mal and malignant plasma cells produce a soluble substance other than immunoglobulin, which regulates the growth of clones of antibody-producing cells. Because of the large number of malignant plasma cells in patients with myeloma and in mice with PC, a larger quantity of this chalone-like substance might be produced, and this might account for the antibody deficiency syndrome that is so characteristic of this malignant state.

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Single Cell Layered Heart: Electromechanical Properties of the Heart of Boltenia ovifera

Abstract. *The heart of* Boltenia ovifera *(the sea potato) is a tubular structure formed by a single layer of myocardial cells. Electron microscopic studies show that each cell contains a single myofibril located adjacent to the luminal surface of the cell. Electrical and mechanical measurement of a cannulated perfused heart demonstrate that only the luminal membrane is excitable and elicits contraction on depolarization. Calcium and magnesium exert antagonistic effects on tension, and potassium depolarizes the myocardium and produces contractures when the luminal membrane is exposed to various concentrations of these ions. The extraluminal membrane does not respond electrically or mechanically to calcium, magnesium, or potassium, and its potential seems to be effectively "clamped" by the luminal membrane. Functionally, therefore, this heart consists of a single active membrane with the adjacent contractile apparatus.*

Boltenia ovifera is a species of tunicate inhabiting the coastal waters of northern Maine at depths of 60 to 90 m. Secured to the ocean floor by a 20- to 30-cm stalk, the sea potato filters seawater for nutrients through two siphons. Tunicates are classified as chordates and are considered the immediate precursors of vertebrates (*1*).

The heart of the sea potato is 0.3 cm in diameter and 5 cm long, and consists of a straight, valveless tube which runs the length of the animal. It lies enclosed in another fluid-filled tube, the pericardium, to which it is attached by a longitudinal raphe. Light microscopy (serial sections) in our laboratory showed that the myocardium of this animal is formed by a single layer of tightly packed cells which are fused together at the raphe by connective tissue. There is no evidence that nonmyocardial cells exist at any site other than the raphe, as has been reported in other species of tunicates (*2*). Electron microscopic studies (Fig. 1b) indicate that each cell contains a single myofibril located near the luminal surface. The luminal membrane of the cells is lined with an electron-opaque material; in contrast, the extraluminal membrane is smooth and devoid of any covering. Adjacent cells are interconnected by specialized junctions located primarily near the extraluminal surface, which appear to seal the luminal from the extraluminal aspects of the cells. In other species of tunicates, junctions similar in appearance have been demonstrated to meet the criteria for tight junctions (*3*).

The beating myocardium is readily visible as the thick outer tunic of the sea potato is dissected away. The heart can be excised intact inside the pericardium after it has been ligated at both ends. To permit continuous perfusion of the inside and outside of the

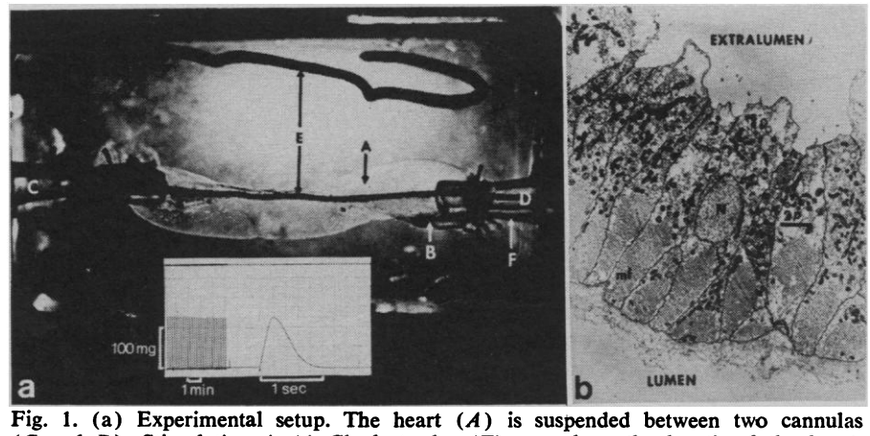

Fig. 1. (a) Experimental setup. The heart (*A*) is suspended between two cannulas (*C* and *D*). Stimulating Ag/AgCl electrodes (*E*) run along the length of the heart, and a tension transducer (*F*) is attached to a pericardial fragment adjacent to the raphe (*B*). (Inset) A typical tension recording at fast and slow time scales. (b) Electron micrograph of the myocardium of the sea potato, which is one cell layer thick. Myofilaments (*mf*) are located along the luminal surface and prominent nuclei (*N*) along the extraluminal surface, with abundant mitochondria scattered throughout.

heart, the pericardium is removed and the heart is cannulated at both ends. During the equilibration period, both surfaces of the heart were perfused with a standard solution of the following composition: 365 m*M* NaCl, 10 m*M* KCl, 10 m*M* $CaCl_2$, and 2 m*M* $MgCl_2$, *p*H 5.5. The choice of the standard solution was based largely on the ionic contents normally found in the pericardial fluid and the blood of the sea potato. Flame photometric measurements of the pericardial fluid from ten hearts gave the following values: 410 ± 8.8 m*M* Na^+; 12 ± 2.8 m*M* K^+; 9 ± 1.1 m*M* Ca^{2+}; 45 ± 4.9 m*M* Mg^{2+}; and 486 ± 25.7 m*M* Cl^-. The ionic content of centrifuged blood from two hearts averaged 380 ± 5.6 m*M* Na^+; 8.8 ± 0.14 m*M* K^+; 7.4 ± 0.7 m*M* Ca^{2+}; 33 ± 1.4 m*M* Mg^{2+}; and 463 ± 3.2 m*M* Cl^-. Smaller concentrations of Mg^{2+} than those directly measured in the animal blood were routinely used, since it was found that the contractile response of the heart diminished in the presence of such large Mg^{2+} concentrations.

The tubular heart of the sea potato in situ beats peristaltically at the rate of 12 to 18 beats per minute. Spontaneous beating generally stops on cannulation and perfusion of the heart. However, small electrical stimuli (0.1 to 0.2 volt and 20 to 50 msec) are capable of eliciting a contractile response in the perfused heart. The myocardium was stimulated by applying suprathreshold current between two Ag/AgCl electrodes, one inserted in the lumen along the total length of the tubular heart and the other running parallel to the first electrode in the bath. This arrangement allowed uniform stimulation of the heart at the desired frequency (usually 6 min^{-1}). The contractile response was measured with a tension transducer attached to the heart by an undissected pericardial fragment. A perfused preparation and its tension record are shown in Fig. 1a. The maximum force of contraction measured under optimal conditions (that is, optimal radial and longitudinal distension of the tube, minimizing the elasticity of the pericardial fragment) was approximately 1 g.

The contractile response of the myocardium was found to be highly sensitive to the intraluminal concentrations of calcium and magnesium. Twitch tension is maximum at $\geqslant 7$ m*M* Ca^{2+}. The force of contraction decreases continuously below 7 m*M*, and the preparation fails to contract in solutions from which calcium has been omitted. Variation in the extraluminal Ca^{2+} concentration has no effect on developed tension. In contrast to the result with calcium, increasing the intraluminal concentrations of magnesium decreases the developed tension markedly. Contraction is maximum at $\leqslant 2$ m*M* Mg^{2+} in the intraluminal solution (from 2 to 160 m*M* tested). Variation in the extraluminal Mg^{2+} concentration has little or no effect on the contractile force. Decreasing the Na^+ concentration does not potentiate tension. Both Ca^{2+} and Mg^{2+} exert their effects by modifying the rate of rise of tension and have no effect on the time to peak of tension.

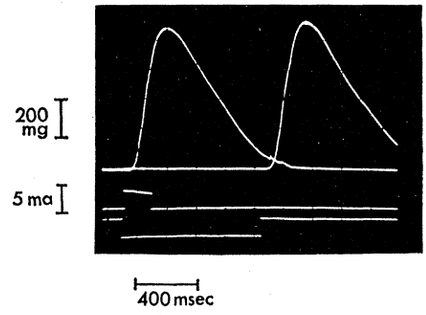

Fig. 2. A comparison of the onset of tension development following a current pulse of short duration (~ 150 msec), depolarizing the luminal membrane (and hyperpolarizing the extraluminal), and a current pulse of longer duration (~ 800 msec) and of the opposite polarity, depolarizing the extraluminal membrane (and hyperpolarizing the luminal). Note that in the first case, the onset of tension occurs immediately (with depolarization of luminal membrane), whereas in the second, tension does not develop until the current pulse is terminated, suggesting anodal break excitation of the luminal membrane.

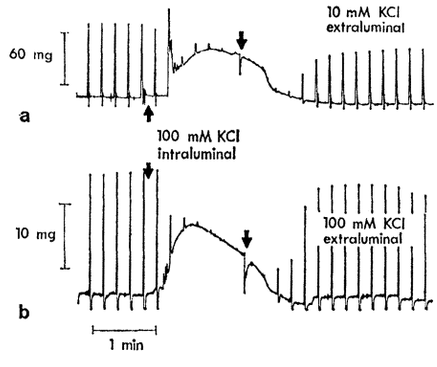

Fig. 3. (a) Contracture recorded when the intraluminal concentration of KCl is isotonically increased from 10 m*M* (the standard) to 100 m*M*. Normal contractility is restored when the 100 m*M* KCl is removed. The extraluminal KCl concentration is 10 m*M*. (b) The extraluminal surface of the heart has been exposed for several minutes to 100 m*M* KCl with no changes in contractility. Increasing the intraluminal concentration of KCl from 10 to 100 m*M* still results in a contracture. Steady state tension is smaller than in (a) because of the gradual decline in the magnitude of tension on repeated exposures of the intraluminal surface to solutions with high KCl concentrations.

In response to changes in the frequency of stimulation (up to 10 min^{-1}) the tunicate heart demonstrated a small but definite staircase effect. Increasing the amplitude or duration of the electrical stimulus produced a graded increase in contractile force in the 160 preparations examined, suggesting a graded, rather than an all-or-none, contractile response.

The tunicate heart responded asymmetrically to the direction of the stimulating current. If current was passed in the direction from the electrode in the bath to the electrode in the lumen, the threshold for contraction was several times less than when current was passed in the opposite direction, suggesting that the luminal and extraluminal membranes have different thresholds of excitability. The nature of these threshold differences was clarified by comparing the time course of the stimulating current pulse with the onset of development of tension. It was found that when current was passed in the direction from the bath electrode to the luminal electrode, tension development coincided with the beginning of the current pulse. In contrast, when current was passed in the opposite direction, tension invariably failed to develop until the current pulse was terminated (Fig. 2), suggesting anodal break excitation of a hyperpolarized membrane. Our interpretation of these findings is that current passed from the bath electrode to the luminal electrode depolarizes the luminal membrane and results in a contraction immediately. Depolarization of the extraluminal membrane by application of current in the opposite direction, on the other hand, does not result in tension development, and the contraction observed when current of this polarity is terminated results from anodal break excitation of the hyperpolarized luminal membrane. Thus, excitation of the luminal membrane only appears to be coupled with the contractile apparatus in these myocardial cells. This finding correlates with the electron microscopic observa-

tion that myofilaments are located near the luminal membrane only.

Additional evidence that the luminal and extraluminal membranes have different properties was derived by comparing the response of the myocardium to alterations in the intraluminal and extraluminal ionic media. Application of isotonic solutions containing high concentrations of KCl (60 to 200 m*M*) to the luminal surface elicited maintained tension (contracture) and rendered the heart unresponsive to current passed in either direction. Exposure of the extraluminal membrane to 100 to 200 m*M* KCl, on the other hand, failed to produce contractures and did not alter the contractile response to current passed in either direction (Fig. 3). Direct measurement of intracellular potential with glass microelectrodes filled with 3*M* KCl showed that the resting potential of the myocardial cells (-65 ± 5 mv) was unaffected by changes in the concentration of extraluminal KCl, whereas the cells promptly depolarized in response to increased luminal KCl. As described above, variation of intraluminal Ca^{2+} and Mg^{2+} altered the contractile response, whereas changes in concentration of these ions extraluminally had no effect on contraction. These observations provide additional evidence that only the luminal membrane actively participates in the excitation-contraction coupling process; the extraluminal membrane appears to be electrically passive and is effectively clamped by the luminal membrane.

Consistent with these findings, direct measurements of intracellular potential in another species (*Chelyosoma productum*) showed that only the luminal membrane is capable of generating an action potential (*4*). In contrast to our findings, Kriebel (*4*) reported that the extraluminal membrane of the myocardial cells depolarized completely when exposed to isotonic 480 m*M* KCl, producing a potential difference of 8 to 15 mv (lumen positive), without eliciting a contracture or any apparent alterations in excitability. If the extraluminal membrane is completely depolarized, any transcellular potential difference recorded must reflect the potential difference across the luminal membrane exclusively. Since the electrically measured threshold for excitation (and resulting contraction) of these myocardial cells is about -50 mv (*4*), it is inconsistent that depolarization of the luminal membrane above the mechanical threshold should not elicit contraction. If, on the other hand, the extracellular membrane is actually insensitive to KCl, as in the sea potato, it is not clear why a transcellular potential was recordable.

In the sea potato, no transcellular potential difference could be measured, even when the luminal or extraluminal surfaces were exposed to 100 m*M* KCl. This finding suggests that the 40- to 50-mv depolarization observed directly with microelectrodes upon exposure of only the intraluminal surface to KCl was being short-circuited through a low-resistance pathway. To test this hypothesis, we measured the transcellular resistance by passing short current pulses (5 msec) between two Ag/AgCl electrodes and recording the voltage difference across another set of Ag/AgCl electrodes. Both ends of the preparation were immersed in oil to insulate the areas of the preparation damaged by the ligatures from the rest of the myocardium. These experiments confirmed a low transcellular resistivity of 200 ohm cm^2. Morphologically, the low-resistance "shunt" pathway may correspond either to extracellular channels between myocardial cells or to the raphe of the heart, where the heart cells are joined together with connective tissue. The presence on electron micrographs of specialized junctions which appear to isolate the luminal from the extraluminal surface may favor the latter possibility. Kriebel (*5*) obtained a similar value for transcellular resistivity in other species of tunicates in which he cut the heart along the raphe to form a sheet and concluded that the tight junctions have an electrically high transverse resistance.

The experiments outlined above show that the heart of the sea potato consists of a single layer of myocardial cells sharing many of the electromechanical properties of vertebrate myocardial tissue. In addition, these experiments strongly suggest that only the luminal membrane of the heart plays an active role in excitation-contraction coupling processes. It appears that the extraluminal membrane has no role in the activation of the contractile apparatus, and its potential is effectively clamped by the luminal membrane. The existence of myofilaments only on the luminal side provides the structural basis for the electromechanical measurements. These properties of this single-layered heart with one active membrane makes it an ideal preparation for a variety of electrophysiological and radioisotope studies.

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Visual Detection of Line Segments: An Object-Superiority Effect

Abstract. *Observers identified a briefly flashed line segment more accurately when it was part of a drawing that looked unitary and three-dimensional than when the line was in one of several less coherent flat designs.*

Many cells in the mammalian visual cortex respond best to straight line segments with a particular orientation in a particular location on the retina (*1*). A number of theoretical models for pattern recognition employ similar oriented-line detectors as an early stage in a hierarchical feature-identification process (*2*).

Our visual detection task used stimuli appropriate for such line detectors: four line segments differing in orientation and location relative to a fixation point, as shown in Fig. 1, a–d. On each trial, one of these target lines, together with one of several context patterns such as Fig. 1e, was flashed briefly on a computer-controlled cathode-ray tube screen, producing the compound patterns shown in Fig. 2.

The viewer's task was always to identify which one of the four diagonal line segments was present in the briefly flashed display. None of the context pat-