could have happened either by a direct effect on tubular transport or as a consequence of the pressor effect of angiotensin. Brull et al. (J. Marine Biol. Assoc. U.K. 32:329-336, 1953) and Brull and Cuypers (J. Marine Biol. Assoc. U.K. 33:733-738, 1954) have described a "pressure diuresis" (sodium excretion was not measured) in isolated perfused goosefish kidneys. Although the kidney in goosefish is perfused by venous blood, portal venous pressure might have increased along with systemic arterial blood pressure when angiotensin II was infused.

This research was supported by a grant from the Skillman Foundation.

THE SARCOMERE LENGTH TENSION RELATION OF THE SINGLE CELL LAYERED HEART OF "SEA POTATO."

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The tubular heart of the "sea potato" (*Boltenia ovifera*) consists of a single layer of spindle shaped cells. The length-tension relation of this myocardium was measured under conditions where servo-control was applied to keep a small segment of the wall isometric (MDIBL Bulletin Vol. 15). The servo-control system was improved and a laser diffraction technique was used in the present study to measure the sarcomere length-tension relation.

The sea potato heart was removed from the animal and after cannulation opened along the raphe to form a sheet of muscular tissue. The tissue was placed over an elongated opening (1.3 mm x 4.5 mm) and tied down along the perimeter of a slightly larger (1.8 mm x 5 mm) circumscribed rectangle, thereby blocking the passage of fluid through the opening from the closed lower section of the chamber to the open upper section. Nylon strings were used to snare down the preparation into two grooves corresponding to the long sides of the rectangle, and the shorter ends were pressed down with two rubber bands. With the pressure of the lower chamber slightly increased, the tissue would bulge up thereby approximating a semicircular cylindrical surface over the central portion of the opening. Both sides of the preparation were perfused with cold filtered seawater. Transepithelial current was applied to elicit contraction.

The experimental set-up is represented schematically in Figure 1. The semicircular arch labeled "muscle" represents a cross section through the bulge shaped preparation. A partially blocked blue light beam is used to measure the height of the bulge, h, and the sarcomere length is estimated from the angular distribution of light diffracted from a red light beam passing through the center of the preparation. Sodium fluorescein (0.2 mM) was added to the solution perfusing the lower chamber. This solution absorbs strongly around 490 nm, thereby effectively blocking the passage of blue light through the bulge, but lets the red light beam through with negligible attenuation. The blue light beam originates from an incandescent lamp equipped with a 488 nm interference filter. The condensed blue light is passed through a rectangular aperture the image of which is focused in a slit (2 mm long and 0.5 mm wide) onto the preparation. The light not blocked by the bulge is measured by a photodiode detector. A second blue interference filter is placed in front of the photodiode in order to block scattered red light and to decrease the effect of varying room light. A second detector measures directly the blue light emitted by the lamp and an amplifier which balances the signals from the two detectors is used to minimize the effect of small variations in the light output from the lamp.

The monochromatic red light (λ = 633 nm) is generated by a 3 watt He-Ne laser and is focused on the muscle in a spot (diameter, 0.4 mm). The diffracted light diverging from the muscle is converted

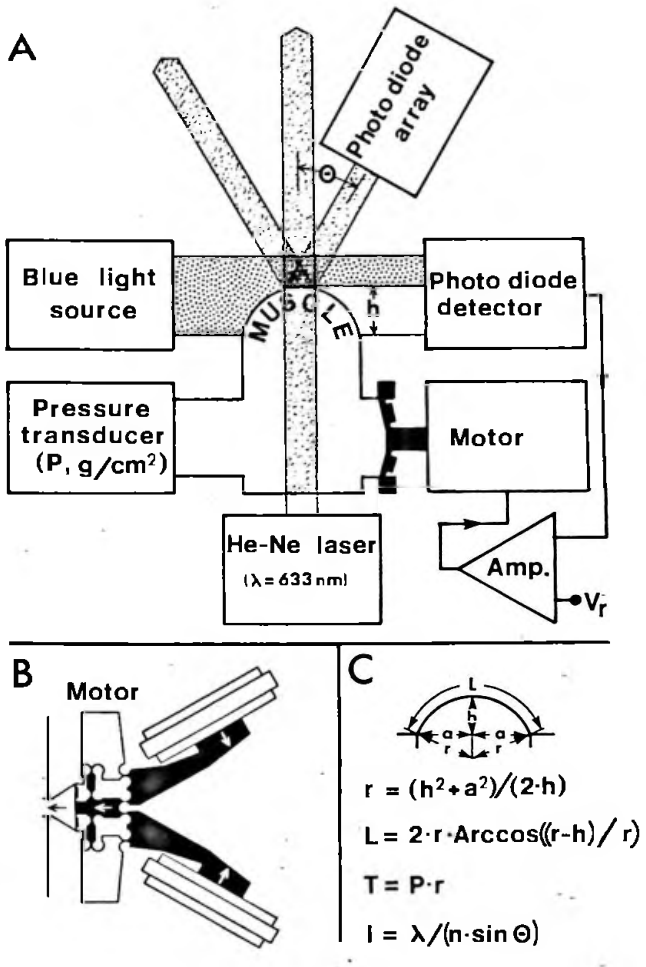

Figure 1. Panel A is a schematic representation of the experimental set-up. The semicircular arch labeled "muscle" represents a cross section through the bulge shaped preparation. The blue light beam measures the bulge height, h. The red light from the He-Ne-laser is scattered by the muscle and the scattering angle, Θ. is monitored with the photodiodide array. The bulge height is servo controlled using a motor which displaces fluid in the closed chamber under the muscle. A pressure transducer monitors the pressure difference across the myocardial wall. The fluid displacing motor is shown in panel B. The length of the preparation, L, the tension in the myocardial wall, T, and the sarcomere length, l, are calculated using the equations in panel C.

to a parallel beam by a lens one side of which is in contact with the fluid. The parallel beam is filtered through a red interference filter and focused with a cylindrical lense on a linear array of 128 photodiodes (Reticon RL128). The interference filter is again necessary to minimize the effect of the blue light and the room light. The linear array of photodiodides and its associated optics can be rotated around the preparation. Calibration is facilitated by a graticule which gives a direct readout of the angular position of the detector.

A pressure transducer (Statham, P23BC) connected to the closed lower chamber served to measure resting pressure and twitch pressure in the frequency range from 0 to 35 Hz. The servo motor was designed to cause rapid fluid displacements in the lower chamber. Linear motion is applied to the central section of a fairly stiff membrane, which constitutes a section in the wall of the lower chamber. The membrane has thin relatively flexible sections forming the pattern of a spoked wheel. The thin sections facilitate a single mode of deformation which couples fluid displacement with motion of the central section of the membrane. The thicker sections between the spokes minimize modes of deformation which add compliance to the lower chamber even when the central section of the membrane is in a fixed position.

Figure 1B shows how two speaker motors through a lever system are attached to the center part of the membrane. The moving parts are in black. The lever system is milled out of a 1 inch plastic plate. The hinges of the lever system are the flexible thin sections which remain between two closely drilled holes. The height of the bulge shaped preparation will usually decrease markedly during contractions and this is counteracted by feedback from the height-measuring light beam to the motor. The height of the bulge can, under optimal conditions, be clamped in 1 - 5 msec. The direclty measured parameters, pressure, P, bulge height, h, and scattering angle, Θ. are converted to wall tension, T, preparation length, L, and sarcomere length, l, using the equations in Figure 1, panel C. The transmitted blue light was in each experiment measured as function of the bulge height which was observed on a calibrated grid in the dissecting microscope. The constants in the equations are: half the width of the preparation, a = 0.9 mm, the wavelength of the red light, λ = 633 nm, and the refractive index which in aqueous solution scales this wavelength down by a factor of n = 1.33. The radius of the curvature, r, is applied in LaPlace's Law calculating the wall tension from the pressure. The relation between the scattering angle and the sarcomere length assumes first order diffraction of a light beam which is perpendicular to the myofibrils.

Figure 2 shows 15 diffraction images and the twitch pressure recorded from a bulge clamped preparation. The diffraction images are recorded with constant intervals before and during the contractqon and indicated by the marker pulses in panel C. The 15 diffraction patterns are (original record, panel A) shifted down over the screen of the oscilloscope. The traces are redrawn in panel B to eliminate some of the noise in the system and to obtain better visual separation of the individual diffraction images. A peak is clearly visible before and toward the end of the contraction. The peak shifts during the development of tension toward a larger scattering angle corresponding to a shorter sarcomere length. The shift is associated with a broadening of the diffraction line and it is difficult to estimate the position during the peak of the contraction. Contributing to this difficulty is a considerable amount of diffracted light which seems to be unrelated to the sarcomere length exhibiting a steadily decreasing intensity with increasing scattering angle. This light may to some extent orginate from the preparation itself but it proved very important to keep the light path free of other scattering objects. Rotation of the detector around its optical axis helped achieve parallel alignment of the photodiode array and the myofilaments. Under favorable conditions the diffraction peak would have an amplitude twice the unwanted diffracted light at the same scattering angle.

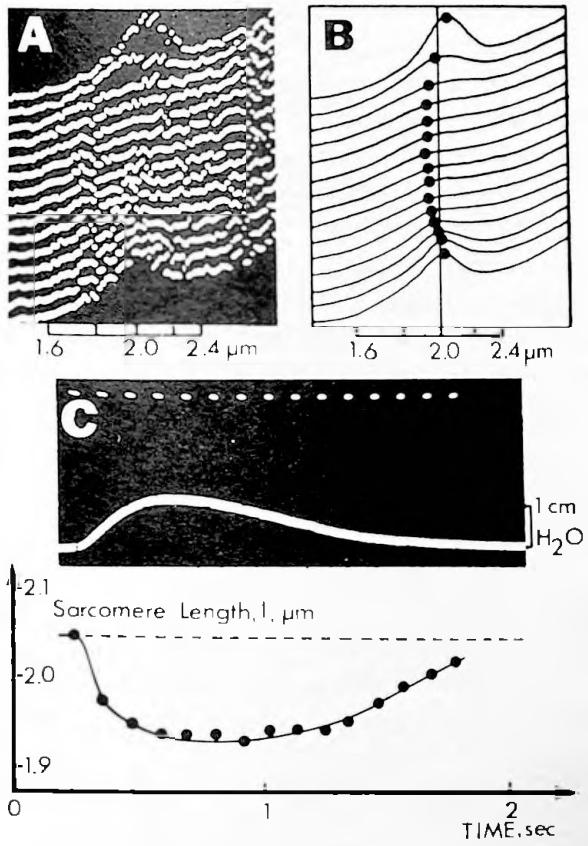

Figure 2. Diffraction patterns (panel A and B) measured during a single isometric contraction (panel C). Panel B is a tracing of the diffraction patterns in panel A. The diffraction patterns are from top to bottom recorded in synchrony with the pulses in panel C. The peak of the individual diffraction images is marked by a dot in panel B and the corresponding sarcomere length is in panel C plotted with the same time base as the isometric contraction.

The sarcomere length was estimated from each of the diffraction images and its change during contraction is indicated in panel C compared to the time course of the twitch. The maximal shortening is approximately 0.1 μm. The return of the sarcomere length to its resting value is slower than that of the isometric tension suggesting a nonlinear relation between the developed tension and the sarcomere shortening. The diffraction peak completely escaped observation during the twitch in some experiments and would in other experiments fade out while a new peak appeared at a scattering angle corresponding to a sarcomere length of approximately 1.6 μm. These phenomena and the broadening of the peak observed during contraction were interpreted to result from nonuniform distribution of sarcomere length.

Figure 3 shows results from an experiment in which the preparation under servo control of the buldge height was subjected to a series of stepwise stretches followed by a series of stepwise releases. Pressure, diffraction patterns, and transmitted blue light were measured when the preparation was stimulated once at each length. The sarcomere shortening during the twitches appeared to be 0.1-0.15 μm. The relation between the transmitted blue light and the bulge height was measured by visual inspection of the bulge height through the dissecting microscope. Using this relation it was possible to calculate the length of the preparation throughout the experiment and compare it to sarcomere length length measured before the twitches. The sarcomere length measured from the position of the individual

diffraction lines deviated less than 0.03 μm from a regression line which correlate sarcomere length with the total length of the preparation. The slope of the regression lines indicates that the strain of the sarcomeres is 92% of the strain of the preparation during the stretch and 115% during the stretch and 115% during the release. Slow leak of the dye from under the preparation may have caused a progressing decrease in the transmission of blue light through the upper chamber. This artifact may have lowered the first value and increased the second value.

In Figure 3, the resting tension (open symbols) and the twitch tension (filled in symbols) is plotted versus the sarcomere length. The arrows indicate the order in which the points are measured. The preparation is first stretched (circles) and then released (squares). There is no significant difference between the passive tension measured during the stretch and during the release. The passive length-tension curve is highly nonlinear, rising steeply around 2.1 μm. The active length-tension relation rises linearly with increasing stretch, reaches a maximum around 2.1 μm and then falls again approximately linearly. During the release, the twitch tension first increases, and then falls toward zero along a lowered and markedly bent curve. Extrapolation of the ascending limb of the length-tension curve seems to intersect the x-axis at 1.6 μm, suggesting that unloaded sarcomeres may shorten to this length when the preparation is stimulated.

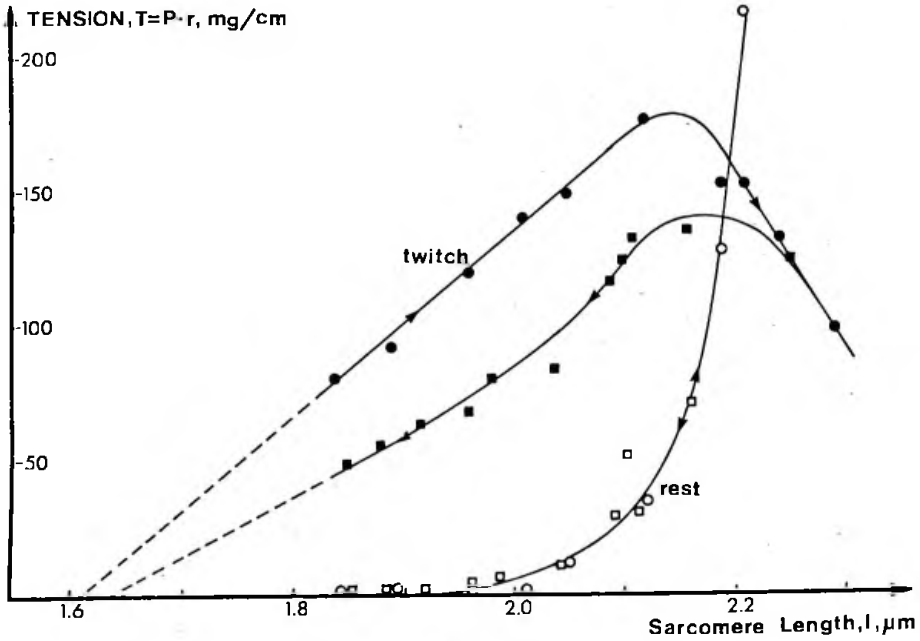

Figure 3. Sarcomere length-tension relation. The resting tension and the twitch tension are measured from the "bulge clamped" myocardium. The sequence of the measurements is indicated by the arrows. The preparation is first stretched (circles) and then released (squares). The sarcomere length is calculated from the angle of the scattered light measured before the twitch.

It is also possible to explain this observation assuming two populations of cells in series. Both populations have the same passive length tension curve which is unchanged throughout the experiments. Only one population of cells develops active tension. There is relatively little sarcomere shortening during contractions when the majority of the cells are in this state, the ascending limb of the length-tension curve is linear, and there is good correlation between the strain of the sarcomeres and the strain of the preparation. With increasing damage caused by the large stretches, an increasing percentage of the cells become incapable of developing active tension. During stimulation the active cells will have to stretch

the inactive cells until there is a balance between the passive force of the inactive cells and the sum of the active and passive force of the active cells. This mechanism explains how increasing damage lowers the peak tension and result in a curved relation for the twitch tension versus the initial sarcomere length. It also explains the nonlinear relation between twitch tensi-n and sarcomere shortening if it is assumed that the red light beam through the center of the preparation is preferentially scattered by undamaged cells. The model described here ascribes the same nonlinear length tension curve for the series and parallel elastic element. The maximum twitch tension is remarkedly constant, 220 ± 40 mg/cm (6 preparations). In this respect, the model predicts significant curvature of the ascending limb with only a small decline in maximum twitch tension. The constancy of the twitch tension in various preparations indicates that there is little variation in the degree of activation from preparation to preparation.

The experiments reported above suggest that laser diffraction images can be used to study the contractile process of the single cell layered myoepithelium at the sarcomere level. The results are similar to those obtained in mammalian heart. Series elastic components seem to exist in varying degrees. Sarcomere length-tension curves were only measured in 3 successful experiments of all which yielded diffraction images of less than optimal quality. The experiments are, however, promising enough to spure continued investigation. An improved design for the laser diffraction system may well improve the quality of the diffraction patterns to a degree where it is possible to clamp the sarcomere length instead of the length of the preparation.

EFFECTS OF MICROIONTOPHORETIC INJECTION OF CALCIUM IONS AND CYCLIC AMP ON CELL SHAPE.

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Fertilized eggs of some mollusks and some annelids undergo a series of shape changes before and during early cleavage, a process known as polar lobe formation. These shape changes are seen in most dramatic form in the eggs of the common marine and snail, *Ilyanassa obsoleta*. A polar lobe results from formation of a constriction slightly below the equator of the fertilized egg. This constriction greatly resembles a cleavage furrow.

Previous work has shown that a ring of microfilaments is present in the cortical cytoplasm at the base of the polar lobe constriction, analogous to the microfilament bundle at the base of a cleavage furrow (Devel. Biol. 36:363-378 (1974)). The microfilament ring is present only when the polar lobe constriction is present and disappears when the constriction relaxes during lobe resorption. Integrity of the microfilaments can be disrupted by treatment with cytochalasin B, with simultaneous regression of the lobe constriction. We wish to determine what normally regulates the constriction of the microfilament ring associated with lobe formation. Previous experiments have shown that, if spherical *Ilyanassa* eggs are placed in high concentrations of exogenous calcium ions, cell shapes change dramatically within 15 min.in a process which appears to mimic normal polar lobe formation (Devel. Biol. 37:280-294 (1974)). Eggs placed in solutions of other ions, such as sodium, potassium, magnesium, or manganese, do not show such shape changes. On the other hand, the normal process of polar lobe formation, as well as of cytokinesis, continues without any inhibition when eggs are placed in calcium-free sea water. This suggests that if calcium ions <u>are</u> involved in regulating the constriction of microfilament rings, they derive from intracellular pools. We therefore wish to know if changes in intracellular concentrations or activities of ions like calcium play a role in normal polar lobe formation.