# ULTRASTRUCTURAL OBSERVATIONS OF VITELLOGENESIS IN THE SPIDER CRAB, *LIBINIA EMARGINATA* L.

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## ABSTRACT

Ovaries from the spider crab, *Libinia emarginata* L. were studied to learn more of vitellogenesis in crustaceans. Oogonia and previtellogenic oocytes were found in the core of the ovaries. Vitellogenic oocytes are located more peripherally. Profiles of the endoplasmic reticulum are abundant in the vitellogenic oocytes. The granular and agranular reticulum as well as the Golgi complex are active in yolk synthesis. As vitellogenesis proceeds, yolk precursors are incorporated into the egg by micropinocytosis at the egg surface. Thus, in *Libinia*, yolk materials appear to be derived from both intra- and extraoocytic sources.

Cytologists have long been interested in the sources of yolk precursors and the mechanisms by which they achieve their definitive forms within the oocyte. The means by which yolk is formed varies among different groups of animals. Such diversity is particularly well documented in the phylum Arthropoda. Recent investigations of numerous insects have implicated extraoocytic sources of yolk materials and activities of the oocyte surface in the vitellogenic process (Anderson, 1964; Roth and Porter, 1964; Stay, 1965; Telfer, 1961). In the crustaceans, there is some evidence of an extraoocytic origin of yolk precursors (Kerr, 1966) as well as active yolk formation by organelles within the oocyte itself (Beams and Kessel, 1963; Kessel 1968).

Since the crustaceans have been less extensively studied than the insects, the present investigation of oogenesis in *Libinia emarginata* was undertaken to learn more about vitellogenesis in this group.

## MATERIALS AND METHODS

Immature females and mature females brooding eggs of *Libinia emarginata* (L) were obtained from the Marine Biological Laboratory, Woods Hole, Mass. Ovaries were fixed for 90 min in either Karnovsky's (1965) paraformaldehyde-glutaraldehyde mixture or 2.5% glutaraldehyde in 0.1 M phosphate buffer. Tissues were washed in 0.1 M phosphate buffer and postfixed in buffered 1% $OsO_4$, pH 7.5. After dehydration through graded concentrations of ethanol, tissues were infiltrated and embedded in Araldite. Sections, stained with uranyl acetate (Watson, 1958) and lead citrate (Venable and Coggeshall, 1965), were examined with a Philips 300 electron microscope.

## OBSERVATIONS

The cylindrical ovaries of *Libinia emarginata* extend the length of the body and are joined by a small band of tissue at a point ventral to the anterior end of the heart. In immature or newly spawned animals the ovaries are small and white. Vitellogenesis occurs as the mature female broods her young (Hinsch, 1968). The greatly enlarged ripe ovaries are a bright orange. Oogonia and previtellogenic oocytes are found in the central regions of the ovaries. As they differentiate, the oocytes and their

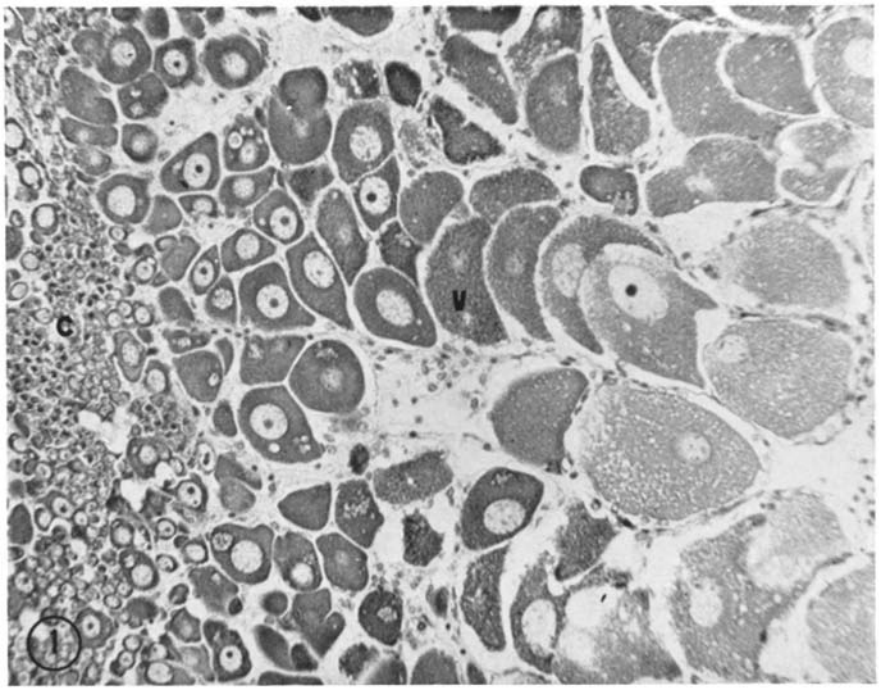



FIGURE 1 Longitudinal section of ovary. Germinal cells and young oocytes are present in the core (c). Larger previtellogenic oocytes are located distal to the core. The vitellogenic oocytes (*v*, and those to the right of it) are greatly enlarged and less basophilic than are the younger oocytes. Nuclei of follicular cells may be seen in spaces between oocytes. Formalin fixation, paraffin embedded, toludine blue. × 312.

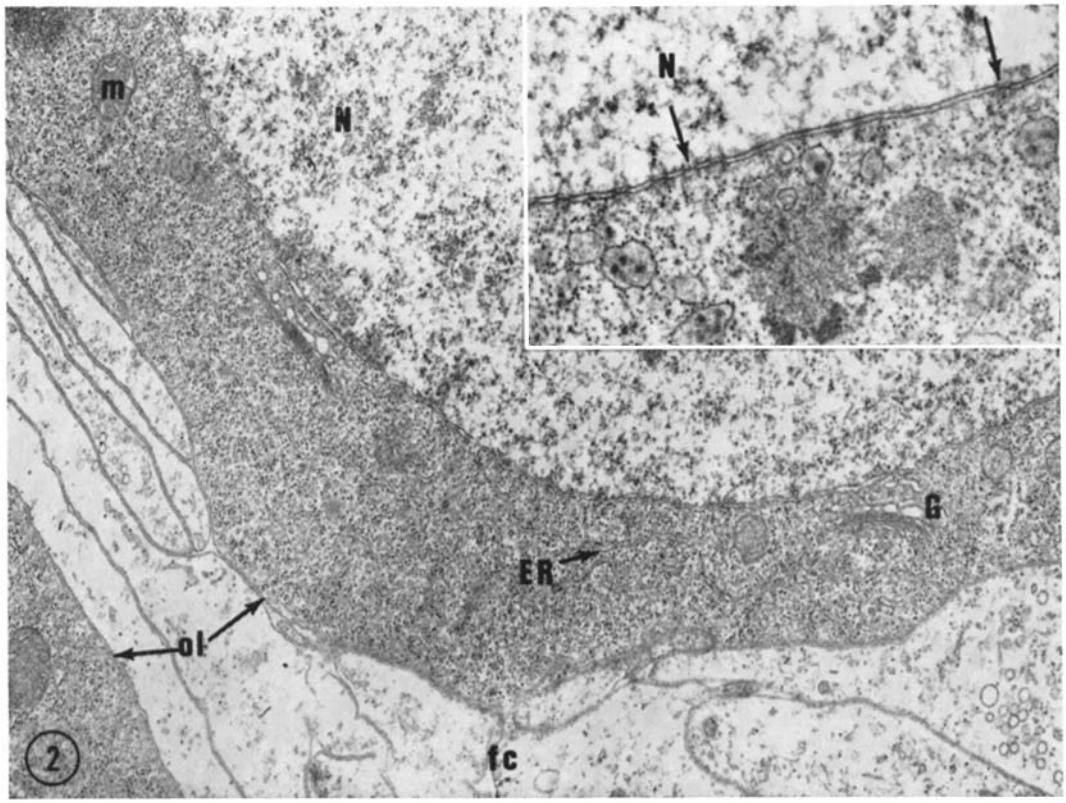



FIGURE 2 Portion of two previtellogenic oocytes and their encompassing follicular cells (*fc*) from the ovary of an immature female. The oolemma (*ol*) is smooth, and the ooplasm contains ribosomes, mitochondria (*m*), Golgi complex (*G*), and narrow cisternae of the endoplasmic reticulum (*ER*). *N*, nucleus. × 13,700. Insert, portions of nucleus (*N*) and cytoplasm of an early vitellogenic oocyte. Several nuclear pores are present (arrows), and dense material, which may have passed through such pores, lies in the adjacent ooplasm (center and arrow at upper right). × 25,800.

encompassing follicular cells become more peripherally located (Fig. 1).

The previtellogenic oocyte has a large germinal vesicle and cytoplasm which contains ribosomes, mitochondria, Golgi elements, and cisternae of the granular endoplasmic reticulum. The oolemma is smooth and at this stage exhibits no particular morphological specialization (Fig. 2). The nuclear envelope is interrupted by numerous pores through which nucleolar material presumably passes into the perinuclear ooplasm (Fig. 2).

In vitellogenic oocytes, the abundance of the endoplasmic reticulum is a particularly striking feature. Profiles of the granular form differ in their shapes and contents (Figs. 3, 5–7). All these profiles contain flocculent material. In addition, most of them contain electron-opaque bodies approximately 25 mμ in diameter, which are similar to the intracisternal granules found in the crayfish oocyte (Beams and Kessel, 1963). The reticulum undergoes progressive differentiation during which the flocculent material and intracisternal granules aggregate to form large yolk bodies. The limiting membranes of the reticulum retain some of their attached ribosomes, at least temporarily (Figs. 5–7). Certain elements of the reticulum are devoid of ribosomes and probably represent portions of the agranular reticulum. Continuity of the agranular reticulum with the granular reticulum and Golgi elements is frequently suggested (Figs. 5–7).

The Golgi complex consists of a variable number of stacked cisternae. Vesicles containing a finely granular content are found at the "forming face" of the Golgi complex, and possible continuities of such vesicles with the endoplasmic reticulum are often seen (Figs. 6 and 7). Small, coated vesicles, often similar in size and density to the intracisternal granules, appear to be derived from cisternae of the "active face" of the Golgi complex (Figs. 5 and 7).

As vitellogenesis proceeds, the oocyte surface becomes irregular with the formation of microvilli and micropinocytotic vesicles. A dense granular material present between the follicular cells and the oocyte collects in the forming micropinocytotic vesicles and can be seen in what appear to be detached vesicles in the cortical ooplasm. The forming and detached vesicles have on their cytoplasmic surfaces "fuzzy coats" which disappear shortly after detachment and internalization. In tangential sections, the membranes of these vesicles exhibit a regular polygonal structure (Figs. 3, 4, and 6). Once in the cortical ooplasm, some of the vesicles appear to fuse to produce smooth membrane-bounded yolk spheres which, at this stage, appear distinct from yolk formed within the endoplasmic reticulum (Fig. 3). The Golgi apparatus may also contribute substances to this micropinocytotically formed yolk material (Figs. 3 and 4).

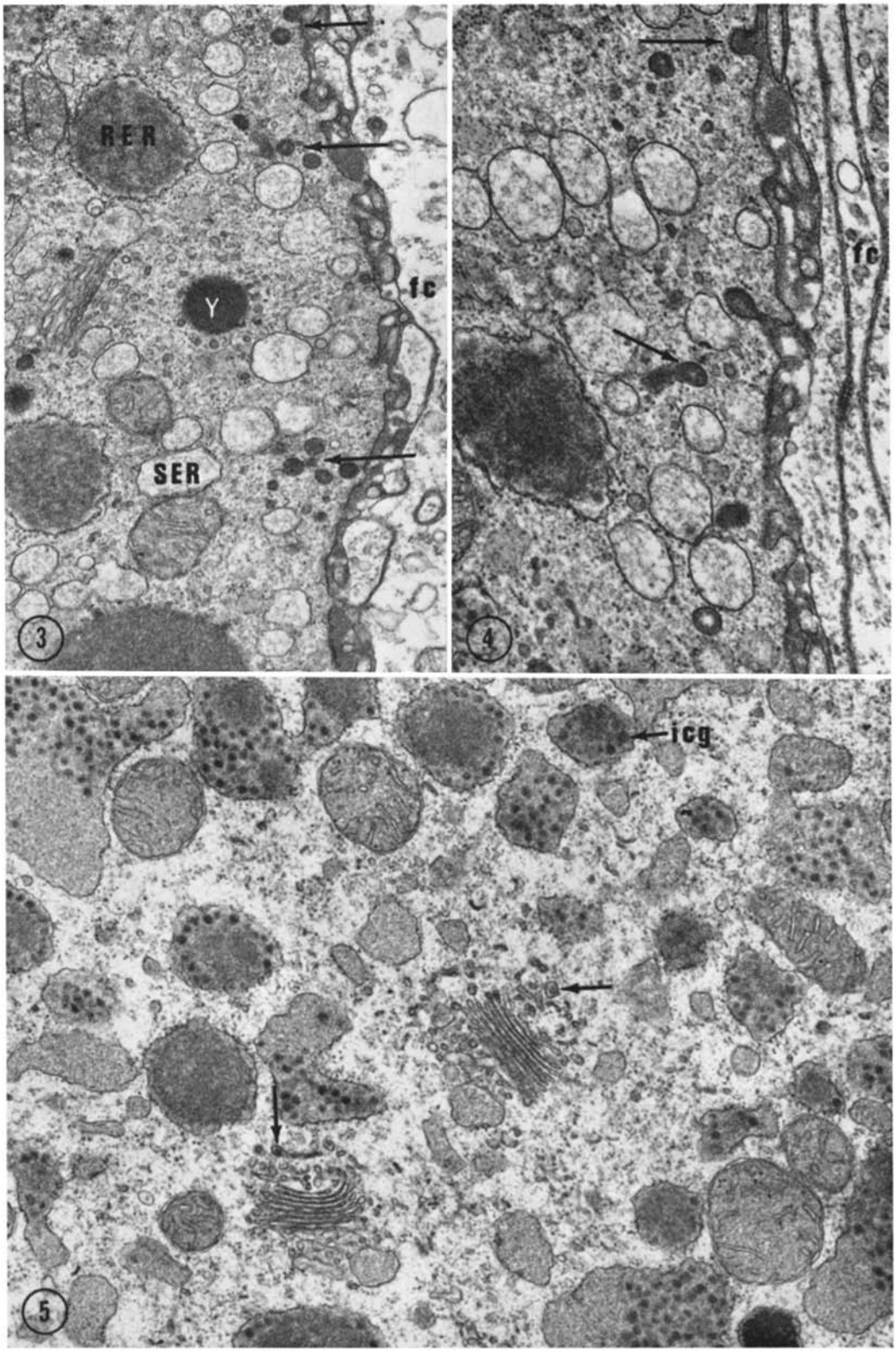
## DISCUSSION

Immunological studies of vitellogenesis in saturniid moths (Telfer, 1965, for review) and ultrastructural studies in *Periplaneta* (Anderson, 1964), *Aedes* (Roth and Porter, 1964), *Cecropia* (Stay, 1965), and *Lepisma* (Cone and Scalzi, 1967) have shown

FIGURE 3 The follicular epithelium (*fe*) and oocyte from ovary of a mature female The oocyte surface with its numerous microvilli and invaginations and the follicular epithelium are separated by dense material which is apparently incorporated into micropinocytotic vesicles (arrows). After internalization, the vesicles lose their bristle-like coats (middle and lower arrows) and probably fuse (lower arrow) to produce immature yolk spheres (*Y*). The vesicles surrounding *Y* may be derived from either micropinocytosis or the Golgi apparatus to the left. Agranular reticulum (*SER*) and granular reticulum (*RER*) containing dense yolk material are seen at the left. × 20,200.

FIGURE 4 Oocyte surface with two attached micropinocytotic vesicles which are coated on their cytoplasmic surfaces (top arrow). Two internalized vesicles are devoid of such coats. The profile at the lower arrow may represent fusion of two vesicles. *fe*, follicular epithelium. × 32,400.

FIGURE 5 Stages in the aggregation of intracisternal granules (*icg*) and homogeneous material within the endoplasmic reticulum of the subcortical region of vitellogenic ooplasm. Vesicles containing dense material (arrows) may be derived from the active faces of two Golgi elements. × 28,300.


RER
Y
fc
SER
3
4
fc
icg
5

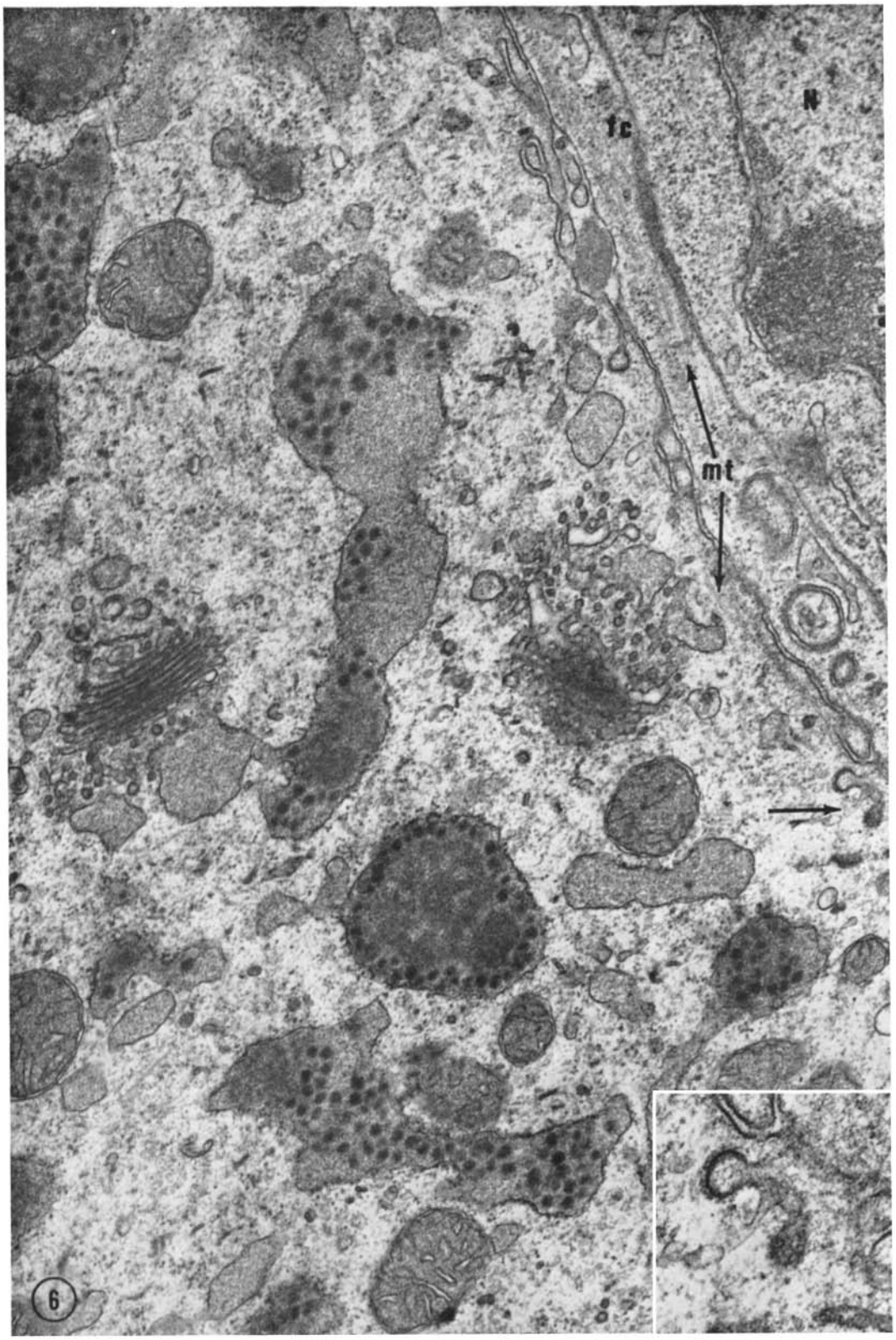



FIGURE 6 The cisternae of the reticulum containing intracisternal granules and aggregating yolk material appear to be continuous, via vesiculation, with the Golgi complex. Microtubules (*mt*) are found in both oocyte and follicular cells. Micropinocytotic vesicles (arrow) are forming at egg surface. *fc*, follicular epithelium; *N*, nucleus. × 31,400. Insert, enlargement of micropinocytotic vesicles showing the bristle-like coat of the upper vesicle and the polygonal surface structure of the lower, tangentially sectioned vesicle. × 62,800.

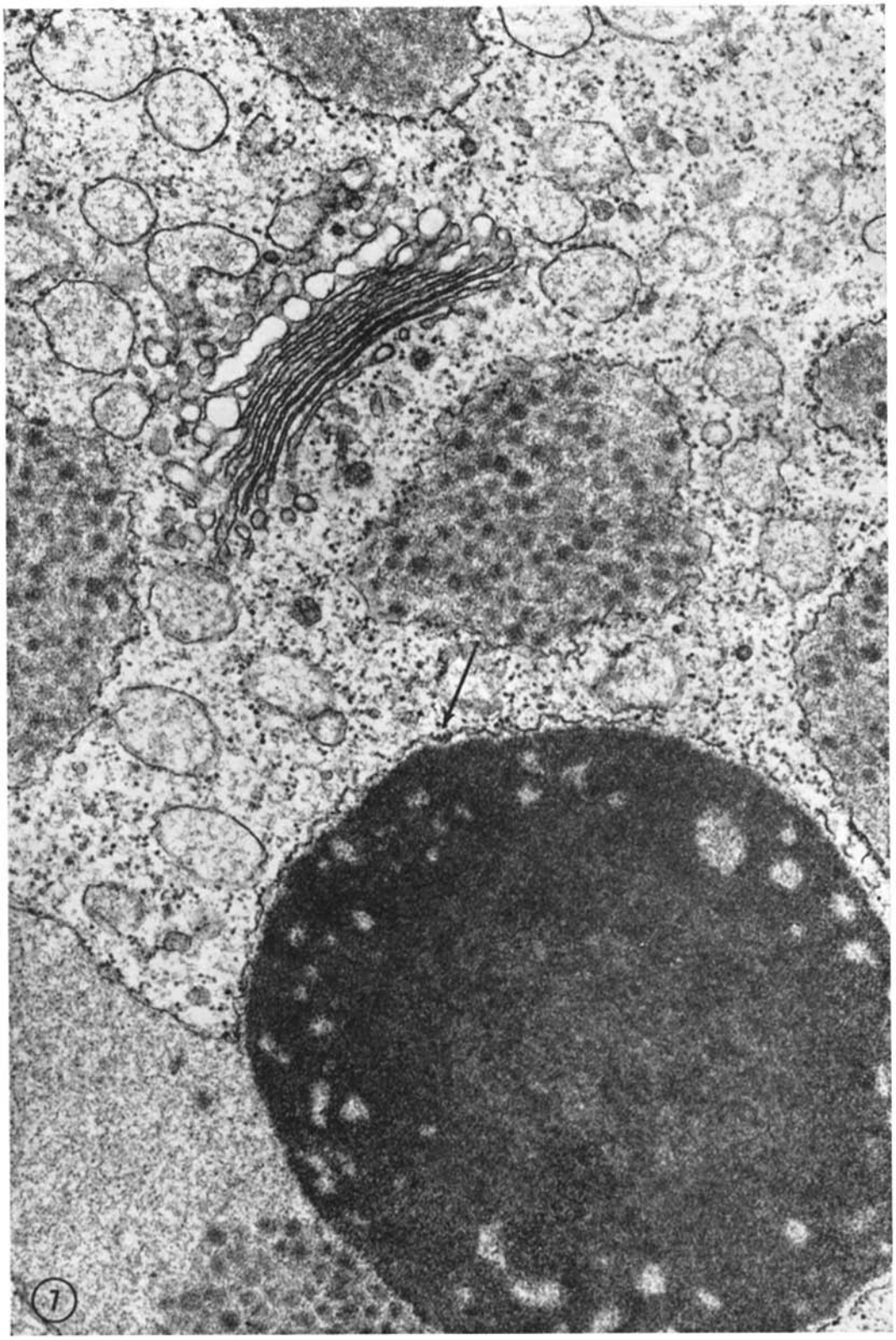



FIGURE 7 A cisterna of the granular reticulum containing flocculent material and intracisternal granules, and a nearly mature yolk sphere. Ribosomes (arrow) are present on the membrane bounding the yolk sphere. The forming face of the Golgi apparatus is closely associated with other elements of the reticulum. × 53,400.

that yolk materials circulating in the hemolymph are incorporated into these oocytes by micropinocytosis. The ultrastructural studies have shown that insect oocytes are characterized by the absence of organelle systems (e.g. endoplasmic reticulum) conventionally associated with synthetic activity. Some degree of oocytic activity is necessary in the assembly and molding of these yolk materials derived from extraoocytic sites.

Although the hepatopancreas has been implicated as the source of extraoocytic yolk materials in the blue crab, *Callinectes* (Kerr, 1966), vitellogenesis in the crustaceans appears to involve a greater degree of intraoocytic synthesis. In the crayfish (Beams and Kessel, 1963), yolk is produced within the highly differentiated system of the endoplasmic reticulum with its complex of interconnected cisternal and tubular elements. The intracisternal granules formed within the stacks of the cisternae of the rough endoplasmic reticulum are transported through the agranular reticulum to different regions of the oocyte where they aggregate to form the definitive yolk. Those authors feel that neither the Golgi complex nor micropinocytosis assumes a significant role in vitellogenesis in the crayfish oocyte.

By contrast, vitellogenesis in *Libinia* appears to involve both intra- and extraoocytic sources of yolk materials. The endoplasmic reticulum is not as highly oriented in its spatial arrangement as is that of the crayfish oocyte. However, the granular reticulum appears active in the synthesis of intracisternal granules which aggregate to produce yolk bodies. The Golgi complex and agranular reticulum appear also to be involved in vitellogenesis, although their precise roles have not been determined with certainty from these ultrastructural observations. They may contribute substances which complex with the materials formed in the granular reticulum. In *Libinia*, unlike the crayfish, micropinocytosis appears to play an important role in the incorporation of yolk materials. The origin of these yolk materials is not known. Yolk formation in *Libinia* resembles that in the horseshoe crab, *Limulus polyphemus*, in which yolk is initially produced by the endoplasmic reticulum and Golgi complex and later is produced by extraoocytic sources and is incorporated into the oocyte by micropinocytosis (Dumont and Anderson, 1967).

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