Histological Observations on Some of the Endocrine Glands in the Remora, *Echeneis naucrates* L., Caught off the Coast of Sado Island in the Japan Sea*

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Summary. Endocrine organs, such as the hypophysis, thyroid, endocrine pancreas, ovary and testis, of two specimens of the remora, *Echeneis naucrates*, were studied histologically to gain some knowledge about comparative anatomy and systematics. The specimens, one female and one male, were caught off the coast of Sado Island in the Japan Sea.

Among four components of the hypophysis, the well developed pars intermedia (PI) is the largest and located in the ventral region of the gland. The PI consists chiefly of two types of glandular cells which are stained distinctly by a combination of dyes. The prolactin, adrenocorticotropic, gonadotropic, somatotropic and thyrotropic cells are also differentiated in the pars distalis.

The thyroid gland, as a compact mass, indicates a fairly hyperactive state. The Brockmann body is composed mainly of three types of cells: A, B and D cells. It is characteristic of this species that each endocrine cell of the body gathers to form large clusters. Both ovary and testis were spent, but a number of young germ cells in various developmental stages were seen. The systematic position of the remora is discussed in comparison with other bony fishes.

The remora or suckerfish belonging to the order Echeneida is well known as having a peculiar sucking disk derived from a modified dorsal fin to attach the animal to larger fishes and turtles. Accordingly, the details of the structural pattern and development of this special organ located on the top of the head have been investigated by several workers (HOUY, 1909; TANING, 1926; BARGMANN, 1973), but histological studies of the internal organs, especially the endocrine and hemopoietic organs, have not been carried out up to the present.

On the other hand, there have been some discussions concerning the systematic position of the remoras: a blood relationship between this group and the cobra (*Rachycentron canadum*) with regard to the resemblance of morphology of the young of the two species, and a similarity to the butterfish family (Stromateidae), in particular *Paliurichthys*, in their internal organs and behavior (GUDGER, 1926; GREGORY, 1959, etc.). In addition, TAKAMATSU (1967) reported a cobra accompanying a large stingray (*Dasyatis maculatus*) in a large circulating channel tank of the Oita Ecological Aquarium. He mentioned that the behavior of this cobra was intermediate between that of the pilot fish (*Naucrates sp.*) and the remora.

On the occasion of preparing the preserved specimens of the sucking disk of the

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remora that were provided for Prof. W. Bargmann at Kiel University, histological observations were undertaken to elucidate the structure of the endocrines, since we had been planning to examine these organs. If special attention will be given to this field, it will be useful to learn the comparative anatomy and systematics among the related fish families, such as cobia, pilotfish and butterfish. Unfortunately, however, no fresh specimens of these species were secured. In spite of this deficiency, the results obtained are described in this paper with special regard to the histology of the pituitary gland.

Material and Methods

Two examples of comparatively large remora, *Echeneis naucrates*, 49 and 50 cm in standard length that were attached to the sailfish, *Istiophorus platypterus*, were caught on July 31, 1972 in a set net. This net was set up off the coast of the Sado Marine Biological Station, Niigata University, located on the west coast of Sado Island in the Japan Sea. Unexpectedly, these were male and female, and were kept in the aquarium of the Station. On October 21, dissection was performed after decapitation, and the brain with hypophysis, thyroid, digestive tube with accessory glands including pancreas, and gonads were removed and immersed in Bouin-Holland-sublimate solution after the external features were observed macroscopically. Small pieces of these organs were embedded in paraplast, cut serially 6 to 8 µ thick in sagittal and transverse directions, and stained by various stainings, such as azan trichrome, PAS, PbH, AF-azan, AF-light green and orange G, PbH-PAS, PAS-light green and orange G, Delafield's hematoxylin-eosin, Hellman and Hellerström's silver impregnation, and azan after being oxidized by dilute potassium permanganate.

Results

Hypothalamo-hypophysial system

Due to the large sucking disk, the head of the remora is much depressed. Since the membrane bone of the cranium is very thin and translucent, the brain is easily seen from outside. The brain is enveloped entirely in a tight membrane of fibrous connective tissue, and the hypophysis is also buried in this connective tissue. The chiasma opticum is thick and stout, and the hypophysis is attached to the floor of the diencephalon near the base of the chiasma. Behind the hypophysis, there is a small saccus vasculosus, ovoid in shape and highly vascularized.

The neurosecretory cells of the nucleus preopticus on either side of the preoptic area are comparatively well developed (Fig. 1). The dorsally shifted pars magnocellularis and ventrally shifted pars parvocellularis cannot be distinguished, because these are continuous with each other. A few of the pars magnocellularis cells lie scattered posteriorly along the ventricles: the cells lying in the more posterior part tend to be larger in size than in the anterior part. Most of the AF stainable material in the cell body, in particular the cell of the pars parvocellularis, is coarse granular or depletive, and only a few cells stained deeply with AF are seen. Near the area of the preopticus, there are found a number of AF stainable axons in many directions. So-called beaded fibers and the large colloid droplets in reddish tint scattered here and there are also seen in these axons (Fig. 2). Noticeably, some of the axons once
Fig. 1. Part of the nucleus preopticus of the female remora, *Echeneis naucrates*. Depletion of the AF stainable material from some of the neurosecretory cells is seen. AF-light green-orange G stain. ×560

Fig. 2. Part of the nucleus preopticus of the female fish showing so-called beaded fiber bearing AF stainable material. AF-light green-orange G stain. ×560

Fig. 3. Part of the RPD of the female fish to show the dark PbH positive corticotropic cells (C) bordering the neural elements (N) and densely packed small cells (=prolactin cells) (P) negative to PbH. PbH stain. ×560
Fig. 4. Part of the PPD of the male fish. In the center of this picture, is a weak basophilic cell mass diagnosed as gonadotrophs (G). The chromophobic cells bordering the gonadotrophs are the somatotrophs (S). AF-azan triple stain. ×450

Fig. 5. Enlarged view of the basophil island of the female fish showing the cell with relatively rich cytoplasm. AF-azan triple stain. ×1,400

Fig. 6. Part of the PPD showing AF (and also PAS) weak positive cells located near the neural elements (N). These cells in wedge shape are diagnosed as thyrotrophs (T). AF-light green-orange G stain. ×450

Fig. 7. Part of the PI consisting of two types of large cells: polygonal, deep basophils (B) and low columnar, deep acidophils (A). Coal-black material in this picture is the AF positive neurosecretory substance (Ns) stored in the neurohypophysial branches. AF-azan triple stain. ×500
ran toward the cephalic portion, and then inclined to the caudal. In the nucleus of the cell body of this nucleus preopticus, a large prominent nucleolus, acidophilic in nature, is recognized, though the chromatin granules are not so clear.

On the other hand, the cells of the nucleus lateralis tuberis located on either side of the margin of the hypothalamus are comparatively small and numerous, and their cytoplasm has a weak affinity to acidic dyes. Rarely, larger cells with rich cytoplasm are seen. Their contour is somewhat irregular, but no cell with a remarkably wavy margin and/or a lobate nucleus is encountered.

The hypophysis is nearly hemispherical in shape measuring 1.8 mm in length and 2.0 mm in width, and is classified roughly as a dorso-lepto basic type. Although the recessus infundibuli is not so deep and narrow, the point of invasion is situated near the anterior to the central portion of the hypophysis. The pituitary stalk is somewhat short and thin containing both AF stainable and acidophilic axons.

The hypophysis is composed of four components: the rostral pars distalis (RPD), proximal pars distalis (PPD), pars intermedia (PI) and pars nervosa (PN) projecting largely into the PI. The PI is well developed and is the largest among the glandular parts, and the volumes of the RPD and PPD appear to be almost equal. However, in either side of the hypophysis, the PPD covering the dorsal part of the PI extends markedly, reaching the level of the posterior end of the PI. Although the pars nervosa sends its highly complicated ramifications into all parts of the adenohypophysis, the neural processes containing AF stainable material are exclusively involved in the PI. Therefore, a large amount of the AF stainable material derived from the nucleus preopticus is stored in the region of the PI. On the contrary, the pars distalis
is invaded by the processes stained with acidic dyes, and the manner of ramifications is more complicated in the RPD than in the PPD.

The margin of the ventral portion of the RPD is occupied mainly by densely packed acidophil cells, the prolactin cells (Fig. 3). These cells, rounded in shape and small in size, have a round nucleus with a prominent nucleolus. In the dorsal region of the RPD, one or more layers of cell cords bordering the neural elements are seen in remarkable convolution (Fig. 3). These cells, high columnar in shape and diagnosed as the corticotrophs, contain an apically placed nucleus, and PbH positive materials are concentrated toward the basal region of the cells. Accordingly, it is easy to distinguish two types of cells in the RPD.

The PPD is situated just beneath the recessus infundibuli in the mediosagittal plane. It is not difficult to discriminate this part from the RPD and PI, in spite of the absence of marked connective tissue septa bordering these glandular parts. The major portion of the PPD is occupied by small chromophobic cells with an indistinct outline. These densely packed cells are regarded as somatotrophs (Fig. 4), and the cell mass of the lateral portion of the PPD extends toward the posterior end of the gland covering the PI. Between the somatotrophs and the RPD, there is a cell mass consisting of AF weak positive and also weak basophilic cells (Fig. 4). These comparatively large cells are roundish polygonal in shape with relatively rich, homogeneous cytoplasm (Fig. 5), and also show a positive response to PAS reaction. They are therefore diagnosed as gonadotrophs. In the dorsal region of the PPD that borders the neural elements, sharp, wedge shaped cells are dispersed (Fig. 6). These cells, corresponding to the thyrotrophs, are weak basophilic and weak PAS and AF

Fig. 9. Section of a small Brockmann body surrounded by the thin layer of exocrine pancreas (E). Masses of the AF positive B cells (B) are surrounded by the A cells (A). AF-light green-orange G stain. × 450
positive, and the stainable granules are distributed toward the basal region of the cell.

The PI is composed of two types of distinct cells which are stained separately and differentially as deep basophils and acidophils in various kinds of dyes (Fig. 7). The basophil is polygonal in shape, and is the largest in size among the glandular cells, while the acidophil is low columnar or polygonal, and the size of the latter is large next to the former. If oxidation is performed before azan or azan-AF stains, the color tone of both cells is more brilliant and distinct, a beautiful blue and orange,
respectively. The nuclei of both types of cells are shifted to the corner of the cells with rich cytoplasm containing fine granules. By light green and orange G stains, the former shows pale green, and the latter orange green tints. Further, the basophil is stained with PbH, and the acidophil reacts positively to PAS. Noticeably, since the basophilic islands of the PI are scattered over the acidophil masses, this appears to be the gonadotrophs' islands of other species of bony fishes.

**Thyroid gland**

The main part of the thyroid gland located on the dorsal surface of the ventral aorta at the bifurcation of the first branchial artery is a considerably compact mass, a flat disc measuring 3 mm in length and 4 mm in width. The gland is enveloped in fibrous connective tissue that is surrounded by loose adipose tissue. The amount of the connective tissue in the interfollicles is relatively small, and the features of the gland are more similar to those found in the higher animals rather than to those of teleosts (Fig. 8). Each follicle, $63 \times 88 \mu$ in mean, is visible to the naked eye, and the largest one attains $242 \mu$ in the longer axis. The shape of the follicle is variable: ovoid, long ovoid, pear-shaped and distorted, but generally the smaller ones are oval to round.

The epithelial cell, columnar in height (mean $8.7 \mu$), contains the PAS positive granules gathered toward the apical portion while the cytoplasm near the basal portion is stained deeply in acidic dyes (Fig. 8). The nucleus is ovoid in shape, and is located in the basal portion of the cell. The colloid in the lumen has a strong positive response to PAS reaction, and shows reddish purple color. The colloid detached from the follicular epithelium shows a wavy outline with some vacuoles (Fig. 8). Sometimes, clumps of liquefied colloid of a bluish purple color are detected. In hematoxylin-eosin stain, on the other hand, the apical portion of the cell has no affinity with dyes showing vacuolization, and the colloid in the lumen is stained weakly with eosin. The picture described here seems to be the active state from a histological criterion.

**Endocrine pancreas**

The exocrine pancreas lies on the ventral surface of the digestive tube from the
cephalic level of the pyloric region covered with the hepatic lobe to the caudal level of the posterior tip of the spleen. The bodies of Brockmann in various size are arranged along this cord-like pancreas either contiguous to each other or separated in islands. The number of the bodies in each specimen is 6 to 10. Roughly speaking, each body surrounded by the thin layer of exocrine acini is composed of three types of cells, A, B, and D, with an occasional detection of another unknown type of cell (Fig. 9). It is noted that these three main elements are gathered in large masses or clusters (Fig. 10).

The B cells, elongate in shape, are stained deeply in orange G and are also positive to AP. The nucleus is located in the basal portion of the cell, and the affinity of the cytoplasm for dyes near the nucleus is stronger than in the apical portion. The A cells, short and thick in shape, contain coarse granules stained by azocarmine, and the nucleus is also situated in the basal portion. There is found a striking cell mass which is stained in an orange-brown tint rather than pale blue by azan trichrome, and in brilliant light green by AF-light green and orange G. A small number of free cells of this nature are also detected. After the AF-light green-orange G stained preparations were decolorized with bleach, they were silver-impregnated, and it
turned out that only the cells tinged in light green were argyrophil. Therefore, these cells were diagnosed as the D type (Fig. 11). Among the masses of the A cells, small but polygonal cells with indistinct outlines are sometimes seen. These cells are usually chromophobic or only pale bluish orange, and are named tentatively C cells. Necrotic cells with a colloid formation are seldom encountered in the larger Brockmann body.

**Ovary**

In the female fish a pair of ovaries in contact with each other are located in the region of the rectum. They measure 51 and 56 mm in length respectively and are covered by a thick capsule. The egg grains are easily recognized by the naked eye. Histologically, the eggs mainly represent the yolk vesicle and perinucleolus stages (Fig. 12). Although the diameter of the eggs attains 90 to 120 μm, the ovary is not crowded by these eggs. Various developmental stages of the oocytes in the perinucleolus stage are found, such as oocytes with lampbrush chromosomes, with an ooplasmic circular zone, and with heterogeneous ooplasm. In addition, a small number of ovulation scars in heavy regression are encountered (Fig. 12), so that the present specimen is diagnosed as having spent ovaries.

**Testis**

In the male fish, a pair of testes facing each other are also located in the region of the rectum. They are club-shaped, measuring 55 mm in length. In cross sections, it is evident that the seminiferous tubules are arranged and directed toward the concavity of the central portion of the bean-shaped testis, i.e., the vasa deferentia, to form radial routes (Fig. 13). Each tubule is separated by a thick septum, consisting of the collagenous and muscle fibers, derived from the tunica albuginea. In the vasa deferentia and also the seminiferous tubules near the vasa deferentia, a considerable amount of relict sperm and scattered phagocytes are found (Fig. 13). However, the distal portion of the tubule is occupied by cysts in various developmental stages, from primary spermatogonia to spermatids (Fig. 14). In the portion containing the relict sperm, a layer is seen of the seminiferous epithelium that is lined with the tubular wall. Therefore, the present specimen is also diagnosed as having spent testes.

**Discussion**

We have often proposed that the knowledge of comparative anatomy and ontogenesis of the endocrine organs is important and useful with regard to the consideration of phylogeny of ichthyoform animals (Fujita and Honma, 1966; Honma, 1966, 1969, 1974). It is difficult to discuss the blood relationship between the remora and other species, such as cobia and butterfish, because there has been no chance to examine the latter two species up to the present. Although we have described some peculiarities in several organs, especially the hypophysis of the remora, the hypophysial structure corresponding to that of the remora was not encountered in the examined species of Japanese fishes (Honma, 1960; Kawamoto, 1967). It can only be said that the histological design of the remora is somewhat identical to that of the Korean snakehead fish (Channa argus), in particular, the well-developed and spacious PI that occupies the ventral region of the PPD except in the medio-sagittal plane. In spite of some similarities, the shape and staining properties of the glandular cells
of the *Channa* differ from those of the remora (Honma, 1960; Kawamoto, 1967). As some of the structural patterns of the hypophysis of the remora are also similar to those of the frigate mackerel (*Auxis tpeinosoma*), further studies should be carried out to discuss this situation (Honma, 1960). In brief, although the fishes of the order Echeneidae are placed in the neighborhood of the order Cottidae by many systematists, the present examination reveals that the hypophysis of the remora may not correspond to that of the fishes belonging to the Cottiformes and Pleuronectiformes.

The thyroid gland of the present remora, like that of bonitos and sailfish (order Scombroidei), is a highly compact organ rather similar to that of higher vertebrates (Honma, 1956a, b, c; 1960). The histologic picture described here, i.e., the high columnar epithelial cell with a basal shifted round nucleus, and PAS positive granules near the apical surface of the cell, may be diagnosed as the ingestion of colloid droplets into the epithelial cell based on active hydrolysis of the colloid in the lumen. Moreover, the fact that the cytoplasm adjacent to the nucleus was stained deeply may correspond to the active synthesis of the proteinous granules in relation to the well-developed endoplasmic reticulum and Golgi apparatus near the nucleus as was previously observed electron microscopically by us in other bony fishes (Fujita, Suemasu and Honma, 1966; Suemasu, Honma and Fujita, 1968).

On the other hand, it is of interest to find that Schlumberger and Luke (1948) described 16 cases of thyroid tumors found in the remora, *Echeneis naucrates*, reared for a considerable time in the aquarium. They surmised that the goiters may be a response to some metabolic idiosyncrasy of the remora, because they were never encountered in any other fishes kept under identical conditions.

There is no great difference between the cyto-histological evidence of the endocrine pancreas (Brockmann bodies in this case) of the remora and other bony fishes reported previously. However, it is of interest to know the disposition of the three major components of the cells. Even in the D type, the cells form masses or clusters, the situation of which has not been described in other bony fishes (Brinn, 1973; Epple and Lewis, 1973; Hirano and Honma, 1971, 1972; Honma and Tamura, 1968; Watari, Tsukagoshi and Honma, 1970; Watanabe, 1960; Kobayashi and Takahashi, 1974, etc.).

As a result of checking the ovaries and testes of the present remora, it is determined that both were spent. We have no information concerning the exact breeding season of this species, but the recovery condition of the gonads permits us to surmise that the remora may be able to spawn twice or more during its life-span.
て軸部に生殖腺刺激細胞、成長ホルモン分泌細胞および甲状腺刺激細胞が検出される。
甲状腺はマグロやカジキと同様に、充実した器官で、かなり機能が亢進している像を示した。

脾内分泌組織はA, B, D細胞の3種の細胞から構成されており、数個の独立したブロックマン小体となって配列している。そして好銀性のD細胞が互いに集まって塊状をなしていることに一大特色がみられる。

卵巣には染色体の個数の卵黄胚位の卵巣細胞のほかに、排卵卵が認められた。一方精巣の細精管には精子形成過程の精巣細胞が、そして精管には残存精子がみられた。したがってこの雌雄両魚ともに、すでに放卵放精した個体であることが明らかにされた。

References


