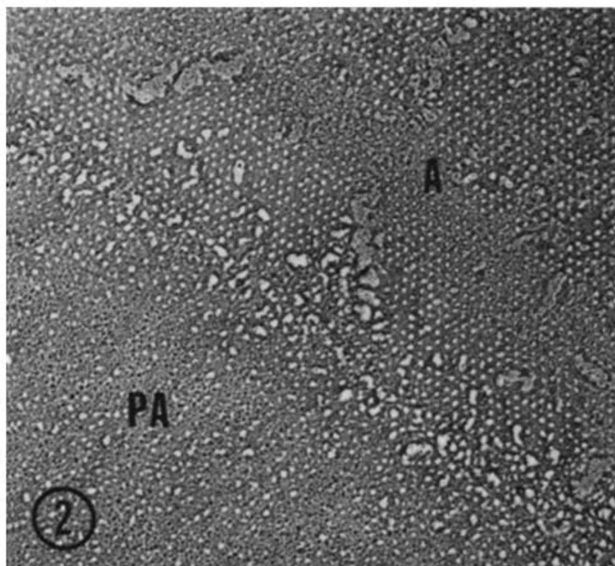
The sperm head offers a fine example of two macrodomains (Fig. 1): one area of the head, the acrosomal-cap portion of the plasma membrane, is highly fusigenic and after appropriate conditioning—that is, capacitation—fuses with the underlying acrosomal membrane. The other area, however, the post-acrosomal segment of the plasma membrane, is nonfusigenic under the same conditions. Only after the acrosome reaction occurs (the fusion of the plasma and acrosomal membranes) does this latter region become capable of fusing with an egg.

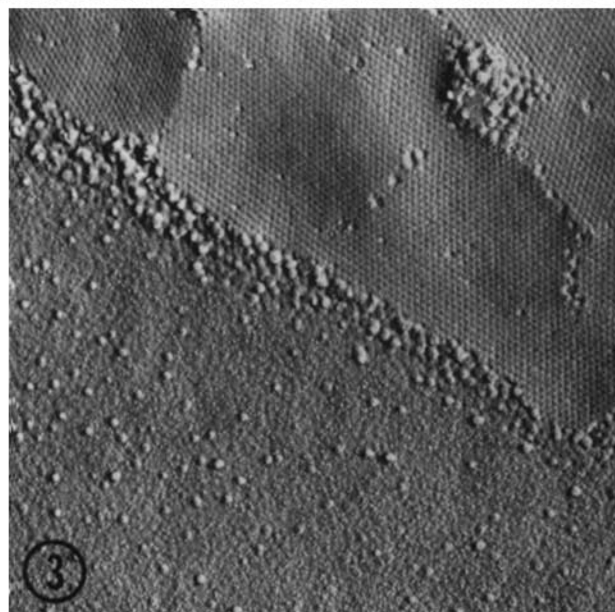
But unlike the experimental situation with other secretory cells, fractionation techniques are not available for cleanly isolating even the membranes of these major domains: their distinctions can only be determined by *in situ* structural criteria. In thin sections, the membranes of the two zones are similarly composed of a trilaminar unit-membrane, although the acrosomal-cap portion does exhibit a thick glycocalyx, itself separable into two layers (14). Freeze-fracture preparations of sperm removed from the tail of the epididymis expose the typical feature of the fusigenic acrosomal cap of the membrane—the quiltlike pattern which mirrors the glycocalyx (Figs. 2 and 3). Random intramembrane particles sprinkle the

This work was presented in a Symposium on Plasma Membrane Diversity at the Twenty-first Annual Meeting of The American Society for Cell Biology, November, 1981.

© The Rockefeller University Press · 0021-9525/82/05/0243/07 $1.00







FIGURES 1–3 Figure 1: Diagram of the guinea pig sperm head incorporating features observed in freeze-fracture and surface replicas. *A*, acrosomal segment. *PA*, post-acrosomal region. Figure 2: The juncture of the acrosomal (*A*) and post-acrosomal (*PA*) portions of the plasma membrane as viewed in a rapidly frozen, rotary-shadowed replica. × 45,000. Figure 3: A freeze-fracture preparation of the same juncture in a fixed, filipin-treated cell. Before the acrosomal area of the plasma membrane is prepared for fusion, filipin/sterol complexes appear only in the aisles between quilt-patterned patches. × 40,000.

post-acrosomal segment (Fig. 3) with several clear-cut particle clusters in front of the striated ring.

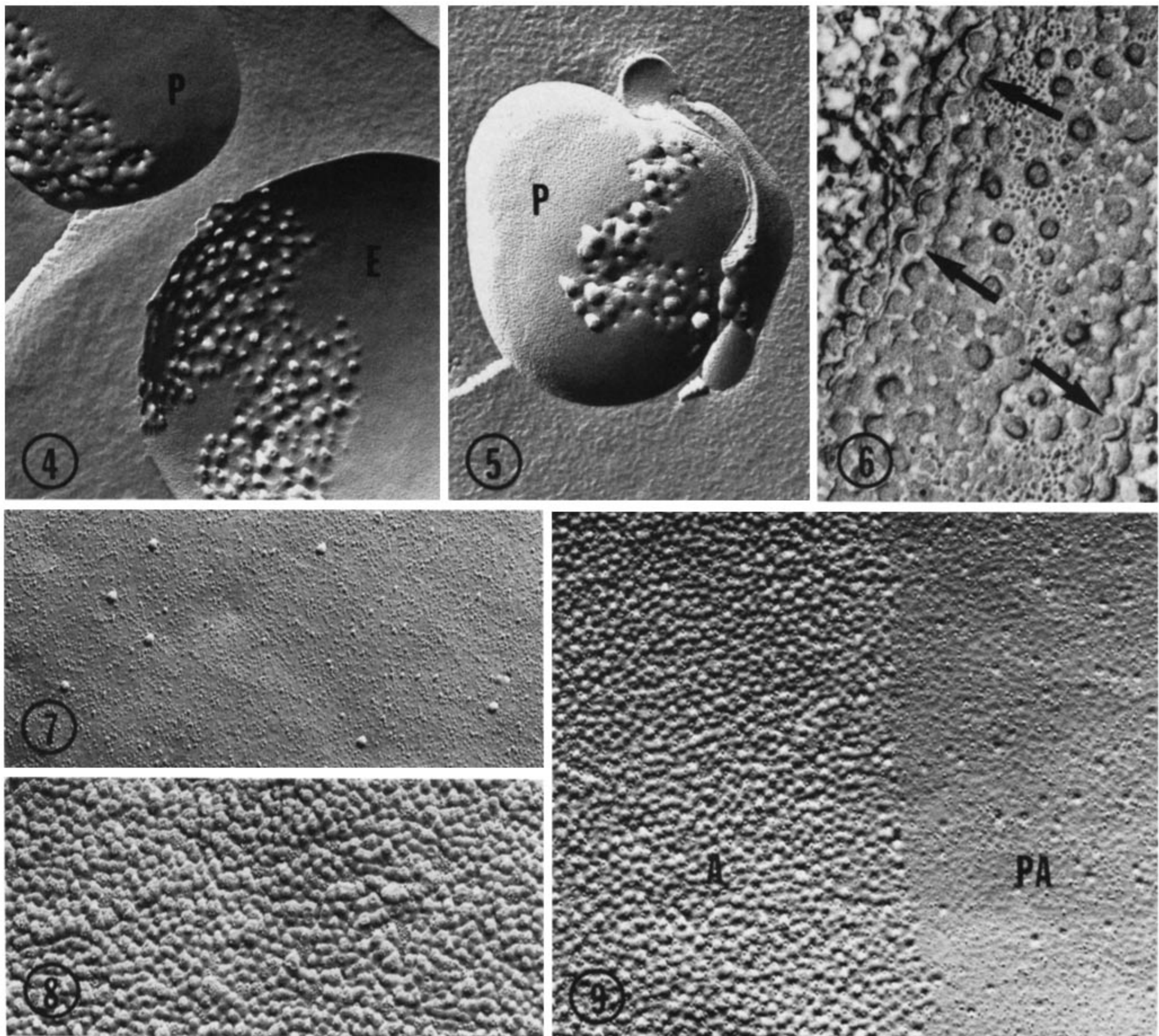
In addition to manifesting differences in freeze-fracture appearance, the fusigenic acrosomal cap and the nonfusigenic post-acrosomal segment of this continuous membrane differ in other parameters. They vary, for instance, in lectin binding, as observed in Koehler's work (15, 16) illustrating the fluorescence of wheatgerm agglutinin (WGA)/FITC conjugate and concanavalin A (Con A)/rhodamine: WGA labels the anterior part of the acrosomal segment but not the post-acrosomal segment—the implication being, of course, that the acrosomal portion contains perceptible glycoconjugates lacking in the post-acrosomal region. This is true even after the glycocalyx has been removed. Con A labels both areas.

Antibodies show a similarly distinctive distribution. At the 1980 American Society for Cell Biology Meeting, Myles et al. (17) presented their evidence of monoclonal antibodies recognizing the two diverse zones of this continuous membrane as well. In fact, in some instances, antibodies are directed only toward antigenic components of the acrosomal cap, while others are aimed exclusively at antigenic sites of the post-acrosomal segment of the plasma membrane. O'Rand and Romrell (18, 19) and Tung et al. (20) have each obtained results with antibodies produced by other methods distinguishing different parts of the sperm cell.

Another disparity between the two sectors of membrane is manifest by the incorporation and fluorescence of merocyanin (21), which measures degrees of membrane fluidity —it selectively partitions into the highly fluid regions. This probe reveals that the fusigenic portion has a greater degree of fluidity than the post-acrosomal segment: fluorescence, initially confined to the tip of the acrosomal cap, extends to the equatorial segment after the cells have been treated with a crude trypsin preparation or have become capacitated (14).

Several known factors may be responsible for this difference in fluidity between the acrosomal and post-acrosomal segments of the membrane. The general parameters important in influencing degrees of fluidity are well established: the amount of cholesterol in the membrane, the length of the fatty acids, and extent of fatty-acid-chain saturation, as well as, less influentially, the types of phospholipid headgroups (22). By electron microscopy cytochemical procedures, some such differences in sterol and anionic phospholipid concentration between these adjacent regions of the membrane are detectable.

The polyene filipin (23, 24, 25), which I will use to demonstrate this disparity in sterol content, is helpful in a couple of ways. K. Hong and D. P. Papahadjopoulos (both of the University of California) and I have used it to determine filipin's sensitivity to the membrane-sidedness of sterols after introducing cholesterol into the lipid bilayer of phosphatidylcholine liposomes. The filipin/sterol complexes indent the membranes of diphosphatidylcholine vesicles right after their exposure to cholesterol and filipin (Fig. 4). We presume that all the cholesterol is in the outer leaflet at this time. Later, when enough seconds (26) have elapsed for a mixing of cholesterol in both leaflets of the bilayer, we see both protrusions and indentations in nearly equal amounts (Fig. 5). These observations support both our theory that the filipin/sterol complexes divert the membrane away from the side of higher sterol concentration (Fig. 6) (25, 27) and the contention of Orci et al. that filipin

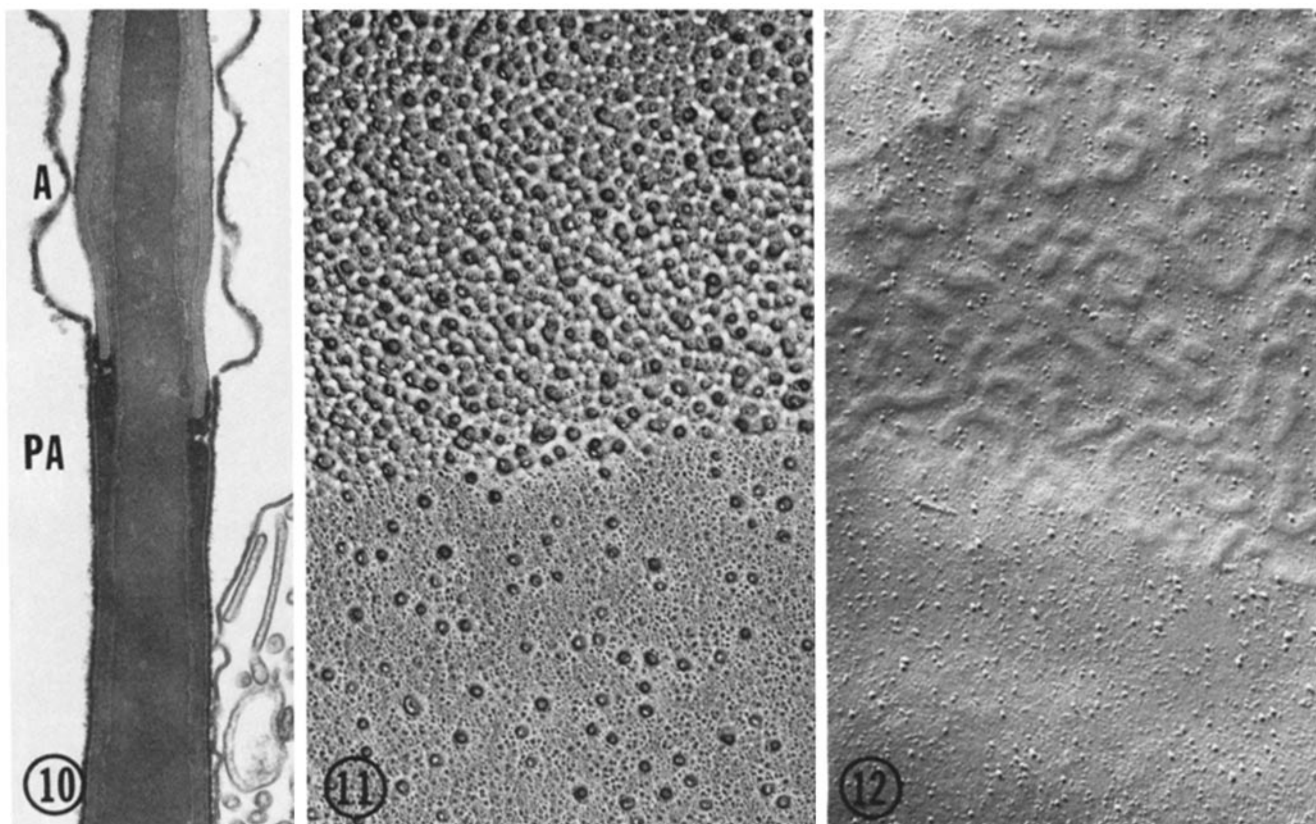


FIGURES 4–9 Figure 4: Filipin-treated phosphatidylcholine liposomes immediately after their incorporation of cholesterol. Filipin/sterol complexes induce indentations on the P-face (*P*) and protrusions on the E-face (*E*). × 53,000. Figure 5: Seconds-to-minutes after sterol incorporation, P-faces (*P*) exhibit both protrusions and indentations. × 56,000. Figure 6: Filipin/sterol complexes of the guinea pig sperm plasma membrane create protrusion of both membrane-leaflets (arrows), an event consistent with a higher sterol concentration in the inner half of the bilayer. × 70,000. Figure 7: Filipin/sterol complexes on the plasmalemmal P fracture-face of a *Drosophila* larval cell from a tissue-culture population of cells containing 1.5×10^{-3} µmol sterol/g protein. × 42,000. Figure 8: Filipin/sterol complexes in the plasma membrane of a larval-cell population which contained 18.4×10^{-3} µmol sterol/g protein. × 44,000. Figure 9: Acrosomal (*A*):post-acrosomal (*PA*) juncture of a capacitated sperm plasma membrane. The fusigenic acrosomal portion contains more than four times the number of sterol/filipin complexes than the stable post-acrosomal segment. Also compare the membrane protrusions in the acrosomal area to the membrane indentations in the post-acrosomal region, indicative of a difference in sterol placement within the bilayer in these contiguous domains. × 32,000.

may be used to determine which half of the bilayer contains the greater concentration of sterol in cell organelles.

We also know from other studies (27, 29) that *Drosophila* cells cultured without sterols subsequently develop increased numbers of filipin/sterol complexes, proportional to the amount of sterol restored to the medium. Likewise, there is a proportional increase in complexes, corresponding to the actual amount of sterol incorporated by the cells (Figs. 7 and 8). Applied to guinea pig sperm, the benefits of these two sets of observations disclose that filipin binding, assayed in freeze-fracture replicas, occurs beneath the membrane's inner leaflet (Fig. 6) and, quantitatively, is far more extensive over the fusigenic acrosomal-cap region than over the post-acrosomal membrane segment (Figs. 3 and 9–11). This latter topographical difference is particularly conspicuous before the quilt forms in the midportion of the epididymis or after it is removed by short incubation in salt solutions (Fig. 9).

Like the sterols, acidic phospholipids demonstrable by polymyxin-B (PXB) binding and membrane perturbation are also far more numerous over the anterior part of the acrosomal cap than over the post-acrosomal sector (14, 30) (Fig. 12). In addition, another technique, recently developed by Bearer (see reference 31), which takes advantage of adriamycin's preferential binding to anionic phospholipids, reveals the antibiotic's



FIGURES 10–12 Figure 10: Thin section through the acrosomal (*A*):post-acrosomal (*PA*) portions of the sperm head. × 40,000. Figure 11: Approximately 500 complexes/μm^2 inhabit the acrosomal portion, while the post-acrosomal segment contains <100 complexes/μm^2 in this rotary-shadowed, freeze-fracture preparation. × 38,000. Figure 12: Polymyxin B crenulates the anionic-phospholipid-rich acrosomal segment of the plasma membrane while sparing the post-acrosomal region. × 46,000.

predilection for the acrosomal cap over the post-acrosomal region.

Among the electron microscope approaches presented so far, the freeze-fracture data provide the most directly interpretable results. Simply put, in sperm taken from the epididymis, the membrane comprising the two macrodomains of the head looks different in each area. After incubation of the cells in an appropriate capacitating medium, the appearance of the membrane in the two sectors is more closely comparable, but each zone retains compositional dissimilarities when probed by lectins, antibodies, and lipid molecule–selective antibiotics. The provocative question here is: Are the observed differences in lipid composition real?

Pertinent to that question in the filipin-labeling of sterols, we have the example of the acrosomal cap, which has no quilt pattern before the sperm reach the midportion of the epididymis. Examining sperm either before the quilt emerges or after it is erased by brief incubation in salt solutions, we find that the membrane labels heavily with filipin complexes (Figs. 9 and 11). But while the quilt is present, it labels only in the aisles between its plaques (Fig. 3). Filipin fluoresces when it combines with sterols. Even in the presence of the quilt, the membrane nevertheless fluoresces uniformly when filipin is applied, hinting that the sterol occupies the entire region, but that the glycocalyx prevents the filipin/sterol membrane perturbation usually seen in freeze-fracture replicas. Although this may be the case, a false freeze-fracture negative does not explain why the neighboring post-acrosomal membrane, with its differing form of glycocalyx, neither fluoresces nor deforms. Therefore, we reason that the difference in sterol concentration between the two segments, deduced from experiments applying the filipin procedure, is real and not a limitation of the technique—particularly since the closely adherent fibrillar matrix of the post-acrosomal segment does not prevent membrane perturbation in that area. Filipin does cause its characteristic bumps and indentations in this segment (Figs. 9 and 11), and, after the acrosome reaction, the post-acrosomal segment, too, labels heavily. We do not know whether this change in labeling reflects an unmasking of free sterols or rather their movement from nearby membrane. We have made the same observations using two other sterol-binding compounds, digitonin and tomatin (25).

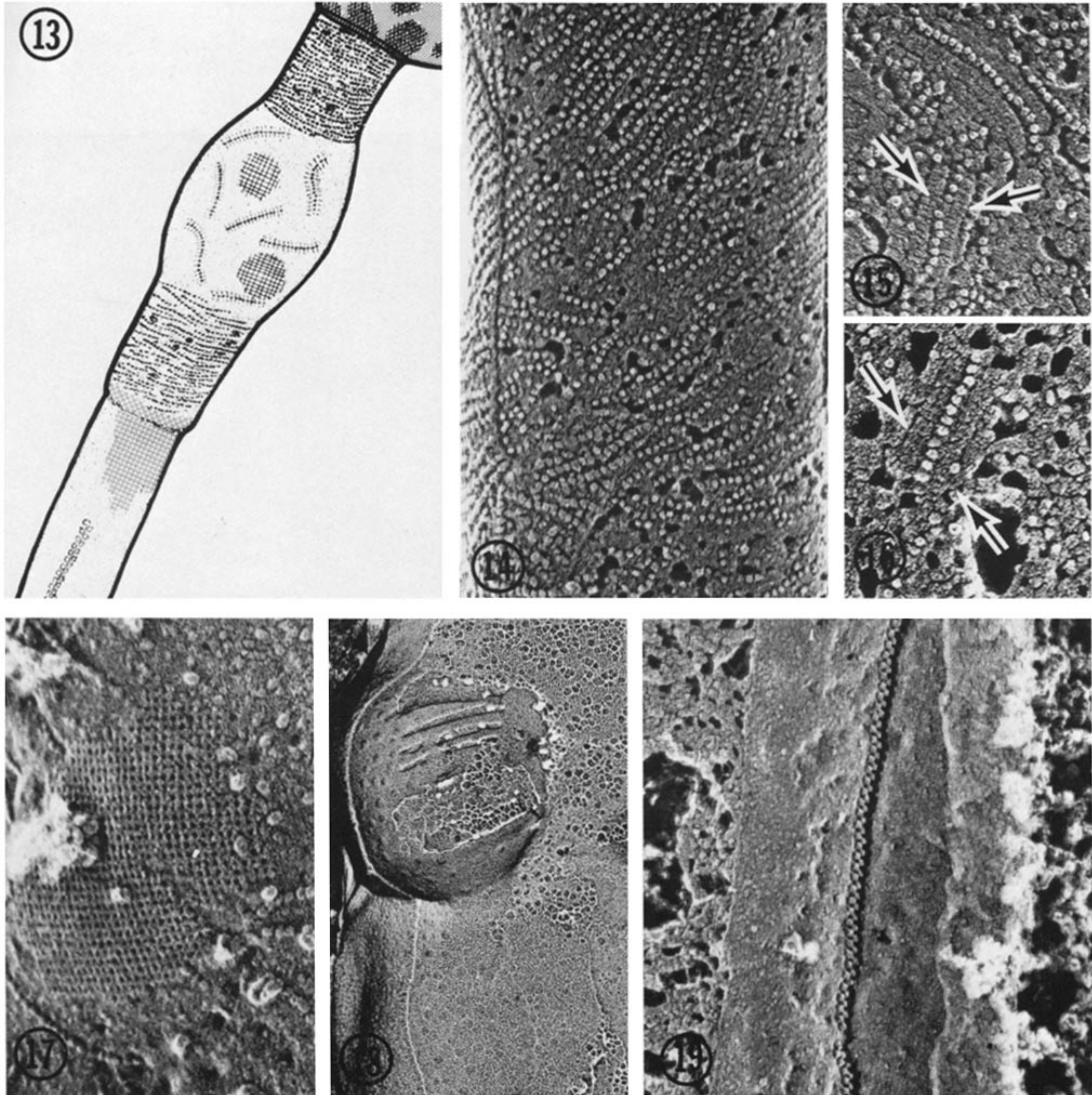
Regarding the probes of anionic lipids, another factor must be considered—that is, which leaflet of the membrane contains the greater concentration of anionic lipid. Since PXB does not cross the hydrophobic region of the bilayer, the demonstration of freeze-fracture cytochemical differences in concentration with PXB relates solely to differences in concentration within the outer half of the membrane—even though total anionic-lipid concentration may be the same in contiguous membrane domains. Therefore, after capacitation, the "flip-flop" of anionic lipids alone could account for the emergence of these lipids in membrane regions where none could be previously observed. But biochemical data suggest that, in addition, concurrent anionic-phospholipid synthesis takes place in sperm (30). And comparing the information derived from PXB binding, which recognizes outer-leaflet lipids, and adriamycin, which does cross the bilayer, we can but conclude that the cytochemically detected differences in anionic-lipid concentration in adjacent domains of the membrane are also real (14, 27, 30) (Fig. 12).

In summary, then, these macrodomains of the plasma mem-

brane composing the sperm head possess some readily discerned differences in freeze-fracture morphology, lectin binding, antibody specificity, and sterol and phospholipid concentration—and, I might add, a difference, most significantly, in fusigenicity upon the addition of calcium ions (32).

These two macrodomains also contain microdomains. The band of membrane fronting the tip of the nucleus is less perturbable by PXB than the surrounding membrane (14, 30) and susceptible to clearing of sterol/filipin complexes and intramembrane particles when glycerinated as a standard freeze-fracture cryoprotectant procedure (12, 14, 33). It does not clear of particles and complexes as readily in rapid-freeze preparations—a point which has been well-documented for other secretory cells (34, 35). Even more obvious are certain intramembrane-particle and lipid microdomains in the sperm tail (Fig. 13).

Strands of particles adorning portions of the plasma membrane which overlie mitochondria (Fig. 14) migrate above the retained cytoplasmic droplet during incubation in capacitating media. In this region, collars of lipid, coplanar but in different



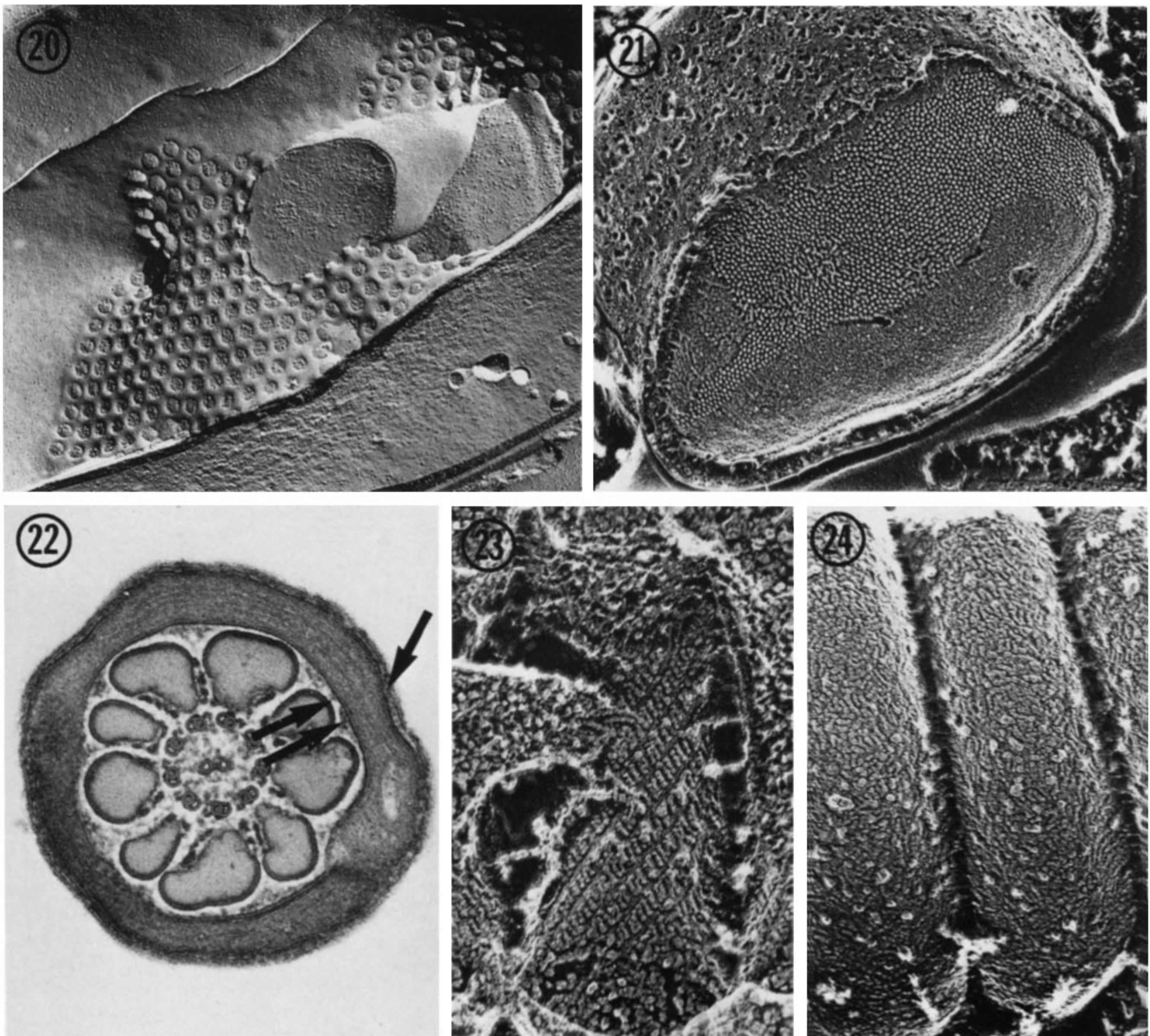
FIGURES 13–19 Figure 13: Diagram of the mid-piece and proximal principal-piece of the sperm tail, depicting microdomains exposed in conventionally and rapidly frozen cell preparations. Figure 14: Rapidly frozen, deep-etched preparations of the mid-piece reveal that strands of particles are present on the surface (left side) as well as in the fracture-face of the membrane. × 120,000. Figures 15 and 16: Similar strands, appearing in the cytoplasmic droplet during capacitation, often develop striated collars of "lipid" (arrows). Fig. 15, × 120,000. Fig. 16, × 170,000. Figure 17: In rapidly frozen preparations of sperm maintained in calcium-poor medium, the membrane comprising the retained cytoplasmic droplet occasionally shows "window-screen" patches. × 140,000. Figure 18: With 5–15 μmol Cal in the solution, cobbled areas appear at the sites where window-screen patches are otherwise seen. × 80,000. Figure 19: A view of the rapidly frozen, deep-etched surface of the principal-piece zipper. × 80,000.

guise than the bulk lipid in the membrane, develop around the ribbons of 80-Å particles (Figs. 15 and 16) (13). These 200-Å annuli are seen only in rapidly frozen, deep-etched sperm preparations.

A window-screen pattern in small patches is another design occasionally observed in rapid-frozen specimens in the realm of the cytoplasmic droplet after incubation of sperm in calcium-poor medium (13) (Fig. 17). They take on a cobbled appearance after the addition of calcium (Fig. 18).

Distinctive particle arrays, more to be expected but also more definitive than patterned lipids, also arise throughout the plasma membrane covering the sperm tail. Although images of both the annulus separating the mid- and principal-pieces of the tail and linear arrays of intramembrane particles have long been in print (10, 11), we have only seen clean surface-images of the zipper, a decoration of the tail's principal piece opposite fiber one by applying the relatively recent technique of rapid-freezing and deep-etching (Fig. 19) (13).

As well as the many recognized plasma-membrane mosaics, a parallel regionality is visible in the membranes of internal organelles. The pores of the nuclear envelope, for example, are confined to one posterior macrodomain (Fig. 20). At a deeper plane, the membranes of the envelope are further modified into a unique-appearing microdomain, the implantation fossa (Fig. 21), which anchors the attachment of head and tail (10, 11). And as a final illustration of mosaicism, particle arrays of the mitochondrial outer membrane facing the axonemal complex are disposed like the rungs of a ladder (Figs. 22, 23), while



FIGURES 20–24 Figure 20: The nuclear-envelope macrodomain of pores. × 26,000. Figure 21: A microdomain of the nuclear envelope—the implantation fossa, seen at a deeper fracture-plane through the envelope depicted in Fig. 20. × 56,000. Figure 22: Cross-section through the sperm tail mid-piece, distinguishing the part of the mitochondrial outer membrane facing the axonemal complex (two arrows) from that part which faces the plasma membrane (one arrow). × 65,000. Figures 23 and 24: Orderly arrays of particle-triplets cover the mitochondrial concave surface (Fig. 23), while the triplets are randomly dispersed over the convex surface (Fig. 24). Fig. 23, × 104,000. Fig. 24, × 90,000.

comparable rungs are haphazardly dispersed on the mitochondrial surface which faces the plasma membrane (Figs. 22 and 24) (13).

CONCLUSIONS

To conclude, as Morris Karnovsky often asks me, "What does all of this mean?", I can only offer the obvious:

(*a*) The guinea pig sperm has the capacity to sequester its membrane components into functionally different groupings which can also be identified morphologically.

(*b*) Although these multiple domains permit the cell to be highly efficient, due to this mosaicism the disturbance of small regions of the membrane can profoundly alter the function of the cell as a whole. For example, sperm to which cholesterol sulfate has been added, presumably intercalating in the sterol-rich acrosomal portion of the plasma membrane, are prevented from undergoing the acrosome reaction, thus precluding the cell's function—fertilization (36, 37).

(*c*) Also, since we see a loss of domain structure accompany cell death, we further suspect that interfering with the cell's energy production compromises the maintenance of domains, so that nonspecific injuries such as anoxia may appear as specific functional deficits, in this case, observed as both decreased cell motility and acrosome reactions.

(*d*) Because many of the domains are very small and may be variable in location, their detection and analysis demand the application of morphological and other *in situ* techniques.

And finally, (*e*) based on our observations of guinea pig sperm, the generalization we would submit about other secretory cells is that the inner leaflet of the plasma membrane prepared for fusion is relatively rich in free sterols, its outer leaflet contains a large proportion of anionic lipids, and the membrane is highly fluid. Conversely, membrane that is resistant to fusion sustains only low levels of free sterols, keeps its anionic lipids tucked in the inner membrane-leaflet, and is less fluid than fusigenic regions.

I thank Elaine L. Bearer, Peter M. Elias, and John E. Heuser, particularly, for their valuable and substantiative contributions to the work summarized in this symposium report. I likewise thank Rosamond Michael for her superior editorial help.

The author acknowledges the reproduction of Figure 12 from reference 14, and the reproduction of Figures 14–17 and 19 from reference 13.

This work was supported by the National Institutes of Health Grant HD 10445.

Received for publication 14 December 1981.

REFERENCES

1. Frye, C. D., and M. Edidin. 1970. The rapid intermixing of cell surface antigens after formation of mouse human heterokaryons. *J. Cell Sci.* 7:319–335.
2. Singer, S. J., and G. L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. *Science (Wash. D. C.).* 175:720–731.
3. Goldstein, J. L., R. G. W. Anderson, and M. S. Brown. 1979. Coated pits, coated vesicles, and receptor-mediated endocytosis. *Nature (Lond.).* 279:679–685.
4. Satir, B., C. Schooley, and P. Satir. 1973. Membrane fusion in a model system. *J. Cell Biol.* 56:153–176.
5. Vik, S. B., G. Georgevich, and R. A. Capaldi. Diphosphatidylglycerol is required for optimal activity of beef heart cytochrome C oxidase. *Proc. Natl. Acad. Sci. U. S. A.* 78:1456–1460.
6. James, H. L., G. A. Clawson, C. H. Chan, and E. A. Smuckler. 1981. Analysis of the phospholipid of the nuclear envelope and endoplasmic reticulum of liver cells by high pressure liquid chromatography. *Lipids.* 16:541–545.
7. Austin, C. R. 1975. Membrane fusion events in fertilization. *J. Reprod. Fertil.* 44:155–166.
8. Bedford, J. M., and G. W. Cooper. 1978. Membrane fusion events in the fertilization of vertebrate eggs. *Cell Surf. Rev.* 5:66–125.
9. Fawcett, D. W. 1975. The mammalian spermatozoon. *Dev. Biol.* 44:394–436.
10. Friend, D. S., and D. W. Fawcett. 1974. Membrane differentiations in freeze-fractured mammalian sperm. *J. Cell Biol.* 63:641–664.
11. Friend, D. S. 1977. Organization of the spermatozoal membrane. *In* Immunobiology of the Gametes. M. Edidin and M. H. Johnson, editors. Alden Press, Cambridge. 5–30.
12. Friend, D. S. 1980. Freeze-fracture alterations in guinea-pig sperm membranes preceding gamete fusion. *Soc. Gen. Physiol. Ser.* 34:153–165.
13. Friend, D. S., and J. E. Heuser. 1981. Orderly particle arrays on the mitochondrial outer membrane of rapidly-frozen sperm. *Anat. Rec.* 199:159–175.
14. Bearer, E. L., and D. S. Friend. 1982. Modifications of anionic-lipid domains preceding membrane fusion in guinea pig sperm. *J. Cell Biol.* 92:604–615.
15. Koehler, J. K. 1978. The mammalian sperm surface: studies with specific labeling techniques. *Int. Rev. Cytol.* 54:73–107.
16. Koehler, J. K. 1981. Lectins as probes of the spermotozoan surface. *Arch. Androl.* 6:197–217.
17. Myles, D. G., P. Primakoff, and A. R. Bellvé. 1981. Surface domains of the guinea pig sperm defined with monoclonal antibodies. *Cell.* 23:433–439.
18. O'Rand, M. G., and L. J. Romrell. 1980. Appearance of regional surface autoantigens during spermatogenesis: comparison of anti-testis and anti-sperm autoantisera. *Dev. Biol.* 75:431–441.
19. O'Rand, M. G., and L. J. Romrell. 1981. Localization of a single sperm membrane autoantigen (RSA-1) on spermatogenic cells and spermatozoa. *Dev. Biol.* 322–331.
20. Tung, K. S. K., A. Okada, and R. Yanagimachi. 1980. Sperm autoantigens and fertilization. I. Effects of antisperm antibodies on rouleaux formation, viability, and acrosome reaction of guinea pig spermatozoa. *Biol. Reprod.* 23:877–886.
21. Williamson, P. L., W. A. Massey, B. M. Phelps, and R. A. Schlegel. 1981. Membrane phase state and the rearrangement of hematopoietic cell surface receptors. *Mol. Cell. Biochem.* 1:128–135.
22. Rothman, J. E. 1980. An overview of membrane structure and biosynthesis. *Soc. Gen. Physiol. Series.* 34:1–9.
23. Bittman, R. 1978. Sterol-polyene complexation: probe of membrane structure. *Lipid.* 13:686–691.
24. Robinson, J. M., and M. J. Karnovsky. 1980. Evaluation of the polyene antibiotic filipin as a cytochemical probe for membrane cholesterol. *J. Histochem. Cytochem.* 28:161–168.
25. Elias, P. M., D. S. Friend, and J. Goerke. 1979. Membrane sterol heterogeneity. Freeze-fracture detection with saponins and filipin. *J. Histochem. Cytochem.* 27:1247–1260.
26. Backer, J. M., and E. A. Davidowicz. 1981. Transmembrane movement of cholesterol in small unilamellar vesicles detected by cholesterol oxidase. *J. Biol. Chem.* 256:586–588.
27. Friend, D. S., and E. L. Bearer. 1981. β-Hydroxysterol distribution as determined by freeze-fracture cytochemistry. *Histochem. J.* 13:535–546.
28. Orci, L., R. G. Miller, R. Montesano, A. Perrelet, M. Amherdt, and P. Vassalli. 1980. Opposite polarity of filipin-induced deformations in the membrane of condensing vacuoles and zymogen granules. *Science (Wash. D. C.).* 210:1019–1021.
29. Friend, D. S., C. M. Havel, M. Silberklang, and J. A. Watson. 1980. Distribution and effects of cholesterol on 3-β-hydroxysterol-free eukaryotic cells. *J. Cell Biol.* 87(2, Pt. 2):196*a* (Abstr.).
30. Bearer, E. L., and D. S. Friend. 1980. Anionic lipid domains: Correlation with functional topography in a mammalian cell membrane. *Proc. Natl. Acad. Sci. U. S. A.* 77:6601–6605.
31. Bearer, E. L., and D. S. Friend. 1981. Maintenance of lipid domains in the guinea pig sperm membrane. *J. Cell Biol.* 91(2, Pt. 2):266*a* (Abstr.).
32. Yanagimachi, R., and N. Usui. 1974. Calcium dependence of the acrosome reaction and activation of guinea pig spermatozoa. *Exp. Cell Res.* 89:161–174.
33. Friend, D. S., L. Orci, A. Perrelet, and R. Yanagimachi. 1977. Membrane particle changes attending the acrosome reaction in guinea pig sperm. *J. Cell Biol.* 74:561–577.
34. Ornberg, R. L., and T. S. Reese. 1981. Beginning of exocytosis captured by rapid-freezing of Limulus amebocytes. *J. Cell Biol.* 90:40–54.
35. Chandler, D. E., and J. E. Heuser. 1980. Arrest of membrane fusion events in mast cells by quick-freezing. *J. Cell Biol.* 86:666–674.
36. Legault, Y., G. Bleau, A. Chapdelaine, and K. D. Roberts. 1979. The binding of sterol sulfates to hamster spermatozoa. *Steroids.* 34:89–99.
37. Davis, B. K. 1980. Interaction of lipids with the plasma membrane of sperm cells. I. The antifertilization action of cholesterol. *Arch. Androl.* 5:249–254.