

The Ultrastructure of Yolk Platelets of Amphibian Oocytes.* BY SAUL WISCHNITZER.† (*From the Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Illinois.*)§

In a number of recent papers (1, 4, 7, 10, 11) it has been shown by electron microscopy that viruses which infect and propagate in HeLa cell cultures, form intranuclear crystalline aggregates. Each of these is composed of a closely packed lattice of regularly ordered spherical viral particles, with a periodicity of about 600 Å. Using an improved shadow casting technique, Labaw and Wyckoff (8) published micrographs of edestin crystals, tobacco protein, and virus protein crystals. Recently Elbers (3), who used sectioned material, showed that the

β -granules (albuminous yolk granules) located in the egg cytoplasm of *Limnaea stagnalis* contain small crystals with a particle size of about 50 Å. In this brief communication, the yolk platelets of amphibian oocytes will be shown to be made up of two components, one of which has the ultrastructure of a crystal-line lattice which appears to be similar to that reported by Elbers.

Oocytes were excised from *Triturus viridescens* and immediately placed in the fixative, which consisted of 1 per cent OsO_4 in 0.7 per cent sodium chloride adjusted to pH 7.4 with McIlvaine's standard buffer solution. After fixation for about 2 hours, dehydration, infiltration, and embedding with a mixture of 4 parts ethyl to 6 parts *n*-butyl methacrylate containing 1 per cent luperco CDB, sections were cut with a Porter-Blum microtome and studied with an RCA-2A electron microscope.

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In a section of a young oocyte in which vitellogenesis has just begun (Fig. 1), the densely granular cytoplasm contains numerous mitochondria and some irregularly shaped lipochondria; pigment granules are absent. The yolk platelets, which may be single or located in clusters (Fig. 2), are oval and measure approximately $0.9 \times 1.1 \mu$. These particles are made up of a central, dense, oval mass surrounded by a less dense outer layer.

In older oocytes (Fig. 3) mitochondria are unchanged in both number and size but the number of lipochondria appears decreased. Pigment is present as clusters of small particles rather than as solitary granules (6). The individual particles, which vary in size from 150 A to 650 A in diameter, are usually circular in shape, but may be irregular (Fig. 3, P). The yolk platelets have increased in size and their two component parts are again evident, with the dense mass having an acentric position. The vacuolated appearance of the cytoplasm is due to numerous light circles with dense peripheries which may represent glycogen granules. Such particles are known to be present in oocyte cytoplasm (2), and in our case their appearance would therefore be due to the extraction of the glycogen in the course of preparation of the material. The light circles, on the other hand, might represent sections through a vacuolar system existing within the cytoplasm. With the evidence at hand it cannot be decided whether these circles represent glycogen granules, sections through a vacuolar system, or both.

When thin sections are examined at higher magnification, the dense oval mass of the yolk platelets has a crystalline ultrastructure (Fig. 4). In this oblique or longitudinal section, the lattice appears as a series of parallel dense linear bands 40 ± 5 A in diameter

(compare with similar type of section (Fig. 3 (8) and Fig. 5 (9)) which is almost identical with particle size of albuminous yolk crystals (3). The distance from center to center of each band measures about 70 A. The crystalline lattice of the yolk platelets constitutes the entire dense, oval mass (Fig. 4), while in the β granules the lattice is in the form of small patches (3).

The suggestion of an oriented ultrastructure was also implied by observations using polarization microscopy (5). Elbers (3) believes that the 50 A particles composing the yolk crystals represent globular protein macromolecules. In view of the presence of very similar crystalline structures in yolk platelets of both molluscan and amphibian oocytes, it is probable that such formations will also be found in eggs from organisms of other phyla.

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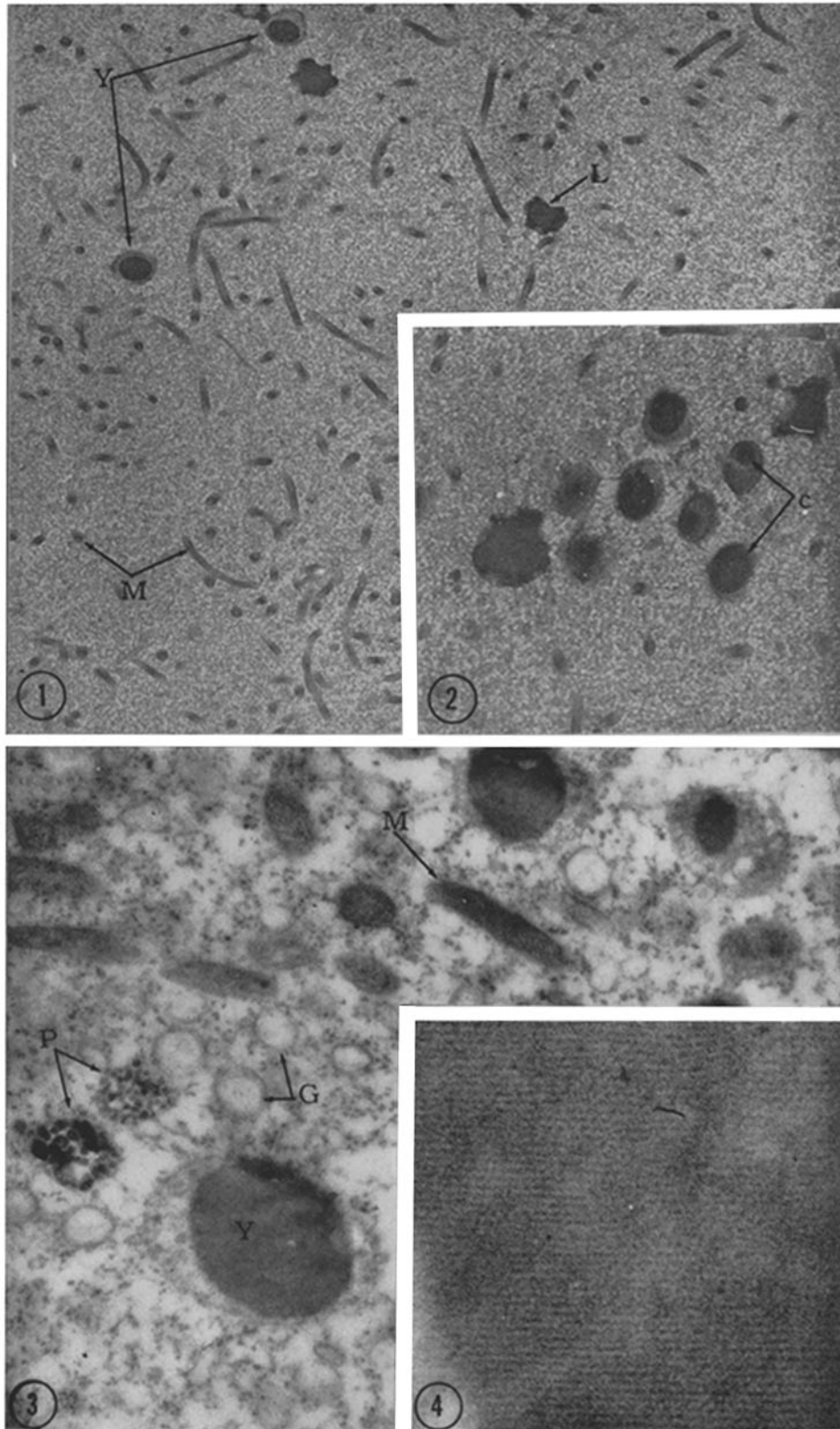
EXPLANATION OF PLATE 320

FIG. 1. View of the cytoplasm and its inclusions of a young oocyte within which vitellogenesis has just begun. Within the dense granular cytoplasm are numerous mitochondria (*M*), some lipochondria (*L*), and yolk platelets (*Y*) which have a dense, oval mass surrounded by a less dense outer layer. $\times 7,000$.

FIG. 2. A cluster of yolk platelets showing variations in the size of the dense mass (*C*), and as a result, comparable variations in the outer layer. $\times 9,500$.

FIG. 3. View of a section from an older oocyte. The cytoplasm appears vacuolated because of the presence of numerous circles with dense peripheries (*G*). Mitochondria (*M*), and pigment, in the form of clusters of particles (*P*), are present within the endoplasm. The two component parts of the yolk platelets (*Y*) are again evident. $\times 42,000$.

FIG. 4. A portion of the crystalline, dense, oval mass of a yolk platelet sectioned at such an angle that superimposition of the macromolecules has resulted in a linear pattern. The lines exhibit an average spacing of 70 A. $\times 170,000$.



(Wischnitzer: Ultrastructure of yolk platelets)