Aggregation of the Smooth Muscle Cells Found in Certain Hemopoietic Organs and Tissues of the Stingray, *Dasyatis akajei* (Elasmobranchii, Chondrichthyes)

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Summary. A combined light and electron microscopic study revealed that there are conspicuous aggregations of smooth muscle cells in several hemopoietic organs and tissues such as in the Leydig (esophageal) and epigonal organs, diencephalic choroid plexus and perihypophyseal connective tissue sheath of the stingray, *Dasyatis akajei*. These cells were gathered in bundles of varying caliber or arranged concentrically around a central focus, but neither innervation nor gap junction was found. In some of the concentrically arranged cells, signs of degeneration were noticed. The possible origin of these structures is discussed in relation to the vascularity of the loose connective tissue.

During the course of our study on the comparative histology of the hemopoietic organs in elasmobranchiate fishes, unusual structures resembling Hassall's corpuscles in the vertebrate thymus were encountered both in the Leydig and epigonal organs of two species of Batoidea (HONMA et al., 1984). Nearly identical structures had already been long recognized as "Muskelspiralen" in the Leydig organ of the skates, *Raja clavata* and *Trygon pastinaca* (KULTSCHITZKY, 1911). Since KULTSCHITZKY's study, much attention has been paid to the structures of the hemopoietic organs of the elasmobranchs (BOLTON, 1927; FÄNGE, 1968; FÄNGE and MATTISON, 1981; ZAPATA, 1981; MATTISON and FÄNGE, 1982, FÄNGE and PULSFORD, 1983; PULSFORD et al., 1984), but further light and electron microscopic studies on such structures have been lacking. Recently, using the stingray, we have demonstrated the Hassall-like structure not only in the hemopoietic organs but also in the choroid plexus of the diencephalon and connective tissue of the hypophysis. In this study, combined light and electron microscopic observations were carried out to clarify its fine structure, and consideration was given to learning the origin of this structure.
MATERIALS AND METHODS

Five specimens of stingray, *Dasyatis akajei* (Müller et Henle), of both sexes ranging from 25 to 50 cm in total length were used in this study. They were collected from the coastal waters in the vicinity of the Sado Marine Biological Station of Niigata University, located on the northwestern part of Sado Island in the Sea of Japan, over a period lasting from May to August, 1982.

For light microscopy, the Leydig and epigonal organs and brains with hypophysis were removed after decapitation. A small portion of the esophageal muscle layer was also dissected out for comparison with these organs and tissues. Pieces of these were immersed in Bouin's fixative, dehydrated through an ethanol series, and embedded in paraffin for histological study.

![Images]

**Fig. 1.** Light micrograph of aggregations of smooth muscle cells in the Leydig organ of the stingray. Hematoxylin-eosin. × 300

**Fig. 2.** Aggregations of smooth muscle cells in the loose connective tissue of the diencephalic choroid plexus. Toluidine blue. × 130

**Fig. 3.** Aggregations of smooth muscle cells in the connective tissue sheath of the hypophysis. Toluidine blue, × 130

**Fig. 4.** Aggregation of smooth muscle cells in a denaturalized condition. Toluidine blue. × 1,000
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Sections 7-8 μm in thickness were cut serially and stained with hematoxylin-eosin and azan trichrome. For electron microscopy, the pieces were immersed in Karnovsky’s solution for 1 or 2 days, postfixed in 1% OsO₄ for 2 hrs, dehydrated in a series of ethanol, and embedded in Epon 812. Semithin sections (1 μm thick) were stained with toluidine blue and examined with a light microscope. Ultrathin sections were double-stained with uranyl acetate and lead nitrate and examined with a Hitachi H-500 electron microscope.

RESULTS

Light microscopy

Peculiar structures were demonstrated as being scattered in: 1) the Leydig and epigonal organs; 2) the loose connective tissue of the diencephalic choroid plexus; and 3) the connective tissue sheaths of the hypophysis (Fig. 1-3). The frequency of occurrence of these structures was very high in the Leydig organ, choroid plexus and perihypophyseal connective tissue sheaths, whereas they were only occasionally encountered in the epigonal organ. The number of the structures varied from fish to fish, and from organ to organ. The entire appearance was spherical or ovoid, surrounded by a delicate capsule and occasionally located in close proximity to blood vessels. The structures were exclusively composed of acidophilic spindle cells assembled to form bundles of varying caliber ranging from 20 to 80 μm, or gathered concentrically around a central focus. The focus was sometimes replaced by a small cavity or capillary. Each cell, 5 μm in mean diameter and more than 50 μm in length, had a centrally shifted elliptic nucleus with one or more nucleoli and a relatively rich cytoplasm occupied by delicate

Fig. 5. Electron micrograph of the aggregation of smooth muscle cells, showing sublemmal dense bodies and caveolar invaginations along the plasma membrane. ×17,000
fibrillar structures disposed longitudinally. In particular, some of the structures appeared to have a resemblance to Hassall’s corpuscles peculiar to the vertebrate thymus, i.e., several denaturalized cells around the central focus appeared as if they had been keratinized or hyalinized (Fig. 4). No macrophage was encountered within the structures.

**Electron microscopy**

The fine structure of the spindle cells in question was almost consistent and identical with that of the smooth muscle cells forming the walls of the esophagus and blood vessels. In cross section, the plasma membrane of the cell usually exhibited a wavy condition (Fig. 5). The greater part of the cytoplasm was occupied by longitudinally shifted thin microfilaments, about 7 nm in diameter (Fig. 6), although their arrangement was irregular near the periphery of the cells. Beside these thin filaments, thick filaments, 12-24 nm (mean 17.4 nm) in diameter, were also demonstrated scattered among the thin filaments (Fig. 6). The density of the thick filaments was greater than the thin filaments. Some obliquely running thick filaments were also seen. In addition, the existence of a number of vesicular caveolae and sublemmal dense bodies located along the inner aspect of the plasma membrane was characteristic of these cells (Fig. 5). The caveolae, sometimes accompanied by vesicular or tubular elements of the agranular endoplasmic reticulum, were absent from the area of membrane occupied with dense bodies (Fig. 6). Some of the thin filaments detected in the periphery of the cell tended to converge on the dense bodies. The juxtanuclear cytoplasm, free of the microfilaments, possessed a small amount of free ribosomes, a few mitochondria, poorly developed Golgi bodies, fragments of granular endoplasmic reticulum, glycogen particles, and a few lysosomes (Fig. 5, 6). There were occasional deep indentations in the nucleus and clumpy heterochromatins along the margin of the nucleus (Fig. 5). Every cell was separated by a generally thick extracellular space occupied by the basal lamina.

![Fig. 6. High power electron micrograph of smooth muscle cells showing thin and thick myofilaments and caveolae in close association with the smooth endoplasmic reticulum. ×35,000](image-url)
with or without thin bundles of collagen fibers. The basal lamina, however, was lack-
ing in several areas where adjoining cells were linked with each other across a very
narrow space of about 15–30 nm (Fig. 6). This structure did not correspond to a gap
junction or nexus, and no desmosomal attachment was demonstrated. The capsule
surrounding these cells was composed of bundles of collagen fibers. Delicate reticular

**Fig. 7.** Part of the smooth muscle cells in a denaturalized condition. Vacuolization of the cyto-
plasm and lysosome, and derangement of the myofilaments are seen. ×17,000

**Fig. 8.** High power electron micrograph of a smooth muscle cell showing the loss and derangement
of the myofilaments. ×110,000
fibers and processes of the reticular cells were also seen in association with the capsule. In spite of careful examination, no nerve terminals were encountered in adjacent portions of the cell. In some of the cells concentrically arranged like Hassall's corpuscles, signs of degeneration were noticed, i.e., the occurrence of vacuoles including several vesicles, flocculent materials, and myelinated bodies in the cytoplasm (Fig. 7). In addition, a reduction in the number of the vesicular caveolae was detected in some degenerating cells. Loss and disintegration of microfilaments (Fig. 8) and pycnosis of the nucleus were also noticed.

**DISCUSSION**

This study evidently shows the occurrence of the smooth muscle cell aggregations in the hemopoietic organ, diencephalic choroid plexus and perihypophyseal connective tissue of the stingray. According to Kulitschitzky (1911), the muscular structures in the Leydig organ of skates took on various appearances as they were called Muskelspirale, Muskelringe and Muskelbündel. He also reported that they were more frequent in the peripheral portion of the organ than in the central portion, and that they sometimes occurred in direct contact with vascular walls. This finding by Kulitschitzky (1911) on the Leydig organ of the skates is thus confirmed by the present study. However, such muscular structures occurring in the hemopoietic organs and loose connective tissues of batoids (skates and rays) have not been reported in the selachii (sharks) (Fänge and Mattison, 1981; Mattison and Fänge, 1982; Fänge and Pulsford, 1983; Honma et al., 1984; Pulsford et al., 1984).

In vertebrates, smooth muscle cells occur widely in the viscera, i.e., the walls of the intestinal tract, blood vessels, genital, urinary and respiratory tracts. In the present specimens, smooth muscle aggregations were found in the loose connective tissue irrespective of the type of organ. They were occasionally seen in conjunction with blood vessels, as reported in the skate (Kulitschitzky, 1911). These findings strongly suggest that the smooth muscle cells may be originated from vascular walls and nearby mesenchymal tissue during early organogenesis. However, the cause of aggregation of the cells is still unknown.

In general, smooth muscle cells in contractive organs and tissues are arranged in layers or bundles, being supplied with autonomic nerves and surrounded by the basement membrane. A gap junction or nexus is common between the adjacent cells. However, the smooth muscle cells shown in this study were lacking in a nerve ending and a gap junction. This finding, in addition to the degenerative changes occasionally manifested in the cells, may be significant when considering the role of the smooth muscle cell aggregations in the loose connective tissue. Further embryological, physiological and experimental studies are necessary to elucidate the functional significance of these smooth muscle cells.

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