

Cytochemical Localisations and Ultrastructure in the Fertilized Unsegmented Egg of *Paracentrotus lividus*

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ABSTRACT

Fertilized, but still unsegmented, eggs of the sea urchin *Paracentrotus lividus* have been centrifugated at 30,000 gravity. A comparison has been made between the cytochemical reactions of the different layers (RNA, polysaccharides, acid phosphatase) and the fine structure as shown in thin sections studied with the electron microscope.

The correlation between the cytochemistry and the cellular ultrastructure is one of the main chapters of the cytology of today (5, 12), and recent work shows that efforts are being made to extend this kind of research to the germinal cells and to eggs in the course of their development (1, 2, 7, 8, 14, 15, 18, 19). It is with this in mind that we have studied the fertilized, but still unsegmented egg of the sea urchin *Paracentrotus lividus*.

A. Cytochemical Studies.—

Studies were carried out on eggs fixed with Baker's calcium formaldehyde (3), (formalin 10 cc., 10 per cent anhydrous calcium chloride 10 cc., distilled water 80 cc.). Some eggs were used whole, others were sectioned at 6 to 8 μ . The following reactions were used: for detection of RNA (test of Unna-Brachet, with control with ribonuclease) (4); for acid phosphatase activity (Gomori's reaction, but applied only to whole eggs, see reference 11); and for detection of polysaccharides (PAS, with and without salivary digestion (10)); and of sulfated mucopolysaccharides (alcian blue at pH 0.2) (17).

In the non-centrifuged egg all these reactions are very intense and show a rather uniform distribution, making localisation of the reacting components diffi-

cult. For this reason we have centrifuged the eggs at 30,000 g for 20 minutes at a temperature of 15–18° C., in order to separate the cellular organelles according to their relative densities. This centrifugation was started 10 minutes after fertilization.

The number and sequence of layers in centrifuged sea urchin eggs vary from one species to another. In particular with *Paracentrotus*, the living egg just after centrifugation shows four layers stratified between the centripetal and the centrifugal pole in the following order:—

1. a lipidic hood
2. a hyaline zone
3. a large zone of big granules corresponding to the yolk granules
4. a thick convex hood, either homogeneous or slightly granular in appearance.

After fixation with calcium formaldehyde, the cytochemical distribution can be established as follows:—

(a) The RNA is abundant but diffuse in zone 2. In zone 3 a fine network can be observed between the yolk granules. The convex hood comprising zone 4 shows a diffuse but extremely intense basophilic character. In the interior part of zone 3 and in zone 4 occur rounded bodies of variable size. Some of them are larger than yolk granules. They have a basophilic appearance resembling that of the nucleolus. The diffuse and localized basophilia of zones 2, 3, and 4 is abolished by ribonuclease.

(b) After salivary digestion, the PAS reaction

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is weak and diffuse in zones 2 and 4, but very strong in the region of the yolk granules (zone 3).

(c) The reaction with alcian blue, pH 0.2, is characteristic of the upper part (centripetal) of zone 3.

(d) The same is true for acid phosphatase studied in eggs incubated for a short period (10 to 20 minutes) and mounted *in toto*. Thus a well defined, phosphatase-active ring appears in the upper zone of the vitelline layer. With longer incubation this ring becomes wider in both directions (centripetal and centrifugal) even up to the point at which the entire egg gives a positive reaction. It is difficult to decide whether the other layers actually possess lower phosphatase activity or whether this slow widespread reaction is an artefact due to focal diffusion.

B. Electron Microscope Studies.—

Studies were done after the eggs had been fixed with buffered osmium tetroxide 1 per cent (pH 7.4) and embedded in *n*-butylmethacrylate. The electron microscope used was the Philips EM 100-B type at a voltage of 60 kv. The ultrathin sections were made with the Porter-Blum microtome.

Isolated eggs were embedded separately and oriented in such a way that serial sections (estimated 150 to 300 Å thick) were made either parallel with, or perpendicular to the stratification. The different zones of the centrifuged egg shows the following structure:

1. In the hood 1, there exists an accumulation of lipoidal droplets all of which are osmiophilic; some mitochondria are also present.

2. Zone 2 is characterized (Fig. 1) by vesicular structures some of which are canalized. The surfaces of these vesicles are surrounded by numerous Palade particles. The structure in this zone can be regarded as characteristic of an atypical form of ergastoplasm.

3. Zone 3 shows vitelline platelets with diameters varying between 0.25 μ and 1 μ (Fig. 2). These platelets are built up of dense grains; they do not have a complete outer membrane; some of them show a notch or indentation and seem to empty themselves into the surrounding cytoplasm.

In the space between the platelets there are three kinds of structures: (a) Golgi bodies (Fig. 2) which have been localised only in the upper or centripetal part of zone 3. (b) The ergastoplasmic vesicles and the grains of Palade analogous to

those of zone 2. (c) A considerable number of annulated lamellae ("periodic lamellae" of Rebhun), the structure of which is similar to that of the nuclear membrane (1).

In the present case, these lamellae must be considered as being isolated and not stacked in successive parallel layers. The individual lamellae appear to undulate through the cytoplasm so that the same structure shows up in different parts of the same section, cut either perpendicularly (Fig. 2) or horizontally (giving typical rings!). At their ends, the double membranes of the lamellae are obviously continuous (Fig. 3, which is only one example of many similar observations) with typical flattened elements of the ergastoplasm.

4. Zone 4 contains the greatest part of the mitochondria of the egg, though, as stated above, a few mitochondria are associated with the lipides of zone 1. In zone 4 the mitochondria are not packed closely together, but are separated by material of low density containing a few very thin filaments. The filaments anastomose to some extent, and seem to be made up of granules the size of which is at about the limit of resolution of the microscope used. They are definitely much smaller than the granules of Palade. In addition there are here and there globular masses, with a structure resembling a vitelline granule, but with a greater density, and often with a larger size, reaching diameters of 3 to 5 μ . (Fig. 4). Some of these structures may be surrounded by an annulated lamella (Fig. 4), but this has been observed only in one single section.

C. *In the Non-Centrifuged Egg.*—The annulated lamellae are situated in the perinuclear area of the non-centrifuged egg. In the spermaster stage they are all radially oriented; *i.e.*, parallel to the aster fibers. These latter have not been observed. The perinuclear area, though poor in platelets, is particularly rich in vesicular ergastoplasm, and shows a few scattered mitochondria. The platelets and the lipidic granules are distributed rather uniformly. The mitochondria are also abundant, and are found either singly or arranged in "rosettes" about individual lipoidal granules, surrounding the lipidic droplets completely in a closely applied layer (Fig. 5).

CONCLUSIONS

1. The annulated lamellae which had been described previously (2, 14, 15, 16, 18) in the un-

fertilized sea urchin egg, in the oocytes of mollusks, the spermatids of the rat, the pancreas of *Amblystoma*, and the cells of the mammalian adenocarcinoma (16) are particularly abundant in the fertilized egg of *Paracentrotus*.

While Afzelius has seen these structures located around the basophilic bodies (heavy bodies) in the unfertilized egg, this association is rather exceptional in the fertilized egg, in which the lamellae are generally free and isolated, but undulating (rippled). We can confirm the opinion of some authors (14, 18), that this is a particular form of ergastoplasm, for in the present material, continuity between an annulated lamella and a typical ergastoplasmic membrane is quite evident (Fig. 3).

2. The strong but diffuse basophilia of the hyaline zone can be attributed to its atypical vesicular ergastoplasm, which is rich in particles of Palade (12, 13). This form of ergastoplasm can probably be compared to the "osmiophilic vesicles" which are present in the polar plasm of *Tubifex* (19) as well as to the vesicular transformation of the ergastoplasm in the mammalian adenocarcinoma cells (6, 16).

3. The strong, diffuse basophilia of the centripetal pole poses a new problem. It may perhaps be due to mitochondria. The space between the mitochondria has structural features which do not correspond to ergastoplasm, and where the particles of Palade, in so far as they are found, are very rare. Nevertheless, this zone contains centrifuged RNA, which thus seems to be related to heavy particles. The structure and nature of these particles should be studied further.

4. Most of the acid phosphatase activity and the strongly acid mucopolysaccharides of the centrifuged egg are located in a zone lying at the

centripetal part of the vitelline layer (zone 3). This zone is differentiated from the underlying layers only by the presence of the Golgi bodies.

5. The lipoidal granules are entirely surrounded by a closely applied crown of mitochondria. Such a disposition indicates a morphological expression of the role of mitochondria in lipide metabolism (*cf.* reference 9).

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EXPLANATION OF PLATES

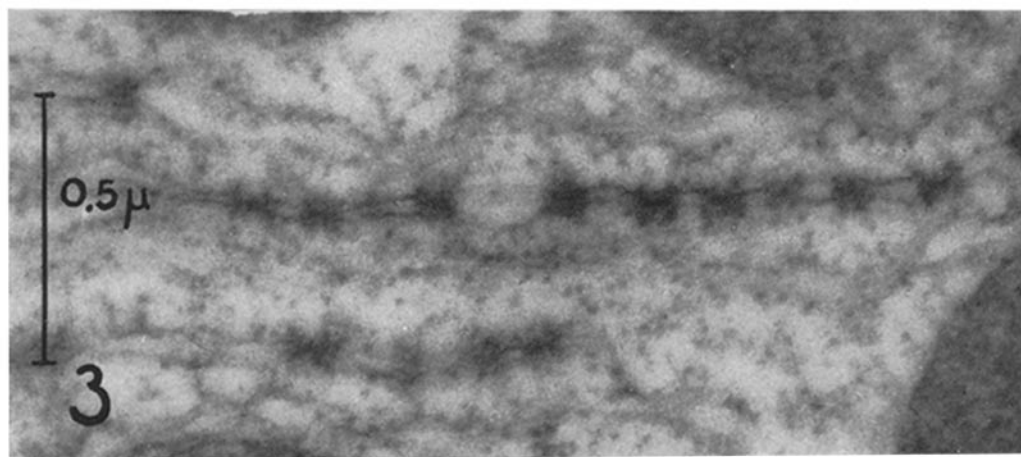
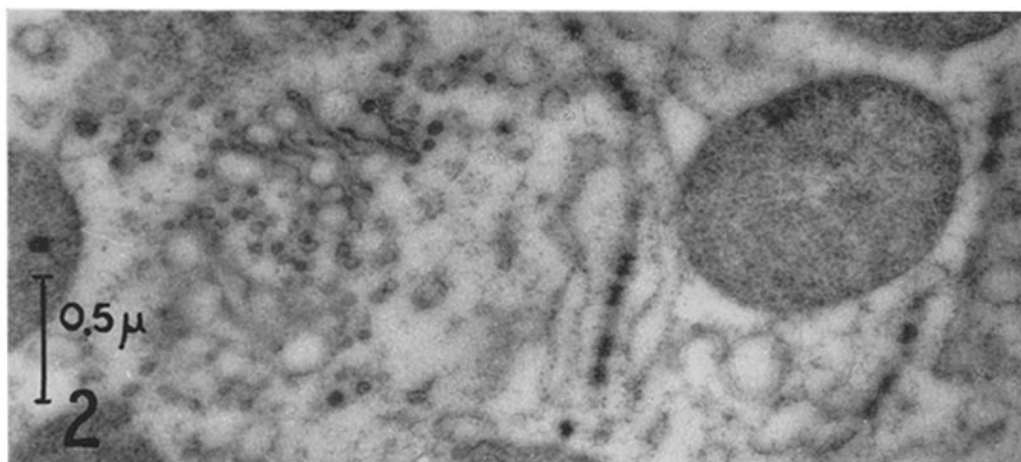
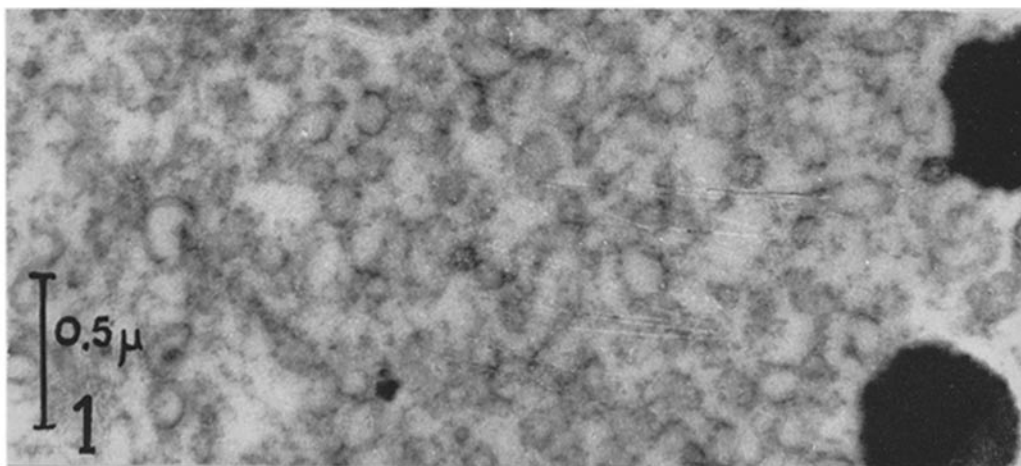
All figures are electron micrographs of thin sections of eggs of *Paracentrotus lividus*.

PLATE 268

FIG. 1. Centrifuged egg showing detail from the hyaline layer. At the extreme right are lipidic droplets. $\times 40,000$.

FIG. 2. Centrifuged egg showing yolk layer. At the left the Golgi complex is evident and at the right a yolk platelet between two annulated membranes. $\times 32,000$.

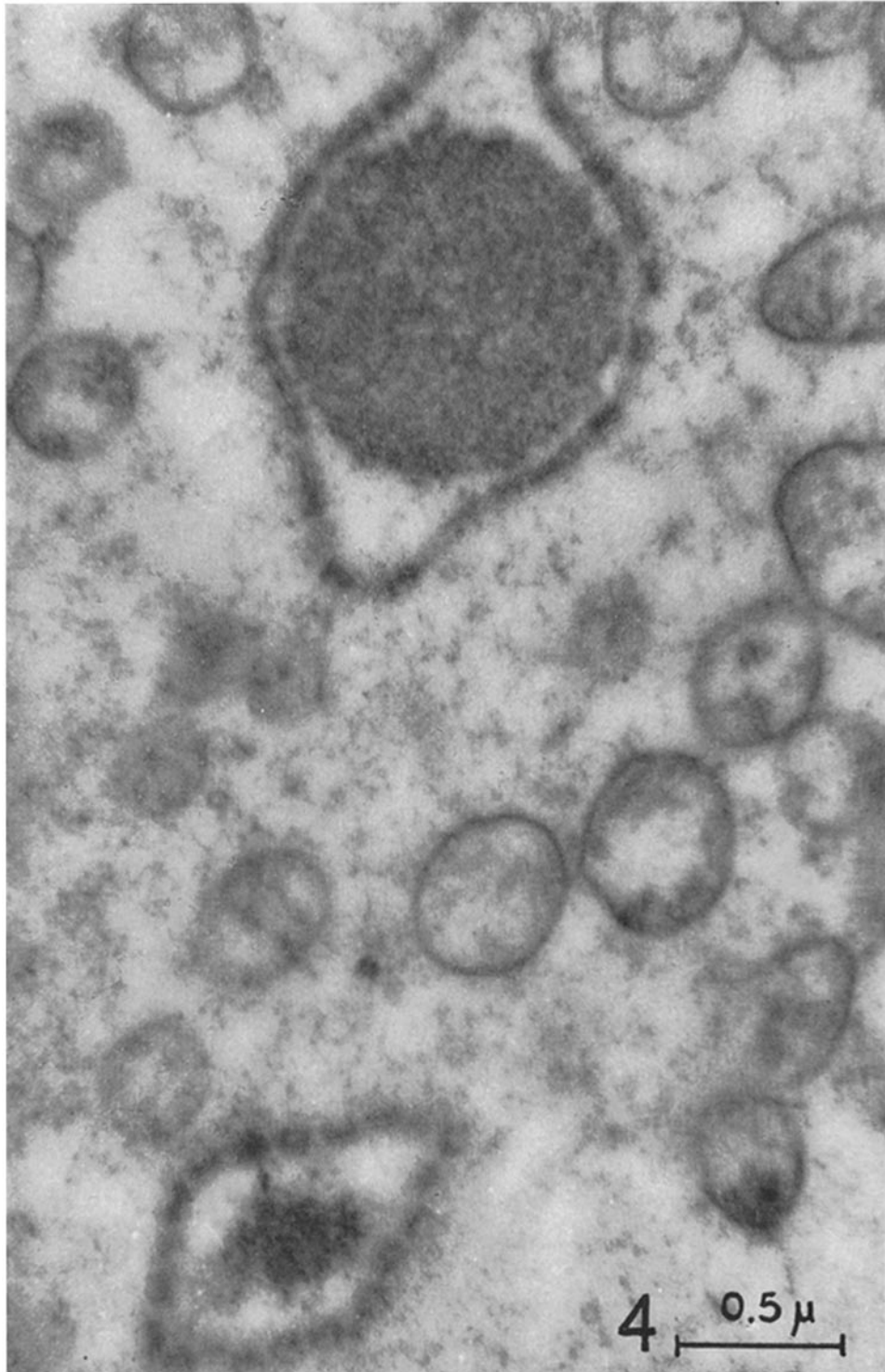
FIG. 3. Centrifuged egg showing yolk layer. Annulated membranes and Palade particles are also shown as well as continuity between an annulated membrane and ergastoplasm (see at the right side). $\times 72,000$.



(Pasteels *et al.*: Fertilized and unsegmented egg of sea urchin)

PLATE 269

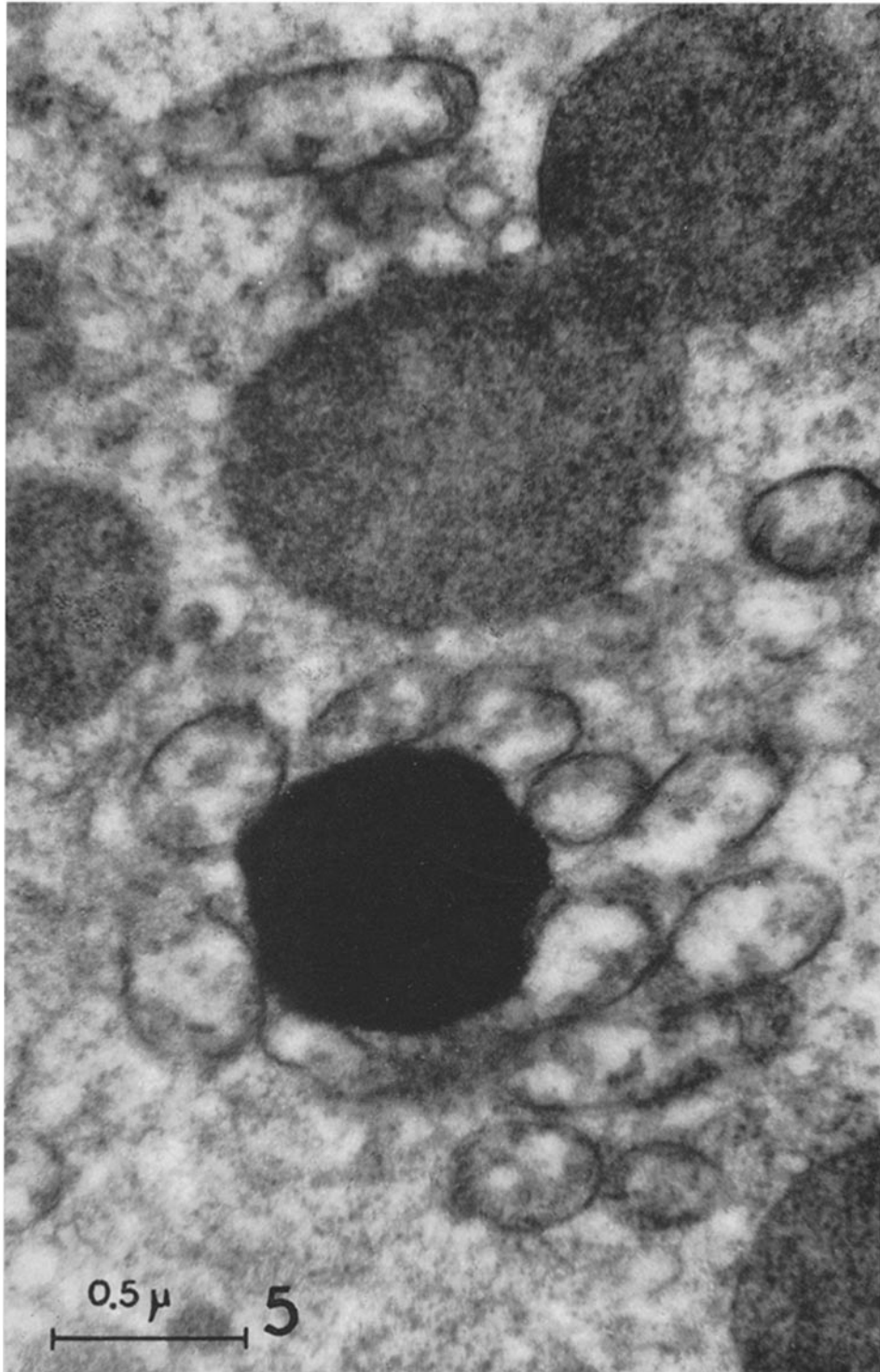
FIG. 4. Section of centrifuged egg including the centrifugal mitochondrial hood. Mitochondria are separated by a fuzzy material (without Palade particles). At the top and bottom of the figure, two heavy bodies are surrounded by an annulated membrane. $\times 48,000$.



(Pasteels *et al.*: Fertilized and unsegmented egg of sea urchin)

PLATE 270

FIG. 5. This depicts a small part of a normal egg. Yolk platelets are evident (at the top) and at the bottom a rosette of mitochondria is pictured in association with a lipidic droplet. $\times 54,000$.



(Pasteels *et al.*: Fertilized and unsegmented egg of sea urchin)