HISTOLOGICAL STUDY ON THE OVIPOSITOR OF THE ROSE BITTERLING, *RHODEUS OCELLATUS**

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The female rose bitterling possesses a protruding urogenital tract, the ovipositor, with which she deposits her eggs inside a fresh-water mussel. This tube changes in length with the maturity of ovaries. Out of the spawning season, the ovipositor always keeps the short length of about 1 to 5 mm. As the breeding season comes near, it grows up to about 10 to 15 mm. During the breeding season the ovipositor thus grown shows a cyclic change closely related to ovarian activity. At ovulation its length is rapidly prolonged to about 50 to 60 mm; after oviposition the organ takes again the basal length at intervals of about 6–9 days (Shirai, 1962).

The relation between these changes and ovarian hormone has already been studied by Bretschneider & De Wit (1947). However, there are no studies on the morphological changes of the genital tract, so far as the author is aware.

Therefore, this study has been undertaken with special attention to histological changes of the ovipositor in various growth phases.

Before going further, the present writer wishes to offer his cordial thanks to Professor Kiichiro Yamamoto for his kind guidance and revision of the manuscript.

Material and Method

Rose bitterling, *Rhodeus ocellatus* collected in Lake Kasumi, Ibaraki Prefecture, were used as material. They were brought to the campus of the Fisheries School of Hokkaido University and kept in a large aquarium set in a green-house.

Ovipositors at the various phases of growth were obtained from the fish. After the measurement of length, ovipositors were fixed in Bouin's solution. Sections prepared by the routine paraffin method were stained with Delafield's haematoxlin and eosin or Heidenhain's iron haematoxylin and light green.

Results

(I) Structure of the ovipositor

The ovipositor, an organ for laying eggs, is a soft tube located between the

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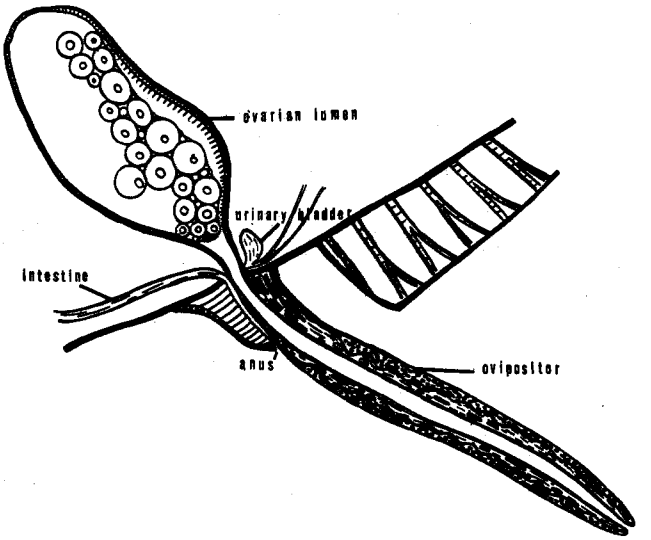

Text-fig. 1. Diagrammatic figure of female reproductive organs

anus and the anal fin, and is developed from the oviduct. The wall of the oviduct is continuous with the luminal epithelium of the ovipositor. The urinary bladder opens into the tube. The anus and the base of the ovipositor are coated with the common muscular layer (Text-fig. 1 and Fig. 1).

The wall of the ovipositor consists of three layers: an outer stratified squamous epithelium covering the whole organ, a middle connective tissue layer with blood and lymph vessels, and an inner stratified epithelium which lines the lumen of the ovipositor.

Two kinds of cells are found in the outer epithelium. One is elliptical in form and has a large nucleus, while the other is round and has a small nucleus, the cytoplasm of which is occupied with numerous granular substances stained deeply with Heidenhain's iron haematoxylin. The latter cells are few in number and mainly localized in the top of the organ (Fig. 2).

The connective tissue layer is loose in texture and composed of collagen fibers, elastic fibers, fibroblasts and macrophages, through which blood and lymph vessels are running. The nearer the connective tissue layer comes to the top of the organ, the thinner it becomes. Numerous melanophores are found in this layer. The basement membrane exists between the covering epithelium and the connective tissue layer.

The luminal epithelium is also composed of two kinds of cells; underlying columnar cells with small nuclei, and superficial large round cells with large nuclei. By its morphological character, this epithelium may be called a stratified

transitional epithelium. In contrast with the ovarian lumen, ciliated cells are missed in the epithelium.

(II) Changes of the ovipositor with its growth

In the post-spawning season, the ovipositor is slender and about 1–5 mm in length. The stratified squamous epithelium makes folds and invades the connective tissue layer as high papillae (Fig. 3). The two kinds of cells in the epithelium have a small amount of cytoplasm. No mitotic figures are found in the epithelial cells. The connective tissue layer is compact and cellular, and consists of fibroblasts and macrophages embedded in a ground substance, but no intercellular fibers. Blood and lymph vessels are not conspicuous. The luminal epithelium consists of many layers of cells. The superficial layer is occupied with large round cells (Fig. 4).

Shortly before the breeding season, the ovipositor gradually gains length (Fig. 5). Many cells in process of mitosis could be encountered in the covering epithelium. The cytoplasm of epithelial cells increases in volume (Fig. 6). At the same time, gradual accumulation of intercellular materials and the appearance of thin fibers occur in the connective tissue layer, and then the layer increasingly becomes fibrous (Figs. 7 and 8).

The ovipositor in the state of the basal length is about 10 to 15 mm in length (Fig. 9). The lower part of the layer of connective tissue is occupied with thick bundles of collagen fibers, and the upper part with elastic fibers. Blood and lymph vessels running through the tissue are not enlarged yet (Fig. 10). Mitotic figures of the epithelial cells are rarely found. The high papillae of the outer epithelium are still found.

At the ovulation phase when maturation and ovulation occur, the ovipositor lengthens rapidly. Histologically, the changes are characterized by the extravasation of tissue fluid into the connective tissue stroma and remarkable vasodilation of the vessels. As a result, the connective tissue layer becomes loose (Figs. 11 and 12). The luminal epithelium becomes thin and consists of one or two layers of cells. The large round cells are found infrequently.

Then, the ovipositor reaches the maximum length at the oviposition phase. The connective tissue layer considerably increases in vascularity and is filled with the tissue fluid (Fig. 13). The collagen fibers become straight in appearance. The papillae of the covering epithelium disappear because of the considerable stretching of the layer (Fig. 14). The upper part of the ovipositor becomes considerably more slender (Fig. 15). Mitotic figures were rarely encountered in the covering epithelium. This state of the ovipositor is maintained so long as the mature ova are present in the ovarian lumen.

After oviposition, the ovipositor rapidly reduces its length and reaches the basal length again. This change is accomplished by evacuation of the tissue fluid from the connective tissue stroma and contraction of the vessels.

After the spawning season, the ovipositor tissue atrophies. The intercellular materials, especially the collagen fibers, gradually diminish and the connective tissue layer becomes compact and cellular (Fig. 16).

Discussion

The growth of the ovipositor consists of two different phases: a slow, steady growth phase in the pre-breeding season and a fluctuation phase in the breeding season.

As demonstrated in the present study, the slow growth is accomplished by the multiplication of outer epithelial cells and development of collagen fibers in the connective tissue stroma, while the fluctuation is achieved exclusively by extravasation of tissue fluid into the connective tissue stroma and remarkable vasodilation of vessels. Therefore, these two phases in the growth of the ovipositor are also characterized by histological changes.

In the river lamprey, *Lampetra fluviatilis*, modifications of the cloacal region occur during the breeding season. Knowles (1939) has suggested that cloacal changes, involving vasodilation, swelling and epithelial changes are in some respect similar to those found in mammals.

In the mammalian endometrial cycle, the endometrium of the uterus shows similar histological changes to those found in the ovipositor of the bitterling. In the follicular phase, all tissues of the endometrium make growth by fibrogenesis and mitosis, and during the progravid phase the thickening of the endometrium is mainly attributed to the increase of secretion from the uterine gland and edema fluid. These growth changes in the endometrium are ascertained to be influenced by estrogen by Fainstat (1962) and Spaziani (1963). Spaziani has reported that the administration of a single dose of estrogen to ovariectomized rat is followed by progressive dilation of large and small uterine vessels and augmented blood flow, responses which coincide with increased extravasation of plasma constituents into the tissue.

Thus, it is possible to suppose that the changes of the ovipositor in the two phases are under the influence of an estrogen-like hormone. However, there is a possibility that in addition to the action of an estrogen-like hormone, another agent also participates in the cyclic changes at the fluctuation phase, because the cyclical changes of the ovipositor take place in a very short time, and further, the rapid elongation of the ovipositor occurrs always in accordance

with ovulation. The agent is supposed to be some hormone secreted cyclically with the occurrence of ovulation. Further research for the elucidation of the problem whether the hormone is one of the pituitary hormones or a kind of ovarian hormone is most desirable.

Summary

1. Histological studies have been performed on the ovipositor changes at the various stages of the sexual cycle.
2. The whole process of ovipositor growth can be divided into two phases: the growing phase in the pre-breeding season, and the fluctuation phase in the breeding season.
3. The growing phase is characterized by the multiplication of the epithelial cells and fibrogenesis in the connective tissue stroma, while the latter phase by edema production and remarkable vasodilation of vessels.
4. The influence of an estrogen-like hormone on the ovipositor changes is discussed.

Literature

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Explanation of Plates

All figures are photomicrographs obtained from sections of the ovipositor of the rose bitterling fixed in Bouin's fluid and stained with Delafield's haematoxylin-eosin and Heidenhain's iron haematoxylin-light green.

PLATE I

Fig. 1. Portion of the anus and the base of the ovipositor, which are surrounded by the common muscular coat. ×14

Fig. 2. Cells of the covering epithelium which are located in the top of the ovipositor and contain numerous granular substances. ×750

Fig. 3. Ovipositor of 5 mm length in the post-spawning season. Many high papillae which invade the connective tissue layer are seen in the covering epithelium. ×22

Fig. 4. Highly magnified figure of the connective tissue layer and the luminal epithelium from the same specimen as above. The connective tissue layer is compact and cellular. Superficial layer of the epithelium is occupied with large round cells. ×330

Fig. 5. Ovipositor of the growing phase, 8 mm in length, obtained in February. ×22

Fig. 6. Mitotic figures in the covering epithelium from the same section as above. ×750

Fig. 7. Ovipositor of 12 mm in length, obtained in March. ×22

Fig. 8. Portion of the same ovipositor as above. Compare with Fig. 4. Notice the collagen fibers which developed in the connective tissue stroma. Several contracted vessels are seen. ×55

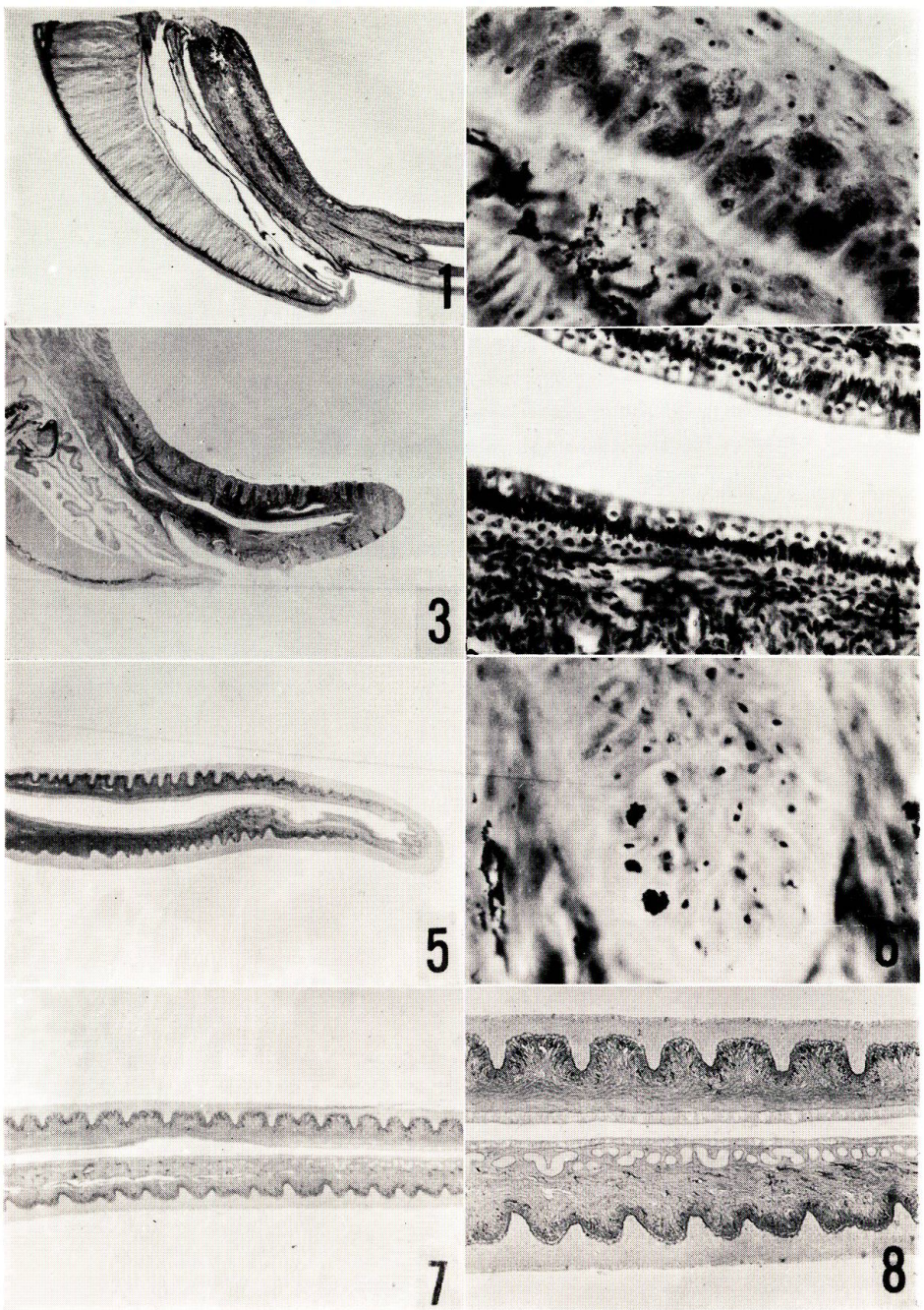

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PLATE II

Fig. 9. Ovipositor at the resting phase, which is 13 mm and represents the basal length. ×22

Fig. 10. Portion of the same ovipositor as above, showing thick collagen bundles in the lower part of the connective tissue layer and contracted blood and lymph vessels passing through the tissue. ×55

Fig. 11. Ovipositor of the ovulation phase, 33 mm in length. ×22

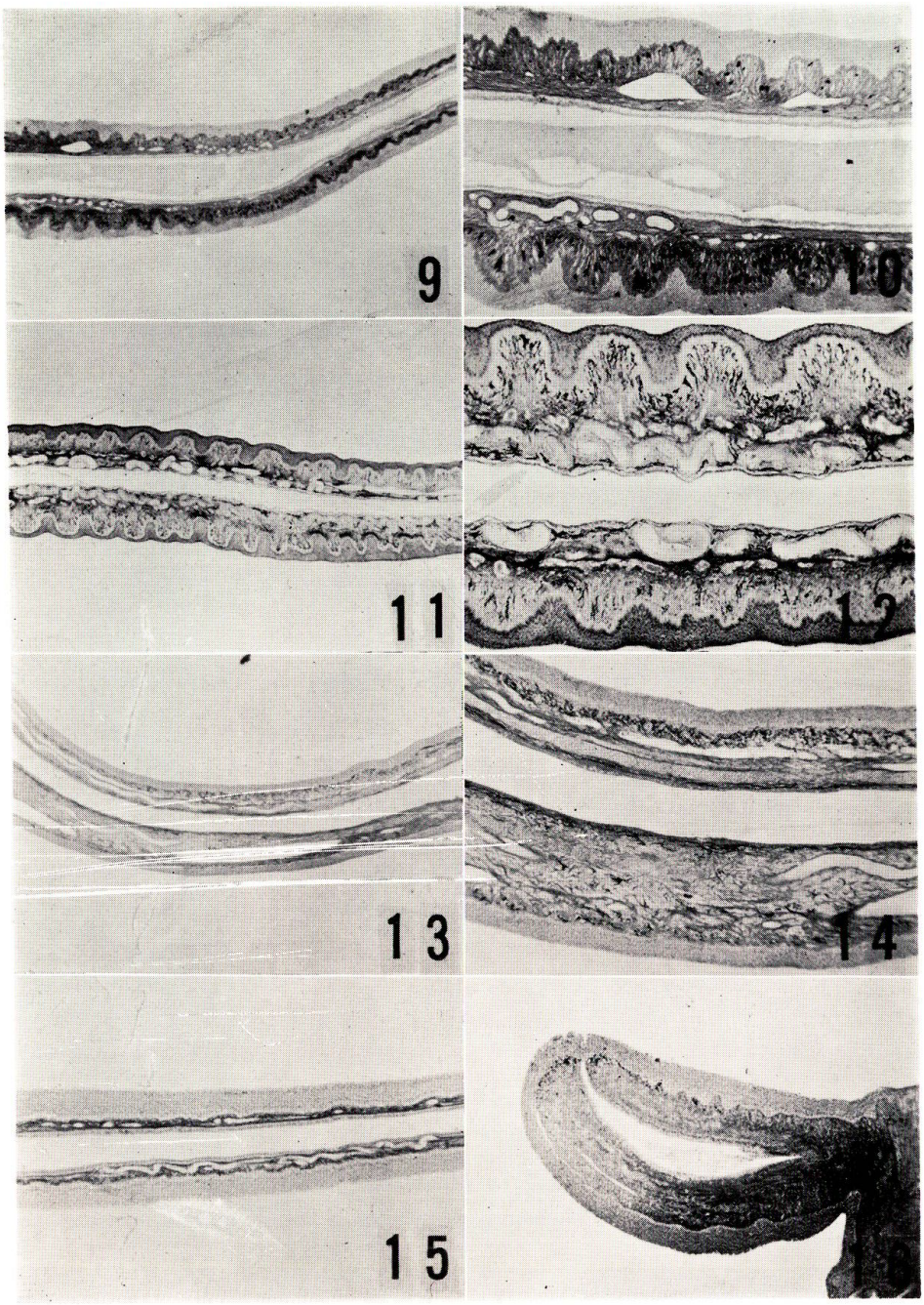
Fig. 12. Portion of the above specimen, showing the remarkable vasodilation of vessels. Notice a loosening of the connective tissue layer. ×55

Fig. 13. Ovipositor at the oviposition phase, being 57 mm in length. ×22

Fig. 14. Magnified portion of Fig. 13. Notice remarkable vasodilation of blood and lymph vessels. Blood vessel is filled with blood cells. The papillae of the covering epithelium disappear. ×55

Fig. 15. Upper part of the ovipositor showing the maximum length of 41 mm. Connective tissue layer is very thin. Vasodilation of blood and lymph vessels is noticed. ×55

Fig. 16. Ovipositor tissue after the spawning season. The connective tissue layer is compact and cellular. ×55



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