Histological Observations of Some Organs in the Triggerfish, Canthidermis rotundatus, Stranded on the Coast of Niigata Facing the Japan Sea

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Abstract The endocrine and internal organs of stranded specimens of the triggerfish, Canthidermis rotundatus, were studied histophysiologically. The specimens of medium size used were stranded on the beach of Niigata facing the Japan Sea. As they were lightly starved, not so severe changes were detected in the digestive tube, exocrine pancreas, kidney, and spleen with the exception of a large amount of fat deposition in the liver. In the endocrine pancreas, i.e., the Brockmann body, three types of cells (A, B, and D cells) were identified. The ovary was crowded with a number of oocytes in the early growth stages, whereas a considerably large portion of the testis was occupied by spermatogonia in addition to a small number of elaborate spermatozoa. The amount of Gomori positive neurosecretory material produced in the cells of the nucleus preopticus varied considerably according to each specimen. On the other hand, moderate amount of this material was deposited in the pars nervosa. The acidophil neurosecretory material stored in the cells of the nucleus lateralis tuberis was scanty. Seven types of glandular cells were demonstrated in the adenohypophysis: among them, the basophils diagnosed as gonadotrophs contained only a small amount of granules. Therefore, it seemed probable that the triggerfish carried by the northward Tsushima Warm Current in winter were the immature fish in the process of abortive migration.

Introduction

It is well known that some marine animals of tropical and subtropical origin, especially teleost fishes, frequently occur in the temperate regions of the comparatively high latitude along the coasts of the Japanese Islands (Honma, 1955, 1956, 1957; Honma and Kitami, 1967, 1970; Honma and Yoshie, 1975; Nishimura, 1958 b; Ueno, 1965; Ueno and Abe, 1966). Among them, the porcupine fish (Diodon holacanthus) and triggerfish (Canthidermis rotundatus) are of particular interest since they, in emaciated condition, are occasionally stranded en masse on the beach after heavy storm during winter season. This peculiar phenomenon was studied extensively in the porcupine fish by Nishimura (1958 a, 1958 b, 1960, 1973) with emphasis on the influences of the Tsushima Current and drift current generated by the heavy seasonal wind of north-west direction. Unfortunately, however, little is known concerning the histological evidences of the drift animals: the dealfish (Tamura and Honma, 1971 b, 1972), the kingof-the-herring (Honma et al., 1973) and the Pacific ridley turtle (Honma and Yoshie, 1975). In order to gain some knowledge about their physical and physiological conditions, histological exmination was carried out on the trigger-fish collected at the beach of Niigata.

Materials and methods

Three fresh individuals stranded on the sandy beach of Niigata City after a heavy storm were selected and used in this study. Date of collection, body size, and sexes of these specimens are shown in Table 1. After decapitation, the organs and tissues were removed, immersed in Bouin-Holland-sublimate solution, dehydrated, embedded in wax, cut serially 6 to 8 μ thick in sagittal and transverse directions,

Table 1. Data of the triggerfish, *Canthidermis* rotundatus, used in this study.

| Specimen number | date of collection | body length (mm) | body weight (g) | sex |
|--------------------|--------------------|------------------------|-----------------------|-----|
| 1 | Dec. 2, 1972 | 201 | 1 | 3 |
| 2 | Nov. 23, 1973 | 211 | 310 | ð |
| 3 | Nov. 23, 1973 | 127 | 220 | 우 |

and stained by various stainings such as Delafield's hematoxylin-eosin, Heidenhain's azan trichrome, aldehyde fuchsin. (AF)-fast greenorange G, alcian blue (AB)-acid fuchsin, periodic acid Schiff's reagent (PAS)-fast green-orange G, AB-PAS-orange G, lead hematoxylin (PbH), and Hellman and Hellerström's silver impregnation.

Results

Stomach

In spite of careful examination the stomach was empty. The gastric mucosa as a stratified epithelium and 25 to 50 μ in thickness was characterized by many mucous cells. The epithelium was thicker in the cardiac portion than in the fundus and the pyrolic portions (Fig. 1). The gastric glands beneath the mucosae had two or more acini consisting of weak acidophil cells with fairly rich cytoplasm (Fig. 2). The mucous neck cells of the gland revealed a foamy appearance. The fibrous submucosa contained thick muscular bundles.

Intestine

The intestinal cavity was also empty, but it was not spacious due to the intricately deep folds of the mucous membrane (Fig. 3). The epithelial cell was high columnar, 45 to 50 μ in height, and had a longish ovoid nucleus with one or two prominent nucleoli. The striated border on the epithelium was distinct, and the goblet and wandering cells were encountered here and there. There was found a comparatively rich vascularization in both lamina propria and submucosa, where lymphocytes occurred sparsely or in clusters. Furthermore, no appreciable change was detected in the layers of circular and longitudinal muscles. Thus, it was assumed that a rather healthy condition was still maintained in the alimentary tract.

Liver and gall bladder

The liver as a lobate organ was classified as the hepatopancreas since the exocrine pancreatic tissues were contained and scattered. Weight of the liver in the No. 2 male fish was 16.5 g, whereas 6.3 g in the female one. The cells of hepatic parenchyma were closely packed around the capillaries. Owing to a large amount of fat deposition, almost all parenchymatous cells showed a markedly vacuolized appearance and the nucleus with a prominent nucleolus was

eccentrically placed in each cell (Fig. 4). The fattiness of the cell was more striking in the male than in the female fish. On the other hand, it was difficult to see the connective tisse stroma since it was scanty and indistinct.

The gall bladder was filled with bile tinted with dark green. No noticeable change was encountered in the bladder wall consisting of a single layer of columnar epithelium and connective tissue capsule.

Exocrine pancreas

The exocrine pancreas was composed of a number of isolated clusters in various sizes, which gathered around the hepatic veins. Each acinar cell, columnar or conical in shape, contained many zymogen granules in its apical portion, and a round nucleus with a prominent nucleolus was seen toward the basal region (Fig. 5).

Brockmann body

Three or four Brockmann bodies as the endocrine pancreas were found on the mesenterium adjacent to the spleen. Each body in ovoid shape was surrounded by a connective tissue sheath, which occasionally included a thin layer of exocrine tissue. Three types of cells, A, B, and D were demonstrated in this body on the basis of staining properties (Fig. 6). No regressive change was seen in these elements, however. The A cells, ovoid or low columnar in shape, were the largest in the size among the endocrine cells and were superior in number to the other elements. The cytoplasm was weakly stained by acid dyes, such as azocarmine and fast green. The AF positive B cells, ovoid in shape, were gathered in small masses remaining somewhat the condition of cell cord. By azan trichrome, the finely granular cytoplasm of this cell was also stained in reddish orange tint. The argyrophil D cells as the third element, fusiform or columnar in shape, were scattered solitary among the A cells. The coarse granules in the cytoplasm of the D cell had a strong affinity for acid dyes.

Testis

A pair of testes were flat and elongate bodies tapering toward the cranial direction, and were located in the dorsal region of the rectum. Since their external appearance were delicate and gonosomatic index was small (0.1 in No. 2 specimen), the testes were diagnosed as immature

stage. In cross section, it was evident that the seminiferous tubules were arranged and directed toward the vasa deferentia running through the central portion of the testis to form radial routes. The proximal portion of the tubule near the vasa deferentia contained a small mass of sperm and a number of elaborate spermatids, whereas the distal major portion was filled with numerous spermatogonia and a small number of spermatocytes.

Ovary

A pair of ovaries in contact with each other were also located in the dorsal region of the rectum. The gonosomatic index indicated a small value (0.5). The ovigerous folds facing the ovarian cavity were laden with a great number of oocytes in early growth stages and nests of oogonia (Fig. 8). The oocytes in the perinucleolus stage and $60 \, \mu$ in maximum diameter were characterized by having ooplasm stained deeply by hematoxylin.

Thyroid gland

The thyroid gland located on the dorsal surface of the ventral aorta was an elongate and compressed mass consisting of dense follicles surrounded by loose connective tissue and capillaries. Each follicle, round or ovoid in shape and 40 to 600 μ in diameter, contained a considerable amount of colloid which has a strong positive response to PAS reaction. The colloid detached from the follicular epithelium showed somewhat wavy margin. The follicular epithelium was constructed from flat to low columnar cells, 4.8 to 17.6 μ in height (mean 8.5 μ) (Fig. 9). Its nucleus occasionally showing a wavy margin was small and round. Moreover, the

cytoplasm above the nucleus was faintly PAS positive. It is assumed that these features indicated a quiescent or hypofunctional state.

Kidney

In the body kidney the renal corpuscles and some segments of renal tubules were surrounded by lymphoid tissue (Fig. 10). The epithelial cell of the proximal segment had a rich granular cytoplasm of acidophil nature, and there was a vesicular nucleus stained lightly with dyes. The brush border was easily visible. The distal segment was composed of cubic cells with relatively rich cytoplasm and irregularly free surface. No abnormality was seen in the glomerular tuft. It was impossible to describe the head kidney, because it was damaged when decapitation was performed to obtain the brain, including hypothalamo-hypophysial complex and the thyroid.

Spleen

The spleen was ovoid in shape measuring 15.0 mm in longer and 8.5 mm in shorter axes and was enveloped with a thin capsule of fibrous connective tissue. The white and red pulps were distinguishable, although they were intermingled with each other (Fig. 11). The white pulp contained a relatively dense mass of lymphoid cells, whereas the fibrous framework of the stroma was thin and less prominent. There was found a highly vascularization with splenic arteries, veins, and sinusoids.

Hypothalamo-hypophysial system

One of the components of this system, the nucleus preopticus, was distributed approximately dorsal to the optic chiasma and was roughly divided into two portions: the ventrally

Fig. 1. Sagittal section of the cardiac portion of stomach. A number of mucous cells arranged in the mucous membrane are noticed. Azan stain. ×200.

Fig. 2. Sagittal section of the fundus of stomach. No abnormality and/or pathologic figure is seen in the gastric mucosa and glands. Azan stain. $\times 120$.

Fig. 3. Cross section of the intestine. No anomalous feature is detected. Azan stain. ×120.

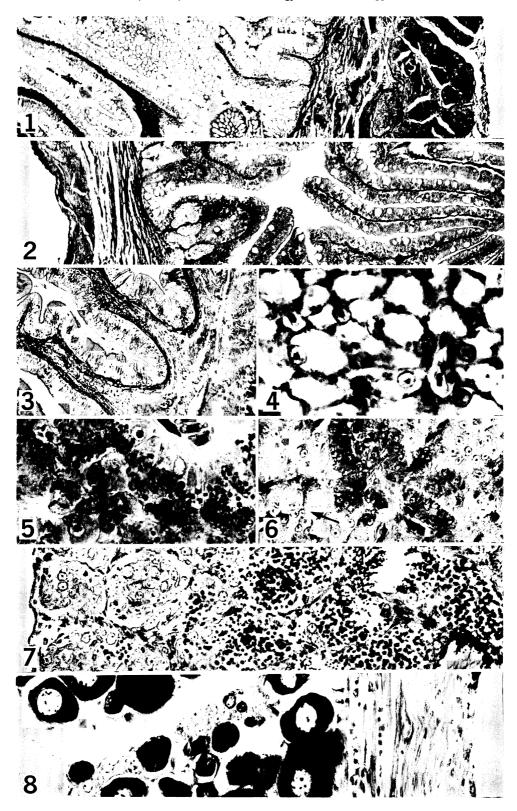
Fig. 4. Enlarged view of the liver. Note a lot of fat deposition in the hepatic cells. Hematoxylineosin stain. $\times 1,000$.

Fig. 5. A part of the exocrine pancreas around the hepatic vein. Many zymogen granules in the acinar cells are noticed. Azan stain. ×1,000.

Fig. 6. A part of the Brockmann body. Three types of cells are distinguished: A cells (light in this picture), B cells (dark), and D cells (by arrows). AF-azan stain. ×400.

Fig. 7. A part of the testis showing spermatogenic cells in various developmental stages. Hematoxylineosin stain. ×400.

Fig. 8. A part of the ovary crowded with many oocytes in the early growth stages. Hematoxylineosin stain. ×400.



shifted pars parvocellularis (10.6 μ in mean cell diameter) and the dorsally shifted pars magnocellularis (19.4 μ). The amount of AF stainable material in the cell body was comparatively large in No. 1 specimen, while rather small in both No. 2 and No. 3 specimens. A marked degranulation in some of the cells were also encountered in the latter two specimens (Fig. 12). Juxtasomal axons of all three specimens were deficient in stainable material. The second component, the nucleus lateralis tuberis, was situated in the floor of the diencephalon just antero-dorsal to the infundibulum. The cell, ovoid in shape and 15.9 μ in mean longer axis, contained a small quantity of acidophil material in its perikaryon (Fig. 13).

The structural pattern and histological design of the hypophysis were in accordance with a brief description of Kawamoto (1967). This gland was composed of four components: the rostral pars distalis (RPD), the proximal pars distalis (PPD), the pars intermedia (PI) and the pars nervosa (PN). In the PN projecting largely into the PI, a moderate amount of Gomori positive material was demonstrated. Noticeably, Gomori negative but acidophil material was detected in the distal portion of the PN bordering the PI. However, these two kinds of materials were poor in the anterior region of the PN covering the pars distalis. The RPD consisted of three types of cells (Fig. 14). The acidophil cells, corresponding to the prolactin cells, were densely packed in the peripheral portion of the RPD. They with relatively rich cytoplasm were stained with azocarmine and also orange G. The weak acidophil cells, columnar in shape, were arranged in one or more layers of cell cords that border the PN. It was noted that there were often found the enlarged nuclei in vesicular condition and degranular cytoplasm in these cells. Therefore, in spite of weak PbH positive reaction, they were regarded as corticotrophs in somewhat hyperfunctional state. Adjacent to the corticotrophs, the basophil cells in wedge shape were detected as small clusters. They, corresponding to the thyrotrophs, included the longish ovoid nuclei and a moderate amount of glycoprotein positive to AF, AB, and PAS in fine granular appearance.

The PPD consisted of two types of cells: dorsally shifted weak acidophils corresponding to the somatotrophs and ventrally shifted basophils equivalent to the gonadotrophs (Fig. 15). In the present specimens, the somatotrophs occupied a major portion of the PPD. Most of the gonadotrophs, polygonal or round in shape, contained a relatively small quantity of granules of glycoproteinous nature, although some of the cells included acidophil globules besides these basophilic granules.

The PI was composed of two types of cells: PAS positive and PAS negative cells. However, small groups of gonadotrophs were occasionally found in the periphery of PI (Fig. 16).

Discussion

Three specimens of medium size examined in the present time revealed that they were immature. The same has already been reported

Fig. 9. A part of the thyroid gland indicating a quiecent condition. Hematoxylin-eosin stain. ×400.

Fig. 10. Cross section of the kidney. No deterioration is recognized. Hematoxylin-eosin stain. ×4

Fig. 11. A part of the spleen. The white and red pulps are discernible. Hematoxylin-eosin stain. $\times 400$.

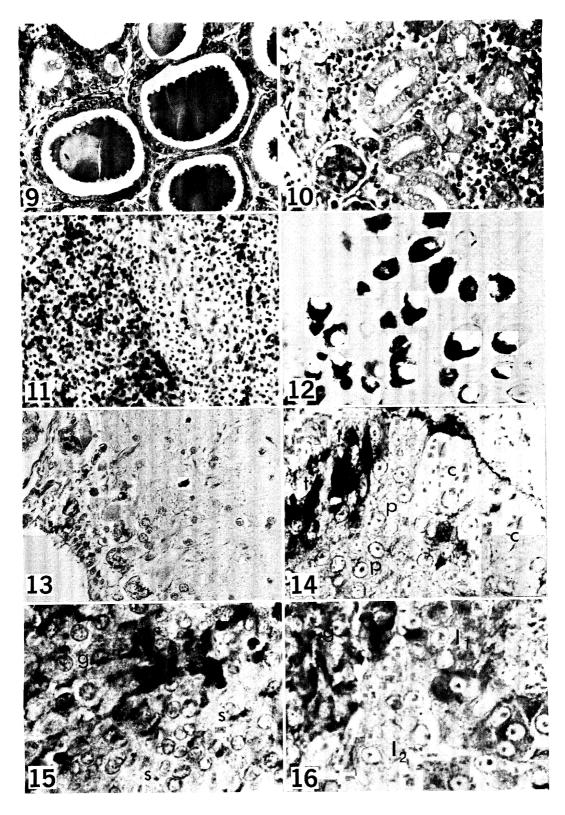
Fig. 12. The pars magnocellularis of the nucleus preopticus of a male fish (No. 2). Several cells under the degranulated condition are encountered. AF stain. ×400.

Fig. 13. The neurosecretory cells of the nucleus lateralis tuberis. A small amount of the acidophil material is contained in the perikarya. AF-azan stain. ×400.

Fig. 14. A part of the rostral pars distalis (RPD) of the hypophysis. Three types of cells, the thyrotrophs (t, dark in this picture), corticotrophs (c, light) under the degranulted condition and prolactin cells (p) packed closely are discernible. PAS-fast green-orange G stain. ×1,000.

Fig. 15. A part of the proximal pars distalis (PPD) consisting of the somatotrophs (s) and gonadotrophs (g) with scanty granules. AF-azan stain. $\times 1,000$.

Fig. 16. A part of the pars intermedia (PI) showing three types of cells: PAS positive cells (I₁, dark in this picture), PAS negative cells (I₂, light) and AB positive cells (gonadotrophs, coal-black). AB-PAS-orange G stain. ×1,000.



in other drift animals such as the porcupinefish without histological criterion (Nishimura, 1960) and the king-of-the-herring (*Trachipterus iris*) based on indirectly histological evidences (Honma, et al., 1973). On the other hand, in the cases of dealfish (*T. ishikawai*) (Tamura and Honma, 1971 b, 1972) and the Pacific ridley turtle (*Lepidochelys olivacea*) (Honma and Yoshie, 1975), post-spawning spent ovaries were secured histologically. These findings on the gonadal condition of the drift animals will be helpful in understanding their life history and mode of migration.

Since some indications of light starvation, such as absence of food particles in the digestive tube and existence of many zymogen granules in the exocrine pancreas, were assumed in the present specimens, not so severe deterioration including regression and atrophy was detected in the digestive tube and its associate glands, nor in the urino-genital organs, the spleen and some of the endocrine organs. These organs maintained still normal and healthy condition. Nearly identical situation was described in the young of the king-of-the-herring (Honma, et al., 1973). On the contrary, a heavily emaciated condition was seen in the spent dealfish indicating close resemblance of histological pictures in the spawning and/or post-spawning salmonid fish (Robertson and Wexler, 1960, 1962), and in the catadromous eel caught in the depth of the Japan Sea (Honma, 1966). In addition to these wild caught fish, a strikingly severe deterioration was reported on a single specimen of eel under the long-term starvation (Honma and Matsui, 1973).

A remarkable fat deposition in the liver of stranded triggerfish is noticed. The same was encountered in experimentally starved goldfish (Yamamoto, 1964), the fish during spawning migration (Robertson and Wexler, 1960, 1962; Honma, 1966; Tamura and Honma, 1971 a), and drift animals (Tamura and Honma, 1972; Honma and Yoshie, 1975). However, it is difficult to elucidate the exact cause of the fat deposition in the present specimens.

In relation to studies on many teleostean species (Ball and Baker, 1969; Sage and Bern, 1971; Chiba and Honma, 1974), the basophil cells in the PPD of the triggerfish hypophysis are diagnosed as gonadotrophs. Differentiation

and secretory activity of the gonadotrophs and the nucleus lateralis tuberis regarded as possible gonatotropic center of the hypothalamus (Billenstien, 1962; Honma and Tamura, 1965) revealed rather hypofunctioning state. This supports the fact that the gonads of the present specimens are in an immature condition.

A hyperplastic interrenal tissue (adrenal cortex) has been reported in the spent dealfish (Tamura and Honma, 1971 b, 1972). In the present triggerfish, however, it is uncertain whether or not a hyperactive condition of the corticotrophs is reflected in the interrenal tissue. Nearly normal picture of the spleen of the triggerfish is also in a striking contrast with the pathologic feature of that of dealfish (Tamura and Honma, 1971 b, 1972). On the other hand, the inactive thyroid and no pathologic endocrine pancreas are common to both cases.

It is important to note that neither the larval fish nor mature adult fish of Canthidermis rotundatus are found in the coasts of Niigata and its adjacent waters located comparatively high latitude of the west side of mainland (Honshu) of Japan (Tanaka, 1915; Honma and Kitami, 1967, unpublished data). Accordingly, the present immature triggerfish without severe derangement as well as the porcupinefish are considered to be the course of an abortive migration carried by the northward warm current. In order to add more accurate information on the vagrant animals, further examination based on more specimens should be carried out in comparison with the porcupinefish.

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(Contributions from the Sado Marine Biological Station, Niigata University, No. 258.)

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新潟海岸へ漂着したアミモンガラの器官組織

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日本海側の新潟海岸へ打上げられたアミモンガラの 新鮮標本3尾を用い、体内諸器官を組織学的に観察した。3尾共空胃であったが、消化管の粘膜上皮、膵外 分泌組織には退化の兆候は無く、肝への脂肪蓄積が著 しい程度で、腎や脾にも異状は認められなかった. 卵 巣は若い卵母細胞のみによって占められていたが、精 巣の大半は精原細胞巣よりなり、一部は精虫細胞に で進んでいた. 視床下部一下垂体系におけるゴモリ陽 性物質の量は、視束前核では細胞体によってまちまして であったが、神経葉には相当量が貯蔵されていた. しい 、隆起部外側核の酸好性神経分泌物の貯留は少たが、塩基好性の生殖腺刺激細胞と同定されるものは、 小形で顆粒に乏しかった. 以上の状態より推案する と、冬季対馬暖流に乗って日本海内部へ運ばれるアミ モンガラは、未熟の若魚で、生殖することなく死滅する無効分散の例であると思われる.

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