**Studies on the Endocrine Glands of a Salmonoid Fish, the Ayu,
Plecoglossus altivelis Temminck et Schlegel—VIII.
Degenerative Changes in the Thymus of Koayu
exposed to Artificial Photoperiods***

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The annual salmonoid fish, Koayu was reared in outdoor hatchery ponds exposed to short (8 hours), long (16 hours), and natural photoperiods (control). These, as well as Otunen-Ayu that survived spawning and was collected in the streams of the suburbs of Niigata City, were examined histologically to elucidate the involution and regression of the thymus. Age involution of this organ commences with gonad maturation. A remarkable decrease in the number of lymphocytes and their mitotic divisions occurs, and at the same time an increase in the amount of reticular and collagenous fibers around the vessels and development of cystic spaces take place. The connective tissues and spaces disappear toward the end of involution. These regressive changes, including the disappearance of the thymus, occurred one month earlier in the short-photoperiod group than in the control group, but under the long photoperiod one to two months later than in the controls. The thymus was absent in all the Otunen-Ayu examined.

A marked involution and loss of hemopoiesis in the thymus and spleen of the ice-goby before and after spawning have been reported by us, using wild-caught fish¹⁾. Nearly identical results has previously been obtained in a series of experiments on various species of migrating Pacific salmon^{2,3)}. Recent studies on the lymphomyeloid organs of higher vertebrates have been directed to explain their role in lymphopoiesis and immune-body formation during early life, but the function of the thymus in lower vertebrates remains obscure both in the fields of cellular and transplantation immunity. For example, CUSHING⁴⁾ has tried to review the immunology of fish and has shown that contributions from thymology are very scarce.

Involution of the thymus in relation to sexual maturity and increasing age has been described in a characid fish, *Astyanax*⁵⁾, and a clariid catfish, *Clarias*⁶⁾. In order to investigate the age-involution of the thymus which may produce a hormone influencing the formation of immune bodies, a study of the annual salmonoid fish, the Koayu, was undertaken.

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Material and Methods

So-called Koayu, *Plecoglossus altivelis* TEMMINCK et SCHLEGEL is a dwarf, land-locked salmonoid found in Lake Biwa. Details of our experimental procedures have been described in a previous paper on the reactions of the fish's hypothalamic neurosecretory system to artificially controlled light⁷⁾. Early in June 1966, 1,500 fish, about 6 cm in length and 2 g in weight, were secured and separated into three outdoor ponds of the hatchery attached to the Niigata Prefectural Inland Water Fisheries Experimental Station. After one month of exposure to natural conditions, group 1 was subjected to a short photoperiod of 8 hours daylight each day by means of a vinyl black sheet. Group 2 was exposed to a long photoperiod, 16 hours/day by means of illumination with fluorescent lamps. Group 3 served as the control and was maintained under natural illumination. The experiment lasted from July 2, 1966 to January 22, 1967.

For the purpose of examining bodily condition and histological changes of the hypothalamic neurosecretory system and thymus of the fish, 5 females and 5 males were selected at monthly intervals. A routine histologic procedure was adopted for fixation with BOUIN's solution, dehydration, embedding and staining. Serial paraffin sections of the thymic region of 5 μ thickness were stained chiefly with hematoxylin-eosin, azan triple, and GIEMSA solutions, and for the PAS reaction. Thymus glands of Otunen-Ayu that had survived over the winter also examined to know the state of old fish.

Results

The thymus of *Plecoglossus altivelis*, like that described from other bony fishes, consists of a pair of small, soft bodies with a bean-shaped appearance that are embedded in the musculature near the dorso-posterior margin of the last branchial arch. The following paragraphs are arranged to facilitate comparison of this paper with the descriptions in the previous one⁷⁾.

Onset of the experiment (July 10, 1966) The thymus of fish with immature gonads was 5.0 mm long, 0.5 mm thick and 1.5 mm wide, with a gradual expansion of the posterior margin. The outer part of the thymus, which faces the branchial cavity, was occupied both by smaller lymphocytes (=thymic cells), 3.5 μ in diameter, and larger lymphoid cells, 6.0 μ in diameter, whereas the inner part, which is slightly embedded in the musculature, contained round mucous cells (14.0 μ), ovoid cells (14.0 $\times$ 7.0 μ), reticular cells, and myoid cells, in addition to lymphoid cells. The cortical (outer) part was packed densely with cells, but the medullary (inner) one was coarse. No clearcut distinction could be made between these two parts, however (Fig. 1 a,b). Some of the cells were in mitosis. At this time, only a single specimen of female fish had a thymus that indicated any regressive change: the mucous cells concentrated in the central part

were in the process of collapse and formed several large cystic spaces (Fig. 2)

One month after the start of the experiment (August 10, 1966)

Control The decrease in the number of lymphocytes and in the frequency of occurrence of mitosis had now become remarkable. The thickness of the thymus was reduced by half (about $250\ \mu$). Although an increase in the amount of reticular and collagenous fibers around the vascular wall had occurred, the general features of atrophic change were apparent. Some of the cystic spaces arising from the fusion of mucous cells had reached a diameter of $90\ \mu$ and formed large lacunae in the central portion (Fig. 3 a, b).

Short photoperiodic regimen A distinct diminution in the size of the thymus was already visible in this experimental group: the thickness of the gland was about half that of the controls (about $150\ \mu$) and there was a sharp decrease in the number of lymphocytes. Some of the large cystic spaces had attained a diameter of $130\ \mu$ by coalescing with one another, and they occupied most of the reduced thymus. In the center of these cystic spaces, a small amount of the debris that originated from the degenerated and collapsed cells was found frequently (Fig. 4 a,b). On the other hand, the well-developed reticular and collagenous fibers, which were derived from the periphery of the vascular walls and had invaded the parenchymous tissue, enfolded the nearby cells, exhibiting a swirling or serpentine appearance (Fig. 5).

Long photoperiodic regimen A slight decrease in the number of lymphocytes was present in this experimental group. Mitotic figures were still detectable. Although most of the mucous cells were scattered about in a solitary manner, some of them formed cystic spaces ($50\ \mu$ in diameter) as a result of coalescence. The development of reticular and collagenous fibers in the periphery of the vascular walls was still poor, and the entire thymus maintained a thickness of $350\ \mu$ (Fig. 6).

In order to show the regression of the thymus, the changes in its thickness were plotted with the value for June considered as 100% (Textfig. 1).

Two months after the start of the experiment (September 10, 1966)

Textfig. 1. Regressive changes of the thymus of *Plecoglossus*.

Control The thickness of the thymus was reduced by half (about $100\ \mu$) from that of the preceding month, and there was a further decrease in the number of lymphocytes. Several large masses of reticular and collagenous fibers were seen. Due to the regression of the thymus as a whole, the cystic spaces appear to fall into a flattened state, occupying the greater part of the gland (Fig. 7).

Short photoperiodic regimen As stated previously, gonadal maturation and concurrent mortality of the fish under this regimen reached the highest peak. By reason of the decrease of the thymus to a thickness of $50\ \mu$, it was very difficult to find any lymphocytes. Cystic spaces had combined in the layer of the mucous cells of the skin and showed a tendency to disappear. A small amount of amorphous debris, derived from thymoma were obtained under this regimen, and these specimens will be described in detail elsewhere.

Long photoperiodic regimen The thymus was about twice as thick as in the controls, although the decrease in number of lymphocytes and mitotic divisions was a gradual one. The fusion of cystic spaces with one another had begun, and some of the spaces had attained a diameter of $150\ \mu$. The reticular and collagenous fibers formed swirls or clumps of various sizes, enfolding the adjacent lymphocytes, mucous and reticular cells. Part of these structures dyed red with azan stain, and also gave a positive PAS reaction. They therefore resembled HASSALL's corpuscles.

Three months after the start of the experiment (October 2, 1966)

Control The highest level of gonadal maturation and mortality was reached in this group, one month later than in the fish exposed to short photoperiods. Accordingly, the thymus revealed a sharp regression and diminution with the loss of almost all lymphocytes (Fig. 10).

Short photoperiodic regimen As regression proceeded further, it became difficult to distinguish the demarcation of the thymus from the surrounding tissues.

Long photoperiodic regimen Although the thickness of the thymus had become reduced by half (about $130\ \mu$) from the preceding period, its structural pattern was still fully retained. There was a noticeable decrease in the number of lymphocytes and hyperplastic features of the mucous epithelial layer of the skin were prominent. Due to atrophy of the thymus as a whole, the cystic spaces were passing into the flattened condition ($100\ \mu$ by $40\ \mu$). In addition, the reticular and collagenous fibers were gradually becoming reduced (Fig. 11).

Four months after the start of the experiment (November 5, 1966)

Control and short photoperiodic regimens It was almost impossible to distinguish the thymus in both groups.

Long photoperiodic regimen The maturation of the gonads and the activity of the hypothalamo-hypophyseal complex appeared to have reached the maximum at this

time. The regression of the thymus was most noticeable and the cystic spaces had attained nearly the same state as in disappearance. The connective tissue fibers had aggregated to form masses.

Five months after the onset of experiment (December 4, 1966)

Control and short photoperiodic regimen The thymus was almost absent in both groups.

Long photoperiodic regimen The number of deaths rose rapidly and the thymus had almost disappeared (Fig. 13).

Six months after the start of the experiment (January 22, 1967) In every one of the surviving fish, it was very difficult to recognize the presence of the thymus. In one fish of short photoperiodic regimen, however, a tumorous growth was seen in the thymic region.

Otunen-Ayu The thymus of the Otunen-Ayu, i.e., fish that had survived for a considerable length of time beyond the spawning season under natural or wild conditions, was examined. The specimens were collected from January to March in a tributary of the River Agano. A remarkable diminution and regression of the thymus of each specimen were seen without exception, and only a small amount of debris from the condensed collagenous fibers could be demonstrated (Fig. 14). Furthermore, a single specimen caught in February had a tumorous growth in the thymic region (Fig. 15).

Discussion

Generally speaking, the differentiation of cortical and medullary layers of the thymus of bony fishes is not as distinct as in the higher vertebrates⁴⁾. On the other hand, two or three zones, such as cortex and medulla, or outer, middle and inner zones, were distinguished in the thymus of several fishes (e.g., VON HAGEN⁸⁾, in *Anguilla*; HAFTER⁵⁾, in *Astyanax*; RIZKALLA⁶⁾, in *Clarias*). In *Plecoglossus* the outer zone is occupied by a considerable number of lymphocytes, while the inner zone, in addition to scattered lymphocytes, contains mucous, reticular, and scanty myoid cells. Nevertheless, it is difficult to distinguish the cortical layer from the medullary one.

It is of significance to mention the structure in fishes that is comparable to HASSALL's corpuscles of higher vertebrates. These concentrically hyalinized bodies of epithelial cells have been considered peculiar to the thymus of homoiothermal animals, although the existence of essentially the same and the unicellular HASSALL bodies of anuran amphibians had been reported by KAPA⁹⁾ and KAPA *et al.*¹⁰⁾. In bony fishes, VON HAGEN⁸⁾ and TOKUHISA¹¹⁾ described HASSALL's corpuscles in European and Japanese eels, and structures relatively similar to HASSALL's corpuscles were described in several species, such as *Astyanax*,⁵⁾ *Clarias*⁶⁾ and *Leucopsarion*.¹⁾ On the other hand, no such description

was offered by ROBERTSON and WEXLER²⁾ in the case of spawning Pacific salmon. Histologically, KAPA⁹⁾ demonstrated much DNA in the central part of the corpuscles, and PAS-positive substance in a small number of cysts around the corpuscles of *Rana* spp. In *Plecoglossus*, several small masses consisting of concentric circles of reticular and collagenous fibers, which involved the mucous cells, reticular cells and lymphocytes, were found. These peculiar structures stained with acidic dyes and showed a positive PAS reaction. Whether or not these are homologous (or correspond) to HASSALL's corpuscles is not definitely known.

With regard to age involution, HAFTER⁵⁾ pointed out that in the thymus of *Astyanax* this begins about the time of sexual maturity with an increase in connective tissue elements and in size of cysts, and a decrease in the number of mitoses of lymphocytes. In contrast, the reduction of the thymus of *Clarias* does not coincide with sexual maturity, and in turn a complete absence of the thymus occurs in aged clariid catfish⁶⁾. The same situation has already been reported in *Salmo*¹²⁾, and *Anguilla*⁸⁾. RASQUIN and ROSENBLOOM¹³⁾ reported that under conditions of complete darkness of more than one year, a remarkable increase in the number of atrophic nuclei of lymphocytes (thymic cells), and a severe involution to complete absence of the thymus occurred. They also pointed out that in the six-year old fish described by HAFTER⁵⁾, the degree of involution was less than that in fish reared in the dark.

During the sexual maturity and senility of the migratory Pacific salmon, the thymic region was filled with monocytes and goblet cells, after a severe regression and diminution of thymic cells²⁾, and in senile kokanee salmon that had been castrated when immature, a complete absence of the thymus was encountered³⁾. Nearly identical degenerative changes have been seen in the spawning ice-goby¹⁾. The same condition has been detected in the present case of *Plecoglossus* reared under the artificially controlled light regimens. Due to sexual maturation caused by the activity of hypothalamo-hypophysial complex, a remarkable diminution of the thymus, including a rapid decrease of mitotic division and the number of lymphocytes, was brought about in addition to the hyperplasia of connective tissue elements around the walls of blood vessels. A close relationship between the reticular fibers and blood vessels has already been described in the thymus of several species of freshwater fishes, in which free fibers were few¹⁴⁾. In contrast to this, VON HAGEN⁸⁾ reported that in the thymus of yellow eels, 15 to 20 cm in length, an increase in the amount of adipose tissue was brought about by age involution.

With increasing age, the size of the cystic spaces in the *Plecoglossus* thymus increases markedly as a result of the collapse of aggregative mucous cells thus forming larger lacunae. At the time of spawning, however, these lacunae disappear as a result of communication with the mucous cells of the epidermis that covers the thymic parenchyma. Moreover, condensed connective tissue fibers were no longer recognized once they had

assumed an amorphous structure.

As mentioned previously, in the spawning, nonmigratory Japanese char, a considerably less pronounced diminution of the thymus was detected—even in the postspawning adult fish¹⁾. Nevertheless, a complete absence of the thymus was seen in the Otunen-Ayu that survived and tided over the winter after their spawning season. It is not known whether these Otunen-Ayu can spawn again and survive, and much more information will be needed to determine the relationship between the age-involution of the thymus and the postspawning death of the fish.

Age involution and associated changes in the thymus of *Plecoglossus* take place in parallel with those of the sexual maturation in fish exposed to artificial photoperiodism⁷⁾, i.e., one month earlier in the short photoperiodic regimen, and one to two months later in the long photoperiodic regimen. It is difficult to decide what is the most critical factor in the death that accompanies the senility, as well as the first-spawning death after sexual maturation, of this annual fish. The exhaustion and severe derangement of the endocrine organs, the considerable regression in the hemopoietic and digestive organs, and the concurrent changes in connective tissue elements and the vascular system are all involved in these aging and maturing involutions. In any case, the great loss of immunity that may follow thymic degeneration could be one of the important causal factors in the death of the species, both annual and migratory-spawning individuals.

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References

- 1) E. TAMURA and Y. HONMA: *This Bull.*, **36**, 661–669 (1970).
- 2) O. H. ROBERTSON and B. C. WEXLER: *Endocrin.*, **66**, 222–239 (1960).
- 3) O. H. ROBERTSON and B. C. WEXLER: *Gen. Comp. Endocrin.*, **2**, 458–472 (1962).
- 4) J. E. CUSHING: in “Fish Physiology” (W. S. HOAR and D. J. RANDALL, eds.), Vol. 4, 465–500, Academic Press, New York (1970).
- 5) E. HAFTER: *J. Morph.*, **90**, 555–582 (1952).
- 6) W. RIZKALLA: *Acta Veter. Acad. Sci. Hung.*, **19**, 331–342 (1969).
- 7) Y. HONMA and A. SUZUKI: *Japan. J. Ichthyol.*, **15**, 11–27 (1968).
- 8) F. VON HAGEN: *Zool. Jahrb.*, **61**, 467–538 (1936).
- 9) E. KAPA: *Acta Morphol. Acad. Sci. Hung.*, **12**, 1–8 (1963).
- 10) E. KAPA, I. OLÁH and I. TÖRÖ: *Acta Biol., Acad. Sci. Hung.*, **19**, 203–213 (1968).
- 11) N. TOKUHISA: *Med. J. Kagoshima Univ.*, **20**, 825–844 (1969).
- 12) R. DEANESLEY: *Quart. J. Microscop. Sci.*, **71**, 113–145 (1928).
- 13) P. RASQUIN and L. ROSENBLUM: *Bull. Amer. Mus. Nat. Hist.*, **104**, 359–426 (1954).
- 14) I. MATSUSHITA: *Med. J. Kagoshima Univ.*, **10**, 65–70 (1958).

Plate I.

- Fig. 1. a. Horizontal section of the thymus of a sexually immature salmonoid fish, *Plecoglossus altivelis*, one month before the start of the experiment (June, 1966). The outer layer of the thymus near the branchial chamber is occupied by dense lymphocytes, while they are sparse in the inner one. Hematoxylin-eosin stain. $\times 100$
b. Enlarged view of a part of Fig. 1 a, showing several mitotic division of lymphocytes and mucous cells. $\times 450$
- Fig. 2. Thymus of a specimen at onset of the experiment (July, 1966). In the central part, the mucous cells have degenerated and formed cystic spaces. Azan stain. $\times 450$
- Fig. 3. a. Thymus of a control specimen, one month after the start of the experiment (August, 1966). The thickness of the gland is half that of specimens killed in June. Hematoxylin-eosin stain. $\times 100$
b. Enlarged view of a part of Fig. 3 a. A decrease in the number of lymphocytes and the large lacunae derived from aggregates of cystic spaces are apparent. Hyperplasia of connective-tissue elements is also marked. $\times 450$
- Fig. 4. a. Thymus of a specimen exposed to the short photoperiod, one month after the start of the experiment (August, 1966). As a result of the notable decrease in lymphocytes, a conspicuous diminution and regression has been brought about, and the thickness of the thymus is only about half that of the controls. Hematoxylin-eosin stain. $\times 100$
b. Higher-power view of a part of Fig. 4 a, to show the enlargement of the cystic spaces (=lacunae) produced by the coalescence of several cystic spaces. Note the debris derived from degenerated cells. $\times 450$
- Fig. 5. a. Another specimen exposed to the short photoperiod (August, 1966). Reticular and collagenous fibers in a state of convolution are well developed, surrounding some of the adjacent cells. Azan stain. $\times 450$.
b. Enlarged view of a part of Fig. 1 a, showing several mitotic division of lymphocytes and mucous cells. $\times 450$
b. Enlarged view of a part of Fig. 3 a. A decrease in the number of lymphocytes and the large lacunae derived from aggregates of cystic spaces are apparent. Hyperplasia of connective-tissue elements is also marked. $\times 450$
- Fig. 6. Thymus of a specimen exposed to the long photoperiod, one month after the start of the experiment (August, 1966). A slight decrease in lymphocytes and an indication of the hyperplasia of connective-tissue elements are present. Hematoxylin-eosin stain. $\times 450$
- Fig. 7. Thymus of a control specimen, two months after the start of the experiment (September, 1966). The involution of the thymus has resulted in a marked decrease of lymphocytes. Cystic spaces or lacunae occupy a great part of the thymus, and a considerable number of convoluted fibers are present. Hematoxylin-eosin stain. $\times 450$
- Fig. 8. Thymus of a specimen exposed to the short photoperiod (September, 1966). After their migration toward the epidermis, the cystic spaces become included in the layer of mucous cells, and then atrophy. At this time, it becomes very difficult to follow the thymus because of its extensive degeneration. Hematoxylin-eosin stain. $\times 450$

Plate II.

- Fig. 9.** Thymus of a specimen exposed to the long photoperiod (September, 1966). The thickness of the thymus is still about twice as great as that of the controls. Cystic spaces including debris, and fibers containing adjacent cells may be noted. Azan stain. $\times 450$
- Fig. 10.** Thymus of a control specimen, three months after the start of the experiment (October, 1966), showing involution with the absence of almost all lymphocytes. Diminution of the cystic spaces is also evident. Hematoxylin-eosin stain. $\times 450$
- Fig. 11.** Thymus of a specimen exposed to the long photoperiod, three months after the start of the experiment (October, 1966). A sharp decrease in lymphocytes is now apparent, and the cystic spaces are in a flattened condition. The degree of involution of the thymus is now marked, and convoluted connective-tissue fibers are well developed. Hyperplasia of the mucous-cell layer of the skin is also apparent. Hematoxyline-eosin stain. $\times 450$
- Fig. 12.** Thymus of a specimen exposed to the long photoperiod, four months after the start of the experiment. Most of the thymus has degenerated (a) ($\times 100$), while a small amount of condensed loosely fibers have been retained (b) ($\times 450$). Hematoxylin-eoson stain.
- Fig. 13.** Thymus of a specimen exposed to the long photoperiod, five months after the start of the experiment. Almost a complete reduction of the thymus has taken place. Hematoxylin-eoson stain. $\times 100$
- Fig. 14.** Thymus of a specimen of Otuneni-Ayu that survived the spawning season. This fish was caught in March, 1967. Without lymphocytes, the thymus is difficult to see. Azan stain. $\times 450$
- Fig. 15.** Thymus of a female fish fixed on January 22, 1967, showing a hyperplastic, tumorous condition. Hematoxylin-eosin stain. $\times 450$