

## Histological Observations of Some Organs in the Porcupine Fish, *Diodon holacanthus*, stranded in Niigata on the Coast of Japan Sea

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**Abstract** The internal and endocrine organs of 8 specimens of the porcupine fish, *Diodon holacanthus*, were studied histologically. They were stranded on the beach of the Japan Sea in Niigata. Although they seemed to be in somewhat starved condition, no drastic emaciation was observed in the digestive tract, exocrine pancreas, kidney, and spleen, except for a large amount of fat deposition in the liver and regressive change in the thymus. The A cell considered to be a glucagon producing cell was predominant in the Brockmann body. The corpuscles of Stannius and the interrenal gland maintained healthy condition, while the thyroid gland showed a hypofunctioning state. The ovary was crowded with a number of previtellogenetic oocytes, whereas a large portion of the testis was occupied by the spermatogonia in addition to a small number of elaborate spermatozoa. A considerable amount of Gomori positive neurosecretory material was detected in the cells of the preoptic nucleus and pars nervosa. The putative gonadotrophs in the hypophysis contained only a small amount of glycoproteinaceous granules. Both the triggerfish *Canthidermis rotundatus* reported by Chiba et al. (1976) and the porcupine fish *D. holacanthus*, carried northward by the Tsushima warm current in winter and speculated upon as being immature fish on the way to an abortive migration, revealed a histologically similar condition.

Frequent occurrences of stranded marine teleost fishes and reptiles of tropical and subtropical origin on beaches at comparatively high latitudes along the coast of the temperate Japanese Islands have been reported by Honma and Yoshie (1975), Honma and Kitami (1976) and Chiba et al. (1976). This phenomenon is particularly remarkable on the beach of the west side of the mainland (Honshû) after heavy storms during the winter. The porcupine fish, *Diodon holacanthus* Linnaeus, is of particular interest from this point of view, and was extensively studied by Nishimura (1958a, b., 1960, 1973). However, histological studies for elucidating the physiological condition of these stranded animals are still scanty. We find such studies made only on the dealfish *Trachipterus ishikawai* by Tamura and Honma (1971, 1972), on the king-of-the-herring *T. iris* by Honma et al. (1973), on the Pacific ridley turtle *Lepidochelys olivacea* by Honma and Yoshie (1975), on the triggerfish *Canthidermis rotundatus* by Chiba et al. (1976), and on the crestfish by Honma and Tsumura (1980).

The present study on the porcupine fish is therefore designed to gain more knowledge about

the stranded animals. A comparison will be made with the triggerfish discussed by Chiba et al. (1976).

### Materials and methods

More than a hundred specimens (96 to 185, mean total length 137.5 mm), stranded on the sandy beach of Niigata City after heavy winter storms, were collected between 1975 and 1978. Eight of them were selected for conducting the present histological study. Records of the specimens, such as body size, gonad weight, date of collection and sexes, are given in Table 1.

The organs, chiefly internal and endocrine, were removed, immersed in Bouin-Hollande-sublimate solution, dehydrated with alcohol, and embedded in paraffin. Sections were cut serially at 7  $\mu$ m thick either sagittally or transversely and stained with various stainings such as Delafield's hematoxylin-eosin, Heidenhain's azan trichrome, aldehyde fuchsin (AF)-fast green-orange G, alcian blue (AB)-acid fuchsin, periodic acid Schiff (PAS)-fast green-orange G, AB-PAS-orange G, lead hematoxylin (PbH), and Hellman and Hellerström's silver impregnation. Moreover, Orth's solution was adopted

to demonstrate the chromaffin cells.

**Results**

**Stomach.** The stomachs were empty. The gastric mucosa was 30 to 43  $\mu\text{m}$  thick and appeared as winding folds. It consisted of mainly two types of PAS positive cells and the unfamiliar round cells (Fig. 1A). The round cells were smaller in size and fewer in number, and were characterized by possessing multistainable coarse granules, positive to PAS, eosin and aniline blue. A flat nucleus was placed near the base of each cell. The gastric gland was hardly visible, but no other degenerative sign was detected in the submucosa and muscle layers.

**Intestine.** The intestines were also empty except in the rectal portion, where a small amount of fecal material was present. The duodenum was formed by intricately deep folds of mucous membrane. The epithelial cell with an elliptic or oval nucleus was high columnar, 42 to 62  $\mu\text{m}$  in height (Fig. 1B). The striated border on the epithelium was distinct, and goblet and wandering cells were frequently encountered. The height and extent of folds in the mucous membrane of the convoluted part of intestine were less than those of the duodenum, although histologic make-up was nearly identical. Lymphoid tissues were found here and there in the intestinal submucosa. There was no appreciable change in the muscle layers. Thus, a rather healthy condition seemed to be maintained in the digestive tract.

**Liver and gall bladder.** The liver was a voluminous organ and a kind of hepatopancreas with scattered exocrine pancreatic tissues. The hepatosomatic index (HSI) is 9.5 for the male and 13.1 for the female (Table 1). The parenchymatous hepatocytes were closely packed around

the capillaries. Due to a large amount of fat deposition, the cell appeared as a marked vacuolization (Fig. 1C). A round nucleus was eccentrically placed in the cell. The amount of stromatous fibrous connective tissue was very small. The gall bladder contained bile in a tint of yellowish green. There was no noteworthy change in the bladder wall that consisted of a single layer of columnar epithelium and connective tissue capsule.

**Exocrine pancreas.** The exocrine pancreas was a diffuse organ scattered over the liver. Many isolated clusters in various sizes gathered around the hepatic veins. A number of zymogen granules were contained in the apical portion of the acinar cell. Contrary, a round nucleus with a conspicuous nucleolus was situated in the basal portion of the cell. The cytoplasm of the juxtannuclear region was intensely stained with hematoxylin (Fig. 1C).

**Thymus.** The thymus consisted of a pair of crescent-shaped bodies embedded in the musculature near the clavicles. The thickness of the thymus was about 350  $\mu\text{m}$ . The parenchymatous tissue was comparatively thin and roughly divisible into two portions: medullary zone rich in epithelial components and cortical zone with lymphocytes (Fig. 1D). The stromatous tissue extended throughout the thymic region. The epithelial components included the reticular, mucous and large round cells. The latter contained the eosinophil coarse granules with agents selectively positive to PAS and AF. The medullary zone was invaded with a small number of round cells, where clumps of small lymphocytes existed together with medium and large lymphocytes. Occasionally, the melanocytes, and possibly phagocytes and small lymphocytes with pycnotic nuclei, were also encountered. These

Table 1. Specimens of porcupine fish, *Diodon holacanthus*, used in this study.

Specimen	Sex	Total length (mm)	Body weight (g)	Gonad weight (g)	Gonosomatic index (GSI)	Hepatosomatic index (HSI)	Date of collection
1	♂	185	200	0.18	0.09	14.2	Nov. 30, 1975
2	♀	137	115	0.01	0.01	—	} Jan. 9, 1976
3		142	121	0.04	0.03	—	
4		132	112	0.02	0.02	—	
5		96	60	0.02	0.03	9.5	
6	♂	102	100	0.09	0.09	12.4	} Jan. 22, 1978
7		108	120	0.12	0.10	12.5	
8		115	130	0.15	0.08	13.1	

features described above showed a process of regression.

**Spleen.** The spleen was an ovoid body measuring 13.0 mm in length and 9.0 mm in short axes. The thin white and thick red pulps were roughly divisible (Fig. 1E). The white pulp containing the lymphoid tissue associated with the splenic arteries was scattered throughout the spleen. The red pulp as a relatively dense mass was occupied exclusively with erythrocytes. Other elements, such as possible phagocytes and clusters of pigment granules in brown color were seen in the parenchyma.

**Kidney.** The renal corpuscles and some segments of renal tubules of the kidney were surrounded by lymphoid tissue consisting mainly of small lymphocytes (Fig. 1F). The epithelial cell of the proximal segment was columnar with a rich granular cytoplasm of acidophil nature. A round nucleus with granular chromatin was cubic or conic with a moderate amount of granular cytoplasm. No abnormality was recognizable in the glomerular tuft. The clusters of pig-

ment granules were seldom encountered in the lymphoid tissue.

**Testis.** The testis was still thin, and was located in the dorsal region of the rectum. The vasa deferentia ran posteriorly on the dorsal surface of the testis and joined to form a common spermiduct running toward the cloaca. Having a delicate appearance and small value of gonosomatic index (mean 0.02) (Table 1), the testis was diagnosed as immature. Cross section revealed that seminiferous tubules were arranged in radial manner and joined to the vasa deferentia at their dorsal region. A major portion of the tubule was occupied by cysts of spermatogonia, while a small amount of elaborate spermatozoa and spermatids were occasionally seen in the proximal portion of the tubule and the narrow cavity of the vasa deferentia (Fig. 1G). The cysts containing the spermatocytes were also visible in the tubules.

**Ovary.** A pair of ovaries was joined at their caudal portion. The gonosomatic index indicated a very small value (mean 0.09) (Table 1).

Fig. 1. A: A portion of the stomach showing the folding mucous membrane. Notice two types of PAS positive cells, large and small. PAS-fast green-orange G stain.  $\times 190$ . B: Enlarged view of the mucous membrane of the duodenum. Numerous goblet cells in the mucous epithelium were found. PAS-fast green-orange G stain.  $\times 630$ . C: A portion of the hepatopancreas showing the exocrine acinar cells and the remarkably vacuolated hepatocytes. Hematoxylin-eosin stain.  $\times 440$ . D: Cross section of the thymus showing a marked diminution of the parenchymatous tissue. Hematoxylin-eosin stain.  $\times 220$ . E: A portion of the spleen showing the intermingled white and red pulps. Azan triple stain.  $\times 200$ . F: Cross section of the kidney showing no deteriorated condition. Notice the presence of a considerable amount of lymphoid tissue. Hematoxylin-eosin stain.  $\times 350$ . G: A portion of the testis showing spermatogenic cells in various developmental stages. Hematoxylin-eosin stain.  $\times 390$ . H: A portion of the ovary crowded with many oocytes in the early growth stages. Hematoxylin-eosin stain.  $\times 390$ . I: A portion of the thyroid gland indicating an inactive state. Hematoxylin-eosin stain.  $\times 230$ .

Fig. 2. A: Cross section of the interrenal gland. Notice the interrenal cells are arranged in cords or lobules and the chromaffin cells intervening among the interrenal cells. Hematoxylin-eosin stain.  $\times 730$ . B: A portion of the corpuscles of Stannius. The endocrine cells arranged in the vesicular cords are stained in various intensity with AF. AF-fast green-orange G stain.  $\times 900$ . C: A portion of the Brockmann body showing two types of cells: A (light) and B (dark). Azan triple stain.  $\times 900$ . Inset shows the argyrophilic D cells interspersed with the A cells. Hellman and Hellerström's silver impregnation.  $\times 900$ . D: Sagittal section of the preoptic nucleus of a female fish. Most of the cells are intensely stained with AF. AF-fast green-orange G stain.  $\times 370$ . E: The neurosecretory cells of the nucleus lateralis tuberis of a female fish. The perikaryon of each cell is thin and scanty in the stainable material. Azan triple stain.  $\times 660$ . F: A portion of the rostral pars distalis (RPD) of the hypophysis showing three types of cells: the thyrotrophs (t, dark in this picture), corticotrophs (c, light cells forming a palisade layer), and prolactin cells (p, closely packed). AF-fast green-orange G stain.  $\times 920$ . Inset shows the PbH stainable corticotrophs. PbH stain.  $\times 640$ . G: Sagittal section of the proximal pars distalis (PPD) showing the predominant somatotrophs (light) and the gonadotrophs (dark) in small group. AB-PAS-orange G stain.  $\times 920$ . H: A portion of the pars intermedia (PI) consisting of the PbH positive (dark) and negative (light) cells. PbH stain.  $\times 920$ .

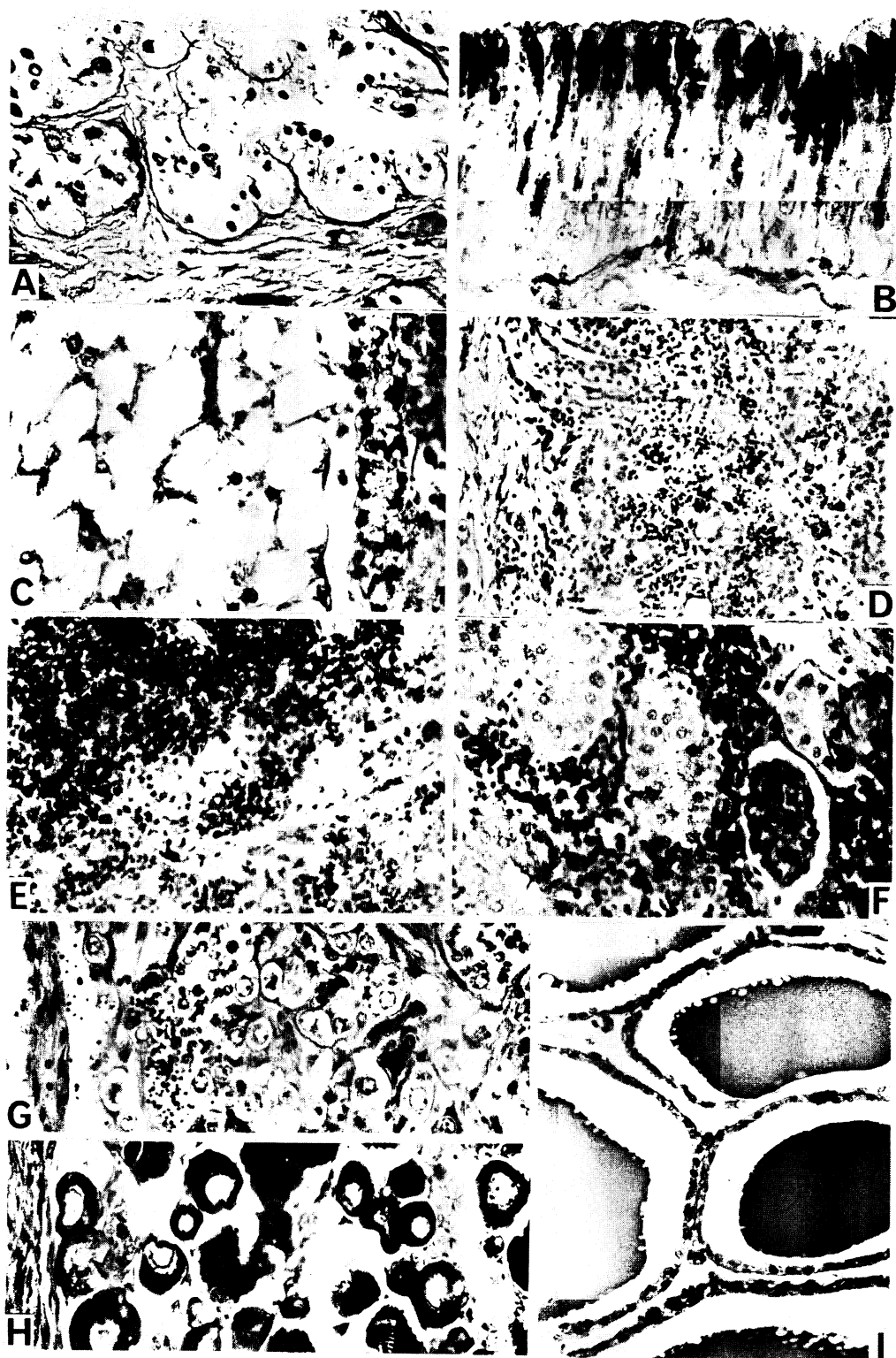


Fig. 1.

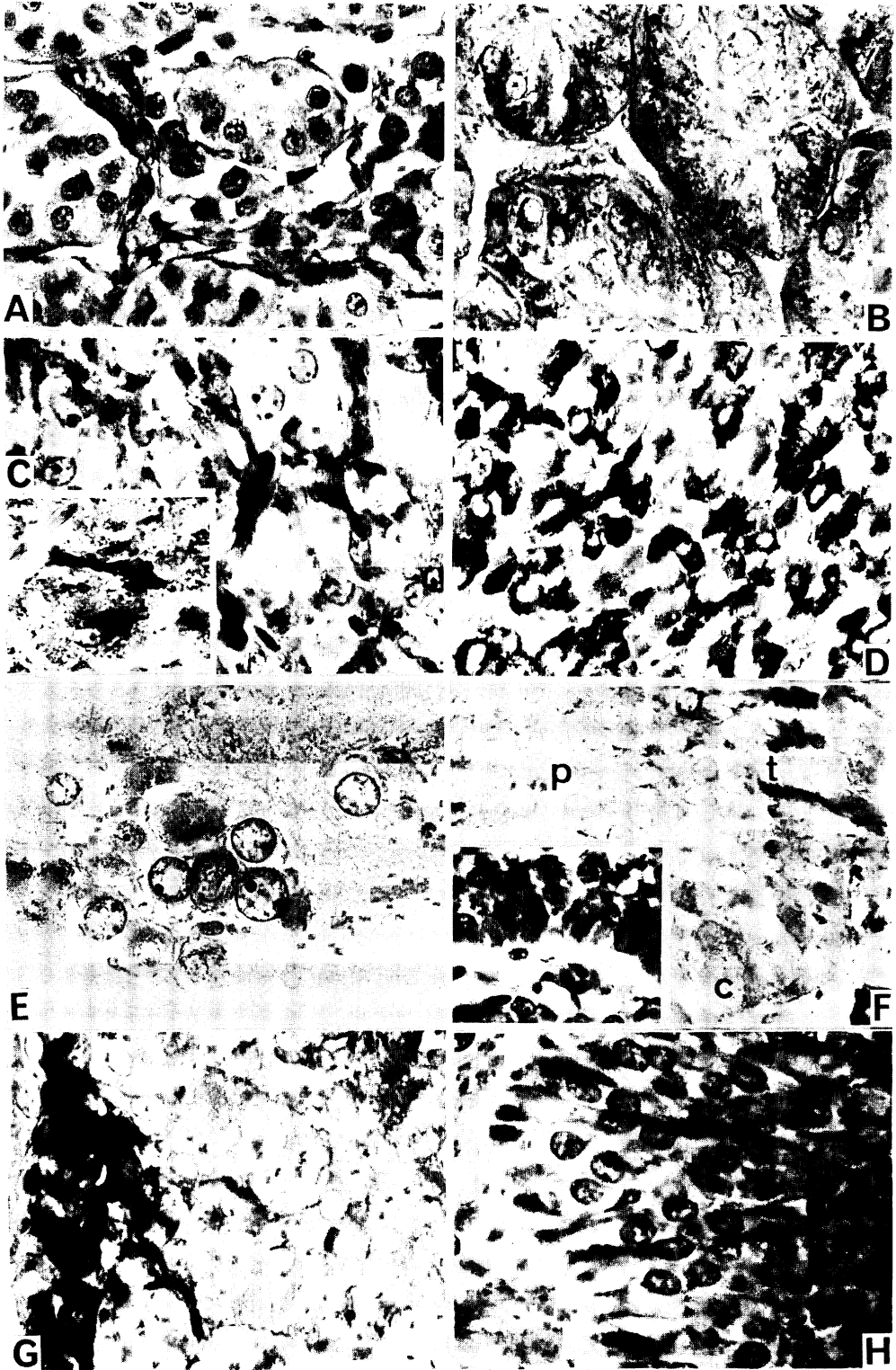


Fig. 2.

The ovigerous folds were crowded with a number of oocytes in their early stage of growth and minute nests of oogonia (Fig. 1H). The ovarian wall was thin, consisting of smooth muscles and fibrous connective tissue together with blood vessels. The oocytes, 100  $\mu\text{m}$  in diameter, were still in the perinucleolus stage and their ooplasm was stained intensely with hematoxylin. Accordingly, an extremely immature condition of the ovary was evident.

**Thyroid.** The thyroid gland existed on the dorsal surface of the ventral aorta as a considerably large, solid mass of the thyroid follicles surrounded by loose connective tissue and capillaries. Each follicle, round or ovoid in shape and 40 to 300 (mean 156)  $\mu\text{m}$  in diameter, contained a large amount of eosinophil colloid in the follicular lumen (Fig. 1I). The colloid was homogeneous, although small vacuoles were frequently seen in the peripheral portion. The follicular epithelium was uniformly thin and composed of flat to cuboid cells, 2 to 7 (mean 2.4)  $\mu\text{m}$  in height. There were a small amount of pale cytoplasm and a flat or ovoid nucleus. These features were indicative of a quiescent or hypo-functioning state.

**Interrenal gland.** The interrenal gland, a compact tubular organ richly supplied with capillaries, was located around the wall of the postcardinal vein in the cephalic portion of the kidney. The gland was accompanied by a very thin layer of lymphoid tissue, although the real head kidney was not produced near the gland. The interrenal cell, low columnar or fusiform in shape, contained finely granular cytoplasm of an eosinophil nature (Fig. 2A). Its round or oval nucleus has a prominent nucleolus. A small number of chromaffin cells were interspersed with the connective tissue between the masses of interrenal cells and, occasionally, in the wall of the postcardinal vein. The cytoplasm of the chromaffin cell appeared chromophobic and slightly foamy.

**The corpuscles of Stannius.** A pair of the corpuscles of Stannius were found on the dorsal portion of the ureter adjacent to the caudal vein. They were small and oval, about  $1.0 \times 0.7$  mm in size, and encapsulated with connective tissue. The glandular cells, fusiform or oval in shape, were gathered in the vesicular and anastomosing cords, which were separated from one another

by loose connective tissue containing capillaries. Most of the cells were stained moderately with AF, whereas the rest were hardly stained (Fig. 2B).

**Brockmann body.** The Brockmann body consisting of one principal body and several minute corpuscles existed on the mesentery adjacent to the gall bladder. The principal body, discoid in shape and 3 mm in maximum diameter, was encapsulated with connective tissue, which occasionally included a thin layer of exocrine tissue. Three types of cells, A, B and D were clearly distinguishable (Fig. 2C). The A cell, ovoid or round in shape and larger outnumbered the others, and was lightly stained with azocarmine and fast green. Its large and round nucleus had a prominent nucleolus. The fusiform AF positive B cell was small and intermingled with the A cells. The coarsely granular cytoplasm of the B cell was stained scarlet tint with azan trichrome. The argyrophil D cell was characterized by having a slender cytoplasmic process toward the basement membrane (Fig. 2C) and scattered solitarily among the A cells.

**Hypothalamo-hypophyseal system.** The nucleus preopticus (NPO) was located on both sides of the preoptic recess and composed of two contiguous portions, the antero-ventrally shifted pars parvocellularis (10.5  $\mu\text{m}$  in mean cell diameter) and the dorso-posteriorly shifted pars magnocellularis (22.0  $\mu\text{m}$ ). The NPO cell, polygonal in shape, contained a round or oval nucleus with a prominent nucleolus. Most of the cells had a perikaryon stained intensely with AF, while the others were devoid of stainable granules (Fig. 2D). The axonal fibers were indistinct. The nucleus lateralis tuberis (NLT) was situated in the postero-ventral region of the hypothalamus just on the antero-lateral part of the tuber cinereum. The polygonal cell of NLT was comparatively large and gathered in small groups (Fig. 2E). The cell had a round nucleus with a prominent nucleolus. The perikaryon of the cell was very thin and feebly stained with azocarmine. The axon was hardly demonstrable.

The hemispherical hypophysis was connected to the diencephalic floor by a slender infundibulum. The pars nervosa sent its complex ramifications deeply into the entire adenohypophysis. A considerable amount of AF stainable neurosecretory material, in the manner of

granules and also so-called Herring bodies, was laden in the pars nervosa. The rostral pars distalis consisted of three main types of cells (Fig. 2F): the PbH stainable cells diagnosed as the corticotrophs, the basophils of possible thyrotrophs located adjacent to the corticotrophs, and the small acidophils of prolactin producing cells. The proximal pars distalis included two types of cells: the dorsally shifted acidophils equivalent to the somatotrophs and the ventrally shifted basophils identical with the gonadotrophs (Fig. 2G). The somatotrophs bordering the pars nervosa were arranged in one or two layers and occupied a major part of this lobe. The small gonadotrophs were gathered in compact cell masses. In addition, clusters of the putative gonadotrophs containing only a small amount of glycoproteinaceous granules were occasionally seen in the marginal portion of other glandular lobes. The pars intermedia was composed of two types of cells: PbH positive and negative cells (Fig. 2H). The former was clavate in shape and extended its basal projection toward the pars nervosa. The latter with homogeneous cytoplasm was low columnar or oval in shape, and concentrated toward the basement membrane.

#### Discussion

The porcupine fish occurring en masse in the Japan Sea had been treated as young or immature fish based mainly on their body size (Nishimura, 1958a, b, 1960, 1961; Tabeta, 1972). Our histological observations have confirmed this previous assumption. However, relationships between the size and maturity in the porcupine fish are still obscure. Sakamoto and Suzuki (1978) reported that in captivity the total lengths of the parental fish which can successfully spawn were 176~259 mm in the male and 210~231 mm in the female. These fish had been kept in aquaria for one or two years since their collection from the Japan Sea coast of Wakasa Bay and the Noto Peninsula. Therefore, according to Nishimura (1961), the fish used in Sakamoto and Suzuki's (1978) study must have been yearlings or older. The gonadal condition of other stranded animals reported by us was variable; the king-of-the-herring *Trachipterus iris* (Honma et al., 1973) and the triggerfish *Canthidermis rotundatus* (Chiba et al., 1976) were sexually imma-

ture, whereas the dealfish *Trachipterus ishikawai* (Tamura and Honma, 1971, 1972), the Pacific ridley turtle *Lepidochelys olivacea* (Honma and Yoshie, 1975), and the crest fish (Honma and Tsumura, 1980) were mature. Moreover, recently a sea snake, *Pelamis platurus*, bearing four fetuses just before parturition was caught off Sado Island in the Japan Sea (Honma et al., in press).

As pointed out previously in reporting the stranded *Trachipterus iris* (Honma et al., 1973) and *Canthidermis rotundatus* (Chiba et al., 1976), no drastic deterioration or atrophy was detected in the digestive tract and its associate glands of the stranded porcupine fish studied. However, a heavy emaciation was noted in the drift-spent dealfish by Tamura and Honma (1971, 1972).

Histological observation of the gonads, the gonadotropic cells in the hypophysis and the thyroid gland revealed that the porcupine fish examined were all sexually immature with an inactive thyroid gland. A similar condition has already been described for *Trachipterus iris* by Honma et al. (1973) and *Canthidermis rotundatus* by Chiba et al. (1976). Except for a regressive change in the thymus, no other notable changes were recognized in the lymphopoietic organs and tissues. However, it is impossible to explain the significance of thymic regression in the porcupine fish without examining specimens at other seasons and ages as conducted by Tamura (1978).

It is rather rare to find a large stranded porcupine fish in the summer (Honma, unpublished record), and neither larvae nor fully ripe fish have ever been obtained on the coast of Niigata and its adjacent waters located in comparatively high latitudes on the west side of mainland Honshū, Japan (Nishimura, 1958a, 1960). Consequently, we would like to suggest that immature porcupine fish without severe derangement as well as the triggerfish reported previously by Chiba et al. (1976) from the coast of Niigata are on the way to an abortive migration, being carried northward by the warm current. To elucidate further, examination of fish in various developmental stages is desirable.

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#### 新潟海岸へ漂着したハリセンボンの器官組織

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日本海側の新潟海岸へ多数漂着するハリセンボンの新鮮標本8尾を用い、各種器官を組織学的に観察した。いずれも軽い飢餓状態にあったが、消化器官、腭外分泌組織、腎臓、脾臓には異常は認められなかった。しかし、肝臓への脂肪蓄積が著しく、胸腺は退化状態にあり、ブロックマン小体にはグルカゴン産生細胞が優勢で、甲状腺は機能低下状態を示した。一方、間腎腺とスタニウス小体は正常と目された。卵巣は卵黄形成前の若い卵母細胞によって占められていたが、精巣の大部分は精原細胞よりなるものの、ごく少数の精子もみられた。視床下部神経葉には相当量の神経分泌物が検出されたが、腺性下垂体の生殖腺刺激細胞はまだ小さく、染色性に乏しかった。冬季に対馬暖流によって日本海の高緯度地域まで運ばれるハリセンボンは、前報(Chiba et al., 1976)したアミモンガラ同様に未熟の若魚で、死滅回遊の過程にあると思われるもので、ほぼ同様の組織像を示していた。

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