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Heart structure and ventricular ultrastructure of hemoglobin- and myoglobin-free icefish *Channichthys rhinoceratus*

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Summary. The structural and ultrastructural characteristics of the heart of *Channichthys rhinoceratus,* an antarctic teleost devoid of respiratory pigments, are described and compared with those obtained from the red-blooded related species *Notothenia rossii.*

The heart of the icefish is characterized by a spongy myocardium supplied with a highly developed arterial coronary system. This vasculature includes a subepicardial system and an extensive intratrabecular capillary network. Arterial hilar network and Thebesian vessels may also be present. The bulbus arteriosus shows unusually large spheroid structures located in the middle layer of the wall.

Both white- and red-blooded species display comparable myocardial cell morphology and organelle distribution. However, the mitochondrial cristae of the former are more densely packed and the sarcolemma possesses numerous caveolae. A large proportion of non-contractile cells is also found in the icefish ventricular wall.

Key words: Heart – Myocardium – Ultrastructure – Antarctic fish – Icefish

Antarctic fish of the family Channichthyidae, commonly named icefish, are unique among vertebrates since they are unable to biosynthesize hemoglobin and myoglobin (Ruud 1954; Hamoir and Gerardin-Otthiers 1980; Walesby et al. 1982). As the colourless blood of these teleosts carries oxygen only in physical solution, the arterial oxygen capacity is reduced to 0.67 vol.%, a value corresponding to one-tenth of that found in the blood of Nototheniidae living in the same waters (Ruud 1965). The red-blooded *Notothenia magellanica* has, in contrast, a high cardiac myoglobin content of 7.7 mg/g tissue wet weight (Feller 1983). It is worth mentioning that these two closely related families, despite such a difference in their respective respiratory pigment concentrations, have comparable feeding and migration habits.

It has been shown (Holeton 1970, 1975; Feller et al. 1983) that Channichthyidae develop special cardio-vascular adaptations to accommodate the lack of hemic pigments: for example, the ventricles of *Chaenocephalus aceratus* and *Channichthys rhinoceratus* are 3 times larger than those of Nototheniidae. In addition, the blood volumes of these species range between 6–7 body weight (Hemmingsen and Douglas 1970) compared to 2–3% body weight in other teleosts.

The large increase in contractile tissue also generates a high cardiac output (5–6 times greater than in the other teleosts) so that the energy cost of cardiac work reaches 27% of the resting metabolic rate in *Chaenocephalus aceratus* (Hemmingsen et al. 1972).

Despite a large blood volume and a high cardiac output, the ventral and dorsal aortic blood pressures are low (Holeton 1970; Hemmingsen and Douglas 1972; Hemmingsen et al. 1972); this is because of a low systemic impedance produced by the very small number of erythrocytes giving hematocrits of 1–10% that of Nototheniidae (Hureau et al. 1977), thereby lowering blood viscosity. An increase in the number and diameter of blood vessels, especially at the capillary level, also contributes to lower the vascular impedance (Steen and Berg 1966; Jakubowski and Byczkowska-Smyk 1970; Kilarksi et al. 1982).

Other physiological adaptations include cutaneous respiration and reduced oxygen consumption, although these points are not well-established (Holeton 1975; Hureau et al. 1977).

Most of the adaptive features of Channichthyidae designed to compensate the limited availability of oxygen are directly related to cardiac function. We have therefore studied histological and cytological aspects of the heart of the bloodless fish *Channichthys rhinoceratus* and compared them with those of the red-blooded *Notothenia rossii.* Our results are discussed in relation to fish heart physiology.

Materials and methods

Specimens of *Channichthys rhinoceratus* and *Notothenia rossii* were caught at 15 m depth in the "Baie du Morbihan" of the Kerguelen Archipelago (49°30′ S; 70° E). Fish were anesthetized with 0.2% tricaine methane sulfonate (MS 222, Sandoz) and their hearts removed.

Light microscopy

Heart ventricles were incised and immersed for 6 days at room temperature into Bouin, Bouin-Hollande, Gendre or Zenker fixatives. The samples were then stored in 70% ethanol V/V.

Inclusion in Paraplast-Piccolyte (90–10%) according to the double-embedding method was carried out one month

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Table 1. Morphological and stereological data of the myocardial cells of two antarctic fish, obtained from electron micrographic analysis

	Channichthys rhinoceratus		*Notothenia rossii*	
	Mean ± S.E.M.	*n*	Mean ± *S.E.M.*	*n*
Cell diameter (μm)	5.7 ± 2.3	27	4.25 ± 1.3	14
Myofibril diameter (μm)	1.8 ± 0.7	26	1.26 ± 0.5	17
Mitochondria (vol%)	24.4 ± 3.3	12	22.8 ± 3.5	6
Myofibrils (vol%)	43.7 ± 6.6	12	44.9 ± 2.2	6
Sarcoplasm (vol%)	31.9 ± 5.1	12	32.3 ± 4.1	6

later in Liège. Slices (5–6 μm thick) were stained either by hematoxylin-eosin, light green, azan, fuchsin-resorcine or with periodic acid-Schiff method.

Electron microscopy

Small samples of approximately 5 mm^3 were (1) fixed for 1 h at 4° C in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, washed with buffer and post-fixed in 1% osmium tetroxide in cacodylate buffer for 1 h, or (2) fixed at 4° C in the following solution: 6 vol. of modified teleost Ringer's solution (Leknes 1980), 2 vols. 0.1 M cacodylate buffer (pH 7.4), 1 vol. 25% glutaraldehyde and 1 vol. 10% formaldehyde, then washed in a solution of 3 vols. Ringer and 1 vol. buffer, and post-fixed in 1% osmium tetroxide for 4 h. Fixed tissues were kept in 70% V/V ethanol or in cacodylate buffer for 1 month before being embedded in Epon 812.

Ultrathin sections obtained with a Reichert OMU3 ultramicrotome were "stained" with uranyl acetate and lead citrate. Observations and micrographs were made with a Philips transmission 301 electron microscope at 80 kV.

Morphology and stereology

Electron micrographs from relaxed ventricular myocardium of *Channichthys rhinoceratus* and *Notothenia rossii* (3 hearts per species) were analyzed with a Leitz ASM morphometer, using a point-counting stereological program.

The number of selected cells is given in Table 1. Volume fractions of subcellular compartments were established by superimposing a double-square lattice with 13 test lines on the micrographs.

Results

In-situ observations

The hearts of both *Channichthys rhinoceratus* and *Notothenia rossii* have a round sac-like ventricle, as described by Santer et al. (1983), with a poorly-marked apex.

The bulk of the large yellow-brown ventricle of *Channichthys rhinoceratus* contracts uniformly in situ; subsequently, the bulbus arteriosus is distended, showing an unusually large flexibility. When the pericardial cavity is opened by incision, the ventricle collapses. This suggests a major involvement of the pericardium in the filling of the heart chambers by intrapericardial pressure effects.

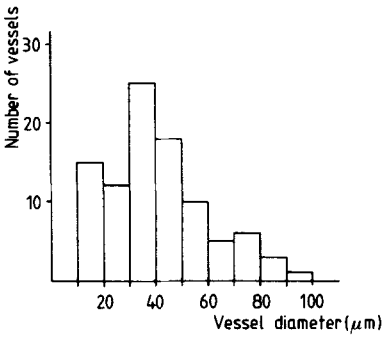


Fig. 2. c Ventricular subepicardial vasculature of *C. rhinoceratus;* coronary vessel diameters were measured at the lumen (data obtained from 1 specimen; weight = 385 gr, length = 31 cm, body surface = 825 cm^2)

Light microscopy

Atrium: The thin-walled atria of *Channichthys rhinoceratus* (Fig. 1) and *Notothenia rossii* consist of atrial myocardium sandwiched between an outer epicardium, composed of a superficial squamous mesothelium and a sub-epicardial layer of connective and elastic tissue, and an inner endothelium. The thin, non-vascularised epicardial core protrudes deeply into the atrial wall. The myocardium forms numerous intertwined trabeculae of various length and thickness. The atrial chamber of *Channichthys rhinoceratus* therefore shows an orthodox tissue organisation and is only distinguishable from that of *Notothenia rossii* because it possesses a squamous endothelial cell lining.

Ventricle: Heart ventricles of both *Channichthys rhinoceratus* and *Notothenia rossii* are of the spongy type and are devoid of an outer compact myocardium as observed in elasmobranchs and some teleosts. In the two species, glycogen was demonstrated in trabeculae by the PAS reaction followed by amylase digestion.

The epicardium covering the ventricle is continuous with that of atrium and bulbus arteriosus. In *Channichthys rhinoceratus*, however, unusually numerous large coronary vessels run in the subepicardial layer (Fig. 2a, b). They push back the epicardium towards the pericardial cavity to an extent dependent on their size; this gives an irregular shape to the ventricle wall. Sometimes they also protrude into the blood lacunary spaces. Although the vessel lumina are large (Fig. 2c), arterioles and venules cannot be distinguished as the tunica media is reduced to one or two smooth muscle cell layers and the adventitia merges with epicardial collagen bundles. These coronary blood vessels are profusely branched and are directly continuous with capillaries of the trabeculae.

The ventricular myoarchitecture of the two species is comparable. Trabeculae of various shapes and sizes fill the chamber, delimiting the venous blood lacunary system. The ventricular lumen is extremely reduced and restricted to a small space between the atrio-ventricular and the bulbo-ventricular junctions.

The most singular feature of the icefish ventricle is the presence of an extensive capillary network within the trabeculae (Fig. 3). In transverse sections, they exhibit a typical vascular-endothelium structure, made up of one or two cells endowed with flat semi-lunar nuclei. They appear highly

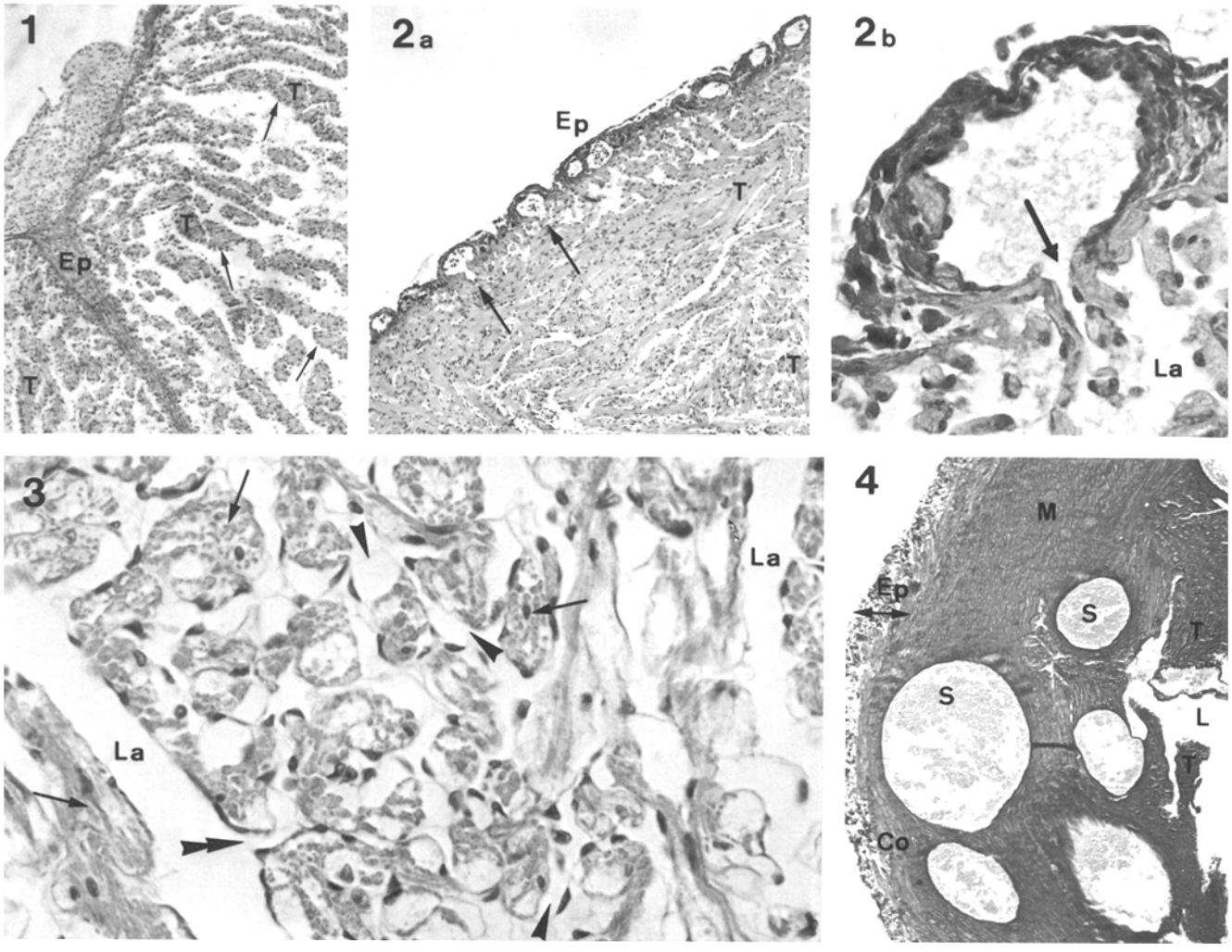


Fig. 1. Light micrograph showing the general structure of the atrium of *C. rhinoceratus*; epicardium (*Ep*), trabeculae (*T*), endothelium of squamous type (*arrow*). × 77

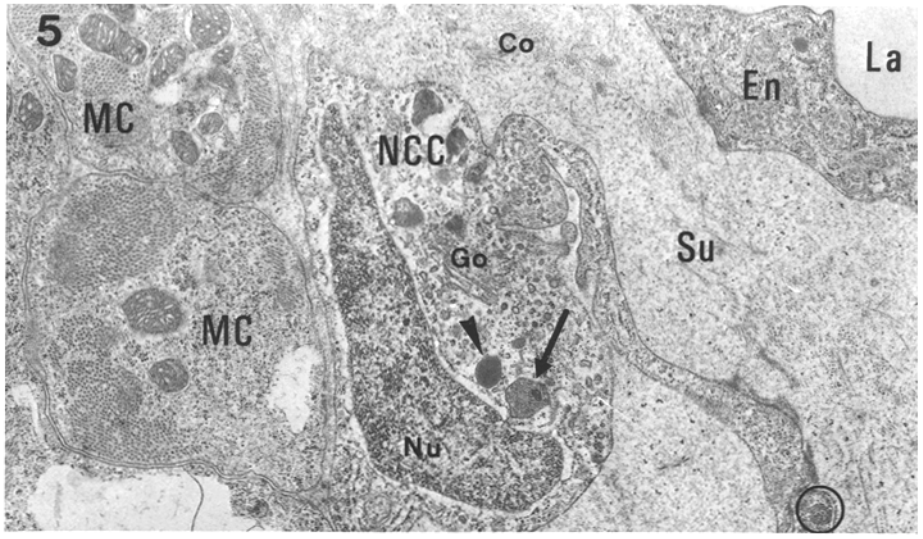
Fig. 2. a, b Ventricular extramural network of *C. rhinoceratus* running under the epicardium (*Ep*). Note the branching sections of these vessels (*arrows*). Trabeculae (*T*) are cut at various angles; lacunary space (*La*). a × 74; b × 407

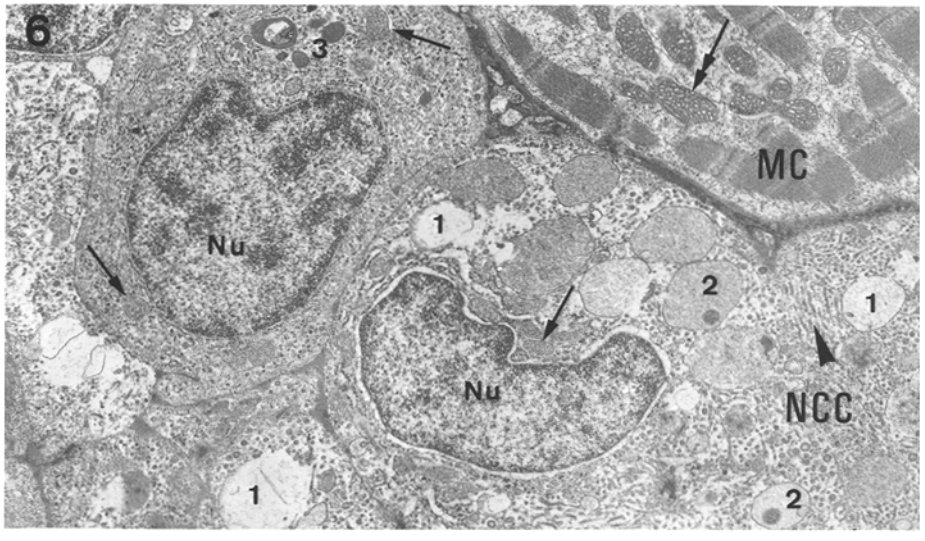
Fig. 3. Ventricular trabeculae from the apex region of *C. rhinoceratus*. Myocardial cells (*arrows*); capillary network (*arrowheads*), lacunary spaces (*La*). Note the importance of the intratrabecular vascularized surface as compared with the contractile surface, and the discontinuities in the endocardial lining (*double arrowhead*). × 517

Fig. 4. Bulbus arteriosus wall of *C. rhinoceratus*. Epicardial layer (*Ep*) and its collagen bundles (*Co*), middle layer (*M*) and inner trabeculae (*T*), bulbar lumen (*L*), spheroid structure (*S*) of middle layer. × 62

deformable, and therefore the shape of the lumen varies with the trabecular contraction level, reaching 15–20 μm in diameter in the relaxed areas but being in a collapsed state in systolic-fixed myocardium. Most of these capillaries are intimately associated with the myocardial cells and are embedded in the muscle bundles, running along them as seen in longitudinal sections. In highly perfused areas, they may be arranged in islets of 2 or 3 capillaries; this appears paradoxical as such an organisation is physiologically less efficient. In addition, some of them are also found between endocardium and muscle layers. Interestingly, the common sequence of arteries-capillaries and venules is not found in the trabecular vasculature of *Channichthys rhinoceratus*. Moreover, discontinuities in the endocardial lining (this appears to be related to the intratrabecular capillaries) are often seen (Fig. 3).

Bulbus arteriosus. In both fishes, the bulbus wall consists of three defined layers (Fig. 4). In *Notothenia rossii*, the innermost endothelial lining is composed of a single layer of tall cuboidal cells; in contrast, the endothelium is of the squamous type in *Channichthys rhinoceratus*. The extensive middle layer of elastic fibers and smooth muscle cells form an inner network of massive trabeculae and an outer core of densely-packed tissue. The third layer covering the bulbus is the epicardium where the collagen bundles are well developed. Here, the bulbus of *Channichthys rhinoceratus* differs because of the presence of large holes in the middle layer often repulsing the connective frame into the lumen and filled with an unidentified material. Serial sections along the bulbus reveals that these cavities are round or fusiform and do not seem to be in register with the bulbus lumen.





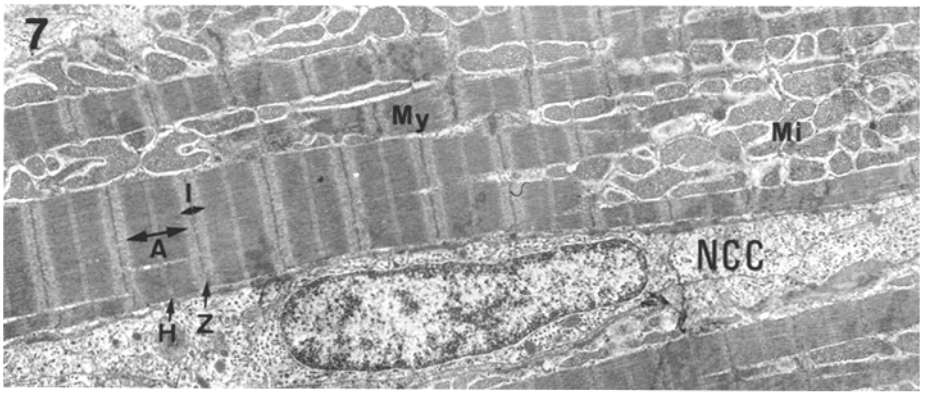


Fig. 5. Electron micrograph illustrating the general cytoarchitecture of a myocardial trabecule of *C. rhinoceratus*. Myocardial cells (*MC*) are isolated from the lacunary system (*La*) by a large subendocardial space (*Su*) containing collagen fibers (*Co*), and a thin endocardial cell lining (*En*). A non-contractile cell (*NCC*) is also seen, showing a large nucleus (*Nu*), Golgi complex (*Go*), an electron-dense body (*arrowhead*), peroxisome (*arrow*), and concentric lamellae of smooth endoplasmic reticulum (*circle*) embedded in a long pseudopod. × 9450

Fig. 6. Non-contractile cells (*NCC*) in the cardiac ventricle of *C. rhinoceratus*. Three types of cytoplasmic vesicles are seen: vesicles containing amorphous material (*1*), peroxisomes (*2*), and electron-dense bodies (*3*). The lamellae of the smooth endoplasmic reticulum appear as rods (*arrowhead*). *Nu* nucleus. The intercellular space between NCC and MC is filled with electron-dense material and microvesicles. The size of mitochondria in NCC (*arrow*) should be compared with those in MC (*double arrow*). × 6800

Fig. 7. Longitudinal section through a myocardial cell and an NCC of *C. rhinoceratus*. Mitochondria (*Mi*) are densely packed between branching myofibrils (*My*). The classical banding pattern of sarcomeres is clearly seen: A-band (*A*), I-band (*I*), H-band (*H*), Z-line (*Z*). × 4560

Electron microscopy

The myocardial endocardium of *Channichthys rhinoceratus* is composed of thin and flattened cells showing bumps at their surface and small basal extensions (Fig. 5). The nucleus is irregular in shape and the cytoplasm contains mainly granular material, some coated vesicles and occasionally small moderately dense bodies (MDB). Under this cell lining, a large and extensively developed subendocardial space filled with small dense granules and some collagen fibers surrounds the trabecular myocardium. In contact with this space, the plasmalemma of the endocardial cells shows numerous maculae adhaerentes and micropinocytotic or exocytotic vesicles.

The bulk of the trabeculae of *Channichthys rhinoceratus* consists of long narrow myocardial cells, including an unusually large proportion of interstitial non-contractile cells (NCC) and large vessels. The NCC are extremely variable in shape and are either arranged in islets (Fig. 6) or individually inserted between the muscle cells (Fig. 7) without any

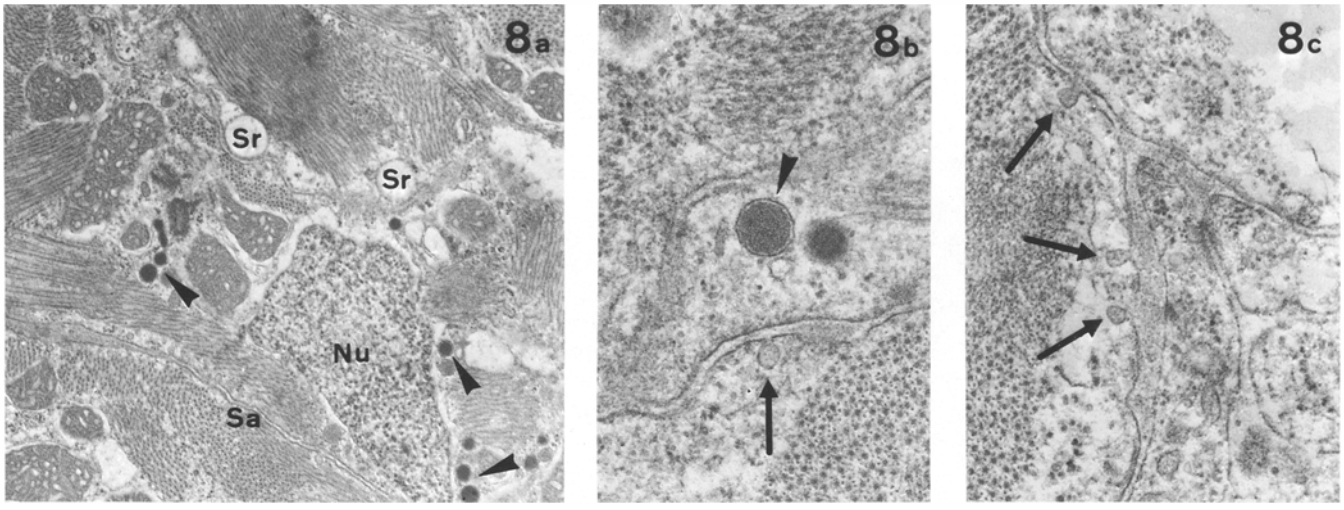
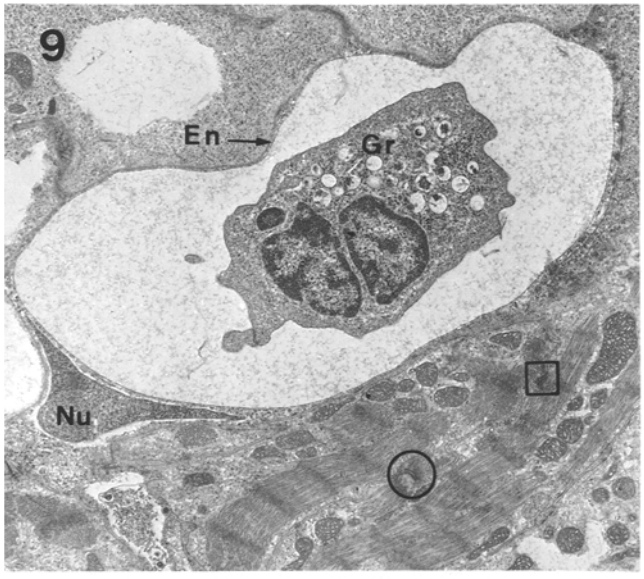


Fig. 8. **a** The myocardial nucleus (*Nu*) is close to the plasma membrane. Some tubules of sarcoplasmic reticulum (*Sr*) run beneath the sarcolemma (*Sa*). Specific heart granules (*arrowhead*). ×11900. **b, c** Higher magnification of the specific heart granules (*arrowhead*); note the presence of numerous sarcolemmal caveolae (*arrow*). ×38500

apparent regularity. They often show long thin cytoplasmic protrusions that are themselves inserted into the myocardium, but can also invaginate the subendocardial space (Fig. 5). NCC usually have small mitochondria, a high glycogen content and a well-developed smooth endoplasmic reticulum either of the rod or onion-like type. An interesting feature of these non-contractile cells is the occurrence of at least three different types of vesicles or bodies in the cytoplasm (Figs. 5, 6). The first type appears almost empty, showing only traces of amorphous material and are surrounded with a membrane-like boundary, probably a result of reticulum condensation.

The membrane-coated vesicles of the second type have a low to moderate electron density and are by far the most numerous. They are unevenly distributed but are particularly abundant near the nucleus. Some of them possess a typical dark granule enclosed in the foamy matrix and thus may be related to peroxisomes. The third type of cytoplasmic component of the NCC is made up of electron-dense, homogeneous granules, most of which are round but variable in size. Our micrographs also reveal micropinocytotic vesicles along the plasmalemma of the non-contractile cells, particularly those cells in close contact with the myocardial cells or the subendocardial space.

Some preliminary observations on the myocardial cells of *Channichthys rhinoceratus* have been reported (Feller et al. 1983). Their diameters (2–8 μm) reflect the dimensions of those cells of irregular shape. Intercellular junctions are frequent (Fig. 9), showing end-to-end contacts (intercalated



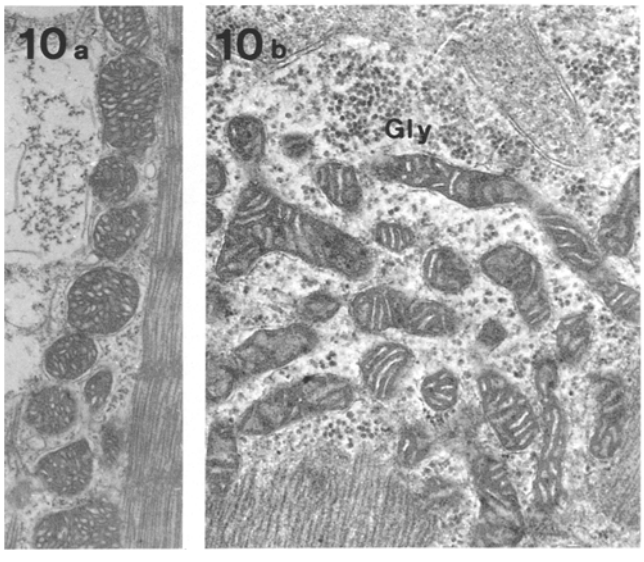


Fig. 9. Large intratrabecular capillary of the ventricular myocardium. It shows a typical flat nucleus (*Nu*), thin endothelium (*En*) and a granulocyte (*Gr*) in the lumen. An intercalated disc (*circle*) and a small lateral desmosome (*square*) are also seen along the cardiac sarcolemma. ×5250

Fig. 10. Myocardial mitochondria illustrating the different morphology and density of cristae in *C. rhinoceratus* (**a**) and *N. rossii* (**b**). Abundant glycogen granules (*Gly*) are also observed in the sarcoplasm of the red-blooded species. a ×9520; b ×19880

discs) and lateral contacts (desmosomes and undifferentiated short junctions). Nexus type junctions have not been observed.

As a rule, the sarcolemma of the cardiac cells is straight but shows a large number of micro-invaginations or caveolae (30–80 nm in diameter, Fig. 8b, c) and some exocytotic vesicles.

The myocardial cells contain 2–5 myofibrils embedded in a large volume of sarcoplasm (Fig. 7); the mitochondria are arranged in rows both in the subsarcolemmal and the intermyofibrillar spaces. The nucleus is located against the sarcolemma; the sarcoplasmic reticulum is scarce and consists of a system of tubules mostly running at the periphery (Fig. 8a) but also entering the myofibrillar space.

The sarcoplasm contains a large number of glycogen granules and the so-called specific heart granules having a mean diameter of 200 nm and an electron-dense granular matrix (Fig. 8a, b). Frequently, lacunae are seen lacking well-defined contours and containing a loose amorphous material.

As already suggested by light microscopy, an extensive capillary network is found either in the bulk of the cardiac muscle (Fig. 9) or beneath the endocardium. As red blood cells are missing, the coronary capillary lumens are filled with plasmatic protein aggregates or sometimes by granulocytes. Interestingly, NCC and enlarged intercellular spaces are often observed in the surroundings of these vessels.

The cardiac ultrastructure of the red-blooded *Notothenia rossii* differs in some aspects, such as the absence of subendocardial space, a higher sarcoplasmic glycogen content and a central nucleus. Although NCC are also observed to a small extent in this species, they do not show the peculiar cytological features of icefish-NCC but rather correspond to the classical interstitial cells of the hearts of other fish.

Stereological analysis (Table 1) does not reveal any significant difference in size or distribution of the major components of cardiac cells from the two species. However, the mitochondrial morphology of *Channichthys rhinoceratus* needs some comments: whereas the cristae in the mitochondria of *Channichthys rhinoceratus* show densely-packed tubular or vesiculated cristae (Fig. 10a), the cristae of *Notothenia rossii* have a villiform appearance (Fig. 10b).

Discussion

Whereas myoglobin is totally lacking in the myocardium of Channichthyidae, the heart ventricle of these antarctic fish presents a brownish colour. This is the result of the presence of lipids, mitochondria, flavoproteins, as is the case for the yellowish oxidative muscles, (Hamoir and Gerardin-Otthiers 1980), and an additional pigment similar to the lipofuscin found in teleost myocardium but usually masked by myoglobin (Torato et al. 1981).

The heart ventricle is about three times larger than that of some red-blooded antarctic fish (Feller et al. 1983). The myocardial cell size and myofibrillar diameters of the two types of fish are not significantly different; the large size of the icefish heart arises mostly because of an increase in cell number rather than an increase in cell volume. Icefish heart morphology also demonstrates that a spongy myocardium is able to generate a high cardiac output and does not require the outer compact layer of myocardium that is usually considered the major generator of cardiac force (Poupa et al. 1981).

How are the contractile elements rapidly activated in a myocardium lacking nodal tissue and T-tubules? It has recently been proposed by Breisch et al. (1983) that caveolae serve as a rudimentary substitute for T-tubules in the heart of tuna, which also displays high cardiac performances. This is consistent with our observations of a high frequency of caveolae along the cardiac plasma membranes; the caveolae provide an appreciable extent of sarcolemmal surface entering the sarcoplasmic space and improve the excitation-contraction coupling.

The bulbus arteriosus is known to protect gill capillaries from large pressure oscillations. In addition, the bulbar trabeculae observed in *Channichthys rhinoceratus* and *Notothenia rossii* presumably contribute to the distensibility of the chamber (Priede 1976). The function, however, of the large spheroid structures present in the bulbar wall of *Channichthys rhinoceratus* is uncertain and the assessment of their role needs further investigation.

The most striking feature of the cardiac ventricle of *Channichthys rhinoceratus* is the development of the coronary system. The angio-architecture of fish heart has recently recevied particular attention since the appearance of a coronary circuit corresponds to an important transitory evolutionary step among vertebrates (Tota et al. 1983; Tota 1983; Poupa and Lindström 1983). These studies have shown that two main coronary networks are usually derived from the hypobranchial arterial vessels: the extrahilar or extramural network consisting of vessels lying beneath the epicardium, and the hilar network consisting of arteries that penetrate the ventricle at the bulbo-ventricular sulcus level. These two arterial systems coexist only in species possessing a mixed type of myocardium (i.e., compacta and spongiosa), whereas trabeculae in spongy heart are supplied only with venous blood returning from body perfusion. In contrast, the heart ventricle of *Channichthys rhinoceratus* displays an unusually well-developed extrahilar system and an extensive intratrabecular capillary network that seems to be a major adaptation to the lack of hemic pigments. This is the first report of a microvasculature in a spongy fish heart.

The extensive branching of the subepicardial vessels indicates that the intratrabecular microvasculature originates, at least partly, from the extramural network. However, the hilar coronary circuit is probably involved in the capillary perfusion of the ventricle of *Channichthys rhinoceratus*, since three other icefishes, *Chaenocephalus aceratus, Champsocephalus gunnari* and *Pseudo-chaenichthys georgianus* also possess a highly developed intratrabecular microvasculature but are devoid of subepicardial vessels (Santer and Feller, unpublished results).

This interesting feature of the icefish coronary circuit can be related to the cardiac physiological conditions, namely a reduced oxygen availability from the blood and the absence of myoglobin in the myocardium. The lack of intracellular respiratory pigment implies that the oxygen flux through the cardiac sarcolemma cannot be regulated and mainly depends upon the oxygen gradient existing between plasma and working mitochondria.

The icefish heart may be assumed to meet 98% of its energy demand by oxidative processes similar to other hearts having low Mb content (Driedzic 1983). It should also be noted that the venous blood of the icefish is desatur-

ated after hypoxia (Hemmingsen and Douglas 1970) thereby preventing oxygenation from the lacunary spaces. Thus, one can conclude that the cardiac work performed by *Channichthys rhinoceratus* is directly related to oxygen delivery by coronaries. Moreover, the cardiac energy cost of *Chaenocephalus aceratus* is 5 times higher in view of the high cardiac output and the large blood volume to be pumped. The lack of oxygen flux regulation, the dependency on arterial perfusion and the elevated cardiac work make necessary an efficient oxygen supply via a highly-developed capillary network.

Interestingly, the classical alignment of the arteriolar-capillary-venous circuit is not found in icefish ventricle; this raised a problem concerning the coronary venous blood return. A shunt between the terminal coronary bed and the lacunary spaces, namely a Thebesian system of arterioluminal vessels (Tota et al. 1983), is suspected to occur in the ventricular wall of *Channichthys rhinoceratus*. This is supported by the numerous capillaries running underneath the endothelium covering the trabeculae and the discontinuities in the endocardial lining. Such an arterioluminal system should ensure the uptake of the coronary venous blood by incorporation to the systemic venous blood flow. As most of the intratrabecular capillaries are collapsed during systole, one can argue that each heart beat acts as a pump improving the coronary arterial blood flow through trabeculae and propelling the venous blood to the ventricular lumen.

As far as we know, there are no metabolic data concerning the preferential use of fuels by icefish for its cardiac energy requirements. Despite the low oxygen level in icefish blood, its heart possesses a normal distribution of mitochondria with, however, densely-packed cristae indicative of the large contribution made by the oxidative pathways to its energy supply. This peculiarity is also observed in the so-called yellowish muscle (Feller and Goessens, unpublished results), suggesting that Channichthyidae mitochondria have a specific morphology at least as far as oxidative muscles are concerned. Furthermore, Johnston et al. (1983) have pointed out that the morphology of the cristae in *Chaenocephalus aceratus* heart can be related to an involvement of mitochondria in lipid metabolism. This seems consistent with the presence of numerous intra- and extracellular vacuoles in *Channichthys rhinoceratus* myocardium; these may arise from lipid extraction when using ethanol as preservative.

On the other hand, energy metabolism in fish heart is highly dependent upon carbohydrates (MacIntyre and Driedzic 1981). The intense PAS reaction of the myocardium of both *Channichthys rhinoceratus* and *Notothenia rossii* might therefore be related to the involvement of polysaccharides in cardiac metabolism.

Driedzic and Steward (1982) have shown that a 16-fold decrease in Mb content does not modify the maximal activities of enzymes associated with cardiac energy metabolism in fish. The perfused hearts met 98% of ATP demand by oxidative mechanisms (Driedzic 1983) and functioned with similar efficiencies under normoxic conditions (Driedzic et al. 1983). During hypoxia, myoglobin-rich hearts are significantly more efficient than myoglobin-poor hearts. Extrapolating these results to icefish heart, the lack of respiratory pigments may be totally compensated by physiological adaptations; the heart of *Channichthys rhinoceratus* may not require modified metabolic pathways for its cardiac requirements. However, icefish myocardium is extremely sensitive to hypoxia as a consequence of the lack of any protective effect of myoglobin.

Another peculiarity of the ventricular wall of *Channichthys rhinoceratus* is the profusion of non-contractile cells. The function of these cells is uncertain but they are probably not related to myoblasts, as stages in myofilament synthesis and sarcomerogenesis have not been observed in these cells. They are similar in appearance to mesenchymal cells, which generate the connective frame in myocardium except that they possess numerous peroxisomes. They may even be related to histiocytes. Further cytochemical analysis is required to resolve this problem.

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