Short Paper

Presence of Ovulation-Inducing Enzymes in the Ovarian Follicles of Loach

The possible role of proteolytic enzymes in the ovulatory process of higher vertebrates has been reported.\(^1,2\) As for fish, however, such investigations have not yet been published in this respect.

Attempts have been made recently to detect the protease(s) which actively take part in ovulation. Gravid female loach, *Misgurnus anguillicaudatus* (CANTOR) were induced to ovulate by injection of HCG (“Puberogen”). After injection those follicles representing various stages of ovulation were removed from the ovaries and protease activity was detected by means of a modified gelatin-silver film method.\(^3\) The follicles were cut at 15 µm on a cryostat and sections were immediately mounted on strips of exposed and developed photographic film and quickly dried. Each section and a large area of the surrounding gelatin was then lightly dampened with either 0.15M phosphate buffer at pH 7.6 or 0.15M acetate buffer at pH 5.0. The preparation was incubated in a water-saturated atmosphere at 37°C for 90 min and immediately stained with Mayer's acid hemalum and picric acid.

Protease activity was revealed as clear, transparent areas where the gelatin had been digested and the silver granules washed out. Prior to ovulation, each oocyte is enveloped within a follicular membrane, composed of a single follicular epithelial layer and a doubled theca layer. In the follicular layer surrounding the ripe egg in which the germinal vesicle has migrated to the animal pole and disappeared, such transparent areas were revealed (Plate I-1). In contrast, however, similar clear areas were not recognized in the corresponding regions of those oocytes which were still distant from ovulation. Upon closer examination the transparent areas were recognized to be composed of oval segments, 50–100 µm in diameter, surrounding the egg cell surface. These segments can possibly be explained by the existence of protease released from “secreting units” which might correspond to certain cells located in the follicular layer (Plate I–2). Based upon the observations presented above, it is possible that protease may play an important role in follicular rupture.

Takashi OSHIRO and Takashi HIBIYA.
Laboratory of Fish Physiology, Fac. of Agr., Univ. of Tokyo, Japan

References


Received October 15, 1974

*ドジョウの卵巣卵胞における排卵誘起酵素の存在 (尾城 隆・日比谷 京：東京大学農学部魚類生理学研究室)*