STUDIES ON THE ENDOCRINE GLANDS OF THE SALMONOID
FISH, THE AYU, *PLECOGLOSSUS ALTIVELIS*
TEMMINCK ET SCHLEGEL
VI. EFFECT OF ARTIFICIAL CONTROLLED LIGHT ON THE
ENDOCRINES OF THE POND-CULTURED FISH

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Introduction

The fact that the photoperiodicity as one of the external factors of the environment plays important role on the maturation of the gonad of Ayu has demonstrated by Nonaka (1957)*. Similar results have been obtained by several investigators, such as the staff of Gifu Prefectural Fisheries Experiment Station (1959, 1960)*, Kobayashi and Matsumoto (1960)*, and Shiraishi and Takeda (1961). The prolonged day light upon the annual Ayu fish induces the delay of maturation of gametes and prolongation of the life span of the fish beyond the natural breeding season. On the contrary, the time of spawning is accelerated one or two months earlier than that of natural spawning after the treatment of short day light.

What mechanism is related to the modification of the rate of maturity of gonad affected with artificial controlled photoperiodicity? In their excellent experiments in birds, for example, Benoit (and his collaborators) (1962) have suggested that the hypothalamo-hypophysial neurosecretory activity stimulated with light rhythm through cerebral cortex has intimate relation to the maturity of gonad. Therefore, the endocrine correlation between the light rhythm entered from the eye and the development of gonad in the Ayu might be intimated. In order to ascertain this problem, the author has examined the fish treated chiefly with prolonged day light histologically.

Materials and Methods

The materials used in this study were offered by a studious fish-culturist Mr. T. Yamada. The fish ponds provided for his experiments are three, 5.4 m², 10.8 m² and 10.8 m² (sq.) respectively. Rich underground spring, 17°C in average water temperature throughout the year, is poured into the ponds with 2 HP pump. About fifteen thousand young fish, the Ayu, *Plecoglossus altivelis* Temminck et Schlegel, caught in the middle of April in the River Naga-kawa of Tokushima Prefecture, Shikoku, were reared in the ponds. However, high mortality was encountered throughout the period

* These publications written in Japanese only were cited from Shiraishi and Takeda (1961).

of experiment. Survival fish have grown satisfactorily. During the middle of May, 1960 to May, 1961, the fish were exposed for 2 or 3 hours every night with two fluorescent lamps (20 W) fitted up at every pond. Next, from June 2 to 13, 1961, the surface of ponds were covered with black shades to induce mature eggs from survivors of the female fish. Afterwards, the fish were liberated from the treatment, and, late in November, 1961, the fish were immersed into Bouin's fixative for histological examination.

Till the end of normal life span of the fish (November, 1960) the males fell down one by one, and all the males have deceased as soon. Anyhow, only dozens of individuals of female fish have survived by the end of the experiment. At that time, the length of body of fish attained to more than 150 mm.

The endocrine glands of the fish were removed and embedded in paraffin, cut serially at 8 to 10 μ and stained with Delafield's hematoxylin-eosin, Heidenhain's hematoxylin-light green, Heidenhain's azan triple stain, periodic acid-Shiff technique as modified and recommended by Wilson and Egrin (1954), Gomori's chromealum hematoxylin-phloxine (CH), Halmi's paraldehyde fuchsin-orange G and light green (AF), May-Grünwald, and May-Giemsa methods.

Results of Observations

Pituitary gland

The external feature of the pituitary gland shows a severe distortion: there are constrictions and grooves in the anterior part of the adenohypophysis, and swollen or protuberant parts are developed here and there. In some preparation, large lacunae containing the colloid like substance in their lumina occupy extensive area of the rostral zone of pars distalis. This substance is stained faintly with anilin blue and AF, indicating a condition of low viscosity and foamy structure (Plate I, fig. 1).

The periphery of the rostral zone of pars distalis (=pro-adenohypophysis) is composed of a single layer of glandular cells. These cells arranged in the cord are cubic or somewhat polygonal in shape. These are small in size with round nuclei in the central region. A small quantity of connective tissue and nerve fiber is found near each basal edge of the glandular cells. The cell cords separate with each other, and the spaces occurred in the cords are invaded by the ramifications of the nerve fibers. Most of these cells are acidophilic, but small number of chromophobes and weak basophiles are also recognized. Furthermore, there have been seen few gigantic cells and the cysts containing small lymphocytes.

In the ventral region of the proximal zone of pars distalis (=meso-adenohypophysis), two types of basophiles in normal appearance were detected. These are: small masses of polygonal basophiles scattered like islands; and high columnar ones arranged in radial direction as the follicles. These are positive faintly with AF and CH, showing homogeneous condition. Nucleus is oval in shape, light in coloration, and is situated in the central region of the cell. Some of basophiles, however, lose

their affinities for dyes. On the other hand, acidophiles lie near basophilic islands, intermingling with each other. The cytoplasm shows the homogeneous or fine granular conditions, and the nucleus is situated in the corner of the cell. Noticeably, there are many gigantic cells, irregular and polygonal in shape, under the process of degeneration. These are in chromophobic, showing a foamy condition (Plate I, figs. 2 and 3). The nucleus, picnotic and crooked in shape, occupies a corner of the cell. Cystic space around each gigantic cell, capillary, sinusoid and connective tissue are well developed. Generally speaking, the dorsal region of the proximal zone indicates a remarkable pale condition, because of the existence of these degenerated cells.

In the pars intermedia (=meta-adenohypophysis) there could be seen small, high columnar weak acidophiles arranged in the heavily convoluted cords. The nucleus of the gland cell is situated in the apical end of the cell. The space between the cell cords is invaded with the capillaries. In some preparations some large lacunae are produced in this lobe.

A part of the pars nervosa (=neurohypophysis) which is surrounded by the pars intermedia is filled with rich neurosecretory granules. In the dorsal zone of pars nervosa, however, many nerve fibers are recognized, and fine AF- and CH-positive granules run along the fibers (=axons), forming the string of beads. Besides, large Herring bodies are encountered near the deteriorated cells. Generally, the shape of cell of the preoptico-nucleus is irregular, and its nucleus is also distorted. The cytoplasm, homogeneous in condition, indicates a weak AF-positive reaction. So that, no striking neurosecretory activity is detected in the cell (Plate I, fig. 4). The same is found in the nucleus tuberis lateralis.

AF-positive materials are also recognized in the vascular sac, although the significance and nature of the materials in this organ are unknown.

Thyroid gland

The epithelium of thyroid follicle is remarkably flat with scanty cytoplasm. The capillaries run the basal margin of the epithelium, some of which invade into the epithelial cells, indicating a feature of the so-called intra cellular capillary. The lumen of follicle is filled with rich PAS-positive colloid, which is also stained deeply with eosin and appeared to be the smooth homogeneous condition without rough wavy margin (Plate I, fig. 5).

There is another type of follicular epithelium, consisted of cubical cells. In this case, a part of colloid contained in the follicular lumen shows the condition of vacuolization and liquefaction. The strange bodies like nuclei, stained with hematoxylin, are scattered in the colloid. These might be derived from the epithelial cells (Plate I, fig. 6). Moreover, the PAS-positive granules are detected in the epithelial cells. Anyhow, the thyroid of the present fish appeared to be the state of

Fig. 1. Rostral zone of pars distalis in the pituitary gland of the senile Ayu fish maintained under the artificial controlled light. A large cyst like area, i.e. lacuna, contains a colloid like substance stained faintly with basic dyes. $\times 300$

Fig. 2. Dorsal region of proximal zone of pars distalis showing degenerating feature of the gland cells. A gigantic or marked dilated cell is detected in the lower left. $\times 400$

Fig. 3. Cells under the course of degeneration in the dorsal region of proximal zone of pars distalis. $\times 400$

Fig. 4. Cells of preoptico-nuclei in the light controlled senile Ayu. No striking secretory phase is detected. $\times 400$

Fig. 5. Thyroid follicles near the ventral aorta of the fish in a condition of hypoactivity. $\times 150$

Fig. 6. Higher magnification of a thyroid follicle. Epithelium is constructed from cubic cells, and colloid in the lumen contains many strange bodies which are referable to the nuclei of epithelial cells. $\times 300$

Fig. 7. Section of head kidney under deterioration. Nephrons in degeneration are seen in the right. $\times 150$

Fig. 8. Interior wall of cardinal vein, showing loose polyhedral cells of connective tissue which might be diagnosed as hemangio-endothelioma. Many blood cells dispersed are found, $\times 300$

hypofunction accompanying the course of degeneration.

Head kidney and adrenal cortical tissue

The head kidney extends its dimensions with marked aberrant contour. In the section, the hyperplastic masses of acidophilic cortical tissue and of basophilic tissue face to each other, placing a cleft between them. However, the origin of the latter mass is not determined. One side of the hyperplastic tissue is surrounded with a layer of connective tissue, and still more a thick layer of serous membrane. A large quantity of tissue under the necrosis is produced, and the tissue runs along another side of the head kidney until the kidney is appeared. Several transections of nephrons in the course of degeneration are found in the area of necrosis. Cystic space is developed around the nephron, and the cytoplasm of epithelial cell of tubule is stained faintly with dyes (Plate I, fig. 7). There could be seen many vacuoles in the cell. Remarkably, loose polyhedral cells form the nest in the cavity (interior wall) of cardinal vein. This structure resembles the beehive, and might be diagnosed as the hemangio-endothelioma (Plate I, fig. 8).

The acidophil, polygonal in shape, is small, and seems to be the adrenal cortical cell. Many vacuoles are seen in the cytoplasm of the cell. These acidophiles constitute a large compact mass, which is divided into many lobules (Plate II, fig. 1). Moreover, some gigantic syncytium scatter here and there. On the other hand, the basophil is large, indicating a remarkable foamy condition with many vacuoles in its cytoplasm. The nucleus of the cell, stained with dyes weakly, is round and small, and occupies the central region of the cell. There is no detectable regular arrangement and lobules in a mass of basophiles. A part of the tissue appeared to be under the process of degeneration and necrosis (Plate II, fig. 2).

As mentioned above, the histologic picture of the adrenal cortical tissue showed a marked aberrant and pathologic condition.

Ovary

The most striking feature in the ovary is an occurrence of the nodules. They are several to scores in number, white in coloration and soft in appearance. In some specimens, however, these nodules unite together with each other, and form a gigantic spongy swelling, yellowish white in color (Plate II, figs. 3 and 4).

From histologic observation, the structure in question is diagnosed as lymphosarcoma. A great deal of fibrous connective tissue and reticulum develops around each degenerated egg, running irregularly or vortically (Plate II, fig. 5). In the fibers of these connective tissue, there found many lymphocytes here and there (Plate II, fig. 6). The lymphocyte has a round nucleus, small quantity of cytoplasm, and indistinct cell outline. Two types of the cells are recognized, larger ones are acidophilic and smaller basophilic. An increase in amount of fibers and lymphocytes takes place following the degeneration of the eggs. In some cases, there have been seen the

Fig. 1. Adrenal cortical tissue of the light controlled senile Ayu. Many vacuoles are recognized in the cytoplasm of the cell. $\times 400$

Fig. 2. Strange basophilic tissue found in the head kidney. Cytoplasm of the cell is in a foamy condition, and size of the cell is larger than that of acidophilic cortical cell. $\times 400$

Fig. 3. General appearance of ovary of light controlled senile Ayu with many lymphosarcomatous nodules, varying in size. $\times 2/3$

Fig. 4. General appearance of ovary suffered from heavy lymphosarcoma. Neoplasm looks like a soft spongy body. $\times 2/3$

Fig. 5. Section of ovary, showing whirling fibrous connective tissue and lymphocytes around an atretic ovum. $\times 150$

Fig. 6. Higher magnification of collagenous fibers and lymphocytes in the neoplastic ovary. $\times 400$

Fig. 7. Necrosis occurring in the central region of ovary under a heavy lymphosarcoma. $\times 150$

Fig. 8. Higher magnification of necrosis occurring in the central region of ovary under a heavy lymphosarcoma. $\times 400$

space between tumorous growth and convolute oolemma under the degeneration. After the ova were disappeared, the tumorous growth increases its volume remarkably, and changes into an almond-like soft body. Relatively, lymphocytes decrease in number and the necrosis occurs in the central region of the growth (Plate II, figs. 7 and 8). Various stages of mitotic figures are encountered in the tumorous cells which are located in the peripheral region of the ovary. As the atresia takes place in the whole ovarian eggs, these are forwarded in the course of pre-ovulation corpora lutea. In each case the yolk is attacked by the invaded and phagocytic granulosa cells. Sometimes, smooth spherical cells containing rich colloidal substance are found near the eggs under the degeneration. The nucleus of the cell is small in size, and is occupies the corner of the cell. It is, therefore, appeared that these are referable to the so-called cell pearls derived from the invaded and luteinized granulosa cells.

Discussion

It is interesting to know the influence of artificial controlled photoperiodicity on the endocrine glands of the Ayu which has survived successively after its natural life span.

The histologic picture of the pituitary gland of the Ayu maintained under the long term photoperiodicity shows the condition of degeneration. There are many lacunae in the rostral zone of pars distalis of the present fish as was described in many kinds of matured teleosts (Honma, 1957). Special mention must be made of the necrosis in the dorsal extensive area of the proximal zone of pars distalis of the present fish. The glandular cells, which seem to be derived from the basophiles, expand remarkably, and the cystic space around each cell is notable. Robertson and Wexler (1962 c) have already stated the identical cytologic figure on the senile castrated kokanee salmon (*Oncorhynchus nerka kennerlyi*), which has survived from one to four years beyond the normal life span of this species. Same findings have also been reported on the mature kokanee salmon, blue back salmon (*O. nerka nerka*), king salmon (*O. tshawytscha*) (1962 b), and rainbow trout (*Salmo gairdnerii*) (1962 a), during the spawning season.

It is said that the hyperadrenocorticism may play a leading role in the post-spawning death of the Pacific salmon, some of trout and Ayu (Robertson and Wexler, 1959; Honma, 1960; Honma and Tamura, 1963). Though Rasquin and Rosenbloom (1954) reported that the adrenal cortical tissue of characin, *Astyanax*, well responds to the stimulation caused by dark-breeding, the tissue of the light controlled Ayu showed a mysterious pathologic condition. Therefore, possible relation between a large quantity of steroid hormone secretion from the adrenal cortical tissue and hypothalamo-hypophysial neurosecretion activated by light rhythm is intimated.

Hoar and Robertson (1959) found that the thyroid activity of goldfish maintained

under the shorter photoperiods was slightly greater than that of the fish under the longer one. On the other hand, the thyroid gland of the present Ayu fish indicated the state of hypofunction.

Although the lymphosarcoma has been found in the many kinds of organs in the vertebrate animals, no reliable report on the lymphosarcoma in the gonad of fish has been appeared. Because the fish (including cyclostome, shark-like fish and bone fish) have no lymph nodes, the hematopoiesis takes place in the spleen, mesonephros, submucosa of intestine, and subcapsular tissue of gonads etc. It is said, however, that the dominant hematopoietic organ in teleost is the mesonephros, and many cases of lymphosarcoma have been reported in the kidney of teleost (Schlumberger and Lucké, 1948; Nigrelli, 1947, 1953). Johnstone (1926) supposed that the primary site of the neoplasm (may be lymphosarcoma) found in the coelom of a herring, *Clupea harengus*, was the subcapsular tissue of the gonad. In the present experiment, it is likely that the lymphosarcomatous growth developed in the ovary of the Ayu is derived from the connective tissue theca of follicular membrane of the atretic egg. The theca is considered as a part of ovigerous folds (=ovarian lamellae) of subcapsular tissue, that is, the splanchnic mesoderm. Recently, Balls (1962) described five cases of spontaneous lymphosarcoma in anuran amphibia, *Xenopus*. It is interesting to find that three of them are detected in the gonad (two in ovary and one in hermaphrodite), since amphibians have no lymph nodes, but have similar hematopoietic organs as seen in the fishes.

In brief, by the artificial controlled light, severe derangement of the hormonal secretion might be brought about in the senile Ayu. However, the cause of the lymphoid growth (=neoplasm) in the ovary of the fish has not been yet elucidated.

Summary

The pond-cultured annual salmonid fish, the Ayu, *Plecoglossus altivelis* T. & S., was maintained under the artificial controlled photoperiodicity, and the survival time of the fish was prolonged considerably beyond the natural life span of this species. In the present paper, merely histological description was given of the endocrine glands of the fish.

The shape of the pituitary gland shows a remarkable deformity, and many lacunae were occurred in the adenohypophysis, particularly in the rostral zone of pars distalis. The glandular cells, including many of dilated cells, located in the dorsal region of the proximal zone of pars distalis, were in the process of marked degeneration. The figure of the thyroid gland appears to be hypofunction and degeneration. Marked expansion of the head kidney was encountered, and the adrenal cortical tissue is composed of hyperplastic masses of acidophiles and of basophiles. The superficial layer of the head kidney indicates the state of severe necrosis. Inside the cardinal vein, there appears a strange structure which might be the hemangio-endothelioma.

Whole ovarian eggs were remained in a state of preovulation, and underwent the process of degeneration. Various stages in the course of formation of corpora lutea were seen. Notable lymphosarcomatous nodules, varying in size, were detected in the ovary. The atretic eggs under the process of formation of corpora lutea were surrounded by fibrous connective tissue and reticulum which maintain many lymphocytes. Sometimes, partial necrosis was developed in the central region of a large tumorous growth. Possible relation between the light rhythm and the endocrine unbalance was discussed.

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