Fine structure and early fertilization changes of the animal pole in eggs of the river lamprey, *Lampetra fluviatilis*

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Fertilization is accompanied by changes in the structure of the egg cytoplasm (cf. Rothschild, 1958; Raven, 1961). At the level of fine structure such changes have mainly been studied in some marine invertebrates with small eggs that can easily be fertilized in vitro (Pasteels & de Harven, 1963; Schäfer, 1966). Vertebrate eggs are less favourable in this respect, but electron microscope studies have been made on eggs of mammals (Fléchon, 1966; Zamboni & Mastroianni, 1966; Zamboni, Mishell, Bell & Baca, 1966) and *Xenopus* (van Gansen, 1966). Changes generally observed soon after fertilization include the formation of polysomes or an increase in their number, a hypertrophy of the Golgi complexes, and the appearance of granulated endoplasmic reticulum and annulate lamellae. Afzelius (1957) observed the dispersal of mitochondria in fertilized sea-urchin eggs. Pasteels & de Harven (1963) reported that the structure and distribution of cytoplasmic organelles in eggs of the bivalve mollusc, *Barnea candida*, are not altered by fertilization. The structural changes produced by fertilization in the small, telolecithal eggs of lampreys were repeatedly studied during the late nineteenth century (cf. Herfort, 1901) and later by Kille (1960). Vivid cortical activity and complicated cytoplasmic movements were observed at the animal pole. Electron-microscope studies of lamprey eggs have apparently been restricted to the yolk platelets (Karasaki, 1967). During a study of sperm penetration in lampreys, the eggs were found to be very favourable for electron microscopy, and some observations of early changes in the thin layer of pole plasm will be reported below. The structure of the egg envelopes, the emptying of cortical vacuoles, and sperm penetration are described in separate papers (Afzelius, Nicander & Sjödén, 1968; Nicander & Sjödén, 1968).

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MATERIALS AND METHODS

Material was collected from two animals caught in the early spawning season and immediately transported to the laboratory for preparation. Eggs were either stripped directly into the fixation fluid or into a small amount of water in porcelain jars, where large amounts of spermatozoa were added and samples for fixation were taken immediately and after $\frac{1}{4}$, 1 and 2 min. Unfertilized eggs were fixed either in a 2 % phosphate-buffered osmium tetroxide solution with sucrose (Millonig, 1961) or in a 3 % cacodylate-buffered glutaraldehyde solution, with subsequent storage overnight in cacodylate buffer with sucrose, followed by osmium tetroxide solution for 1.5 h (Sabatini, Bensch & Barrnett, 1963). All fertilized eggs were fixed in glutaraldehyde. All eggs were embedded in Epon (Luft, 1961). JLL sections of the animal pole were stained with toluidine blue for light microscopy. Thinner sections were collected on copper grids, stained with lead acetate, uranyl acetate, or both consecutively, and examined in a Siemens Elmiskop I at magnifications from 2000 to 14000. Some sections were placed on gold grids and used for the EM cytochemical demonstration of glycogen according to Monneron & Bernhard (1966).

OBSERVATIONS

Unfertilized eggs

Light microscopy shows that a crescent-shaped superficial layer of the cytoplasm, about 2 $\mu$m thick, contains no cortical vacuoles and only a few small yolk bodies. Instead, there are smaller granules arranged in a subsurface layer (Plate 1, fig. A). This cytoplasm will be called the ‘pole plasm’.

Electron microscopy. Plate 2, fig. A shows a cell surface with some very short microvilli. After fixation in glutaraldehyde their tips make contact with the inner chorion, leaving a narrow space between the envelope and the egg surface.

PLATE 1

Photomicrographs of sections stained with toluidine blue.

Fig. A. Unfertilized egg. Survey of animal pole, with the three envelopes: tuft (t), outer chorion (och) and inner chorion (ich). The pole plasm (ppl) forms a sickle-shaped surface layer without cortical vacuoles (cv) and yolk but with small subsurface granules. The ‘endoplasm’ (end) is filled with yolk.

Fig. B. Animal pole 1 min after addition of sperm. The cortical reaction has started and produced a wide ‘perivitelline space’ (pvs) at the periphery of the pole plasm. The small subsurface granules are no longer seen. Distinct surface protrusions (p) are seen near the periphery.

Fig. C. Pole plasm 1 min later, to show the different layers. The ‘hyaline’ cytoplasm (hc) appears granulated and mainly forms a broad column adherent to the inner chorion (ich), as is one of the narrow peripheral projections (p). The wide vesicular layer (vl) borders on a narrow, finely granulated layer (pl), separated from the yolky ‘endoplasm’ (end) by the ‘spongy’ layer (spl).
After osmium fixation, these microvilli are bent and the whole cell surface lies closer to the inner chorion. The plasma membrane is distinctly triple-layered with the inner dense leaflet somewhat denser than the outer one (cf. Plate 4, fig. A). Below the cell surface, there are spherical or oval membrane-bounded bodies with a maximum size of 0.3 by 1.5 μ, arranged in one layer generally with their longitudinal axis perpendicular to the cell surface. These bodies are embedded in a rather dense, somewhat granular cytoplasmic matrix with some very tiny, often elongated vesicles.

The deep layers of the pole plasm are mainly occupied by large vesicles of smooth-surfaced endoplasmic reticulum containing some flocculent material. Near the border with the yolky endoplasm the vesicles are few, but some mitochondria and patches of electron-dense granules are seen. These granules are mainly seen after fixation in glutaraldehyde and show higher contrast after staining with lead acetate than with uranyl acetate. They are digested by amylase and are therefore considered to be glycogen. The mitochondria (Plate 1, fig. B) are somewhat elongated with transverse, regularly spaced cristae in a matrix which contains numerous small, dense granules after fixation in osmium tetroxide. Very few mitochondria were seen in the superficial layers. No typical ribosomes were noticed. Small, rather ill-defined Golgi complexes without vacuoles are scattered in the deep layer of the pole plasm as well as in other parts of the egg. A few vacuoles with a wrinkled boundary membrane and rather electron-transparent contents are sometimes present. They probably represent immature cortical alveoli.

The endoplasm contains large amounts of yolk. The proteinaceous yolk platelets, up to 7 μ in diameter, have a loose cortex and a crystalline core as recently described by Karasaki (1967). The smaller (0.5–2 μ) more numerous round granules are obviously lipid yolk. The cytoplasm between the yolk granules contains numerous mitochondria and much glycogen similar to that in the pole plasm.

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**PLATE 2**

Electron micrographs.

Fig. A. Part of pole plasm before fertilization. The short microvilli of the egg surface are not adherent to the inner chorion (ich). Opaque granules (arrows) are seen under the surface together with a few lipid droplets. The remaining pole plasm contains vesicles of the endoplasmic reticulum (er), patches with glycogen granules (gl), and mitochondria (m) near the yolk bodies (y). Fixation: glutaraldehyde, stained with uranyl acetate.

Fig. B. A Golgi complex (Gc) surrounded by mitochondria (m) with small internal granules. Part of a lipid yolk droplets (ly) is seen. Osmium fixation, double staining.

Fig. C. Survey of pole plasm 1 min after the addition of sperm. One large, coarsely granular surface protrusion (p) partly adheres to the inner chorion. The superficial layer of hyaline cytoplasm forms numerous smaller irregular protrusions into the perivitelline space (pvs). Below this layer mitochondria and large vesicles of the endoplasmic reticulum (er) are seen. Some narrow, regular cytoplasmic septa (arrows) are seen bordering some vesicles. Glutaraldehyde, double staining.
Eggs fixed 1 min after the addition of sperm

Distinct structural changes have occurred in the pole plasm at this stage, though none were seen in eggs fixed after 30 sec.

Light microscopy showed the formation of a distinct perivitelline space separating the peripheral layer of the pole plasm from the chorion at the start of the cortical reaction (Plate 1, fig. B). Protrusions from the cell surface were seen in this area. No subsurface granules could be discerned.

Electron microscopy showed a highly irregular cell surface in the peripheral areas (Plate 2, fig. C). The subsurface cytoplasm appeared hyaline without any opaque bodies. It consisted of a granular matrix with less electron-dense areas, particularly in the longest protrusions, which sometimes reached the chorion and appeared adherent to it. The deeper layers showed a smooth surface (as in Plate 3, fig. A). Interior from the surface layer the pole plasm contained mitochondria and vesicles of the endoplasmic reticulum, the latter sometimes separated from each other by a narrow, curved wall of regular width. Patches of glycogen granules were less conspicuous, but it could not be decided whether the glycogen had diminished in amount or only dispersed. No changes were observed at the border to or within the endoplasm.

Eggs fixed 2 min after the addition of sperm

Light microscopy showed marked changes in the central area of the animal pole. The amount of pole plasm seemed to have increased and the perivitelline space had widened, but a large central cylinder of dark, finely granulated cytoplasm was adherent to the inner surface of the chorion (Plate 1, fig. C). The periphery still showed many slender protrusions. The bulk of the pole plasm was electron-transparent and seemed to accumulate near the centre of the pole. It was subdivided into two light layers by a darker, narrow band of diffusely granulated cytoplasm.

Electron microscopy confirmed the apparent adherence of the central cylinder to the inner chorion. Laterally the surface of this cylinder displayed numerous very small folds (Plate 4, fig. B). Its substance was opaque, with a diffusely granular texture, some tiny vesicles and many small patches of more distinct

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Plate 3

Electron micrographs of eggs fixed in glutaraldehyde 2 min after the addition of sperm. Fig. A. Survey picture of all layers near the periphery of the animal pole, with 'hyaline' cytoplasm (hc) in a thick layer bordering on the perivitelline space (pvs), vesicular layer (vl), particle layer (pl), and a rim of the 'spongy' layer (spl). Double staining.

Fig. B. Higher magnification of the three deep layers. A structure similar to a small cortical vacuole (x) and a lipid droplet (l) are seen among the vesicles of the endoplasmic reticulum. The particle layer (pl) is characterized by less endoplasmic reticulum and many small, dense, very polymorphic particles. The vesicles of the 'spongy' layer have lighter contents and are separated by narrow cytoplasmic strands of regular width, curved so that ring-shaped cross-sections (arrows) are often seen. Double staining.
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electron-dense granules, probably glycogen. These patches obviously correspond to the granules seen in the light microscope after staining with toluidine blue. The remaining pole plasm showed a smooth surface near the cylinder (Plate 3, fig. A) and many folds and protrusions near the periphery (Plate 4, fig. A). The fine structure was similar to that in the cylinder, but patches with dense granules were not present in the peripheral pole plasm. A few tiny, generally elongated vesicles were often seen. The outer light layer consisted of numerous large vesicles of the endoplasmic reticulum similar to those in the unfertilized egg (Plate 3). A few vacuoles similar to small cortical vacuoles were sometimes seen, but mitochondria and Golgi complexes were rare, as in the rest of the pole plasm. The band of dark cytoplasm also showed many of these vesicles, while the cytoplasm separating them contained small, very electron-dense and polymorphic particles (Plate 4, fig. C) with homogeneous contents and a boundary membrane similar to that of the vesicles. Their origin could not be traced. The inner light layer bordering on the endoplasm was spongy on account of the many vesicles, obviously smooth endoplasmic reticulum but with less flocculent contents than the vesicles of the outer light layer. The narrow walls between adjacent vesicles very often formed highly curved septa of constant width and had a rather opaque cytoplasmic matrix (Plate 4, fig. D). Their curvature was sometimes so marked that ring-shaped cross-sections were seen (Plate 3, fig. B). Some glycogen granules were present singly or in small groups, especially near the periphery of the endoplasm. The last-mentioned region did not show any distinct changes that could explain the increase in volume of the pole plasm.

DISCUSSION

The observations here reported obviously have a preliminary character. Material has to be collected at shorter time intervals and for a longer period.

PLATE 4

Electron micrographs of eggs similar to those in Plate 3, higher magnifications.

Fig. A. Periphery of the pole plasm, superficial layer. The protrusions (p) into the perivitelline space (pvs) contain a very finely granulated cytoplasmic matrix with lighter areas. The plasma membrane is an asymmetric "unit membrane" (arrows). One protrusion is closely applied to the inner chorion (ich). A mitochondrion (m) and some small, elongated vesicles are seen. Double staining.

Fig. B. Part of the column of "hyaline" cytoplasm (hc) shown in Plate 1, fig. C. The surface bordering on the perivitelline space (pvs) shows many small invaginations (arrows). The cytoplasmic matrix contains granular material (gl), probably glycogen. Stained with lead acetate.

Fig. C. Dense, polymorphic particles (arrows) between the vesicles (v) of endoplasmic reticulum in the particle layer. Double staining.

Fig. D. Small area of the "spongy" layer, showing the narrow, curved, septa of dense cytoplasm of regular width (arrows) between many vesicles (v) of the endoplasmic reticulum. Some glycogen granules (gl) are present. Stained with lead acetate.
after fertilization before the origin and fate of some structures can be traced and their functional meaning understood. The apparent absence of ribosomes is one enigmatic feature, as other egg types are reported to show free ribosomes and even polysomes, at least after fertilization (Pasteels & de Harven, 1963; Fléchon, 1966; van Gansen, 1966; Schäfer, 1966). Karasaki (1967) also mentioned ribosomes in eggs from other lamprey species. However, the ribosomes described in this literature are remarkably large and have sometimes been observed very near yolk platelets like many glycogen granules in lamprey eggs. Similar observations to ours have recently been reported for *Xenopus* eggs (Hay, 1966). Moreover, the articles mentioned above obviously follow the development after fertilization for a much longer time than the present report. At the latest stage studied by us, 2 min after the addition of spermatozoa to the egg, the sperm nucleus has not even entered the pole plasm (Kille, 1960, 1961; Nicander & Sjödén, 1968).

The appearance of the ‘spongy’ layer may be the first step in a differentiation of the endoplasmic reticulum such as observed in blastomeres of a bony fish (Lentz & Trinkaus, 1967) and in fertilized eggs of mammals (Zamboni & Mastroianni, 1966). This spongy layer was obviously observed by Herfort (1901), who interpreted it as participating in the mobilization of yolk. No such function could be deduced from the observations described here. The increase in the amount of pole plasm evident after 2 min might be caused by a streaming of cytoplasm from between the yolk inclusions of the endoplasm to the animal pole, though this interpretation does not explain the paucity of mitochondria in the pole plasm at this stage.

A vivid surface activity at the animal pole was reported already by Herfort (1901) and further studied by Kille (1960). They interpreted the adherence of protrusions and the hyaline cylinder to the chorion as the persistence of a condition already present in the unfertilized egg. The electron micrographs appear to invalidate this interpretation, as the contacts are mainly made by protrusions which are formed after activation of the egg and show a specialized fine structure. A parallel study of sperm penetration (Nicander & Sjödén, 1967) confirmed Kille's (1960) observation that the fertilizing sperm nucleus, preceded by the head filament, enters the egg through one of the peripheral protrusions and not through the central wide cylinder. Thus, the last-mentioned transitory structure does not correspond to the reception cone of the sea-urchin egg (cf. Rothschild, 1956, 1958; Raven, 1961), which, instead, seems to be analogous to that peripheral protrusion of the lamprey egg which leads the spermatozoon into the egg. The cause of these surface changes may be the enlargement of the egg surface suddenly taking place due to the emptying of the cortical vacuoles (cf. Afzelius *et al.* 1968).

The stratification of the animal pole plasm seen after 2 min is remarkably similar to the ‘phase separation’ produced in fertilized sea-urchin eggs by pretreatment with trypsin (Runnström, 1963). Obviously, the many marked changes
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in fine structure taking place in a rapid sequence just after activation add to other features which make the lamprey egg a very favourable object for the study of fertilization in a vertebrate.

SUMMARY

1. The specialized cytoplasm of the animal pole of mature lamprey eggs contains some very short microvilli, a row of subsurface bodies in a granular cytoplasmic matrix, large vesicles of smooth endoplasmic reticulum, some very small vesicles, mitochondria, a few ill-defined Golgi complexes, and many accumulations of glycogen granules, but no typical ribosomes.

2. One minute after the addition of sperm to the eggs, the peripheral surface layer shows long protrusions containing dense cytoplasmic matrix and often adherent to the chorion. The subsurface bodies have disappeared and the smooth endoplasmic reticulum shows some signs of differentiation.

3. After 2 min a broad central cylinder of granular cytoplasmic matrix also adheres to the chorion. The endoplasmic reticulum occupies a very wide zone with an outer, undifferentiated layer separated from an inner ‘spongy’ layer by a narrow band containing small, very dense and polymorphic bodies of unknown origin.

4. The yolky ‘endoplasm’ occupying the bulk of the egg shows large composite yolk platelets and smaller droplets of lipid yolk, separated by cytoplasmic strands rich in mitochondria and glycogen granules. No early fertilization changes were observed in the endoplasm.

RÉSUMÉ

Structure fine et évolution du pôle animal pendant les premières phases de la fécondation chez la Lamproie de rivière, Lampetra fluviatilis.

1. Le cytoplasme spécialisé du pôle animal des œufs murs de Lamproie contient quelques très courtes microvillosités, une rangée de corps subcorticaux dans une matrice cytoplasmique granulaire, de larges vésicules de reticulum endoplasmique lisse, quelques petites vésicules, des mitochondries, quelques complexes golgiens mal définis et de nombreuses accumulations de granules glycopéniques, mais pas de ribosomes typiques.

2. Une minute après l’insémination, la couche périphérique de surface montre de longues protrusions qui contiennent une matrice cytoplasmique dense et qui adhèrent souvent au chorion. Les corps subcorticaux ont disparu et le réticulum endoplasmique lisse montre certains signes de différenciation.

3. Après 2 min, un large cylindre central de matrice cytoplasmique granulaire adhère au chorion. Le reticulum endoplasmique occupe une très large zone se composant d’une couche externe indifférenciée qui est séparée d’une zone interne ‘spongieuse’ par une bande étroite contenant des corps denses et polymorphes dont l’origine est inconnue.
4. ‘L’endoplasme’ vitellin occupant la masse de l’œuf montre de grosses plaquettes composites et de plus petites gouttes de vitellus lipidique, lesquelles sont séparées par des travées cytoplasmiques riches en mitochondries et en granules de glycogène. On n’a pu observer aucun changement dans cet endoplasme à la suite de la fécondation.

REFERENCES


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