On the Nature and Behaviour of Secretory Granules in Hatching Gland Cells of the Fresh-water Teleost, *Odontobutis obscura*

By

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(*With 9 Text-figures*)

Several reports have hitherto been published on the morphological study of hatching glands of teleosts (Ishida 1944, 1948; Yanai 1953; Ouji 1955; Yanai, Ouji and Iga 1956), reporting that the hatching gland is of endodermal origin in *Oryzias latipes*, while it is ectodermal in origin in *Hypomesus olidus*, *Salanx microdon*, *Cyprinus auratus* and *Zacco platypus*. Working on the development of the hatching gland of *Oryzias latipes*, Ishida (1944) has reported the disappearance of both nuclei and secretory granules in hatching gland cells at the hatching stage.

The present author, in collaboration with Messrs. Sasaki and Ono, from the cytochemical view point studied cells forming the hatching gland of *Odontobutis obscura* and *Zacco platypus*. It was concluded that the secretory (or hatching) granules of the gland cells are double in structure, the outer part showing a reaction indicating the occurrence of ribonucleic protein, while the inner part reacted in such a way as to indicate it is provided with lipoid, hyaluronic acid and a complex acid mucopolysaccharide. The present study deals with the nature and extrusion of secretory granules from the hatching gland at the hatching stage in *Odontobutis obscura*.

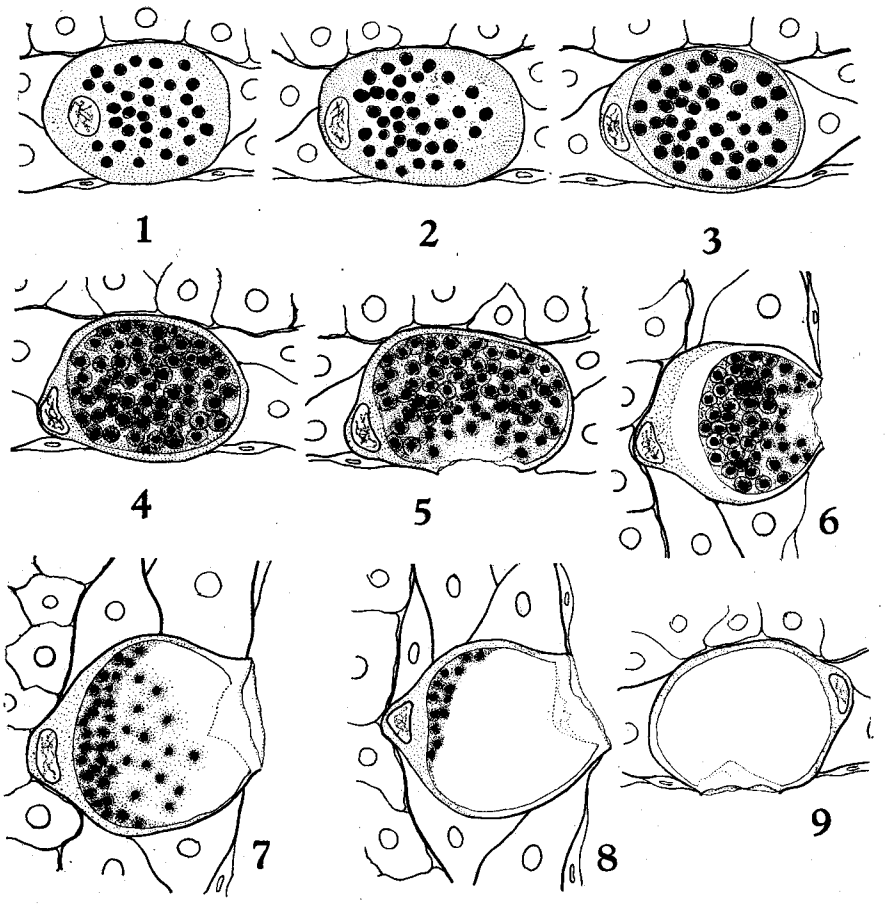
Before going further, the author wishes to express his cordial thanks to Professor Sajiro Makino, Hokkaido University who showed a keen interest in this subject and improved the manuscript. The technical assistance given by Messrs. M. Sasaki and S. Ono is likewise greatly appreciated.

Material and Methods: Embryos of *Odontobutis obscura* were fixed in Bouin's fluid at the lens-formative stage, mouth-opening stage and just before and after the hatching stage. They were serially sectioned at 8 micra and stained with picro-blue-black, haemalaum-eosin, haemalaum and sudan black B. They were observed under the magnification of 600 or 1200 times.

Observations

1). *Hatching gland at the lens-formative stage*: The cells forming the hatching gland are oval in shape, and contain 30 to 35 secretory (or hatching) granules in each. The nucleus is of ovoidal shape. The outer part of the granule which showed a reaction of ribonucleic protein is 1.0 to 1.2 micra in diameter; it was

stained by both eosin and picro-blue-black. The inner part, showing the reactions of lipoids, hyaluronic acid and complex acid mucopolysaccharide is about 0.7 to 0.8 micron in diameter and stained with haemalaum (Fig. 1).



Figs. 1–9. Change of secretory granules in the hatching gland cells.

2). *Hatching gland at the mouth-opening stage*: The cells of the hatching gland at this stage are also of oval shape; they show an increase in volume. They each contain 30 to 40 secretory granules. The membrane-like element surrounding the granules along the inner side of each cell is clearly visible; it shows a lipoid reaction. They showed also rather intense affinity to both eosin and picro-blue-black (Figs. 2–3).

3) *Hacthing gland just before hacthing*: The secretory granules are 40 to

45 in number per gland cell just before hatching when observed in haemalaum preparations. Each granule is 1.5 micra in entire diameter, while its inner part has a diameter of 0.8 micron. At this stage, the granules generally show affinity to neither eosin nor picro-blue-black. The cells lying close to the surface of the gland begin to break down in the latter part of this stage. In these cells, the granules show remarkable disintegration (Figs. 4–6). Those which undergo breaking down each contain a nucleus, irregular in shape.

4) Hatching gland just after hatching: Most cells of the hatching gland at this stage undergo breaking down. In these cells the granules decrease strikingly in number (Figs. 7–8). In an extreme case, no granules are observed in the cell body (Fig. 9). In every such cell there is a nucleus of irregular form at an eccentric corner (Figs. 7–9).

At the onset of breaking down, the granule becomes indistinct in appearance. With the passage of time, the granular body becomes vague in outline resulting in its final disintegration. The debris from the degenerating granules is extruded out of cells in the perivitelline space.

Discussion

Ishida (1944) reported that the nuclei of the hatching gland cells underwent disintegration slightly before the hatching stage in *Oryzias latipes*. It has generally been observed that the secretory (or hatching) granules undergo disintegration at the hatching stage. The results of the present study have revealed that the secretory granules undergo disintegration and extrusion from cells after hatching.

Ouji, Sasaki and Ono conpublishad have found that the secretory granules have a double structure, consisting of outer and inner parts which differ in chemical constitutents. No attention has been paid by most investigators to the double nature of the secretory granules as above; they have observed no detailed process of distintegration of the granules. As shown in *Odontobutis obscura*, the secretory granules show no affinity to picro-blue-black in the later part of the hatching stage, while they do show affinity to haemalaum. This seems to mean that the secretory granules undergo a change (or changes) in their chemical nature during the later part of the hatching stage. Further, the present sutdy indicates that at the time of disintegration, the secretory granules show an increase in volume of the outer part. This seems to suggest also the alteration occurring in the nature of the granules.

Summary

The present study deals with some morphological and cytochemical observations on the nature and behavior of the secretory (or hatching) granules in cells forming the hatching gland of *Odontobutis obscura*.

It was found that the secretory granules have a double structure in their

chemical constitution. The granules seemed to undergo a change (or changes) in both morphological and chemical nature during the later part of the hatching stage, and undergo disintegration and extrusion from cells after hatching.

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