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A FUNCTIONAL ANALYSIS OF THE RELATIONSHIP BETWEEN ENVELOPE CELLS AND OOCYTES IN THE TELEOSTEAN OVARY

by

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1. Abstract

Structural and functional relationships between oocytes and their follicular envelopes were studied with electron microscope for three teleost species: Anguilla anguilla, Sparus aurata, and Aphanius dispar. Live fish were injected intravenously with a marker-enzyme, horseradish peroxidase, in order to follow its distribution in the ovarian tissue. In the injected fish, the marker first appears in the capillaries and the pericapillary spaces of the stroma. It then enters the basement membrane space system between the granulosa and theca cells. The marker penetrates further through the inter-granulosa cell region and the zona radiata channels, thus surrounding the microvilli which traverse these channels. Finally, the marker reaches the surface of the oocytes, which proceed to take up the enzyme by micropinocytosis. The frequency of peroxidase-filled pinocytotic vesicles in the oocytes can be used as an indicator of the extent of the vitellogenic process. We are currently investigating the possibility of using the accumulation of these markerfilled vesicles as a quantitative bioassay of hormones or drugs injected to induce vitellogenesis.

2. Introduction

During oogenesis two cell types of different ontogenic history, the oocytes and the follicle cells, come in close contact and display signs of interaction which last until the end of vitellogenesis. At the end of the leptotene stage of the first meiotic division, the oocytes are already enveloped by a layer of follicular cells. The role of the follicular cells and the functional value of the interaction between them and the oocytes are not clear. Follicular cells may be involved in various physiological activities occurring during oogenesis such as transfer of metabolites to the growing oocyte, the formation of the zona radiata and/or the synthesis of ovarian hormones.

A detailed knowledge of folliculogenesis may contribute to mariculture. The normal features of ovarian function in relation to artificial control of fecundity present problems where knowledge of folliculogenesis may be important in the design of the experiments. A frequent problem is the efficiency of the hormones administered in order to induce vitellogenesis. In the present paper, a bioassay is proposed for measuring the intensity of the vitellogenic process in fish submitted to gonadotropic stimulation. In essence, the proposed bioassay is as follows: adult female fish specimens are injected with the marker-enzyme horseradish perocidase and the frequency of pinocytotic uptake of the marker by the oocyte is evaluated as an index of vitellogenic activity. This bioassay and the stages of normal folliculogenic process are discussed.

3. Materials and Methods

The fish used in this study were female specimens of various ages of Anguilla anguilla, Sparus aurata, and Aphanius mento. The eels were obtained from commercial sources in Germany and kept in the laboratories of the Institut für Küsten- und Binnenfischerei in Ahrensburg, Germany. The Sparus specimens were brought as fingerlings from the Bardawill lagoon in Northern Sinai and raised in outdoor tanks with running seawater in the Mariculture Laboratory in Eilat, Israel. Aphanius mento were caught in freshwater sources near the Dead Sea and kept in the laboratory of the Zoological Department of the Hebrew University in Jerusalem.

For the EM studies, the fish were killed by decapitation and the ovaries dissected. Pieces of ovary were fixed by immersion in either a paraformaldehyde-glutaraldehyde fixative (Karnovsky, 1965) or a 0.1 M cacodylate buffered, 2-5% glutaraldehyde for varying periods, postfixed in Osmium tetroxide, and then dehydrated and embedded in Epon. In the experiments with horseradish peroxidase, the fish were injected under the dorsal fin with different amounts of peroxidase (type II, Sigma Chem. Comp., St. Louis) over periods of 10 to 60 minutes before being killed. The ovaries were fixed in 3% glutaraldehyde or in Karnovsky's fixative for one hour, removed and cut into about 100 µm sections either freehand or with a Smith and Farquhar tissue sectioner. The sections were treated according to the techniques of Graham and Karnovsky (1966) with diaminobenzidine and H_2O_2, postfixed with Osmium tetroxide, stained with 2% Uranyl acetate in bulk, and embedded in Epon. Thin sections were cut with an Ultrotome (LKB - Stockholm). Contrasted and non-contrasted thin sections were studied with Philips 300 and JEM 100 CX electron microscopes.

4. Results

During the chromatin nucleolus stage, in young oocytes, the oolemma and the plasmalemma of the granulose (i.e. follicular) cells are separated by 20 nm. At the perinucleolar stage, the oolemma exhibits small protrusions and invaginations. At the end of this stage, microvilli supported by microfilaments protrude from the oocyte surface (Figs. 1, 2). The granulosa cells and oocytes are now about 250 nm apart, and the microvilli protrude into an apparently "empty" space. An electron-dense secretion of oocytal origin forms the first deposit of the zona radiata at the base of the microvilli. A so called "basement membrane" becomes apparent between the granulosa and theca cells. This "membrane" is about 200-300 nm thick. It actually consists of a) a basement lamina closely adhering to the granulosa cells and continuing unbroken at the junction of adjacent granulosa cells, and b) an area filled with collagen fibers (Figs. 1-4).

In sections where capillaries of both the stroma and oocytes can be seen, the collagen-filled spaces and the pericapillary spaces appear to be linked (Fig. 3). The next stage, still in early vitellogenesis, microvilli originating in the granulosa cells appear. A granulosa cell secretion is deposited around the follicular microvilli (Fig. 4). In the region where microvilli coming from the oocyte and those coming from the granulosa cells meet, a low electron-dense secretion could be observed. Its origin was not determined (Fig. 4). Granulosa cells are rich in rough endoplasmic reticulum, free ribosomes, mitochondria and Golgi structures. There were no signs of organelles characteristic of steroidogenic cells, such as smooth endoplasmic reticulum and tubular mitochondria.

In specimens injected with horseradish peroxidase, the marker first appears in the capillaries and the pericapillary space of the stroma. It then enters the basement membrane space system and continues into the area surrounding the granulosa cells (Fig. 5). Between these cells, microvilli of both follicular and oocytal origin are outlined by the marker, which penetrates further via the channels of the zona radiata, there surrounding the microvilli traversing these channels (Figs. 5, 6).

The marker finally reaches the surface of the oocyte into which it is incorporated through micropinocytotic invaginations (Fig. 6). Pinocytotic vesicles filled with the marker are found at the periphery of the oocyte throughout the vitellogenic process. Neither the granulosa cells, nor the microvilli take up the enzyme, which proceeds externally along the microvilli until it reaches the oocyte surface. Only here is the marker taken up by pinocytosis.

5. Discussion

Phases of folliculogenesis and the pathway of horseradish peroxidase from capillaries to oocytes. The follicle cells form a continuous follicular epithelium which retains its single-layer structure throughout the oocyte growth (Kraft and Peters, 1963; Götting, 1967). The first traces of the zona radiata can be seen at the bases of the microvilli (Flügel, 1967) which protrude from the oocyte.

Concomitantly with the growth of the oocyte, the follicle cells, too, develop cytoplasmic processes. The horseradish peroxidase marker first appears in the pericapillary spaces of the ovarian stroma and then penetrates into the space between granulosa and theca cells. Thereafter it continues between the granulosa cells and along both follicular and oocytal microvilly until it reaches the oocyte surface where it is taken up by the oocyte. Our research has led to the following working hypotheses:

1. The marker pathway between the pericapillary space and oocyte may represent the pathway of heterosynthetic vitellogenin (originating in the liver) into the oocyte.
2. Peroxidase (and possibly vitellogenin) is not taken up into the granulosa cells prior to being incorporated into the oocyte. The granulosa cells, frequently mentioned in literature for their "nutritional activity", may not be directly involved in the transfer of vitellogenin into the oocytes (However, according to Guraya (1978), part of the proteins synthesized in the follicular epithelium are transported into the oocyte and the follicular microvilli may be involved in the transport of substances synthesized in the following epithelium).
3. The microvilli do not take up peroxidase. The marker surrounds the microvilli, and proceeds along them externally until it reaches the oocyte surface. Only here is the marker ingested by pinocytosis.

The frequency of peroxidase-filled pinocytotic vesicles in the oocytes as an indicator of the intensity of the vitellogenic process. In mariculture, gonadal development is usually induced either by changes in the physical environment (such as light periodicity, temperature or salinity of the water) or by treating the fish with drugs or hormones. In mature fish, the treatment may lead to ovulation, and then by stripping the fish, the ovulated eggs are extruded. The end result of the treatment, i.e. the production of ripe eggs is easily assessed. If fecundable eggs are not obtained within 1-2 days, the treatment may be regarded as having been ineffective. In non-mature fish, the results of a treatment to induce ovarian development cannot be easily assessed. Wiley and Dumont (1978)

measured the extent of gonadatropic stimulation in Xenopus by comparing the uptake of radioactive vitellogenin in the oocytes of stimulated and non-stimulated toads. In mariculture, various techniques of oocytes sampling could be used. The increase in oocyte diameter may indicate the intensity of the gonadotropic stimulus; however, a measurable effect cannot be obtained before the elapse of several weeks of treatment, and even when biopsy techniques of the ovary are available, a fairly large number of oocytes must be sampled in order to obtain a statistically satisfactory answer.

The possibility of using the uptake of peroxidase into the oocyte as a quantitative bioassay for assessing vetellogenic activity is now being studied in our laboratories. We are considering a test for vitellogenic stimulation by estimation of the pinocytotic activity of the oocytes in fish injected with the gonadatropic drug. A series of preliminary experiments have already been carried out to ascertain the feasibility of such an assay. Fish were injected with different amounts of carp pituitary homogenates, and at varying time intervals thereafter, they were injected with horseradish peroxidase. After sectioning the Epon-blocks, the frequency of the pinocytotic activity was evaluated with the electron microscope and the results were used as an indicator of the extent of the vitellogenic process. Our preliminary experiments indicate that the proposed test is a suitable bioassay for vitellogenesis. However, before the test can be proposed as a practical assay for maricultural scientists certain difficulties need to be overcome. One of these is to find out in practice the optimal time schedule between gonadotropin administration, horseradish peroxidase injection, and sampling of the oocytes. We are currently investigating these problems in our laboratories.

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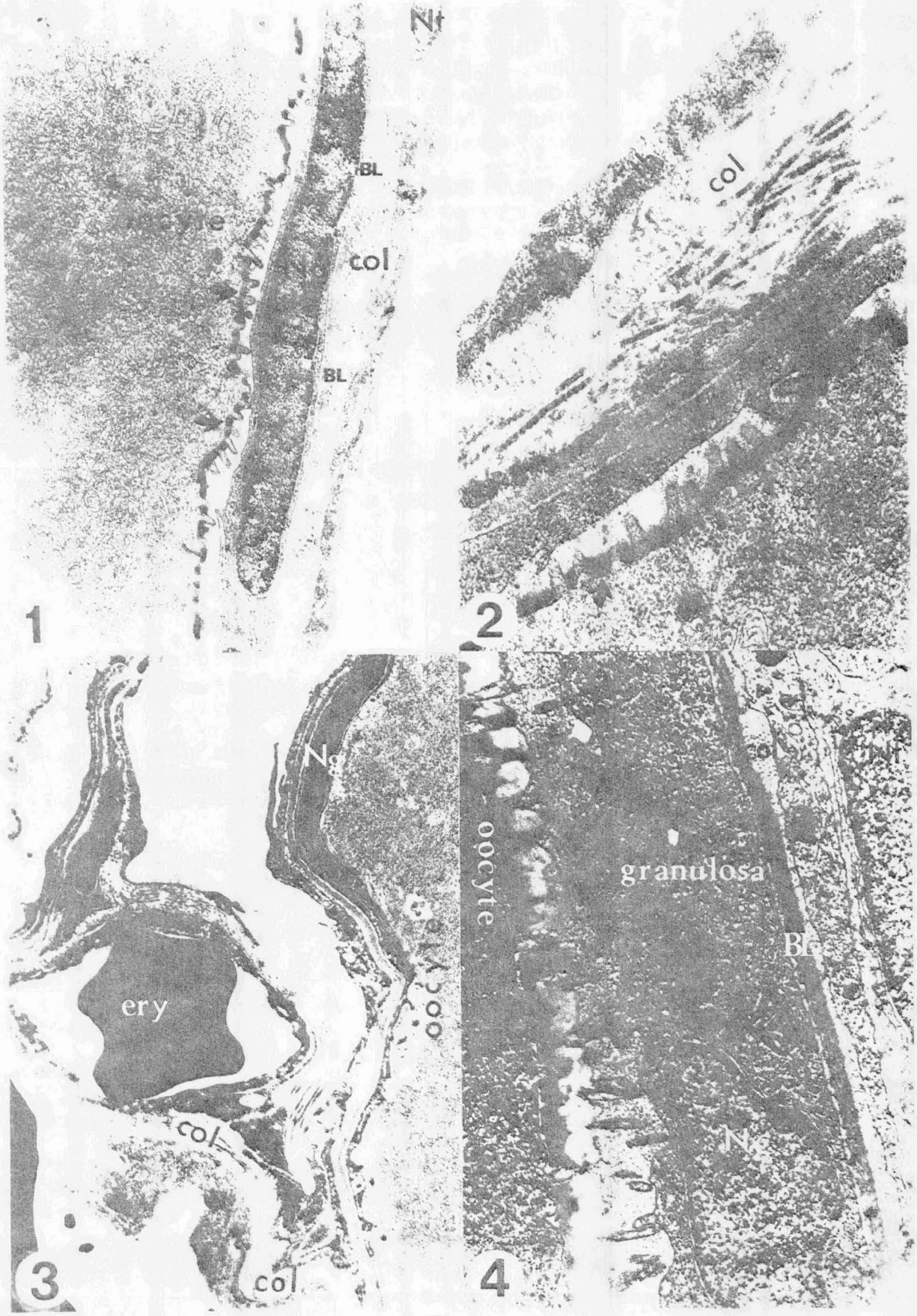
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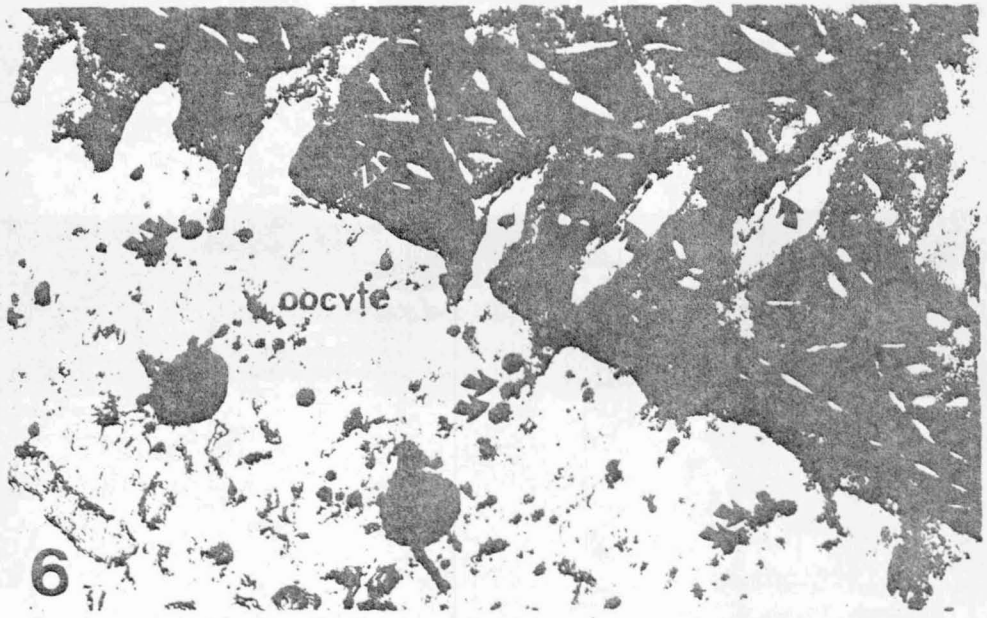

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