Muscle Volume Changes

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ABSTRACT Measurements have been made of the volume changes accompanying single isometric and isotonic twitches of frog sartorius muscle. The volume change consists of a rapid increase, a subsequent decrease, and a return to the initial volume; the order of magnitude of increase and decrease is 10^{-5} cc/g of muscle. This volume change is length-dependent: the initial increase becomes more pronounced as the initial length of the muscle is decreased, while the volume decrease is greatest at reference length and is diminished for longer and shorter initial lengths. Muscle volume changes are also dependent upon temperature and amount of shortening: the return phase is prolonged as the temperature is lowered; and, in an isotonic twitch, a volume increase accompanying muscle shortening is superimposed upon the volume change described for an isometric twitch. These "shortening volume changes" may correspond to the volume decrease observed in frog muscle under a passive stretch. If the active state is prolonged by the use of a frog Ringer solution in which iodide ions have been substituted for chloride ions, the time course of the volume decrease is likewise prolonged; this suggests a relationship between the volume decrease and the active state of the muscle.

INTRODUCTION

A consideration of the existence of a change in muscle volume during contraction has proceeded since the 17th century. In spite of this long history of concern over the existence of muscle volume changes many aspects of these changes have not been resolved. Abbott and Baskin (1962) have discussed the various attempts to measure changes in muscle volume during contraction. The major reason for the difficulty in measuring muscle volume changes lies first in the rapid time course of contraction and second in the extremely small size of the volume changes. The volume changes for a 100 mg frog sartorius muscle are about 10^{-6} cc. In order to measure small volume changes which occur very rapidly it is necessary to use a sensitive recording system. Such a system is also sensitive to mechanical vibration and artifact. The problem becomes more complex if one attempts to study volume changes in a muscle that is contracting isotonically. In this case a system is required which will allow the measurements of volume change without at the same time causing a (artifact) volume change due to the recording system. Such a system will be described in this paper.

Baskin (1960) and Abbott and Baskin (1962) developed a sensitive system capable of detecting volume changes as small as 10^{-7} cc. The response to a sudden mechanically induced change was 90% complete within 2 msec. This method, as did preceding methods, involved recording the change in height of a liquid surface in a capillary. The improved sensitivity of this instrument over preceding instruments allowed the use of frog sartorius in place of gastrocnemius muscle. In the sartorius muscle fibers are arranged parallel to the direction of contraction; there is no significant force component developed perpendicular to the axis of the muscle. Consequently, volume decrease resulting from a development of internal pressure does not occur (as it does in the gastrocnemius).

Abbott and Baskin reported a three phase volume change accompanying an isometric twitch of sartorius muscle, an initial volume increase, a volume decrease, and a return phase. At 20°C there was a latent period of 2 to 3 msec before the onset of the increase. Volume change attained a maximum increase of 2.5×10^{-6} cc/g of muscle about 6 msec after stimulus and then a maximum decrease of 5×10^{-6} cc/g of muscle in about 10 msec. The return of the volume decrease was complete within 55 msec after stimulus. At 2°C there was no difference in the magnitudes of the volume increase and the volume decrease but the latent period lasted 5 msec, while the times of maximum volume increase and minimum volume decrease were 10 and 35 msec respectively. At 2°C there was a delay of several seconds in the return of the decrease to the base line. The magnitude of the early volume increase was found to be strongly dependent upon muscle length, becoming smaller at lengths above reference length and larger at lengths below reference length.

Abbott and Baskin suggested that the volume increase was in some way related to the process linking excitation to contraction of the muscle. The time course of the length dependence of the increase was shown to parallel the behavior of the latency relaxation (Abbott and Ritchie, 1951).

Baskin (1963) introduced a modification in his earlier muscle chamber design to allow a coupling between the muscle and external loads and transducers. A chamber was designed such that when the muscle contracted it exerted a torque about a pulley interior to the chamber. The pulley was mounted on a shaft which extended through a water-tight seal to the outside. The shaft was coupled by means of a chain to an isotonic lever or to a tension transducer. Rotation of the shaft varied the initial length of the muscle without introducing a volume change artifact.

The chamber used in the experiments described in this paper incorporated the above facilities to permit isotonic measurements (Baskin and Paolini, 1965). Volume changes are monitored by a pressure transducer mounted in a wall of the chamber and not by a proximity transducer close to the surface of a fluid continuous with the fluid surrounding the muscle. For the very

small volume changes to be measured it is assumed that the pressure measurement in such a closed vessel is equivalent to the volume change that would be measured if the muscle were in an open vessel.

A major difficulty encountered with the proximity transducer of the earlier system was its susceptibility to oscillation upon mechanical shock caused by sudden displacement of load. There is no such difficulty with the pressure transducer. Also the positioning of the proximity transducer wire over the liquid surface was extremely critical and often required a great deal of time for its proper setting. This problem is also avoided with the present pressure transducer.

In an article on muscle volume changes Ernst (1962) makes certain objections to the work of Abbott and Baskin (1962). In particular he is critical of the lack of an "original calibration curve" and photographic recordings of the muscle volume change results. In this article Ernst continues to express his belief that the existence of a volume increase preceding the volume decrease in muscle has not been adequately proven. This paper will consider these objections and will also present the results of measurements of muscle volume changes under isotonic conditions. The results of this work have a bearing on the current theories of muscle contraction. In particular the sliding filament model of Huxley and Hanson (1954) and the molecular theory of muscle contraction by R. E. Davies (1963) will be discussed in the light of the new information from the volume change experiments.

METHODS

Muscle Chamber

The muscle chamber and its associated transducers are shown in Fig. 1. All parts of the chamber are made of brass, with the exception of the front cover and the electrode grid assembly. These two components are made of lucite in order to minimize shunting between the electrode wires and the grounded chamber during stimulus.

A sartorius muscle (*A*) is shown mounted within the muscle chamber (*B*). The piece of pubic bone left attached to the proximal end of the muscle is held by a small bone clamp (*C*). The muscle lies against an electrode grid (*D*) consisting of a lucite block in which are imbedded nine exposed lengths of 16 gauge platinum-iridium wire spaced at 5 mm intervals. Alternate lengths of this wire are connected to the positive and negative terminals of the stimulus circuitry by means of the shielded cable (*E*) which passes out the back of the chamber.

The thread tied to the distal end of the muscle is secured to a small hole in the rim of the pulley (*F*); this pulley is mounted on a shaft (*R*) (Fig. 2) which passes through the back wall of the chamber by way of a water-tight ball-bearing seal (*S*). A fine jeweler's chain (*G*) is wrapped around a second pulley (*T*) mounted on this shaft at the back of the chamber. This second pulley is stabilized on either side of the shaft by ball bearings (*U*) mounted in the support (*V*). This arrangement allows the length of the muscle to be increased by drawing on the chain from outside the chamber.

The chamber is sealed with a lucite cover (*H*) which fits over the front of the chamber and is secured with seven thumbscrews. A micrometer adjustment (*I*) is connected to a No. 59 drill rod (*J*) which is inserted through the hole (*K*) used for filling the chamber with Ringer's solution. A 5 psi pressure transducer (*L*) is threaded into the top of the chamber so that its diaphragm is flush with the chamber's inner wall.

The silver chain from the pulley behind the chamber passes through a groove in the support (*V*) and is placed over the large pulley (*M*) of the length transducer (*N*). For isotonic measurements, the chain was secured to an isotonic lever (*O*) by means of a setscrew (*P*). A load was supported by a pan suspended close to the bearing of the lever in order to minimize inertial effects when the mass is accelerated by the muscle. The screws (*Q*) could be set to limit the shortening of the muscle.

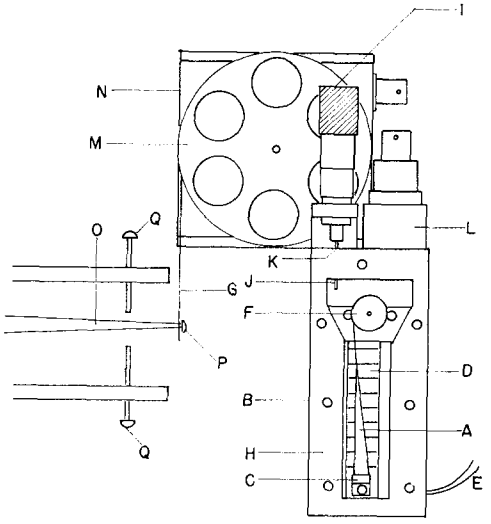

FIGURE 1. Muscle chamber. *A*, muscle; *B*, chamber; *C*, bone clamp; *D*, electrode grid; *E*, stimulus cable; *F*, pulley; *G*, chain; *H*, cover; *I*, micrometer adjustment; *J*, drill rod; *K*, fluid input hole; *L*, pressure transducer; *M*, length transducer pulley; *N*, length transducer optics; *O*, isotonic lever; *P*, setscrew; *Q*, screws for limiting lever travel.

The entire isotonic lever assembly was mounted on a rack and pinion (not illustrated) so that the lever could be moved along the vertical axis in order to take up the slack in the silver chain.

In the case of isometric measurements, the entire length transducer assembly and isotonic lever were moved away and a tension transducer assembly was secured to the length transducer support (*V*). This assembly consisted of a mechanoelectric transducer held in a rack and pinion (again to allow slack in the chain to be taken up and the length of the muscle to be set).

Fig. 2 shows a cross-section view of the length transducer. Movement of the silver chain causes rotation of the transducer pulley (*M*). Mounted concentrically with this pulley is the vane (*W*) whose shape is complementary to a translucent pattern on a lucite sheet (*X*). The vane and lucite sheet constitute a shutter which admits an intensity of light from the rear to the front of the transducer unit in exact proportion to the angular position of the large pulley. Two 6 V lamps (*Y*) are mounted at the back of the transducer enclosure. Four photocells (*Z*) are positioned at the front of the

enclosure to receive maximum intensity of light admitted through the shutter. Over a short range, the DC voltage output variation of the photocells is linear with variation in the intensity of incident light. Within this range, the DC output voltage of this transducer is directly proportional to the rotation of its pulley and therefore to the length of the muscle in the chamber.

Pressure Transducer System

The transducer diaphragm and an insulated electrode within the transducer shell form an electrical capacitor; the value of the capacitance changes proportionally to the change in pressure on the diaphragm. This capacitor and a fixed inductor comprise a tank circuit which modulates a 700 kc oscillator. The oscillator output is

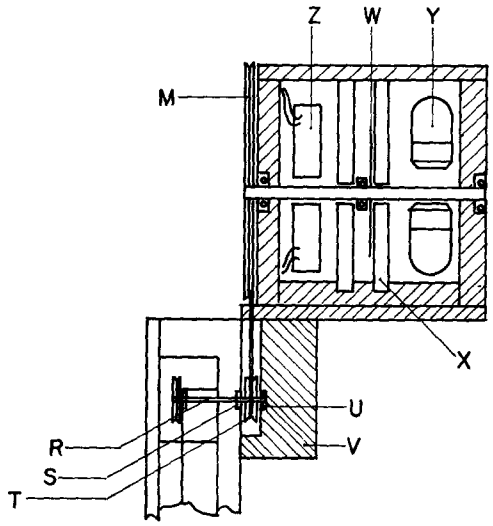

FIGURE 2. Length transducer. *M*, pulley; *W*, vane; *X*, lucite shutter; *Y*, lamps; *Z*, photocells (4); *R*, shaft; *S*, water-tight bearing; *T*, rear pulley; *U*, ball bearings; *V*, support block.

"detected;" i.e., changes in the oscillator frequency cause linear changes in the detector impedance. The detector output is amplified and subjected to a low-pass filter (a circuit which attenuates the carrier frequency much more than the modulating frequency). The output of the detector is therefore a DC voltage proportional to the pressure on the transducer diaphragm.

A signal-averaging digital computer was utilized to improve the signal-to-noise ratio of low level data. The computer digitized transient waveforms (corresponding to volume changes accompanying single muscle twitches) and stored these waveforms in a magnetic core memory. Successive waveforms are digitized and added to the content of information in memory, so that as more waveforms are added, the random noise in the input tends to average to zero while the repetitive signal of interest adds linearly.

The automatic statistical averaging performed by the computer allowed a substantial improvement in the quality of the volume change waveforms; the magnitude

of volume change in sartorius muscle was so small that the change was masked by random noise superimposed on the base line of the transducer output.

Photographs of volume changes accompanying single muscle contractions were taken from an oscilloscope as were recordings of averaged volume waveforms (i.e., computer memory content).

PROCEDURE

Muscle Dissection

The sartorii of *Rana pipiens* were measured *in vivo* to the nearest 0.5 mm. Varying the orientation of the limb affected the measurement, so all measurements were taken with the legs laid out in a line. Sartorius muscles were dissected out with very fine iridectomy scissors; during the dissection the muscles were washed constantly with cold, oxygenated Ringer's solution. A fragment of the pubic bone was left attached to the sartorius. A fine thread was secured to the distal tendon.

Before use, muscles were soaked for several hours in oxygenated Ringer's solution at about 4°C. The composition of the Ringer solution was as follows: NaCl, 0.115 M; KCl, 0.002 M; $CaCl_2$, 0.0018 M; $NaHCO_3$, 0.0024 M; pH adjusted to 7.2.

Average sartorius reference length and weight for one hundred muscles were 41 mm and 140 mg.

The thread attached to each muscle was passed through the hole in the rim of the pulley inside the muscle chamber. The bone fragment was secured by tightening the screw in the clamp at the bottom of the chamber. The thread was tied to the pulley at the approximate length indicated in Fig. 1. For isotonic measurements the initial length of the muscle was set first by the coarse vertical adjustment (the rack and pinion) and then by the screws (*Q*, Fig. 1) in the isotonic lever assembly.

The front edges of the open chamber were coated lightly with silicone stopcock grease and the lucite cover was secured to the chamber with thumbscrews. The chamber was filled with oxygenated Ringer's solution through the hole (*K*, Fig. 1) at the top of the chamber. Great care was exercised not to allow air bubbles to be trapped in the chamber. Gases being more compressible than liquids, any appreciable amount of retained air would "delay recording" the pressure differential created by the change in muscle volume.

The drill rod was then inserted in the hole and the attached micrometer adjustment secured by a thumbscrew in a supporting bracket. This micrometer adjustment was originally intended for calibration of the pressure transducer response to a manually introduced volume change. It also served as a pressure transducer circuitry base line control, since any change in the depth of the rod caused a change of the pressure level within the chamber. The chamber was then immersed (to the level of the bottom of the length transducer) in a specially constructed water bath enclosure. The enclosure was made of brass (so that it would be at some uniform temperature) and was itself enclosed by a lucite chamber. Water at a preset temperature was pumped through the outer enclosure by a thermoregulated refrigeration unit.

The muscles were stimulated with single 10 to 15 v square wave pulses of very fast (less than 0.1 μsec) risetime and a duration of 3 msec.

Studies were also made of the effect on the volume change of changing the extra-

cellular fluid (Baskin and Paolini, 1965). A Ringer solution was made with an equivalent amount of sodium iodide substituted for the usual sodium chloride.

Calibration of the Pressure Transducer

Two different methods were used to calibrate the pressure transducer system. First, a device was constructed which applied a rapid step function volume change (1 or 2 msec risetime and approx 30 msec duration) into the chamber. A second calibration apparatus introduced an approximate ramp function volume change into the chamber, the rate of rise of the ramp corresponding to a typical rate of volume change in a muscle. The second calibration method was employed to test whether or not the

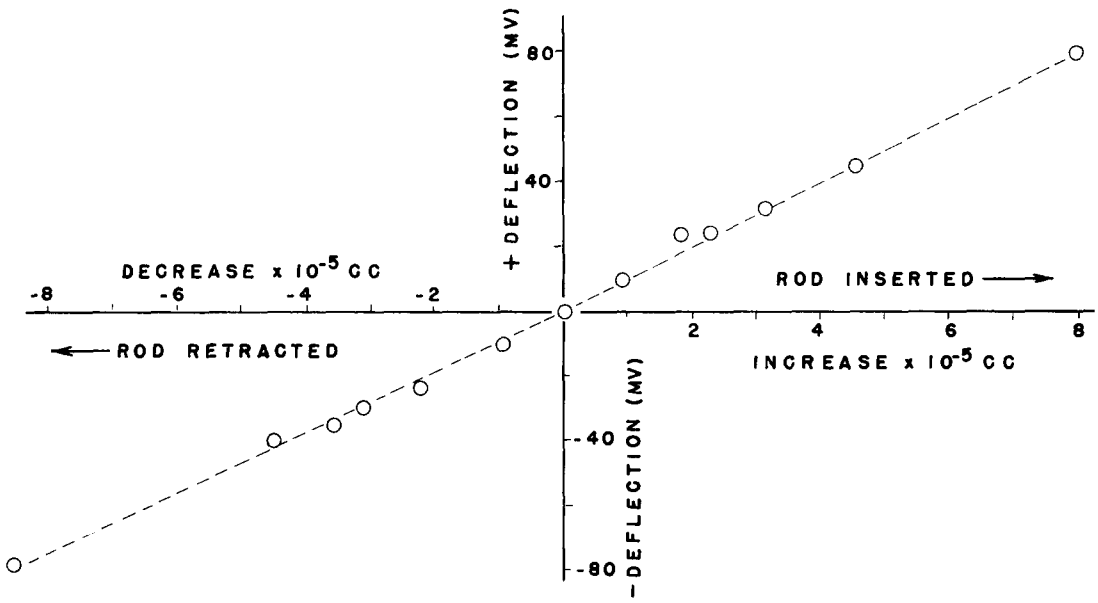

FIGURE 3. Volume calibration curve: response to a step function.

transducer response was a function of the rate of the volume change; the excellent agreement between results from the two calibration methods suggests strongly that it is not.

The first method of calibration utilized a miniature loudspeaker mounted on the face of the volume chamber cover. A small drill rod fastened to the center of the loudspeaker's field coil was inserted through a hole in the cover and sealed against leakage by stopcock grease. Application of a voltage step function to the coil caused a rapid insertion or withdrawal (depending upon the step function polarity) of the rod. The magnitude of volume change introduced into the chamber was controlled by a micrometer adjustment which limited the rod's movement. The distance of travel of the rod was observed through a × 30 field dissection microscope.

Fig. 3 summarizes the results of one experiment in which the step function response of the transducer was recorded; oscilloscope deflection in millivolts has been

plotted as a function of the volume change caused by rod movement. The slope of this plot is defined as the calibration constant K and for this graph $K = 1.05 \times 10^{-6}$ cc/mv. The mean value for three such experiments, and the value used in the reduction of data reported herein, was

$$K = 1.13 \times 10^{-6} \text{ cc/mv}$$

Fig. 4 is a record of the transducer output response (A) to a calibrator square wave pulse (B) of 35 msec duration. (C) shows the unhindered mechanical movement of

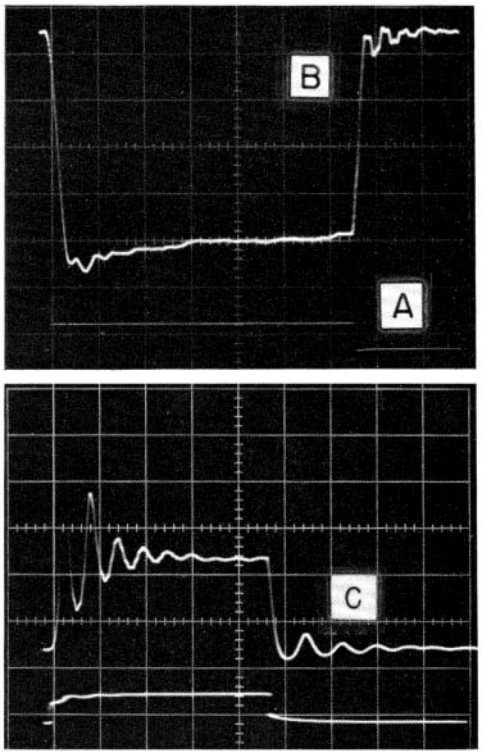

FIGURE 4. Transducer step function response. *A*, 35 msec square wave applied to loudspeaker coil; *B*, pressure transducer circuit output; *C*, loudspeaker response in air. Sweep time = 5 msec/cm.

the calibrator rod (i.e. in air, where it is not damped by the fluid in the chamber or by friction at the hole through which it passes). The calibrator's "overshoot" and ringing in air are much less noticeable under damping; however, the initial rise and decay times of the response in air and in the chamber are about the same: 2 msec. The response of the pressure transducer to a calibrated volume change is therefore effectively instantaneous.

The third calibrator apparatus utilized a relay with a spring-damped reed; a rod mounted on the reed was inserted through a hole in the chamber cover, as in the case of the calibrator described above. A voltage step function applied to the relay coil caused the rod to be inserted into the chamber at a relatively constant velocity.

Fig. 5 shows the comparison between this "ramp function generator" and the

pressure transducer output. The two response curves have been normalized: one curve corresponds to the rod displacement with time, while the other curve indicates the transducer output DC voltage level change with time. Data for this graph consisted of digital output from the signal-averaging computer, and correspond to an average of 64 responses of the system to a ramp function. The positive and negative deviations of the pressure transducer output from the calibrator rod movement are equal, suggesting that the pressure transducer responds quite closely to the introduced volume change, for a rate of change typically occurring in muscle. The value of K determined by this method was 1.12×10^{-6} cc/mv.

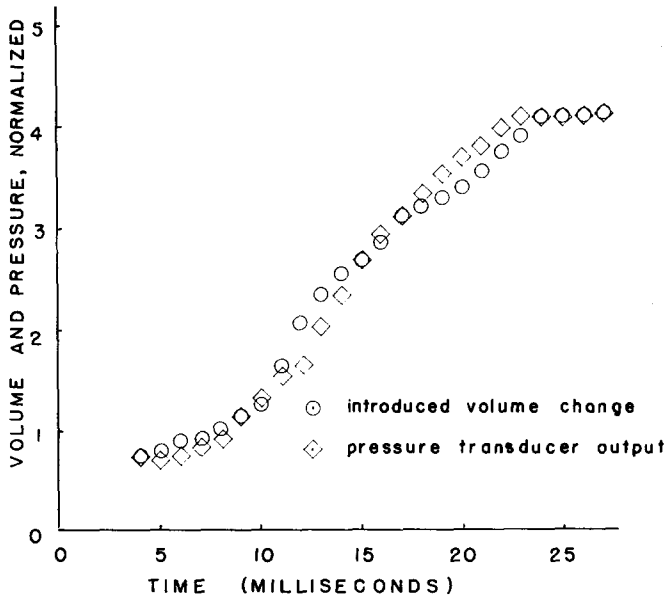

FIGURE 5. Calibrator and transducer response to a ramp function.

The possibility has been considered that these recorded waveforms correspond to shock waves arising from mechanical movement of the muscle, rather than to volume changes internal to the muscle. The following experiments have resulted in the conclusion that the waveforms illustrated here represent true volume changes and not a mechanical artifact.

A wooden vane was attached to the pulley (Fig. 1, *F*) of the muscle chamber. A frog sartorius muscle was mounted on a frame external to the chamber; upon stimulation this muscle shortened against the chain (Fig. 1, *B*) attached to the pulley inside the chamber. Thus, a single twitch of the muscle was accompanied by an abrupt counter-clockwise rotation of the pulley. The time course and magnitude of the pulley rotation in this experiment and in the muscle volume change experiments were equivalent. Stimulation of the externally mounted muscle (there was no muscle within the chamber) introduced movement into the fluid within the chamber but there was no measurable "volume change." In another experiment volume changes were re-

corded from a muscle contracting isotonically. In this case there was a considerably greater amount of muscle movement than in the isometric case and the resultant waveform showed less volume change.

This recording system does not differ in principle from that used by earlier investigators (Abbott and Baskin, 1962; Baskin and Paolini, 1964). The conclusion of their experiments and of the present investigation is that such records represent true volume changes and are not the result of mechanical artifact caused by muscle movement.

The Length Transducer

The transducer illustrated in Fig. 2 could be expected to produce a linear output voltage change with rotation if the photocells used in the transducer had a linear response. But the over-all photocell response to varying light intensity is decidedly not linear; it is asymptotic, approaching a limiting value of 120 or 130 mv per cell.

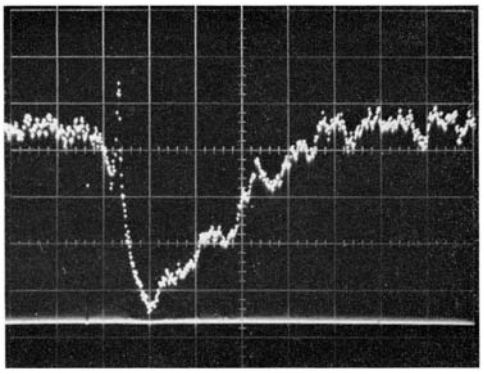

FIGURE 6. Volume change in frog sartorius muscle: isometric twitch at reference length = 45 mm. Muscle weight = 310 mg; temperature, 24°C. Sweep time = 10 msec/cm. Volume increase magnitude = 0.8×10^{-5} cc; decrease magnitude = 2.4×10^{-5} cc. Average of 25 waveforms.

The usable (i.e. linear) region of operation of the transducer corresponded to an allowable muscle shortening of about 15 mm. The calibration constant was determined to be 42 mv/mm.

The Tension Transducer

The circuit incorporating the mechanoelectronic transducer is a conventional triode amplifier. For calibration of this transducer, weights were hung on the inner pulley in the muscle chamber, and the corresponding output voltage change was recorded. The calibration constant was 21.4 mv/g.

RESULTS

If a muscle initially at reference length is preshortened by several individual twitches and allowed to recover at the preshortened length, and is then subsequently pulled back to reference length, an accompanying volume decrease occurs (Baskin and Paolini, 1964). This volume decrease is dependent only upon the distance the muscle is stretched and is not a function of velocity or tension under which it is returned to reference length. This is a "passive

stretch" back to reference length since no appreciable amount of tension is developed. This indicates the possible presence of molecular rearrangement or a shift in ionized groups occurring during the stretch; this shift accompanies a change in length of a muscle even in the absence of appreciable tension development.

Active volume changes (measured when the muscle is stimulated) have been studied using two types of recording conditions, isometric and isotonic. In addition, such factors as temperature, composition of Ringer's solution, and initial length of the muscle have been carefully controlled and in some cases varied.

The volume changes accompanying an isometric twitch at reference length in a single sartorius muscle from the frog have been discussed previously

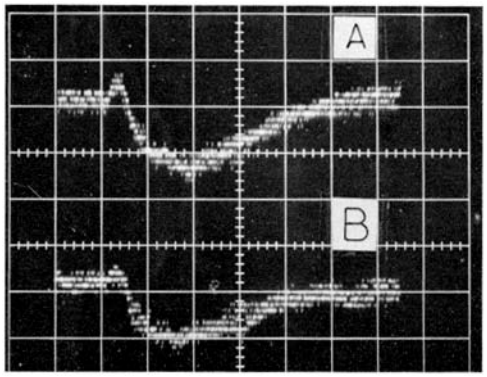

FIGURE 7. Volume changes in frog sartorius muscle: prestretched case. Isometric twitch at 24°C at (A) 8% and (B) 13% above reference length. Average of 9 waveforms. Sweep time = 10 msec/cm.

(Abbott and Baskin, 1962). The present investigation, using a different chamber and recording transducer, has given the same results as reported earlier. With the muscle mounted isometrically at reference length one initially records a volume increase about 2.5×10^{-5} cc/g of muscle in magnitude and a subsequent volume decrease which is 3 times the size of the volume increase (Fig. 6). These isometric volume changes have been investigated at lengths other than reference length. If a single frog sartorius muscle is prestretched between 1 and 6 mm above reference length before stimulation, upon stimulation a change in the characteristic appearance of the volume changes is observed (Fig. 7). When the muscle before stimulation, is held above reference length the volume increase upon stimulation is small. It is followed by a large volume decrease.

If the muscle is initially preshortened and allowed to contract isometrically at lengths below reference length, the volume change picture is again different (Fig. 8). As one goes successively to shorter and shorter lengths the magnitude of the volume increase becomes larger and larger and the volume decrease appears later. This was found in the previous investigation (Abbott and

Baskin, 1962) where in an end-free contraction a large volume increase and a very small volume decrease resulted. This picture becomes more pronounced as the muscle is allowed to go to shorter and shorter initial lengths until at about 6 mm below reference length the volume increase attains a large magnitude, about 5×10^{-5} cc/g of muscle, and the volume decrease measured from the zero base line becomes extremely small, less than 1×10^{-6} cc/g (Fig. 9).

The effects of change in length of an isometrically contracting muscle can

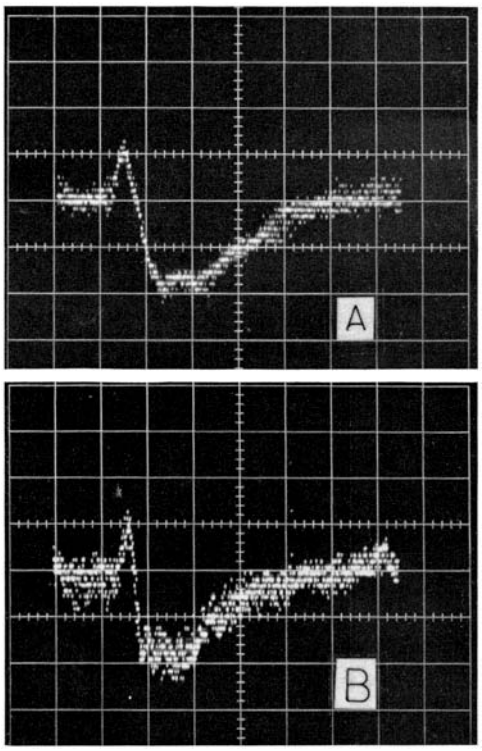

FIGURE 8. Volume changes in frog sartorius muscle: preshortened case. Isometric twitch at 24°C at (A) 95% and (B) 88% of reference length. Average of 9 waveforms. Sweep time = 10 msec/cm.

be studied in a different but analogous way. If a muscle, initially at reference length, is unfastened at one end, and is then given a series of single shocks, the volume change picture recorded for each shock shows a progressive change from a small increase followed by a large decrease to a large increase followed by a small decrease in volume.

Volume changes have also been studied using an isotonic recording system. The first result (Fig. 10) that emerges from the isotonic studies is that the volume changes (both the initial increase and decrease) are not significantly affected by the load. This is explicable since the volume increase occurs before the muscle actually lifts the load and therefore the magnitude of the volume increase is determined principally by the initial length of the muscle at the

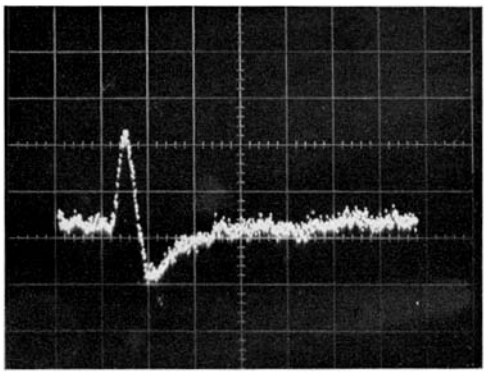

FIGURE 9. Volume changes in frog sartorius muscle: end-free case. 24°C. Average of 36 waveforms. Sweep duration = 125 msec.

time of stimulation. The volume decrease magnitude can be prolonged slightly with heavier loads but the effect is small.

Under light loads where a large amount of shortening occurs one observes a volume increase accompanying the shortening. This is the reverse of the process which occurs when a preshortened muscle is passively stretched. As the load stretches the muscle a volume decrease occurs. A typical isotonic volume change record shows a volume increase which was dependent on the initial length of the muscle, a volume decrease whose magnitude is inde-

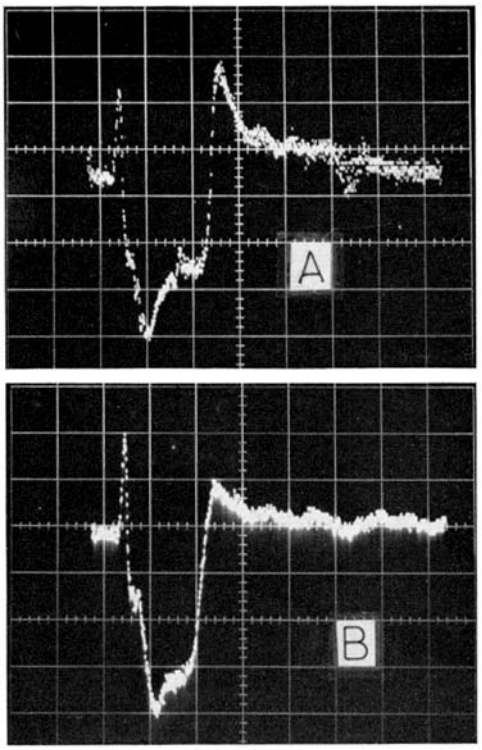

FIGURE 10. Volume changes in frog sartorius muscle: isotonic case, 24°C. (A) 25 g load; (B) 40 g load. Averages of 16 waveforms. Sweep duration = 125 msec.

pendent of load but whose duration can be prolonged with heavy loads, a volume increase accompanying the actual shortening of the muscle, and a volume decrease as the muscle returns to its initial length (Fig. 10). The isotonic results suggest three different processes causing volume changes: an initial excitation-contraction process resulting in the volume increase, a tension development process resulting in the volume decrease, and the shortening process itself resulting in a volume increase during shortening and a volume decrease during lengthening.

The temperature dependence of these volume changes has also been studied. Temperature dependence of the volume changes is approximately the same

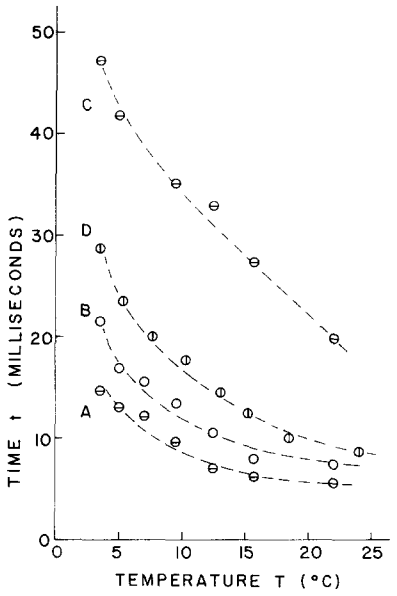

FIGURE 11. The time course of volume changes in a frog sartorius muscle during an isometric twitch: the time dependence of volume increase onset (A), maximum increase (B), and minimum decrease (C) upon the temperature T. Time t is given in milliseconds after stimulus. The temperature dependence of the time of tension onset (D) is shown for comparison.

as the temperature dependence of tension development in the muscle (Fig. 11).

In order to explore the possibility of a connection between the active state process and the volume decrease, experiments were performed in which the muscle under investigation was presoaked in Ringer's solution containing an equivalent number of iodide ions substituted for chloride ions. A portion of the results of this experiment have been previously reported (Baskin and Paolini, 1965). The time course of the volume decrease in those muscles that had been presoaked in iodide-Ringer's solution (Fig. 12) showed the same sort of prolongation that has been reported for the active state (Sandow, 1958).

The effect of iodide-Ringer's solution upon the change in volume was threefold; although the time of onset of the increase was not affected, the rate

of rise of increase was decreased; the time at which the muscle attained maximum volume was delayed; and the time at which the minimum volume occurred was delayed. That is, iodide-Ringer's solution delayed the time course of the decrease in volume.

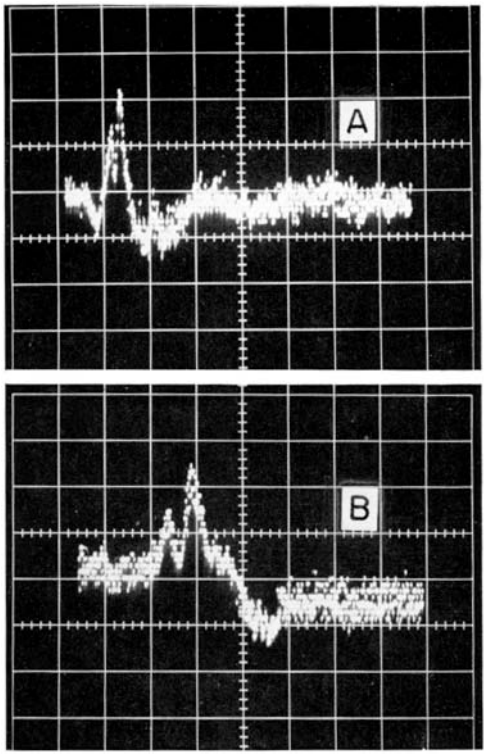

FIGURE 12.—Volume changes during an end-free twitch of frog sartorius muscle at 24°C. (A) in chloride-Ringer's; (B) following a 6.5 min soaking in 60% iodide-Ringer's. Amplitude of increase, approximately 2.5×10^{-5} cc/g of muscle. Sweep duration = 125 msec.

DISCUSSION

The Volume Increase

The volume increase appears to be related to the process which links excitation to contraction. This relationship seems plausible due to the following observations. If, during a single twitch of a frog sartorius muscle, a second stimulus is applied shortly after the first (within about 10 msec), a second volume increase appears (Abbott and Baskin, 1962). The volume decrease resulting from the first stimulus is not appreciably affected by the second stimulus, as long as it is applied no later than about 10 msec after the first. If a second stimulus is applied 13 msec or later after the first stimulus, a second volume increase and decrease result. The volume increase starts about the same time that latency relaxation occurs in frog sartorius muscle. The recorded magnitude of latency relaxation varies as a function of the initial

length of the muscle, being greater the longer the initial muscle length (Abbott and Ritchie, 1951). However, as the muscle is stretched its tension increases, and the effect of a tension relaxation is more pronounced. Thus the magnitude of the "process" causing latency relaxation may be the same per twitch, the variable being the "ability" to record the tension decrease. (The problem arises because of the fact that a muscle can pull but not push.)

While the magnitude of the volume increase varies with initial length (becoming greater at initial lengths below reference length), its initial time course is the same for all lengths. This suggests that the volume increase may tend to the same value (per twitch), its apparent variation being due to the time of onset of the volume decrease.

The Volume Decrease

Evidence for a relationship between the volume decrease and the process called the "active state" is provided by two observations. First, the time course of the volume decrease is approximately the same as that postulated for the active state (Sandow, 1958). Second, the variation in the volume decrease with iodide-Ringer's substituted for normal Ringer's is similar to the effect of iodide-Ringer's on the active state (Baskin and Paolini, 1965).

The volume decrease reaches its minimum value before the tension developed is at a maximum. This is true for both isometric and isotonic twitches but is most prominent and easily measured in isometric contractions. The magnitude of the volume decrease varies with initial length of the muscle, becoming greater for a greater initial prestretch. An explanation for this result is provided by the recent work of A. V. Hill (1964), who has shown that increasing the tension development in a muscle prolongs the duration of the active state. In the case of muscles which are prestretched, tension is initially high and is maintained longer than in muscles which are initially below reference length. Thus variation in the volume decrease with length (tension) is the same as that predicted for the active state and suggests the existence of a relationship between the two processes.

On the basis of the previous arguments it appears that the rise of active state is accompanied by a volume decrease of approximately 5×10^{-5} cc/g of muscle. In the theory of muscle contraction advanced by Davies (1963) a volume decrease would be related to bond formation between nucleotides. It must be noted, however, that the results presented in this investigation are of a nature such that it is not possible to ascribe them to a particular molecular change.

The Shortening Volume Changes

A volume decrease accompanying passive stretch of a preshortened resting muscle has been previously described (Baskin and Paolini, 1964). An analo-

gous (if not identical) volume change was observed in this investigation. In single isotonic twitches, in addition to the volume increase and decrease previously described, a second set of volume changes was observed (Fig. 10). A volume increase accompanied muscle shortening and a decrease (return to base line) accompanied the lengthening of the muscle by the load. These volume changes were a function mainly of the distance shortened by the muscle and were only slightly affected by load. These volume changes exhibit properties similar to shortening heat described by A. V. Hill (1964) and an analogy between the two phenomena may be warranted. The present investigation has established the existence of a volume increase accompanying shortening in the absence of any change in tension, and of a volume decrease accompanying the return of the muscle to its initial length, also in the absence of any appreciable tension change.

The magnitude of the (initial) volume decrease has been shown to be related to the amount of tension developed in a twitch. This relationship suggests a common molecular origin for the two phenomena. The sliding filament model of muscle contraction (Huxley and Hanson, 1954) relates tension development to degree of overlap between thick and thin filaments. A difficulty arises in this concept, however, since as a muscle shortens the degree of filament overlap increases whereas tension developed decreases. Any attempt to relate volume changes to filament overlap encounters this same problem.

The speed with which these volume changes occur during a single twitch suggests that they are indicative of molecular (structural) changes occurring in the muscle proteins. This may not be strictly true in the case of the initial volume increase. This volume change may be due to changes in ion binding as a result of excitation. In any event it is unlikely that the volume changes described in this investigation are related to slower chemical changes such as CrP breakdown.

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BIBLIOGRAPHY

ABBOTT, B. C., and BASKIN, R. J., 1962, *J. Physiol.*, **161**, 379.
ABBOTT, B. C., AND RITCHIE, J. M., 1951, *J. Physiol.*, **113**, 330.
BASKIN, R. J., 1960, Rapid volume changes in frog muscle, Ph.D. Thesis, University of California, Los Angeles.
BASKIN, R. J., 1963, *Biophysic. Abstr.*, 1963, MD13.
BASKIN, R. J., and PAOLINI, P. J., 1964, *Nature*, **204**, 694.
BASKIN, R. J., and PAOLINI, P. J., 1965, *Science*, **148**, 971.
DAVIES, R. E., 1963, *Nature*, **199**, 1068.

ERNST, E., 1962, *Rev. Canad. Biol.*, **21**, 249.
HILL, A. V., 1964, *Proc. Roy. Soc. London, Series B*, **159**, 589.
HUXLEY, H., and HANSON, J., 1954, *Nature*, **173**, 973.
SANDOW, A., 1958, *Science*, **127**, 760.