The average time elapsed between phase I and II was 24.5 minutes and between II and III, eight minutes at an initial perfusion rate of 14/nl/min. Four samples of perfusion fluid from the dye-diluted sections of the coiled segments were collected. The average inulin C^{14} concentration was 54 percent of the perfusate concentration indicating a dilution between the two puncture sites. Because of possible contamination with interstitial fluid this figure is only suggestive. A more direct assessment of the amount of dilution is necessary.

The relationship between passage time and half-time of reabsorption determines the fractional fluid reabsorption in the segment under consideration. Deetjen *et. al.* (Bull. MDIBL 10:5-7, 1970) found a passage time through segments III-VI (which correspond to phase I and II in our study) of 17 minutes. With a perfusion rate of 14 nl/min we have found a passage time of 24.5 minutes. This figure in combination with the half-time value of 8.3 minutes indicate a reabsorption of 75-87.5 percent of the filtered fluid. Micropuncture studies of Stolte *et. al.* (Bull. MDIBL 11:91-93) have shown that in segments III and V the tubular fluid osmolarity is equal to the plasma value. This leads to the conclusion that urea permeability is high and allows a rapid transport rate in comparison with the passage time of tubular fluid. Fractional urea reabsorption may therefore be of the same order of magnitude as fluid reabsorption and is in good agreement with the urea excretion rates found in clearance studies. The observed countercurrent concentration-dilution system (Figure 2) may be the site of net fluid secretion into the tubular fluid. Another possibility is that the concentration of the dye is reduced by a passive inward movement of water due to high water permeability in this part of the coiled segment. There is at present no experimental evidence favoring one or the other of these two possibilities. The localization of the 'diluting' segment near the end of the nephron is in good agreement with a hypothetical net fluid secretion into tubular urine and could account for a final U/P urea <1 without active transport of this substance. Supported by NIH grant 5R01-AM-14424. Dr. von Baeyer is a Fellow of the National Kidney Foundation and of the Henry C. and Bertha H. Buswell Endowment.

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ELECTROMECHANICAL STUDIES IN THE SINGLE CELL LAYERED HEART OF *Boltenia ovifera*

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The heart of the tunicate *Boltenia ovifera* consists of a straight one-cell layer thick tube which propels blood through the circulatory system by means of peristaltic contractions. In the MDIBL Bulletin of 1972 we reported on some of the contractile properties of this singular myocardium. In this report we are concerned with electrical as well as mechanical characteristics of this heart.

The excised heart was cannulated at both ends (see Figure 1). The cannulae were in turn fixed to two micromanipulators adjusted to hold the heart at constant length in a tissue bath. Through the cannulae the luminal surface of the heart could be perfused with different solutions in quick succession at constant pressure. The extraluminal surface of the heart was also perfused with different solutions through an arrangement of holes in the side of the chamber. The standard intra- and

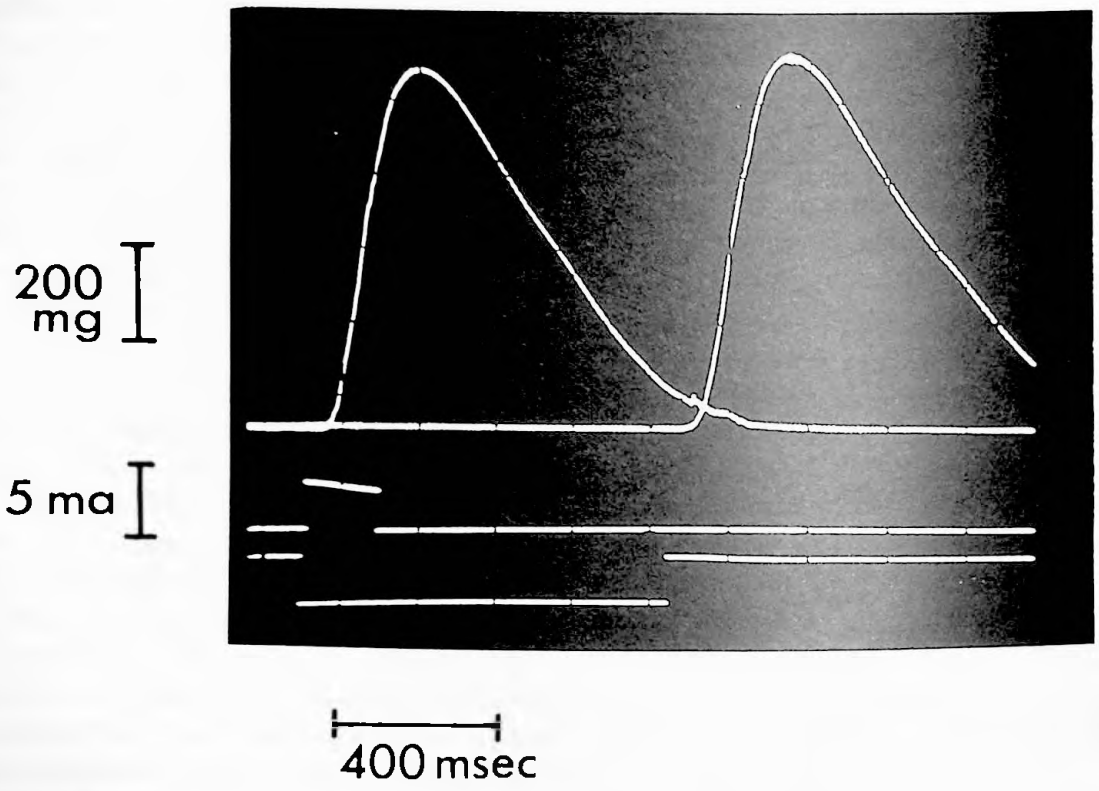

Figure 1: The experimental setup for tension measurement. A. the heart. B. pericardial fragments and the raphe. C. the inflow cannula. D. the outflow cannula. E. stimulating Ag/AgCl electrodes. F. the transducer wire. Insert: a typical example of a tension record.

extraluminal perfusate consisted of a solution containing 365 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$ and 2 mM $MgCl_2$. Stimulating Ag/AgCl electrodes were passed along the entire length of the heart within the lumen and along the heart in the extraluminal compartment so that upon electrical stimulation the whole heart contracted uniformly. When tension was measured an isometric tension transducer was attached to one end of the heart. A glass microelectrode filled with three M KCl was used to measure the transmembrane potential.

A number of results obtained during the past summer (1972, MDIBL Bulletin) were reconfirmed. Increasing the intraluminal concentrations of Mg^{+2} suppressed tension, while increasing $[Ca^{+2}]$ potentiated tension. More careful experiments testing the effects of these ions on the extraluminal surface of the myocardium showed that they have little or no effect on development of tension in this preparation. Application of high concentrations of KC1 (150-300 mM) intraluminally resulted in development of contracture which subsequently relaxed despite the continued presence of the high concentrations of KCl. Extraluminal variations of KCl had little or no effect on development of twitch or contracture tension. The tunicate heart responds asymetrically to the direction of stimulating current. When current was passed in such a direction as to depolarize the inner (luminal) membrane of the preparation (and hyperpolarize the outer extraluminal membrane) the threshold for excitation was lower than when current passed was in the opposite direction. Examination of the relation between the onset and the duration of stimulating current and the onset of development of tension demonstrated that depolarization is followed by a contrac-

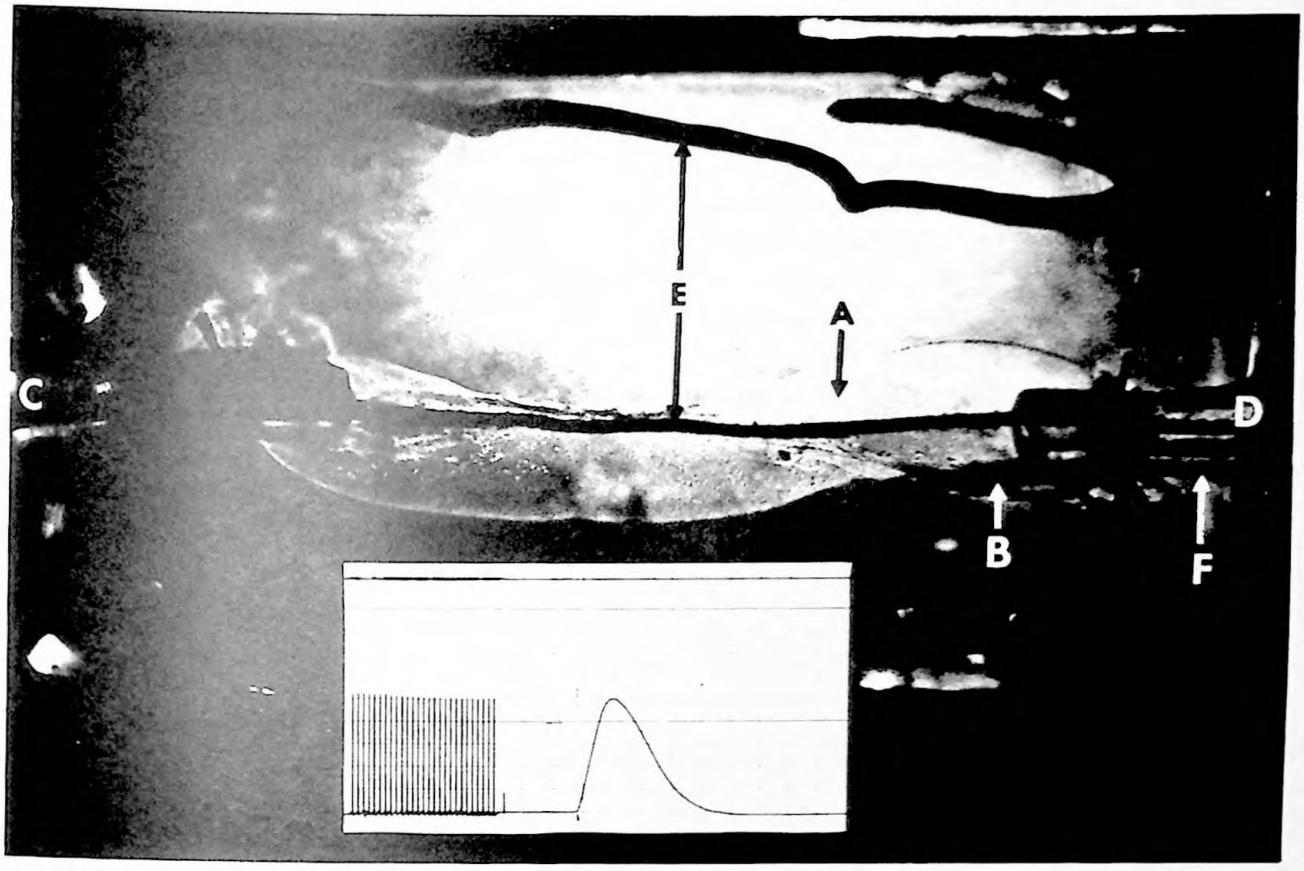

Figure 2. The time course of the onset of tension in response to stimulating current in the direction which depolarizes the extraluminal membrane (upper carrier trace); in comparison with the response to stimulating current in the direction which hyperpolarizes the inner membrane and depolarizes the outer membrane (lower current trace).

tion with no apparent delay but when the stimulating current is such as to hyperpolarize the inner membrane, the onset of contraction coincides with the termination of hyperpolarizing current (Figure 2). This observation suggests that only the inner membrane is coupled to the contractile process and that tension developed following a current pulse which hyperpolarizes the inner membrane and depolarizes the outer membrane occurs via the anodal break excitation of the inner membrane. Tunicate myocardium also shows a graded contractile response to variations in the duration and amplitude of the stimulating current pulse, i.e., the myocardium does not exhibit an "all-or-none" contractile response similar to that observed in the mammalian or amphibian heart.

Transluminal electrical resistance of the preparation was measured by passing a small pulse of current between two Ag/AgCl electrodes placed in the lumen and bath respectively and recording the resulting voltage drop between another set of Ag/AgCl electrodes placed across the lumen. The value of the resistivity obtained by this method was 200 ohm-cm^2. Also it was found that with the standard perfusate intra- and extraluminally no significant potential difference existed across the lumen. In fact when 100 mM KCl was placed either intra- or extraluminally only a very small potential difference (4 mV maximum) developed transluminally. These observations led to the hypothesis

that a shunt pathway was short-circuiting the potential. Morphologically this shunt may correspond to either extracellular channels between the myocardial cells or else to the longitudinal raphe of the heart where the myocardial cells form a seam from which arises the pericardium.

Numerous micropunctures of myocardial cells with a glass microelectrode demonstrated an intracellular resting potential of -65 ± 5 mV. When high concentrations of KCl were applied to the extraluminal surface of the heart, the resting potential was unaffected. With high concentrations of KCl intraluminally however the myocardial cells promptly depolarized. On the basis of these observations we constructed an equivalent circuit for the myocardium. The component of the circuit had the following values: R_m = 1000 ohm-cm^2, R_s = 220 ohm-cm^2, and E_m = -65 mV.

In addition to confirming more accurately our impression that the tunicate myocardium shares most of the major electromechanical properties of mammalian myocardium our experiments have more precisely characterized the nature of the tunicate myocardium. In particular excitation of the inner (luminal) membrane only of this preparation is coupled to the contractile process whereas the outer (extraluminal) membrane is relatively inert. The graded contractile response is unique among the myocardial preparations studied so far. Supported by USPHS HL 13288 - 03 and HL 16152 - 01.

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COMPARISON OF RENAL CLEARANCE OF SEVERAL GLOMERULAR MARKERS IN WINTER FLOUNDER

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The previously reported observation of significant differences between inulin and ^{14}C-polyethyleneglycol (PEG) clearances in southern flounder (Hickman *et. al.* Bull. MDIBL 12:47-50, 1972) and the demonstration of cellular accumulation of ^{3}H-inulin, ^{14}C-inulin, and ^{3}H-PEG but not ^{14}C-PEG in kidney and other tissues of winter flounder (Hickman *et. al.* and Schmidt-Nielsen *et. al.* Bull. MDIBL 12:99-103, 1972) have raised a serious question of whether inulin is a reliable measure of glomerular filtration in renal clearance studies in fish. In the present investigation this problem was further studied by comparing the clearance of four compounds namely non-labelled inulin, ^{3}H-inulin, ^{14}C-PEG, and creatinine in winter flounder.

The flounder were kept in aquaria equipped with running sea water for four to 14 days before experimental use. Proocedures for handling the fish were essentially those of Maack and Kinter (Am. J. Physiol. 216:1034-1043, 1969). Bladder urine was collected for three to four consecutive two-hour periods through a polyethylene catheter (PE 90) inserted into the urinary papilla and tied securely in place. Gentle pressure was applied externally over the bladder at hourly intervals to assure expression of urine not spontaneously voided. Blood samples, 0.4 ml., were obtained by caudal vein puncture at the beginning, midpoint, and end of each experiment. Plasma concentrations at the midpoint of each urine collection period were determined by interpolation. All test compounds were obtained commercially, ^{14}C-PEG (mol. wt. 4000) from New England Nuclear Corp. (Boston), ^{3}H-inulin from Amersham-Searle Corp. (Chicago), the non-labelled inulin, and creatinine