impalements of the cells of this epithelium with the microelectrodes available. In contrast, in one study of the rectal gland of the spiny dogfish (*Squalus acanthias*) cellular impalements of slices of tissues gave stable negative intracellular potentials between 90 and 103 mV. (Supported by USPHS AM 16663, National Institute of Arthritis, Metabolism and Digestive Diseases.)

MECHANICS OF THE SINGLE CELL LAYERED HEART OF "SEA POTATO"

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The heart of the "sea potato" is a tubular structure which consists of a single layer of cells. Previous experiments from our lab have shown that laser diffraction methods (Cleemann, Dillon and Morad, MDIBL Bull., 16:8-13, 1976), can be used to measure the sarcomere length from a sheet of myocardial tissue. The sheet is fastened along the perimeter of a rectangular aperture and a servo-control device was used to bulge-up the tissue and keep it length clamped during the time course of contraction. In the experiments described below the measurements of sarcomere dynamics were continued and the contraction response to rapid stretch and release were recorded. These measurements obtained from a small segment of the myocardial wall were then compared to measurements obtained using the intact tubular heart.

Measurement of Sarcomere Dynamics: In 1976 we succeeded in measuring the passive and active sarcomere length-tension relationship in this tissue. In order to follow and servo-control the sarcomere length during the contraction the instrumentation was altered to increase the angular resolution of the light detector. Figure 1 panel A shows a diffraction pattern obtained after such improvements. The width of the central diffraction peak corresponds to a sarcomere length change less than 0.1 μm, suggesting an improvement over previous measurements. As an experiment progressed, it was consistently noticed that the diffraction peaks become broader and less defined as the twitch tension decreased. These observations suggest that the isolated sheeth preparation deteriorates because of cell injury and that the absence of signal was not limited by the resolution of the optical instrumentation.

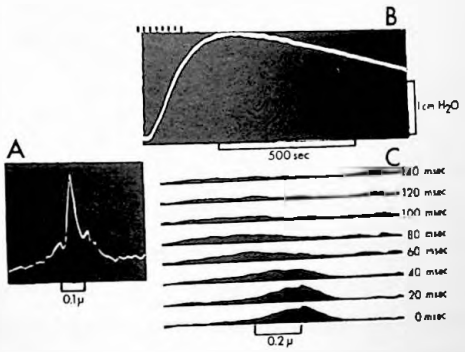

Figure 1. Measurement of sarcomere length using diffraction patterns. Panels A and B show diffraction patterns, i.e., the intensity of light as function of the scattered angle. The left side of the panels corresponds to a large diffraction angle and a short sarcomere length and the right side corresponds to a small diffraction angle and long sarcomeres. The diffraction patterns in panel C are measured at the points indicated during the twitch pressure shown in panel B. The diffraction patterns in panel C have been traced from oscilloscope recordings and are shown with subtraction of a common baseline scatter.

Figure 1 panel B shows the twitch pressure measured from a length-clamped preparation during the early part of a twitch. Diffraction patterns are measured at 20 msec intervals (Figure 1C). The first two diffraction patterns (labeled 0 msec and 20 msec) are recorded before the contraction and have peaks of about 0.2 μm width and 2 μm sarcomere length. A broadening of the peak and a shift to the left (sarcomere shortening) is observed in the next diffraction pattern (40 msec). After an additional 20 msec the peak of the diffraction pattern (60 msec) has shifted about 0.2 μm from the original location. The following diffraction patterns show that the peak continues to broaden until it virtually disappears. In the later diffraction patterns there is also some indication of appearance of a secondary peak at a location corresponding to about 2.4 μm (panel C). This observation suggests that sarcomeres not only shorten against the lengthening damaged ends of the preparation, but also against another inactive sarcomere population in the possibly damaged center of the preparation.

Stretch and Release Experiments: Figure 2 shows results from an experiment where the servo-control was used for rapid stretch and release. The upper traces show the bulge height (roughly proportional to the length of the preparation) and the lower traces show pressure response recorded during a twitch. Two twitch pressures are recorded under isometric conditions and the length of the preparation was changed by about 10% between the twitches. A third recording was obtained where the change in length is applied during the time course of the twitch (first stretched 10% and 0.6 sec later released again). Notice that the stretch or release are complete within 20 msec and that the pressure

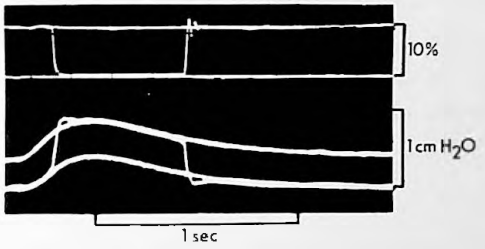

Figure 2. Stretch and release experiments. Upper traces show the bulge height (downward deflexion indicates an increase in bulge height and an increase in the length of the preparation) and lower traces show the pressure recorded during twitches. Two twitches are recorded under isometric conditions at two lengths. A third twitch is recorded while the length is changed rapidly forth and back between these two lengths.

trace within 50 msec reaches the value which was measured at the same time and length during the isometric contraction. A faster response could be obtained by increasing the loop gain of the servo-control system, but this tended to cause oscillations especially following release steps (Figure 2). The present experimental arrangement was not sufficiently fast on application of stretch or release steps required to study cross-bridge dynamics.

Measurement of the Peristaltic Contraction of the Intact Heart: Having studied the contractile properties of a small segment of the myocardial wall it was of interest to relate these findings to the performance of the intact heart. The experimental arrangement shown in Figure 3 was used for this

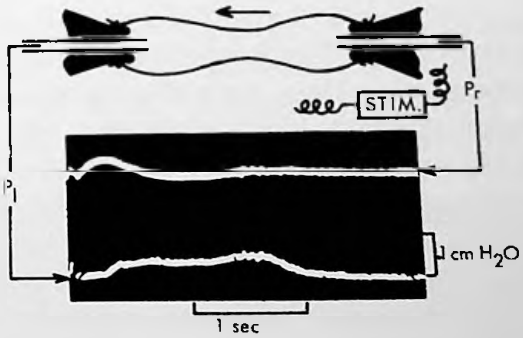

Figure 3. Recording of pressure in the intact heart. The heart is tied at either end to a wide tapered cannula. Thinner cannulas are advanced through the lumen of the tapered cannulas into the lumen of the heart and are connected to pressure transducers. A stimulating current pulse is applied at one end. The lower panel shows the pressures recorded at the left (P_l) and right (P_r) end.

purpose. The tubular heart was tied at either end to a wide cannula (1.5 mm) and an inert fluorescent dye (Na fluorosine) was perfused through the lumen of the heart. The length of the heart between the

two cannulas was about 40 mm. The dye made it possible to observe the peristaltic wave of contraction more clearly and to take motion pictures of the process. The current necessary to stimulate the preparations was passed through the lumen of one of the cannulas. Figure 3 shows the time course of pressure change at two ends of the beat as the right end of the heart is stimulated. Two thinner cannulas connected directly to pressure transducers were advanced through the lumen of the wider cannula until the opening was extended about 3 mm into the heart. The illustrated pressure traces correspond to a clearly propagated wave of contraction. The pressure at the stimulated right end of the heart (P_r) increased transiently for about 0.5 sec while the pressure at the left end (P_l) continued to increase until the contractile wave reached the unstimulated end. Notice also that there is a few hundred msec delay between the initiation of the contraction of the left end and the first indication of contraction at the right end. These observations may be explained on the basis of the propagation of two pressure waves: a pressure wave which is passively propagated, and a dynamic pressure wave which travels with the speed of the electrical excitation (1-2 cm/sec).

Conclusions: Experiments of the type illustrated in Figures 1 and 2 indicate that a 10% change sarcomere length or total length are completed within about 50 msec. This time interval is short compared to both the rising and falling phase of the twitch suggesting that the time course of the twitch tension corresponds to changes in degree of activation of the myofilaments. Figure 2 further suggests that a first approximation of the developed wall tension may be obtained considering only the present length and the time since stimulation (i.e., disregarding the preceding changes in length). A computer simulation along these lines is presently being attempted and the results are being compared to the results obtained with the intact heart (Figure 3). Figure 3 shows that the heart can develop a pressure difference between its two ends. In this respect the computer simulation may help to elucidate how factors such as viscosity, speed of propagation and diastolic pressure determine the efficiency of the valveless tunicate heart.

HISTOCHEMICAL AND ULTRASTRUCTURAL CHARACTERISTICS OF RED AND WHITE MUSCLE OF THE SPINY DOGFISH, *Squalus acanthias*

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The spiny dogfish, *Squalus acanthias*, like many other poikilotherms is able to survive for many weeks without food. During this period it must rely on endogenous stores. Fuel utilization has been poorly studied in fish but a high degree of dependence on lipid metabolism during starvation is to be expected. This study examines the possibility that lipid may be a significant fuel for skeletal muscle of starving fish.

To begin analysis of the specific properties of shark muscle that might be correlated with its biochemical and physiologic properties, a morphologic survey was undertaken utilizing standard methods. Portions of both red and white striated muscle were preserved in a fixative containing 2 parts 4% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.2), 1 part 8% paraformaldehyde and 1 part elasmobranch saline, and other pieces were snap-frozen and maintained in the frozen state at -30°C. Tissue for ultrastructure examination was embedded in Epon 812 and semi-thin sections were stained with Toluidine blue and examined in the light microscope for selection of areas to be thin-sectioned. Frozen sections cut at 6 μ were stained for lactic (LDH) and succinic dehydrogenase (SDH) activity by the azo dye method using a chromogenic substrate, and magnesium ATPase (ATP-ase) activity was demonstrated by a standard method (Brooke and Kaiser, Arch. Neurol. 23:369, 1970).

Hematoxylin and eosin staining revealed differences between the red and white muscle. The red muscle has loose myofiber texture with clusters of mitochondria and lipid granules lying between the