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Histochemical Changes in Activities of Dehydrogenases Related to Steroidogenesis in Salmonid Fishes (Genus *Oncorhynchus*) during Sexual Maturation and Spawning

Gulroo Begum SUFI*, Katsuyoshi MORI and Ryuhei SATO

Department of Fishery Science, Faculty of Agriculture, Tohoku University, Sendai, Japan

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Summary

Studies on 3β-hydroxysteroid dehydrogenase (3β-DH) and 17β-hydroxysteroid dehydrogenase (17β-DH) related to steroidogenesis were undertaken to ascertain the distribution of these enzymes and also the variation in their activities. The interrenal glands, gonads and livers of chum salmon, *Oncorhynchus keta*, and masu salmon, *Oncorhynchus masou*, were used in the present experiment. In the interrenal gland, the distribution of these enzymes was similar in both fishes. A gradual increase in the level of 3β-DH activity was observed in the interrenal tissue of chum salmon from the early to late period of sexual maturation, and a subsequent decline in the same activity was noticed in the spawned fish. Moreover, the rise in the 3β-DH activity in the interrenal gland seemed to be parallel to the increase in size of nuclear diameter of the interrenal tissue. A similar observation was obtained in masu salmon during sexual maturation and spawning. However, the intensity of 3β-DH activity in the interrenal gland was much lower in masu salmon than that of chum salmon.

The glucose-6-phosphate dehydrogenase (G-6-PDH) showed a distribution similar to that of 3β-DH, but gave a more intense reaction. In addition to the interrenal tissue, G-6-PDH activity was also found in the lymphopoietic tissue of the head kidney. No variation in the distribution of this enzyme in the interrenal tissue of these fishes was observed during maturation and spawning. However, this enzyme also showed a parallel variation to the activity of 3β-DH.

Besides the interrenal gland, the ovary and testis of chum salmon showed considerable 3β-DH and G-6-PDH activities. Although a tendency towards a gradual increase in these activities was observed in the gonads of chum salmon from the early to late period of sexual maturation, the intensity of activity in the gonads was very low as compared with that in the interrenal gland. The pattern of distribution of these enzymes in the gonads (ovary and testis) of masu salmon was almost the same as that of the chum salmon. However, the intensity of activity of these enzymes in the interrenals and gonads of masu salmon was much lower than that of the chum salmon during various stages of their life.

The activity for 17β-DH was not detected in any tested organs of maturing and spawned chum salmon except in the testis tubule of mature fish. On the other

* Present Address: Department of Zoology, Dacca University, Dacca-2, Bangladesh.

hand, a positive activity of this enzyme was observed in the liver and ovary of maturing masu salmon as well as in the liver of spawned fish. However, the testis and its tubule did not show any kind of activity for 17β-DH.

It is interesting to note that the activity of 3β-DH and G-6-PDH was reduced considerably in all the tested organs of the spawned fish of both chum and masu salmons. Moreover, 17β-DH also showed a slight reduction in the rate of its activity in the spawned liver of masu salmon.

The results of the present investigation indicated a close relationship in the three parameters, nuclear diameter, 3β-DH activity and G-6-PDH activity, in the interrenal glands of chum and masu salmons during sexual maturation.

The interrenal gland probably participates in certain stages of the reproductive function, and it is also assumed that the secretions of corticosteroids are activated in maturation, ovulation and spawning processes of various species (1–4). The fact that the luteinizing hormone promotes corticosteroidogenesis in the interrenal tissue of *Heteropneustus fossilis* has already been demonstrated (5). These observations indicate that maturation, ovulation and spawning can be modulated by certain steroidal hormones.

It is also known that the spawning migration of Pacific salmon is accompanied by morphological, physiological characteristics required in the transition from the sea to fresh water environment (6, 7). Hyperplasia as well as degeneration of the interrenal tissue of salmon were observed in the head kidney during the breeding season (8–10). The hyperplasia was accompanied by increased corticosteroid titers in the peripheral blood of migrating salmon during pre- and post-spawning periods (11, 12). The structural alterations of the interrenal gland and biochemical changes in the plasma of Pacific salmon, steelhead trout and sockeye salmon are supposed to occur under the stimuli of starvation and gonad maturation.

Considerable work has been done on plasma of sockeye salmon by Idler and his collaborators (13) who found cortisol, cortisone and traces of corticosterone in plasma of mature animals of both sexes. Other steroids, 17β-hydroxyprogesterone and 17β-, 20β-dihydroxyprogesterone, were also found in plasma of both male and female mature salmon. 11-Ketotestosterone isolated from male sockeye salmon has been demonstrated to have androgenic activity (14).

The application of enzyme-histochemical methods to the endocrinological study of fish organs has been infrequent.

Species variations in the activities of dehydrogenases related to steroidogenesis have been observed in the adrenal, ovary and testis of mammals. Conflicting results have been reported concerning the Δ^5-3β-hydroxysteroid dehydrogenase activity in the interrenal tissue of man, and these findings generally differ from the observations reported in rodents and other mammals (15). The composition of the incubation media as well as other experimental conditions vary in many of these reports, and comparative analysis of the results is therefore almost impossible. Similar situations regarding the presence or absence of a steroid dehydrogenase system in young stage salmon have also been reported (16, 17).

The present investigation is planned to characterize the steroid hormone producing tissue of Japanese main Pacific salmons.

Materials and Methods

The adult chum salmon (3–4 years old, average length 68 cm, and weight 3.36 kg) used in this study were obtained from Tsugaruishi Salmon Hatchery, Miyako City, Iwate Prefecture, Japan on January 14, 1977 and January 18, 1978. The juvenile chum salmon (10 months old, length 17.5 cm, weight 28.8 g) were collected from the Matsushima Aquarium, Matsushima Town, Miyagi Prefecture, Japan.

Masu salmon (1–2 years old, average length 15–22 cm, average weight 58–134 g) were secured from Niinomi Trout Culture Farm, Zao, Miyagi Prefecture, on July 25, 1977 and September 15, 1977.

Determination of Dehydrogenase Activity

The procedure and the substrates used for the demonstration of enzyme histochemical activity and the histological study were the same as described previously (16–18).

In the case of histochemical procedures, the incubations were terminated by fixation in 10% formalin at room temperature for 10–15 minutes. The tissue sections were then rinsed in distilled water, counter-stained with Kernechtrot, and mounted in Canada balsam after being dehydrated in graded series of alcohols.

1. *Hydroxysteroid dehydrogenase* (3β–, 17β-*DH*)

The 3β-DH is a nicotinamide-adenine dinucleotide (NAD) dependent dehydrogenase capable of oxidizing Δ^5-3β-hydroxysteroids to Δ^4-3-ketosteroids (19, 20). 3β-Hydroxy-5β-androstan-17-one and dehydroepiandrosterone were used as substrate for the demonstration of 3β-DH, testosterone propionate and estradiol-17β for 17β-DH enzyme reaction.

2. *Glucose-6-phosphate dehydrogenase* (*G-6-PDH*)

The substrate used for the demonstration of G-6PDH was D-glucose-6-phosphate (monosodium salt).

Control sections were incubated in a medium from which the substrates had been omitted.

The fresh tissue of the liver of gold fish *Carassius auratus* was also studied to evaluate the 17β-DH technique used by the present authors.

The intensity of dehydrogenase reactions as judged microscopically by the intensity of intracellular formazan granule deposition was rated from – or 0 (negative reaction) to +++++++ or 8 (maximum reaction).

PROCESSING OF TISSUE

For the histological study, the head kidney and liver were fixed in Helly's fixative fluid. The gonads were stained with Mayer's hematoxylin and eosin, following the procedure described in the previous publications (17, 18).

Mean nuclear diameters of the interrenal cells were determined by measuring mutually perpendicular diameters of 50 nuclei selected at random from 3–4 cluster of cells.

Results

A. HISTOCHEMICAL CHANGES

1. Dehydrogenase Activities in the Sexually Immature Salmon

a) 3β-DH

Interrenal glands: In chum salmon and also masu salmon, 3β-DH activity appeared to be strictly limited to the interrenal tissue (Fig. 1). Using the

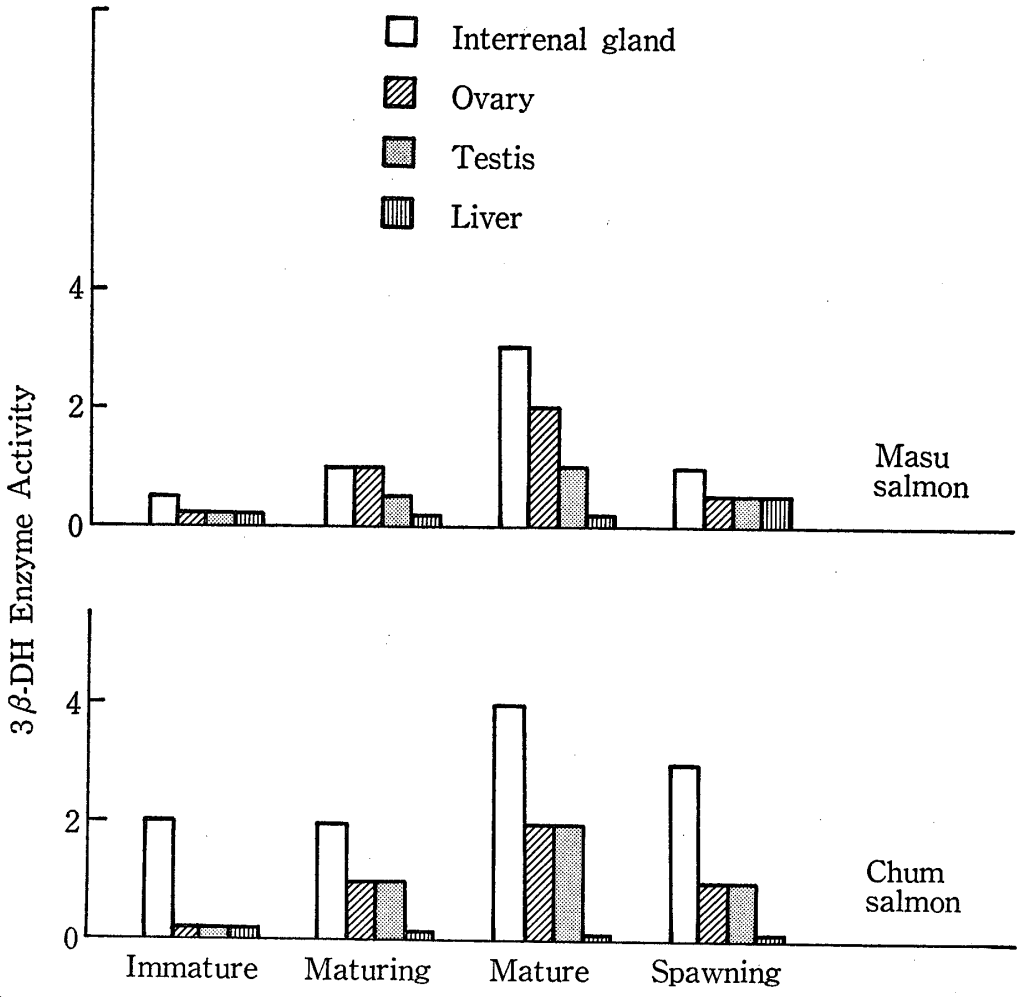

FIG. 1. Variations in 3β-hydroxysteroid dehydrogenase activity in the different organs of chum and masu salmons during various stages of life. Incubation time is 2 hours at 37°C with DHA and NAD.

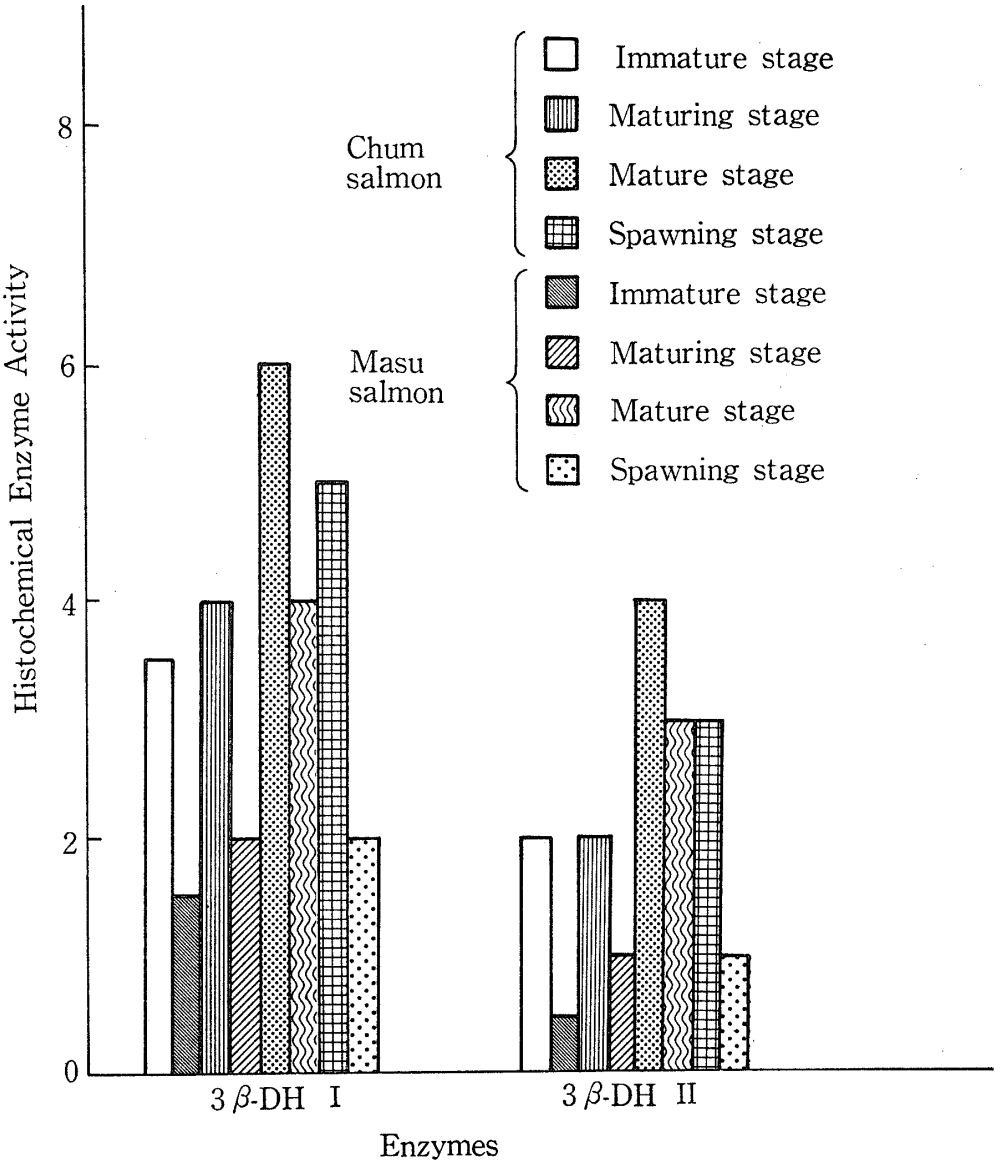

FIG. 2. A comparison of Δ^5-3β-hydroxysteroid dehydrogenase activity with different substrates in the interrenal glands of chum and masu salmons during various stages of life. Incubation time is 2 hours at 37°C with 3β-hydroxysteroids and NAD. 3β-DH I: 3β-hydroxy-5β-androstan-17-one, 3β-DH II: dehydroepiandrosterone. The intensity of reaction is graded from 0 to 8; 0 denotes the absence of reaction; 8: a maximal reaction.

substrates 3β-hydroxy-5β-androstan-17-one and dehydroepiandrosterone, the enzyme activity was found to be strong with the former and moderate with the latter (Fig. 2). There was no activity in other parts of the head kidney. The gland of male and female fish did not differ much in its rate of intensity of activity, although a difference in the intensity of activity was found in these two species (Fig. 2). Almost no 3β-DH activity was observed in the ovaries, testes and livers of either species of fishes (Fig. 1).

b) 17β-DH

Interrenal glands: A completely negative reaction with testosterone propionate and estradiol-17β was obtained in the interrenal of both species.

There were no reactions of 17β-DH in the ovaries, testes and livers of chum and masu salmons (Fig. 3).

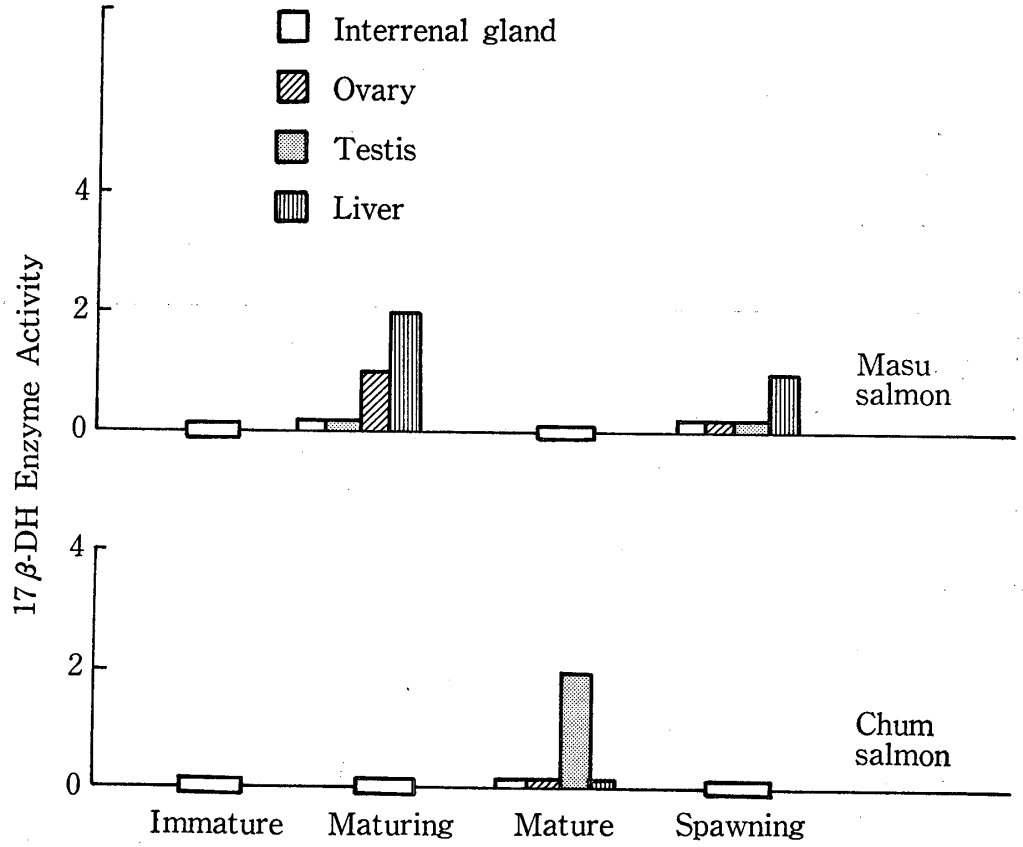

FIG. 3. Variations in 17β-hydroxysteroid dehydrogenase activity in different organs of chum and masu salmons during various stages of life. Incubation time is 2 hours at 37°C with testosterone or 17β-estradiol and NAD.

c) G-6-PDH

Interrenal glands: The reaction of NADP dependent G–6–PDH was observed in the interrenal tissue of both chum and masu salmons, but a variation in the rate of activity was noticed in these species (Fig. 4). The lymphoid tissue of the head kidney also showed a positive reaction for this enzyme which is, however, insignificant as compared with the interrenal tissue.

Ovaries: Activity of G–6–PDH was restricted to the granulosa cell and theca cell layers. Although the pattern of distribution of activity was similar in masu salmon and chum salmon, the intensity of activity in the two species of fishes was different (Fig. 4). The distribution of G–6–PDH activity in the granulosa cells was the same as that of 3β-DH enzyme activity in both species of fishes, although the former showed a more intense reaction.

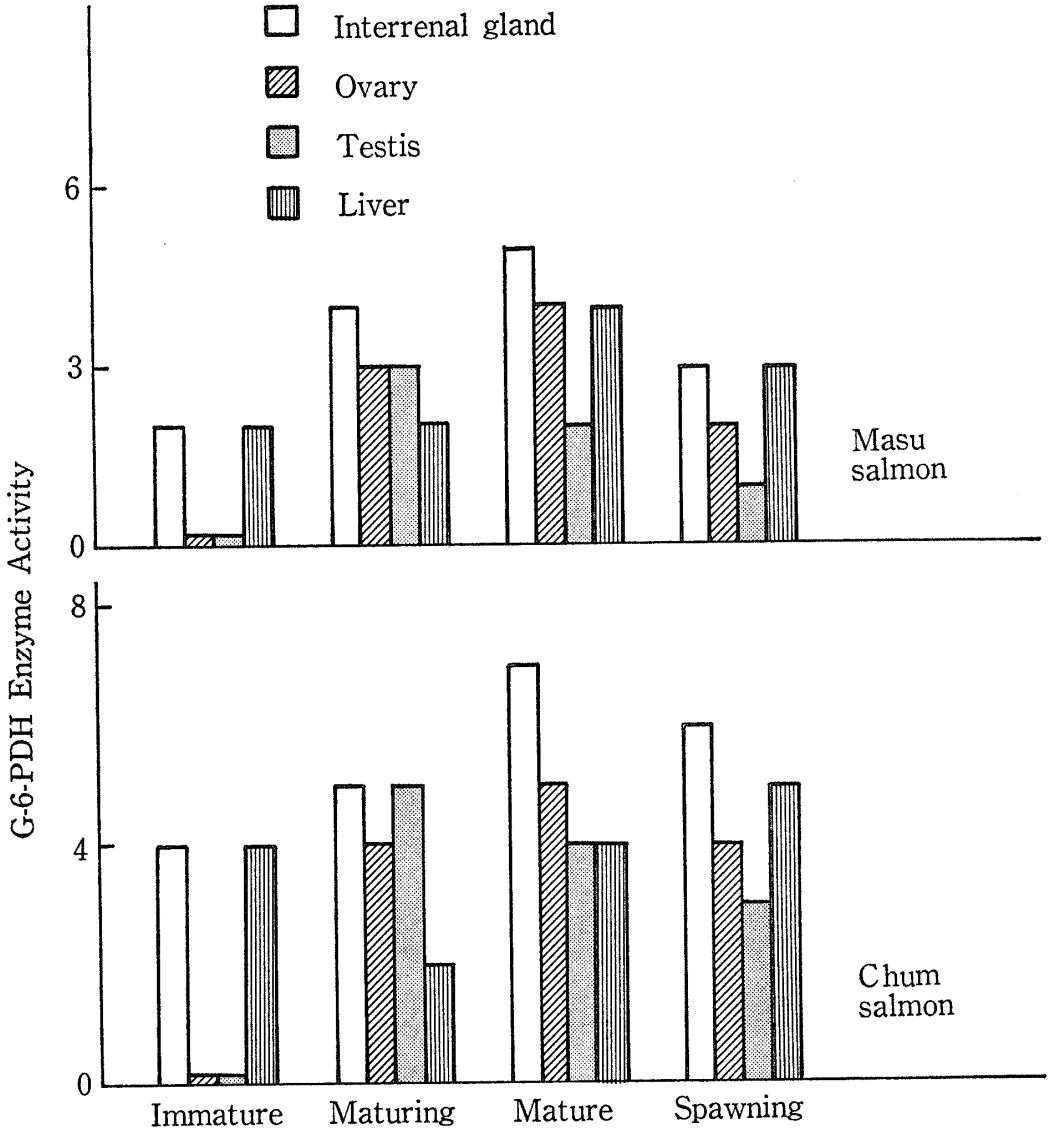

FIG. 4. Variations in glucose-6-phosphate dehydrogenase activity in the different organs of chum and masu salmons during various stages of life. Incubation time: 1 hour at 37°C with glucose-6-phosphate.

2. Dehydrogenase Activities during Maturation

a) 3β-DH

Interrenal glands: In maturing chum salmon and masu salmon, 3β-DH activity appeared to be restricted to the interrenal tissue. The variability of intensity of this enzyme in chum and masu salmons is shown in Fig. 1.

Ovaries: Enzymatic activities were far more variable in location of the ovary than in the cases of interrenal gland and testis. The ovaries of chum salmon during maturation showed activity of 3β-DH in the theca and granulosa cells of follicles (Plate 1, Fig. 1) and also at the periphery of oocytes. Although the pattern of distribution of 3β-DH was similar to that of masu salmon, the

overall reactivity seemed slightly lower in masu salmon (Fig. 1).

Testes: In chum salmon, the activity of 3β-DH was found only in the interstitial cells (Plate 1, Fig. 2). The reaction was moderate with 3β-hydroxy-5β-androstan-17-one, but weak with dehydroepiandrosterone substrate. In masu salmon, the location of activity of this enzyme was similar to chum salmon. However, the intensity of activity was comparatively less than that of chum salmon (Fig. 1). This low activity appears to be due to the presence of a small number of interstitial cells in the lobule boundary.

In the maturing fish of masu salmon the relative volume of interstitial cells in the testis was smaller, and hence the overall enzyme reaction seemed to be weaker.

No reaction of 3β-DH enzyme was obtained in other parts of testis and livers of the experimental fish.

b) 17β-DH

Interrenal glands: No activity of this enzyme occurred in the interrenal gland of both species (Fig. 3).

Ovaries: No reaction was obtained when testosterone propionate or estradiol-17β was used as substrate in the ovary of chum salmon. The masu salmon ovary (several fish), however, showed a moderate reaction with these substrates in the follicle cell (Plate 1, Fig. 3) layer as well as at the periphery of the oocytes. No reaction was found in any other parts of masu salmon ovary. The reaction for 17β-DH was of equal magnitude and sensitivity in both kinds of substrates used in the present experiment.

Testes: A negative reaction for 17β-DH enzyme was observed not only in the testis tubule of chum salmon but also in other part of testis.

Livers: The 17β-DH enzyme did not show any trace of activity in the parenchymal tissue of the liver of chum salmon. However, a positive reaction of this enzyme was obtained in the same organ of masu salmon (Plate 1, Fig. 4).

c) G-6-PDH

Interrenal glands: Although a very strong reaction of glucose-6-PDH was obtained in the interrenal tissue of both chum and masu salmons, a slight variation in the intensity of activity was noticed in these two species (Fig. 4). A positive reaction was also found in the lymphopoietic tissue of the head kidney of both species of fishes.

Ovaries: A strong reaction of G-6-PDH was observed in the granulosa and also at the periphery of oocytes, but a weak reaction occurred in the theca of the follicle and the main body of the oocytes. A difference in the intensity was observed between masu salmon and chum salmon (Fig. 4), although a similar pattern of distribution was found.

Testes: Only the interstitial cells of the testis of chum salmon showed a strong activity of G-6-PDH. In masu salmon also, the same kind of tissue

reacted with glucose-6-phosphate, but the degree of activity of this enzyme was lesser to that of chum salmon (Fig. 4).

Livers: A reaction for G–6–PDH was noticed in the liver of both chum and masu salmons. However, a great variation in the intensity of activity of this enzyme was present in the tested animals (Fig. 4).

3. Dehydrogenase Activities at Full Maturity

a) 3β-DH

Interrenal glands: The variation in the intensity of activity of 3β-DH with different substrates was almost the same in both species of fishes. A very strong activity was obtained with 3β-hydroxy-5β-androstan-17-one whereas a comparatively low, but strong activity was also detected while using dehydroepiandrosterone as a substrate (Plate 2, Figs. 5 and 6). These activities were confined only in the interrenal cells (Fig. 1).

Ovaries: A strong activity of 3β-DH was observed in the granulosa as well as in the theca of follicles in chum salmon. The pattern of distribution of this enzyme was almost the same in both fish but masu salmon showed a comparatively low level of activity under microscopic observation (Fig. 2).

Testes: The activity of 3β-DH both in the interstitial cells of testis lobule and testis tubule in chum salmon was strong to moderate. A strong activity was found in the interstitial tissue of the testis lobule in masu salmon whereas a weak activity was observed in the testis tubule. However, this enzymatic activity of the testis tubule was observed only in very few number of specimens of chum and masu salmons.

Livers: No positive activity of 3β-DH was noticed in any case.

b) 17β–DH

Interrenal glands, ovaries and livers: Histochemically these organs were tested for 17β-DH. A negative reaction was found in all cases.

Testes: A moderate activity of 17β-DH was observed only in the wall of testis tubule of chum salmon (Plate 2, Fig. 7), but an almost complete negative reaction was found in the testis and also in the testis tubule of masu salmon.

c) G–6–PDH

Interrenal glands: A strong positive activity of G–6–PDH occurred in the interrenal tissue and a weak activity in the hematopoietic tissue of both fishes. However, the degree of intensity of this activity was much lower in masu salmon than that of the chum salmon (Fig. 4).

Ovaries: In a mature ovary, the G–6–PDH activity occurred mainly in the granulosa and the theca cells. However, the periphery of the oocytes and also the main body showed this enzyme activity. The theca of the oocytes reacted

weakly, but the granulosa and the periphery of the oocytes reacted strongly in both fishes. A slight variation in the level activity obtained in these species are shown in Fig. 4.

Testes: The activity of G–6–PDH was very strong in the interstitial cells of testis in chum salmon. A strong reaction for this enzyme was also found in the wall of testis tubule. In masu salmon, the pattern of this reaction was the same as that of chum salmon, although a less intensive activity was observed in the same organ of masu salmon (Fig. 4).

Livers: A strong G–6–PDH enzymatic activity was observed with G–6–P in both fishes.

4. Dehydrogenase Activities after Natural Spawning

a) 3β–DH

Interrenal glands: Interrenal cells of the spent masu salmon showed reaction with all the steroids used such as 3β-hydroxy-5β-androstan-17-one and dehydroepiandrosterone. Although a moderate activity was obtained in few sections, but mostly a trace activity was observed with dehydroepiandrosterone for the localization of 3β-DH. The interrenal cells showed very low activity in comparison with that of mature fish (Plate 2, Fig. 8). This activity in the interrenal of spent chum salmon was much stronger than that in the masu salmon.

Ovaries: A positive activity of 3β-DH was detected in very small patches of modified granulosa cells or single cell (seemed to be interstitial cells) after an usual incubation period of 2 hours (Plate 2, Fig. 9).

Testes: In spent testis of masu salmon, a weak activity of 3β–DH was found in the interstitial cells. In the present study, the relative staining with different 3β-hydroxysteroids was the same although the overall reactions with dehydroepiandrosterone was slightly less than that of 3β-hydroxy-5β-androstan-17-one. No spent testes of chum salmon were studied.

Livers: No activity was observed in both species of fishes.

b) 17β–DH

In case of masu salmon, interrenal, testis and ovary failed to show any activity of this enzyme. A weak activity was found, however, in the liver of a few female fish. On the other hand, a total absence of this enzyme was noticed in case of chum salmon.

c) G–6–PDH

Interrenal gland: A moderately strong G–6–PDH enzyme activity in the interrenal tissue and a weak activity in the lymphopoietic tissue of head kidney were observed in both species of fishes.

Ovaries: The pattern of G–6–PDH in the spent ovary of masu salmon was

very much similar to that of the 3β–DH enzyme, but the degree of intensity was slightly stronger in case of the former enzyme. A few young oocytes also showed activity for G–6–PDH. However, the rate of activity of this enzyme was stronger in chum salmon than in masu salmon.

Testes: This activity was confined to a few number of interstitial cells. The intensity of activity was very weak in masu salmon. No observation was made with chum salmon.

Livers: Like interrenal gland, the liver of masu and chum salmon also reacted strongly to G–6–P substrate.

B. Histological Evaluation

1. Structural Changes of the Interrenal Gland of Sexually Immature Salmon

a) Juvenile stage of chum salmon

The interrenal cells occurred in small elongated clumps adjacent to or surrounding the vein and venules. These clumps were 2–3 cells in thickness. The cells were characterized by a round nucleus. Most of the nuclei contained two or three nucleoli which were found near the nuclear membrane. The average size of the nuclei was about 5.5 μm (Fig. 5).

b) Sexually immature stage of masu salmon

The interrenal cells were few in number which stained with acid dyes and contained large round or elliptical nuclei. The size of nuclear diameter was about 4.7 μm (Fig. 5).

c) Maturing stage of chum salmon

The number of interrenal tissue was more than that of the immature stage of fish. A few number of nucleoli were found in the nucleus. Small patches of interrenal cells were found to be scattered in the hematopoietic tissue, near the branches of cardinal veins and also around the main vein. The mean diameter of these nuclei was 6.9 μm (Fig. 5).

d) Maturing stage of masu salmon

There was an increase in number of the interrenal tissue during this stage of fish. Although the interrenal cells and chromaffin cells were found to be present separately, a close arrangement between these two types of cells were observed along the cardinal veins and their branches. The nuclear diameter was about 5.9 μm (Fig. 5).

e) Sexually mature stage of chum salmon

A marked hyperplasia of the interrenal tissue was observable in all the specimens studied. The volume of this tissue is many times larger than that present in the maturing stage of this fish. The mean diameter of the nuclei was 8.1 μm. The

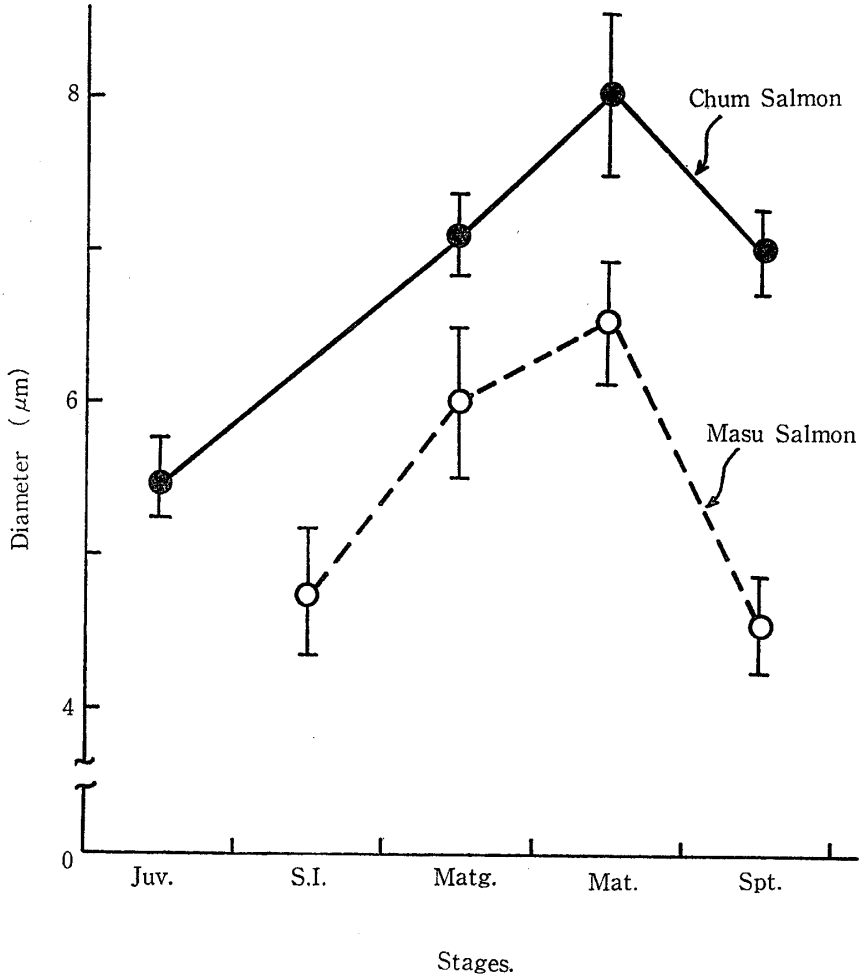

FIG. 5. Changes in the nuclear diameters of the interrenal cells of chum and masu salmons during various stages of their life-cycle. The circles are the mean values and the vertical lines are the standard deviations. Juv.: juvenile; S.I.: sexually immature; Matg.: maturing; Mat.; mature; Spt.: spent.

activity of the tissue was indicated by the presence of large spherical nuclei, frothy and granular cytoplasm and group-cell arrangement (Fig. 5).

f) *Sexually mature stage of masu salmon*

At full maturity, the vascularity of the interrenal tissue had developed along the development of sinusoid structures. The hyperplasia of these cells was very prominent. The mean diameter of the nuclei was about 6.5 μm (Fig. 5).

g) *Spawned stage of chum salmon*

In spawned fish, the process of degeneration was initiated by the vascularization of the interrenal tissue and the development of edema. The cytoplasm of these cells appeared to be vacuolated. In the hematopoietic tissue, large hemorrhages were observed. The mean size of the nuclei was 6.9 μm (Fig. 5).

h) Spawned stage of masu salmon

A degenerative stage of the interrenal gland was noticed. Vascularization and vacuolization were the main characteristic of this organ after spawning. The nuclear diameters showed a marked decrease as compared with the mature fish. The mean value of nuclear diameters was 4.5 μm (Fig. 5).

Discussion

The activities of 3β-DH and G–6–PDH in the interrenal gland of chum salmon were stronger as denoted by the extremely dense staining at the time of full maturity than at the period of early maturation (Plate 3, Figs. 10–13). Along with the rise in the level of activities of these enzymes, nuclei and nucleoli of the interrenal tissue were found in hypertrophy and the cytoplasm was in hyperactive conditions. The active state of the interrenal tissue (secretion of steroid hormones) seems to be related to the influence of physiological stress caused by different environmental factors (external and internal). This idea is deduced from the fact that with the approach of spawning phase, a rise in the level of activities of 3β-DH and G–6–PDH in the interrenal tissue was found in accord with the change in size of nuclei (Figs. 6 and 7), as well as with the volume of cytoplasm. On the other hand, the activities of these enzymes decline to a considerable degree in the interrenal of spawned fish in both species. The nuclear diameter of the interrenal tissue (Plate 3, Figs. 14 and 15) also showed a parallel correlation to the decreased activity (Figs. 6 and 7) of these enzymes. The vacuolation of the nucleus indicates the inactive stage of the cell (Plate 3, Fig. 16). Since it is known from the results of Goswami and Sundararaj (20) and also Jalabert (21), that maturation, ovulation, and spawning can be modulated by hormonal steroids, the increased histochemical enzyme activity of the interrenal gland at the time of full maturity indicates the possibility of this organ to take part either directly or indirectly in the process of maturation or ovulation in chum salmon as well as in masu salmon. The correlation with the rise and fall in these dehydrogenase activities and the corresponding changes in nuclear diameters of the interrenal cells suggest that a close relationship is present among these three parameters during different phases of their life. As indicated in Figs. 6 and 7, the two peaks of the nuclear diameters, one at the age of 3–4 years in chum salmon and other at the age of 1–2 years in masu salmon coincided with the highest levels of dehydrogenase activities at the time of full maturity. Again the low points of nuclear diameters at the time of spawning were found to be related to the decreased activities of the same dehydrogenase enzymes. The histological result agrees with the observations of Robertson and Wexler (9) in Pacific salmon and rainbow trout in which they found hyperplasia of the interrenal tissue during late period of maturation, and partly hyperplasia and partly degeneration of the same tissue at the time of spawning. On the contrary, the present histochemical results did not completely agree with the

biochemical observations on the Atlantic salmon by Fontaine and Hatey (24) who found high concentrations of adrenal corticosteroids in maturing as well as in spawning fish. The results of Fontaine and Hatey (24), and Robertson and Wexler (9) together indicate that the high concentrations of adrenal corticosteroids in the plasma at the time of full maturity were the result of hyperplasia of the interrenal in Pacific or Atlantic salmon. The present observations related to the rise in 3β-DH and G–6–PDH enzymes activities in the interrenal tissue were found to be parallel to the change in nuclear diameter of the same tissue. However, the results related to the high concentrations in the plasma of Pacific and Atlantic salmons after spawning are not very clear, because the data on nuclear diameters

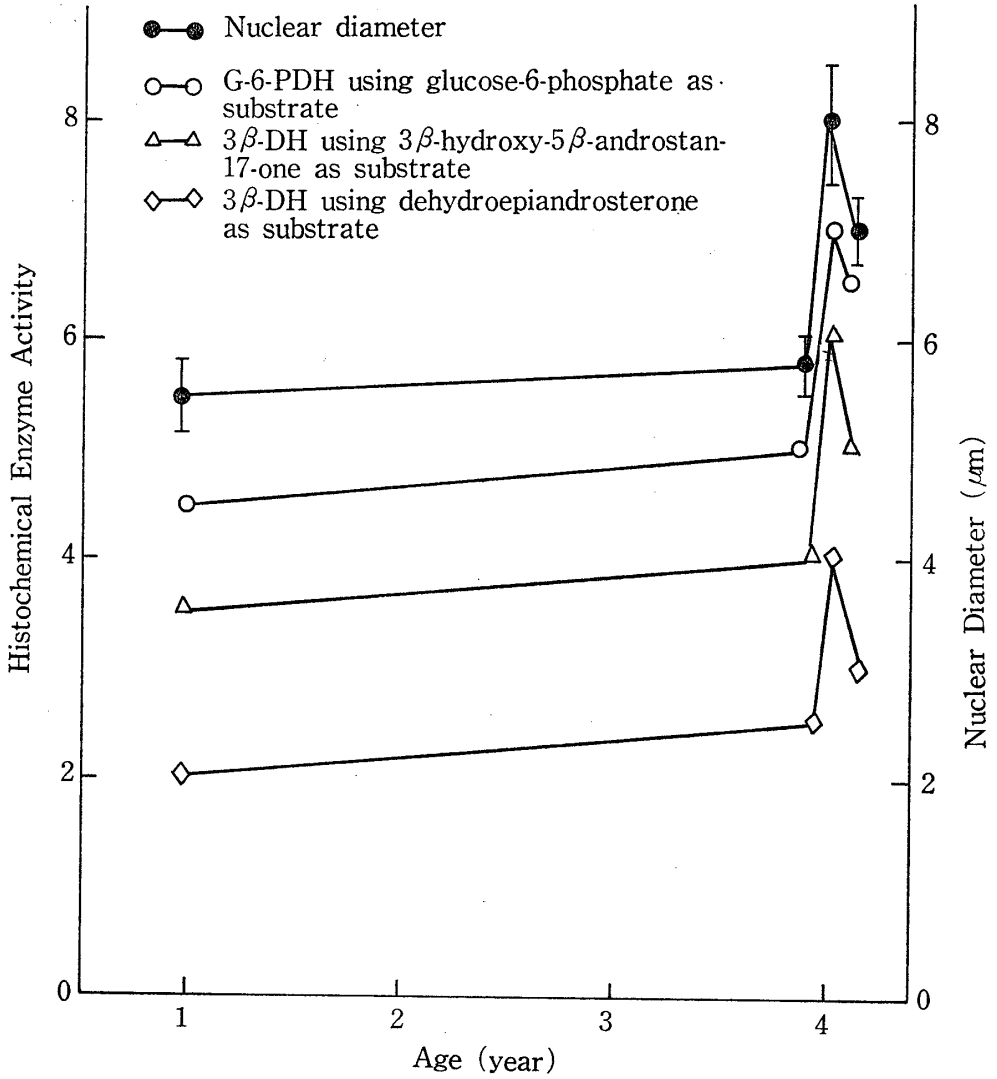

FIG. 6. Variations in the nuclear diameter of the interrenal cells, and the activities of 3β-hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase in chum salmon of I–4 years. The circles are the mean values and the vertical bars are the standard deviations. O: no reaction; 8: maximum reaction.

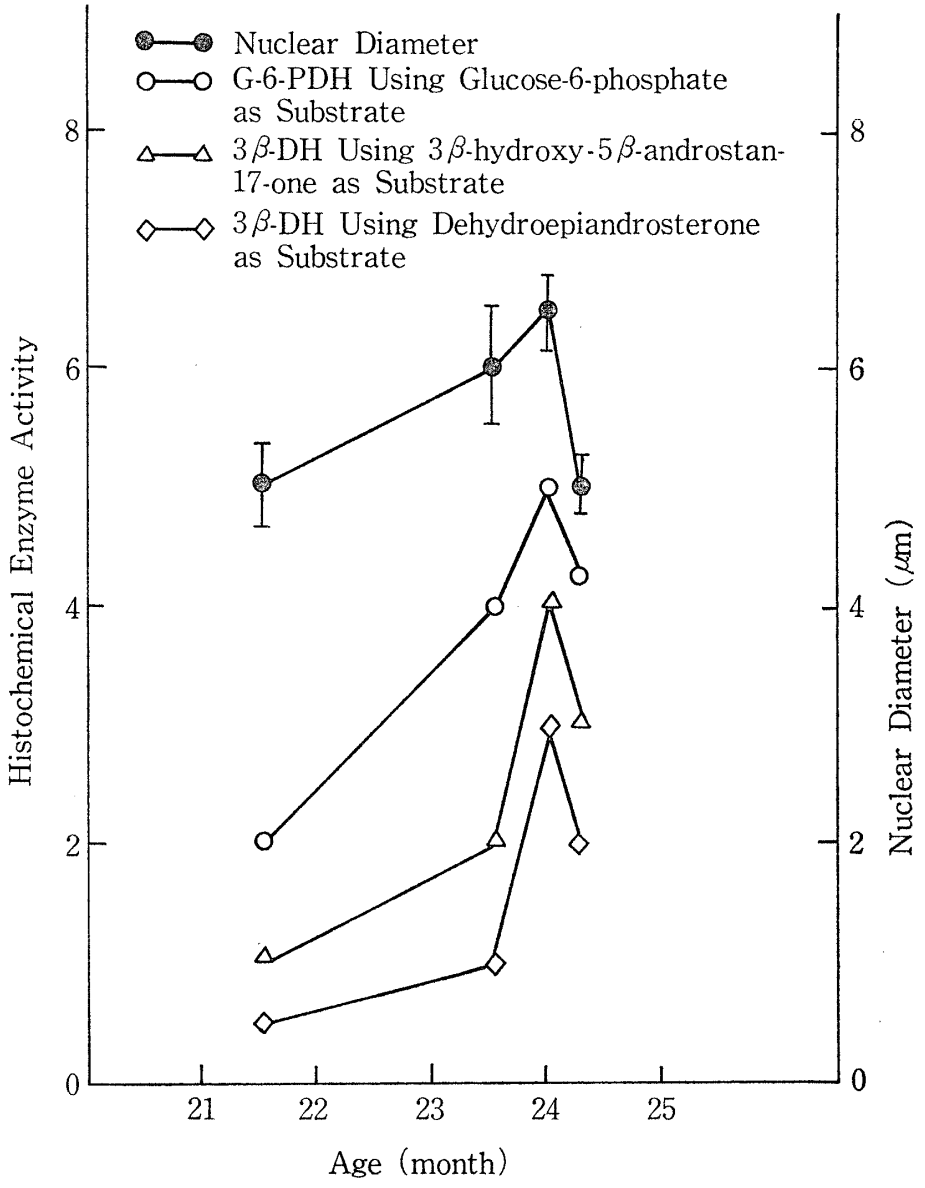

FIG. 7. Variations in the nuclear diameter of the interrenal cells, and the activities of 3β-hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase in masu salmon of 21–25 months. The circles are the mean values and the vertical bars are the standard deviations. The rate of intensity of activity is graded in the same way as in Fig. 7.

and also dehydrogenase activities of the interrenal tissue showed decrease in their respective functions. Again the histological structure of the interrenal gland of Pacific salmon observed by Robertson and Wexler (9) also showed degeneration in the interrenal tissue at the time of spawning. All these observations suggest that the results of Fontaine and Hatey can be supported on the basis of the data obtained by Sandberg *et al.* (25), who demonstrated that an impaired metabolism and reduction in the clearance rate of corticosteroids could account for the elevated hormone level as observed in moribund human. Idler and Truscott (26),

also found an impaired hormone clearance in the mature and spawned Pacific salmon, *Oncorhynchus nerka*. According to Donaldson and Fagerlund (12), there was an increased clearance rate of cortisol in mature or post spawning fish compared with the fish with immature gonads. This observation may be due to the hyperactive state of some other organs producing steroids after spawning. The result on the dehydrogenase activities of the gonad showed decreased activity in the fish after 5–7 days of spawning.

Next to the interrenal gland the ovary and the testis are supposed to be the important organs in the synthesis of steroid hormones in the maturing fish, since activities of 3β-hydroxysteroid dehydrogenase and G–6–PDH were demonstrated in the gonadal tissues throughout the present experiment (Figs. 1 and 4). However, the activities of these enzymes were found far lower than those of the interrenal gland. A gradual rise in the activities was noticed in the gonads of maturing and mature fish but a subsequent decrease in the activities of the same organs was observed in spent fish.

The ovary granulosa and theca cells of follicles seemed to be the primary site of the distribution of 3β-DH and G–6–PDH. However, the additional site is supposed to be a periphery region, since a few slides showed these activities in the periphery of the oocytes. Therefore, it can be concluded that theca and granulosa cells of the normal follicles of these fishes are able to produce steroid hormones. Bara (27) also demonstrated the 3β-DH activity to the theca cells of follicle of mackerel, *Scomber scomber*; Hardisty and Barnes (28) reported the positive reaction of 3β-DH activity in the ovarian granulosa cells of the cyclostome during the breeding season. The reacting theca cells of the present investigation were morphologically slightly different from the unreacted cells in the thecal layers.

In case of the male gonad, the activities of 3β-DH and G–6–PDH were found to be restricted mainly to the interstitial cells of testis lobule and testis tubule (Plate 1, Fig. 2 and Plate 2, Fig. 7). The present studies showed the presence of activities of the above mentioned enzymes in the interstitial tissue of lobule, suggesting their ability to synthesize steroid hormones.

The intense formazan deposition was obtained with 3β-hydroxy-5β-androstan-17-one in the testes of both species. This is similar to that of the lizard reported by Erpino (29) and the mackerel reproted by Bara (30) and in amphibians as reported by Saidpur and Nadkarni (31). This intense formazan deposition is supposed to be due to the oxidation of the 5β-position of a steroid in addition to the 3β-position as described by Baillie *et al.* (32). In chum salmon, the testis tubule also showed some difference in the rate of activities of 3β-DH using different 3β-hydroxysteroids as substrates. It is concluded that 3β-hydroxy-5β-androstan-17-one is the most useful substrate for the demonstration of 3β–DH activity in the endocrine organs of chum and masu salmons.

According to the present results it appears that in masu salmon, the post

ovulatory follicle also posesses the capacity to synthesize steroid hormones since the activities of 3β-DH and G-6-PDH were detected in the tissue. This result coincides with recent observation by Kagawa and Takano*1 in *Salvelinus leucomaenis* and by Nagahama*2 in *Oncorhynchus kisutch*. These activities were found to reduce gradually in the post spawning stage. The reduction in activity of these enzymes is associated with degeneration of these cells. The reactions for dehydrogenases were intense in the chum salmon which had just ovulated (a few hours before) whereas these activities were quite low in masu salmon which had ovulated about 5–7 days ago. In all these cases the location of activity was found generally, in the follicular granulosa tissue or some type of modified granulosa cells. The post spermiated testis of masu salmon also showed 3β-DH and G-6-PDH activities but the activity of 3β-DH was more or less untraceable when compared with that of G-6-PDH activity. The presence of 3β-DH and G-6-PDH activites in the post ovulatory and post spermiatory gonads suggests the potentiality for steroidogenesis but unlikely to contribute to it, because the weak activity of 3β-DH disappears as the post ovulation or the post spermiation period advances.

17β-DH was totally absent from the interrenal tissue of chum and masu salmon. The negative results can not be explained on the basis of the present technique since this enzyme activity has been demonstrated in the ovary and liver of rat (33) and in the livers of gold fish and masu salmon with the same procedures used by the present authors. It is thought that the absence of 17β-DH in the interrenal tissue of masu salmon is similar to the result obtained by Pearson and Grose in rat adrenal (34).

The positive reaction of 17β-DH enzyme was found to be restricted to the granulosa cells and the periphery of the oocytes of guppy, *Poecilia reticulata* (35), the results in masu salmon also showed the distribution of this enzyme to the same regions as observed by Lambert (35). No 17β-DH activity was detected in any ovarian sections of maturing, mature and spawned chum salmon. This is surprising in view of the biochemical analysis of testosterone and estradiol in plasma of mature sockeye salmon, suggesting significant 17β-DH activity either in the gonads or in the interrenal gland. The absence of this activity in chum salmon may be suggested to be due to the difference in functional state of this organ from that of masu salmon at the time of this study or the presence of low activity of this enzyme which is undetectable by the present procedure. Another reason may be that there exists some other pathway for the production of testosterone. The absence of this enzyme indicates that testosterone is not the principal androgen in chum and masu salmon as suggeted by Idler *et al.* (14) in sockeye salmon where 11-ketotestosterone was found 10 times more effective than testosterone.

17β-DH activity has been biochemically shown in the livers of trout and rat

*1 and *2 The oral presentation of this report was given in the Spring Meeting of Japan. Soc. Sci. Fish. at Tokyo, April, 1979.

(36, 34) and histochemically in the liver of gold fish (32). However, the absence of this enzyme in the liver of chum salmon is difficult to explain in light of the results of the present investigation.

From the results of the present investigations it is clear that on one hand the fluctuations in the 3β-DH activity is parallel to the fluctuations in the G-6-PDH activity, on the other hand, the fluctuations of both dehydrogenase activities are parallel to the fluctuation in the size of the nuclear diameter of interrenal cells. A close relationship was observed among the three parameters of nuclear diameter, 3β-DH activity and G-6-PDH activity in the interrenal glands of both chum and masu salmons under different environmental conditions. The range of activity recorded in the various organs at the different period of their life is an indication of true variation in the rate of steroid secretion under different physiological activities, occurring in the body during early and late periods of maturation and also after spawning under different environmental conditions.

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PLATE 1.

1. Localization of 3β-hydroxysteroid dehydrogenase (3β-DH) activity in the granulosa and theca cells of the mature masu salmon. × 210
2. Localization of 3β-DH activity in the interstitial cells of testis of mature masu salmon. ×210
3. Localization of 17β-hydroxysteroid dehydrogenase (17β-DH) activity in the granulosa cells of the follicle of maturing masu salmon. × 210
4. Localization of 17β-DH activity in the liver cells of the maturing masu salmon. × 210

PLATE 2.

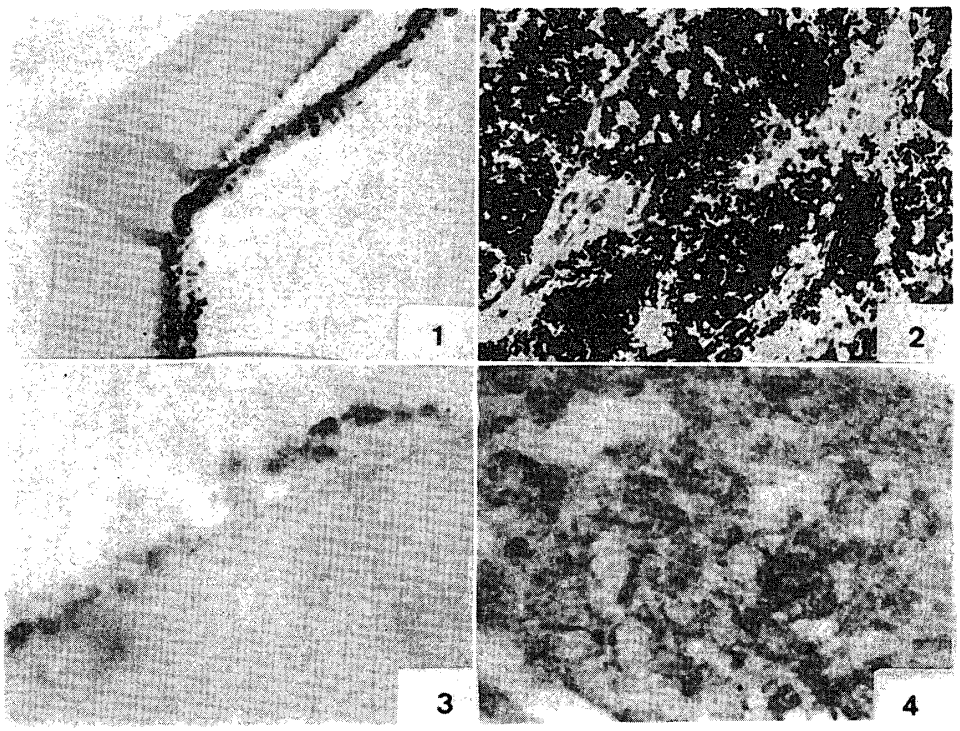
Light micrographs of materials fixed in Bouin's or Helly's fixative fluid, embedded in paraffin, and treated with potassium iodate, then stained with hematoxylin and eosin. Cross sections of the interrenal glands of chum and masu salmons.

5. Interrenal tissue of the head kidney of mature chum salmon, showing reaction with 3β-hydroxy-5β-androstan-17-one. × 210
6. Interrenal tissue of the head kidney of mature chum salmon, showing reaction with dehydroepiandrosterone. × 210
7. Testis tubule of mature chum salmon, showing reaction with estradiol-17β. × 840
8. Interrenal tissue of the head kidney of artificially spawned chum salmon, showing reaction with 3β-hydroxy-5β-androstan-17-one. × 210
9. Naturally spawned post ovulatory gonad, showing reaction with 3β-hydroxy-5β-androstan-17-one in the granulosa cells. × 210

PLATE 3.

Light micrographs of materials fixed in Bouin's or Helly's fixative fluid, embedded in paraffin and treated with potassium iodate, then stained with hematoxylin and eosin. Cross sections of the interrenal glands of chum and masu salmons.

10. Low magnification of the head kidney of the juvenile chum salmon, showing patch of interrenal tissue. H-E stain. × 210
11. Low magnification of the head kidney of the sexually immature masu salmon, showing interrenal tissue of 3–4 cells in thickness. H-E stain. × 210
12. High magnification of the head kidney of the mature chum salmon, showing hyperplasia and hypertrophy of the interrenal cells. H-E stain. × 840
13. High magnification of the head kidney of the mature masu salmon, showing hyperplasia and hypertrophy of the interrenal tissue. H-E stain. × 840
14. High magnification of the interrenal tissue of the spawned chum salmon, showing vacuolization of the cytoplasm and hypoactive state of the interrenal cells. H-E stain. × 840
15. High magnification of the interrenal tissue of the spawned masu salmon, showing vacuolization of the cytoplasm and hypoactive state of the cells. H-E stain. × 840
16. High magnification of the interrenal tissue of the full mature chum salmon, showing vacuolization of the cytoplasm and the nuclei. H-E stain. × 840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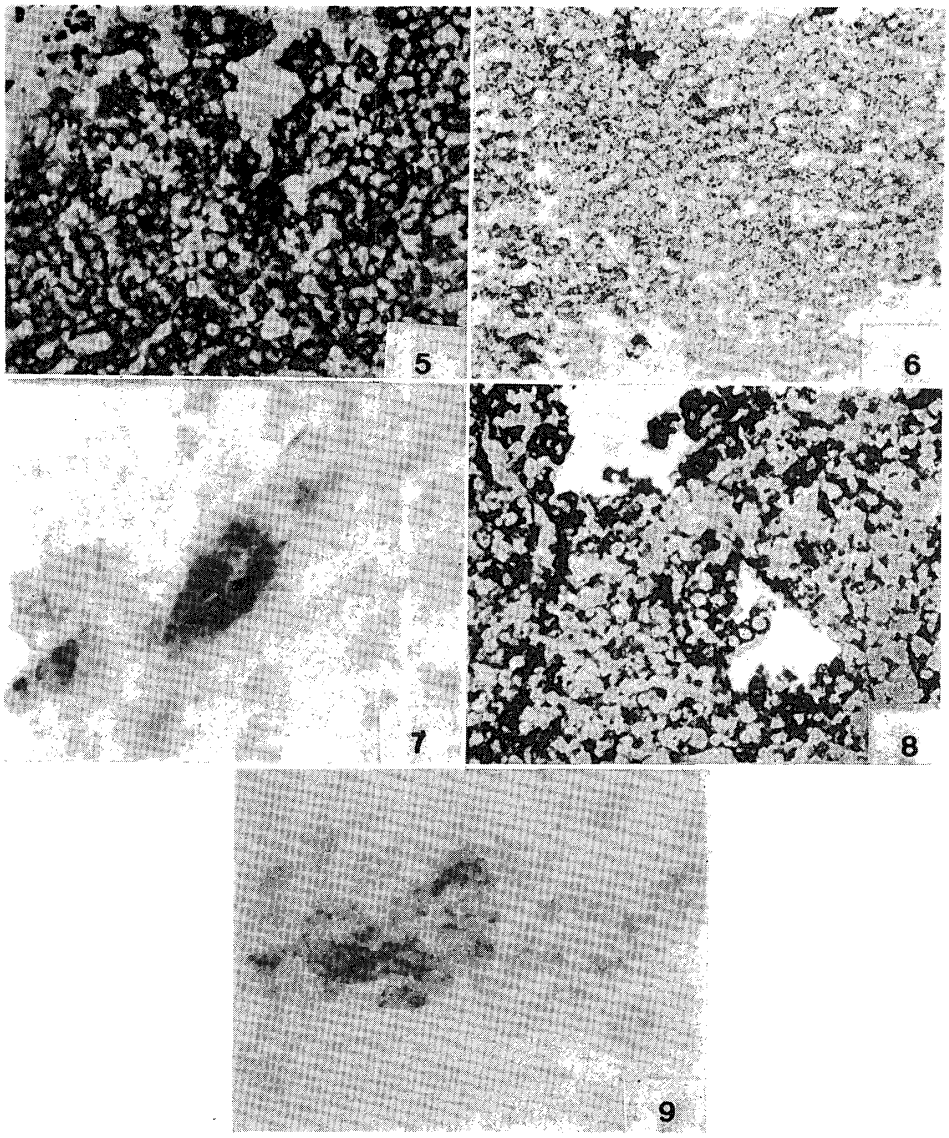
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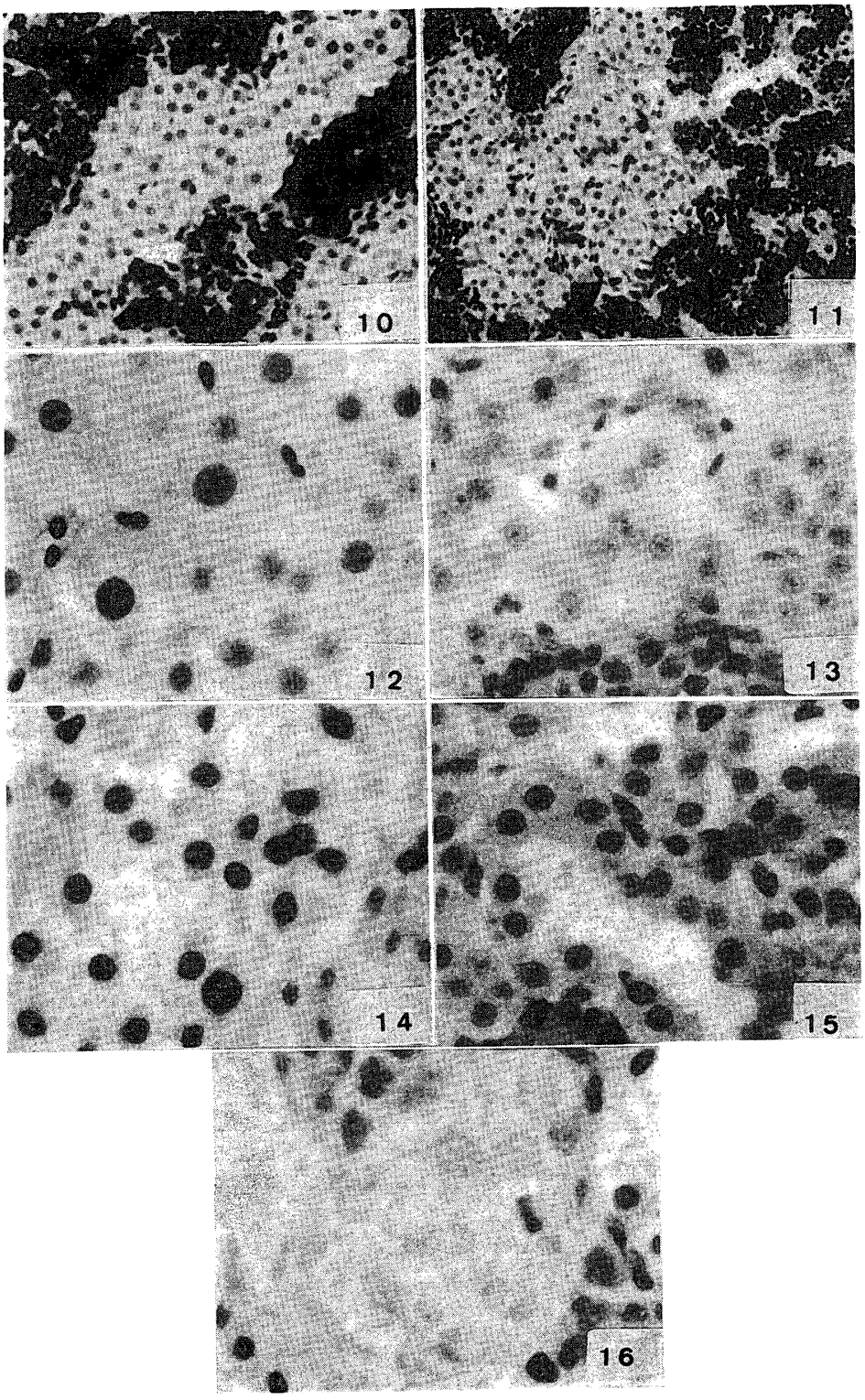
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