In the central region of the retina at stage 20, many more pigment granules are within the cells of the pigment epithelium than were observed in this tissue at the previous stage and only a few granules remain in the space between the epithelial layers. Most of these extracellular granules appear to be actively engulfed by cytoplasmic processes from the pigment epithelium (Figure 3). Inside the pigment epithelium, some of the pigment granules aggregate to form a larger unit. Up to 18 granules have been observed in some sections but usually only 3 to 6 granule profiles are observed. Never were aggregates of egg pigment observed in the retinal neuroepithelial cells or in the space between the epithelial layers.

The rod shaped melanosome (2.0–2.5 μm long, 0.6–0.7 μm wide) typically found in the pigment epithelium of this animal[8–10] first appears during late stage 20. At this time there are 2 types of melanin granules in the pigment epithelium: one produced during the development of the egg and the other synthesized de novo by the pigment epithelium. These are the melanosomes of 2 different morphologies that have been observed in the pigment epithelium of the tadpole[11].

A complete electroretinogram (ERG) can be recorded from the developing eye of this animal only when both receptor outer segments and receptor synapses have developed[10]. In my material both these structures are present at stage 24. Also at this stage the differentiating retina and intraepithelial space are free of pigment except for a few granules which persist in the cells at the margin where the retina merges with the pigment epithelium. Thus, by the time the retina has differentiated to the degree that it responds to light with the usual pattern of electrical activity, most of the egg melanin has been eliminated from its cells.

The early elimination of egg pigment from the neural retina is in sharp contrast to the time this pigment is eliminated from neurons of the brain and spinal cord. In the central nervous system of young tadpoles I have found large amounts of pigment within neurons 2 to 3 weeks after the retina is free of pigment. In some species of frogs, egg pigment is present in the central nervous system well after metamorphosis[12].

The role of the pigment epithelium in removal of egg pigment eliminated by the embryonic retina offers a preview to a role of this tissue in the adult. In the mature retina, protein is assembled in the cell body and added at the base of the rod outer segments. The material lost from the outer segment tips is engulfed and destroyed by the pigment epithelium[8, 9, 13, 14].

Zusammenfassung. Die während der Oogenese entstandenen Melaningranula werden aus dem neuralen Netzhautanteil ausgeschieden und vom Pigmentepithel aufgenommen. Dies geschieht, bevor das Zentralnervensystem pigmentfrei geworden ist, und ermöglicht wirkungsvolle Lichtaufnahme in den Photorezeptoren.

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Scanning Electron Microscopy of Golden Hamster Spermatozoa Before and During Fertilization

Recent development of scanning electron microscopy has made possible the investigation of details of cellular surface topography, which would be very difficult or impossible to examine by conventional light microscopy or transmission electron microscopy. This relatively new technique has apparently not been fully applied for studies of morphology of mammalian gametes[1–4]. This paper reports the result of our scanning electron microscopic observations on golden hamster spermatozoa before and during fertilization. The details of the internal anatomy of the golden hamster spermatozoon and its changes during fertilization have been published[5–10].

Fresh spermatozoa were obtained from the caudae epididymides of fertile golden hamster males. Capacitated spermatozoa were prepared as described by Yanagimachi[11]. The spermatozoa were spread over clean slides, quickly air-dried, and fixed 1 h in cold 2.5% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.4). After thorough rinsing in 0.1 *M* phosphate buffer (pH 7.4), the sperm smears were dehydrated with a graded acetone series. After complete dehydration with 100% acetone, the smears were allowed to dry in air. The smear-holding slides were then cut into small pieces (1 × 1 cm^2) and with smear surface up each piece was glued to an aluminum stub using an electron conductive cement (silpaint; Fansteel Electric Material Lab.). The specimen stubs were placed in a vacuum evaporator (JEOLCO, model 4B) and a thin film of gold was evaporated at a high vacuum (10^{-4} mm Hg) onto the specimens, which were rotated constantly so that the gold coated all parts of the specimens. The coating process was stopped when the slides turned slightly bluish-green. Observations and photography were

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made using a JEOLCO model JSM-U3 scanning electron microscope.

For examination of spermatozoa entering the egg cytoplasm, recently ovulated, unfertilized eggs of the golden hamster were freed from the cumulus oophorus and zona pellucida by means of hyaluronidase and trypsin respectively[10,12], and inseminated in vitro with fully capacitated spermatozoa. At varying time between 2 and 5 min after insemination, the eggs were fixed 1 h in cold 2.5% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.4), rinsed with 0.1 *M* phosphate buffer (pH 7.4) and dehydrated with a graded acetone series. After complete dehydration with 100% acetone, the eggs were transferred onto pieces of clean slides (1 × 1 cm²) and allowed to dry in air. The egg surfaces were coated with gold and examined with the scanning electron microscope, using the same procedures as those used for the spermatozoa.

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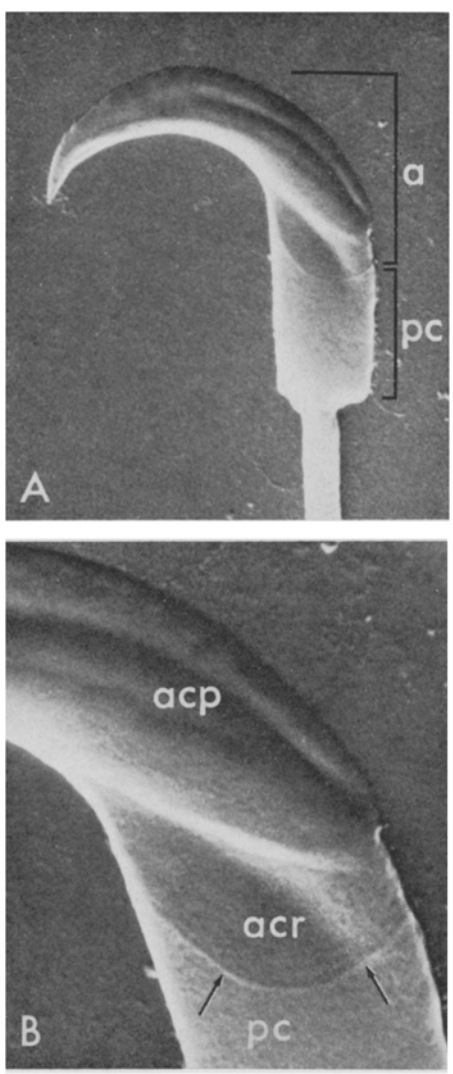

Fig. 1. Head of an intact epididymal spermatozoon: a, acrosomal region; acp, acrosomal cap region; acr, acrosomal collar region; pc, postacrosomal region. Arrows (B) indicate the posterior border of the acrosomal collar. A) ×5,900; B) ×14,000.

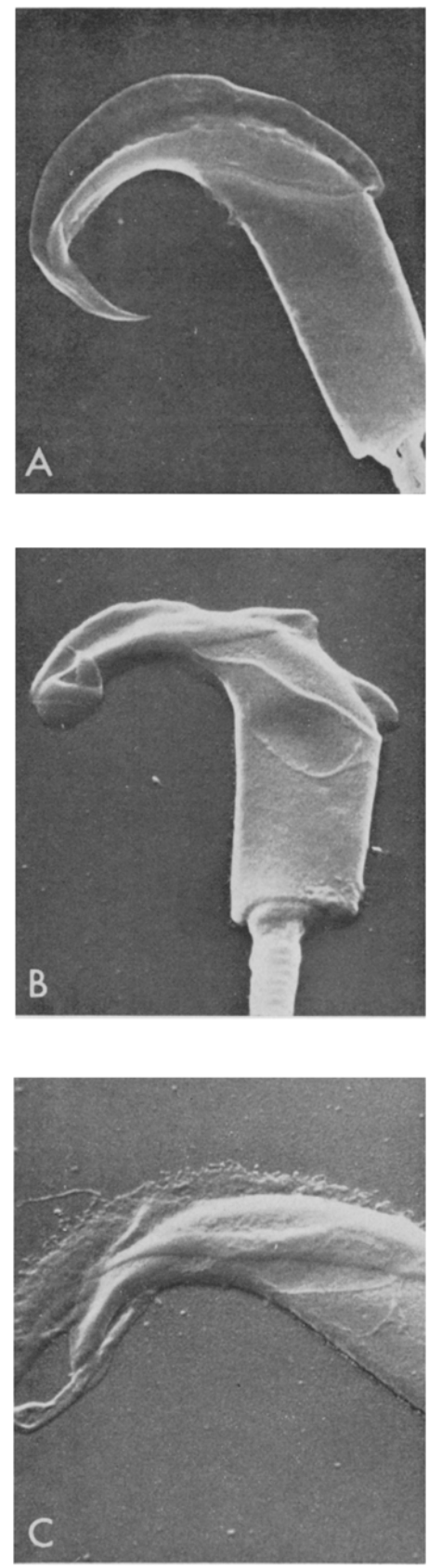

Fig. 2. Capacitated spermatozoa with acrosomal caps swollen (A), collapsed (B), and possibly vesiculated (C). A) ×6,800; B) ×7,200; C) ×7,700.

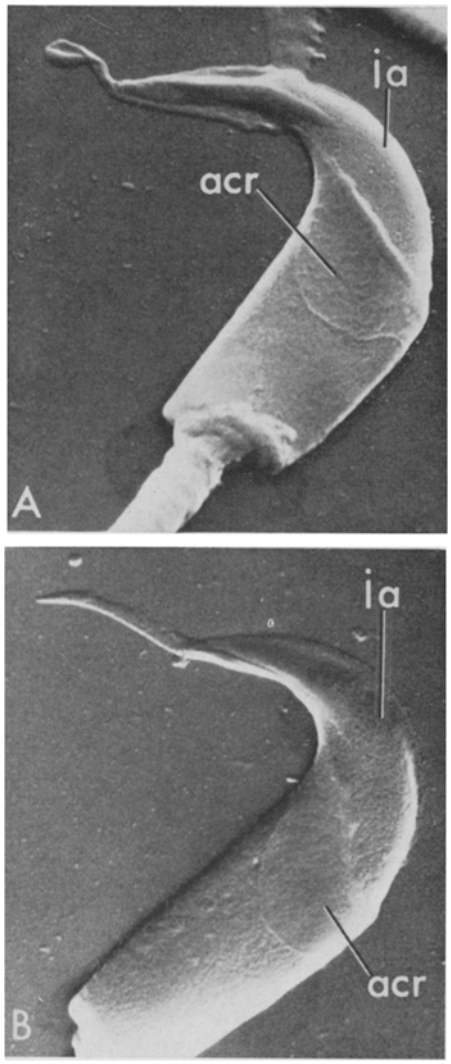

Fig. 3. Capacitated spermatozoa of which acrosomal caps have disappeared. Note that the acrosomal collar (acr) remains in situ; ia, inner acrosomal membrane. A) ×7,800; B) ×8,300.

Figures 1A and B show the head of a fresh epididymal spermatozoon. Two distinct parts of the acrosome, the acrosomal cap and the acrosomal collar, can be clearly seen. The former is relatively thick and occupies the strongly curved, apical part of the sperm head; the latter is thin and flat, covering the equatorial part of the sperm head. The posterior half of the sperm head is called the postacrosomal region (= the postnuclear cap region). In ram spermatozoa, the material of the postacrosomal region (postacrosomal dense lamina[13]) overlaps the posterior edge of the acrosomal collar, forming regularly spaced serrations[14]. A series of depressions along the posterior border of the acrosomal collar of ram and bull spermatozoa observed by DOTT[2] using the scanning electron microscope, and the precisely serrated belt-like boundary described in the rabbit by KOEHLER[15,16] using a freeze-etching technique, could be caused by this overlapping postacrosomal material. Previous transmission electron microscopic studies of the ultrastructure of the hamster sperm head[7,8] have not reported any such overlapping, and no regularly spaced depressions along the posterior border of the acrosomal collar were detected in the present study with scanning electron microscopy. The ridge-like elevation of this area as seen in Figure 1B (arrows) to us appears to represent a specialized acrosomal border not easily flattened by the dehydration process used in this study.

Capacitated spermatozoa showed various stages of acrosome reaction. In some spermatozoa the acrosomal caps were extensively swollen (Figure 2A) or collapsed (Figure 2B). In the spermatozoon shown in Figure 2C, the acrosomal cap seemed to have vesiculated possibly due to multiple unions between plasma and outer acrosomal membranes[5,7,9]. In other spermatozoa the acrosomal caps had completely disappeared, exposing the inner acrosomal membrane directly to the outside (Figures 3A and B). It appeared that the inner acrosomal membrane has a rougher surface as compared with the plasma membrane covering the rest of the spermatozoon. Ridge-like areas along the anterior border of the acrosomal collar and along the concave surface of the sperm head (Figure 3A) seem to represent the areas where subacrosomal material is most abundant[7,8]. The rostral end of the sperm head was straight in some spermatozoa (Figure 3B) and bent in others (Figure 3A), suggesting that this part of the sperm head is not rigid, but rather flexible[17]. Vesiculation of the acrosomal collar as reported by BARROS and FRANKLIN[6] and YANAGIMACHI and NODA[9], was not detected by the scanning electron microscope technique used.

Figures 4 and 5 show hamster spermatozoa recently attached to the surface of the egg cytoplasm. Although numerous egg microvilli were flattened down and clamped together apparently due to shrinkage of specimens during dehydration, these micrographs nevertheless show active participation of the egg microvilli in sperm-egg association. The microvilli seem to grasp the sperm head and immediately fuse with the plasma membrane of the spermatozoon. A wave-like protrusion of the egg cytoplasm covering the head as seen by transmission electron microscopy [9,10] is well illustrated three dimensionally in the micrograph of Figure 5.

Since the scanning electron microscope can show us only the surface views of the spermatozoon and egg, comprehensive information about the ultrastructural events occuring in these cells should be obtained by combining both scanning and conventional transmission electron microscopies. Figure 6 illustrates our interpretation of the changes in the hamster sperm head before and during its contact with the surface of the egg cytoplasm, based on the information obtained with scanning electron microscopy (the present study) and transmission electron microscopy[8–10,18]. We believe that scanning combined with transmission electron microscopy, would greatly facilitate the elucidation of the many as yet unclarified details of the dynamic changes in mammalian gametes at the time of fertilization[19].

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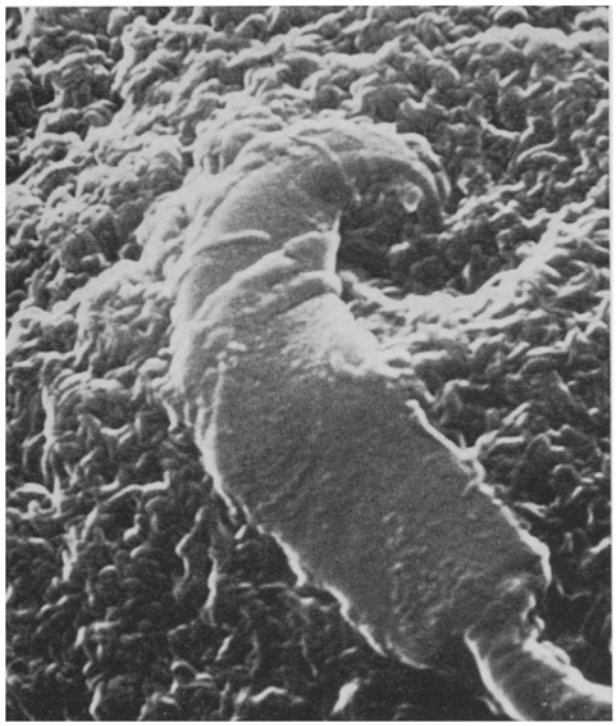

Fig. 4. Sperm head on egg surface about 2 min after insemination. Note egg microvilli trapping head of the spermatozoon. ×12,000.

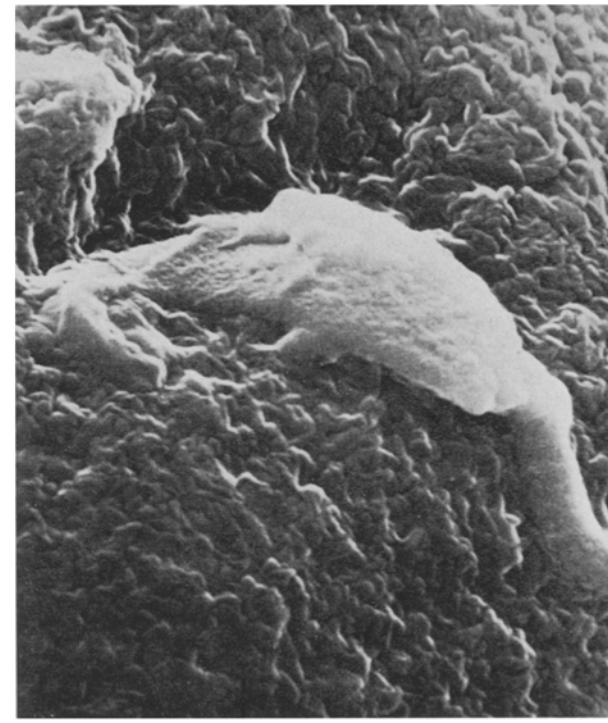

Fig. 5. Sperm head being incorporated into the egg cytoplasm about 4 min after insemination. ×10,600.

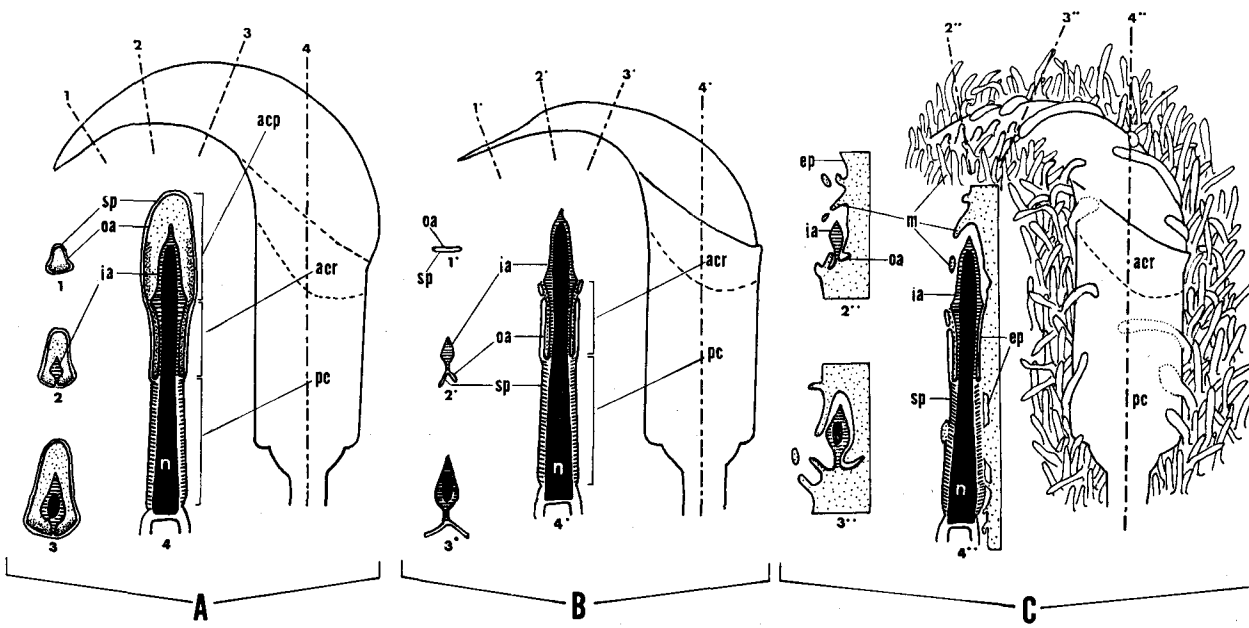

Fig. 6. Diagrams of 3 representative stages of changes in hamster spermatozoon before and during fertilization. A, intact epidiymal spermatozoon; B), capacitated spermatozoon of which acrosomal cap has disappeared; C, capacitated spermatozoon in an initial stage of incorporation into the egg cytoplasm. Acp, acrosomal cap; acr, acrosomal collar; ep, egg plasma membrane; ia, inner acrosomal membrane; m, egg microvilli; n, nucleus; oa, outer acrosomal membrane; pc, postacrosomal region; sp, sperm plasma membrane.

Résumé. L'observation directe de la surface du sperme et de l'œuf au microscope électronique «scanning» a montré une série de changements dans le spermatozoïde du Hamster doré avant la fertilisation ainsi que la relation topographique entre le spermatosoïde et l'œuf au moment de la fertilisation. Il semble que les microvilli de l'œuf participent activement à l'incorporation de la tête du sperme dans le cytoplasme de l'œuf.

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