Osmium Zinc Iodide Staining of Golgi Elements in Oocytes of *Triturus cristatus*

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Summary. Developing oocytes of the newt *Triturus cristatus* were studied in order to clarify the role played by the Golgi apparatus in the formation of yolk. The cytochemical method used for this purpose was that of Maillet (1968) which employs an Osmium Zinc Iodide (OZI) complex.

Previtellogenic oocytes reveal a pattern of OZI staining only after hormonal (HCG) stimulation, following which both the Golgi apparatus and the multi-vesicular bodies are stained.

Vitellogenic oocytes taken from non-hormonally stimulated females reveal OZI deposits in a number of vesicles peripheral to the Golgi apparatus as well as within the superficial layer of the forming yolk platelets. Following hormone stimulation, many of the Golgi apparatus located in the central ooplasm of vitellogenic oocytes have all their cisternae blackened by the OZI deposits; other apparatuses, more peripherally located, remain essentially unchanged in their staining pattern. Further, a large number of OZI stained vesicles becomes visible in the vicinity of the Golgi apparatus and within the superficial layer of the forming yolk platelets.

The present findings are interpreted as indicating the occurrence of fusion between Golgi derived vesicles and forming yolk platelets. It is also suggested that the vesicles in question function as carriers of Golgi produced enzymes which are presumably required to accomplish the final elaboration of the yolk material.

Key words: Yolk — Oocyte (*Triturus cristatus*) — Vitellogenesis — Pinocytosis — Cytochemistry

Introduction

It is now firmly established, mainly by biochemical work, that isolated oocytes in both insects (Anderson, 1971) and amphibians (Wallace et al., 1970, 1973), can...
sequester yolk proteins from the external medium. This is taken to indicate that most of the material forming the mature yolk platelets has an exogenous origin, and, therefore, that a tissue other than the ovary synthesizes yolk precursors.

A number of ultrastructural studies have further shown that yolk precursors can enter vitellogenic oocytes by means of pinocytotic uptake (Roth and Porter, 1964; Anderson, 1969; Dumont, 1969; Kress and Spornitz, 1972; Spornitz and Kress, 1973). Support for this view has also come from several cytochemical studies in which various extracellular tracers were used (Wartenberg, 1964; Dumont and Wallace, 1968; Anderson and Spielman, 1971). For instance, peroxidase has been located within the pits of the oolemma, coated vesicles and forming yolk platelets, all of which were previously assumed, on morphological grounds, to be involved in the process of yolk formation (Anderson and Spielman, 1971).

More recently, Massover (1971) has called attention to the structural complexity of the forming yolk, referred to as nascent yolk platelets by him, and has also added that material from several sources may ultimately be sequestered into the yolk platelets. It is conceivable that yolk platelets may grow not only through a progressive fusion of pinocytotic vesicles, but also by a contribution of vesicles of endogenous origin (Spornitz and Kress, 1973).

The aim of the present investigation was to clarify this point, and in particular, to establish whether or not Golgi system derived vesicles may fuse with the forming yolk platelets so as to contribute to the final maturation of the yolk material. This question was investigated by means of the Osmium Tetroxide – Zinc Iodide (OZI) method (Maillet, 1963, 1968) which has been shown to stain selectively the Golgi elements.

Material and Methods

The specimens of the newt *Triturus cristatus carnifex* (Laurenti, 1768) used in the present investigation were collected around Pisa during the winter.

The OZI complex was prepared and used as previously reported by Niebauer et al. (1969). The pH of the final solution, however, was adjusted to 7.0 with 0.1 N NaOH as suggested by Elias et al. (1972). This procedure for preparing the OZI complex is said to yield a greater staining specificity for the Golgi elements than the complex prepared as previously described.

Previtellogenic (less than 100 μ in diameter) and vitellogenic (ranging from 300 to 500 μ in diameter) oocytes were both dissected from adult females and fixed in the OZI complex for periods of 2, 4, 8 or 24 h. Control specimens were fixed for the same periods in 0.2% unbuffered osmium tetroxide. A few oocytes were also prefixed in a mixture of 5% glutaraldehyde – 5% formaldehyde in 0.05 M phosphate buffer at pH 7.2 before treatment with the OZI complex for the same length of time.

In another series of experiments, previtellogenic and vitellogenic oocytes were collected from females which had previously received two injections of Human Chorionic Gonadotropin (HCG Profasi, Serono, 2,000 U.I.) and fixed in the OZI complex as before. The injections, of 0.20 ml each, were given at an interval of 24 h.

In order to verify Maillet's (1968) assumption that the staining specificity of the OZI complex may primarily depend upon a lipid component, two lines of experiment were pursued. Firstly, several oocytes in either previtellogenic or vitellogenic stages, were treated with chloroform/methanol for 1 h before fixation in the OZI complex. Secondly, thick sections of Epon-Araldite embedded oocytes, previously fixed with the OZI complex, were treated with a diluted epoxy solution. According to Eurenius and Jarsskar (1970), this procedure extracts lipids in a selective manner from sections of material embedded in epoxy resins.