In summary, this comparative approach has been of great value in showing that fish and mammalian (or amphibian) lens have similar Na^+-K^+ transport systems and rates of ion transport) although the fish tissue lacks carbonic anhydrase. The data suggest that the role of lens enzyme is in dissipation of metabolic CO_2.

This work was supported by NIH grants EY-02227 and NRSA EY-05378. We thank the following colleagues for making these measurements: Martin Morad (lens potential); Larry Reinking (urea); Jill Eveloff and David Miller (ATPase); Ness Pessah and Richard Solomon (electrolytes); Robert Rosenthal (computer and graphics).

EXCITATION-CONTRACTION COUPLING IN VENTRICULAR MUSCLE OF DOGFISH (SQUALUS ACANTHIAS)

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Comparative ultrastructural and electrophysiological studies in amphibian and mammalian ventricular muscle suggest that the development of t-tubules and the sarcoplasmic reticulum (SR) parallels the development of internal and releasable Ca^{2+} stores. Thus in the absence of T-system and a rudimentary SR the frog heart contrasts with that of mammalian heart by showing no phasic tension, no post-extrasystolic potentiation, and no post-clamp potentiation. In the experiments to be described below we compare and contrast the structure function characteristics of the dogfish heart (Squalus acanthias). The results provide further support for the hypothesis that t-tubular system and the adjoining SR is required for the development of inotropic characteristics of the heart associated with internal release and re-circulation of the activator Ca^{2+}.

Ultrastructural studies. Exposed trabeculae on the ventricular wall of dogfish heart were fixed in 3% glutaraldehyde buffered with 0.2 M cacodylate with 0.2 mM $CaCl_2$. After 2-3 hours the specimens were rinsed in buffer, and post-fixed for 1 hour in 1% osmium tetroxide and 1.5% potassium ferrocyanide in cacodylate buffer (Karnovsky, Abstracts of Papers of 11th Ann. Mtg. for Cell Biol., 284:146, 1971), then dehydrated in ethanol and propylene oxide and embedded in Epon. Thin sections were stained with uranyl acetate and lead salts and examined in Jeol 100B and 100S electron microscopes.

The ventricular fibers of shark myocardium are fusiform in shape with an average diameter of 4-6 μm at their greatest width (Fig. 1, panels A & B). The cells are tightly packed and connected to each other at their tapering ends by intercalated discs. The extracellular space between fibers appears as a continuous electrondense matrix often crossed by prominent bundles of collagen fibrils. The contractile material is located at the periphery of the cells whereas the nucleus and mitochondria occupy the central core of the fibers. The sarcolemma frequently invaginates into the cytoplasm as small flask shaped inpocketings (caveolae) of uniform size; they all appear to c communicate with the extracellular space through a narrow ostium.

Sarcolemmal invaginations of t-tubules were absent but internal membrane systems resembling SR were frequently observed (Fig. 1, panels B & C). These membraneous tubules seem to be qualitatively less organized than in the mammalian heart. Specialized enlargements of the SR into flattened cisternae were not directly observed but saccules adjacent to the sarcolemma resembling SR cisternae were often observed (panels B & D). These couplings were always present at the level of the Z-lines or at A-I junctions (panel D).. Regularly spaced densities spanning the gap between the cisterns and plasma membrane were not observed. In general the lateral couplings seem to be less specialized as compared to those found in the mammalian ventricle. Free running trabecula 0.3 to 0.6 mm in diameter were isolated from the ventricular wall and mounted in a single sucrose gap setup. The shark Ringers had the following composition in mM: NaCl 280, KCl 6, $CaCl_2$ 5, $MgCl_2$ 3, Na_2So_4 0.5, NaH_2PO_4 1, urea 350, 99% 0_2.

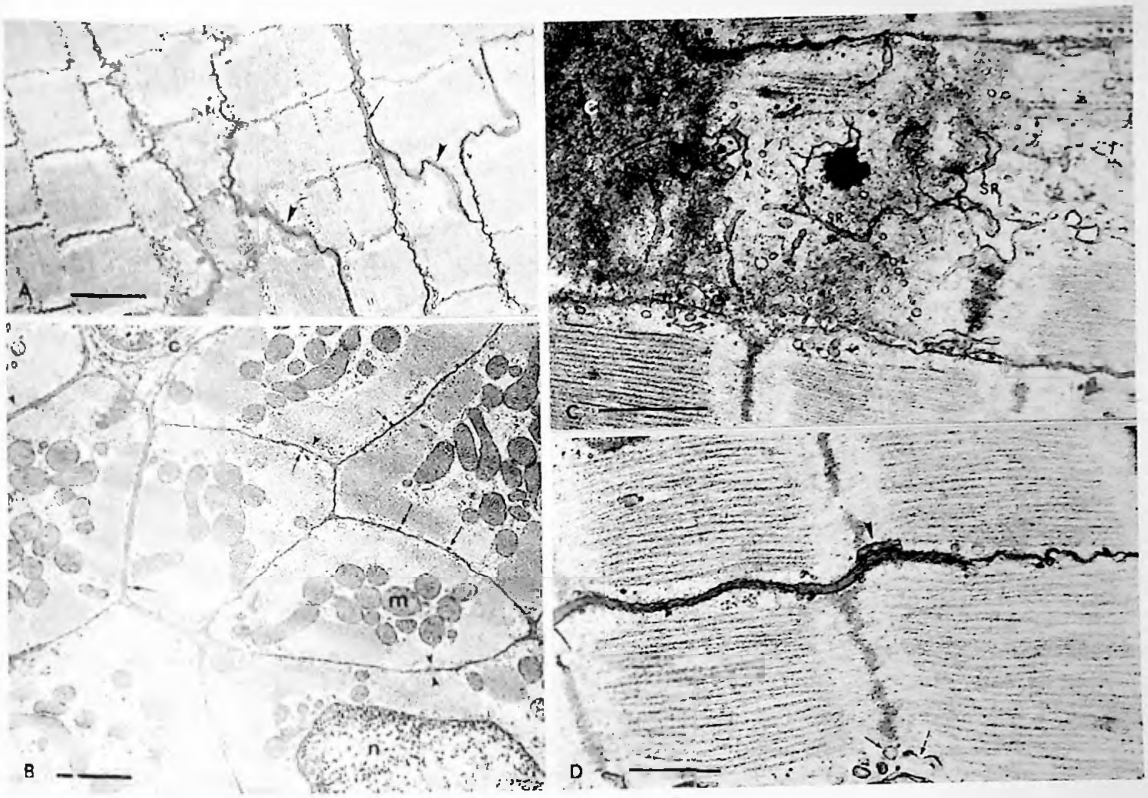

Figure 1. Electron-micrographs of shark heart. Panel A shows a longitudinal section of a bundle of ventricular fibers. The extracellular space surrounding each cell is filled with an electron-dense matrix (arrow). Intercalated discs (arrowhead) join adjacent cells. Calibration bar represents 1 micron. Panel B is a transverse section showing the peripheral location of the contractile material. Enlarged sacs (arrowhead) are seen close to the sarcolemma. Caveolae (arrows) are present. c = collagen; m = mitochondria; n = nucleus; calibration bar = 1 micron. Panel C is a longitudinal section showing the presence of an internal tubular network resembling SR. Arrowheads = caveolae; s = sarcolemma in tangential section; c = collagen fibrils; calibration bar = 1 micron. Panel D shows a magnified longitudinal section of two adjacent cells with an apparent lateral sac (arrowhead) in close association with the sarcolemma. Calibration bar represents 0.5 microns.

Figure 2 shows the dependence of peak tension on the $[Ca]_o$. The inset in Figure 2 shows that dP/dt and peak tension rapidly increased while the time-to-peak decreased when $[Ca]_o$ was changed from 1.0 to 10 mM. The decrease in time-to-peak of tension is thought to result from the shortening effect of high $[Ca]_o$ on the action potential. This observation is consistent with those found in the frog heart.

Several procedures were used to test for the presence of internal and releasable Ca^{2+} stores in the shart heart. For instance, extrasystoles produced by paired pulse stimulation resulted in no post-extrasystolic potentiation (Fig. 3 Similarly, clamping the membrane potential to +5 mV for 3 seconds although producing large tension did not cause post-clamp potentiation of tension (Fig.). The time course of tension during the clamp was monotonic lacking the phasic component seen in the mammalian ventricle. Note large depolarization induced by K^+ - accumulation in the extracellular space quite similar to those found in frog heart (Cleemann & Morad, Science 191:90-92, 1976).

The frequency dependence of contractility was examined in Figure 3 and peak tension was plotted as a function of rate of stimulation. Following a change in the rate of stimulation, tension immediately increased (or decreased) in the first beat and then slowly increased (or decreased) over the next 20 to 100 beats. The dependence of peak tension on the frequency of stimulation is small probably because the $[Ca]_o$ of 5 mMolar is sufficiently high

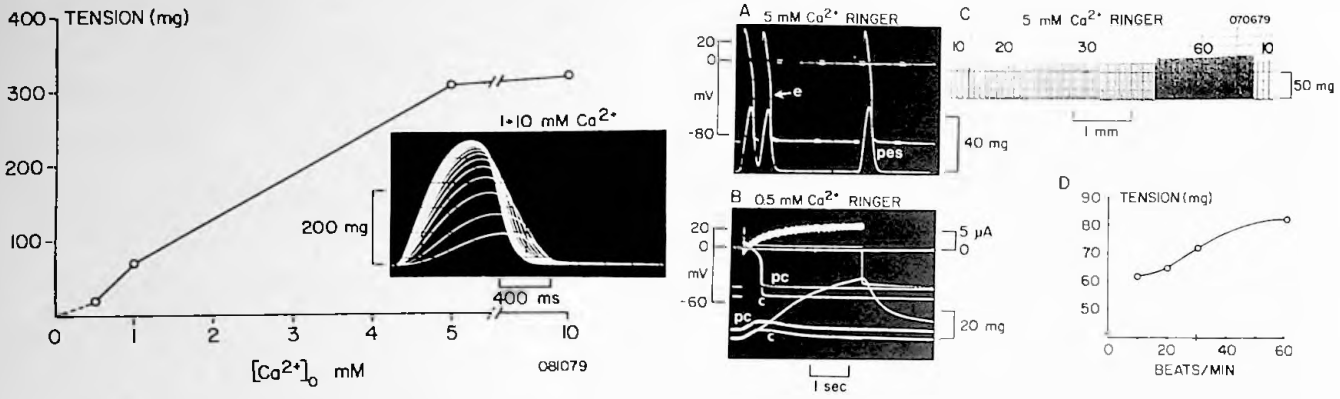

Figure 2. Dependence of contractility on external calcium. Plotted is peak tension as a function of the external calcium concentration. Inset shows continuous recordings of twitch tension during an increase in external calcium from 1.0 to 10 mM.

Figure 3. Panel A demonstrates lack of post-extrasystolic potentiation (PES) in response to an extrasystole, 4. Intracellular recording of membrane potential (upper trace) and tension (lower trace). Normal stimulus interval was 3 sec. Panel B shows an example of a voltage clamp to +5 mV (middle trace). Tension development (lower trace) during the clamp was monotonic and there was no post-clamp potentiation (pc). The resting potential following the clamp (PC) was depolarized due to K^+ accumulation in the extracellular space as a result of the voltage clamp. Current is shown on the upper trace. Panel C shows a record demonstrating the slow rate staircase. Plotted in D is steady state peak tension as a function of rate of stimulation in panel C.

to suppress the rate-staircase phenomenon (compare with Fig. 2). This slow rate staircase is similar to that observed in frog heart, as are all of the other inotropic interventions illustrated in Figure 3. In Figure 4 we examined the process of restitution following a normal contraction. Panel B shows superimposed traces demonstrating that con-

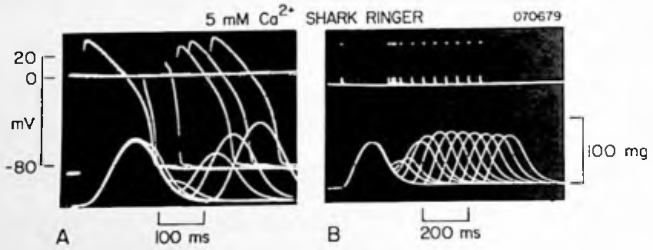

Figure 4. Restitution of contractility in shark heart as a function of the paired pulse interval. Panel A demonstrates that the recovery of the action potential plateau and duration (top traces) follows the restitution of tension (bottom trace) as the paired pulse interval is increased. Panel B shows the restitution of tension on a slower time scale. Top trace shows stimulus pulse for timing.

tractility sharply increased as the stimulus interval was increased from 400 to 600 ms and then slowly decreased back to control values as the stimulus interval was further increased. Panel A demonstrates that the restitution of contractility is dependent on recovery of the action potential plateau in which short stimulus intervals resulted in action potentials with suppressed plateau and of short duration. Stimulus intervals greater than 600 ms resulted in action potentials of normal configuration concurrent with control twitch tensions. These studies show that the contractility of the

dogfish heart is highly sensitive to $[Ca]_o$ and to the duration of the action potential. There appeared to be no internal releasable stores of Ca^{2+} as demonstrated by the lack of post-extrasystolic potentiation, post-clamp potentiation, and the lack of a phasic component of tension during long voltage clamp steps. These findings are consistent with the ultrastructural findings that peripheral couplings and the SR are poorly developed and the t-tubular system is absent. The relation between structure and function in the shark heart is similar to that of the frog heart supporting the hypothesis of Morad & Goldman (Prog. Biophys. & Molec. Biol., 27:257-313, 1973), that in the absence of peripheral couplings and t-tubular system the heart cells rely primarily on the electrical activity of the surface membrane to transport the activator Ca^{2+} from an external source and thus to directly control contraction.

INOTROPIC EFFECTS OF ADRENALINE IN DOGFISH HEART (SQUALUS ACANTHIAS)

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In amphibian and mammalian hearts epinephrine markedly potentiates tension and suppresses contracture tension (Kavaler & Morad, Circ. Res. 18:492-501, 1966; Morad & Rolett, J. Physiol. 224:537-558, 1972). In the dogfish heart catecholamines are thought to have little or no positive inotropic effect. In the experiments to be described below the positive and the relaxant effects of adrenaline on the isolated ventricular trabeculae were studied. Adrenaline was found to have both a positive inotropic and a relaxant effect on the ventricular trabeculae in a manner quite similar to mammalian and amphibian hearts.

Positive inotropic effect at various $[Ca]_o$. Figure 1 shows the effect of adrenaline on a ventricular strip bathed in solutions containing three different Ca^{2+} concentrations. The upper left panel of Figure 1 shows that adrenaline markedly potentiates tension and prolongs the duration of developed tension when added to a strip bathed in 1 mM Ca^{2+} containing shark Ringers in mMole/l (NaCl 280, Kcl 6, $MgCl_2$ 3, Na_2So_4 0.5, NaH_2PO_4 1, urea 350, $NaHCO_3$ 8, glucose 5, at pH 7.4). As the rate of rise of tension increases the peak tension is potentiated and prolonged. Addition

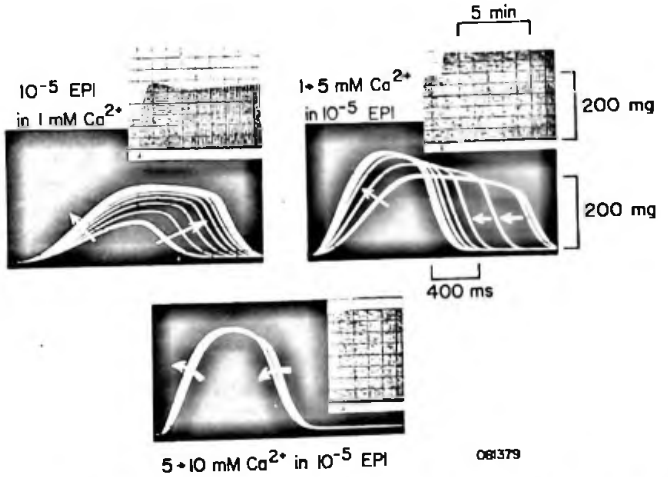

Figure 1. Effect of 10^{-5} g/L of epinephrine on contractility. Each panel shows continuous recording of tension at a fast sweep speed to observe the change in dP/dt (rate of tension development) and the duration of developed tension as indicated by the direction of the arrows. The inset in each panel shows the time course of action on peak tension at a slow chart speed. Addition of epinephrine in 1 mM Ca^{2+} Ringers produced a marked potentiation of twitch tension (left panel) by increasing dP/dt and duration of developed tension. Increasing the Ca^{2+} from 1 mM to 5 mM (right panel) and 5 mM to 10 mM (lower panel) in the presence of epinephrine further potentiated twitch tension by increased dP/dt but the duration of developed tension decreased. This effect was most dramatic when the Ca^{2+} was changed from 1 mM to 5 mM.