Ultrastructural Evidence for a Direct Connection between the Myocardial Granules and the Sarcoplasmic Reticulum in the Cardiac Ventricle of *Myxine glutinosa* (L.)

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**Summary.** The occurrence of structural connections between myocardial granules and tubular elements of the sarcoplasmic reticulum in the cardiac ventricle of the Atlantic hagfish is described. The core substance of the myocardial granules is shown to be uranophilic and in this respect similar to the granular cores within the lumen of the sarcoplasmic reticulum.

After application of CaCl$_2$-containing fixatives, the ultrastructure of the core substance of these organelles resembles that described for calcium-containing structures in vertebrate glial cells. Incidences of uranophilic core substance in the extracellular space suggest a secretory function of the myocardial granules. Possible implications of the sarcoplasmic reticulum and the myocardial granules in storage, intracellular transport, and secretion of bound calcium are discussed.

**Key words:** Specific heart granules (Cyclostome) — Atrial granules — Calcium — Uranophilic core substance — Electron microscopy.

**Introduction**

In the sarcoplasm of cardiac muscle fibers, moderately electron-dense granules occur as characteristic structural elements. These organelles were first seen in atrial fibers of the rat (Palade, 1961) and have since been detected in the hearts...
of a number of vertebrate species, ranging from the cyclostomes to man (Bloom et al., 1961; Fawcett and McNutt, 1969; Huet et al., 1974; Jamieson and Palade, 1964; Sommer and Johnson, 1968). In the rat, the purified atrial granules have been shown to be devoid of catecholamines (De Bold and Bencosme, 1973a, b). In situ the specific heart granules contain a uranophilic, proteinaceous matrix substance (Berger and Bencosme, 1971; Kuhn and Tranzer, 1974), and an uptake mechanism for metallic ions has been demonstrated (Baux and Nicaise, 1971). However, the functional role of these granules has remained obscure, although it has been suggested that they represent sites of Ca\(^2+\) bound in a nonionic form (Sommer and Johnson, 1968).

Pervious observations on the systemic hearts of the hagfish have shown that in this species the myocardial granules often occur in close proximity to the tubular elements of the sarcoplasmic reticulum (Helle and Lønning, 1973). In frog and chicken the myocardial granules have been observed within segments of the sarcoplasmic reticulum (Sommer and Johnson, 1969). However, a direct connection between the tubular elements and the myocardial granules was not detected in our earlier studies of the hagfish myocardium. Our aim of the present investigation has been to elucidate further the structural relationship between the elements of the sarcoplasmic reticulum and the myocardial granules in the cardiac ventricle of the Atlantic hagfish.

Materials and Methods

Specimens of *Myxine glutinosa* (L.) were kindly supplied by the Biological Station, University of Bergen, Espegrend. The animals were kept in aerated sea water at 4°C in the dark until used.

Cardiac ventricles were dissected, trimmed, and rinsed in the buffered medium before preincubation and fixation. The following media were used:

1. **Buffered sucrose**: 0.6M sucrose in 20 mM Hepes buffered at pH 7.4.
2. **Buffered sucrose/calcium/oxalate**: 0.6M sucrose in 20 mM Hepes pH 7.4, 4 mM CaCl\(_2\), 4mM EGTA, 4mM NaOH, 5 mM K\(_2\)(CO\(_3\))\(_2\) (Agostini and Hasselbach, 1972).
3. **Buffered sucrose/calcium**: 0.6M sucrose in 30 mM Hepes pH 6.5, 50 mM CaCl\(_2\).
4. **Buffered sea water/calcium**: Sea water was diluted 2:1 (vol/vol) with distilled water and buffered at pH 6.5 with 30 mM Hepes in presence of 50 mM CaCl\(_2\).

Preincubation of the tissue was carried out in some experiments by one of the two procedures:

1. The ventricles were each cut into four pieces and incubated at room temperature in 20% glycerol in the buffered sucrose/calcium/oxalate medium for 30 min, followed by incubation for 60 min in 4 mM ATP in the same medium; or
2. The ventricles were cut open and incubated at room temperature for 60 min in the buffered sucrose/calcium or the buffered sea water/calcium.

The tissue was fixed for 2 hrs at room temperature in 2.5% glutaraldehyde in the respective media. The fixed pieces were postosmicated for 1 hr in 1% OsO\(_4\) in the buffered calcium-containing media and in most experiments stained en bloc with uranyl acetate (2%) for 1 hr. In other cases, the tissue pieces were prepared for electron microscopy as earlier described (Helle and Lønning, 1973; Helle et al., 1972). The electron micrographs were obtained by the use of the Siemens and the Philipps 300 electron microscopes.

Definitions and Criteria

A detailed description of the morphologic terms used in the present study was given in our earlier work (Helle and Lønning, 1973) but will be briefly summarized here: *Sarcoplasmic reticulum* is