BEHAVIOR OF THE GAMETE MEMBRANES DURING SPERM ENTRY INTO THE MAMMALIAN EGG

C. BARROS and L. E. FRANKLIN. From Tulane University, Delta Regional Primate Research Center, Covington, Louisiana 70433. Dr. Barros' present address is the Departmento de Biologia, Escuela de Medicina, Universidad Catolica de Chile, Santiago, Chile

Techniques for fertilizing golden hamster eggs in vitro have recently been developed (4, 14). Consequently, sperm entry into the egg can be followed with the phase-contrast microscope, and after semination eggs can be fixed at desired intervals for subsequent examination with the electron microscope.

The probability of obtaining thin sections that demonstrate sperm penetration of an egg is enhanced if the eggs are polyspermic. Phase-contrast microscope observations show that monospermic entry and polyspermic entry into eggs does not differ with respect to behavior of the spermatozoa. Compare Figs. 1, 6, and 7 to photographs in earlier studies (1, 2) of monospermic, in vivo fertilized eggs (also see reference 9 for discussion). Thus, in the present study use was made of polyspermic eggs obtained from a preparation set up for fertilization in vitro as described by Barros and Austin (4) and Barros (3). Eggs were fixed either with osmium tetroxide alone or with glutaraldehyde followed by osmium tetroxide.

Recent evidence (5, 6) has shown that the acrosome reaction of mammalian spermatozoa involves extensive vesiculation between the sperm plasma membrane and the outer acrosomal membrane except in that portion covering the posterior region of the acrosome, the equatorial segment. In some specimens the acrosomal material of this segment has a septate appearance. Most of the equatorial segment persists on spermatozoa that have crossed the zona pellucida, whereas the rest of the acrosome remains outside. Some spermatozoa in the zona or in the perivitelline space show vesiculation (as described above) in the equatorial segment, but whether these sperm enter the egg is unknown.

As previously indicated in a study of sperm penetration in the rat (11), the mid-lateral region of the sperm head enters the egg first (Figs. 1 and 2). Entry of the sperm head involves fusion of the plasma membranes of the sperm and egg (Fig. 3) as was first demonstrated in invertebrates by Colwin and Colwin (7, 8) and in mammals by Szollosi and Ris (12). However, as has not been previously recognized in mammals, incorporation of the sperm head progresses from the mid-region toward the anterior and posterior ends, the anterior end entering the egg last. Incorporation of the posterior region of the sperm head involves a degree of invagination of the fused cell membranes of the sperm and egg (Figs. 2 and 3), but apparently follows the pattern of sperm entry first described in the annelid, *Hydroides hexagonus* (7, 8). However, the anterior end of the sperm head is drawn below the egg surface within a channel. The channel may later become a true phagocytotic vesicle, but this has not yet been ascertained (Fig. 4). The vesicle or channel originates from the anterior limit of the equatorial segment of the sperm head (Fig. 5). Any portion of the sperm head which lies anterior to the equatorial segment appears to be enveloped by an intact egg cell-membrane. A large part of the equatorial segment is seen within the egg cortex (Figs. 4 and 5), and the structural organization of that region resembles that of a "septate" desmosome (see reference 13). The origin of the outer membrane (*om*, Fig. 5) of the desmosome-like region has not yet been determined. If the vesiculation observed at the equatorial segment is complete before sperm penetration, the outer membrane is of egg origin, but if the equatorial segment persists intact until after sperm entry the outer membrane is of sperm origin. This problem of membrane identification does not exist anterior to the equatorial segment (Fig. 5), the anterior limit of which is indicated by a ring of subacrosomal material (Fig. 5). The equatorial segment is a characteristic structure of mammalian spermatozoa (10) and probably plays a significant

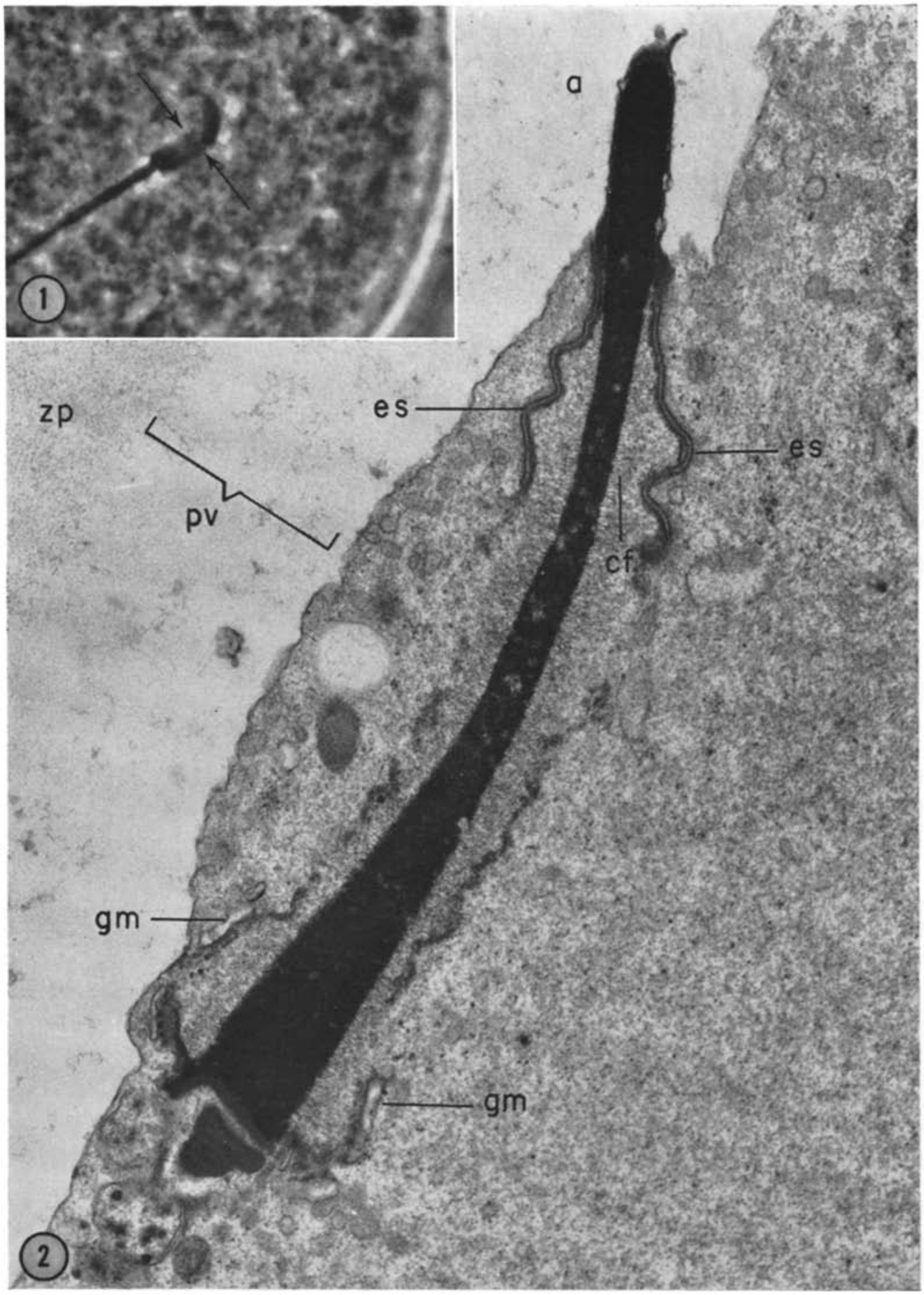


FIGURE 1 Early stage of sperm entry into a living egg. The mid-lateral region of the sperm head (arrows) has entered the egg, and the nucleus has begun to disperse in that region. Phase-contrast micrograph. × 1,250.

FIGURE 2 Penetrating spermatozoon. Only the anterior end of the sperm head is outside the egg (*a*). Invaginated regions of the fused cell membranes of the sperm and egg (*gm*) are shown at the base of the sperm nucleus. Note filamentous elements of the dispersing nucleus (*cf*). Equatorial region, *es*; zona pellucida, *zp*; perivitelline space, *pv*. × 18,000.

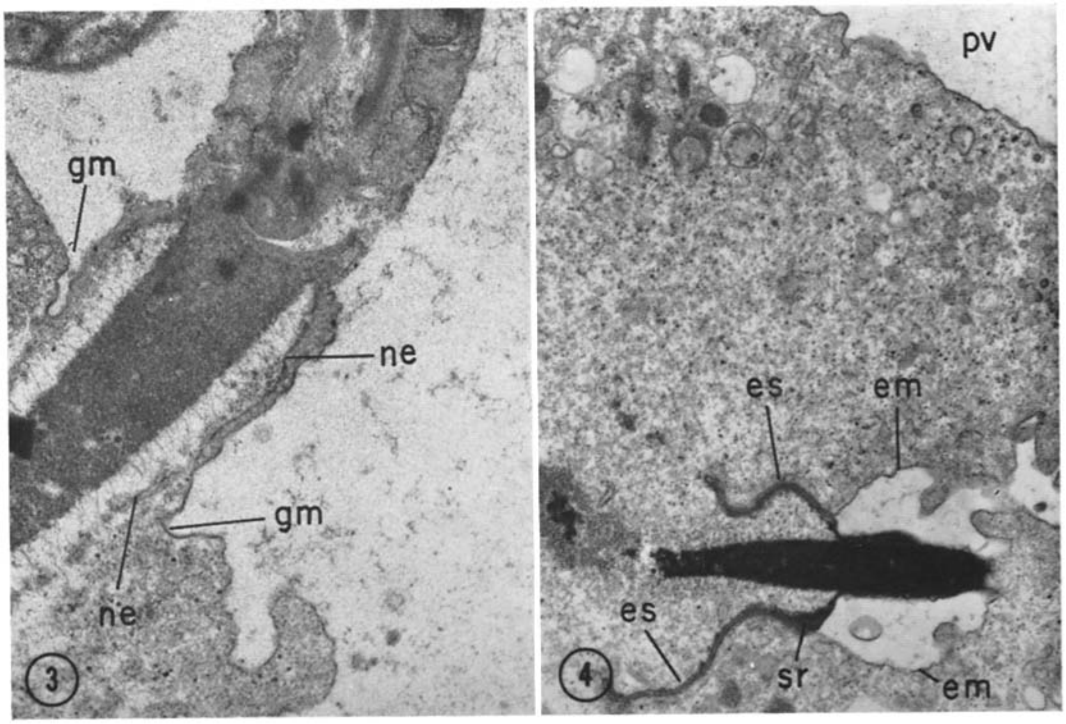


FIGURE 3 Entry of the posterior end of the sperm head. The anterior end (not shown) is still outside the egg. The fused cell membranes of the sperm and egg (*gm*) appear invaginated in this stage. Nuclear envelope remnants (*ne*). × 20,000.

FIGURE 4 Anterior region of a penetrated sperm head. The region of the sperm head anterior to the subacrosomal ring (*sr*) lies within a space bounded by the egg plasma membrane (*em*). Perivitelline space (*pv*). The equatorial region (*es*) shows a septate fine structure. × 11,000.

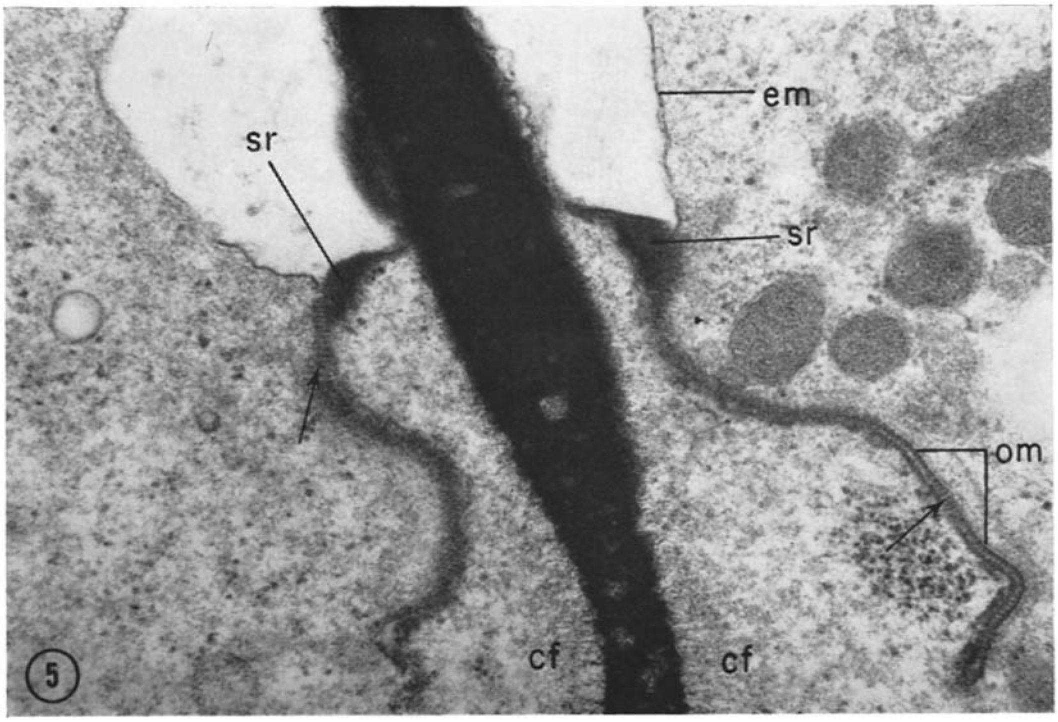
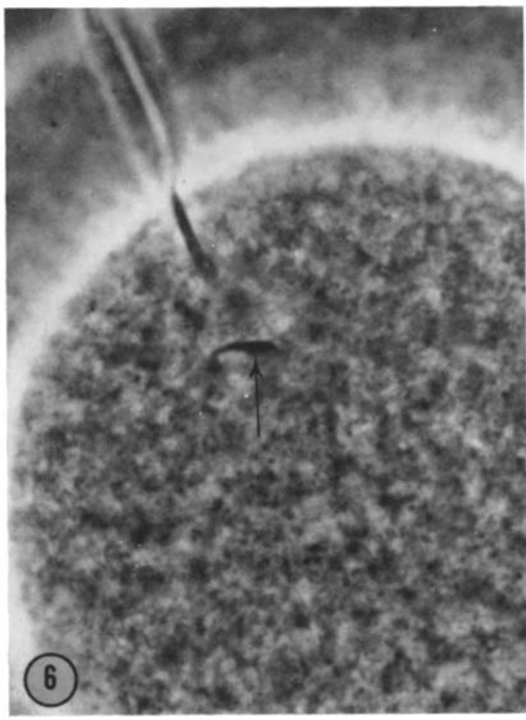


FIGURE 5 Anterior region of a penetrated sperm head. The structural organization of the equatorial region resembles that of a septate desmosome (arrows). The "outer membrane" (*om*) of the desmosome-like region is discussed in the text. Egg plasma membrane (*em*), subacrosomal ring (*sr*), filamentous elements of the dispersing nucleus (*cf*). × 41,000.



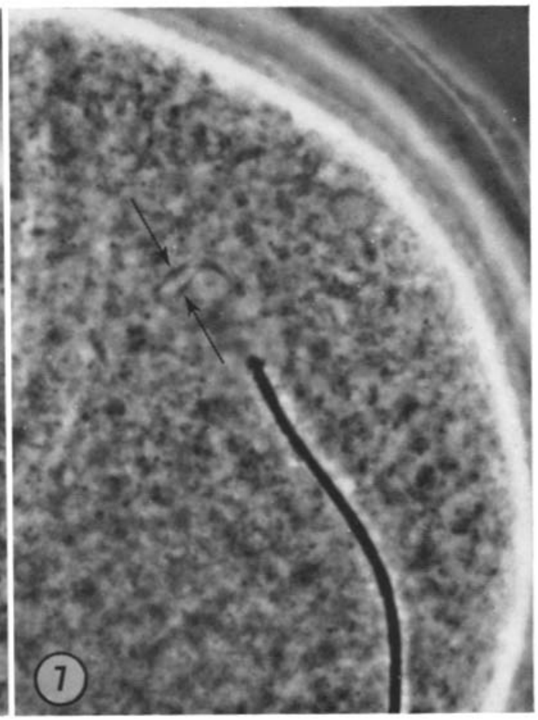


FIGURE 6 Phase-contrast micrograph of a living egg. The anterior part of the sperm nucleus (arrow) has not yet dispersed. $\times$ 1,250.

FIGURE 7 Phase-contrast micrograph of a living egg. The *entire* sperm nucleus has dispersed. The subacrosomal region (arrows), comprised of subacrosomal material and bounded by the inner acrosomal and egg plasma membranes, is the last part of the sperm head to be incorporated into the vitellus. $\times$ 1,250.

role in sperm entry. It is now evident that the manner of sperm entry in hamsters, and very likely in all mammals, differs markedly from the *Hydroides-Saccoglossus* pattern which is widespread in invertebrates (9).

Szollosi and Ris (12) observed that the nucleus of rat spermatozoa has a granular appearance, like that of a late spermatid, after penetrating the egg. Those authors also reported that sperm chromatin is dispersed into fine components, showing fibrils, near the caudal region of the nucleus. The present study shows that dispersion of the sperm chromatin, which progresses from the periphery of the nucleus to its core, occurs first in the mid-region of the nucleus, then progresses toward both ends, the anterior end being affected last (Figs. 2, 4, and 5). Phase-contrast microscope observations also show that the sperm nucleus disperses first in the mid-region, then in the caudal region, and finally at the anterior end (Figs. 1, 6, and 7).

This work was supported by the United States Public Health Service grants No. FR00164 and GM13226 from the National Institutes of Health.

Received for publication 13 March 1968.

REFERENCES

1. Austin, C. R. 1956. Ovulation, fertilization, and early cleavage in the Hamster (*Mesocricetus auratus*). *J. Roy. Microscop. Soc.* **75**:141.
2. Austin, C. R., and M. W. H. Bishop. 1958. Role of the rodent acrosome and perforatorium. *Proc. Roy. Soc. (London) Ser. B.* **149**:241.
3. Barros, C. 1968. Capacitation *in vitro* of golden hamster spermatozoa. *Anat. Record.* In press. (Abstr.)
4. Barros, C., and C. R. Austin. 1967. *In vitro* fertilization and the sperm acrosome reaction in the hamster. *J. Exptl. Zool.* **166**:317.
5. Barros, C., J. M. Bedford, L. E. Franklin, and C. R. Austin. 1967. Membrane vesiculation as a feature of the mammalian acrosome reaction. *J. Cell Biol.* **34**:C1.
6. Bedford, J. M. 1967. Experimental requirement for capacitation and observations on ultrastructural changes in rabbit spermatozoa during fertilization. *J. Reprod. Fertility.* 2 (Suppl.): 35.
7. Colwin, A. L. and L. H. Colwin. 1960. Fine structure studies of fertilization with special reference to the role of the acrosomal region of the spermatozoon during penetration of the egg (*Hydroides hexagonus*-Annelida). *In* Symposium on the Germ Cells and Earliest Stages of Development, Institut International d'Embryologie and Fondazione. A. Baselli, Milano. 220.
8. Colwin, A. L., and L. H. Colwin. 1961. Changes in the spermatozoon during fertilization in *Hydroides hexagonus* (Annelida). II. Incorporation with the egg. *J. Biophys. Biochem. Cytol.* **10**:255.
9. Colwin, L. H., and A. L. Colwin. 1967. Membrane fusion in relation to sperm-egg association. *In* Fertilization. C. B. Metz and A. Monroy, editors. Academic Press Inc., New York. **1**:7.
10. Nicander, L., and A. Bane. 1966. Fine structure of the sperm head in some mammals with particular reference to the acrosome and the subacrosomal substance. *Z. Zellforsch. Mikroskop. Anat.* **72**:496.
11. Piko, L. and A. Tyler. 1964. Fine structural studies of sperm penetration in th rat. *Intern. Congr. Animal Reprod. Artificial Insemination 5th Trento.* **2,** 372.
12. Szollosi, D., and H. Ris. 1961. Observations on sperm penetration in the rat. *J. Biophys. Biochem. Cytol.* **10**:275.
13. Wood, R. 1959. Intercellular attachment in the epithelium of *Hydra* as revealed by electron microscopy. *J. Biophys. Biochem. Cytol.* **6**:343.
14. Yanagimachi, R., and M. C. Chang. 1964. *In vitro* fertilization of golden hamster ova. *J. Exptl. Zool.* **156**:361.