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Cardiac ultrastructure in the ringed seal, *Phoca hispida* and harp seal, *Phoca groenlandica*

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Summary

The present data describe for the first time a number of the cardiac ultrastructural features in the ringed seal (*Phoca hispida*) and harp seal (*Phoca groenlandica*). By light microscopy, the ringed seal and harp seal hearts closely resembled the hearts of similar-sized terrestrial mammals, but at the ultrastructural level, several distinctive features were observed. The fine structure of myocyte Z, H, I and A bands and M lines was conventional, and a well-developed transverse tubular system was present in the cardiac muscle. Unusual features included enlarged cytoplasmic reservoirs of glycogen in ventricular myocytes, sacculations of the mitochondrial cristae (ringed seal), and a thickened endocardium. The transverse tubules, which showed much pinocytotic activity, traversed the glycogen reservoirs and extensively invested vascular elements within the cardiac muscle. Electron dense endocrine granules were observed in some cardiomyocytes, and these appeared similar to atrial natriuretic polypeptide granules reported in other species. Capillary endothelial cells demonstrated typical high levels of pinocytotic activity. Generally, the hearts of these seals were morphologically similar to those of non-diving, terrestrial mammals, but their blunt gross configuration and enlarged stores of subcellular glycogen appear to be adaptations to diving and in this respect are similar to features reported in cetacean hearts.

Introduction

The remarkable diving abilities of seals have long been appreciated, and some species, such as the Weddell seal, *Leptonychotes weddelli* are capable of descending to 600 m and can remain submerged for 70 min (Kooyman, 1966; Kooyman *et al.*, 1970). Even greater diving capabilities have been reported for northern elephant seals, which may dive deeper than 1000 m (Le Boeuf, 1990). Consequently, biologists have studied a variety of morphologic and physiologic adaptations of a number of pinniped species which can support such diving behavior. It has been determined that pinnipeds have a characteristic high blood volume (Simpson *et al.*, 1970) which increases their oxygen transport capacity. Further, the seal heart can maintain mechanical function with minimal (10%) coronary perfusion during dives (Blix *et al.*, 1976; Elsner *et al.*, 1985). Heart rates, which reduce during diving, varied inversely with the length of dives in Weddell seals (Kooyman & Campbell, 1972). Although early observations suggested a proportional large bulk of the heart in the sea lion, *Otaria jubata* (Murie, 1874), seal hearts have been shown not to be disproportionately large in comparison to hearts of terrestrial mammals (Drabek, 1977). The heart of the ringed seal, *Phoca hispida*, comprises 0.7% of the animal weight, which is typical of seals generally (Bryden, 1972). Comparative studies on the gross anatomy of seal hearts have shown, however, that phocid hearts tend to be broader and flatter than terrestrial mammalian hearts (Müller, 1940; Drabek, 1975, 1977; Bisaillon, 1982), a feature which has been considered an adaptation to the extreme hydrostatic pressures to which seals are subjected during deep dives (Drabek, 1975). Although a preliminary study on the ultrastructural morphology of the heart of a cetacean, the bowhead whale, (*Balaena mysticetus*), has been recently reported (Pfeiffer, 1990), pinniped hearts have not yet been described at the subcellular level.

The ringed seal is a small-sized, wide ranging hair seal inhabiting arctic and subarctic waters. In spite of its importance to the Eskimo native economy and recent interest in its population dynamics (Frost *et al.*, 1988), comprehensive biologic study of this species remains incomplete (McLaren, 1958) and its cardiovascular system has not been subject to detailed study. Further, the cardiac ultrastructure of harp seals has previously received little attention. As part of our investigation of

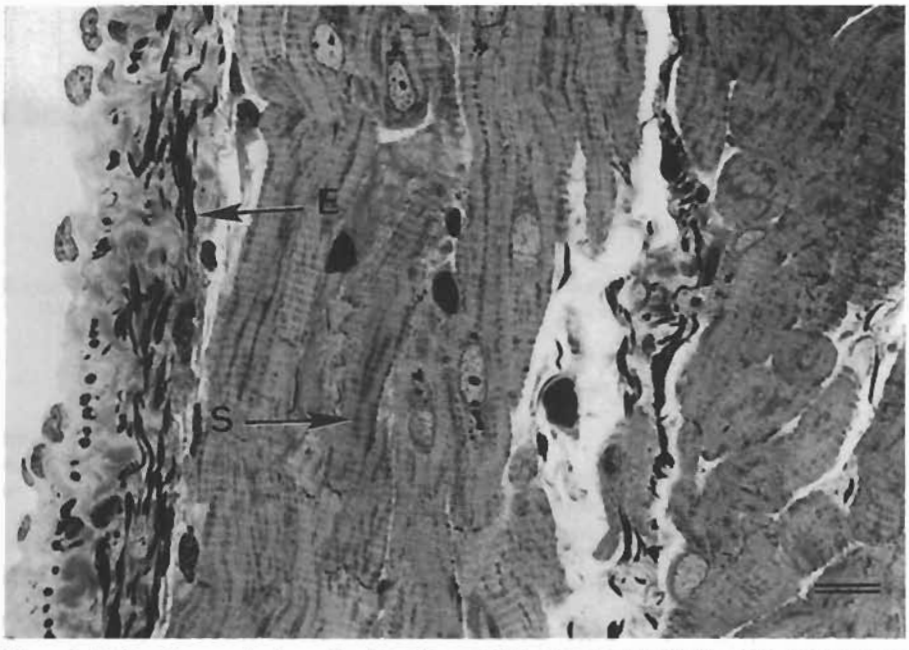

Figure 1. Light microscopic view of endocardium of the ringed seal ventricle. The lumen of the ventricle is shown on the left. Note that the endocardium contains elastic fibers (E) which run in perpendicular directions. Striations (S) can be seen in the myocytes. 900 ×, Scale bar = 12 μm.

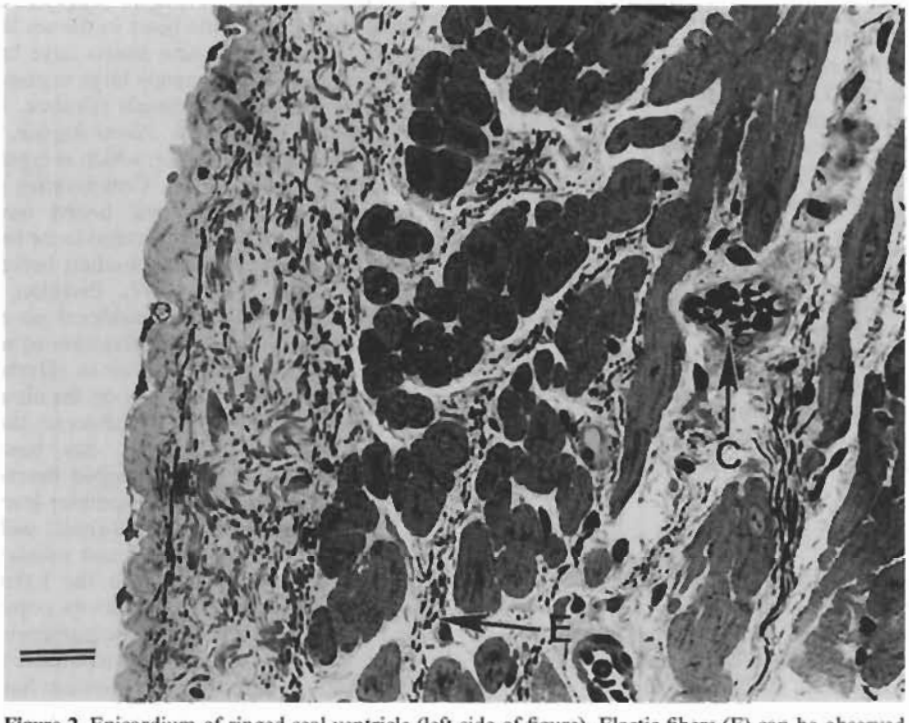

Figure 2. Epicardium of ringed seal ventricle (left side of figure). Elastic fibers (E) can be observed intermingled with muscle fibers as well as in the epicardium. Myocardial capillaries (C) are also illustrated. 500 ×, Scale bar = 20 μm.

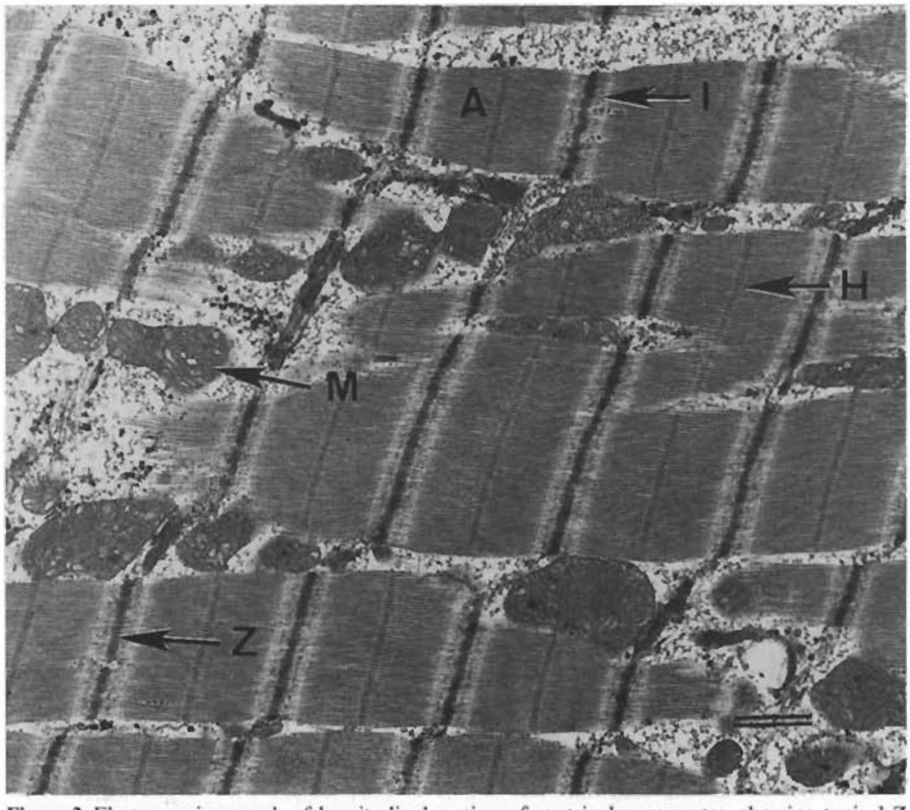

Figure 3. Electron micrograph of longitudinal section of ventricular myocytes, showing typical Z lines (Z) and (H), (I), and (A) bands. Note that cristae of mitochondria (M) are sacculated. 11 900 ×, Scale bar=840 nm.

comparative cardiology and ultrastructure of diverse species (Uehara *et al.*, 1989; Pfeiffer, 1990; Pfeiffer *et al.*, 1990; Pfeiffer & Keith, 1993), we have studied the ringed and harp seals, and the present communication is the first report on some aspects of the subcellular cardiac architecture of these species.

Materials and methods

Animals:
Hearts from four ringed seals were obtained from Alaska through the courtesy of Dr T. F. Albert under the National Marine Fisheries Service Permit No. 519. Two of these were from juvenile males (7 kg and 10 kg body wt) trapped in fishing nets by Eskimo hunters and were available *in situ* in frozen animals. The other two hearts were obtained already removed from female seals caught by Eskimo hunters and were received initially fixed in 10% buffered formalin. Ventricular and atrial cardiac samples were obtained in the field fresh from the heart of one of the latter two animals listed above and were fixed in cold, buffered glutaraldehyde, described below, for electron microscopic study. A heart from one moribund adult male harp seal which was stranded on the north Atlantic shore was also obtained through courtesy of the stranding network and was fixed similarly.

Light and electron microscopy:
Light microscopy was done on fresh-fixed, resin-embedded samples. The tissue was fixed for a minimum of 24 h in 5% glutaraldehyde/4.4% formaldehyde/2.75% picric acid in 0.05 M cacodylate buffer at pH 7.4. The fixed specimens were washed in 0.1 M sodium cacodylate buffer at pH 7.4, postfixed in 1% osmium tetroxide in 0.1 M sodium cacodylate for 1 h, washed again in buffer, dehydrated in increasing ethanol concentrations, and embedded in Poly/Bed 812 resin. Freshly cut semi-thin (1 μ) sections were placed on a clean glass slide and allowed to dry on a hot plate for at least 5 min at 60–120°C prior to staining. Sections were flooded with equal amounts of filtered methylene blue/Azure II stains (1% methylene blue/sodium

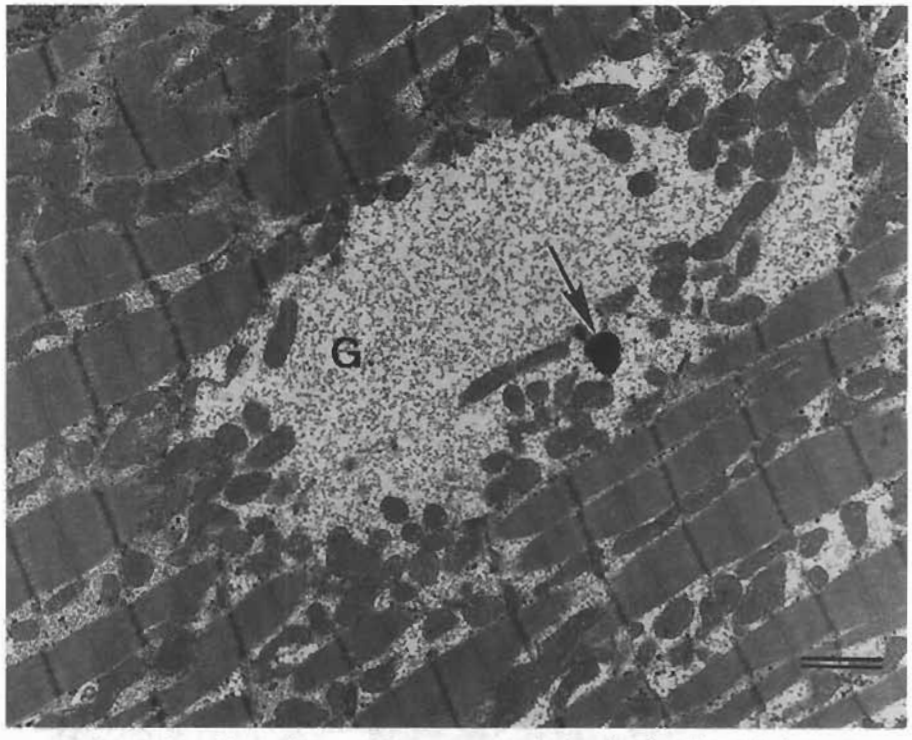

Figure 4. Reservoirs of beta glycogen (G) were present in the ringed seal ventricular myocytes, usually in close proximity to the nucleus. This electron micrograph shows the large size of these pockets. A single, electron dense endocrine granule (arrow) is present in this section. 6800 ×, Scale bar = 1.47 μm.

borate in distilled water and 1% azure II in distilled water) and allowed to incubate on the hot plate for approximately 15 to 20 s. After thoroughly washing the slide with distilled water, the sections were counterstained with 4 parts of 0.65 sodium borate in distilled water to 1 part 0.5% basic Fuschin for approximately 5–10 s on the hot plate, washed, dried, and coverslipped for viewing. The samples fixed initially in 10% formalin were either embedded in paraffin and stained by hematoxylin and eosin, or were postfixed in the glutaraldehyde/formaldehyde/picric acid fixative, processed, and embedded as described above.

Electron microscopy was done on ultrathin sections of tissue samples fixed and resin-embedded as described above. The sections were doubly stained with uranyl acetate and lead citrate (Reynolds, 1963) and studied with a JEOL 100 CX-II transmission electron microscope operating at 80 KV.

Results

Light microscopy:

The histology of the seal heart, as revealed by light microscopy, will not be extensively described in this report, but the ventricular endocardium and longitudinally sectioned myocytes are shown in Figure 1, and epicardium and cross-sectioned ventricular myocytes are illustrated in Figure 2 for the ringed seal. Note that the endocardium is relatively thick for this marine mammal. Capillaries were particularly numerous throughout the cardiac muscle, and many can be observed in Figures 1 and 2.

Ultrastructure:

Myocytes in ringed seal ventricle and atrium were generally similar to cardiomyocytes described for many terrestrial mammals with respect to ultrastructure, although a few distinctions were observed. Distribution of myocyte mitochondria in the sarcoplasmic reticulum between striated myofibrils was not atypical. Longitudinal sections of ventricular myocytes revealed the usual sarcomere architecture with Z, H, I and A bands, and relatively wide M lines within the A bands (Fig. 3). Beta glycogen (20 nm particle size) was found between the myofibrillar contractile elements, along with the mitochondria, as in hearts of terrestrial mammals but, in addition, larger reservoirs of glycogen were also noted (Figure 4). As observed by higher

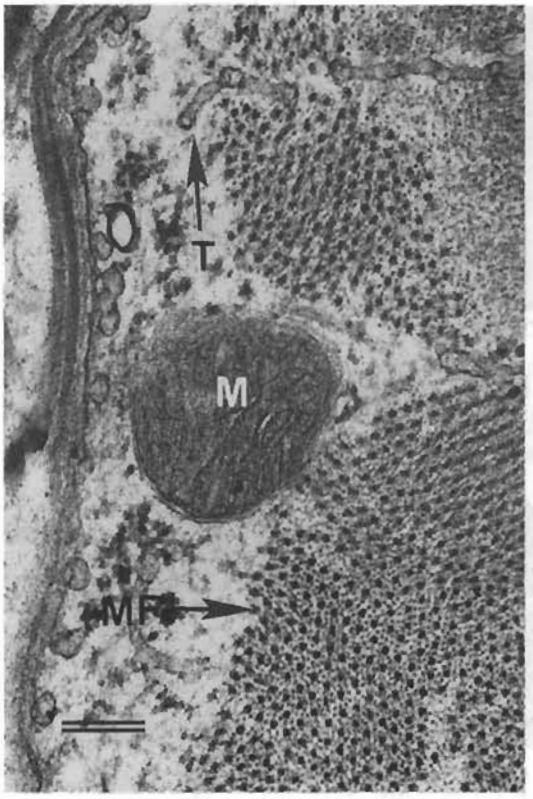

Figure 5. Cross section of ventricular myocyte, showing thick myosin fibrils (MF) which are surrounded by thin actin fibrils. A mitochondrion (M) is on the left, and a transverse tubule (T) can be observed at the top. 50 000 ×, Scale bar = 200 nm.

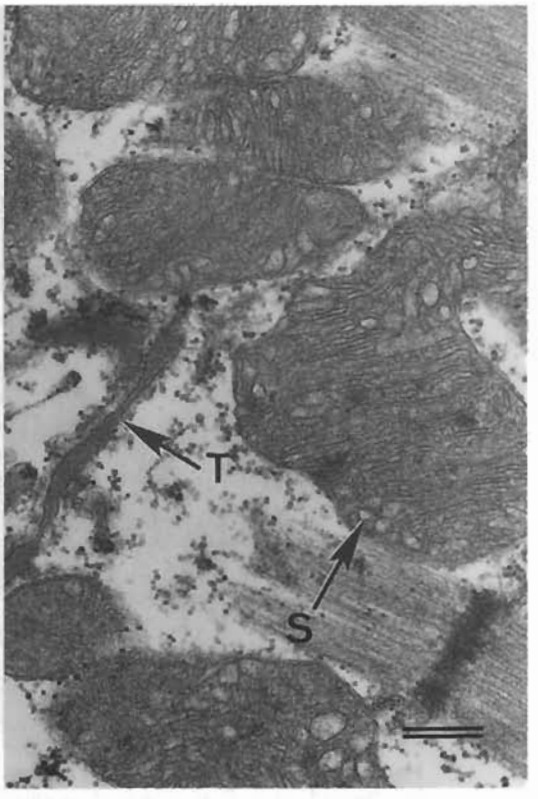

Figure 6. This electron micrograph illustrates the sacculations (s) of mitochondrial cristae, as well as transverse tubules (T) of the ventricular myocyte. 30 200 ×, Scale bar = 331 nm.

magnification of cross sections (Fig. 5), the myofibrils were composed of typical thick (200 Å) myosin filaments surrounded by thin (60 Å) actin filaments. The mitochondria of ventricular myocytes were ovoid or irregular-shaped and contained densely packed cristae with prominent sacculations (Fig. 6) in the ringed seal. In the harp seal, sacculations of mitochondrial cristae were not observed (Fig. 7), and there was considerable variation in density of intracellular packing of myofibrils (Fig. 7).

A well-developed transverse tubular system was present in the seal myocardium. Its lumen was filled with a moderately electron dense homogeneous material (Figs. 6, 8), and numerous pinocytotic vesicles were observed in some regions of the tubular system (Fig. 8) in the ringed seal. The tubular system was continuous with the Z lines in common with all other mammals, but in the ringed seal, this transport system had a particularly well developed association with the capillaries, as seen in Figure 9. Also, the transverse tubular system traversed the enlarged glycogen reservoirs and much pinocytotic activity was observed in this zone (Fig. 9). Pinocytotic vesicles were also prevalent in the capillary endothelial cells between the cardiomyocytes of both species, such as illustrated in Figure 10 of the harp seal.

Endocrine granules were occasionally observed in the perinuclear region of ventricular myocytes. These granules were very electron dense, of irregular shape, and of variable size but similar to mitochondrial size as shown for the ringed seal (Fig. 11). They were membrane-bound (Fig. 12).

The epicardium consisted of a loose network of both collagenous fibers and widely spaced elastic fibers, each of which were nondistinctive at the ultrastructural level.

Discussion and conclusions

Many structural and physiologic adaptations exist in diving mammals and a number of these have

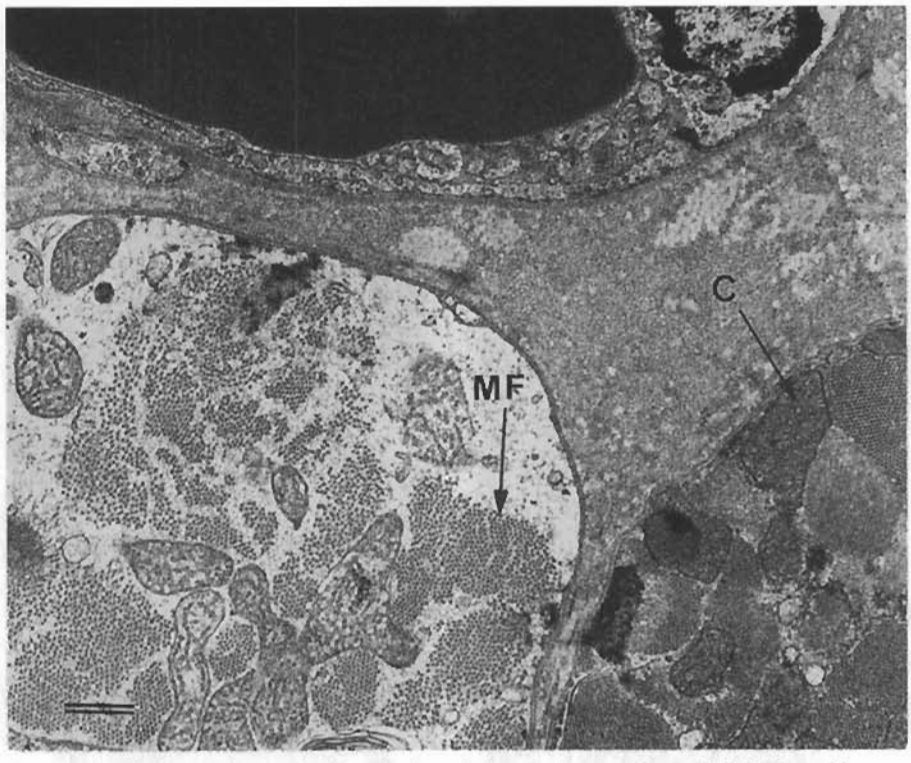

Figure 7. In this figure (harp seal, left atrium), variations in density of myofibril (MF) packing can be observed, as well as a lack of sacculations in mitochondrial cristae (C). 15 700 ×, Scale bar = 638 nm.

evolved in the cardiovascular system. Although few studies have focused on these adaptations at the subcellular level, we have earlier reported several such changes in cardiac or vascular structure in bowhead whales *B. mysticetus* (Pfeiffer, 1990; Pfeiffer & Kinkead, 1990). It is important also to examine the pinniped heart from this perspective, and the present data are believed to be the first ultrastructural information on the seal heart.

A few cardiac subcellular features appear to be distinctive for the seal relative to the typical terrestrial mammalian heart. These include the large reservoirs of glycogen in the cardiomyocytes. Glycogen particles in the seal heart were structurally similar to beta glycogen observed in the canine heart (Rybicka, 1979). The stores of glycogen in the ringed seal heart were contiguous with the more usual deposits of free glycogen observed between the myofibrils but also were intimately associated with the transverse (T) tubular system of the cardiomyocytes. In the latter regard, metabolite transport between these glycogen reservoirs and the capillary lumen could readily occur via the T-tubular system as morphologic evidence was found of extensive pinocytotic activity on T-tubules communicating between both the glycogen reservoirs and the pericapillary region. It is noteworthy that the presence of enhanced glycogen storage was also observed in the narwhal, *Monodon monoceros*, arterial retia (Vogl & Fisher, 1976) and bowhead whale heart (Pfeiffer, 1990), although the T-tubular system was not as extensively developed in the bowhead whale heart. The metabolic activities of the heart of the diving seal have been examined by earlier workers. In artificial diving experiments, cardiac output in grey seals (*Halichoerus grypus*) was reduced and associated with a 90 percent reduction of coronary blood flow (Blix *et al.*, 1976; Schytte *et al.*, 1976; Blix *et al.*, 1983). During hypoxic conditions, anerobic glycolysis in the heart may become essential in the submerged seal, and Kerem and associates (1973) reported cardiac glycogen levels were increased two–three-fold in the Weddell seal (*L. weddelli*) compared to various terrestrial mammals. Furthermore, the heart of the Weddell seal contains elevated levels of lactate dehydrogenase (Murphy *et al.*, 1980). Hormonally mediated glucose conservation is also involved in the regulation of carbohydrate metabolism during diving and postdiving recovery in the

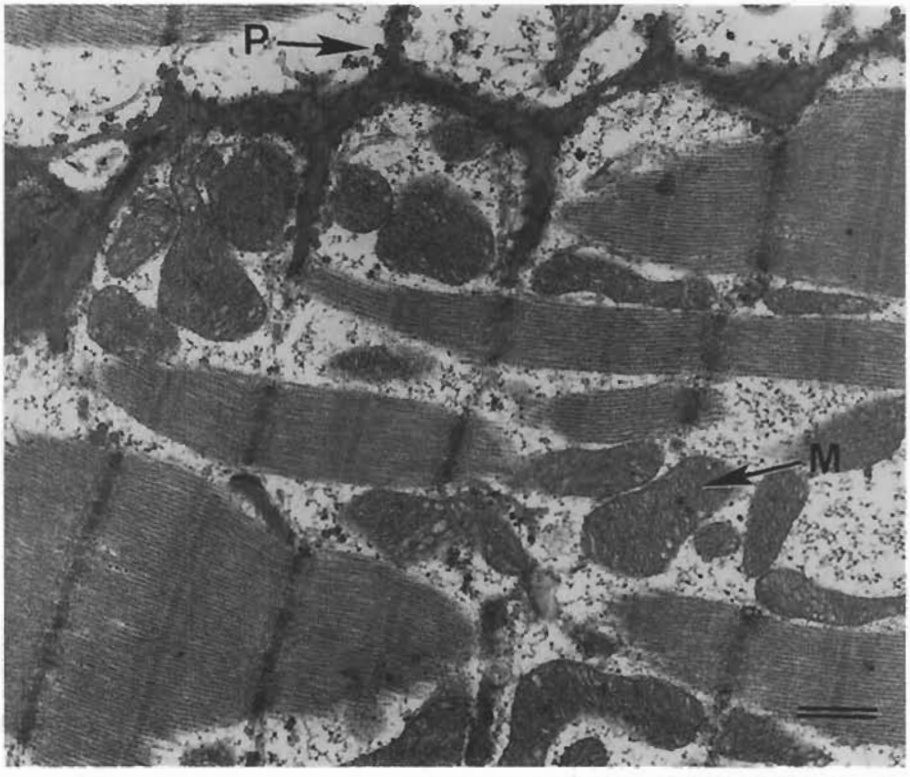

Figure 8. This electron micrograph of the ringed seal ventricular myocyte shows the extensive pinocytotic activity (P) on the transverse tubules, and tightly packed and sacculated cristae of mitochondria (M). 21 700 ×, Scale bar=451 nm.

harbor seal, *Phoca vitulina* (Robin *et al.*, 1981; Davis, 1983).

Another distinctive fine structural feature of the ringed seal heart was the presence of sacculations on the mitochondrial cristae. This characteristic has not been noted in cardiomyocyte mitochondria of other mammals but is similar to the sacculation apparent on mitochondrial cristae of rat adrenocortical cells (Andreis *et al.*, 1989). The heart of the seal also had a thickened endocardium, a feature observed in the bowhead whale, but somewhat distinct from the thinner endocardium common in terrestrial mammals of comparable size to the seal. The physiologic significance of both the thickened endocardium and the mitochondrial cristae sacculations of the ringed seal, to diving or other functions, is obscure at the present time, and the latter feature was not seen in the harp seal.

Cardiomyocytes of the seal heart were generally similar to those observed for terrestrial mammals (Kisch, 1956; Sommer & Johnson, 1968), except for features mentioned above. As reviewed elsewhere (Hirakow, 1970; Forbes & Sperelakis, 1984), mammalian cardiac ultrastructure reveals a transverse tubular system in contrast to lower vertebrate hearts, which do not possess this structure. The seal heart contains, however, fewer associated structures at the Z band level than the mouse heart (Forbes & Sperelakis, 1980), but still the T-tubules are prominently connected with the Z band in the seal. Conductile components of the seal heart, such as Purkinje tracts, have not yet been studied, so further detailed investigations will be required to elucidate this important component of the seal heart.

In recent years the heart has been recognized as an endocrine organ, and endocrine granules have now been identified within atrial and ventricular myocytes of most mammals that have been carefully studied by electron microscopy. The endocrine granules observed in the present investigation closely resemble the endocrine granules, in electron density, size, and subcellular location, as reported for other mammals (Lanning & Zaki, 1966; Otsuka *et al.*, 1969; Didio *et al.*, 1987). We have not investigated the immunoreactivity of the cardiac

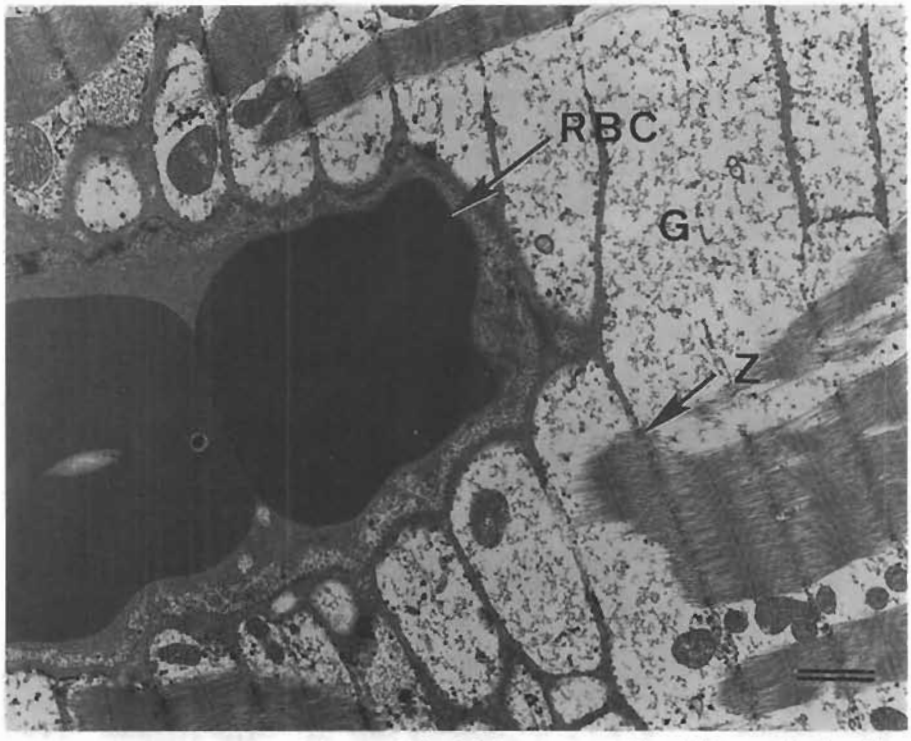

Figure 9. This figure shows the intimate relationship between a capillary (with contained erythrocytes (rbc)) and the transverse tubular system of a ventricular myocyte. The tubular system invests the capillary and extends through a glycogen reservoir (G) to the Z lines (Z) of the contractile elements. 8100 ×, Scale bar = 1.23 μm.

endocrine granules in the seal, but it is likely they contain various natriuretic polypeptides such as the atrial natriuretic factors (ANF) described and purified by other workers (Geller *et al.*, 1984; De Bold, 1985; Laragh, 1985).

Further investigation beyond the present study will be required to elucidate additional details of the pinniped heart, including studies also at the macroscopic level. It will be of particular interest to investigate the subcellular morphology and biochemistry of the Weddell seal and California sea lion, as these species undertake the most extreme diving behavior. Since marine mammals are rigidly protected for conservation purposes, thus limiting physiologic experimentation, future cardiac information derived from direct experimental studies will likely be more limited than future morphologic studies.

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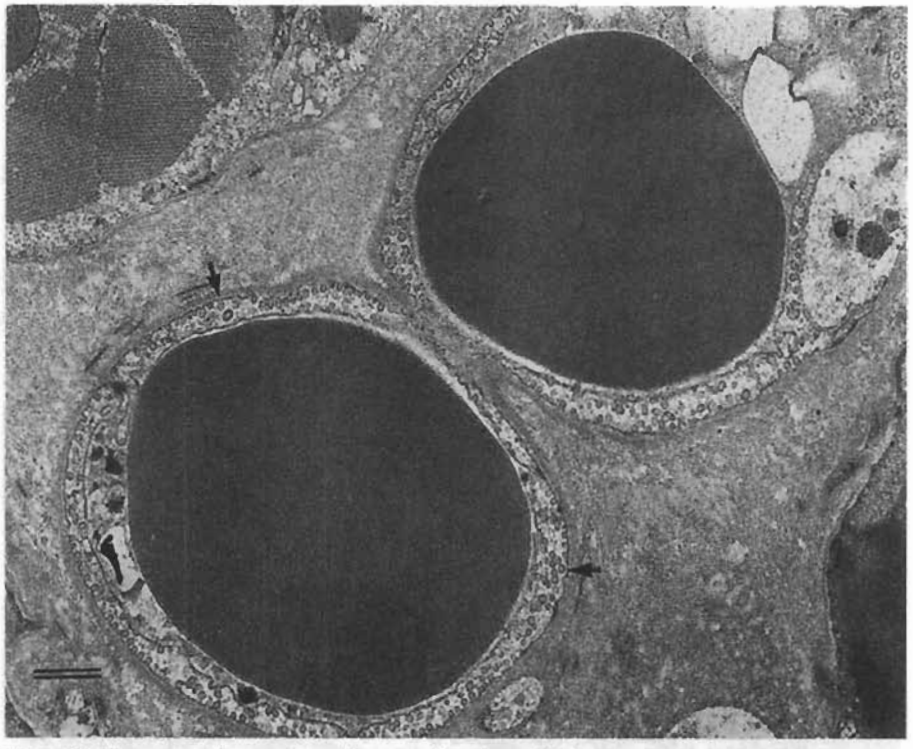

Figure 10. Numerous pinocytotic vesicles are evident in capillaries (arrows). Myofibrils are shown in cross section in the upper left corner. Harp seal atrium. 15 650 ×, Scale bar = 640 nm.

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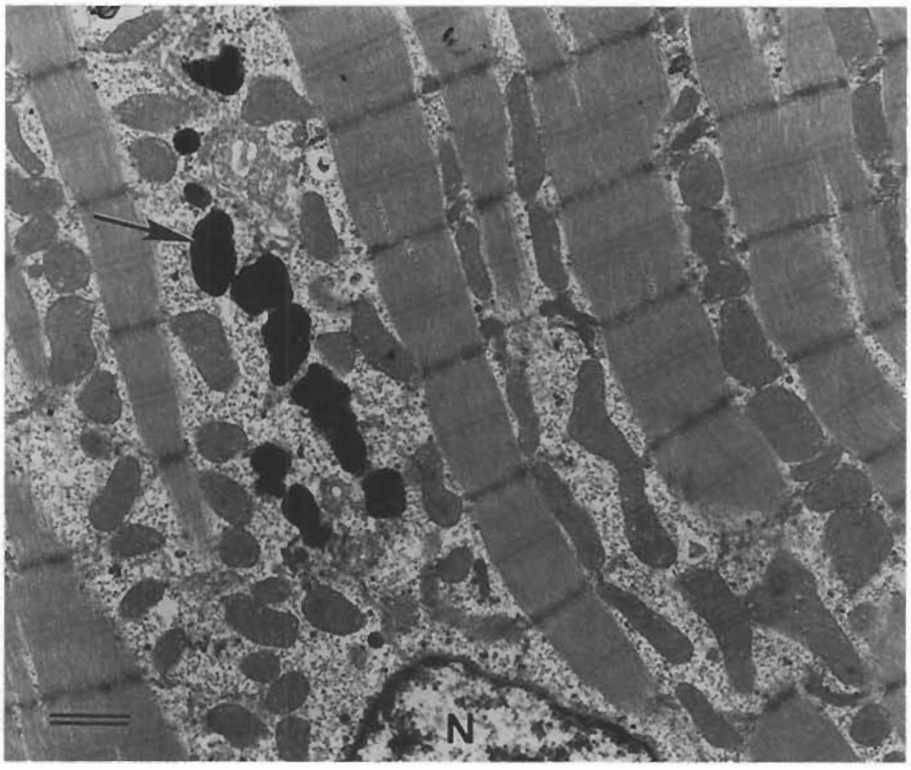

Figure 11. Endocrine granules (arrow) were observed in the myoplasm near the nuclear (N) region in the ringed seal ventricular myocyte. These electron dense granules are a likely source of natriuretic polypeptide. 10 250 ×, Scale bar = 976 nm.

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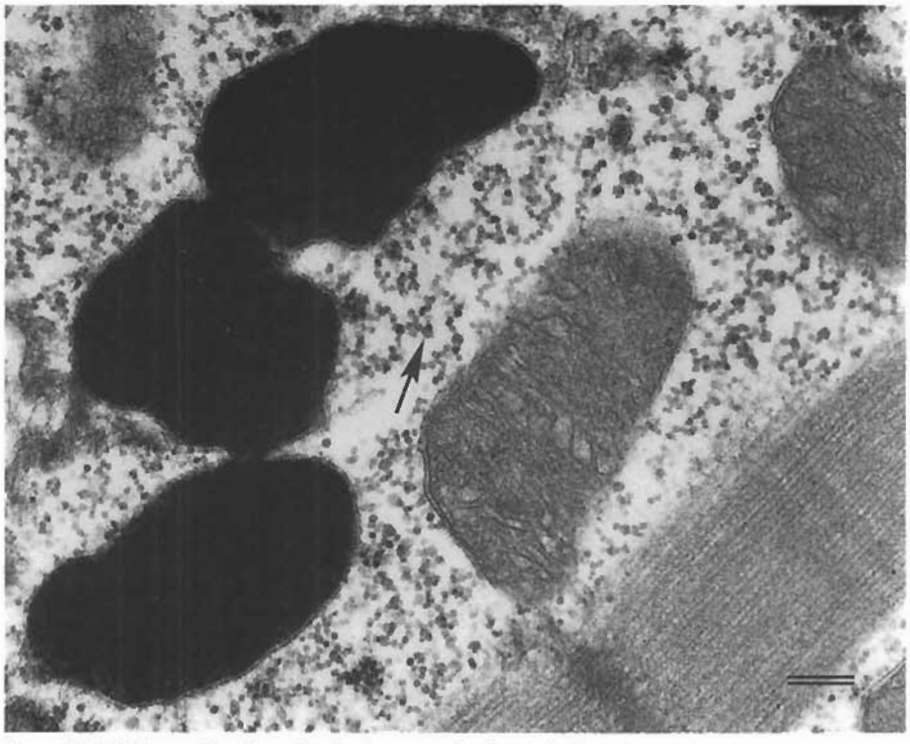

Figure 12. High magnification of endocrine granules shown in Fig. 10, showing they are membrane-bound. The small granules (arrows) are beta glycogen. 51 250 ×, Scale bar = 195 nm.

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