Movement of $NaH^{14}CO_3$ from the bag also yielded $k_{out} \cong 0.12\ min^{-1}$. Efflux from lens was much slower, however, with $k_{out} \cong 0.023\ min^{-1}$. This is close to the value obtained for rabbit lens by Kinsey (vide supra).

These very preliminary data suggest experiments to find whether HCO_3^- efflux may be linked to the active efflux of sodium. In lenses containing carbonic anhydrase, the enzyme may speed formation of HCO_3^- from CO_2 to subserve Na transport. It is of particular interest that the pentose phosphate pathway in lens provides a continuous source of CO_2. (Kinoshita and Wachtel, J. Biol. Chem. 233:5, 1958.) Supported by NIH Grant EY 02227.

ULTRASTRUCTURAL STUDIES ON THE HEART OF *Boltenia ovifera* (SEA POTATO)

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In the past few years we have examined and confirmed that the heart of *Boltenia ovifera* is composed of single cell-layered myoepithelium. Tight junctions separate the luminal and junctional surfaces from the surface facing the pericardial cavity. The luminal membrane generates action potential and seems to be the site of E-C coupling (Weiss, Goldman and Morad, J. Gen. Physiol. 68:503-518, 1976). The extraluminal membrane is insensitive to ionic or drug variations and does not seem to play a prominent role in generation of excitation or excitation-contraction coupling. It would be of interest to examine whether the functional differences in these two membranes are represented by corresponding structural differences. In this report we have carried out an ultrastructural analysis to examine this possibility. Freeze-fracture, transmission and scanning electron microscopy were used to examine the cell surface topography as well as the molecular architecture of the luminal and extraluminal membranes.

Methods

The tubular hearts were dissected free of pericardium and fixed in 1% glutaraldehyde in 0.2 M phosphate buffer, pH 7.4 with added H_2O_2 (Peracchia and Mittler, J. Cell Biol. 53:236-238, 1972). NaCl

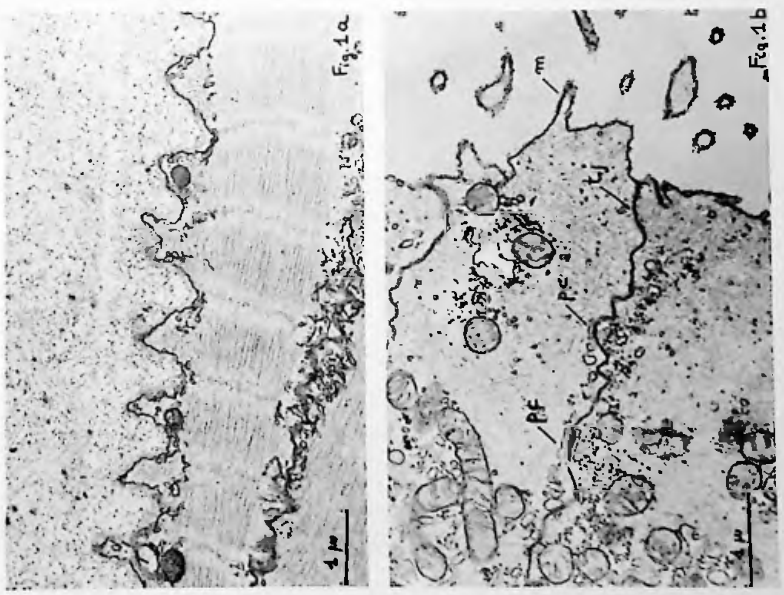

Figure 1a,b. Thin sections illustrating the different features of myocardial cells, at the level of the heart lumen and pericardial cavity, respectively. m = microvilli; pc = peripheral coupling; tj = tight junction; scale marker = 1 µm.

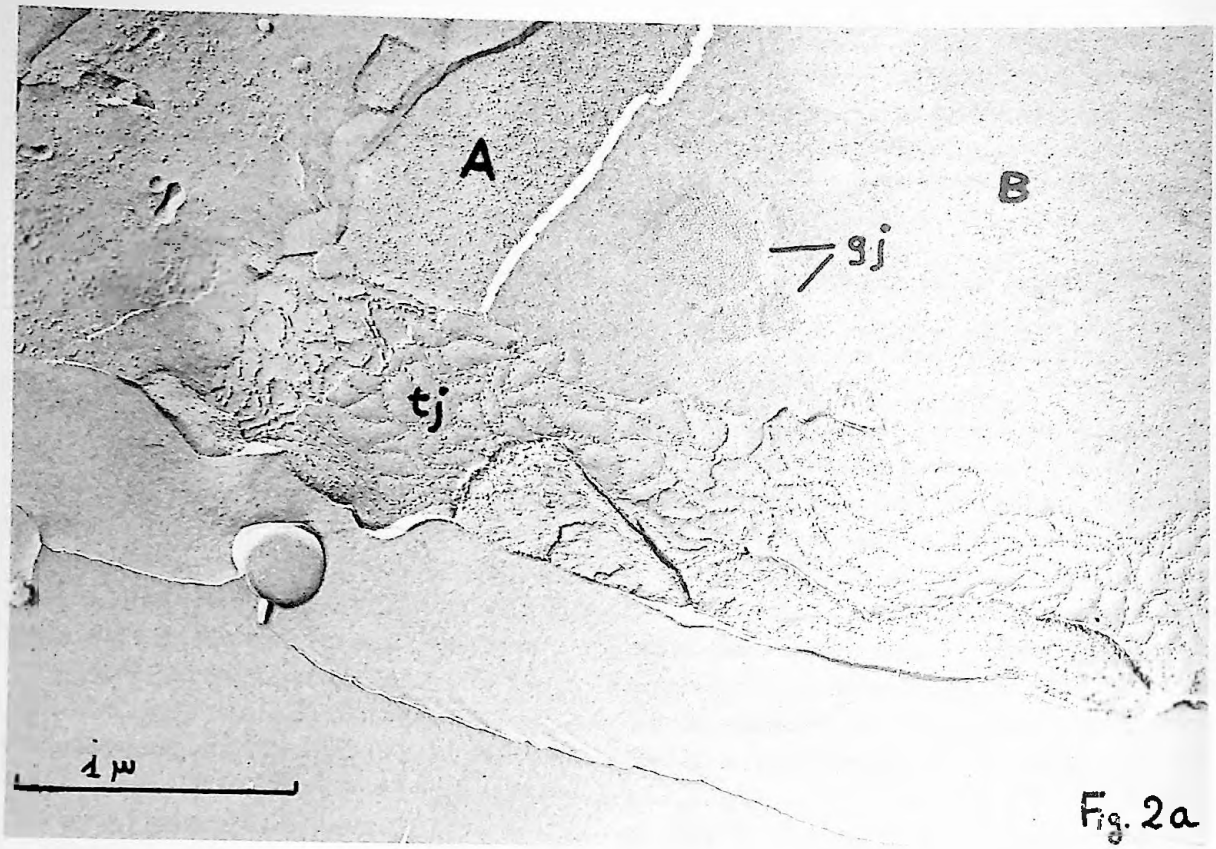

Fig. 2a

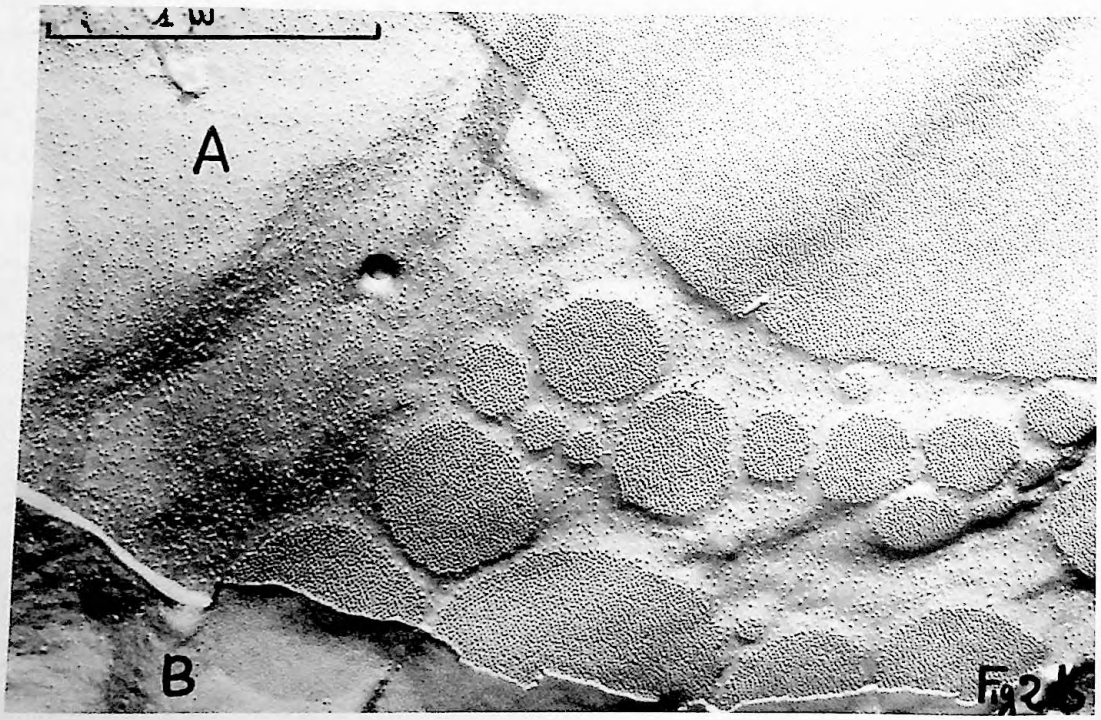

Fig 2b

Figure 2a,b. Freeze-fracture appearance of tight and gap junctions in myocardial cells. The fracture plane exposes both the cytoplasmic (A) and external (B) leaflet of the sarcolemma. gj = gap junction; Tj = tight junction; scale marker = 1 µm.

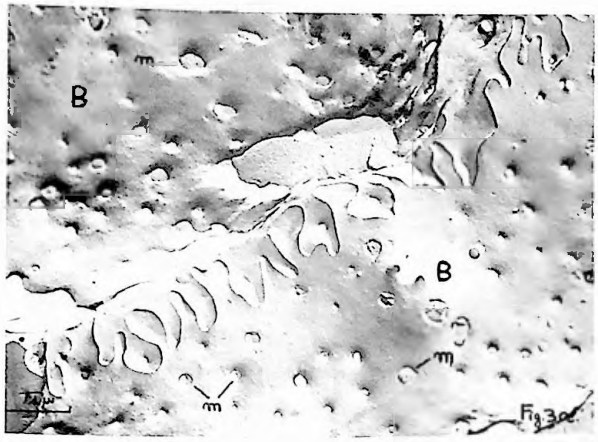

Figure 3a. Replica of freeze-fractures B faces of the extraluminal sarcolemma of interconnecting cells. The cross-fractured microvilli are seen as large indentations of the membrane. m = microvilli, scale marker = 1 µm.

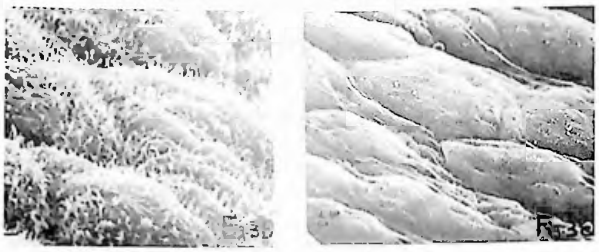

Figure 3b,c. Scanning electron micrographs showing two different regions of the myocardial wall as regards the presence of microvilli. Scale marker = 1 µm.

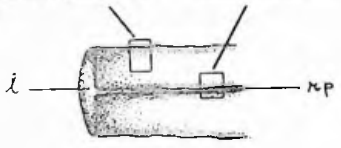

Fig. 3d.

Figure 3d. Schematic representation of a segment of myocardium with two areas corresponding to the above scanning micrographs. l = heart lumen; rp = raphe.

was added to adjust the final osmolality to 600 mOsm. For transmission electron microscopy the fixation was followed by 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.4. Sections were stained with uranyl acetate and lead citrate. For freeze-fracture replicas the specimens after fixation and infiltration in glycerol up 30% were cooled in liquid Freon-22 at a temperature slightly above that of liquid nitrogen. Fracture, shadowing, and replication were performed in a Denton DV-502 freeze-etch unit using the double replica method. For scanning microscopy the heart was cannulated with a glass rod, fixed in glutaraldehyde and osmium tetroxide and then dehydrated in methanol. The critical drying point was carried out in a Sorvall device using 100% liquid CO_2. The specimens were coated with gold-palladium to a film thickness of approximately 100 Å. Specimens were then examined in a AMR 1000 A scanning electron microscope.

Results

Figure 1a illustrates a longitudinal thin section of the luminal segment of the myocardium. The luminal surface is covered by a thick layer (2-3 µm) of fibrillar material closely apposing the sarcolemma. Electron dense granules were found in the mesh-work of the extracellular coating. Cytoplasmic evaginations extend into the lumen and are often found to contain groups of myofilament perpendicularly oriented to the main longitudinal myofibril. This different orientation of myofilaments may provide for the twisting characteristic of the propagation of the peristaltic wave in the tunicate heart.

The extraluminal segment of myocardial cells is shown in Figure 1b. The plasma membrane is underlaid by a thin strand of electron-dense, "fuzzy" material, and displays numerous microvilli protruding into the pericardial cavity. Tight junctions are always found near the extraluminal border of the junctional surface. Numerous peripheral couplings (pc) occur on the junctional membrane surfaces. These peripheral couplings are mostly found close to the extraluminal surface and often appear directly opposite in adjacent cells. It is intriguing to attribute a functional role to these "couplings" based on structure-function studies made on vertebrate skeletal muscle (Spray, Waugh and Sommer, J. Cell Biol. 62:223-227, 1974). However, it should be remembered that in this preparation the E-C coupling processes seem to occur at the luminal surface. The appearance of these surface couplings away from the luminal surface may suggest that these structures may serve as steady state Ca^{2+}-exchange sites rather than Ca-release sites.

Freeze-fracture was used in order to study the molecular architecture of the plasma membrane of the myocardial cells. The plane of the fracture follows a path inside the unit membrane revealing the cytoplasmic and external leaflets of the plasma membrane (Pinto da Silva and Branton, J. Cell Biol. 45:598-605, 1970). In the case of myocardium an extensive view of the sarcolemma may thus be obtained. Figure 2a shows a freeze-fracture replica through the junctional sarcolemma toward the pericardial cavity. The cytoplasmic leaflet (face A) contains a great number of intramembranous particles whereas the complementary external leaflet (face B) is fairly devoid of membrane particles. The junctional region (tj) is characterized by a belt-like tight junction which appears as a complex network of ridges on the A fracture face and of furrows on the complementary B fracture face. Gap-junctions (gj) were also found often in close proximity to the tight junctions. On the A face the junctional particles are closely packed together leaving an ordered array of pits on the complementary B face. Gap junctions seem to aggregate forming extensive areas of cellular communication (Figure 2b). These extensive areas of gap junctions suggest low resistant-pathways for flow of current between cells.

The extraluminal sarcolemma is easily identified in freeze-fracture replicas by the presence of numerous cross-fractured microvilli (m, Figure 3a). The presence of microvilli on the extraluminal surface is also confirmed by scanning preparations (Figure 3b). However, regions of the myocardial wall appear to be fairly smooth (Figure 3c) revealing a diversity of the density in microvilli of some areas of the myocardium, as illustrated in the drawing (Figure 3d). The significance of this heterogeneous distribution is not as yet clear. It is intriguing to speculate on the appearance of microvilli only on the extraluminal surface. The existence of microvilli on this surface may be consistent with lack of excitability or E-C coupling processes on this membrane. It is conceivable that this surface is involved in transport of metabolites and ions across the cell surface.

Summary and Conclusions

Although no clear-cut molecular evidence could be found to distinguish the luminal membrane, considerable ultrastructural evidence was found which is consistent with the physiological findings. Scanning electron microscopy clearly shows that the extraluminal membrane is rich in microvilli, which may be implicated in trans-myoepithelial transport of metabolites and ions. Freeze-fracture studies show that the junctional membrane is rich in gap junctions and could serve as the site of low resistant electrical couplings. Large and fairly complex arrays of tight junctions were also found in the junctional membrane which may serve as an effective barrier for ionic diffusion between the myocardial cavity and pericardiac sac. Peripheral couplings were also found on the junctional membrane. No direct evidence is as yet available as to the functional roles of these couplings. The segment of the membrane apposing the luminal surface could not be identified from the freeze-fracture experiments and must remain for future studies. Acknowledgment. We wish to thank Dr. L. Peachey and Dr. C. Franzini-Armstrong for use of the electron microscope.