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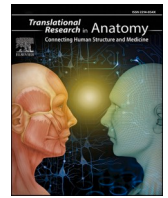
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Endocrine and scavenging cell functions of a juvenile fish heart atrium: Fine structure aspects and translating conjectures

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ABSTRACT

Background: The atrium of the juvenile teleost, smallmouth bass, *Micropterus dolomieu* Lacépède showed a pigmented endocardium. Our goal was to use fine structure to survey this fish heart tissues and verify the content responsible for this shrouding and discuss mammalian, translational conjectures about this cardiac structure.

Methods: Using electron microscopy, heart pericardium, myocardium, and endocardium were analyzed, including atrial peptide immunolabeling.

Results: The endocardium endothelial cells of the atrium revealed pinocytosis and endocytosis activities that resulted in accumulated electron-contrasted secondary lysosomes and lipofuscin bodies not found in the ventricle endocardium. This endothelium contacted subjacent atrial and ventricular myocardial cells producing immunolabeled atrial peptide-containing vesicles. Other migrating cells, including melano-macrophages, were noticed in the atrial sub endocardium.

Conclusions: The endocardium functioned as a potent blood-heart barrier where transcytosis and dispatching of numerous materials, including those atrial peptides, between myocardium and the circulation occur. Peculiarly, this juvenile endothelium revealed abundant lysosomal processing of scavenged circulation materials like in a reticuloendothelial tissue. These juvenile cell's accumulated 'aging' lipofuscin bodies as organized deposits into a network with other organelles, especially mitochondria without evident disposal. Additionally, other cells, including melano-macrophages roamed both sub endocardium and ventricle; those also can influence the viewed pigmentation. Some of these data comforted the endocardial mesodermal lineage, still sketchy for all vertebrates while fish heart development is still used. These observations could raise questions: Does turnover of lipofuscin and associated captures make endocardial cells to model other mammalian pathologies, including those associated with heart failure?

1. Introduction

During comparative studies of cardiac structures and development dealing with atrial structures and peptides [1–6] and cardiomyopathy [7], If our curiosity made us to survey the expression of cardiac peptides (abbreviated ANF or ANP) in the cardiac tissues of the smallmouth bass, a teleost fish, a peculiar internal brownish hue was observed. A cursory light microscopy appraisal made us realize that, like in many other fish freshwater and seawater species, a series of research reports had

described the endocardium endothelium, the thin serous membrane of the heart, to be often the main tissue pigmented and surveyed with light microscopy and histochemistry. However, those studied dealt with adult fish, including some with interest as recreational species [8–22] and others that had interest in fisheries [10–12,16,18–22]. Other related studies revealed the endocardium scavenging features with or without fine structure that could involve nucleic acid [23] while most other authors have supported endocardial captures of circulating ferritin or other circulation products because of injections of hemal compounds

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[24–35], a few had suggested hyaluronan capture by endocardium [34]. Furthermore, several of these reports also included another endocardial cell type, lining the endocardium, named melano-macrophage cells, and responsible for the heart pigmentation [24–32,35]. However, these cell's location still bore confusions about the components of the endocardium cells.

Fish cardiovascular developmental and functional aspects have been reported [36–45] and, in recent years, fish models had and are still used to understand some of the early mammalian heart development, recapitulating some primitive structures viewed in those models because the endocardium early development in vertebrates, including mammals, steered into crucial cardiac tissues that are still enduring unresolved questions in normal and pathology outlooks as well as those that regard homeostatic salt balance [44–60].

Our aim was thus to verify with fine structure the heart layers of a juvenile teleost, the smallmouth bass, to further describe the cells and the cytologic features responsible for its pigmentation, likely located at the endocardium level.

This report revealed the species pericardium and the myocardial cytology. The myocardium is marked by the production of cardiac peptides while the endocardial endothelial cells had a peculiar content that resembled accumulated lipofuscin 'aging' bodies and constituted a pigmented inner cardiac layer. It developed from pinocytosis and endocytic captures of undisclosed circulation products out of lysosomal production and processing into secondary lysosomes, then stowed as lipofuscin-like bodies. It seemed to function as a sort of reticuloendothelial tissue, creating excessive long term waste loads and/or a protective shield. Other wandering cells of the subendocardial endothelium were noted, including the so-called melano-macrophages whose organelles resembled melanosomes.

2. Materials and methods

2.1. Ethical concerns

Procedure and sacrifice of fishes were approved through the Animal Ethic Committee of the College of Medicine (1987–1991) and the Department of Comparative Medicine of NEOUCOM (now NeoMED), Rootstown Ohio, USA.

2.2. The fish collection

Eight juvenile smallmouth bass fishes, *Micropterus dolomieu* Lacépède, a teleost Perciform, 9–10 cm long (about 11-month-old), were purchased from a private fish farm, Green Ohio, USA [60,61]. They were kept at the same freshwater and temperature as collected (between 12 and 14 °C), in an insulated container for transport to a small laboratory aquarium for a 2-day period adjustment before they were anesthetized by a 75 mg dose/L of according to specific consideration also noted in a U.S. Fish and Wildlife Service report [62–64].

2.3. Light (LM) and Transmission electron microscopy (TEM)

Four of the small bass were intraventricularly perfused with a 0.1 M cacodylate buffered 3.5% glutaraldehyde solution (pH = 7.2) during 5-min. After heart excision, atria and ventricles were immersed 2-h long in the same fixative solution at 4 °C. Samples were washed for 1h duration in cacodylate buffer and postfixed for 2h in aqueous 2% osmium tetroxide. After a wash of 1,5h in the same buffer samples were processed in PolyBed epoxy resin (Polysciences, Warrington, PA.). Examination of 1-µm thin sections, stained with toluidine blue, allowed to select areas to cut ultrathin (50–65 nm) sections to be collected on 50 and 100 mesh hexagonal copper grids (SPI, West Chester, PA) and contrasted with uranyl acetate and lead citrate [1,2].

2.4. Immunoelectron microscopy

Four fish specimens anesthetized as described above had heart excised and components fixed by 1% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2–7.3) for 2 h at room temperature before rinsed in the same buffer, dehydrated, and processed in LX11 embedding epoxy medium (Ladd Res Ind, Burlington VT). No osmium tetroxide post fixation was used. Ultrathin grey sections were cut, collected on nickel grids, and treated according to De Mey et al. [65, 66]; incubated for 1 h with normal goat serum diluted 1:3 by Tris-NaCl buffer, pH 8.2 and without rinsing, sections were immersed in rabbit ANF antiserum #10-5 (1:2000 dilution; 12 h at 4 °C). After rinsed with Tris buffer, the sections were now incubated 1h at room temperature in goat anti-rabbit IgG coupled with colloidal gold (15 nm average particle size; Janssen Pharmaceutica, Beerse Belgium) diluted 1:20 by 0.1 M Tris with 10% goat serum -NaCl buffer. Sections were then contrasted with uranyl acetate and lead citrate, and carbon-coated before they were observed with the electron microscope. Controls included substitution of the primary antiserum with #10-6 M atriopeptin III (5-25) or with alpha-human ANF (Bachem AG, Bubendorf, Switzerland). In addition, substitution of the primary antiserum by rabbit anti-VIP, -NPY, -substance P, or -CCK antisera did label atrial granules or of any part of the sarcoplasm by immunogold particles. Preabsorption of the antibody with synthetic ANF(1–28) or substitution of the primary antiserum by normal rabbit serum resulted in the absence of immunogold labeling [4–6]. The ANF antiserum #10-5: Anti-ANF serum was obtained by immunization of a rabbit with synthetic atrial natriuretic peptide (rat 5–28; Bachem AG) that was coupled to keyhole limpet hemocyanin via 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide-HCl [67]. The antiserum (10#5) recognizes atrial natriuretic peptide(5–15), ANF (1-28) and beta-rANF (17–48). These data suggested that the antiserum #10-5 binds to the free active ANF hormone as well as to ANF portion of the free active precursor molecule. Because the antibody does not recognize the ANF fragment (18–28), it is suggested that an intact cystine bridge is required for binding or the portion of the peptide close to the N-terminus is the site that is recognized by the antibody [4,5].

2.5. Ultrastructure analyses

Examination of ultrathin sections were done after all were contrasted with both uranyl acetate and lead citrate. prior to examination in a Jeol 100 S 100S (Jeol Inc, Peabody, MA USA) electron microscope and a Siemens Elmiskop (Mechelen, Belgium). From both ultrastructure observations, Kodak 4489 EM negative films were printed, and digitized images were recorded with an Epson V550 home scanner for our collection.

3. Results

3.1. The teleost cardiovascular functional anatomy

The cardiovascular functional anatomy of a teleost fish can be summarized as made of two main chambers, the atrium, and a ventricle. When venous blood arrives from the body with both common cardinal and hepatic veins, it first reaches a thin-walled confluent sinus venosus that carries it into the atrium and then the ventricle where it is pumped to the bulbous arteriosus and out, it reaches the gills capillary beds before distributed to the general capillary beds of the body tissues. Anatomically, the young bass ventricle wall is the thickest of all structures considered as verified with histology and fine structure. The collected micrographs were organized into panes to suggest sequentially the dynamics about the intracellular cytology of intracellular stowage, unusually kept in this juvenile tissue.

3.2. The juvenile smallmouth bass heart histology and ANF immunolabeling

The atrial histology appeared fragile and more finely trabeculated than that of the ventricle, especially when one considered its internal muscle composition of thicker fascicles in cardiomyocytes (Fig. 1A and 2A vs Fig. 1E and F). Even though the tissues collected are still undergoing growth, considering the age of the animal (less than 1-year old), one can recognize with both light microscopy and at a low ultrastructure magnification that these cardiac walls encompassed all the three tissue layers of the heart of all the vertebrates from outside in: epicardium, myocardium, and endocardium (Fig. 1A and 2A). Each layer contains their proper cell types and both epithelial inner and outer layer show their basement membrane, consisting of both basal laminae and connective components issued with the network of organized cardiomyocytes myocytes from the developed mesoderm. We shall describe our observations from outside to inside structures, as the paragraphs to follow.

3.2.1. The pericardium or pericardial layer

The extension of a mesothelium layer corresponded with the outermost squamous epithelium that typically covered the heart, encompassed by a thickness comprised between 0.9 and 3.05 μm. It covered a connective layer that ranged from 5.0 to 9.0 μm in thickness that comprised connective fibrillar and fibrous collagenic bundles where sustaining cells, the telocytes, can be found in most random sections. These cells can be recognized within the pericardial layer by their

peculiar stroma morphology, nucleus and perinuclear cytoplasm displayed a triangular to ovoid shape radiating slim, moniliform cytoplasmic extensions or telopodes where thin segments (podomeres) and dilated portions (podoms) reached out a fibrous connective matrix in the epicardial fibrous layer (Fig. 1D and 2A).

3.2.2. The myocardium or myocardial layer and the atrial natriuretic peptide or factor (ANF)

The myocardial layer contained cardiomyocytes whose morphologic aspects were always recognizable in random sections, either illustrated by sarcomere segments of myofibrils that revealed typical myofiber aspect: a central nucleus, surrounded by organelles and sleeves of myofibrils that demonstrated their sarcomere components and intercellular junctions (tight, desmosome-like and gap junctions) in diverse orientations, according to random planes of sectioning whether for atrium (Fig. 1D, 2B-D) or ventricle (Fig. 2E, F, 11E and H). All the atrium wall myocytes were organized into delicate, trabecular bundles, and with random sections, appeared sticking out into the heart blood circulation lumen. They displayed round to oblong mitochondria profiles amongst and between myofibrils along with dispersed glycogen and ribonucleoprotein granules.

In comparison to the atrium, the ventricle myocardium is seemingly contracted by complex, thick, entwined trabecules apparently devoid of protruding extensions. The cardiomyocytes typically depicted a central sarcoplasm region where large number of elongated mitochondria packed, surrounded by one or two myofibril sleeves. These cells did not show lysosome-related structures and deposits.

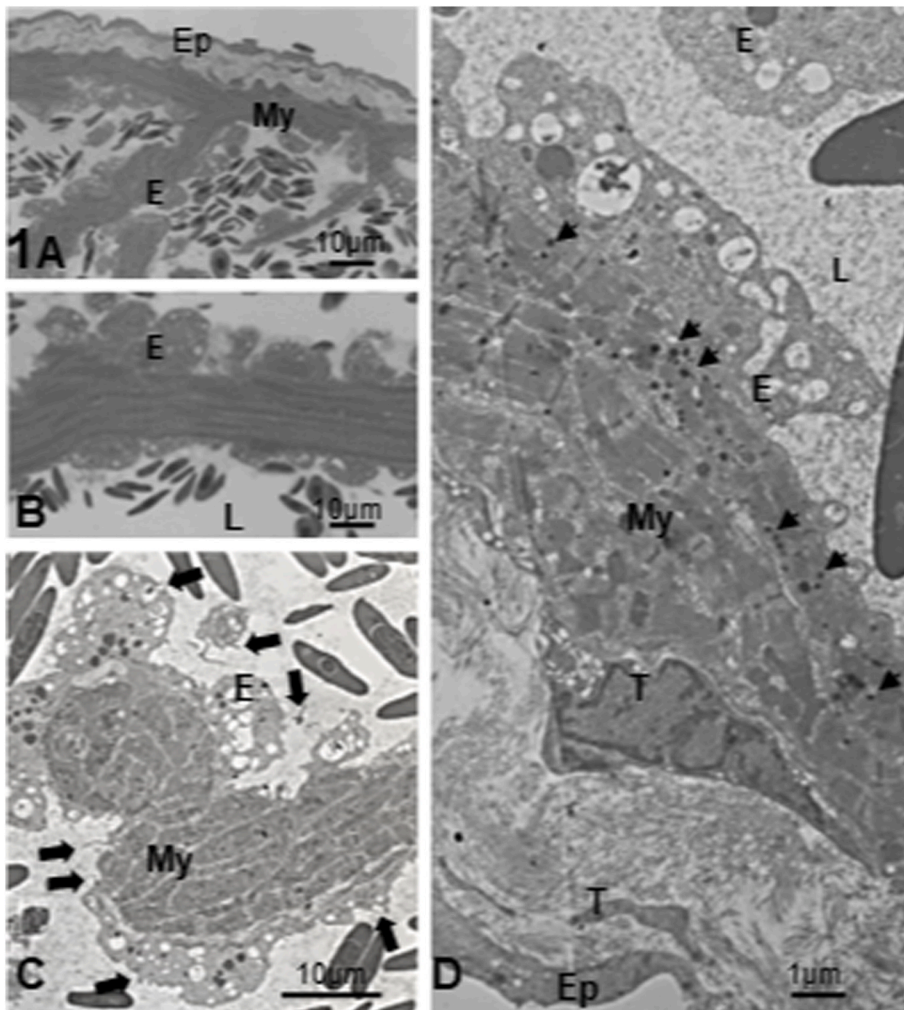


Fig. 1A-D. Heart of juvenile smallmouth bass (*Micropterus dolomieu* Lacépède). A-B: One-μm epoxy thick sections, toluidine-blue stained of cardiac atrium wall in cross and oblique sections. Note the muscular trabeculated aspect of the myocardium. C-D: Fine structure featured endocardial pinocytosis, pseudopodia (black arrows) and vacuoles formed by endocytosis. D reveals the ultrastructure of part of A and series of small arrows mark atrial hormone-containing vesicles. E: endocardium, Ep: Epicardium, L: lumen/blood space with erythrocytes; My: myocardium cardiomyocytes; T: Telocyte; v: vascular structure.

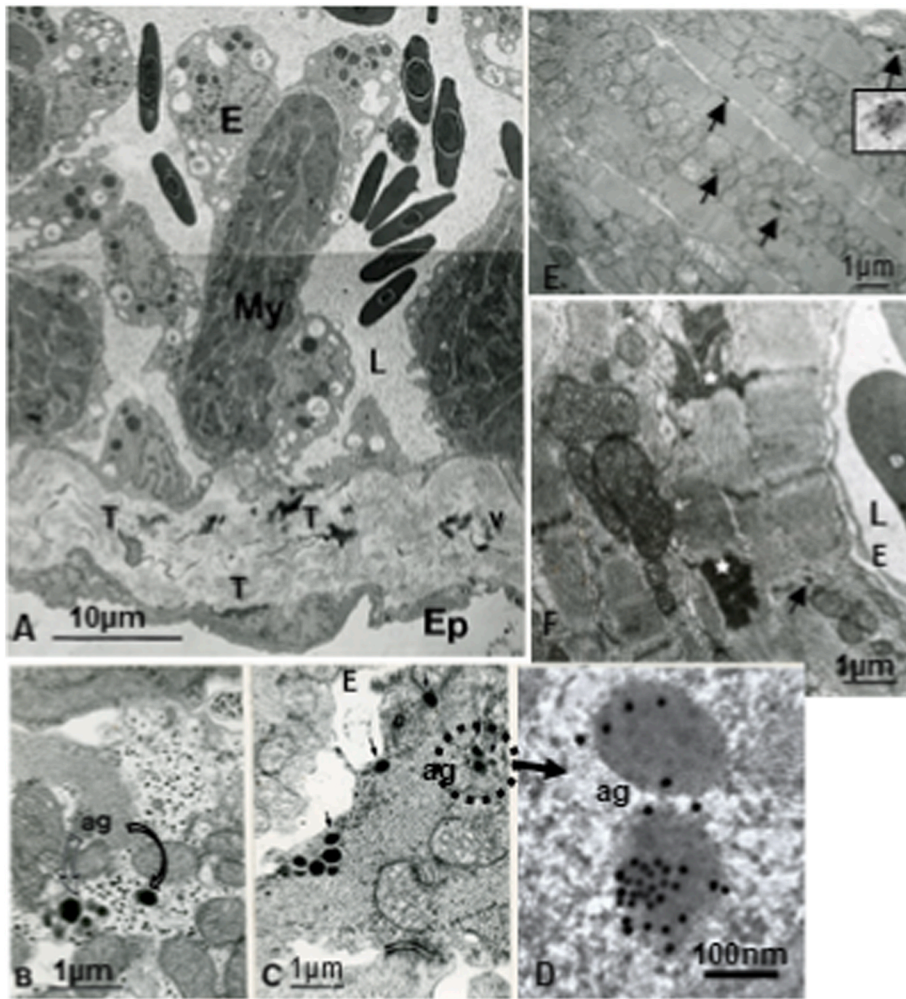


Fig. 2A-F. TEM views of parts of the trabeculated, atrial myocardium of the young smallmouth bass (*Micropterus dolomieu* Lacépède). A-D: The spongy, finely branching myocardium (My) is surrounded by a narrow endocardium layer (m) with dense vesicles and surface projections toward the lumen supported by the epicardial (Ep) collagen bundled fibres containing telocytes (T). Specific atrial vesicles said 'granules' are shown in B-D amongst ribonucleoproteins in myocyte's sarcoplasm; in D, ANF content of 2 adjacent vesicles, almost similar to those viewed in C, immunolabeled by 15nm gold particles (My). E-F: Ventricular myocytes: tightly packed mitochondria in their cores with loosely groups of glycogen granules; there, typical sarcomere specialization contacts (* and white arrows). L: blood lumina lined by a thin endocardial layer (E) devoid of electron dense bodies. Note ventricular myocyte atrial vesicles (arrows) and insert with example of one ventricle vesicle content as immunolabeled.

According to random sectioning, both atrium and ventricle myocytes contained variable numbers of round to and oblong electron dense vesicles that reached 50–200 nm in diameter; they scattered in the free subsarcolemma or the intermyofibrillar sarcoplasm. These vesicles recognized as atrial 'granules' contained the natriuretic peptide or factor (abbreviated ANF; Fig. 2A–C) produced by the Golgi apparatus to be released in the circulation. Their content was also identified by the immunogold labeled antibodies against the atrial natriuretic peptide or factor (both Fig. 2D and E, insert E and F).

3.2.3. The endocardial endothelium

Classically, the most inner heart endothelial layer or blood-heart barrier is constituted as a simple squamous-like cell layer. In this fish, eyesight view showed a darkened, almost blackish layer and this feature appeared with light microscopy as tall, cuboidal-like cells with a large subcentral nucleus perikaryal zone decorated with unusually contrasted organelles. Low magnification ultrastructure verified that most cells prominently bulged into the circulation space or lumina (Fig. 1A–D, 2A, 3 A-B) and higher magnifications made these electron dense ones to show either vacuolated or massively contrasted contents, as in lipofuscin bodies. Golgi apparatus were noticed and other organelles surrounded by innumerable ribonucleoproteins or fixed as rough ER, where glycogen stores remained undetected, compared with an abundant stowage of the adjacent cardiomyocytes (Fig. 7B). The subsurface zones of the same endocardial cells featured both pinocytosis and endocytosis structures. Numerous tubular-like parts of the smooth endoplasmic reticulum were issued from the rough one and the reticulum also connected few

mitochondria outer membrane along with some altered ones (Fig. 5A–B, 7B). Morphology alone without markers showed the Golgi distal saccules clearly formed primary lysosomes as well as diverse vesicles. The small ones populated the subsurface cell regions and after capture some circulation products constituted secondary lysosomes, and residual bodies, revealed as those heavily contrasted oblong-shaped ones with complex content, classically described as lipofuscin bodies in eukaryote cells. These organelles were parts of cell's narrow extensions (0.2–0.6 µm in thickness) and contacted similar adjacent cells by slender cell edges with poorly defined junctional complexes (Fig. 2A) and the same cells bore delicate extensions, almost appearing as 'invadosomes' that ran underneath endocardial adjacent cells (i.e., Fig. 3A). Mitochondria, and, among them, some appeared altered. Scattered particles as ribonucleoproteins (ribosomes) appeared throughout without glycogen, unless part as dispersed, with similar size as the isolated ribosomes found in myocytes where the clumped glycogen and dispersed showed in discrete zones (Fig. 5C). Ribosomes showed as strings along mRNA throughout the cytoplasm (Fig. 5D and 6A and B), or attached to the endoplasmic reticulum as RER (Fig. 5D and 6A, C) that sometimes extended into smooth ER (Fig. 6C). In all the observations, the basal lamina was poorly or not clearly detected (Fig. 4 B–C, 5 A-B, 6, 7 S, 11 D, E, G); instead, a poorly contrasted meshwork of fibrillar materials or an occasionally thick bundle of collagen bundle was seen surrounded by endocardial extensions and telocytes that, altogether, depicted a separation between this endocardium from the myocardium (Fig. 11 E). In comparison, the endothelial endocardial layer covering the ventricle myocardium, was slim (0.1–0.5 µm and did not reveal those intracellular

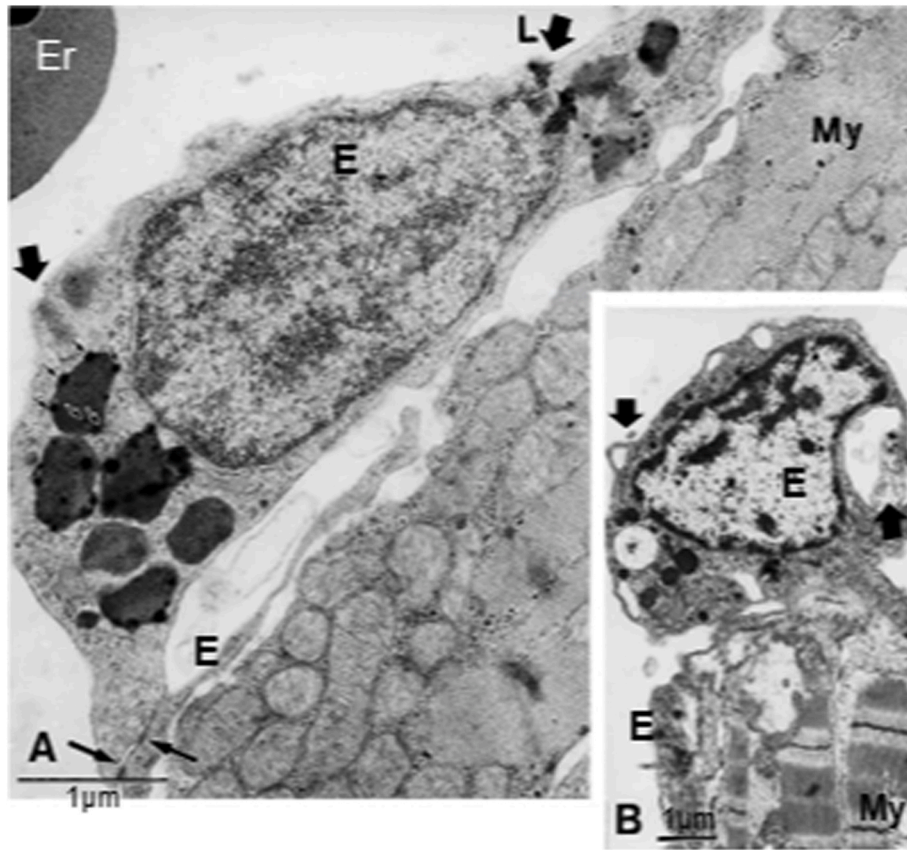


Fig. 3A-B. TEM aspects of endocardial cells (E) of atrium of juvenile smallmouth bass covering the myocardium whose content in endocytosis activities were verified by large lysosomal bodies and vacuoles resulted from captures from circulation components (thick arrows). Junctions between endocardium and myocardium are noted by thin arrows. L: atrial lumina.

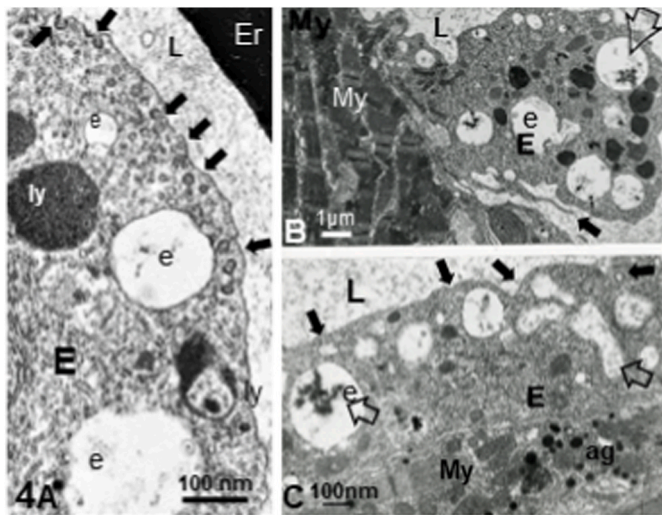


Fig. 4A-C. TEM enlarged aspects of subsurface parts of endocardial cells of a juvenile smallmouth bass. A: Numerous pinocytosis activities (small arrows) and B-C: vacuoles with diverse inner content (e for endosome, and some open arrows), resulting from endocytosis captures among contrasted lysosomal bodies. In C: ag: atrial granules of cardiac peptide; My: cardiomyocyte; L: blood lumen.

deposits (Fig. 2E-F). Other sub endocardial wandering cells are described in Fig. 11 B-H and in a further paragraph.

3.2.4. The endocardial endothelium phagocytosis activities

3.2.4.1. Pinocytosis. Light microscopy revealed numerous round profile vesicles (Fig. 1B), and, with fine structure, the same endocardial superficial cytoplasm revealed countless small vesicles that ranged from 15 to 25 nm in diameter issued from the plasma membrane rim as pits with or without coat. According to random observations, a cell section can contain several hundred of them while adjacent cells had few. (Fig. 4 A, 6, 7A, B and C).

3.2.4.2. Endocytosis. Some light and fine structure micrographs revealed extended endocardial cell pseudopodia into the circulation swooping fluid content and creating endosome wide to narrow invaginated grooves or coves that made phagosome vesicles (Fig. 4 A, B and C) with the contribution of membranes out of the Golgi apparatus (Fig. 5-B-7B) and clear lysosome formation and growth (Fig. 4A, 5A-B, 6A, 7D).

3.2.4.3. Processing, lysosomes, and deposits. Merged pinocytotic vesicles and other wide vacuoles resulted in electron contrasted bodies, made of heterogeneous floccular and granular content that haphazardly filled in part these vacuoles, delineated by a typical (double leaflet) plasma membrane, and ranged from 50 to 1800 nm in diameter as endosomes. These also appeared out of the Golgi apparatus zones, made of 3 or more evident cisterna distributed as more or less pileups or stacks. Toward these zones, vesicles can be seen and reached *cis* sides while from the outermost edges of *trans* cisternae erupted other vesicles either emptied-like or, like cherries, lysosomes bore an electron dense contrast out of

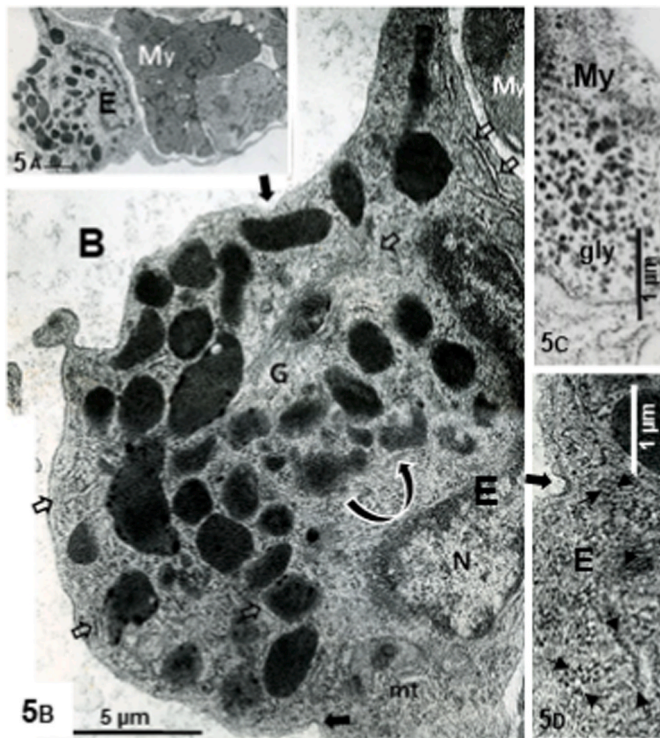


Fig. 5A-D. TEM of an endocardial endothelial cell of juvenile smallmouth bass (*Micropterus dolomieu* Lacépède). A: Bulging cytoplasm loaded by lysosomal bodies and extensions covering subjacent myocardial fibers (My). B: Enlarged view of A demonstrating the prominent perikaryal zone (N: nucleus) filled by crowded organelles: rough and smooth endoplasmic reticulum (open arrows) squeezed among a Golgi (G) forming out of lysosomes secondary lysosomes. Coated tiny pinocytotic and some pitted invaginations (black arrows) accumulated as electron-dense bodies (curved arrows), of heterogeneous content that showed oblong shapes. C: Enlargement of glycogen reserves displaying aggregates of diverse size in kept apart cytoplasmic zones of endocardial cells (in 6A) while, in D: Ribosomes of endocardial cells appeared either attached to endoplasm to become RER or to strings of mRNA making polyribosomes.

their acidic pH content (Fig. 7C–D).

The observed endosomes seemed originated from the poorly contrasted Golgi vesicles that filled up progressively with contrasted compounds captured from the circulation joined by pinocytosis and endocytosis. These events can make with fine structure a partly granular to cloudy electron dense components with large surroundings of the vacuoles that resulted from these captured circulating plasma compounds (Fig. 1 D and 4 A-C). Meanwhile, these large, poorly filled vesicles, appeared as endosomes (Fig. 1B–D, 2A, 3B, 4A-C, 6A, 8A). Can these become progressively filled to appear later with light to heavily contrasted, round, and elongated bodies that ranged from 30 to 750 nm? (Fig. 5 A-B, 6A, 7A, C and D). Some views could suggest their expansion from fusion between small ones and small ones with large ones (Figs. 4A, 6A and 7A). In most endocardial endothelial cells, out of these endocytosis activities lysosomes as primary ones, through their ‘digestive’ activities became entirely filled secondary lysosomes; they showed with high electron contrast as round to oblong, fusiform profiles that can reach from about 100 nm to 4.5 μm in size (Fig. 3A, 5A-B, 6A, 7C-D). Among these huge, contrasted secondary lysosomes, some bodies can become named lipofuscin bodies for some that contained 50 nm wide, round, opaque droplets [Fig. 6A and 7A, C and D, 8A-B, 9A-D) and further heterogeneous low contrast, fibrillar-like line up packed structures that either straighten out, bent, or twisted along the axis parts of the deposits; each with less than 10 nm in thickness (Fig. 8A and 9A-D). Particulate and round internal contrasted parts of the deposits usually located in the outskirts of these contrasted bodies (Fig. 3A, 5B and 8A-

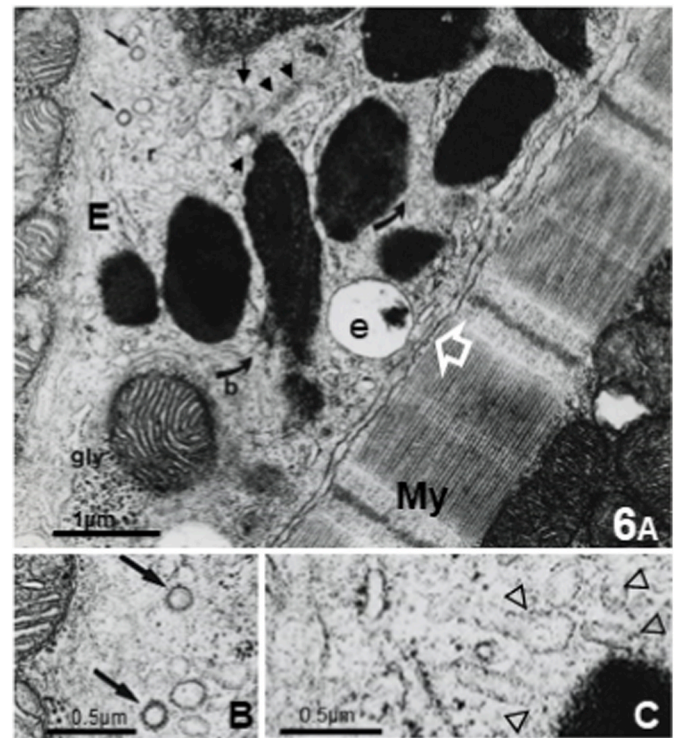


Fig. 6A-C. Smallmouth bass oblong, interconnected lipofuscin-like bodies in the endocardial endothelium cytoplasm, in close contact with an adjacent myocardial fiber (My), marked by the white open arrow. Note the abundant rough endoplasmic reticulum and glycogen (gly) associated with dense vesicles among mitochondrial profiles and an endosome (e) that captured some endocytosed compounds carried by coated vesicles (e.g., small arrows).

B, and 9 A-D). All the organelles, especially mitochondria with all the lysosomal bodies and those that can appear as lipofuscin bodies, displayed connections with one another through membrane-like channels, as a contiguous and continuous networks, as exemplified in Figs. 3A, 5B and 6A and C, 7C, 8A-B, 9 A-D. In 8 A and B, one can see the complex interconnected assemblies of phagosomes that could facilitated fusion with one another, as suggested by curved arrows, producing further enlargement of these lipofuscin, heterogeneous bodies. A diagrammatic representation of the viewed pinocytosis and cytophagocytosis is attached Fig. 10.

4. Other sub endocardial cells, including the melanomacrophages

Even though the circulating cells of the juvenile smallmouth bass were not the topic of our investigation, loitered cells in the vicinity of the endocardium can be reported here. Few leukocytes, possibly lymphocytes, were noted within the blood circulation compartment (Fig. 11 A). Other cells were found in the sub endocardial lining that were like the plasma cells of other fishes, depicting a large network of rough endoplasmic reticulum throughout the perikaryal regions, sometimes with dilated aspect (Fig. 11B–C). Further cells were either single or grouped, could be named lymphocytic macrophages, like the one exemplified in A, contained lysosome bodies that have accrued intercellular materials (Fig. 11D–E). Lastly, and depicted in Fig. 11 F to H and Fig. 12, wandering cells that contained lysosomal-like bodies with quasi whorls, like the melanogenic depots and melanosomes of higher vertebrates, were also in the sub endothelial spaces. They differed from the endothelial cells that never contained such coiled aspect associated with fibrillar and other components that congregated into vesicles constituted by a sort of polymerization, into an eye sighted ‘black’ pigment.

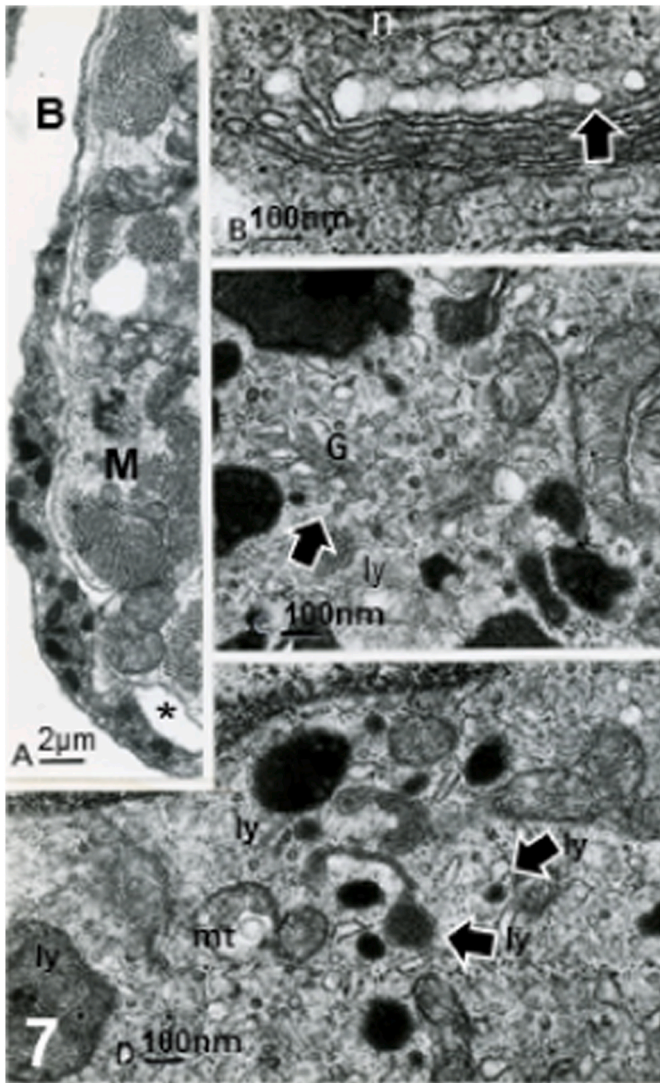


Fig. 7A-D. Ultrastructural aspects of the endocardial endothelium cells of juvenile smallmouth (bass) fish (*Micropterus dolomieu* Lacépède) and its phagocytotic content. A: Thin endocardial endothelial aspect whose extensions covered subjacent cardiomyocytes (M). B: A large perikaryon (n: nucleus) Golgi apparatus part is noted by its *cis* wide, adjacent piled cisternae where a series of constitutive vesicles erupted. C: Numerous small, coated vesicles and lysosomes (ly) fused as multivesicular bodies forming progressively enlarged, heterogeneous, phagosomes. D: Lysosomes issued out *trans* Golgi complex (matured like suspended by the neck of narrowed cistern; arrows) formed secondary lysosomes or phagosomes network out of fusion with small, coated vesicles and other transfer of endocytosis vesicles.

5. Discussion

The smallmouth bass is a benthopelagic freshwater fish, Actinopterygian Perciform, out of Centrarchidae family (Sunfishes); it usually inhabits shallow rocky-or gravel-bottom runs near flowing pools of rivers, or other streams and reservoirs fed by such streams. Juvenile bass feed on plankton and insect larvae while adult become carnivorous [60–62]. Juvenile fish organs, especially dealing with fine structure have yet to be compared to aged ones, like in mammals.

The opportunity of investigating the cardiac fine structure and atrial peptides of this young smallmouth bass, a Teleost fish, made us able to describe and identify this peptide secretion in this low vertebrate organ and to reveal about the pericardium, myocardium, and endocardium components, and to go beyond in revealing peculiar structures that could relate to still unresolved mammalian homology. Heart and other

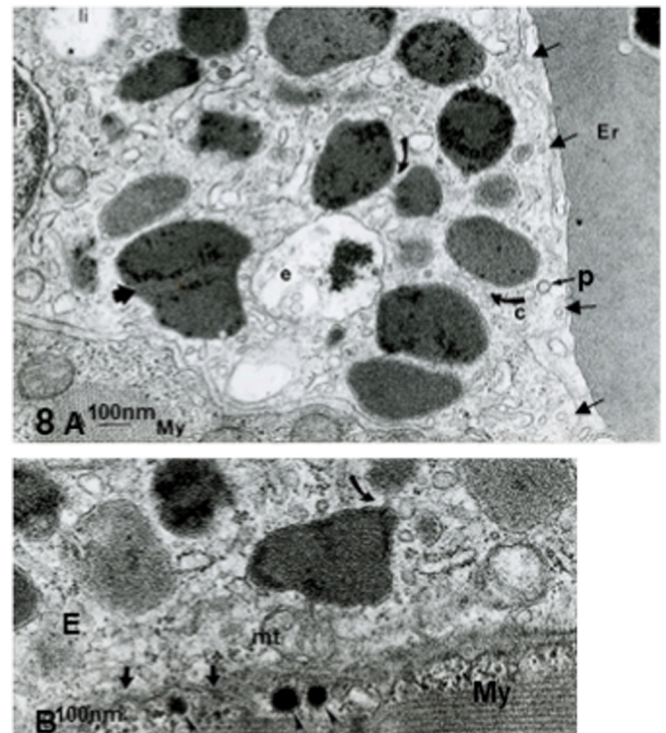


Fig. 8A-B. TEM aspects of endocardial endothelial cells loaded with secondary lysosomes in juvenile *Micropterus dolomieu* Lacépède. A: Pinocytosis (small arrows) and a large endosome (e) with loosely contrasted accumulation. Large accumulated secondary lysosomes, with increased electron density, surrounded by scattered ribonucleoproteins, and smooth endoplasmic reticulum seen in both A with B, extended as interconnecting channels between organelles, including lipofuscin bodies (curved arrows) where a thick arrow marks an example of mature body containing a centrally located score of poorly contrasted bundle, also found in Fig. 9. E: endocardial endothelial cell nucleus; li: lipid droplet; My: cardiomyocyte. B: Phagosomes suggested size and content thickened and channel (curved arrow), caused their electron dense, heterogeneous content. Small contact zones with adjacent sarcoplasm of an adjacent myocyte are viewed (small arrows). Arrowheads in adjacent cardiomyocyte (My) contained cardiac atrial granules.

vascular structures have been studied, reported in Refs. [36–44], and recalled some of the initial cardiovascular development of the mammals, including humans. If the pericardium showed a typical and similar general histology of any vertebrate, both atrium and ventricle revealed some new features and could favor other translational research studies especially pertaining to the endocardium heart-blood barrier, its development and maturation, and those other derived key functional specializations, such as the conducting system, and the valves [45–47]. For these developmental aspects and for some clarifications deemed of interest for mammals, a couple fish species had and are still more favorable used, such as the so-called zebrafish (*Danio rerio*) and the Japanese Medaka (*Oryzias latipes*) [e.g., 9, 16, 42, 46, 49, 51, 52, 53, 54, 55, 56, 57, 58, 59].

5.1. Pericardium

This cardiac layer, as a mesothelium component, did show similar components as those noted in mammals [68–74] or fish species [75–77] and, if one considers all proportions and size, the cell’s connective constituents had telocytes [78]. This fish pericardial connective tissue should be fused with that of the depressor-pharyngeal muscles, the body wall muscles or the peritoneum. and this semi-rigid ‘box-like’ and may not allow inwards cardiac bulge during systole but, instead, into the pericardial cavity [75,79,80]. The pericardium could function as in

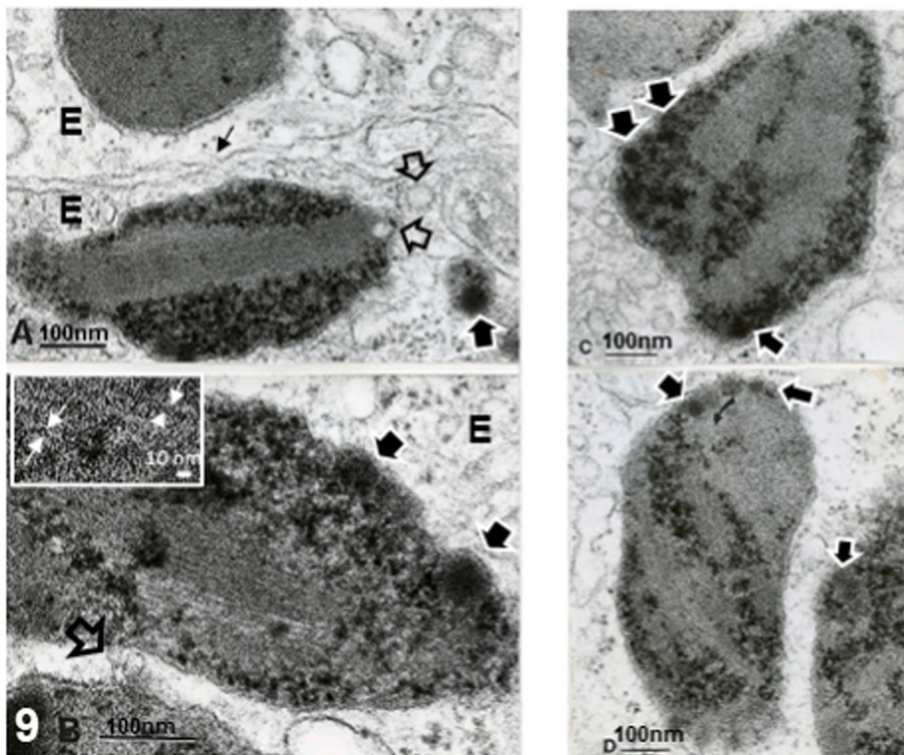


Fig. 9A-D. Enlarged random view of 50 nm thick section of some of the mature lipofuscin bodies in endocardial cells of a juvenile smallmouth bass fish (*Micropterus dolomieu* Lacépède). A: Adjacent endocardial cells revealed phagosome heterogeneous content and shapes. In the largest bodies, fibrous bundles were centrally located, surrounded by disseminated heavily contrasted particulate bodies. B: Another view of a similar body as in A where an insert depicts the details of the fibrillar-like structure, made of less than 10 nm thick material appearing as fibrils or plate-like components, marked by white arrows. C: Lipofuscin or residual phagosome with multiple fibrous bundles; Both C and D: Fibrillar bundles across lipofuscin bodies, including twisted one (arrows). Note that some round content parts, revealed at outskirts heavily contrasted content that could suggest lipid components (open and black arrows) admixed and getting coalesced into the 'black' pigment aspect seen at low magnifications.

mammals as protective, mechanical, and related sensing device, ruled by the Frank-Starling mechanism [79–81] but little is known [81].

5.2. Myocardium and natriuretic peptide hormones

This muscular layer wall of the heart atrium is delicately trabeculated with only, at the found stage of growth, made of 2–6 myocytes thick versus the ventricle where at least 5 to 20 myocytes constructed the chamber wall thickness. In both atrium and ventricle, cardiomyocytes linked with specialized, intercalated junctions as described classically as in mammals [e. g., Refs. [82–86]] with little endomysium and a perimysium and epimysium equivalent made by the telocytes and the associated connective fibers and extracellular matrix associated as found in the pericardium layer [78]. No clear elastin components were noted in these juvenile hearts. Most cardiomyocytes reveal tiny to elongated gap-like junctions with the endocardial layer, as already described [e. g., [55,87]], and also reviewed in Ref. [55].

In fishes, the gills, the kidneys, and the digestive tract are the main organs involved in the maintenance of body fluid balance or osmoregulation that are controlled by interconnected endocrine and autocrine feedbacks [88–95]. Among those organs, the gills, acting as a high vertebrate 'lung', can be modulated by local innervation [96,97] as well as, in the freshwater species, by the activities of thyroid-prolactin-cortisol hormonal interactions [95,98,99]. Furthermore, the heart, like in all vertebrates, acts as a pump [36–41,47] and functions also as an autocrine and endocrine organ, secreting its hormones toward those above-cited organs involved in electrolyte homeostasis [93,98,99]. One can think about the Na^+/K^+ ATPases as the most important ion pumps essentials to provide homeostatic balance for the maintenance of many organ cells that included the cardiac myocytes in normal development and growth, including in fish [94,98,100,101]. This Na^+/K^+ balance included the influences of the cardiac endocrine and autocrine secretions, discovered as atrial peptides or 'factors' in the rodent heart atrium by both Canadian groups of De Bold and Cantin [102–106] that have shown clues by hormone's blood levels with cardiac regulation and atrial normal and pathology conditions [107–110].

The secretory content of these cardiomyocyte vesicles shows a co-location of a A-form as 'atrial' abbreviated ANP co-expressed with a 'brain peptide' or B- peptide abbreviated BNP, found in the pig brain [102,107,109] can be produced in excess in failing mammalian and human heart, especially atrium and ventricle peculiar re-expression [1, 100,104,107,109,110]. Both A and B, made by the myocyte's Golgi apparatus, can be delivered as secretory pro-hormones according to blood and osmotic pressure of the cardiac surrounding fluids and associated tissues [3,4]. It is after transcytosis via the endocardium [3,4] or any other endothelial barriers of the intravascular and lymphatics that the final, shorter active peptide can be recognized by its respective distant or and auto-receptors [4]. If A-form is essentially made in the mature normal, mammalian heart right ventricle myocytes [102], B-form can be found in lower vertebrates, including fish [5,111–116]. Another C-form (C for 'for cytoplasmic' or sarcoplasm) noted as 4 CNPs are produced by the fish heart atrium and ventricle [112–117]; they can regulate body osmolarity homeostasis and have heart autocrine influences. The B-type can modulate autocrine and autonomic functions [118,117]. Meanwhile, autocrine functions cannot be ignored for the sake of translational applications into clinics as this C-peptide exhibits other favorable modulating actions on cardiac structure and functions, including post-trauma fibrous healing [118,117], of autonomic system, the central nervous system, osteochondral system, and vascular system [119], as shown in fish [120–123].

5.2.1. The endocardium layer

Most fishes, like in this juvenile one, the atrium wall is thinner than all the other cardiac walls [36–41,44] and the endocardial endothelium of the atrium, whether examined with light or electron microscopy should have resembled a typical squamous tissue as a 'blood-heart barrier', like most of the other vascular endothelia, but instead is thicker [8–12,19]. One can recall that this layer constitutes a selective and potent homeostatic control fence for circulating compounds as well for endocrine transcytosis, including making the necessary processing passageway for the atrial peptides towards the circulation, as we had described with immunolabels previously in an adult mammal [1–4].

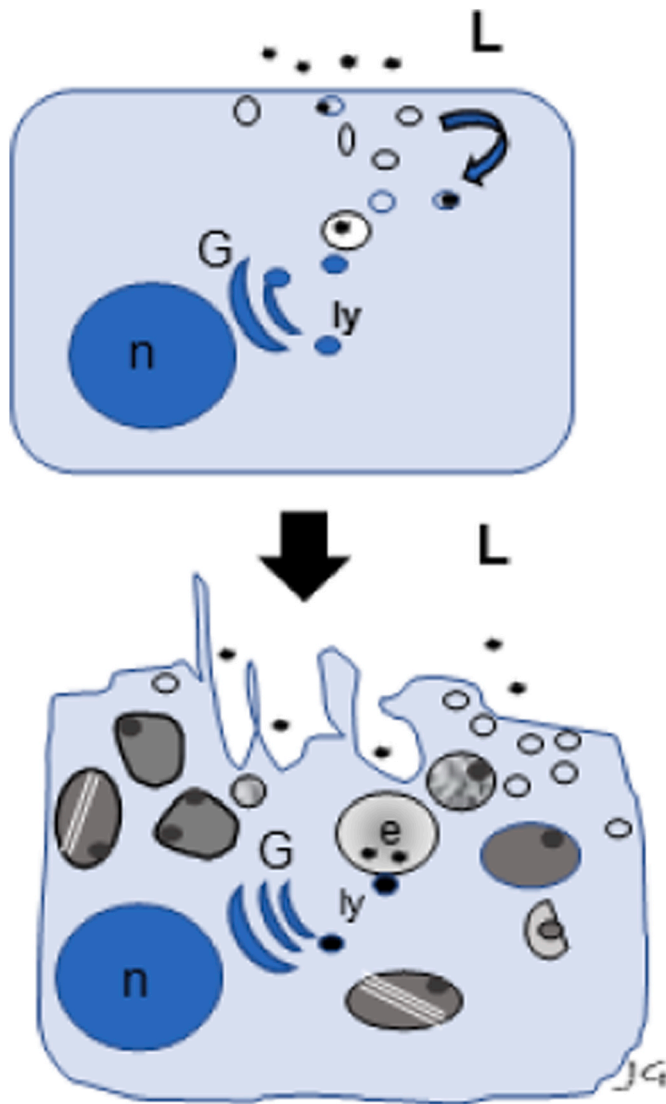


Fig. 10. Diagrammatic representation of endocardial cells with both pinocytosis and endocytosis resulting in phagosomes and lipofuscin bodies. The former are small vesicles while the latter are the result of cell's extensions captures that form content highlighted with grey to black and eventually striped. G: Golgi; L: circulation lumen; ly: lysosome; n: nucleus.

Some aspects of endocardium endothelium studied in development with fine structure in both fish, mammalian heart, including human, showed discontinuities [1,124–128], incurred with cardiomyopathy [127] can show interest for further studies about permeability of endothelium and pathology [126,127,129,130] and as a selective filter and a capture wall for circulating compounds after development [130–139], unless damaged in myopathy [126–130]. Out of all mentioned above, fish models have been and is astonishingly used for progress in understanding mammalian development and lineage [42–45,49–55,136,139–141] for pacemaker (Purkinje) cells [48], valves [46–50,52,131,132,134,135,142,143], the coronary endothelium and pericytes, arteriogenesis after myocardial infarct, and the resulted, healed interstitium fibrogenesis and adipogenesis [132,135,144–146] and even myocardium [143]. Many aspects are still in need for further clarification, especially considering the complexity of the ‘barrier’ components [134,137–139], the involvement in the embryonal endocardium to grow the liver vasculature and some hematopoietic aspects [132,142–147]. Altogether, this topic may also bring interest with new SARS pandemics [148,149]. However, the basic life cycle and renewal of

developed endocardium is poorly known [142] and appeared to last almost the entire life in mice [150].

This ‘barrier’ is also formed and located along the ventricle but there, it developed without revealing prominent cell size and those peculiar intracellular deposits [138], as in Fig. 2 E-F.

Furthermore, as we illustrated, the fish sub endocardial region revealed delicate, telocytes as final support of matrix as in mammalian heart [126,127], as Gherghiceanu [151], These cells extended in the matrix between myocytes as ‘endomysium-like’ and can also contain other wandering cells and, among them, those named melano-macrophages (see 4th paragraph).

5.2.2. Accumulated residual bodies aka lipofuscin bodies: aging wastes or valuable products?

Similarly, as many other authors cited in the Introduction, our observations fitted with the repeated scheme found in many fish species previously studied, that captures by the layer of endocardial cells of the heart contributed to retain or eliminate foreign circulating particles, altered blood cells, other increased amounts of circulating ionic or macromolecular species as in reticuloendothelial tissues [8–19]. These observations [17,18,20,21,23] or experiments [24,27–29] comforted those and can bring about translational aspects to understand how, in animal and human pathology, circulating compounds could favor some atrial alterations and pathologies as found in other vertebrates, including mammals and thus, humans. In overload cardiomyopathies.

The fish atrial endocardium contained abundant electron dense bodies [74]. These were associated with few mitochondria and because glycogen stores were not found here, compared with an abundant stowage of the adjacent cardiomyocytes, the endocardium lining would get nutrients through diffusion of circulated nutrients, making unwarranted (or warranted) storage of basic foodstuffs. Golgi saccules noted were providing diverse cell's vesicles and, with the endoplasmic reticulum, constituted an active vacuome that consisted in innumerable pinocytotic coated and uncoated vesicles, (i.e., receptor-mediated endocytosis [152]) associated with typical endocytosis viewed as cell's extensions formed by the apical surfaces. Both pinocytosis (the tiny vacuome parts) and endocytosis (the largest protrusions) activities got collected in endosomes, located deep in the perikaryon, as first described [153], with co-operation of Golgi [153,154], and further reviewed with molecular aspects [155–161], including morphologic aspects [153,157–167]. In our observations, the endosomes featured as dilated vacuoles with small, contrasted content. These structures probably accommodated heavily osmotic species that could include iron (hemal or ferritin) species - where granular content appeared to fit with - due to possible, diverse metabolism along with other circulated, organic chelated metals as shown by many experiments [18–35], similar to iron found with microanalyses that seemed to stabilize the lipofuscin deposits [168,169]. Issued from residues of intracellular ‘digestive’ processing [159,160,167,170] as captured compounds can be used by the cell's stomachs, i.e., the lysosomes, as addressed by transporting signals to be degraded as found with biochemistry [159–163] and ultrastructure [160,164–167] or as it seemed for many cells, remained ‘undigested’ that lead to accumulated contrasted bodies after involvement of the Golgi apparatus that evidently formed these primary lysosomes. Because these bodies whether small or large did not bore a double or concentric surrounding membrane, thus were not the result of autophagosome processing. Instead, bodies could be suggested out of in vivo ‘dehydration’ and fusion with lysosomes formed in their perikaryal proximities, after capture of some circulation products both pinocytosis and endocytotic small vesicles, constituted and managed by the coated cytoskeleton in a reverse transport towards captures [153,158], thus making them secondary lysosomes [158–161], and, later, as stowed, residual bodies or lipofuscin bodies, revealed as those heavily oblong-shaped contrasted ones with complex content as those classically described in eukaryote cells by DeDuve and others [162–167]. Similar heterogeneous lipofuscin bodies, loaded with cholesteric compounds were noted

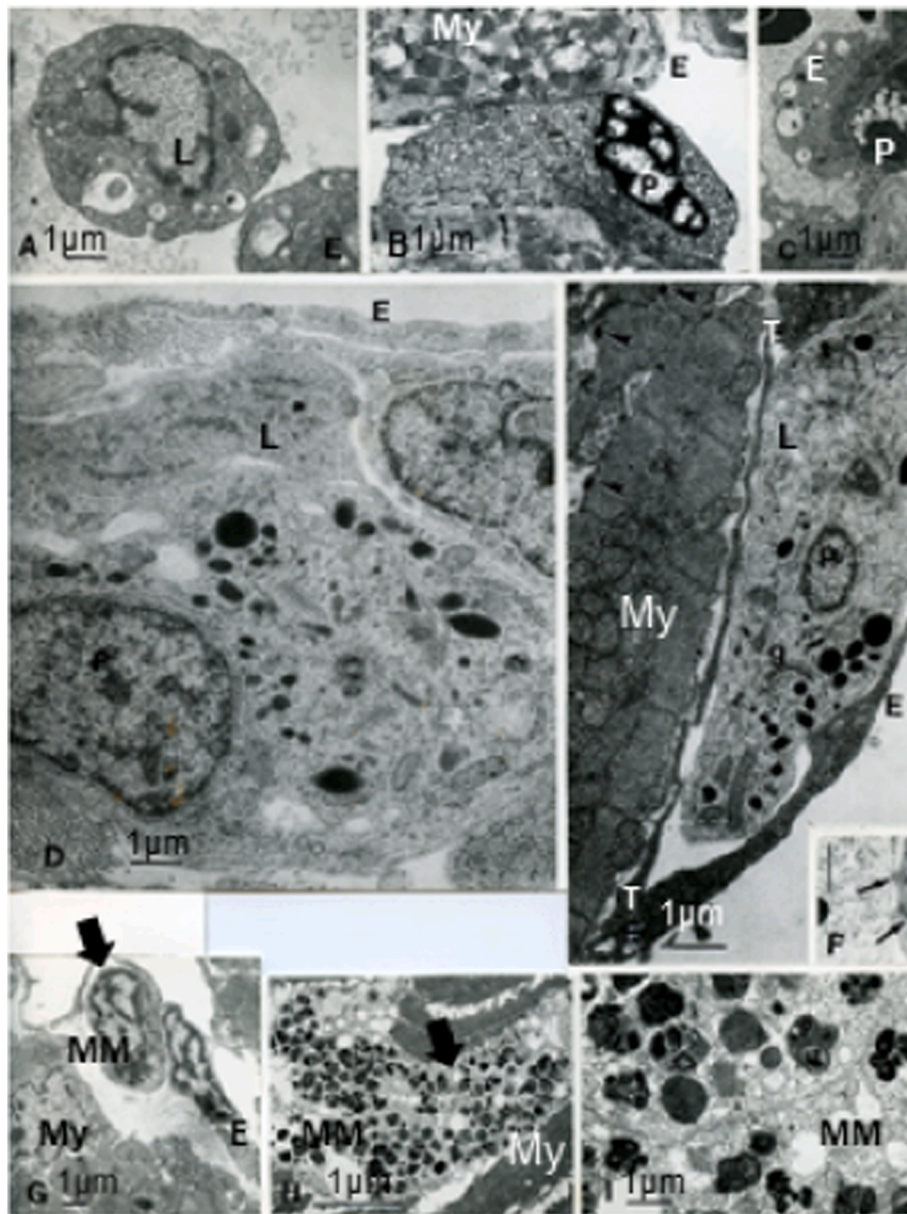


Fig. 11A-H. TEM of smallmouth bass blood and sub endocardium cells A: Circulating leucocyte (L); B and C: plasma cells; E: endocardial endothelium. In D: group of wandering macrophages and single one under the endocardium lining (E). In F, G and H: melano-macrophage cells.

previously as additional intracellular and captured turnover lipid species probably resulted in round-shaped internal contrasted substructures within the lipofuscin bodies (sphingosine ceramides?) as in Refs. [171–178] and other saturated or non-polar lipids contents (cholesterol metabolites, would form the other fibrillar-like, poorly contrasted and striated components that made long linear to twisted components as found in complex lipofuscin bodies elsewhere [165,176–179]. These poorly or ‘undigested’ components could recall about lesser active lysosome enzymatic species such as lipases as reported in Refs. [161–164,166,180,181], thus resulting in lipid-like ‘islands’ [179] within lipofuscin bodies.

5.2.3. Lipofuscin, a cardiac scourge in mammals but a bearable waste in a juvenile teleost?

Known to be formed out of accumulated products resulting from reactive oxygen species (ROS) cytotoxic activities that included metabolic ‘injuries’ of proteins, associated with overloads of proteosomes and lysosomes (as noted above) and, thus, linked to ageing in cardiac tissues,

known deposits in the cardiomyocytes [170,182–185]. Mainly caused by mitochondrial degradations [185–188], along with similar deposits found in other tissues with ageing [171,173–178,188–194], these accumulated residual bodies, issued from ‘maturation’ of secondary lysosomes, connected through a network of structured channels linking them with smooth ER as well as mitochondrial outer membranes [195–197]. These verified that not only contiguity, but certainly with free functional, spatial continuity between organelles in the endocardial cells. These fine structures do not appeared artifacts and found and preserved throughout all the endothelial cells. This morpho-structural network aspect has been part subject of others [198], including the book of Reinert [199]. Further interdisciplinary research could be conducted to understand heart cell’s functions and other associated pathologies involving organelle interactions with channels as biochemistry, including molecular ones [200–204] because among membrane (i.e. microsomal) fractions or supernatants from broths of organelles are analyzed, in bulk, without considering some of these submicroscopic associations. Nowadays, the advent of experienced

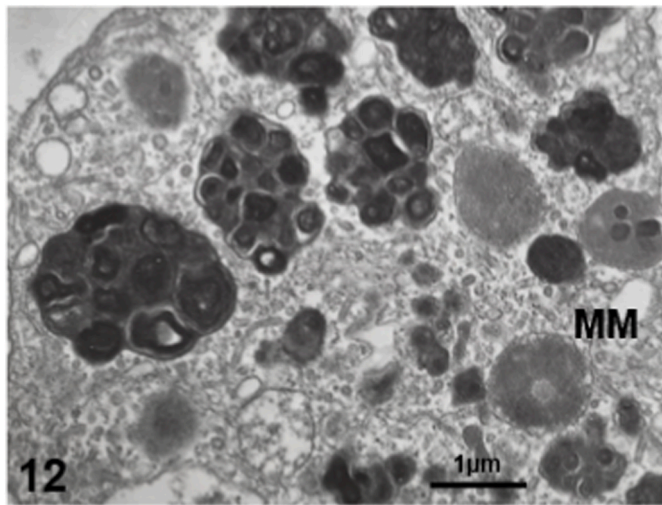


Fig. 12. TEM high magnification of similar melano-macrophage cells as in 11H and I. Note the whorl-like depots resembling those of melanogenesis and mature melanosomes.

molecular analyses and specific markers could further reveal some of those missing 'links.' [Strangely, peroxisomes have not been recognized with fine structure throughout, as we had compared our data with others that have dealt with fish tissues besides only a few ancient biochemistry studies (available, but not cited) that have dealt with fish peroxisome activities [205,206].

Can these waste collections expel from these juvenile cells [207] ? Out of all collected micrographs, we have not observed any, considering the tissue processing, one cannot be sure, as these events could be speedy. Only one recent report [208] (not peer reviewed) has claimed such process however, according to the micrographs of the report, the process seemed slow to occur.

Thus, if these deposits can become cytotoxic [e.g., Refs. [174,175,180,192,194,209,210]] and favor a cell death claimed as necroptosis [211] there was no such event found in the tissue examined. Alternatively, could parts of these stowed, even 'apparently' waste products be useful to still be used for some energy source for the cells? Can these accumulated products really alter the normal functions of these juvenile cells and tissues? [212] If the tenet can hold in this young fish structure, juveniles, collected at random from a fish farm tank, holding hundreds of similar ones, that would survive and usually grow as adult, and populate fishponds, what do we know about these cells turn over? Nothing, and the same for most other animals but a handful [142,150].

5.2.4. The lipofuscin and atrial peptides

If the atrial peptides made by the myocyte's Golgi apparatus can be secreted pro-hormones according to homeostatic osmotic pressure of the cardiac surrounding fluids and bodily tissues [3,4], the obligated transcytosis via the saturated endocardial cells with lipofuscin and the other endothelial barriers of the intravascular and lymphatics of the heart of a long peptide to obtain the final, shorter active peptides do not appear to be altered, considering the fish growth in the wild or in freshwater ponds. But in human, would an altered or pathologic atrial lining function adequately or not? Studies in old (with lipofuscin -the so-called aged pigment- or altered) mammalian endocardium are yet to be observed and published.

6. Cells of the sub endocardium

If the heart endothelium as endocardium captures and stows in a same manner of a reticuloendothelial tissue (see Introduction and e.g., [213–217]), some other cells have been noted wandering in the connective layer from the circulation with fine morphology different than

all endocardial cells [35,218–221]. Be as it may, one has compared them with those of specialists in fish investigations and recognized to be circulating leucocyte, plasma cells, in view of their nucleus/cytoplasm [219,220,222–224]. However, among those wandering cells, those one were called melano-macrophage cells [e.g., Refs. [17–19,21–23,29,30,34,35,214,215,220]] have facilitated their identification by their abundant organelles whose organelle's content (derived from early lysosomes) revealed whorl-like depots resembling those of found in melanogenesis and melanosomes [51,225–227]. They are always located under the endocardial endothelium and certainly would contribute to the pigmentation of the same atrium wall. However, besides showing fine structure differences from the surface endocardium layer, they were not investigated further. They likely contribute to form a screen pigmentation of heart structures originate from developmental stages involving the neural crest [45,46,49,228,229] building a 'protective' layer against excess UV B radiation while the maturing juveniles inhabit shallow waters [60–62] were melanic pigments can be associated with other functions [229–231], including pathology [232] as well as in human [51–56,200–204,228,233–236] where fish has been used because some features, such as those associated with the endocardium unknown deposits could also associate with heart development or failure and its complications [231,232,237–239]

7. Conclusions: translational aspects of endocardium changes and cardiac pathology

In view of the complex functionalities of an endothelium barrier and its specializations found in the heart, where most clinical data arose from clinics, one can mention pathologies can occur in young mammals and human hearts can also result from accumulated deposits still uncharacterized of endocardium. At the same time, the atrial peptides, markers for heart functions or defects, after production of precursors by and in the cardiomyocytes need to be dispatched across the endocardium to circulate [4]. If the endocardium 'intact' functions get compromised by perturbing deposits [239–241], can these peptides secrete in the circulation with normal flow and levels? As fish heart development can be easily studied, would this low vertebrate still a far-fetched model for some mammalian defects?

In all things of nature there is, you see, something amazing.

Ἐν παντί γὰρ τοῖς φυσικοῖς ἔνεστί τι θαυμαστόν [242].

Declaration of scientific writing

The authors have not used AI tools to write, analyse and draw insights from data as part of the research process.

Ethical concerns

Procedure and sacrifice of fishes were approved through the Animal Ethic Committee of the College of Medicine (1987–1991) and the Department of Comparative Medicine of NEOUCOM (now NeoMED), Rootstown Ohio, USA.

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School of Medicine, Newcastle upon Tyne, United Kingdom, and Scientific Collaborator at URPhyM, Narilis, Université de Namur, Namur, Belgium.

CRedit authorship contribution statement

Jacques Gilloteaux: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Methodology. **Lothar Jennes:** Methodology. **Jean-Jacques Vanderhaeghen:** Funding acquisition, Methodology.

Declaration of Competing interest

The authors report no conflicts of interest. The authors alone are responsible for the content of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tria.2023.100252>.

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