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Comparative Histology of the Leydig and Epigonal Organs in Some Elasmobranchs

Yoshiharu Honma, Kazuyuki Okabe and Akira Chiba

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Abstract Species differences of external features and cellular components of the Leydig and epigonal organs in the elasmobranchs were studied macroscopically and histologically using 5 species of Japanese fishes. The mutual relationship between the epigonal organs and the gonads is roughly divided into 3 types, while no noticeable difference is detected in the Leydig organs among 5 species. As the posterior lobe of the epigonal organ is elongated to reach the area of rectal gland, the term "subgonal" is proposed to the cases of smoothhound and its relatives. Both organs consisted of many eosinophilic granulocytes in various immature stages, and a small number of heterophilic granulocytes, lymphocytes and plasma cells. The cells with large vacuoles originated from the granulocytes in the process of degeneration were also found in addition to some lymphoid and/or blast cells. Hassall's corpuscle-like structures were infrequently demonstrated in both Leydig and epigonal organs of the sting ray and skate. The presence of lymphomyeloid cells in both organs may support their possible role in the immune response and the process of inflammation.

In the elasmobranchiate fishes without bone marrow and lymph nodes, the Leydig organ around the esophageal wall has been known as one of the peculiar and large lymphoid organ (Leydig, 1857; Bolton, 1927). Another outstanding lymphoid organ has also been discovered and named as the epigonal organ which exists over the ovary and testis (Matthews, 1950). Histological observations of these two hemopoietic organs were made by several investigators using the European and American species (Fänge, 1968; Zapata, 1981; Fänge and Mattisson, 1981; Mattisson and Fänge, 1982; Fänge and Pulsford, 1983). On the other hand, in Japan, there were only two papers with a brief description on 2 species (Kanesada, 1956; Oguri, 1983), and suprisingly, the specialists of elasmobranchiate fishes have mentioned that the epigonal organ is a part of reproductive systems (Mizue, 1978; Teshima, 1978, 1981). Gohar and Mazhar (1964) and Fänge and Mattisson (1981) reported that either of the two organs is lacking in several species, and holocephalians, another subclass of Chondrichthyes, lack both organs (Stahl, 1967). Therefore, this study was undertaken to define the comparative anatomy and cellular structure of both Leydig and epigonal organs in Japanese elasmobranchiate fishes.

Materials and methods

Three species of sharks, the smoothhound (*Mustelus manazo*), banded dogfish (*Triakis scyllia*) and cloudy dogfish (*Scyliorhinus torazame*), 1 species of sting ray (*Dasyatis akajei*) and 1 species of skate (*Raja porosa*) were used in this study. They were collected in the coastal waters in the vicinity of Sado Marine Biological Station, Niigata University, located on the northwestern part of Sado Island in the Japan Sea. From May to October, 1982, more than 30 specimens in various stages of growth and maturation were collected, consisting of both sexes and ranging from 30 to 130 cm in total length.

After measurements of fish body, both Leydig and epigonal organs were excised, and these external features were observed macroscopically with respect to the disposition of other organs. The epigonal organ was weighed in order to make comparison with the weight of spleen.

For light microscopy, pieces of both organs were immersed in Bouin's fixative, dehydrated through alcohol or butanol series, and embedded in paraplast. Serial sections were made at a thickness of 8 μm, and stained with hematoxylin-eosin, azan trichrome, May-Grünwald or routine Giemsa. For electron microscopy,

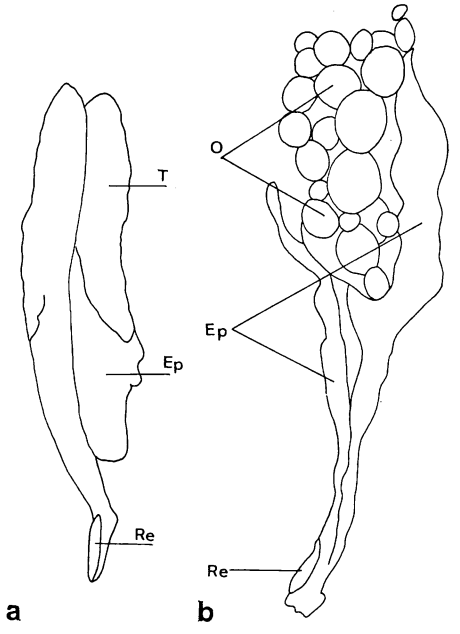

Fig. 1. Diagram of the epigonal organ of smoothhound, *Mustelus manazo*. a, male; b, female. T, testis; O, ovary; Ep, epigonal organ; Re, rectal gland.

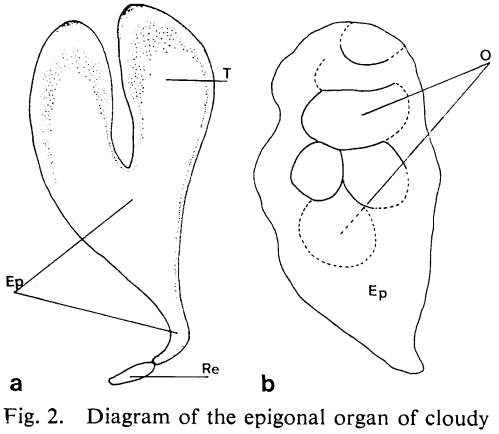

Fig. 2. Diagram of the epigonal organ of cloudy dogfish, *Scyliorhinus torazame*. a, male; b, female.

tiny pieces were first immersed in 2% glutaraldehyde solution in 0.1 M cacodylate buffer (pH 7.4) for 2 days, and postfixed in 1% OsO_4 in the same buffer for 2 hours. The specimens were dehydrated and embedded in epon 812. Semithin sections (1 μm thick) were stained with toluidine blue and observed with a light microscope. Ultrathin sections were stained with uranyl acetate and lead nitrate, and observed with a Hitachi H-500 electron microscope.

Results

Macroscopic observations. In the present examination all 5 species of both sexes possess Leydig and epigonal organs. Above all, the development of the epigonal organ is great, the relative weight of which to body weight is 0.68–6.43%, while the weight of spleen, which is a comparatively large hemopoietic organ in the elasmobranchs, is 0.30–1.25%.

Leydig and epigonal organs are both white in color, and are easily recognizable by the naked eye. The feature of contact of the epigonal organ with the gonads were somewhat different from species to species, but were classified roughly into 3 types.

1. Smoothhound type. The smoothhound and the banded dogfish belong to this type. In both species, the epigonal organs are connected with the posterior part of gonads and projected further to the rectal gland. As the left ovary of the smoothhound became atrophied and degenerated with the growth of fish, the development of epigonal organ is synchronized to this process, i.e., regression in the left lobe of epigonal organ is prominent. However, such sexual difference was not seen in the banded dogfish. Thus, it is suggested by the appearance of this type that the term epigonal should be replaced by the subgonal (Fig. 1a, b).

2. Sting ray type. The sting ray and the skate belong to this type. The epigonal organs envelop entirely the ventral surface of both gonads, and parts of the organs reach and cover the dorsal surface. However, the tip of the organ does not project caudally across the posterior end of the gonads. There are no morphological and sexual difference between both species and both sexes.

3. Cloudy dogfish type. The general feature of this type appears the intermediate between the type 1 and type 2. The epigonal organ covers the gonads and tip of epigonal organ extends and reaches to the level of the rectum (Fig. 2a, b).

Histological observations

Light microscopy. The Leydig organ of every species existed beneath the mucous membrane

Fig. 3. a, Leydig organ cross-sectioned throughout the esophagus of a sting ray, *Dasyatis akajei*. The organ (L) consists of two halves. Azan trichrome. ×10. b, epigonal organ of female smooth-hound showing the eosinophilic granulocytes and the granuloblasts. H-E stain. ×1,500. c, epigonal organ of a male banded dogfish, *Triakis scyllia*. It consists of various types of cells. Toluidine blue stain. P, plasma cell. ×1,000. d, epigonal organ of a male cloudy dogfish showing a mass of lymphocytes. H-E stain. ×1,500. e, a Hassall's corpuscle-like structure demonstrated in the epigonal organ of a sting ray. Azan trichrome. ×1,000.

of the esophagus, and is divided into two parts: dorsal and ventral patches. However, the grade of partition is variable among the species. In the sting ray two distinct lobes are seen, whereas in the banded dogfish each lobe is further subdivided into several lobules (Fig. 3a).

The histological designs of Leydig and epigonal organs are similar to each other, and the pictures of these two organs apparently resemble those of the bone marrow of higher vertebrates (Fig. 3b). However, both organs peculiar to elasmobranchs are different from the latter in lack of the erythroblasts and fat cells. The parenchyma of both organs, supplied by a small quantity of capillaries, consisted mainly of leucocytes in various maturing stages. Most of the leucocytes are eosinophilic granulocytes having the acidophilic (=eosinophilic) granules and a round, oval or somewhat indented nuclei (Fig. 3b). Noticeably, almost all leucocytes are in the immature stages belonging to the myelocytes and metamyelocytes.

Other components of the parenchyma are a small number of cells with basophilic cytoplasm and a comparatively large and round nucleus. However, it was difficult to decide whether these cells are granuloblasts or lymphoblasts by the aid of routine light microscopy. The semithin section (and also ultrathin section) revealed that they are plasma cells characterized by well developed rough endoplasmic reticulum (ER) and an eccentrically located spherical nucleus with heterochromatin attached in coarse clumps to the inner surface of the nuclear membrane (Fig. 3c).

In several places of the periphery of the blood vessels, concentration of lymphocytes with scanty cytoplasm stained deeply with hematoxylin, aniline blue and toluidine blue was detected (Fig. 3d). There were no essential differences of histological structure in lymphomyeloid elements among both sexes of every species. In addition, a very curious structure was detected in 2 species of batoids. This structure is closely similar to that of Hassall's corpuscles exclusively found in the thymus of all classes of vertebrates: the epithelial cells in denaturalized condition are gathered concentrically around a central focus (Fig. 3e). This focus is sometimes pale forming a small lumen, and the whole corpuscles appears to be keratinized. The diameter of corpuscle is about 5 times larger than that of common eosinophilic granulocytes. Although the corpuscle is very scarce, it is found here and there in both Leydig and epigonal organs of the sting ray and skate.

Electron microscopy. Most of the granulocytes are also diagnosed as the immature eosinophilic granulocytes. The cells are characterized by having comparatively large, homogeneous electron dense spherules in a small number, only several to score in one section, while a lot of minute vesicles scattered throughout the entire cytoplasm. No crystalline structure was recognized in the core of spherules. The well-developed Golgi apparatus and a few rough ER surrounding the spherules are seen. The size of cell measures 10–12 μm, and the size of spherule varies from 125 to 1,700 nm. The nucleus, indented, dumbbell, constricted or highly lobated in shape, has a comparatively large amount of heterochromatin sticking in the periphery of nucleus (Fig. 4a).

There are a small number of agranular cells characterized by thin cytoplasm thought to be small lymphocytes, and so-called blast cells. The former, 6–7 μm in size, has a rich peripherally shifted heterochromatin and a dense content of free ribosomes (Fig. 4b), whereas the latter, 10–13 μm in long axis, has a comparatively large amount of cytoplasm containing the rich ribosomes (Fig. 4c).

Among these cells mentioned above, several kinds of peculiar cells are seen. The cell with many large lysosomes in a spherule or long ovoid form and comparatively dense cytoplasm is defined (Fig. 4a). Another cell is characterized by the existence of numerous vacuoles and rough and lucent cytoplasm, some vacuoles of which communicate with each other. There are several spherical granules (500–1,000 nm in size) with limited membrane. This cell with vacuoles is considered to be in the process of degeneration, collapse and finally burst (Fig. 4d). Although not so many typical and mature heterophilic granulocytes (10–12 μm in size with spherules, 500–850 nm in long axis) are found (Fig. 5a), the cell in exceedingly immature state corresponding to promyelocyte is infrequently recognized among eosinophilic granulocytes (Fig. 5b).

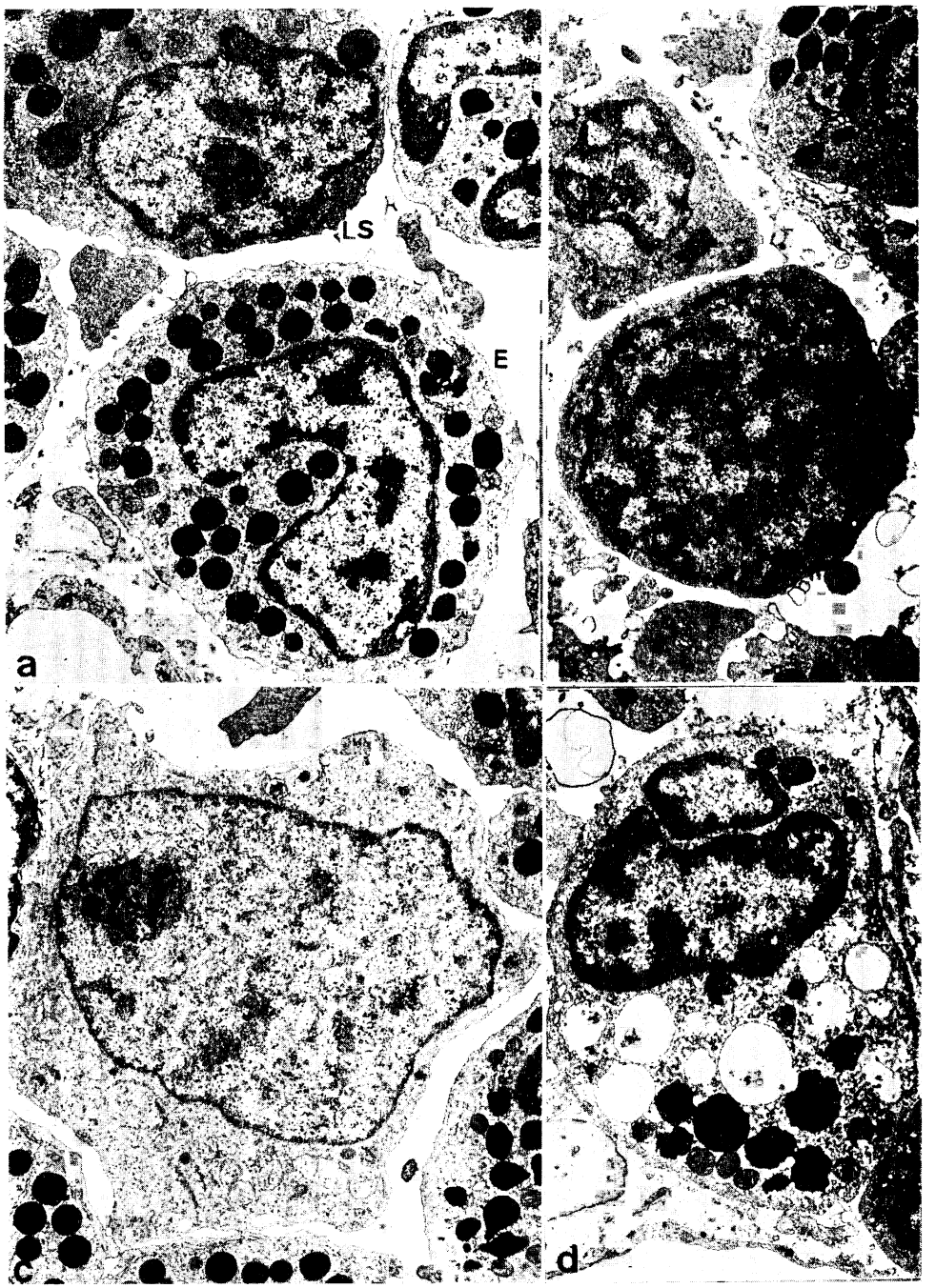

Fig. 4. Cell types comprising the epigonal organ of a banded dogfish. ×5,900. a, an eosinophilic granulocytes (E) with homogeneous, electron dense spherules and a dumbbell form nucleus. The cell with lysosomes (LS) is also seen in this picture. b, a small lymphocyte with thin cytoplasm. c, a blast cell with comparatively thick cytoplasm. d, a cell with vacuoles and lysosomes. The cell may be in the process of degeneration.

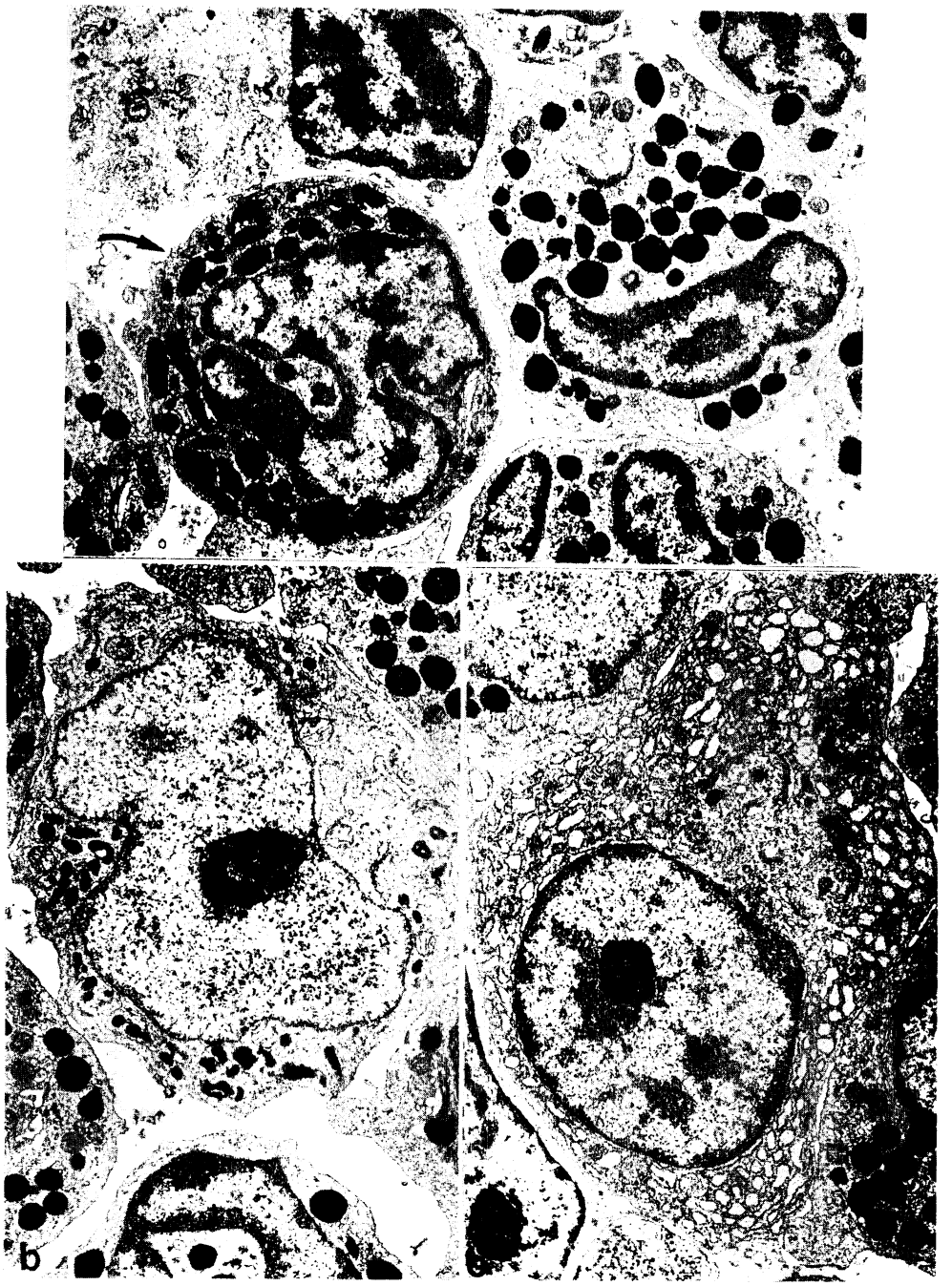

Fig. 5. Cell types comprising the epigonal organ of a banded dogfish. ×5,900. a, a heterophilic granulocyte (arrow) with elongated or rod-shaped heterogenous granules. b, an immature heterophilic granulocyte with small rod-shaped granules. This cell corresponding to the promyelocyte has a comparatively large nucleus with a large nucleolus, and well-developed Golgi apparatus. c, a plasma cell with eccentrically placed round nucleus.

The plasma cell, another component of Leydig and epigonal organs, is also detected in a small number. This cell, 10–14 μm in size, has a somewhat eccentrically placed round nucleus with a peripheral attachement of a thin frame of heterochromatin and a centrally localized dense one. The dense distribution of ER whose cisternae are distended by accumulation of moderately dense secretory material was noticed. Moreover, the well-developed Golgi apparatus in the process of elaboration of inclusions existed near the nucleus (Fig. 5c).

Discussion

In the chondrichthyans hemopoiesis occurs in several organs and tissues, such as the spleen, thymus, intestinal submucosa, Leydig, epigonal and meninges. Among others, the epigonal organ is the largest lymphomyelopoietic organ (Fänge, 1977), or approximately the same weight as the spleen (Fänge and Pulsford, 1983), and the present study has also demonstrated this situation. This organ is known as the prominent gonad-associated tissue occurring exclusively in elasmobranchs. This envelops entirely not only ovary or testis but also the posterior region of intestine and rectal gland in some species, such as smoothhound and banded dogfish. This feature convinced us that the term epigonal should be replaced by subgonal.

Histological evidences revealed that the main function of Leydig and epigonal organs may be concerned on the production and store of granular leucocytes, especially the eosinophilic granulocytes, in addition to lymphopoiesis. This myeloid nature has already been stated by Zapata (1981), Fänge and Mattisson (1981), Mattisson and Fänge (1982) and Fänge and Pulsford (1983). However, as reported by Morrow and Pulsford (1980) and Mattisson and Fänge (1982), the granules of eosinophils are different from those of mammalian eosinophils by having no crystalline structure.

Although the term "heterophil" was adopted and recommended by Fey (1965, 1966), the corresponding cells of elasmobranchs seemed to be functionally and morphologically analogous to the neutrophils of higher vertebrates. The heterophilic granulocytes were dominant elements in the elasmobranch lymphomyeloid organs (Zapata, 1981; Fänge and Mattisson, 1981; Mattisson and Fänge, 1982), and were subdivided into three types (Zapata, 1981; Mattisson and Fänge, 1982). From the electron micrographs of the peripheral Type II cells of the dogfish (Morrow and Pulsford, 1980) and also the peripheral neutrophils of the plaice (Ferguson, 1976), these were considered heterophilic. On the other hand, these cells were scarecely recognized in the present examination of 5 species of Japanese elasmobranchs, and merely the occurrence of an exceedingly immature stage of heterophils with small ovoid granules was demonstrated. Kanesada (1956), using 2 species of Japanese chondrichthyans, mentioned that the granulopoiesis is involved in Leydig and epigonal organs. He also found the small and large lymphocytes, thrombocytes and erythroblasts in lesser number. However, our preparation failed to demonstrate the thrombocytes and erythroblasts.

Zapata (1981), Mattisson and Fänge (1982) and Fänge and Pulsford (1983) described the presence of some blast-like cells corresponding to several kinds of myeloblasts and lymphomyeloblasts. The present electron microscopy also revealed a few blast cells, other than a large number of eosinophilic metamyelocytes and a very few heterophilic granulocytes. The structures similar to the Hassall's corpuscles described here have never been reported in the lymphomyeloid organs and tissues other than the thymus. Further studies are now in progress to elucidate the detail of these corpuscles. Further studies from inflammatory and immunological view points are necessary to define the real function of Leydig and epigonal organs.

Literature cited

Bolton, L. L. 1927. Note on the structure of the lymphoid organ (organ of Leydig) and spleen of *Hexanchus corius*. J. Anat., 61: 60–63.

Fänge, R. 1968. The formation of eosinophilic granulocytes in the oesophageal lymphomyeloid tissue of the elasmobranchs. Acta Zool. (Stockholm), 49: 155–161.

Fänge, R. 1977. Size relations of lymphomyeloid organs in some cartilagenous fish. Acta Zool. (Stockholm), 58: 125–128.

Fänge, R. and A. Mattisson. 1981. The lymphomyeloid (hemopoietic) system of the Atlantic nurse shark, *Ginglymostoma cirrhatum*. Biol. Bull., 160: 240–249.

Fänge, R. and A. Pulsford. 1983. Structural studies on lymphomyeloid tissue of the dogfish, *Scyliorhinus canicula* L. Cell Tissue Res., 230: 337–351.

Ferguson, H. W. 1976. The ultrastructure of plaice (*Pleuronectes platessa*) leucocytes. J. Fish Biol., 8: 139–142, XII pls.

Fey, F. 1965. Hämatologische Untersuchungen der blutbildenden Gewebe niederer Wirbeltiere. Folia Haematol., 84: 122–146.

Fey, F. 1966. Vergleichende Hämozytologie niederer Vertebraten. III. Granulozyten. Folia Haematol., 86: 1–20.

Gohar, H.A.F. and F.M. Mazhar. 1964. Internal anatomy of selachians from the northwestern Red Sea. Publ. Mar. Biol. Stat., Al-Ghardaqa (Egypt), 13: 145–240.

Kanesada, A. 1956. A phylogenetic survey of hemocytopoietic tissue in submammalian vertebrates. Bull. Yamaguchi Med. Sch., 4: 1–21.

Leydig, F. 1857. Lehrbuch der Histologie. Meidinger, Sohn, and Comp., Frankfurt A.M.

Matthews, L. H. 1950. Reproduction in the basking shark, *Cetorhinus maximus* (Gunner). Phil. Trans. Roy. Soc. Lond., B, (Biol. Sci.), 234: 247–299.

Mattisson, A. and R. Fänge. 1982. The cellular structure of the Leydig organ in the shark, *Etmopterus spinax* (L.). Biol. Bull., 162: 182–194.

Mizue, K. 1978. Reproductive organs in male selachians. Kaiyo-kagaku (Ocean Science), 10 (2), 19–25. (In Japanese with English summary).

Morrow, W. J. W. and A. Pulsford. 1980. Identification of peripheral blood leucocytes of the dogfish (*Scyliorhinus canicula* L.) by electron microscopy. J. Fish Biol., 17: 461–475.

Oguri, M. 1983. On the Leydig organ in the esophagus of some elasmobranchs. Bull. Japan. Soc. Sci. Fish., 49: 989–991.

Stahl, B. J. 1967. Morphology and relationships of the Holocephali with special reference to the venous system. Bull. Mus. Comp. Zool., 135: 141–213.

Teshima, K. 1978. Reproduction of the female sharks. Kaiyo-kagaku (Ocean Science), 10 (2), 39–44. (In Japanese with English summary)

Teshima, K. 1981. Studies on the reproduction of Japanese smooth dogfishes, *Mustelus manazo* and *M. griseus*. J. Shimonoseki Univ. Fish., 29: 113–199.

Zapata, A. 1981. Ultrastructure of elasmobranch lymphoid tissue. 2. Leydig's and epigonal organs. Develop. Comp. Immunol., 5: 43–52.

(YH and KO: Faculty of Science, Niigata University, 8050 Igarashi-ninocho, Niigata 950–21, Japan; AC: Biological Laboratory, Nippon Dental University, 1–8 Hamaura-cho, Niigata 951, Japan)

日本産軟骨魚類のライディヒ器官とエピゴナル器官の比較組織学的研究

本間義治・岡部和之・千葉　晃

ライディヒ器官とエピゴナル器官は，軟骨魚類に特異的に存在する造血器官である．これら両器官の種間における差異を明らかにするため，5 種について，外形と構成細胞を光学顕微鏡ならびに電子顕微鏡により比較検討した．用いた種はホシザメ，ドチザメ，トラザメ，アカエイおよびコモンカスベである．エピゴナル器官と生殖腺との接触の状態は，3 型に大別できるが，ライディヒ器官には明瞭な種間差がみられない．ホシザメやドチザメのエピゴナル器官は，生殖腺の中程よりはるか後方に延び，直腸腺にまで達していた．したがって，サブゴナルという名称の方がふさわしい状態にあった．5 種共に，両器官はいずれも大半が種々の発達段階にある好酸球から成り，その他少数の好異球，リンパ球，形質細胞なども存在する．さらに，若干のリンパ芽細胞ないし芽球の外，上記果粒球から由来したと思われる大型空胞をもつ退化中の細胞も見出された．また，従来胸腺にのみ存在することが知られていたハッサル小体に極めて良く似た構造が，アカエイとコモンカスベにみられた．上述の細胞構成から推察して，軟骨魚類における両器官の免疫応答や炎症過程に果す役割が追求されねばならない．

(本間・岡部: 950–21 新潟市五十嵐二ノ町 8050 新潟大学理学部; 千葉: 951 新潟市浜浦町 1–8　日本歯科大学新潟歯学部