

INTRACISTERNAL GRANULES OF THE ENDOPLASMIC RETICULUM IN THE CRAYFISH OÖCYTE

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The early literature on the origin of yolk contains numerous studies which assert that a close morphological relationship exists between the developing yolk masses and certain cellular organelles. The nucleus, mitochondria, Golgi material, and ergastoplasm have all, at one time or other, been implicated in the synthesis of yolk and of other types of secretion as well (*cf.* Bowen, 2; Wilson, 12). The description of the fine structure of the ergastoplasm (endoplasmic reticulum), coupled with the firm evidence for the synthesis of protein involving ribosomes, has led investigators to look for evidence of a close morphological relationship between the genesis of the protein-rich secretion granules and the elements of the endoplasmic reticulum. Studies have been reported where large secretion masses appear within the cisternae of the endoplasmic reticulum (*cf.* Porter, 8, for literature). However, the best documented studies which seem to involve the endoplasmic reticulum in the synthesis of protein secretion are those of Palade (7) and Siekevitz and Palade (9-11). They present evidence which suggests that in the guinea pig pancreas the ribosomes synthesize the enzymes which traverse the cisternal membranes and gave rise to the relatively large intracisternal granules; the latter probably have chemical properties similar to those of the definitive zymogen granules. How the intracisternal granules are delivered to other regions of the cytoplasm is not clear; they may exit in some way through the cisternae of the Golgi complex. It is interesting to

note that similar intracisternal granules are not found in the pancreas of the mouse (5) or rat (7). Large, dense granules of unknown significance have also been reported within the cisternae of the endoplasmic reticulum of the spider oocyte (1).

In the course of a preliminary study on the fine structure of the crayfish egg it was noted that the endoplasmic reticulum possessed sharply defined intracisternal granules. The fact that this condition is infrequently found in other cells seemed to warrant a description of it here.

MATERIALS AND METHODS

Crayfish (*Cambarus virilis*) were collected locally during the spring and summer and pieces of the ovary fixed for 1 hour in cold Palade's solution buffered at pH 7.5 (6). The specimens were then dehydrated rapidly, embedded in methacrylate, and sectioned with a Porter-Blum microtome. The sections were stained with lead hydroxide (4) and examined with an RCA EMU 3D electron microscope.

DESCRIPTION

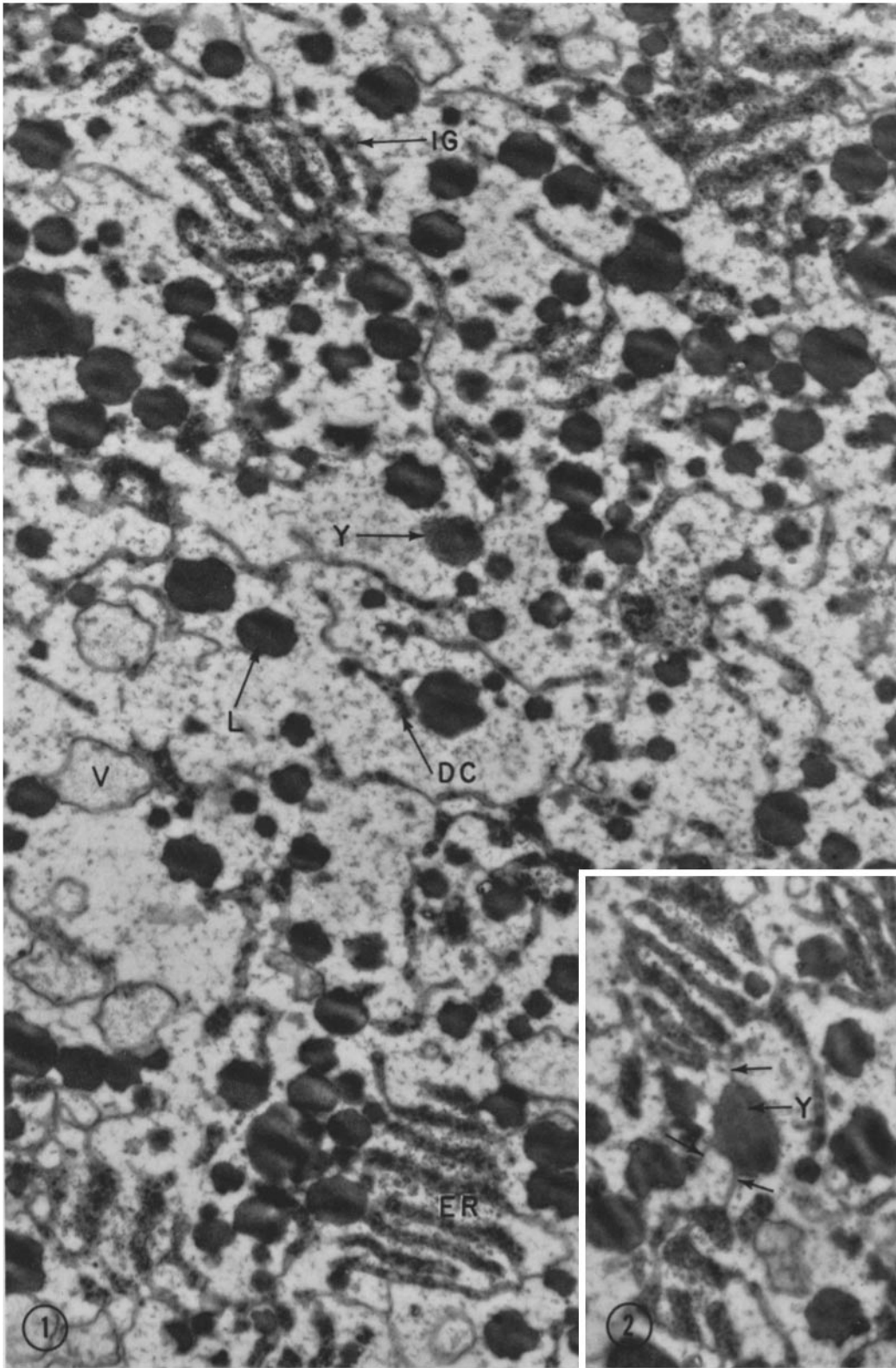
The season in which the ovaries were fixed is one in which rapid growth of the egg occurs, that is, a rapid synthesis and deposition of materials such as lipids, glycogen, and yolk; the last is often of a heterogeneous nature, but generally is composed of a high concentration of protein (3). An analysis of the biochemistry of the crayfish egg has not to our knowledge been made and the identity of the

FIGURE 1

Survey electron micrograph showing form and distribution of the cisternal stacks of endoplasmic reticulum (*ER*) and lipid droplets (*L*). The groups of parallel cisternae are limited by membranes bearing ribosomes and contain numerous intracisternal granules (*IG*). Intracisternal granules are also found in certain dilated regions of the loosely arranged and branching cisternae (*DC*). Note dense yolk body (*Y*) and unidentified vesicle (*V*). $\times 14,000$.

FIGURE 2

Dense yolk body (*Y*) which appears to be surrounded by membranes of the endoplasmic reticulum (arrows). $\times 14,000$.



various fine structural components is based upon their morphology alone.

A low power electron micrograph of a growing oöcyte is illustrated in Fig. 1. The two most striking components of the cytoplasm, namely, the endoplasmic reticulum (*ER*) and lipid droplets (*L*), are easily recognizable by their characteristic appearance. These are embedded in a continuous hyaline and homogeneous appearing cytoplasmic ground substance. The endoplasmic reticulum extends throughout the ground cytoplasm and is composed, in places, of stacks of parallel cisternae that are limited by membranes having great numbers of ribosomes attached to their outer surface (Figs. 3 to 6). The groups of closely packed parallel cisternae are connected at their ends with other cisternae of similar shape and structure, but without preferred orientation; instead, they extend in all directions from the stacked cisternae, branching and anastomosing freely so that the entire system of membrane-limited cisternae appears interconnected, a morphological condition suitable to serve as the basis for an active transport system (Fig. 1).

From the description noted above it is clear that the fine structure of the endoplasmic reticulum in the developing crayfish egg is, to some degree, similar to that in many other types of cells. However, it differs from the condition in most other cells in its distribution and in possessing clearly defined, well developed intracisternal granules (Figs. 1, 5, 6, *IG*). The granules, as well as the entire content of the cisternae, appear more dense than the surrounding cytoplasmic matrix. They are relatively numerous, discrete, and measure

about 40 to 60 μ in diameter. In some preparations parallel lines (lamellae?) of a greater density than the remaining part of the granule are observed (Fig. 6).

The parallel cisternae appear somewhat distended possibly due to the accumulation of granules within them. The more highly magnified electron micrographs (Figs. 5, 6) show clearly the ribosomes arranged on the outer surface of the membranes bounding the cisternae and the rosettes of ribosomes located in the space between the membranes of the stacked cisternae (Fig. 5, *RR*). Ribosomes attached to the membranes and in the rosettes measure about 15 μ in diameter.

The loosely arranged network portion of the endoplasmic reticulum which permeates throughout the ground cytoplasm seems to have a structure similar to that of the parallel cisternae; *i.e.*, composed of flattened vesicles that are limited by membranes. However, one important difference is noted, namely, that fewer ribosomes are present on the outer surface of the membranes limiting the randomly arranged cisternae than are present on membranes of the parallel, stacked cisternae. In many instances, the randomly arranged cisternae appear to be devoid of ribosomes. The loosely arranged network of endoplasmic reticulum also shows variation in the width of its branching cisternae; the more dilated regions contain intracisternal granules (Fig. 1, *DC*) while the remaining portions often appear collapsed.

At this stage in the growth of the egg, no large yolk spheres are present in the cytoplasm as is the case in the more mature oöcyte. However, dense bodies are seen which are probably yolk (Figs.

FIGURE 3

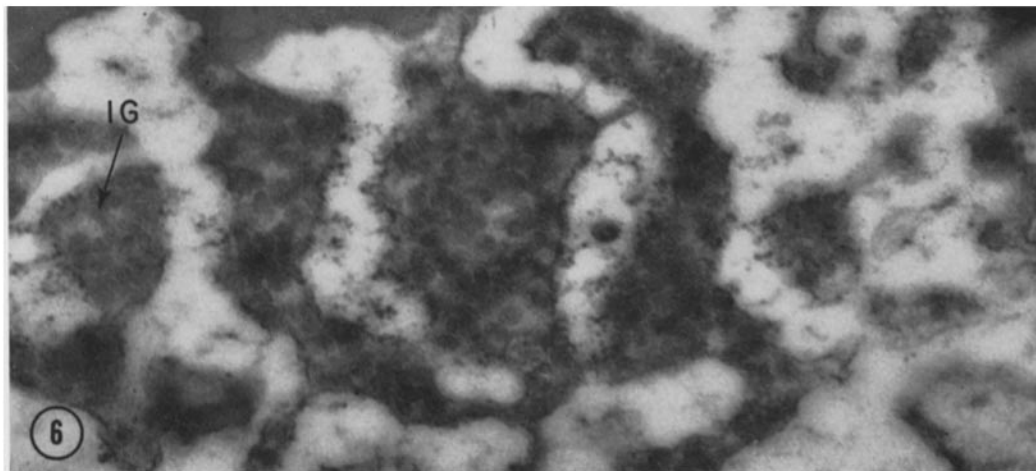
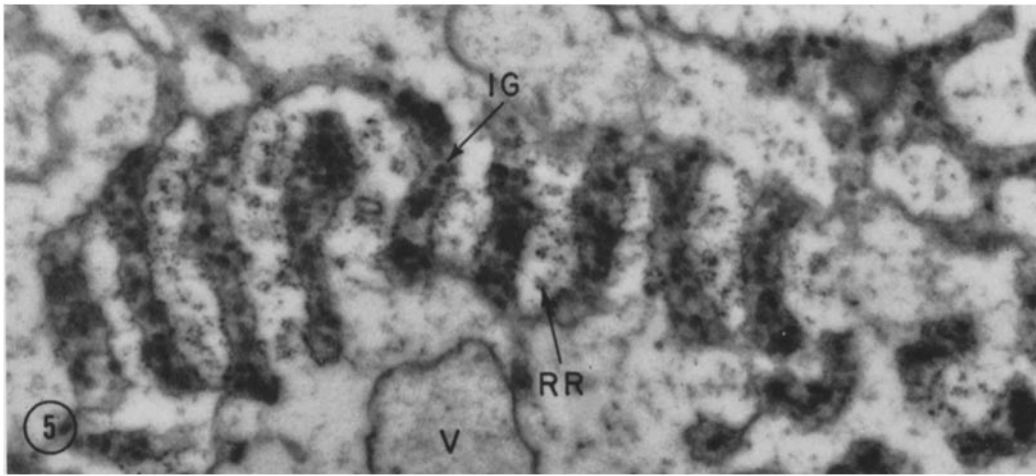
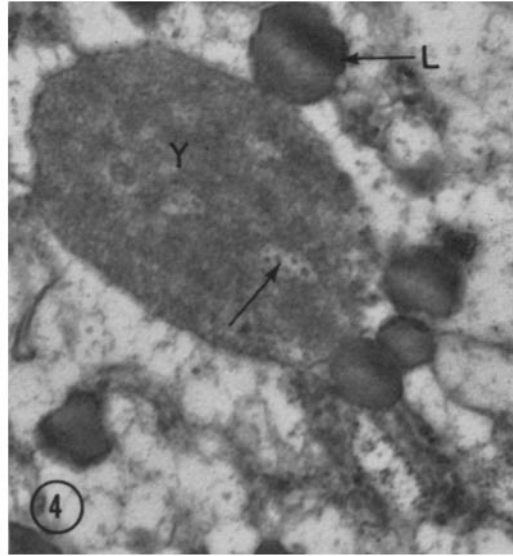
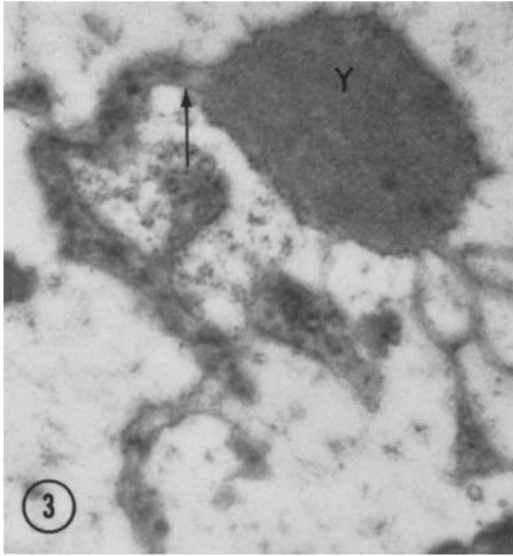
A yolk body (*Y*) showing continuity (arrow) with a granule-containing cisterna of the endoplasmic reticulum. $\times 38,000$.

FIGURE 4

Yolk body (*Y*) in which scattered patches of discrete granules are apparent (arrow). The granules are similar to those within cisternae of endoplasmic reticulum. *L*, lipid droplet. $\times 32,000$.

FIGURES 5 AND 6

Sections through groups of cisternal stacks. The membranes bear ribosomes on their outer surface. Ribosomes are also found arranged as rosettes between the parallel cisternae (*RR*). Within the cisternae are intracisternal granules (*IG*). A portion of a vesicle (*V*) is seen in Fig. 5. Fig. 5, $\times 32,000$; Fig. 6, $\times 47,000$.



1 to 4, *Y*). These bodies seem to be located within the cavities of the endoplasmic reticulum as judged by the fact that cisternal membranes appear to surround them (Figs. 2, 3, arrow). In fact, in Fig. 3 a continuity is demonstrated between the yolk body and a granule-containing cisterna of the endoplasmic reticulum. It is also possible to observe instances in which the forming yolk body contains granules similar to the intracisternal granules of the endoplasmic reticulum (Fig. 4, arrow). These appear to be undergoing a dissolution process which results in the eventual formation of a more homogeneous yolk body.

Membrane-bounded vesicles (Figs. 1, 2, *V*) were observed in the cytoplasm. Their identity is unknown; they do not appear to be connected with the endoplasmic reticulum.

It is interesting to note that both the mitochondria and the Golgi material are relatively sparse at this stage in the developing crayfish egg.

SUMMARY

This note records an additional observation of well defined granules of uniform size within the cisternae of the endoplasmic reticulum. In the absence of biochemical studies, it is impossible to state the nature or significance of the intracisternal granules. In the light of the work of Palade (7) and Siekevitz and Palade (9-11) noted above, it seems reasonable to think that they represent a product associated in some way with the synthesis and deposition of yolk. Thus, the hypothesis is advanced that the intracisternal granules, believed to represent the precursors of the proteinaceous yolk, arise chiefly in the region of the oriented cisternae possibly under the influence of

ribosomes. They then "flow" into and along the unoriented cisternae to regions or pockets where they collect, expand the cisternae, and undergo transformation into a finely granular, relatively large yolk body which may or may not remain associated with the cisternae.

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