Electromechanical Properties of the Single Cell-Layered Heart of the Tunicate *Boltenia ovifera* (Sea Potato)

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**ABSTRACT** The tubular heart of the sea potato is composed of a single layer of myoepithelial cells interconnected near the extraluminal surface by specialized junctions. If these junctions are used as the border which separates the luminal from extraluminal membrane, the surface area ratio, luminal:extraluminal, is approximately 12:1. A single myofibril is located near the luminal surface in each cell. Current passed across the heart wall in the direction that depolarizes the luminal membrane and hyperpolarizes the extraluminal membrane immediately produces “all-or-none” action potentials and contractions. Current passed in the opposite direction fails to produce action potentials until after the break of the stimulus, suggesting anodal break excitation of the hyperpolarized luminal membrane. High potassium solutions depolarized the myoepithelium and produced contractions only when applied to the luminal surface of the heart. [Ca]₀ increases and [Mg]₀ decreases twitch tension only on the luminal surface of the heart. The transwall resistivity is low (50–100 Ω/cm²) due to an extracellular shunt. Because of this shunt and the larger surface area of the luminal membrane, the extraluminal membrane is effectively clamped to the potential of the luminal membrane and is not capable of directly influencing excitation-contraction coupling. These findings suggest that only the luminal membrane of the sea potato myoepithelium is capable of generating an action potential and triggering contraction.

**INTRODUCTION**

*Boltenia ovifera,* commonly known as the “sea potato,” is a species of tunicate inhabiting the deep coastal waters of North America. Secured to the bottom of the sea by means of a long stalk (20–30 cm), the sea potato filters seawater for nutrients through two siphons. Tunicates are classified as chordates and are considered to be closely related to the immediate precursors of vertebrate forms (Wilson, 1973).

The heart of the sea potato consists of a straight valveless tube (diameter 3 mm, length 5 cm) which runs nearly the length of the animal. It lies suspended by a raphe in another fluid-filled tube, the pericardium. A single blood vessel exists from each end of the heart, to the stomach and pharynx, respectively, eventually anastomosing in a closed microcirculation. Blood is pumped through the circulatory system by means of peristaltic contractions (12–48/min) originat-
ing at one end of the heart. The direction of peristalsis and the blood flow reverses periodically.

A number of microscope studies on other species of tunicates have shown that the hearts of these animals consist of a single layer of myoepithelial cells (Lorber and Rayns, 1972; Jones, 1971; Millar, 1953). Electron microscope examination of the heart has demonstrated the presence of tight junctions between adjacent cells which create a diffusion barrier between the luminal and extraluminal aspects of the heart for specific cations such as lanthanum (Lorber and Rayns, 1972). Recent electrophysiological evidence (Kriebel, 1973; Weiss and Morad, 1974) suggests that the electrical properties of the luminal and extraluminal surface membranes are different. For instance, in several species only the luminal membrane appears to be capable of generating an action potential.

In the experiments reported here, the structure and physiology of the heart of a large tunicate, the sea potato, were investigated. The heart of the sea potato is a single cell-layered myoepithelium. Each cell contains a single myofibril located near the luminal surface. Only the luminal membrane is capable of generating an action potential. The experimental results suggest that only the luminal membrane is involved in excitation-contraction coupling. A preliminary report of this study has been presented (Weiss and Morad, 1974).

**METHODS**

Sea potatoes were obtained at depths of approximately 300 feet in coastal waters of Maine and were maintained in running seawater tanks (temperature, 14°C). Specimens averaged 7-12 cm in body length and 3-5 cm in diameter (Fig. 1 A). To remove the heart, the thick outer tunic of the sea potato was peeled off to expose a thin translucent membrane which encloses the viscera (Fig. 1 B). With the “inner tunic” intact, the beating heart was easily visualized close to the surface near the midline of the animal and adjacent to the prominent gonads. Parallel incisions were made through the inner tunic alongside the pericardial tube (premature puncturing of the inner tunic was unrewarding). Special care was again taken to avoid puncturing the fluid-filled pericardial tube during this procedure. A 2-3-cm section of the pericardial tube containing the heart was then isolated between two ligatures and removed from the animal (Fig. 1 C). The pericardium was opened and was partially removed. The preparation now consisted of the heart and two pericardial flaps joined at the raphe (Fig. 1 D). This tubular preparation either was directly cannulated or was opened into a rectangular sheet by a longitudinal cut through the raphe.

**Cannulated Preparation**

The tubular heart was cannulated at both ends, permitting rapid independent perfusion of luminal and extraluminal surfaces of the heart. The presence of gross leaks between the two surfaces of the heart was detected by perfusion of methylene blue dye through the lumen of the heart.

To stimulate the heart uniformly, an Ag/AgCl electrode was passed along the length of the preparation through the tip of one cannula. Another Ag/AgCl electrode was placed in the bath parallel to the electrode in the lumen of the heart. Stimulus current was measured as the voltage drop across a 10 kΩ resistor placed in series with the Ag/AgCl electrodes.

An isometric tension transducer (Endevco 8102, Endevco, Becton, Dickinson & Co., San Juan Capistrano, Calif.) was hooked onto a pericardial flap near one cannula to
record contractile activity. Twitch tension was maximized by adjusting the position of the perfusing cannulae, the stretch of the raphe by the transducer wire, and the perfusion pressure. Contractions of 50–1,000 mg were generally recorded. Most of the tension appeared to be generated by a twisting motion of the myocardial tube about its longitudinal axis.

Tension, current, and membrane potential were recorded on a Gould Brush 220 (Gould, Inc., Instrument Systems Div., Cleveland, Ohio) and a Tektronix (Beaverton, Ore.) (5113) storage oscilloscope. Intracellular potential was measured by impaling individual myoepithelial cells with a 3 M KCl-filled microelectrode (tip resistance 10–20 MΩ). In the cannulated preparation it was difficult to maintain a continuous impalement during contraction.

**Sheet Preparation**

The heart was cut along the raphe to form a flat sheet of tissue, approximately 5 × 10 mm. The sheet of myocardial tissue was then positioned over a 2 × 3 mm aperture connecting the two compartments of a lucite chamber (Fig. 2) and was held in place.

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**Figure 1.** A, Photograph of a typical sea potato demonstrating the stalk and two siphons. B, After peeling back the thick outer tunic (OT), the tubular heart (H) inside the pericardium (P) becomes visible beneath the taut inner tunic (IT) located adjacent to the prominent gonads (G). Single muscle strands (M) can be seen coursing through the inner tunic. C, Incisions are made through the inner tunic along the outlines of the pericardial tube, and the heart inside the pericardium is removed after being ligated at both ends. D, The bulk of the pericardium is removed. The preparation now consists of the tubular heart and two pericardial flaps joined together by the raphe (R) (see cross-section detail).
between two thin ridges of silicone grease by a plastic clamp. The edges of the preparation outside the clamp were also covered with silicone grease in order to prevent current from leaking underneath the clamp. Both the upper and lower compartments of the chamber could be perfused independently and their contents rapidly exchanged. Two Ag/AgCl electrodes were placed in each compartment. One set of electrodes was used to pass current, and the other set to measure the voltage difference between the two compartments. When a piece of Parafilm was mounted over the aperture in place of the heart, the resistance between the two compartments was greater than 100 MΩ. To measure intracellular potential in the "sheet" preparation, the heart was supported over the aperture by a rectangular patch of sheer nylon stocking. This arrangement immobilized the heart sufficiently so that intracellular potential could be monitored during contraction. Microelectrode impalements were significantly more successful through the extraluminal membrane than the luminal membrane.

**Solutions**

The standard perfusate consisted of a solution containing 365 mM NaCl, 10 mM KCl, 10 mM CaCl₂, and either 2 mM or 10 mM MgCl₂, pH 5.5. The choice of the standard solution was based largely on the ionic contents found normally in the pericardial fluid and blood of the sea potato. Smaller concentrations of Mg²⁺ than those directly measured in the blood (see Results) were routinely used since it was found that the contractile response was diminished in the presence of such high Mg²⁺ concentrations. For measurement of tension in the cannulated preparation the standard Mg²⁺ concentration chosen

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**Figure 2.** The sheet preparation. The tubular heart is cut along the raphe to form a sheet which is then positioned over the aperture connecting the two compartments of a lucite chamber. The heart (h) is held in place between two ridges of silicon grease (v) by a plastic ring (r). Both upper and lower compartments can be perfused independently (U₁, U₂ and L₁, L₂, respectively). Current is passed between two Ag/AgCl electrodes (S₁ and S₂) and potential difference measured across an independent set of Ag/AgCl electrodes (R₁ and R₂). Intracellular potential is recorded with a 3 M KCl-filled glass microelectrode. **RC** = recorder; **OS** = oscilloscope; **GC** = ground clamp; **CF** = cathode follower.
was 2 mM. For monitoring electrical activity in the sheet preparation, 10 mM Mg++ was used. In some experiments Ca++ and Mg++ concentrations were varied in the perfusing solution, with no correction for tonicity changes. High KCl solutions were made by replacing sodium with potassium mole for mole.

Microscopy

Sea potato hearts were dissected from the animal and placed whole in a fixative solution of 4% glutaraldehyde, 0.2 M phosphate buffer, and sucrose made up to an osmolarity of 900 osmol. The hearts were cut into 2-mm length sections and fixed for 2 h at 0°C. The tissue was postfixed in 1% OsO₄ for 30 min and then dehydrated with a graded water-alcohol mixture and embedded in Epon. Sections were cut at 800 Å and stained with lead citrate and uranyl acetate. The sections were examined in a JEOL electron microscope. Thick sections were also examined under a light microscope.

RESULTS

The heart of the sea potato within the pericardial sac was tied at each end, trapping a small volume of blood and pericardial fluid. Na⁺ and K⁺ concentrations were measured by flame photometry (Instrumentation Laboratory Inc., Lexington, Mass., model 343). Cl⁻ was determined by coulometric titration (Buchler-Cotlove chloridometer, Buchler Instruments Div., Searle Analytic, Inc., Fort Lee, N. J.) and Ca++ and Mg++ concentrations by atomic absorption spectrophotometry (model 107, Perkin-Elmer Corp., Norwalk, Conn.). The ionic content of blood from two hearts in millimoles per liter averaged 380 ± 5 SD Na⁺; 8.8 ± 0.1 SD K⁺; 7.4 ± 0.7 SD Ca⁺⁺; 33 ± 1 SD Mg⁺⁺; and 463 ± 9 SD Cl⁻. The ionic content of pericardial fluid from 10 hearts in millimoles per liter averaged 410 ± 9 SD Na⁺; 12 ± 2 SD K⁺; 9 ± 1 SD Ca⁺⁺; 45 ± 5 SD Mg⁺⁺; 486 ± 26 SD Cl⁻. The wet weights of the hearts averaged 10–15 mg. The heart survived in vitro significantly better at lower temperatures (15°C) than at room temperature.

Structure of Sea Potato Heart

Light microscope examination of the sectioned material revealed that the heart of the sea potato consists of a single layer of tightly packed myoepithelial cells fused together at the raphe by a thin layer of connective tissue. The myoepithelial cells were uniform in appearance throughout the myocardium. The cell borders on the extraluminal surface were oval shaped and measured approximately 8 × 10 μm. On the luminal surface, the cells appeared spindle shaped and measured 2 × 60 μm. Striated myofibrils were clearly visible over the luminal cell surface. No evidence for an "undifferentiated line" of cells opposite the raphe was found (Millar, 1953).

Electron microscope examination of the tissue indicated that the myoepithelium ranged from 20 to 30 μm in thickness. Fig. 3 A shows a cross section of heart wall with most cells extending from the luminal to the extraluminal side. Because of the spindle shape of the myoepithelial cells not every cell extends across the heart wall in every thin section. The luminal surface of the myoepithelial cells was covered by a 1-2-μm thick layer of densely staining material, whereas the extraluminal surface was comparatively smooth and devoid of any ground substance (Fig. 3 A). Adjacent myoepithelial cells were interconnected by
specialized junctions located near the extraluminal surface (Fig. 3 A, arrows, and Fig. 3 B, arrows). By using the location of these junctions to demarcate the luminal from the extraluminal membrane of the heart, the ratio of luminal to extraluminal membrane surface area in the sea potato myocardium was estimated to be about 12:1. In this estimation the luminal membrane area includes the surface of the cell in contact with adjacent cells.

Each myoepithelial cell contained a single myofibril measuring approximately 2 μm × 4 μm × 60 μm, located near the luminal membrane. Since no attempt was made to stretch the heart tissue during fixation, sarcomeres generally appeared contracted. Intracellular membranous vesicles were present throughout the cell. Mitochondria were mostly located adjacent to the myofibril and throughout the central portion of the cell. Nuclei were usually located near the extraluminal surface.
PASSIVE PROPERTIES In both the cannulated and sheet preparations, no significant resting potential difference across the heart wall was recorded. A small potential difference of 3 mV developed when either the luminal or extraluminal surface of the heart was exposed to an isotonic solution containing 100 mM KCl.

Resting potentials of −50 to −70 mV were obtained by impaling individual myoepithelial cells with KCl-filled glass microelectrodes. The membrane time constant, determined by recording the decay of intracellular potential back to the resting potential after 5- or 10-ms current pulses passing across the heart, averaged 30 ms in 12 preparations.

The transwall time constant was also measured by passing a 5- or 10-ms current pulse across one set of Ag/AgCl electrodes and observing the decay of potential across another set of extracellular electrodes after the cessation of current. The transwall time constant was much shorter than the membrane time constant and averaged 2–3 ms in three preparations. In the sheet preparation the transwall resistance was measured and the transwall resistivity calculated after the area of the myocardial sheet had been estimated. The resistivity ranged from 50 to 100 Ω/cm². In the cannulated preparation the resistivity of the myocardial sheet generally was higher (100–200 Ω/cm²; Weiss and Morad, 1974).

In order to determine if this low transwall resistivity was due to injury of the preparation, an attempt was made to map the spatial current distribution during a 5-ms transwall current pulse. The local electrical field over the myocardial sheet was measured with two small potential sensing electrodes (Frömter, 1972). No regions of high current density which would indicate localized injury were identified in these experiments. However, these measurements were difficult to obtain reproducibly and areas of microinjury may have remained undetected.

ACTIVE ELECTRICAL PROPERTIES Upon stimulation of the heart, a small (less than 5 mV) transwall action potential, positive with respect to the extraluminal fluid regardless of the direction of the stimulating current, was recorded. Intracellular potential was measured with respect to the luminal bath. When the direction of the stimulating current (Iₐ) was from extraluminal to luminal surface, an all-or-none action potential (Vₐ) was recorded (Fig. 4). The action potentials typically attained an amplitude of 60–80 mV, a duration of 150–250 ms, and an overshoot of 5–15 mV. The action potential often had a characteristic plateau, but sometimes repolarization was rapid without a plateau region. The current passed in the direction from the extraluminal to the luminal surface should depolarize the luminal membrane and hyperpolarize the extraluminal membrane. When current was passed in the opposite direction, i.e. to hyperpolarize the luminal membrane and to depolarize the extraluminal membrane, the upstroke of the action potential occurred upon the break of the stimulus (Fig. 4, V₈). Even with long stimuli (up to 200 ms), action potentials never developed until after the current pulse had been terminated. In contrast, long current pulses depolarizing the luminal membrane always produced action potentials during the time course of the stimulus. This observation suggests that depolar-
zation of luminal membrane generates an action potential, whereas the depolarization of extraluminal membrane is ineffective. The action potential observed after a stimulus depolarizing the extraluminal membrane results from the anodal break excitation of the hyperpolarized luminal membrane. Furthermore, the extraluminal membrane seems to follow the potential of the luminal membrane since only a small (2.5 mV) net transwall action potential was recorded.

After prolonged perfusion of the sheet preparation, slowly decaying electrotonic depolarizations (lasting up to 400 ms) were often observed in response to 5-ms subthreshold stimuli (Fig. 5). These "potential tails" were generally nonexponential and could not be attributed to the 30-ms time constant of the cell membrane. With sufficiently large stimuli, normal action potentials could still be elicited (Fig. 5). These slowly decaying potential tails may represent decrementally conducted action potentials generated in another segment of the preparation.

![Figure 4](image)

**Figure 4.** Comparison of an action potential (VA) generated by a current pulse (IA) passed in the direction from extraluminal to luminal surface (depolarizing the luminal membrane and hyperpolarizing the extraluminal membrane), and an action potential (Vn) after a current pulse (In) passed in the opposite direction (hyperpolarizing the luminal membrane). Note that the action potential VA occurs immediately after the stimulus, whereas the action potential Vn does not occur until well after the stimulus (In) has been terminated. During the period of delay the luminal membrane slowly depolarizes before finally breaking into an action potential.

**Excitation-Contraction Coupling**

**EFFECT OF STIMULUS CURRENT ON CONTRACTION** Fig. 6 shows the contractile response of the cannulated preparation to two suprathreshold stimuli of opposite polarities. For a short pulse which depolarizes the luminal membrane (plotted upward) contraction occurs immediately. For pulses of opposite polarity (which hyperpolarize the luminal membrane) tension develops only after termination of the pulse. These observations are consistent with intracellular electrical measurements, showing that only the luminal membrane generates action potentials and triggers contraction (Fig. 4).

The electrical response of an individual myoepithelial cell (impaled by microelectrode) to current depolarizing or hyperpolarizing the luminal membrane is
all-or-none in character. The contractile response measured in the cannulated preparation was often graded with respect to changes in the magnitude or duration of stimuli of either polarity. In several of the freshly mounted sheet preparations, visually monitored contractions appeared to be all-or-none. The contractile response, however, became graded in all preparations during the time course of the experiment. The graded nature of contraction may partly be due to impairment of electrical conduction after prolonged perfusion.

In the cannulated preparation the magnitude of steady-state contraction depends on the frequency of stimulation. Fig. 7A shows a typical interval-strength relation. Contraction increased in strength up to frequencies of about 10/min. Higher stimulus frequencies resulted in less than maximal tension. The cannulated sea potato heart also demonstrated a small but definite potentiation in contraction after resumption of stimulation of a resting preparation (Bowditch, 1871).

**K-induced contractures** When the luminal surface of the cannulated heart was exposed to isotonic solutions containing high concentrations of potassium (60–200 mM K$^+$ replacing Na$^+$), the myoepithelial cells depolarized and a state of maintained tension (contracture) developed (Fig. 8, upper trace). During the contracture the heart was electrically and mechanically inexorable in response to current passed in either direction. Upon removal of the high K$^+$ solutions, the myoepithelial cells repolarized to their resting potential and...
contractility resumed. Twitch tension did not recover completely to the precontracture level after several exposures to high K⁺ solutions.

Exposure of the extraluminal surface of the myocardium to high concentrations of K⁺ did not alter significantly the resting potential of the myoepithelial cells, and failed to produce contractures or any other alterations in the contractile response to current passed in either direction. Fig. 8 (lower tracing) shows

![Diagram](image)

**Figure 7.** (a) Interval-strength relationship in a typical cannulated preparation. Steady-state twitch tension continued to increase until the rate of stimulation reached 10 beats/min. (b) Demonstration of the Bowditch staircase effect at two different luminal Ca⁺⁺ concentrations (10 mM and 2 mM). At the downward arrow, the stimulus is interrupted. Upon resumption of the stimulus (upward arrow), twitch tension builds up gradually in a staircase fashion before reaching the previous steady-state level. In 10 mM Ca⁺⁺ the effect is considerably more prominent than in 2 mM Ca⁺⁺ (note that twitch tension also falls off markedly in the lower Ca concentration).

the tension recorded from a cannulated preparation in which the extraluminal surface has been exposed to isotonic 100 mM KCl solution for over 10 min without significant change in twitch tension. Increasing the luminal concentration of KCl from 10 mM to 100 mM produced a typical contracture. The decline in magnitude of the steady-state twitch tension between upper and lower traces of Fig. 8 resulted from repeated exposure of the luminal membrane to the high
concentrations of KCl. Thus, KCl depolarizes the myoepithelial cells and causes contraction only when applied to the luminal surface.

EFFECT OF Ca\(^{++}\), Mg\(^{++}\) AND Na\(^{+}\) ON DEVELOPMENT OF TENSION

The cannulated myoepithelium produced little or no tension when Ca\(^{++}\)-free solutions were applied to the luminal surface. Fig. 9A shows that increasing the Ca\(^{++}\) concentration up to 7 mM on the luminal perfusate potentiates twitch tension. Higher calcium concentrations (>20 mM) resulted in development of less than maximal tension. The time course of changes in tension after alteration of luminal calcium concentration is shown in Fig. 9B. Twitch tension declines steadily to a very low level as calcium is washed out from the lumen. The rate of

decline of tension depends solely on the luminal perfusion rate and is unrelated to the frequency of stimulation. Variation in the extraluminal calcium concentration had no effect on development of tension.

In contrast to calcium, increasing the luminal Mg\(^{++}\) concentrations decreased twitch tension markedly. As shown in Fig. 10A, the contractile force was maximum at Mg\(^{++}\) \(\leq 2\) mM (0-160 mM tested). Fig. 10B shows the effect of increasing luminal Mg\(^{++}\) concentration from 2 to 32 mM. Variation in the extraluminal Mg\(^{++}\) concentration had no effect on development of tension. Both Ca\(^{++}\) and Mg\(^{++}\) exerted their effects by modifying the rate of rise of tension and did not affect the time to peak of tension. Lowering of the Na\(^{+}\) concentration to 50% (Na replaced by sucrose) either in the luminal or the extraluminal space did not affect significantly the development of tension.
FIGURE 9. The effect of luminal Ca$^{++}$ concentration on twitch tension in the cannulated preparation. In (a) the Ca$^{++}$ concentration of the luminal perfusate is reduced from 10 mM (the standard) to 0 (first arrow). Tension falls markedly to a very low level, and then quickly returns to its previous level as 10 mM Ca$^{++}$ is readmitted (second arrow). The rate of decline and re-establishment of tension were dependent only on the rate of perfusion of the new solution through the lumen. In (b) the steady-state twitch tension is plotted as a function of the luminal Ca$^{++}$ concentration. Note that twitch tension reaches a maximum at [Ca] = 10 mM, but then falls off at very high Ca$^{++}$ concentrations.

FIGURE 10. The effect of luminal Mg$^{++}$ concentration on twitch tension in the cannulated preparation. In (a) the Mg$^{++}$ concentration of the luminal perfusate is increased from 2 mM (the standard) to 32 mM (first arrow). Twitch tension is depressed until the Mg$^{++}$ concentration is again reduced to 2 mM (second arrow). The rate of decline and re-establishment of tension are dependent only on the rate of perfusion of the new solution through the lumen. Twitch tension is maximum at [Mg] < 2 mM.
DISCUSSION

The sea potato heart is a tubular single cell-layered myocardium with the "functionally" excitable membrane located on the luminal surface. Excitation-contraction coupling occurs exclusively at the luminal surface of the cell, where the single myofibril is located. Specialized junctions (Fig. 4) encircling the myoepithelial cell near the extraluminal surface were assumed to be the border between the luminal and extraluminal membrane. The estimated ratio of the surface area of the luminal to extraluminal membranes defined in this way was 12:1. The justification for including the area of the membrane between adjacent myoepithelial cells as a part of the luminal membrane surface area was based partly on the observation that in other species of tunicates the specialized junctions form a diffusion barrier for La^{3+} (Lorber and Rayns, 1972).

Several structural and physiological observations suggest that the luminal and extraluminal membranes have different properties. Unlike the luminal membrane, the extraluminal membrane is devoid of a covering ground substance. Depolarization of the luminal membrane immediately elicits an action potential and contraction, whereas the depolarization of the extraluminal membrane does not generate an action potential or contraction.

Application of high K^+ solutions to the luminal membrane depolarizes the myocardium and produces contractures, while at the extraluminal surface high K^+ solutions do not significantly alter the electromechanical activity of the heart. Similarly, variation of Ca^{2+} or Mg^{2+} concentrations is ineffective on the extraluminal membrane but has a marked effect on the contractile response of the myoepithelium when applied to the luminal surface. These observations uniformly support the assertion that the luminal membrane is the exclusive site of excitation and electromechanical coupling.

Similar electrical differences between the luminal and extraluminal membranes in another species of tunicate have been observed (Kriebel, 1973). In Chelysoma productum depolarization of only the luminal membrane produces an action potential. However, no anodal break excitation or contractions were reported. This discrepancy from our observations may have been due to the higher threshold for anodal break excitation than for direct depolarization. In Chelysoma productum elevation of K^+ in either the luminal or the extraluminal perfusate depolarized the myoepithelium and produced a transwall potential difference of 8-15 mV. However, visually monitored contractures were observed only when high K^+ solutions were applied to the luminal surface (Kriebel, 1973) as in the sea potato (see Fig. 8). If high K^+ solution applied extraluminally completely depolarized the extraluminal membrane and the transwall potential was 8-15 mV, then the luminal membrane potential could not be more negative than -15 mV. Under these conditions it is unclear why contraction was not observed and the preparation remained excitable, since the measured threshold for excitation was -50 mV (Kriebel, 1973). In the sea potato this problem does not arise because the extraluminally applied high K^+ solutions neither depolarize the cells nor initiate contractures. Thus, the available evidence indicates that only the luminal membrane supports excitation.

Although no action potentials were observed after depolarizing stimuli of the extraluminal membrane, these findings do not definitely rule out a regenerative
depolarizing system in the extraluminal membrane. Since the transwall resistance in this tissue is quite low (50-100 \( \Omega/\text{cm}^2 \)), the transwall potential is effectively short-circuited under all experimental conditions. The potential generated by the larger surface area of the luminal membrane may voltage clamp the potential of the extraluminal membrane through the paracellular shunt (see Fig. 11). It is, therefore, conceivable that any existing regenerative activity in the extraluminal membrane is masked by the shunting capability of the luminal membrane.

**Figure 11.** An equivalent electrical circuit for the sea potato. DC analysis of circuit component of tunicate cells shows that the above schema is consistent with the experimental observations described above. \( R_1 \) (membrane resistance), \( E_1 \) (membrane potential), and \( C_1 \) (membrane capacitance) represent electrical components of the extraluminal membrane. \( R_2, E_2, \) and \( C_2 \) represent the components of the luminal membrane. \( E_1 = E_2 \cdot C_2 \) was assumed to be equal to 12C on the basis of the surface area measurement of the luminal and extraluminal membrane from electron microscopy. \( R_s \) is shunt resistance across the tunicate heart.

**Excitation-Contraction Coupling in Sea Potato Myoepithelium**

The sea potato heart has a number of physiological properties in common with the vertebrate myocardium. A small but definite rate staircase effect is present. Calcium increases and magnesium decreases twitch tension, when applied to the luminal surface of the heart. Luminal KCl concentrations greater than 30 mM depolarize the myoepithelial cells and produce contractures. Accurate voltage tension relations could not be obtained with KCl depolarization since repeated exposure to high KCl concentrations seemed to damage the preparation. The action potential of the sea potato heart is similar to the vertebrate cardiac action potential. Electrical activity is all-or-none in character with a sharply defined threshold for excitation.
Several physiological differences between the sea potato and the vertebrate heart were also found. Sea potato heart did not appear to be mechanically sensitive to lowered Na concentration or application of epinephrine or acetylcholine. Another property of the sea potato heart not shared with vertebrate myocardium is the graded nature of contractile activity in cannulated preparations. The smooth gradation in the force of contraction which is observed as the stimulus strength is increased cannot be attributed directly to changes in the action potential. However, graded contraction may be an artifact due to impaired conduction in these preparations, since in several freshly mounted sheet preparations the contractions appear to be all-or-none. It is conceivable that injured regions of myocardium contribute to twitch tension in a graded fashion as they are electrotonically depolarized progressively with increased stimulus strength (Fig. 6). Myoepithelial cells producing normal action potentials may generate tension on an all-or-none basis.

An Electrical Model for the Sea Potato Heart

A simplified equivalent circuit for the sea potato myocardium consists of the series combination of the luminal and extraluminal membrane resistances and batteries (opposing each other) all in parallel with an extracellular shunt resistance (Fig. 11). The sum of the membrane resistances in this equivalent circuit is considerably greater than the shunt resistance since the intracellular action potential (70–80 mV) is attenuated by a factor of 30 to produce the transwall action potential (2.5 mV). Thus, the shunt resistivity is almost equal to the total transmyoepithelial resistivity (50–100 Ω/cm²). The sum of the membrane resistivities ranges between 1.5 and 3 k Ω/cm². The surface area of the luminal membrane, however, is approximately 12 times larger than that of the extraluminal membrane (if one assumes that the border between the two membranes is the specialized junction near the extraluminal surface). If the resistivities of the luminal and extraluminal membranes are approximately equal, then the luminal membrane contributes only 8–9% to the total membrane resistivity.

Morphologically, the shunt resistance could occur either through paracellular channels or at discrete sites of inadvertently created microinjury. Attempts to identify sites of microinjury by measuring the current density over the surface of the myoepithelium with a roving microelectrode showed that no significant loci of injury were present. Thus, it is likely that shunt resistance is paracellular and the sea potato myoepithelium may be classified as "leaky" epithelium.

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REFERENCES

BOWDITCH, H. P. 1871. Über die Eigentümlichkeiten der Reizbarkeit, welche die Muskelfasern Herzens zeigen. Ber. Verh. Saechs. Ges. Akad. Wiss. Leipzig. 652–689.

FRÖMTER, E. 1972. The route of passive ion movement through the epithelium of Necturus gallbladder. J. Membr. Biol. 8:259–301.
JONES, J. C. 1971. On the heart of the orange tunicate, *Ecteinascidia turbinata* herdman. *Biol. Bull. (Woods Hole).* 141:130.

KRIEBEL, M. E. 1973. Action potentials occur only on the lumen surface of tunicate myocardial cells. *Comp. Biochem. Physiol.* 46A:463-468.

LORBER, V., and D. RAYNS. 1972. Cellular junctions in the tunicate heart. *J. Cell Sci.* 10:211.

MILLAR, T. 1953. Ciona Liverpool Marine Biological Committee Memoirs on Typical British Marine Plants and Animals. No. 4.1–123.

WEISS, J., and M. MORAD. 1974. Single-cell layered heart: electromechanical properties of the heart of *Boltenia ovifera*. *Science (Wash. D. C.)*. 186:750–752.

WILSON, E. L. 1973. Life on Earth. Sinaver, Stamford, Conn. 752.