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Effects of Steroid Hormones on Spawning Death and Endocrine Functions in Masu Salmon

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Summary

An attempt was made to investigate the effects of steroid hormones (estradiol benzoate, cortisol acetate and testosterone) on the changes in activities of 3β- and 17β-hydroxysteroid dehydrogenases (3β-DH and 17β-DH) and glucose-6-phosphate dehydrogenase (G-6-PDH) of several endocrine organs of maturing masu salmon, *Oncorhynchus masou*. The activities of 3β-DH and G-6-PDH in the interrenal organ, testis and ovary were reduced by estradiol benzoate treatment. The effect of this hormone was more pronounced in the change of the activity of 3β-DH than in that of the activity of G-6-PDH. The activity of 17β-DH was very low in the liver of estradiol benzoate-treated and saline-treated fish. However, from a histological study it was clear that estradiol benzoate had a stimulatory effect on the liver cells of early maturing fish. The estrogen increased the weight of the liver during early maturation. On the other hand cortisol acetate and testosterone had no pronounced effect on the activity changes in dehydrogenases. These two hormones accelerated the degenerative changes in the lymphoid organs and liver at full maturity.

Estradiol benzoate could induce 80% mortality in the sexually immature group and 30% mortality in the early maturing group, whereas saline treatment induced 2.5% in the former group and 70% in the latter group. Testosterone caused 32% and 70% mortalities in the above groups, whereas cortisol acetate treatment resulted in 100% mortality in both the sexually immature and early maturing groups at the end of this experiment.

These results suggest that, among the steroid hormones treated, exogenous estrogens inhibit thymic involution which may be connected with the spawning death. The mechanism of spawning death is discussed.

Robertson and Wexler (1) observed that the sexually mature fishes of blueback salmon, *Oncorhynchus nerka nerka*, and kokanee salmon, *Oncorhynchus nerka kennerlyi*, exhibited extensive histological alterations in various tissues and organs. Robertson *et al.* (2) observed also progressive hyperplasia of the adrenocortical tissues of the Pacific salmon, *Oncorhynchus tshawytscha* during its migration. This

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hyperplasia was accompanied by a marked increase in concentration of 17-hydroxy-corticosteroids (17-OHCS) in the blood and pronounced anatomical changes. The physiological changes generally started to develop during migration when full sexual maturity was not attained. Progressing slowly these changes reached their maximum at the time of full maturation. At full maturity the adrenocortical cells were in various stages of degeneration, yet the level of plasma 17-OHCS remained high during the spawning and post spawning periods (2). It seemed probable that the catabolic action of higher levels of plasma 17-OHCS for a prolonged period, in combination with the stress of starvation, migration and spawning, causes the deterioration of the internal organs at the end of their life cycle (3).

The situation in the case of migrating Atlantic salmon was different from that of non-migratory rainbow trout. The latter mostly survived after spawning, suffering only partial degeneration of the interrenal tissue, whereas the former suffered complete degeneration and the majority died within a few weeks after spawning (3). Sundararaj and Goswami (5) showed that corticosteroids were effective in inducing oocyte maturation in catfish. Hirose (6) observed that both cortisol and progesterone took part in oocyte maturation in medaka, *Oryzias latipes*. Fostier and Breton (7) assumed that these corticosteroids were probably produced mainly by the interrenal organ. Sufi *et al.* (8) had revealed that the activities of 3ß-DH and G-6-PDH increased in the interrenal organ of masu and chum salmon during maturation. The histological study of the interrenal organ of these fishes supported this histochemical observation (9, 10). The thymus showed an involution in intact or adrenalectomized animals on administration of corticosteroids (11). Corticosteroids might also produce their physiological effects by regulating the level or the activity of specified enzymes (12).

All of the above findings in different types of fishes and higher mammals led us to investigate the relation between the function of the endocrine organs and the first spawning death of masu salmon. Since masu salmon are land-locked fish which are not under the stress of migration and complete starvation (because they eat occasionally during maturation), it is thought that the changed endocrine functions at the time of spawning are not related to the influence of the stress of migration but mainly to gonadal maturation and partly to starvation. Both chum and masu salmon meet their final spawning death, although a difference related to stress is observed between them.

Materials and Methods

Masu salmon were collected from Iwate Prefectural Trout Culture Station, Matsuo, Iwate Prefecture, Japan. The average body weight varied from 105 g to 135.5 g and fork length from 19.5 cm to 21 cm. The gonadal weight of these fish was recorded to estimate the reproductive status of each group. The fish collected

TABLE 1. *Steroid Hormone Treatments in Masu Salmon*

Treatment No.	Time of injection	Group	No. of fish	Hormone treatment	Weekly dose per g wt.		Total dose received per g wt.	
					(μg)	(μl)	(μg)	(μl)
I	Middle of July, *a	Sexually Immature	58	Saline		5.1		10.2
			58	Cortisol acetate	51		102	
		Testis: transparent	58	Testosterone	51		102	
		Ovary: white	58	Estradiol benzoate	51		102	
II	End of August, *b	Early Maturing	58	Saline		4.5		4.5
			58	Cortisol acetate	45		45	
		Testis: slightly white	58	Testosterone	45		45	
		Ovary: light pinkish	58	Estradiol benzoate	45		45	

*a Sampling: (1) at the end of July
(2) at the end of August
*b Sampling: (1) in the middle of September
(2) at the end of October

in the middle of July and at the end of August had an average gonadosomatic index of 0.5% and 2%, respectively. The former was classified as immature and the latter as at the stage of early maturation.

The head kidney, ovary, testis and liver were embedded in blocks of albumen and maintained on dry ice until sectioned. The frozen tissues were sectioned at 12 μm on a rotary freezing microtome maintained at –25°C. For histochemical visualization of the activities of 3β-DH, 17β-DH and G-6-PDH, the frozen sections were incubated in the incubation medium for 120, 120 and 60 minutes, respectively. The sections were then counterstained with Kernechtrot and mounted in Canada balsam after being dehydrated in a graded series of alcohol and xylene. Steroid substrates used were dehydroepiandrosterone, estradiol-17β and D-glucose-6-phosphate. Nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate were used as cofactors. A few sections were incubated in a medium lacking the steroid substrate as a control.

The schedule of hormone treatment is shown in Table 1. In the middle of July, 232 masu salmon, which had a mean body weight of 105 g and fork length of

19.5 cm, were divided into four groups of 58 fishes. The first group received 5.1 μl physiological saline per gram body weight once a week for two weeks. The second group received two intraperitoneal injections of 51 μg cortisol acetate, the third group 51 μg testosterone and the fourth group the same dose of 51 μg estradiol benzoate per gram body weight once a week for two weeks, respectively.

The second treatment was performed at the end of August. The average body weight was 135.2 g and fork length 21 cm. The number of groups and kinds of hormones were similar to the first treatment. The dose applied only once was 45 μg steroid per gram body weight of the fish. The control was treated with 4.5 μl physiological saline per gram body weight.

The preparation of the hormones and physiological saline was similar to the one mentioned in the thesis of Sufi (10). Injections were made intraperitoneally just anterior to the cloaca with a 2 ml syringe and No. 1 needle. After the injection, the fishes were treated with Furanace (Dainihon Pharmaceutical Co., Japan) and malachite green to prevent bacterial infection.

The reagents used for the experiment were as follows: testosterone, Fluka; nitro-blue tetrazolium (nitro-BT), Oriental Yeast Co.; dehydroepiandrosterone (DHA), estradiol benzoate, cortisol (hydrocortisone)-21-acetate, D-glucose-6-phosphate; nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), Sigma Chem. Co.

The weight of the gonad and liver was recorded at the time of the sampling of tissues for histological and histochemical observations. Gonadosomatic indices (GSI) and hepatosomatic indices (HSI) were calculated respectively. Head kidney, thymus and liver were used as histological materials. Tissues were fixed in Bouin and stained with haematoxylin and eosin. Mean nuclear diameter (ND) of the interrenal cell was determined by measuring mutually perpendicular diameters of 50 nuclei at random from four cell clusters. The measurement of the thickness of the thymus was made across the widest area of its serial transverse sections. Experimental results for the various groups of fish were expressed as mean±standard deviation. The intensity of the dehydrogenase reactions was judged microscopically and rated from − (negative) to ± (trace), + (low), ++ ~ +++ (medium), and +++ ~ ++++ (strong).

Results

A. NON-TREATED FISH

1. Histochemical Activity

The change in 3ß-DH activity was noticed in the different organs of non-treated masu salmon during various stages of maturation (8).

a) *Sexually immature fish*

The activity of 3ß-DH was found to be confined to the interrenal tissue only.

The ovary, testis and liver showed a negative reaction.

b) *Maturing fish*

3ß-DH activity was present in all the endocrine organs examined. In case of the interrenal organ, the activity was confined to interrenal cells; in the ovary to the theca cells; and in the testis to the interstitial cells.

c) *Mature fish*

A strong activity of 3ß-DH was obtained with DHA as substrate in the interrenal, ovary and testis when compared with the maturing fish. The location of activity was similar to the maturing group.

d) *Spawned fish*

The activity of 3ß-DH was lower in the different organs than in the mature

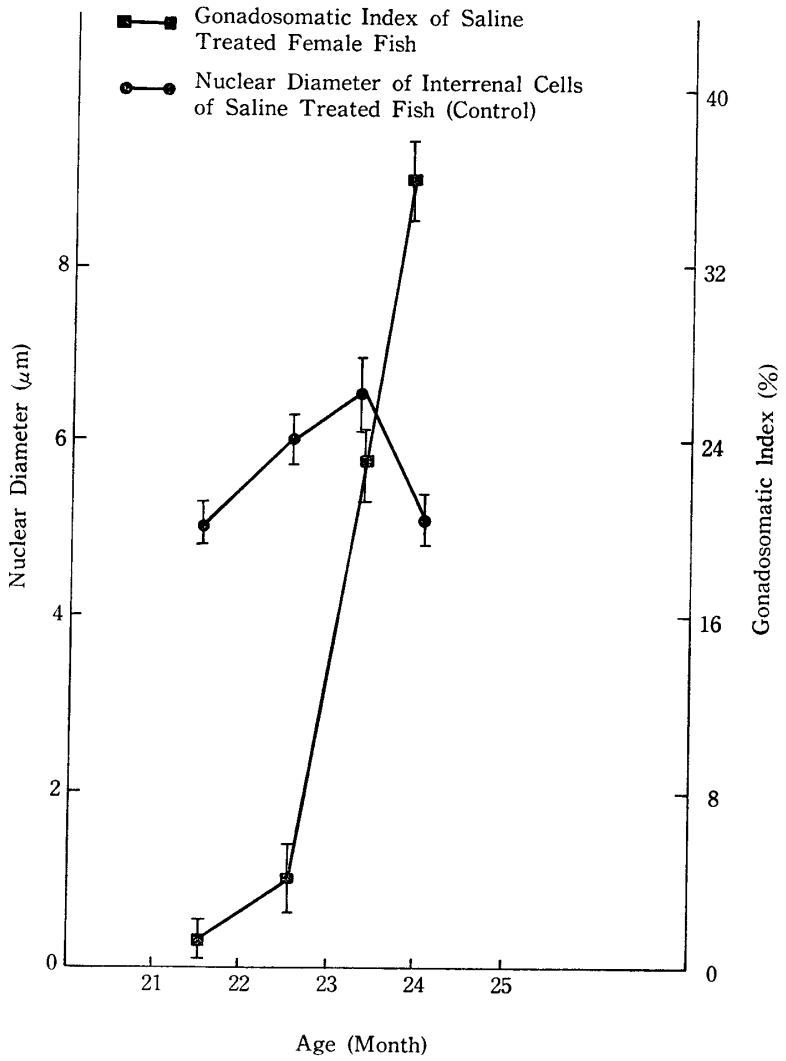

FIG. 1. Changes in the interrenal nuclear diameters and gonadosomatic indices of female masu salmon during various stages of its life. The circles and squares are the mean values, and vertical bars are the standard deviations.

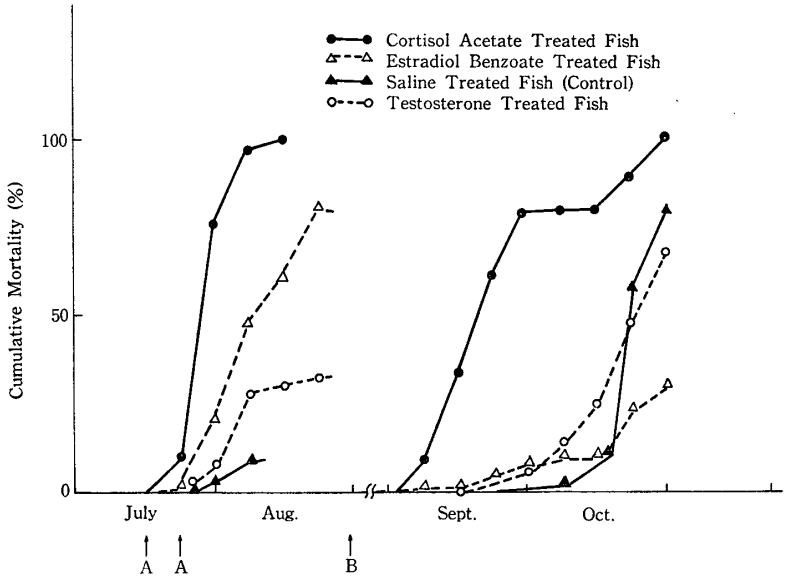

FIG. 2. Cumulative mortality curves of sexually immature and early maturing masu salmon after intraperitoneal injection of steroid hormones or saline. Arrows (A) indicate time when steroid hormone of 51 μg/g or saline of 5.1 μl/g were injected in hormone treated and saline treated group, respectively. Arrow (B) indicates time when steroid hormone of 45 μg/g or saline of 4.5 μl/g were injected in hormone treated and saline treated group, respectively.

TABLE 2. *Histochemical Reactions of Dehydrogenases Related to Steroidogenesis in Different Organs of Steroid Hormone Treated and Saline Treated Masu Salmon During Immature Stage (incubation time is 2 hours for 3β- and 17β-hydroxysteroid dehydrogenases and 1 hour for glucose-6-phosphate dehydrogenase at 37°C)*

Treatment	Dehydrogenase	Immature Stage				Early Maturing Stage	
		Interrenal tissue	Liver	Testis	Ovary	Interrenal tissue	Liver
Estradiol benzoate	3β-DH	++	−	−	−	++	−
	17β-DH	−	±～+	−	−	−	−
	G-6-PDH	+++	+++	±	±	+++	+
Testosterone	3β-DH	++	−	−	−	+++	−
	17β-DH	−	−	−	−	−	+
	G-6-PDH	+++	+++	±	+～±	++++	+
Cortisol acetate	3β-DH	++	−	−	−	+++	−
	17β-DH	−	−	−	−	−	−
	G-6-PDH	++++	++～+++	±	+	++++	++++
Saline	3β-DH	++	−	−	−	+++	−
	17β-DH	−	±～+	−	−	−	±
	G-6-PDH	++++	+++	+	+	++++	++

−: no reaction. ±: slight reaction. ++++: maximum reaction.
3β-DH=3β-hydroxysteroid dehydrogenase
17β-DH=17β-hydroxysteroid dehydrogenase
G-6-PDH=glucose-6-phosphate dehydrogenase

fish. The positive activity of this enzyme was detected in a few modified granulosa cells. In a few specimens, liver sections showed 3ß-DH activity.

2. Nuclear Diameter

A gradual increase in size of the ND of interrenal tissue was noticed from the maturing to the full mature stage with a decrease in ND of the same kind of cells in spawned fish (Fig. 1).

3. Gonadosomatic Index

An increase in GSI of non-treated masu salmon was observed (Fig. 1). A pronounced change in the weight of the gonad was found from the end of August to the end of October.

4. Mortality

The cumulative mortality rates of saline treated and steroid hormone treated fish were shown in Fig. 2. In the control fish, the mortality rate was 2.5% in the sexually immature group and 70% in the maturing one.

B. HORMONE-TREATED FISH

1. Histochemical Activity

The variation of staining intensity for 3ß-DH with different steroids was shown in Table 2. Estradiol benzoate treatment produced a definite inhibitory effect on the rate of reaction for 3ß-DH in the interrenal and ovary of maturing fish at the end of October (at full maturity). A lower inhibitory effect of the above hormone was observed in early maturing fish (Tables 2 and 3).

2. Nuclear Diameter and Gonadosomatic Index

A direct relationship was clearly observed between the increased ND and GSI of the non-treated fish in the early stage of gonadal development (Fig. 1). Fig. 3 clearly demonstrated the inhibitory effect of estradiol benzoate on the ND and GSI when compared with that of the control (Fig. 1). The effects of cortisol acetate (Fig. 4) and testosterone were not inhibitory on the above mentioned parameters.

3. Mortality

The rate of mortality by cortisol acetate was shown in Fig. 2. One hundred percent mortality was induced in masu salmon of both sexually immature and early maturing groups by cortisol acetate. The sexually immature group of testosterone treated fish had 32% mortality at the end of August and 70% mortality at the end of October (Fig. 2).

In the estradiol benzoate treated sexually immature fish, cumulative mortality rates of 3%, 22%, 48%, 62% and 80% were observed after the first, second, third,

TABLE 3. *Histochemical Reactions of Dehydrogenases Related to Steroidogenesis in Different Organs of Steroid Hormone Treated and Saline Treated Masu Salmon During Mature Stage (incubation time is 2 hours for 3β- and 17β-hydroxysteroid dehydrogenases and 1 hour for glucose-6-phosphate dehydrogenase at 37°C)*

Treatment	Dehydrogenase	Early Maturing Stage		Mature Stage			
		Testis	Ovary	Interrenal tissue	Liver	Testis	Ovary
Estradiol benzoate	3β-DH	+	+	++	−	±	±~+
	17β-DH	−	−	−	−	−	−
	G-6-PDH	+	++	+++	+	+	±~+
Testosterone	3β-DH	+	±	+++	−	++	++~+++
	17β-DH	−	−	−	±~+	−	−
	G-6-PDH	++	++	+++	+	+++	+++
Cortisol acetate	3β-DH	++	+~++	+++	−	+++	++~+++
	17β-DH	−	−	−	−	−	−
	G-6-PDH	+++	++	+++	++	+++	+++
Saline	3β-DH	++	+~++	+++	−	+++	+~+++
	17β-DH	−	−	−	−	−	−
	G-6-PDH	+++	+++	+++	+++	+++	+++

Expression of the intensity of the dehydrogenase reaction is the same as in Table 2.

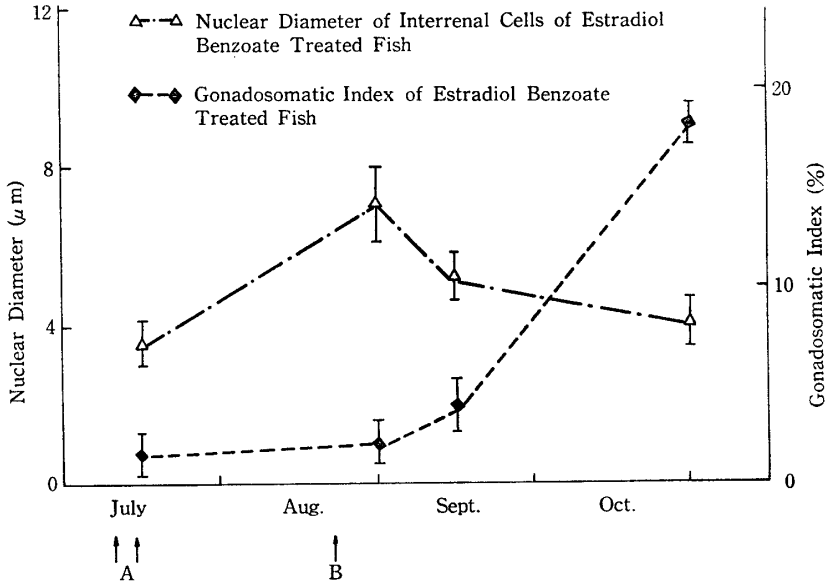

FIG. 3. Changes in interrenal nuclear diameter and gonadosomatic index of masu salmon after treatment with estradiol benzoate. Arrows (A) and (B) indicate the time when steroid hormone or saline were injected. The applied doses were the same as in Fig. 2. The triangles and the squares are the mean values and vertical bars denote the standard deviations.

fourth, and fifth week of injection, respectively. In the case of early maturing fish, the estradiol benzoate treated fish had the lowest mortality rate at 30% when compared with that of the control which was 70% (Fig. 2).

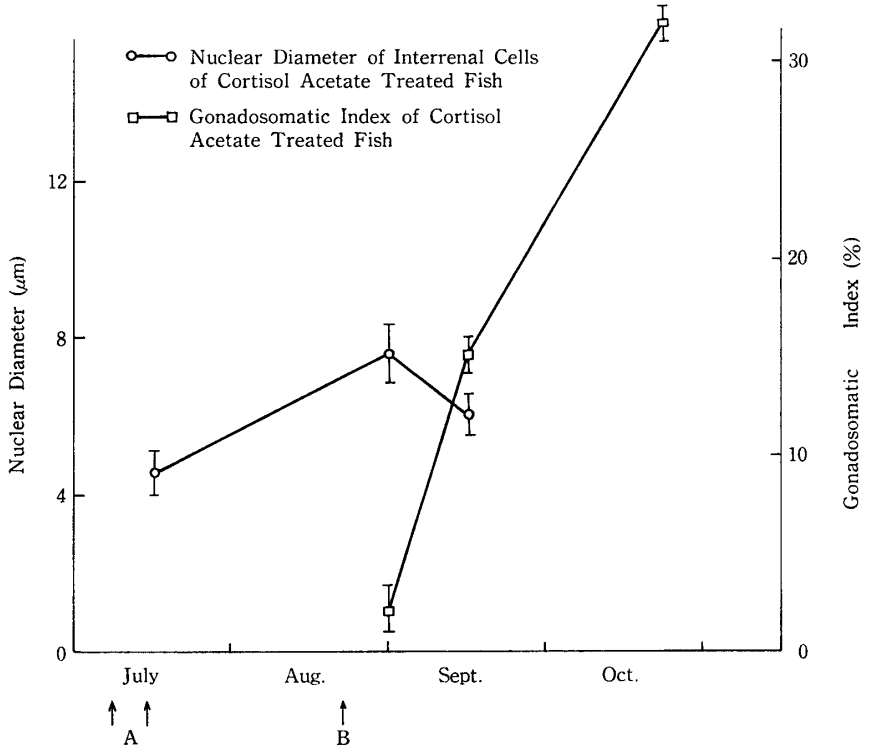

FIG. 4. Changes in interrenal nuclear diameter and gonadosomatic index of masu salmon after cortisol acetate treatment. Arrows (A) and (B) indicate injection time. The condition of injection was the same as in Fig. 2. The GSI of October group of cortisol acetate treated fish was recorded from the dead fish. Interrenal nuclear diameter could not be measured due to the death of all fish by that period. The circles and squares are the mean values and vertical bars denote the standard deviations.

4. Thymic Thickness

Table 4 showed the influence of corticosteroid and sex steroid hormones on the involution of the thymus during different stages of the maturing group. In the middle of September, the cortisol acetate treated group exhibited a distinct decrease of thymic thickness from 122 μm (female) and 102 μm (male), whereas the estradiol benzoate treated group had a thymic thickness of 398 μm for female and 349 μm for male. The thickness of thymus in the testosterone treated group was 290 μm for female and 265 μm for male. In the saline treated group (control) the thymus reduced further to 295 μm in the case of females and 234 μm in males. At the end of October, a further decrease in thymic thickness was observed in all groups of fishes.

In the middle of September, the cortisol acetate treated fish developed fungus-like patches on their bodies. Control and other steroid treated groups had no fungus patches on any part of their bodies (Table 4). However, at the end of October, the control as well as all hormone treated groups except estradiol benzoate showed severe fungus patches on the external surfaces of their bodies.

TABLE 4. *The Thymolytic Activity of Corticosteroid and Sex Steroid Hormones in Masu Salmon During Maturation*

Time of observation	No. of fish	Steroid injection	Total dose		Thymus thickness (mean μm±S.D.*)		Condition of fish
			(mg)	(ml)	female	male	
Middle of July	5	non-treated	—	—	580±58.2	530±38.6	No fungus
End of July	5	S		1	380±42.2	300±58.7	No fungus
	5	C	10		305±95.5	235±85.5	Slight fungus
	5	T	10		350±39.7	280±65.6	No fungus
	5	E	10		320±32.6	290±48.5	Fungus
Middle of September	5	S		0.5	295±48.8	234±55.3	No fungus
	5	C	5		122±17.8*2	102±25.5*2	Slight fungus
	5	T	5		290±52.5	265±69.1	No fungus
	5	E	5		398±39.2	349±12.5	No fungus
End of October	5	S		0.5	92±15.8	55±10.3	Severe fungus
	5	C	5		All dead by this time		Severe fungus
	5	T	5		75±12.6	105±22.9	Severe fungus
	5	E	5		165±25.2*2	202±85.3*2	No fungus or slight fungus

S: Saline C: Cortisol acetate
T: Testosterone E: Estradiol benzoate
* S.D.: standard deviation
*2: significantly different to the saline injected group ($p<0.005$)

5. Hepatosomatic Index

A general decreasing tendency was noticed in the liver weight at the time of spawning in both sexes (Fig. 5). Cortisol acetate and testosterone treatments did not inhibit the reduction of liver weight when compared with that of the control. However, an estradiol benzoate injection prevented masu salmon from losing the liver weight (Fig. 5).

6. Histology

The changes in the histological structure of the thymus, head kidney and liver of the non-treated, cortisol acetate and testosterone treated fishes revealed a heavy degeneration of the lymphocytes, vacuolation of the cytoplasm, and pycnosis of the nuclei at the time of full maturity. Estradiol benzoate treatment, however, induced an inhibitory effect on the above parameters and caused degeneration of lymphocytes from the thymus to a great extent, if not completely, at the same time (Plates 1 and 2).

Discussion

The results of Goswami and Sundararaj (13) in catfish and Jalabert (14) in trout suggested that maturation, ovulation and spawning can be modulated by steroid hormones. The highest 3ß-DH activity in the endocrine organs (interrenal

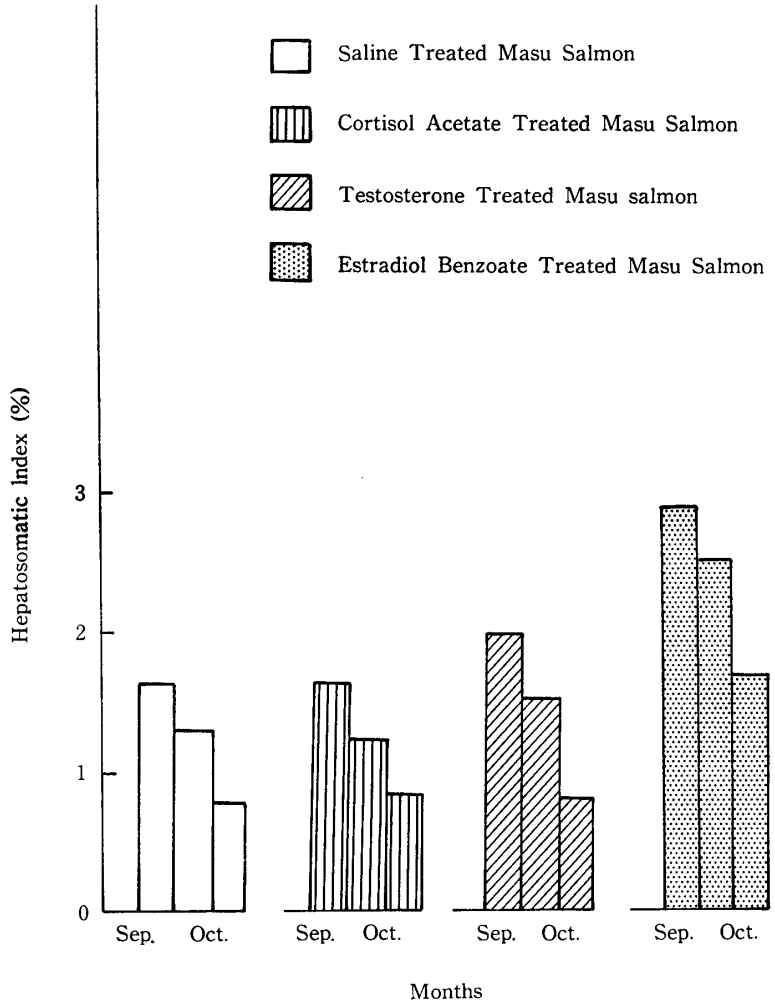

FIG. 5. Changes in the hepatosomatic index of maturing masu salmon after the intraperitoneal injection of steroid hormones at the end of August. The condition of injection is the same as in Fig. 2.

and gonad) of masu salmon at the time of full maturity (8) indicated the possibility of either a direct or indirect role of the endocrine organs over the process of maturation. From the results of hormonal treatment of masu salmon, it was observed that cortisol acetate had some stimulatory effect on the development of gonad (Fig. 4), although this effect was slightly lower than that of the control group (Fig. 1). It was therefore suggested that cortisol was one of the main hormones necessary for gonadal maturation, but not the only one. Participation of corticoids in ovulation had been reported in catfish (29) and medaka (30). Some other steroids might be necessary to maintain the normal gonadal growth rate as observed in the control fish. The latter idea agrees with the results of Fostier and Breton (7) in trout, in which they suggested that some steroids other than cortisol were necessary for the development of gonad. It is thought that the effect of progestagens should be tested in future study. The effect of testosterone

was not very clear, but it was observed that testosterone did not have much inhibitory effect on the weight gain of the maturing gonad. It seemed that the inhibitory effect of this hormone was lower than that of the estrogen.

In most cases, the estradiol benzoate injection induced some definite inhibitory effect on the weight gain of gonad and on the ND of the interrenal organ (Fig. 3). However, this hormone had a stimulatory effect on the weight gain of the liver during early period of maturation. Estradiol benzoate failed to induce a stimulatory effect on the maturation of gonad. It might be due to negative feedback control. This results was similar to the results of Jalabert (14) and Yamazaki (15) in trout or goldfish. The result of the stimulatory effect of this hormone on the weight of the liver during early maturation, indicated that the liver might take part in vitellogenesis (Fig. 5). This idea was based on the observation of Aida *et al.* (16) in *Gadus morrhua*. They showed that hypertrophied nuclei of the liver parenchymal cells were related to the synthesis of protein such as female specific protein. The histological study of the estradiol benzoate treated livers of maturing fish revealed both hyper- and hypoactive conditions of the parenchymal cells during the late period of maturation. The former condition was related to protein synthesis and the latter might be the result of enhanced yolk synthesis followed by a slight suppression of this process. This suppression of vitellogenesis might be due to the inhibitory effect of estradiol benzoate on the lower secretion of gonadotropin. Campbell and Idler (17) observed a light depression in levels of radioactive yolk in the liver of hypophysectomized fish, *Pleuronectes americanus*. The present data of the liver weight and histology of the liver of masu salmon

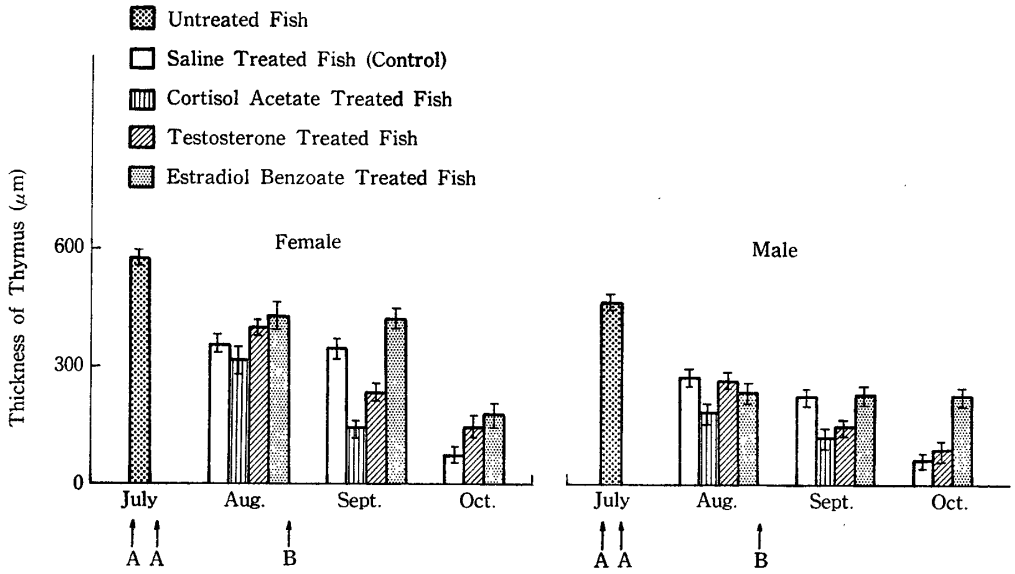

FIG. 6. Changes in the thickness of thymus following steroid or saline treatment in masu salmon of both sexes at the various reproductive stages. The doses of steroid and saline are the same as in Fig. 2. Arrows indicate injection time. The vertical bars are the standard deviations.

during maturation agree with the observations of Campbell and Idler (17) in winter flounder.

The application of estrogen prevented the reduction in the thickness of the thymus from September to October in both sexes (Fig. 6). Moreover, estradiol benzoate treatment in an early maturing fish group prevented the rate of mortality during its first spawning to a great extent (Fig. 2). The results of the prolongation of the life span in masu salmon were similar to the observations of Larsen (18) in lamprey. She succeeded in extending the life span of lamprey with estradiol-17ß treatment. According to her, the reason for the death of lamprey during spawning was not a simple consequence of sexual maturation, because lamprey were under the stress of migration, starvation and gonad maturation. However, in the case of masu salmon there was low or no stress from migration, because this fish was a landlocked fish and was provided with food throughout its life. They ate food occasionally during gonadal maturation. Therefore, it is thought that the stress of starvation was either absent or partial. Masu salmon face death during their first spawning as do chum salmon and lamprey, it was already known that stimuli such as genetical as well as environmental factors were operating in fish during maturation (19, 20). The observations of Frank *et al.* (21) and Dougherty (22) in rats, mice and human beings showed that the degeneration of lymphocytes from lymphoid organs was accompanied by increased secretion of adrenocortical hormones, inanition etc. The data of the enzymatic changes in rat thymus suggested that cortisol brings about its effect on protein catabolism by enhancing the transamination processes (23). The degenerative changes of the lymphoid organs (thymus and head kidney) in the sexually immature and maturing masu salmon might be the result of the chronic treatment with corticosteroids, and those in the spawned fish (control) might be due mainly to the endogenously secreted corticosteroid hormones and gonadal hormones during starvation and maturation.

On the basis of the previous information and hypotheses in different higher animals and the results of the present experiments, a hypothetic schematic diagram of the hormonal control of the internal organs in masu salmon has been proposed (10), and more simply modified in Fig. 7. As suggested before, in control maturing fish, the external as well as the internal factors (stress) induce higher secretion of corticosteroids. This results in the degeneration of the lymphoid organs and inactivation of the liver. The lymphocytes of the lymphoid organs are the seat for antibody production in fish (24). As the degeneration occurs in the lymphoid organs of masu salmon as well as in chum salmon (28), it is suggested that antibody producing cells are also destroyed. As a result resistance power also decreases to a great extent in these fishes. The degenerative changes of the lymphoid organs are reduced by the application of estradiol benzoate. This might be explained on the basis of lower secretion of adrenocorticotropin (ACTH) and

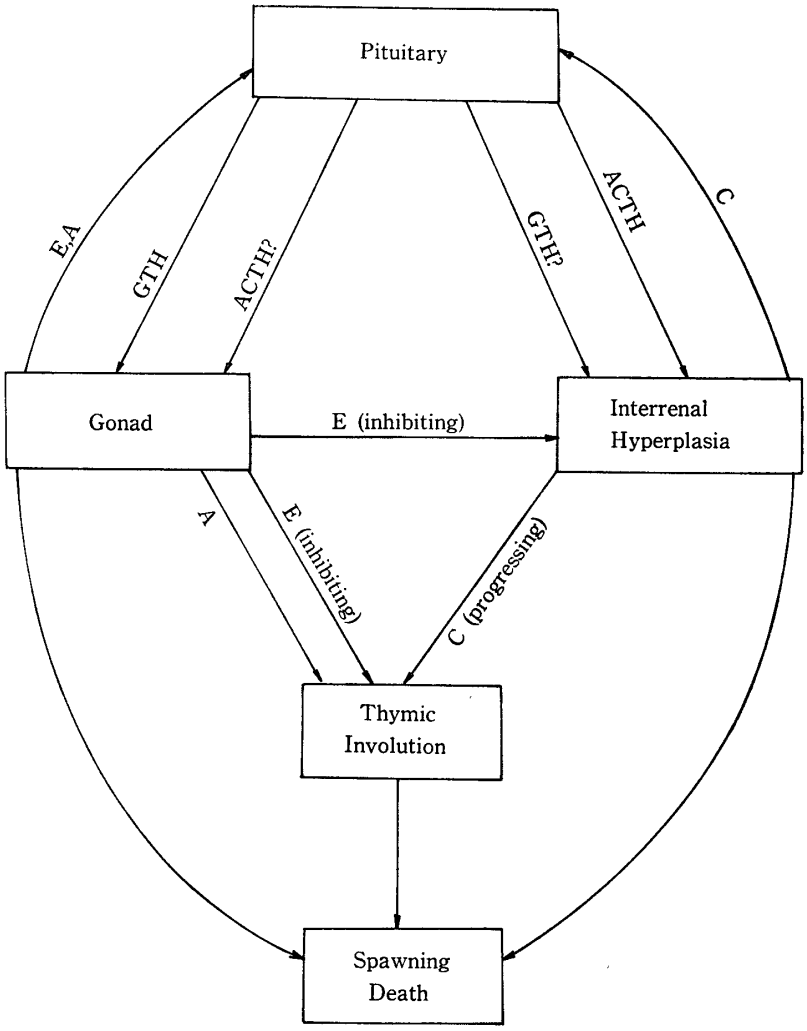

FIG. 7. A hypothetic scheme of the hormone control in the different internal organs of masu salmon towards spawning death. A: androgens, C: corticoids, E: estrogens

gonadotropin (GTH) from the pituitary gland. It has been known that glucocorticoids stimulate gluconeogenesis in the liver and other tissues and also prevent the rate of utilization of carbohydrates (25, 26). It seems that the lower secretion of ACTH and GTH not only reduces the rate of secretion of corticosteroids and sex steroids from the endocrine organs, but also prevents the development of any drastic change in the degeneration of lymphocytes. Moreover, the lower secretion of corticosteroid hormone fails to induce gluconeogenesis. This, in turn, prevents energy transformation from different parts of the body to the gonad. The lower secretion of sex steroids fails to stimulate sufficient production of female specific plasma protein. Thus, gonadal development is slow, in other words, the maturation process is going slower than that of the control. The presence of a sufficient number of lymphocytes (antibody producing cells) or their precursors might help the fish to tolerate the stress accompanied with changed environmental

factors (pathogenic invasion). This, in turn, causes reduction in the mortality rate.

The data on the mortality of corticosteroid treated masu salmon was consistent with the hypothesis of Robertson and Wexler (27) that high plasma corticosteroid in salmon was related to the death at the time of their first spawning. In this experiment, the estrogen treatment could reduce the rate of mortality (Fig. 2) and the increased activity of the dehydrogenases in endocrine organs of maturing masu salmon (Table 3). Since it is known that gonadal hormones have lower potency in degeneration of lymphocytes than that of the corticosteroids in rats (22), it is suggested that in masu salmon also exogenously applied estradiol benzoate failed to induce severe degeneration of lymphocytes and thus preserved greater tolerance against disease. Moreover, this hormone appears to prevent the higher rate of secretion of corticosteroid which could have caused degeneration of lymphocytes.

In addition to the idea that natural death may be postponed by treatment with estrogens (18, 31, 32), to which we consent, the authors stress here the significance of possible involvement of the thymus on the mechanism of spawning death in salmonid fishes.

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Explanation of Plates

Tissues of thymus, liver, and head kidney were fixed in Bouin's or Helly's fixative fluid and embedded in paraffin. Transverse sections (T.S.) of the tissues were treated with potassium iodate, then stained with haematoxylin and eosin (H.E.); HL: heavy degeneration of lymphocytes, T.L.: thymic layer, T: thymocytes, VC: vacuolated cytoplasm, NC: non-vacuolated cytoplasm, DL: degeneration of lymphocytes, HH: higher degeneration of haematopoietic tissue, LH: lower degeneration of haematopoietic tissue, N: necrosis, and DH: degeneration of haematopoietic tissue.

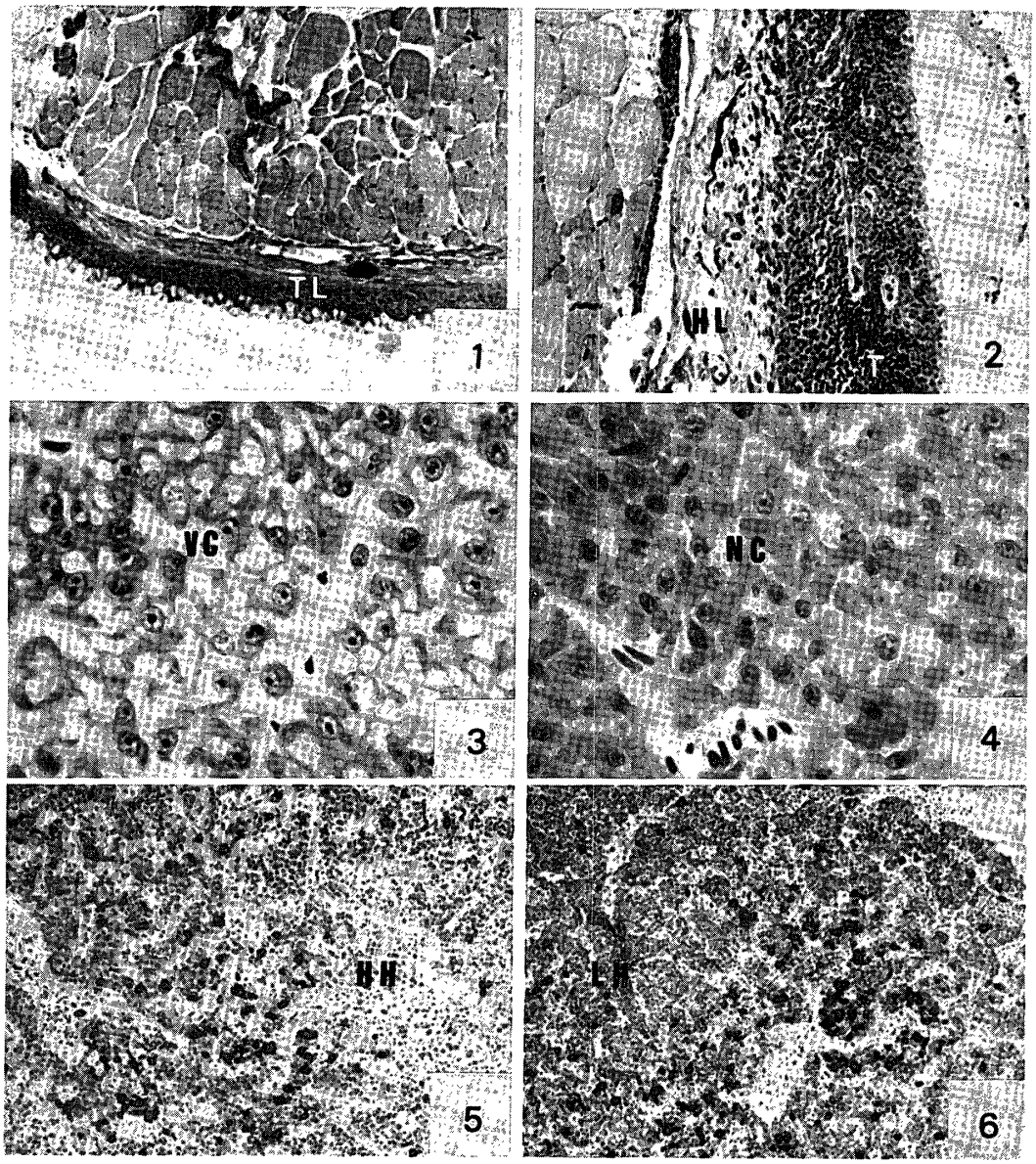

PLATE 1

1. T.S. of the thymus of saline treated spawned masu salmon showing heavy degeneration of lymphocytes (HL). Thymocytes (T) have shrunk to a narrow thymic layer (TL). H.E. stain. × 210
2. T.S. of the thymus of estradiol benzoate treated maturing masu salmon, showing lower degeneration of lymphocytes. H.E. stain. × 210
3. T.S. of the liver of saline treated spawned masu salmon, showing vacuolated cytoplasm (VC). H.E. stain. × 840
4. T.S. of the liver of estradiol benzoate treated maturing masu salmon, showing non-vacuolated cytoplasm (NC) and lower active hepatic nuclei. H.E. stain. × 840
5. T.S. of the head kidney of saline treated spawned masu salmon, showing heavy degeneration of haematopoietic tissue (HH). H.E. stain. × 210
6. T.S. of the head kidney of estradiol benzoate treated maturing masu salmon, showing lower degeneration of haematopoietic tissue (LH). H.E. stain. × 210

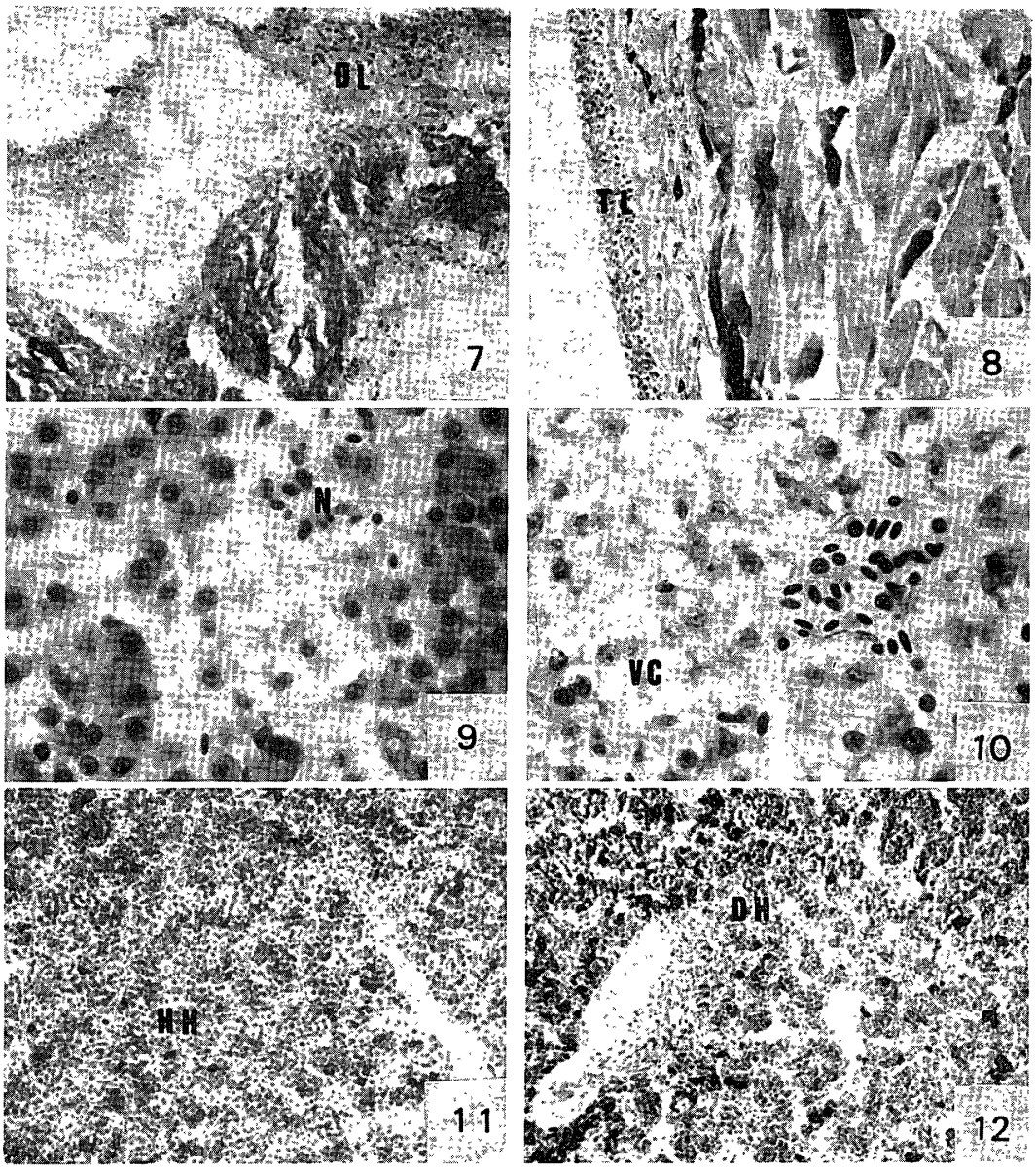

PLATE 2

7. T.S. of the thymus of cortisol acetate treated maturing masu salmon, showing degeneration of lymphocytes (DL) and vacuolation of cytoplasm. H.E. stain. × 210
8. T.S. of the thymus of testosterone treated mature masu salmon, showing heavy degeneration of the lymphocytes. Thymocytes (T) have shrunken to a narrow thymic layer (TL). H.E. stain. × 210
9. T.S. of the liver of cortisol acetate treated maturing masu salmon, showing necrosis (N). H.E. stain. × 840
10. T.S. of the liver of testosterone treated mature masu salmon, showing vacuolation of the cytoplasm (VC). H.E. stain. × 840
11. T.S. of the head kidney of the cortisol acetate treated maturing masu salmon, showing heavy degeneration of haematopoietic tissue (HH) and vacuolation of the cytoplasm. H.E. stain. × 210
12. T.S. of the head kidney of the testosterone treated mature masu salmon, showing degeneration of haematopoietic tissue (DH). H.E. stain. × 210