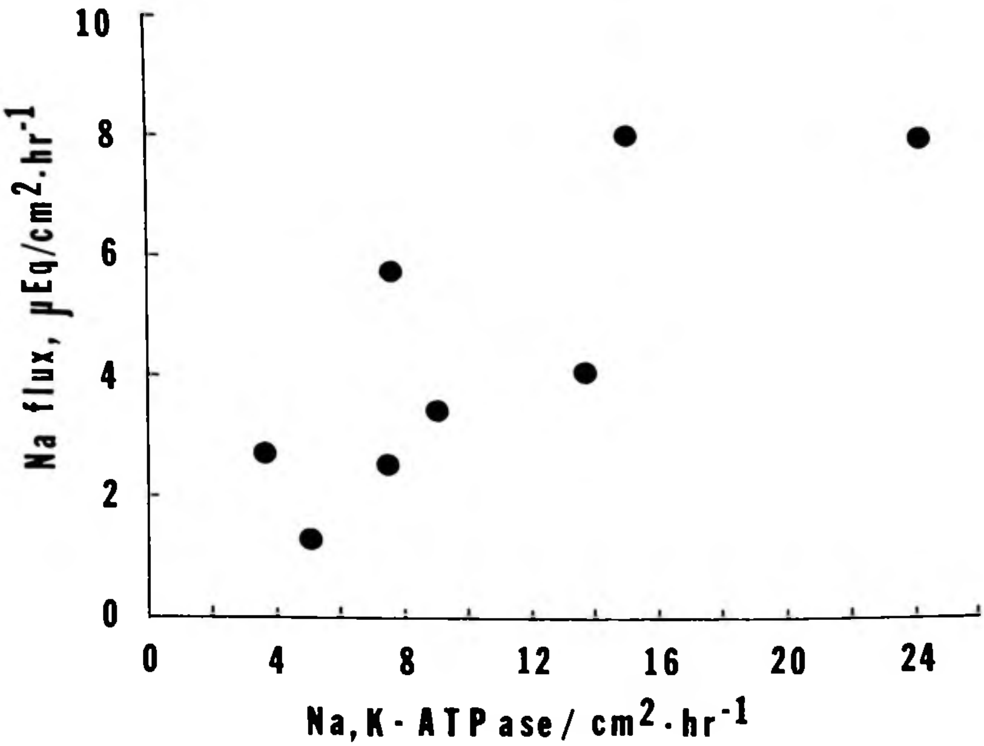

Figure 2. Relationship between Na-K-ATPase activity and unidirectional mucosal to serosal sodium flux in the isolated perfused urinary bladder of the winter flounder. ATPase assays (standard reaction mixture) as described in Figure 1. Sodium flux was determined with identical solutions (modified Forster's saline) bathing serosa and mucosa under open circuited conditions. Units of Na-K-ATPase activity are µM P_i liberated/$cm^2 \cdot hr^{-1}$.

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VOLTAGE CLAMP STUDIES IN VENTRICULAR TRABECULAE FROM DOGFISH *Squalus acanthias.*

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The link between excitation of the myocardium and the initiation of contraction has been extensively studied in various mammalian and amphibian species. The general picture emerging from a combination of electrophysiological and electron microscopic studies is that specific subcellular structures can account for specialized functional developments in the myocardium. For instance in the amphibian (frog) heart, which has a relatively simple, subcellular structure with no t-tubules and little sarcoplasmic reticulum, the surface membrane electrical activity (action potential) directly controls the availability of the activator Ca^{+2} and therefore the development and maintenance of tension. On the other hand the mammalian (cat, sheep, dog) heart, which shows an extensive and complex intracellular membrane system (t-tubules and sacroplasmic reticulum), responds indirectly to the surface membrane electrical activity. That is the action potential serves: 1) as a "trigger" which releases intracellularly stored activator Ca $^{+2}$; 2) as a replenisher of Ca^{+2} to the stores; and 3) direct control of transport of Ca^{+2} across the surface membrane. This indirect control of contraction by the action potential in the mammalian myocardium enables this tissue to manifest complex contractile responses such as rate staircase and post-extrasystolic potentiation.

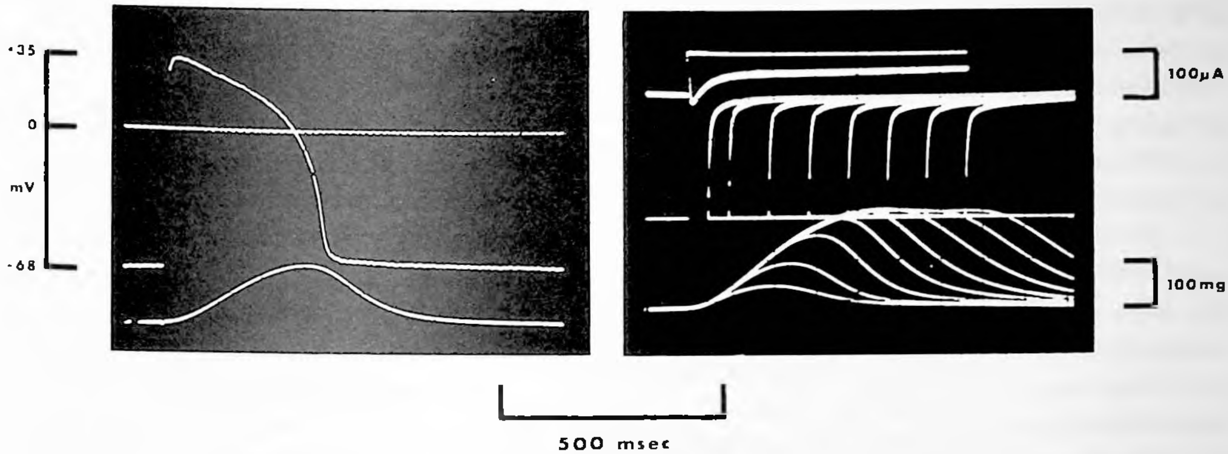

Figure 1. Left panel, action potential and contraction recorded from a dogfish ventricular strip, diameter 0.5 mm, $[Ca^{++}]_o$ 10 mM. Right panel, superimposed voltage clamp pulses of various durations and accompanying contractions and membrane currents. Outward current is plotted in the upward direction. Note that the contraction is maintained for the duration of depolarization.

The results of the structure-function studies apply not only across species boundaries but within a species during development. For a more extensive discussion of the excitation-contraction coupling processes in the heart muscle see Morad and Goldman, Prog. Biophys. Mol. Biol. 27:257-313 (1973). Quantitative data on the relation between action potential and contraction and structure-function information of marine life are lacking. Electromechanical coupling processes in dogfish myocardium were studied using a hybrid sucrose gap voltage clamp method similar to that described by Morad and Orkand (J. Physiol. 219:167-189 (1971). Ventricular trabecular 500 µ in diameter and 3 mm long were drawn through small holes in two latex membranes which separated the preparation into three compartments. The central compartment was bathed with isosmotic sucrose-urea solution which electrically isolated the extracellular spaces of the muscle in the two outer compartments. The left outer compartment contained the physiologic portion of the ventricular strip which was attached to an isometric tension transducer and was bathed with a solution containing in mM /L: NaCl,280; KCl, 8.0; $CaCl_2$, 3.78; $NaHCO_3$, 2.0; urea, 362. Membrane potential was measured with a glass microelectrode (tip diameter 0.1 micron) filled with 3 M KCl. The right outer compartment was perfused with a depolarizing solution of isotonic KCl. This arrangement allowed for a quantitative spatial and temporal control of the surface membrane potential with simultaneous measurement of membrane current and contraction of the shark heart trabeculum.

Measurement of intracellular electrical activity showed a resting potential of -75 mV, action potential overshoot of +35 to +40 mV, and action potential duration of 350-400 msec at room temperature. The rate of rise of the upstroke of the action potential was about 10 V/sec. The action potential was found to be highly sensitive to the presence of sucrose in the central compartment. In fact the preparation showed deterioration signs such as shortening of action potential duration and appearance of graded contractile response within 30 minutes of exposure to the sucrose solution.

Prolongation of the action potential with the voltage clamp prolonged the contraction. The developed tension was maintained for the duration of depolarization. Tension developed at membrane potentials around –40 mV and the voltage tension relationship plateaued around +40 to +60 mV. No post-extrasystolic or post-clamp potentiation was observed. Such a direct relation between the membrane depolarization and development of tension suggests that movements of activator Ca^{+2} are under direct control of the surface membrane. Although in some preparations a phasic component of tension was recorded it could be shown that in these preparations the clamped potential was not spatially homogenous.

These results suggest that the contractile response of the dogfish myocardium is under direct control of surface membrane potential and that release and transport of activator Ca^{+2} occurs across the surface membrane. Although no structural results are as yet available specimens were embedded in collaboration with Dr. Karl Karnaky for electron microscopic examination. Based on the voltage clamp studies we predict that the dogfish myocardium will have no t-tubules and will show limited quantity of sarcoplasmic reticulum.

The voltage clamp experiments also revealed the existence of a slow inward phasic current which seemed to be responsible for the upstroke of the action potential. This current as well as the upstroke of the action potential were found to be insensitive to high concentrations of tetrodotoxin (10^{-5}M). These observations suggest the possibility that the inward current in this preparation may be carried by Ca^{+2}. If this finding is substantiated by future experiments it may reveal a mechanism for the transport of activator calcium across the cell membrane.

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THE CEREBRAL VENTRICULAR SYSTEM OF *Myxine glutinosa*

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Early anatomical reports suggested that the hagfish *Myxine glutinosa* differs from other vertebrates in lacking a well-developed ventricular system and a choroid plexus. As part of an effort to relate the structure of the hagfish brain to the physiology of the cerebrospinal fluid we have examined the ventricular system in a series of adult hagfish.

Most animals were fixed by vascular perfusion with phosphate buffered glutarldehyde-paraformaldehyde fixative for electron microscopy or with 10 percent formalin or Bouin's solution for light microscopy. Serial sections were made from brains processed for light microscopy.

The ventricular system consists of a much-reduced central canal extending from the spinal cord to the midbrain and two diencephalic cavities, the preoptic and the infundibular recesses (Figure 1).