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Primordial Germ Cells and Lymphomyeloid System in the Embryos of the Aleutian Skate, *Bathyraja aleutica*

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Abstract Organogenesis in embryos (1 and 5 mm-disk widths) of the Aleutian skate, *Bathyraja aleutica* was described histologically in complete serial paraffin sections. Special attention was paid to localization of primordial germ cells (PGC) and development of lymphoid tissues. The embryo of 1 mm-disk width was at the somite stage with no evidence of organogenesis. PGC, gathered in the genital ridge, were seen in the somatic mesodermal layer as well as in the mesenchyme between the endoderm and splanchnic mesoderm of the 1 mm-embryo. Most of the visceral organs were developing in the embryo of 5 mm-disk width. With respect to the development of immune system, two pairs of thymus anlagen, filled with numerous thymic lymphocytes, were recognized in the pharyngeal region. A small focus of numerous immature blood cells appeared to be an anlage of the spleen was found between the stomach and the liver.

Aspects of reproductive and developmental biology of cartilaginous fishes have been reviewed extensively by Wourms (1977). In his review we noted that although general features of embryology in elasmobranchs were firmly established by Balfour in 1885, information on embryogenesis of each species tends to be incomplete, since the reproductive and developmental patterns are diverse among species. We recently had the opportunity to obtain embryos of *Bathyraja aleutica* aboard the research vessel in the eastern Bering Sea. Two of these embryos were at early developmental stages with 1 and 5 mm-disk width. In this report we will describe histological characteristics of two *B. aleutica* embryos with respect to primordial germ cells (PGC), viscera, thymus and spleen.

Materials and methods

Two embryos, with 1 and 5 mm-disk width of the Aleutian skate, *Bathyraja aleutica*, found in egg capsules, were taken together with free living specimens through trawl operations during a cooperative groundfish survey by the Fisheries Agency of Japan and the U.S. National Marine Fisheries Service in the eastern Bering Sea in 1982. The 1 and 5 mm-embryos were taken respectively from the ground of 445 and 523 m in depth at 56°34′N, 172°51′W and 55°14′N, 167°58′W on 3 and 21 October in 1982. The embryos with their egg capsules were fixed in 10% formalin aboard the vessel and brought to the laboratory for histological examinations. After the embryos were dehydrated in alchohol series and embedded in paraffin, complete serial cross sections of about 6 μm thick were stained with haematoxylin and eosin.

Abbreviations. A, a thin membrane, presumably an anal fin; bc, blood cell; bi, blood island in yolk sac membrane; ec, ectoderm; eg, external gill; en, endoderm; ga, ganglion; gr, genital ridge; in, intestine; l, liver; n, notochord; nt, neural tube; P, pectoral fin; pc, pharyngeal cleft; pgc, primordial germ cell; pp, pharyngeal pouch; pv, post-cardinal vein; px, pharynx; s, somite; sa, supposed spleen anlage; so, somatic mesoderm; sp, splanchnic mesoderm; st, stomach; t1 and t2, thymus; ur, urinary ridge; V, ventral fin; ys, yolk sac.

Results

Macroscopic features. The embryo of 1 mm-disk width corresponded approximately to stage G defined by Balfour in the shark embryo (see Dean, 1906), with numerous somites (not counted). Head, optic vesicles and mandibula were seen but gill slits were not distinguishable (Fig. 1). The embryo of 5 mm-disk width, corresponding to Balfour's stage N in the shark (see Dean, 1906), showed the following characteristics (see Fig. 2). The mouth was widely open, and well developed external gills extended from the five branchial

arches. Pectoral, ventral and two dorsal fins were distinguishable. A thin membrane was recognized on the ventral side of the tail between the ventral and the 1st dorsal fins as shown in Fig. 2(A). This membrane was considered to be an embryonic anal fin. The membrane was not found in any embryos of more than 90–100 mm in disk width or in adult fish, thus suggesting that the anal fin might have disappeared during ontogenesis.

General histological characters. In the embryo of 1 mm-disk width, neural tube, notochord, somites with dermatomes and myotomes, dorsal aorta, endodermal (or gut) tube and embryonic coelom (or mesodermal tube) consisting of the splanchnic and somatic mesodermal layers, were observed. However, no visceral organs were developing yet (Fig. 3). The external yolk sac membrane consisted of four layers (Fig. 4), continuous with the four embryonic epithelial layers (i.e. endoderm, splanchnic mesoderm, somatic mesoderm and ectoderm) at a region where the yolk sac stalk was attached to the embryo.

On the other hand, in the embryo of 5 mm-disk width, most of the visceral organs were in the process of development. Three regions of the digestive tract, i.e. esophagus, stomach and intestine, and rather large liver were seen in the abdominal cavity (Figs. 5–10), and the mesogastrium and mesenterium were already formed (Figs. 5, 7). The digestive tract was composed of three layers; 1) columnar lining epithelial cells, 2) subepithelial mesenchyme in which smooth muscle cells seemed to be not yet developed, and 3) visceral peritoneal epithelial cells. Spiral valve formation was recognized in the developing intestine (Figs. 7–10). The epithelial layer forming the convex part of the lumen was generally thicker than that on the concave side, and a much denser cell population was observed in the subepithelial mesenchyme close to the convex side (Figs. 9, 10), suggesting that asymmetrical cell proliferation might contribute to formation of the spiral valve. A pair of urinary ridges containing nephritic tubules and ducts protruded into the abdominal cavity (Figs. 5, 7, 8). Medial to the urinary ridges a pair of genital ridges showed prominent projections (Figs. 7, 8). The rectal gland which was composed of a large number of acinous cells with acidophilic cytoplasm was already well differentiated in the dorso-caudal part of the abdominal cavity. The heart and major blood vessels were found as components of the circulatory system. The external gill filament was recognized as a capillary loop covered with a thin epithelial layer (Figs. 17, 18).

Development of primordial germ cells (PGC). Large cells characterized by having a round nucleus with dispersed chromatin and numerous yolk granules were located in the somatic mesodermal layer and mesenchyme between the endoderm and splanchnic mesoderm in the embryos of 1 mm-disk width (Figs. 11, 12). As will be discussed later, these large cells were considered to be primordial germ cells (PGC) by their cytological characteristics. The PGC were found in the middle to caudal region of the trunk but not in the cranial part of the embryo. At the stage of 5 mm-disk width, the PGC were found only in the genital ridges (Figs. 13, 14). In the ridge, the lateral epithelial cell layer was thicker than that of the medial one, and the most of the PGC were seen in the former layer, though a small number of PGC were also observed in the mesenchyme of the genital ridge.

Appearance of lymphomyeloid system. In the embryo of 1 mm-disk width, the lymphomyeloid system was not formed yet, but hemopoietic foci occurred in the yolk sac membrane, and they were localized between two mesodermal cell layers (Fig. 4). The major cell type appeared to be of the erythrocytic series. However, at the stage of 5 mm-disk width, two kinds of lymphomyeloid tissue, i.e. thymus and probably spleen appeared to be developing. Two pairs of thymus anlagen were observed in the pharyngeal region associated with the first two pharyngeal pouches and clefts (Figs. 15–18). Cuboidal epithelial cells surrounded the anlage in which many thymic lymphocytes were already found. In the sections, the thymus anlagen appeared to be embedded in mesenchyme and isolated from the epithelial layer of the pharyngeal region. Observations on serial sections, however, indicate that the anlage was continuous three-dimensionally with the epithelial layers of both the pharyngeal cleft and the pharyngeal pouch (Figs. 15–18). Medial to the thymus anlage, the ganglion of the cranial nerve was noticed and the post-cardinal vein was also consistently found in the area dorsal to the anlage (Figs. 15–18). A small mass of tissue with many immature hemopoietic cells appearing to be an

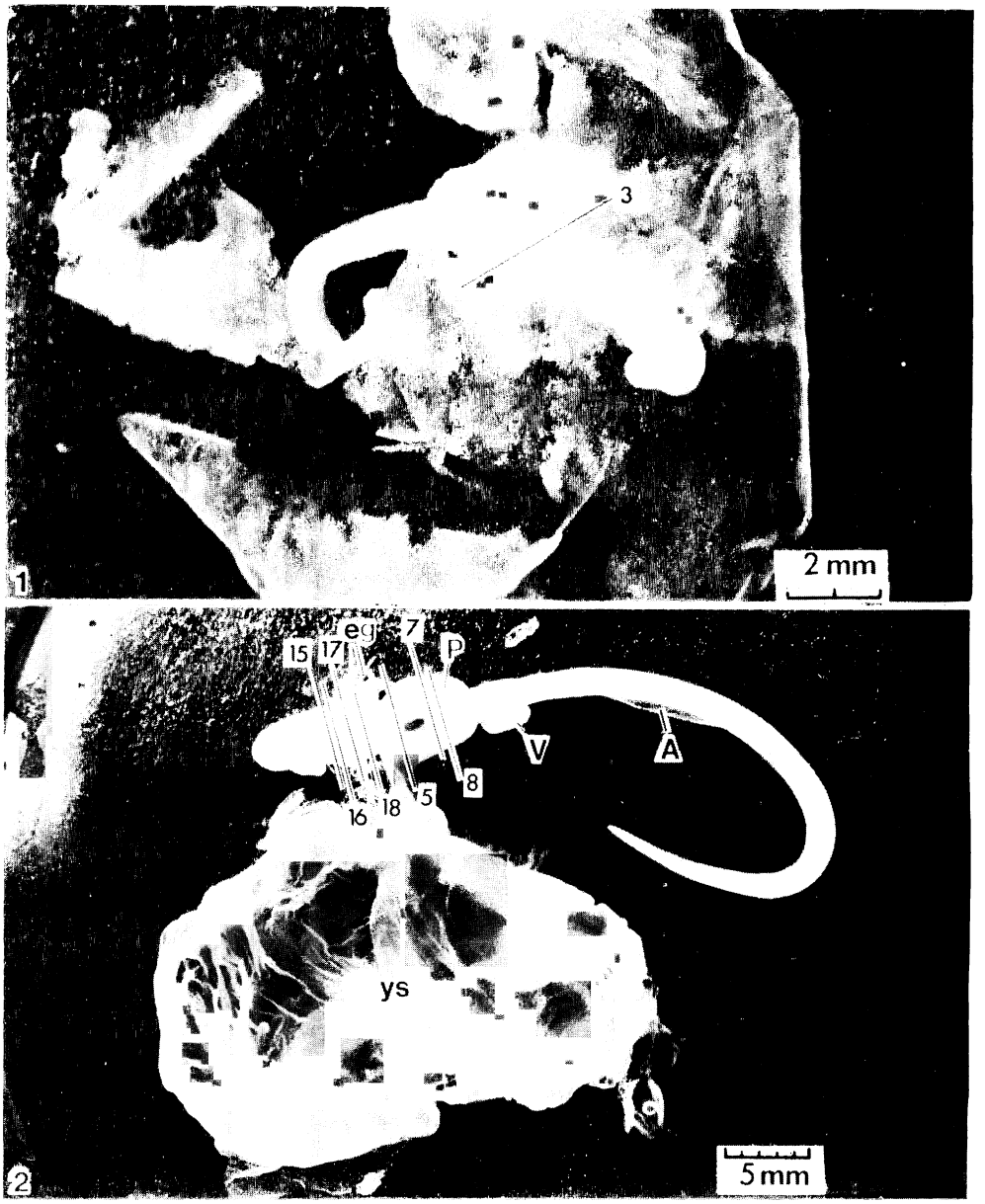

Fig. 1. Lateral view of the Aleutian skate, *Bathyraja aleutica*, embryo of 1 mm-disk width. A bar with a number indicates cutting direction of Fig. 3.

Fig. 2. Ventral view of an embryo of 5 mm-disk width. An anal fin (A) was found on the ventral side of tail. Bars with numbers indicate cutting directions of Figs. 5, 7, 8, 15–18.

anlage of the spleen was found between the stomach and the liver (Figs. 5, 6). The Leydig organ and the epigonal organ, which were expected to become hemopoietic tissues in submucosa of the esophagus and in the abdominal cavity associated with the gonad respectively in the later stage of embryos and also in adult fish, were not recognized at this stage.

Discussion

In this paper, we were able to describe the developmental features of visceral organs, PGC and components of the lymphomyeloid system, mainly the thymus and spleen of *Bathyraja aleutica*

embryos. We identified PGC based upon Hardisty's description (1967) that PGC are characterized by their large size and low nucleo-cytoplasmic ratios, by a vesicular nucleus with a sharply defined nuclear membrane, and by their tendency to retain yolk inclusions long after these have disappeared from somatic cells. In the present Aleutian skate embryos, PGC were found only in the genital ridges of the 5 mm-disk width embryo though they were distributed rather widely in the mesodermal layer and mesenchyme when the embryo was at 1 mm-disk width. This observation suggests that PGC may have migrated into the genital ridge from other parts of the more undeveloped embryos. A phenomenon of PGC migration is now generally accepted in most vertebrate classes though the origin of these cells and their migratory patterns are variable among animal species and group (Nelsen, 1953; Fujimoto *et al.*, 1976, 1977).

In the historical context, cell migrations were originally described in the dogfish, *Squalus acanthias*, by Woods (1902). According to his observation, PGC segregate from primitive endoderm during early development and migrate via the mesoderm into the site of the developing gonad. Localization of PGC in sites other than the genital ridge was also reported in various embryonic stages of *Raja batis* by Beard (1903–04). At present we have no conclusive information on the origin of PGC and their migratory patterns except that PGC in *B. aleutica* surely migrated into the genital ridge.

With respect to the lymphomyeloid system, we recognized two pairs of solitary masses of thymus anlagen, already filled with a number of thymic lymphocytes, in the embryos of 5 mm-disk width. However, it is not certain whether more of the anlagen would develop in advanced stages or whether two pairs of anlagen grew to become a large tissue without additional anlage formation. Nevertheless, in later stages of embryos, e.g. embryos of 87 and 134 mm-disk widths, the thymus was a large mass with many lobules; it was localized in the dorso-medial area of the pharyngeal region between the skin and gill arches (unpublished observation). Thymus formation during early embryonic development in elasmobranchs has been studied extensively in *R. batis* by Beard (1903), who concluded that the production and source of leucocytes for the body is the sole function of the thymus. The former conclusion, i.e. production of leucocytes, is unique and historically brilliant. The latter, i.e. the sole source of leucocytes, however, certainly needs modification since he observed only early embryonic stages, in which other hemopoietic tissues, i.e. the spleen, epigonal organ and Leydig organ of the esophagus were not developed.

It is known that the spleen has well-developed lymphoid tissues in elasmobranchs (Yoffy, 1929; Kanesada, 1956; Good *et al.*, 1966; Fänge, 1977; Fänge and Pulsford, 1983; Honma *et al.*, 1984; Tomonaga *et al.*, 1984a; Kobayashi *et al.*, 1985). We recently observed that the spleen of adult *B. aleutica* has an extraordinally numerous number of immunoglobulin-forming cells (Tomonaga *et al.*, 1984b; Kobayashi *et al.*, 1985), and the spleen of late embryonic stages of 40–134 mm-disk widths, has already immunoglobulin-forming cells. Moreover, a population of immunoglobulin-forming cells of the embryos contained two classes of immunoglobulins in single cells (Kobayashi *et al.*, 1985). These observations supported our conclusion that the spleen of *B. aleutica* might be the primary lymphoid organ for so-called B cell differentiation (Kobayashi *et al.*, 1985). To analyse in more detail cell differentiation of the immune system in this unique experimental animal (Tomonaga and Kobayashi, 1985), establishment of embryologic sequences is necessary. In the present study a small tissue with immature hemopoietic cells was found in the abdominal cavity between the stomach and the liver, suggesting that

Fig. 3. A cross section of the embryo of 1 mm-disk width. Yolk sac (ys) was not attached to the embryo body in this section. ×25. Approximate level of the section is shown in Fig. 1 as a bar with number 3. Figs. 3, 4, 11, 12: An embryo of 1 mm-disk width.

Fig. 4. Enlarged view of Fig. 3 showing four layers of yolk sac membrane and yolk sac hemopoiesis. Immature blood cells (bc) were seen between somatic (so) and splanchnic (sp) mesodermal layers. ×100.

Fig. 5. Stomach (st), liver (l) and urinary ridge (ur) were seen. A haemopoietic tissue, presumably the anlage of the spleen (sa), was seen between the stomach and liver. ×25. Figs. 5–10, 13–18: An embryo of 5 mm-disk width.

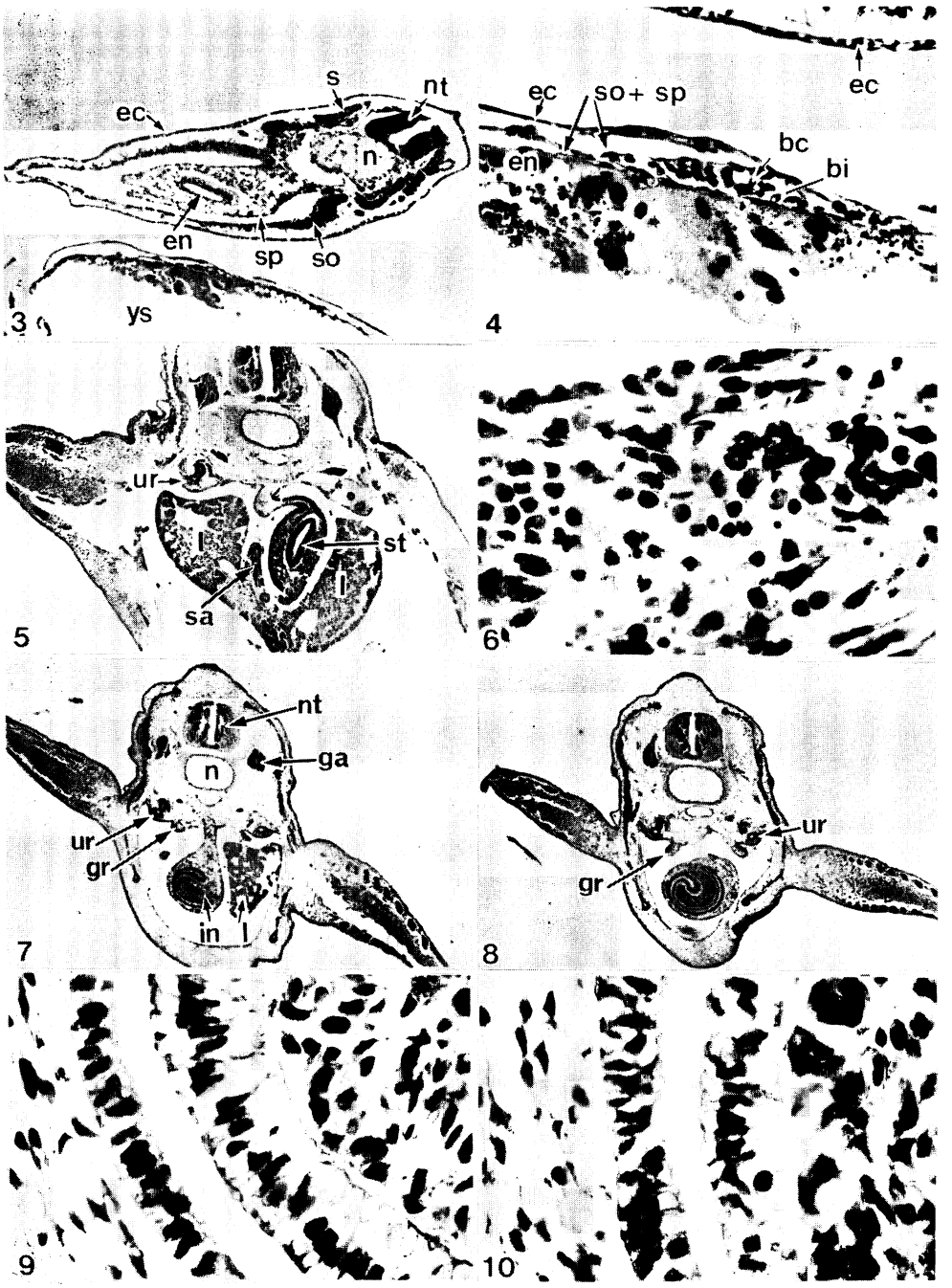

Fig. 6. High power view of the hemopoietic tissue (sa) in Fig. 5. ×250.

Figs. 7, 8. Spiral valve formation in the intestine. Note horse shoe (Fig. 7) and spiral (Fig. 8) shaped intestinal lumen. Urinary (ur) and genital ridges (gr) were seen in the dorsal wall of the abdominal cavity. The mesenterium was shown in Fig. 7, but it was broken artificially in Fig. 8. ×100.

Figs. 9, 10. Higher power views of the epithelial and subepithelial layers of the developing intestine. Note the difference of cell height of gut epithelial layers and that of cellular density of subepithelial mesenchyme in opposite (each) sides of the lumen. ×250.

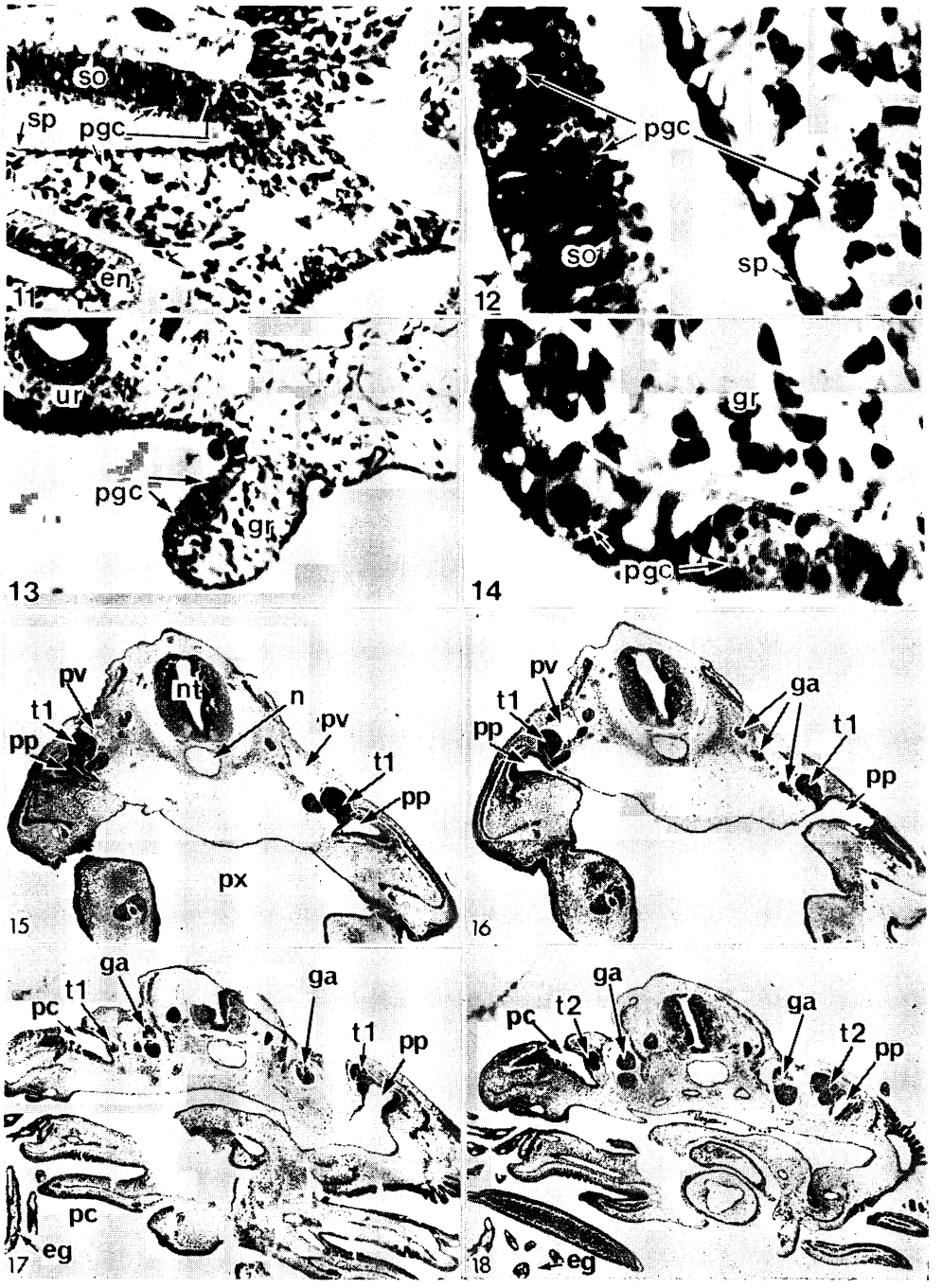

Fig. 11. Enlarged view of Fig. 3. Primordial germ cells (pgc) were seen in the somatic mesodermal layer (so) and in the mesenchyme between the endoderm (en) and the splanchnic mesoderm (sp). ×100.
Fig. 12. A higher magnification of Fig. 11. Note the primordial germ cells (pgc) with a number of yolk granules. ×250.
Fig. 13. Showing genital (gr) and urinary ridges (ur). ×100.
Fig. 14. Enlarged view of the genital ridge (gr). Note primordial germ cells (pgc) with numerous yolk granules. ×250.
Figs. 15–18. Two pairs of thymus anlagen (t1, t2) were found in the pharyngeal region. ×10.

differentiation of the spleen might occur during that stage of development. Information obtained in the present study though not extensive was fragmented since the material was limited. Further analyses using various developmental stages and more animals are needed to clarify embryogenesis and organogenesis of the Aleutian skate.

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アラスカカスベの始原生殖細胞とリンパ組織の分化

手島和之・友永　進

体盤幅 1 mm と 5 mm のアラスカカスベ *Bathyraja aleutica* の胚のパラフィン連続切片を作成し，それらの組織発生を調べた．材料は，1982 年に東部ベーリング海大陸斜面の海底域からトロール網によって漁獲された卵殻内より採集されたもので，主として，始原生殖細胞の出現部位とリンパ組織の発達を観察した．体盤幅 1 mm の胚で，神経管，脊索，体節，原始腸管，中胚葉側板などが観察されたが，器官形成はまだ行われていなかった．始原生殖細胞は，体壁中胚葉及び内胚葉と内臓中胚葉の間にある間葉に存在していることが分かった．一方，体盤幅 5 mm の胚では，形成過程にある多くの

内臓諸器官がみられた．始原生殖細胞は，前段階の部位には認められず，生殖隆起表層内に集合していた．二対の胸腺原基は咽頭域に存在しており，リンパ球で充満していた．また，多数の未熟な血液細胞からなる脾臓原基と思われる小型の組織が認められた．

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