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Involution of Thymus in Relation to Sexual Maturity and Steroid Hormone Treatments in Salmonid Fish

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Summary

The thymus of young chum salmon, *Oncorhynchus keta*, increased in size gradually with general growth during the immature stage, and it slowly began to atrophy and almost disappeared in the post-spawned fish. The thymus of alevin of the chum salmon was recognized as a swelling of 125 μm in thickness located in the same region as in the adult. In the fry, the thymus ranged from 130 μm to 245 μm. No clear-cut distinction between cortex and medulla regions were observed, although the number of small lymphocytes was large as compared with that of the large lymphocytes and reticular epithelial cells. The thymus of juvenile chum salmon was about two times thicker (435 μm) than in the fry stage. Medulla and cortex regions could be distinguished, and blood vascular systems were well developed. In four-year-old spawned fish the thymus reduced to the almost undetectable size of 50 μm.

In the masu salmon, *Oncorhynchus masou*, the thymus showed a gradual decrease in thickness from sexually immature to spawning stage. The thickness of thymus of the sexually immature fish was about 554 μm. The number of small lymphocytes was large. Distinct regions for cortex and medulla were observed. In the case of maturing fish, the thymic thickness reduced to 435 μm. A decrease in number of the small lymphocytes was noticed. With the advancement of maturation the thymus showed a decline in its thickness to 115 μm and a conspicuous increase in connective tissue layers. A further reduction in size of the thymus from 115 μm to 50 μm was observed in one-year-old fish at the time of its first spawning.

A study was made on the thymic thickness developing in the immature and maturing masu salmon in response to steroid hormones. The fish was injected intraperitoneally with cortisol acetate, testosterone and estradiol benzoate. In the sexually immature and maturing masu salmon (male and female) cortisol acetate injection induced a marked involution of thymus. Estradiol-17β showed a weaker response in the induction of thymic involution as compared with the response of the cortisol acetate. The effect of testosterone on the thickness of thymus could not be clearly observed in these fish.

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A marked involution and loss of lymphopoiesis of the thymus in ice-goby before and after spawning was reported by Tamura and Honma (1). Similar results have also been obtained in various species of Pacific salmons such as *Oncorhynchus tshawytscha*, *O. nerka*, and *O. nerka kennerlyi* (2). Involution of the thymus in relation to sexual maturity and increasing age has been described for a characid fish, *Astyanax* and a clarid fish, *Clarias* (3, 4). Although a gradual diminution of the thymus with increasing age has been demonstrated in several species of fishes, the commencement of involution differs among the various species (1, 4, 5).

Among the endogenously formed adrenocortical hormones which produce karyorrhexis of lymphocytes, cortisol has been found to be the most effective in mammals (6). No marked thymolytic effect could be demonstrated for estrogens, estradiol-17β and stilbestrol, but testosterone and progesterone produced conspicuous and limited involution at relatively high dose levels in higher vertebrates (7).

Miller *et al.* (8) observed in rats that thymic lymphocytes did not take part in the production of antibody, but the recovery of lymphopoietic and immune functions in an irradiated adult mouse was still dependent on a thymus controlled mechanism.

Recently Tamura (9) has investigated the morphology of the thymus in some Japanese fishes. The presence of immunoglobulin bearing lymphocytes in the adult skate thymus suggests that the thymus may be a primary lymphoid organ for the production of both T and B lymphocyte analogues (10). The evidence to date suggests that the lymphocytes of fish probably play a role similar to

TABLE 1. *Thymic Involution by Steroid*

Experiment	Time of injection and observation	No. of fish	Steroid injection
I	Middle of July 1978	5	non-treated
	End of August 1978	5	S
		5	C
		5	T
		5	E
II	End of August 1978	5	non-treated
	Middle of September, 1978	5	S
		5	C
		5	T
		5	E
	End of October, 1978	5	S
		5	C
		5	T
		5	E

S: Saline C: Cortisol acetate
T: Testosterone E: Estradiol benzoate

lymphocytes in higher vertebrates, i.e. they are immunocompetent cells (11).

Before proceeding with this type of investigations in fish, it is necessary to gather ideas relevant to the changes in size of the thymus during different stages of its life, as well as to ascertain the effects of steroid hormones on the degeneration of lymphocytes in thymus (12).

Materials and Methods

Histology

Thymi of the alevins, fry, juveniles and adults of chum salmon, *Oncorhynchus keta*, sexually immature and maturing adults of masu salmon, *Oncorhynchus masou*, were fixed in Bouin's or Helly's solution.

Paraffin sections of 5 to 7 μm in thickness were prepared by routine methods and stained with Mayer's hematoxylin and eosin (H-E stain). The measurements of the thickness of thymus were made across the widest area of its serial transverse sections.

With individual fish gross observations were made on the external features to detect symptoms of diseases.

Hormone treatments

The schedule of hormone treatment is shown in Table 1.

Experiment I. The first treatment was performed on July 1978. Eighty masu salmon which had a mean body weight of 99 g and fork length of 19.2 cm

Hormone Treatments in Masu Salmon

Total (mg)	Dose (ml)	Thymus thickness (μm±S. D.*)	Condition of fish
—	—	554±48.4	
	1	296±55.3	
10		279±87.4	Fungus
10		315±52.6	
10		332±11.6	Slight fungus
—	—	430±59.3	
	0.5	262±43.4	
5		112±14.6*2	Fungus
5		277±60.8	
5		373±36.0	
	0.5	73±10.5	Severe fungus
5		All dead by this time	Severe fungus
5		90±17.7	Severe fungus
5		183±21.0*2	Slight fungus

*S.D.: Standard deviation

*2: Significantly different to the saline injected group ($p<0.005$)

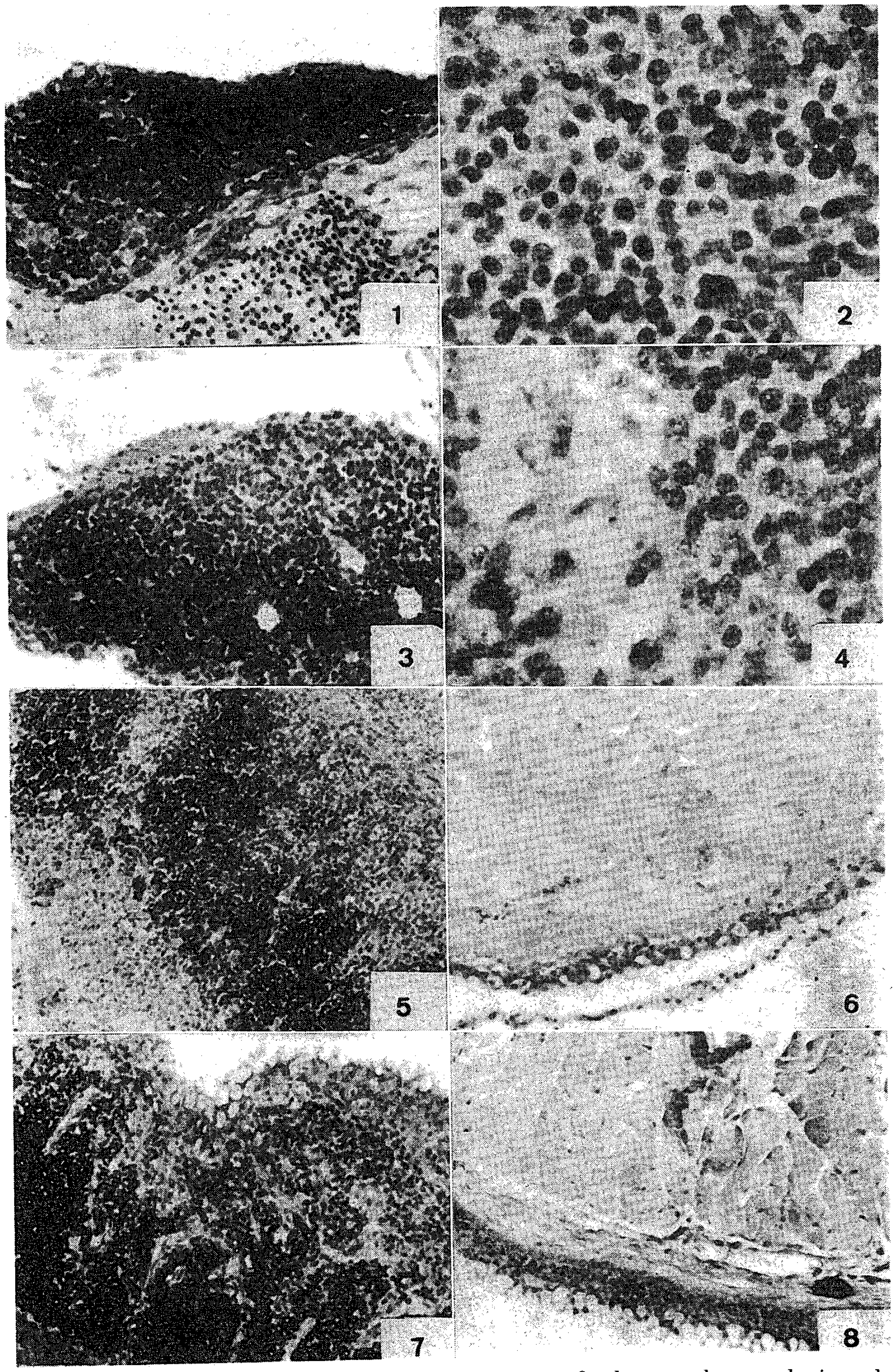

FIG. 1. T.S. (transverse section) of the thymus of chum salmon alevin, showing undeveloped cortex and medulla regions. H-E stain. × 210

FIG. 2. T.S. of the high magnification of the medulla of alevin thymus, showing reticular cells, epithelial cells and large lymphocytes. H-E stain. × 480

FIG. 3. T.S. of the thymus of four-month-old fry of chum salmon, showing developed cortex and medulla regions. H-E stain. × 210

FIG. 4. T.S. of the high magnification of the medulla of fry shown in Fig. 3. Epithelial and reticular cells are visible. H-E stain. × 480

were divided into four groups of 20 fish. The first group was given 0.5 ml physiological saline (S) per fish once a week for the first two weeks, and the second group was given one intraperitoneal injections of 5 mg cortisol acetate (C) per fish once a week for the first two weeks; the third and fourth groups were given testosterone (T) and estradiol benzoate (E), respectively. The dose for the third and fourth groups was the same as that for the second group.

Experiment II. The second treatment was done on August 1978. The number of groups and the kinds of treatments were similar to the first treatment. The only dose applied was 5 mg steroid per fish. The average body weight was 109 g and fork length 19.6 cm. The control group was also treated with one dose of 0.5 ml physiological saline per fish.

The dose was injected intraperitoneally just anterior to cloaca with a 2 ml syringe and No. 1 needle.

Results

Size and morphology of the thymus of untreated fish

The thymus of alevin of the chum salmon (one-month-old) consisted of a number of lymphocytes (Fig. 1). There were many large lymphocytes, reticular epithelial cells (Fig. 2), while small lymphocytes were few in number. Several mitotic figures which appeared to be an indication of multiplication of cells, were observed in these sections. The thymus measured about 125 μm. In fry, an increase in number of small lymphocytes was noticed (Fig. 3), but was not remarkable when compared with that of the juveniles (Fig. 5). Large lymphocytes and reticular epithelial cells were found in the section (Fig. 4) of fry thymus. However, cortex and medulla regions were more distinct in fry than in alevin. The thymus size varied from 130 μm to 245 μm. The thymi of juveniles reached a maximum thickness of 437 μm, and the increase in number of small lymphocytes was found to be conspicuous (Fig. 5). Mitotic figures were frequently detected in this period. The vascular system containing the connective tissue was found to be scattered among the thymi of juveniles. At the age of four years just before spawning, the decrease in number of thymic lymphocytes was remarkable, and the diminution in thickness reached 25 μm. This stage of involution was accompanied by an increase in amount of connective tissues in the basal part of the thymus (Fig. 6). The thymus shrank to a thin surface layer, and the lymphocytes disappeared. By

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FIG. 5. T.S. of the thymus of juvenile chum salmon, showing hyperplasia of the lymphocytes of cortex and medulla regions. H-E stain. × 210

FIG. 6. T.S. of the thymus of adult mature chum salmon, showing shrunken thymic tissue to narrow layer of cells and thick connective tissue layer. H-E stain. × 210

FIG. 7. T.S. of the thymus of sexually immature masu salmon, showing hyperplasia of small and large lymphocytes. H-E stain. × 210

FIG. 8. T.S. of the thymus of spawned masu salmon, showing degeneration of lymphocytes. Thymic cells have shrunken to narrow layer. H-E stain. × 210

the time of spawning a row of cells on the basement membrane was observed to be similar to the appearance of epithelial cells.

The thymus of sexually immature masu salmon collected in the first sampling was about 554 μm in thickness. Clear distinction could be made between the cortex and medulla regions. The cortex of the thymus consisted of both large and small lymphocytes, whereas the medulla contained mucous cells, reticular cells and myoid cells in addition to lymphocytes (Fig. 7).

The thymus of masu salmon collected in the second sampling was about 430 μm which was smaller in size than that of the first sampling. The structure of the thymus in the maturing group was very similar to the sexually immature group. A further decrease in size of this gland was also observed after two weeks, and almost a complete depletion of lymphocytes of this gland was found after two months. As a result the thymus became 73 μm thick (Fig. 8). This involution was accompanied by an increase in amount of connective tissue in the basal regions of the thymus that was similar to that of the chum salmon.

Thymic size of hormone treated fish

Table 1 illustrates the influence of corticosteroid and sex steroid hormones on the thymus of sexually immature fish (Experiment I). The thickness of the thymus decreased from 554 μm to 279 μm in the second group of fish treated with cortisol acetate, whereas the thymic thickness of saline treated and testosterone treated groups decreased to 296 μm and 315 μm respectively. This indicates a very slight inhibitory effect of the androgen on the involution of thymus. The thymus of the estradiol benzoate treated group exhibited a reduction in size of the thymus to 332 μm. In all these cases, the changes observed in the lymphoid tissues following the injection of the steroid hormones were degeneration of lymphocytes and inhibition of mitotic figures.

In Experiment II, the cortisol acetate treated group had a marked reduction in size of the thymus from 430 μm to 112 μm after two weeks of hormone injection. However, in the estradiol benzoate treated group the thymus size decreased from 430 μm to 373 μm, whereas the size of thymus became 277 μm and 262 μm in the testosterone and saline treated groups, respectively. In another observation (Experiment II) at the end of two months (Table 1), a further reduction in size of the thymus to 183 μm was noted in the estradiol benzoate treated fish along with a conspicuous shrinkage of this organ. A more definite reduction in size of the thymus to 90 μm and 73 μm was observed in testosterone and saline treated groups, respectively.

Discussion

A rapid prepuberal gain and the following loss of lymphatic organ weight are the characteristics of the thymus in aging vertebrates, as described by Miller (8). Also in the present study a gradual increase in size of the thymus was observed from alevin to juvenile and then a decrease in the same parameter of the adult spawned fish (Figs. 1–6). Moreover, the thymus of masu salmon also exhibited a gradual decrease in thickness from the early maturing stage (Fig. 7) to the spawning stage (Fig. 8). In spawned fish an increase in the basal layers of connective tissues was noticed along with the decrease in number of the lymphocytes. This indicates that some drastic physiological changes are progressing in the body.

According to Ellis (13) all lymphocytes in the blood and tissues of plaice including the thymus carry immunoglobulin on their surface. Therefore, it is thought that the decrease in number of the lymphocytes in thymi of chum and masu salmons indicates the reduction in antibody producing cells or their precursors. This suggests that increased susceptibility to disease occurs in these fishes.

Mammalian studies have indicated that adrenocorticosteroids regulate the synthesis or secretion of pituitary adrenocorticotropin (ACTH) (14, 15). It is already known that 17-hydroxycorticosteroids increase in the plasma of maturing and spawned Pacific salmon (16). Jalabert (17) had shown that cortisol, cortisone and testosterone increase the effectiveness of gonadotropin on intrafollicular maturation *in vitro* of the oocytes of trout. Estradiol benzoate had almost no effect on it. In catfish, gonadotropin exhibits a much greater adrenocorticotropic effect than ACTH (18). An inhibitory effect of estrogen on the interrenal tissue in eel was reported by Hanke and Chester Jones (19). It was also reported in the gonadal development in amphibians by Follet *et al.* (20) and in *Notemogonus crysoleucas* fish by De Vlaming (21). Moreover, it is known that administration of estrogen in humans causes the increased production of corticosteroid binding globulin or transcortin. The protein bound corticosteroids are inactive, whereas the free form of corticosteroid remain physiologically active (22). In the rat the corticosteroid binding globulin concentration increases after adrenalectomy and hypophysectomy (23). A striking rise in the cortisol binding capacity of human plasma during pregnancy or estrogen treatment was observed by Sandberg and Slaunhite (24). Accordingly, it is suggested that the treatment of estradiol benzoate in masu salmon produces lower secretions of gonadotropin (GTH) or ACTH from the pituitary gland, and subsequently reduces the secretion of corticosteroid hormones. It is also thought that estradiol benzoate injection induces the production of corticosteroid binding proteins or the rise in cortisol binding capacity.

Estrogen (estradiol benzoate) showed a marked effect on the prevention of thymic involution in the maturing group (Exp. II) as compared with the sexually immature group (Exp. I). The reasons of varying effects of estrogen on thymic involution of sexually immature fish were believed to be:

1) the absence of enough endogenous estrogen due to immaturity and thus the exogenous estrogen stimulated the secretion of corticosteroid hormones from the endocrine organs in some fish, or

2) the impairment to the secretion of ACTH or GTH from the pituitary due to insensitivity of this organ to estrogen.

The depletion of lymphocytes from the thymus and the fungal development on the body surface were noticed in dead fish of the cortisol acetate treated groups of both sexually immature and maturing stages (Table 1). The sexually immature testosterone treated group showed no conspicuous thymic involution when compared with that of the cortisol acetate and estradiol benzoate treated fish of the same group. Moreover, the maturing group exhibited a thymic inolvution which was more or less similar to the control group (Table 1). As it is already known that testosterone is effective when used at a higher dose in trout (17), it is thought that the present dose is on the lower side. It seems that some higher dose is necessary to get a pronounced effect of thymic involution by testosterone.

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