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Light Microscopy of the Structural Changes of the Interrenal Gland of Chum Salmon during the Various Stages of Its Life

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Summary

A study was conducted on the structural changes in the interrenal gland of chum salmon, *Oncorhynchus keta*, at the successive stages of its life cycle. In adult chum salmon, the head kidney was triangular in shape and morphologically different from the rest of the kidney. It was composed primarily of lymphoid tissues, uriniferous tubules, thyroid follicles, the cardinal vein and its tributaries, and a few glomeruli. The interrenal gland was situated in the head kidney, and was composed of interrenal and chromaffin cells which correspond to the adrenal cortex and medulla in mammals. These two kinds of cells such as the interrenal (adrenal cortex) tissue and the chromaffin (medullary) tissue were found to remain always separate throughout their life. The interrenal cells were basophilic in nature, whereas the chromaffin cells were non-basophilic. The former type of cells were usually found around the cardinal vein and its branches, whereas the latter type of cells in the vein wall and also in small groups in the hematopoietic tissue.

Apparently, the structure of the interrenal gland of chum salmon was completely different from that of mammals. However, it was shown that there were analogies to the different cell types found in the adrenal gland of mammals.

The interrenal gland which is the homologue of the mammalian adrenal gland, is morphologically extremely diverse in fish. In mammals, adrenal glands are necessary for the regulation of water mineral and carbohydrate metabolism and for releasing immune bodies (1). Knowledge about the fundamental function of the fish's interrenal gland, which takes part in the production of steroid hormones is not sufficient. Since it seemed possible that the hyperactivity of the adrenal gland might play a major role in bringing about the marked deterioration and death of the Pacific salmon which occur after its first spawning (2), many workers have already reported on the effects of hypophysectomy and ACTH treatment on the histology of the teleostean interrenal tissue (3). It seems, therefore, that if the activity of the interrenal gland in fish plays some role at the various stages of its life, it may be expected that some change in the histological pictures of this gland will be observed.

Although a histological study of the interrenal gland of a few teleosts including adult chum salmon has already been reported, there is no systematic study of this gland to date. Therefore, a systematic microscopical examination of the interrenal gland from the fry to adult stages of chum salmon has been undertaken to elucidate the histological changes of this gland associated with the various stages of its life and also to find out whether or not any structural similarities are present between the chum salmon and higher vertebrates.

Materials and Methods

Four stages of chum salmon were used as materials. The fry, the sexually mature and spawned salmon were collected from the Tsugaruishi River Salmon Hatchery, Miyako City, Iwate Prefecture and the juveniles were secured from the Matsushima Aquarium, Matsushima Town, Miyagi Prefecture, Japan. The ages and sizes are represented in Table 1.

TABLE 1. *Records of experimental materials, O. keta*

Stage	Age of fish after hatching	Body	
		Length	Weight
Alevin	30 days	2.8 cm	0.3 g
Fry	45-120	3.9-8.3	0.5-17.5
Juvenile	300	17.5	28.8
Adult	3-4 years	66.0	3.0 kg

The fry from 30–120 days of age were cut into small pieces and fixed in Bouin's or Helly's fixative fluid. The latter fixative contains potassium dichromate used to determine the distribution of the chromaffin cells. Specimens of the juvenile and adult were killed, the head kidney was separated from the kidney properly by a transverse incision at a point where the two arms of the cross join the body kidney and then fixed in the above-mentioned fixative fluids. Serial sections of 7 μm thickness were prepared after the materials had been dehydrated, and embedded in paraffin wax (m.p. 60°C). Before staining with Mayer's hematoxylin and eosin, the sections were passed through Lugol's iodine and 5% aqueous sodium thiosulfate solutions.

Results

Histological characteristics of the interrenal gland at the various stages of the life cycle of the chum salmon were as follows:

In general, the adult head kidney of chum salmon, which was triangular in shape and morphologically different from the rest of the kidney, contained uriniferous tubules, hematopoietic tissue with predominantly lymphoid tissue, interrenal

tissue, chromaffin tissue, ganglionic structure, a few scattered thyroid follicles and glomeruli.

(a) *Alevin (30 days of age).*

In alevin, the interrenal gland was composed of a small number of interrenal and chromaffin cells. These interrenal cells were found to be arranged in small patches or as narrow collars of one-cell in thickness around the cardinal vein and its tributaries in the hematopoietic tissue (Figs. 1 and 2). The average size of the interrenal cells and their nuclei were 11.2 μm and 4.1 μm respectively. The nucleoplasm was no longer clear, but was dark with irregular chromatin bodies. With eosin, the cytoplasm of the interrenal cells was stained more deeply than the cytoplasm of adjacent kidney cells. Only a few chromaffin cells were observable in the vein wall.

(b) *Fry (45–120 days of age).*

In fry, the number of interrenal cells was small and found to be present as narrow collars of one or two cells in thickness, almost the same as in alevin (Figs. 3 and 4). However, with the advancement of age this arrangement of the interrenal cells changed from one-cell layer to irregular compact clumps or collars of one or two-cells in thickness, which found adjacent to the cardinal vein and venules. In general, these cells contained circular nuclei, but there were a few elliptical nuclei among these circular nuclei. The cytoplasm of these cells was stained paler than that of the adjacent kidney cells. The nuclear diameter of these cells varied from 4.8–5.9 μm, and the cell size varied from 11.5 μm to 12.8 μm. The chromaffin cells (adrenal medulla) occurred in the vein wall and were oblong or spherical in shape. These cells were always found to remain away from the interrenal cells. A few number of chromaffin cells were found to be scattered in the hematopoietic tissue (Fig. 7).

(c) *Juvenile (300 days of age).*

Juveniles of chum salmon, with infantile gonads, were collected from the seawater pond at Matsushima Aquarium. In this stage, the interrenals occurred in small elongated clumps adjacent to or surrounding the vein and the venules. The clumps were two, three or more cells in thickness (Fig. 5). These cells were characterized by a round nucleus with a prominent nucleolus. Most of the nuclei contained two or more small nucleoli (Fig. 6). With eosin, these cells were stained more pale than that of the kidney cells. The average size of these cells and nuclei was 14.8 μm and 6.8 μm respectively. The chromaffin cells were found to be present in the vein wall and its tributaries (Fig. 8). Hemosiderin patches were observed in the head kidney of the juvenile salmon.

(d) *Sexually Mature and Spawned Salmon (3–4 years of age).*

By time full sexual maturity was reached, the vascularity of the interrenal

tissue developed along with the development of sinusoid-like structures. As a result of the development of these sinusoids the shape of the interrenal tissue became distorted. However, a marked hyperplasia of the interrenal tissue was observable in all the specimens studied. The volume of this newly-formed tissue was several times larger than that which was present in the immature specimens. The diameter of the nuclei was quite uniform in size, about 8.1 μm. The activation of the interrenal cells, which was indicated by the presence of large spherical nuclei, frothy and granular cytoplasm and group-cell arrangement, was significantly greater than in the fry and juvenile salmon (Figs. 9 and 10). A quite large number of hemosiderin patches were found in the head kidney. The chromaffin cells were found to be present in groups of 4–6 cells in the vein wall (Fig. 16).

In the case of spawned salmon, degenerative changes of the interrenal gland were observed. Usually this process of degeneration was initiated by the vascularization of the interrenal tissue and the vacuolization of the nuclei (Figs. 13 and 14) and the disappearance of blood cells from the vein and venules (Fig. 15). A large number of narrow blood channels, which developed within the interrenal tissue, distorted the compact mass of this tissue into many small groups. The cytoplasm of these cells appeared to be vacuolated (Figs. 11 and 12). In the hematopoietic tissue, large hemorrhage was observed. The average cell and nuclear size were found to be 7.0 μm and 17.5 μm respectively. The cell area did not decrease in size along with the nuclear size, which did decrease. As degeneration had progressed significantly, perhaps by the time of calculation it was not possible to obtain a correct measurement of cell area, due to the occurrence of post-degenerative empty spaces. In a few cases, arteriosclerosis was found with a somewhat thickened adventitia due to the increased amount of collagen, and accompanied with the destruction of the underlying internal elastic membrane.

Discussion

In alevin, the interrenal gland was composed of a very small number of interrenal and chromaffin cells (Figs. 1 and 2). The number as well as the size of these cells increased with the advancement of age (Figs. 3 and 4). A gradual change in the structure of this gland from loose layers to irregular clumps was observed from the fry to juveniles, whereas in the adults, the hyperplasia of interrenal tissue was accompanied by the development of sinusoid-like structures and vascularization. The interrenal cells were eosinophilic in nature, and during the inactive stage these cells became dark pink when eosin was applied. However, in the active stage they were more pale pink in contrast to the surrounding kidney cells. The chromaffin cells which were larger in size than the interrenal cells, were non-eosinophilic (Figs. 7, 8 and 16), utilizing potassium iodate, the cytoplasm of chromaffin cells became brown in colour but the nucleoplasm remained pale. The interrenal cells

of the fry and juvenile were small and densely granulated resembling the cells of the mammalian zona glomerulosa.

Our histological observations on the hyperactivity of interrenal tissue of the fry at the age of 120 days, seem to be similar to the observations of Fontaine and Olivereau (4).

The results on the hyperplasia of interrenal gland of adult chum salmon during migration (Figs. 9 and 10) agree with those of Robertson and Wexler (5) and Homma (6). According to them the interrenal tissue undergoes a marked hyperplasia following the anadromous breeding migration under the stress of starvation and gonad maturation. These large hyperplastic cells in chum salmon, appear to have some similarity to the fasciculata type of mammals.

The microscopical results on the structure of interrenal tissue in spawned chum salmon (Figs. 11 and 13) coincide with those found in Ayu (6) and in Pacific salmon (5). A marked hemorrhage in the hematopoietic tissue and the disappearance of blood cells from veins (Fig. 15) and venules were the common characteristics observed in the spawned salmon. The degeneration of these cells was accompanied by vascularization (Fig. 13), vacuolated cytoplasm (Fig. 11) and distorted nuclei (Fig. 11). This degenerative stage of the interrenal tissue is somewhat similar to the reticularis zone of mammals.

The structural changes in the histology of the interrenal gland at various stages of life throw some light on the necessity for such changes because of the alterations in the level of circulating corticosteroids due to various kinds of physiological changes that are developing under the influence of internal as well as external factors of environment.

In conclusion, our observations point out that the variations in number and structure of the interrenal cells are related to the physiological changes connected with the various kinds of requirements of the body. The structure of the interrenal gland of this fish is completely different from that of mammals, however, it is shown that there are analogies to the different cell types found in the adrenal gland of mammals.

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PLATE 1

FIGURES. 1–8 are 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 section of the interrenal gland of chum salmon.

FIG. 1. Low magnification of the head kidney of the alevin, showing interrenal gland (IG), which is composed of one-cell layer in thickness. Hematoxylin-eosin (H-E) stain. ×210

FIG. 2. High magnification of the head kidney of the alevin, showing the interrenal gland (IG), which is composed of one-cell layer in thickness. H-E stain. ×840

FIG. 3. Low magnification of the head kidney of the fry, showing interrenal gland, which is composed of one or two cells in thickness. H-E stain. ×210

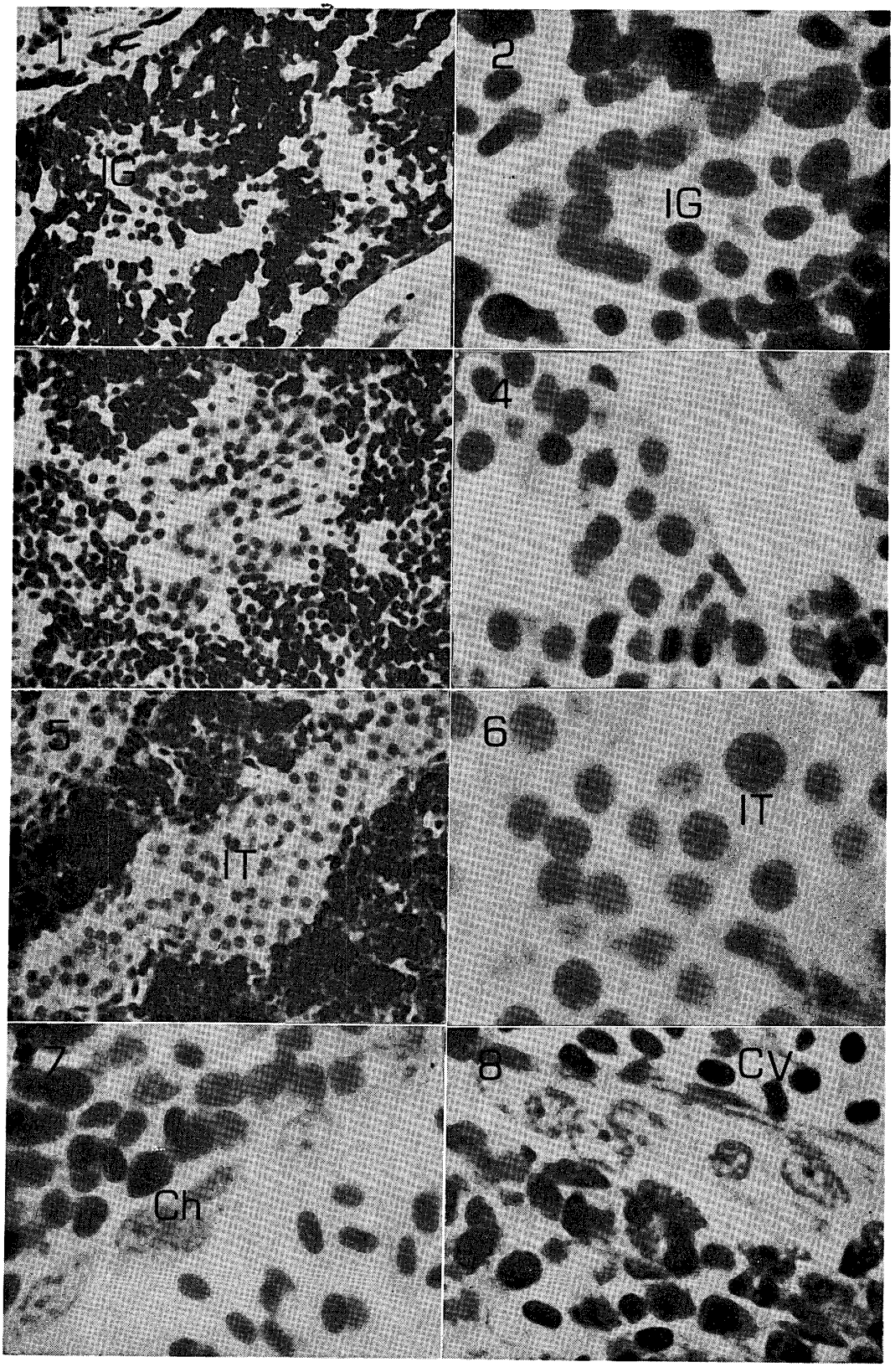
FIG. 4. High magnification of the head kidney of the fry, showing interrenal gland, which is composed of one or two cells in thickness. H-E stain. ×840

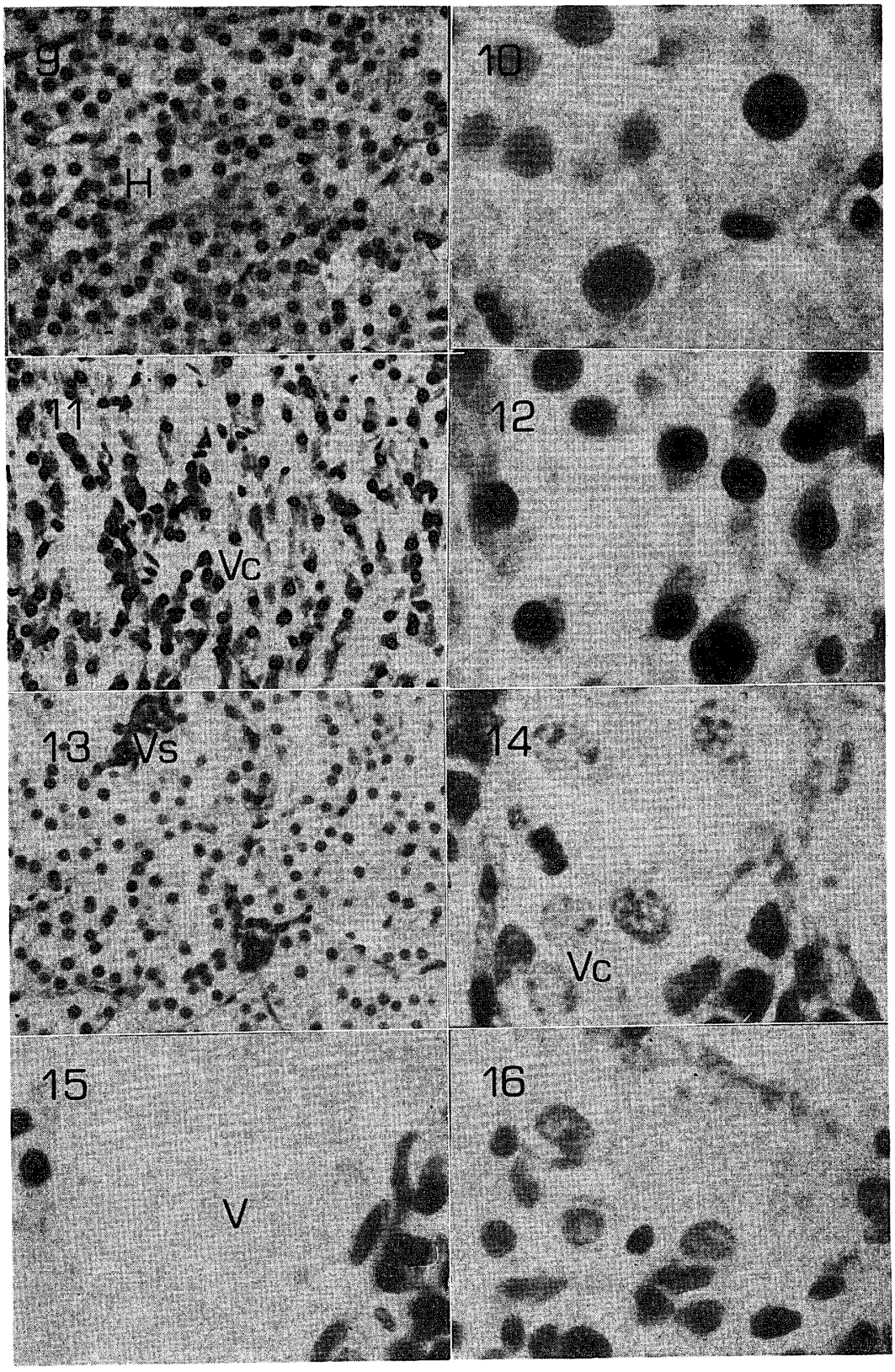
FIG. 5. Low magnification of the head kidney of the juvenile, showing interrenal tissue (IT) of two or three cells in thickness. H-E stain. ×210

FIG. 6. High magnification of the head kidney of the juvenile, showing interrenal tissue (IT) which is composed of two or three cells in thickness. H-E stain. ×840

FIG. 7. High magnification of the fry, showing chromaffin cells (Ch) along the cardinal vein and in the hematopoietic tissue. Potassium iodate (PI) treatment. ×840

FIG. 8. High magnification of the head kidney of the juvenile, showing chromaffin cells along the cardinal vein (CV). PI treatment. ×840


1
IG
2
IG
4
5
IT
6
IT
7
Ch
8
CV


9
H
10
11
Vc
12
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Vs
14
Vc
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V
16

PLATE 2

FIGURES 9–16 are 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 section of the interrenal gland of chum salmon.

FIG. 9. Low magnification of the head kidney of the mature chum salmon, showing hyperplasia (H) of the interrenal tissue. H-E stain. ×210

FIG. 10. High magnification of the head kidney of the mature chum salmon, showing hyperplasia of the interrenal gland. H-E stain. ×840

FIG. 11. Low magnification of the head kidney of the spawned chum salmon, showing vacuolization of the cytoplasm (Vc) of interrenal tissue. H-E stain. ×210

FIG. 12. High magnification of the head kidney of the spawned chum salmon, showing vacuolization of the cytoplasm of interrenal tissue. H-E stain. ×840

FIG. 13. Low magnification of the head kidney of the spawned chum salmon, showing vacuolization and vascularization (Vs) of the interrenal tissue. H-E stain. ×210

FIG. 14. High magnification of the head kidney of the spawned chum salmon, showing vacuolization of the cytoplasm and the nuclei. H-E stain. ×840

FIG. 15. High magnification of the head kidney of the spawned chum salmon, showing the disappearance of blood cells from vein (V). H-E stain. ×840

FIG. 16. High magnification of the head kidney of the adult chum salmon, showing chromaffin cells embedded in the connective tissue of the vein. PI treatment. ×840