

The Primitive Spleen of the Hagfish

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ABSTRACT

The structure of hemocytopoietic islands in the primitive spleen of the hagfish, *Eptatretus burgeri*, was examined by light and electron microscopy and autoradiography. It was found that the most abundant cell population in the primitive spleen is a granulocytic series. Erythrocytopoiesis was not recognized in the hemocytopoietic islands.

The primitive spleen of the hagfish is an important hemocytopoietic tissue; however, views on cell constituents in the spleen are divergent (Jordan and Speidel 1930, Finstad *et al.* 1964). In this research the structure of the primitive spleen and the distribution of the other hemocytopoietic tissues in the hagfish were studied with light and electron microscopy. About 30 specimens of *Eptatretus burgeri* ranging in body length from 15 cm to 63 cm were used as material. Methods for electron microscopy were described elsewhere (Tomonaga *et al.*, 1973). For photomicrography, the intestine was fixed in 10% neutral formalin or 2.5% glutaraldehyde, mounted in paraffin and sectioned. Sections were stained with haematoxylin and eosin. For autoradiography, 30 μ Ci of ^{59}Fe , in the form of mixture of ferric chloride and ferric acetate, was dissolved in 1 ml of saline and injected intraperitoneally or subcutaneously into the hagfish 1, 3, 6, 24, 48 and 72 hours before sacrifice. The hagfish were sacrificed by urethan anesthesia. Sections of the intestine

were prepared as mentioned above. The radioautograph was prepared by the dipping method.

Active hemocytopoiesis was observed only in the submucosa of the intestine. Tissues showing active hemocytopoiesis (primitive spleen) were distributed throughout the submucosa of the intestine, and had an island-like appearance (Fig. 1). Electron micrograph clearly showed that the most abundant cell population in the primitive spleen was made up of developing granulocytes which contained many fine granules (Fig. 2). Undifferentiated immature blood cells, lymphoid cells, and reticulum cells were also observed though they were limited in number.

Immature granulocytes (promyelocytes and myelocytes) enclosed the abundant rough endoplasmic reticulum and the prominent Golgi complex was surrounded by membrane-bounded spherical granules in various developmental stages (Figs. 3 and 4). The number of spherical granules decreased and the number of rod-shaped granules increased with granulocyte maturation. Fully matured cells had only rod-shaped granules. These observations suggest that spherical granules may change to rod-shaped granules. These granulocytic series correspond to those of neutrophil leukocytes in other vertebrates.

Erythrocytic series, thrombocytes and spindle cells were not found in the hemocytopoietic islands of the primitive spleen. In addition, incorporation of ^{59}Fe into erythroblasts was not detected in the hemocytopoietic islands by autoradiography. These findings indicate that cell proliferation and cell differentiation of the erythrocytic series does not occur in the primitive spleen. It probably occurs only in the peripheral blood in the species (Tomonaga *et al.* 1973).

Hemocytopoietic tissues of the primitive spleen surrounded the sinusoid (Fig. 4). Lining cells of the sinusoid are very thin and partially lacked basal lamina. These structures resemble the figures of bone marrow sinusoids in vertebrates higher than amphibians (Campbell 1970, Zamboni and Pease 1961).

Hemocytopoietic tissues of the primitive

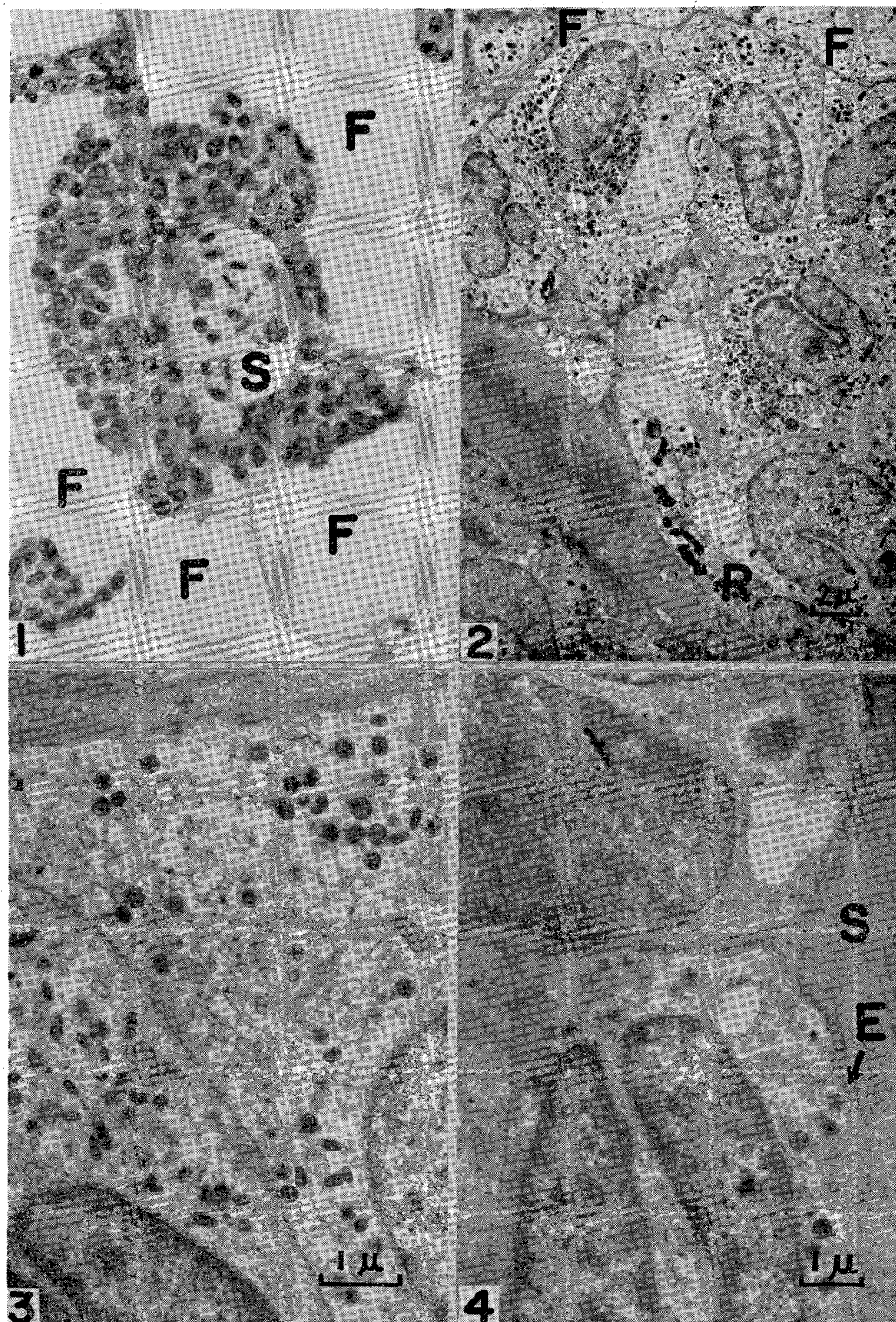


Fig. 1. Hemocytopoietic island of primitive spleen in the submucosa of the hagfish intestine. Cell of the spleen surround the sinusoid (S), and are encircled by large fat cells (F) (Hematoxylin and Eosin). $\times 400$.

Figs. 2-4. Electron micrographs of the primitive spleen of the hagfish. Fixed with 2.5% glutaraldehyde and 1% osmic acid, and stained with uranyl acetate and lead citrate.

2. Low power view of the spleen. Reticulum cell (R) and fat cells (F), are shown encircling hemocytopoietic tissue. $\times 4,000$. 3. Promyelocyte in the spleen. $\times 13,000$. 4. Part of hemocytopoietic tissue of the spleen facing sinusoid (S). No basal lamina is present there beneath the endothelium (E). $\times 10,000$.

spleen of old hagfish showed involution. In senile hagfish, the primitive spleen degenerated and was replaced by collagenous bundles.

In the liver of young hagfish, aggregations of mononucleated cells were observed. However, no mitotic figures were observed even in an animal injected with 0.01 or 0.1 mg of colchicine per g body weight 4 or 6 hours before sacrifice. Therefore, it is not clear whether these structures represent true hemocytopoietic tissues or not.

References

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