J. Physiol. (1962), **161**, *pp.* 379–391
With 5 text-figures
Printed in Great Britain

VOLUME CHANGES IN FROG MUSCLE DURING CONTRACTION

BY B. C. ABBOTT AND R. J. BASKIN*
From the Department of Zoology, University of California, Los Angeles 24, *California, U.S.A.*

(*Received* 24 *April* 1961)

The variation in volume when a muscle contracts was the subject of much discussion towards the end of the 17th century, and more recently by Fulton (1926) and by Ernst (1958). Many scholars expected an increase in volume as the vital spirits moved from the nerve into the muscle and induced activation: but Swammerdam (*ca.* 1660) experimented with an isolated muscle contained in a jar with a fine-bore tube attached and in fact observed a slight decrease in volume. Ernst (1925) first demonstrated the decrease with certainty and set the stage for many investigations of volume change in frog striated muscle. Meyerhof & Hartmann (1934) showed that the volume decrease during tetanic contraction parallelled the development of tension, and that a further decrease accompanied maintenance of the tension. They also made precise studies of the slow changes in volume which occur following mechanical activity. These were related quantitatively to the formation of lactic acid under anaerobic conditions and to the break-down of creatine phosphate in an iodoacetate-poisoned muscle. Fischer (1941), on the other hand, reported the occurrence of a volume increase as well as a decrease during contraction, but this was not confirmed by Ernst. All the authors found a volume decrease of about 0·002 % of the muscle volume during a short tetanus, but the response of the apparatus was too slow to record the precise time relationships of the change during contraction. A tetanus was necessary in order to obtain a change large enough to record; the muscles used were almost always large gastrocnemii and usually several muscles were necessary. Hill (1948) has criticized this choice: the fibres in the gastrocnemius muscle do not run parallel through the length of the muscle but instead are arranged at an angle to the axis of the muscle, so that during contraction a portion of the muscle tension would be directed at right angles to the axis of the muscle, thus producing an internal pressure. This was demonstrated by Hill and can also be substantiated by the observation in mammals that during

* Now at Department of Biology, Rensselaer Polytechnic Institute, Troy, New York.

contraction the blood flow to certain skeletal muscles is slowed and in some cases is even stopped. Such internal pressure can itself produce a volume decrease.

Ernst, Tigyi & László (1954) have recently developed a new technique for measuring volume changes, in which the gastrocnemius muscle is enclosed in a sealed chamber, one end of which is a piezo crystal. The volume changes produce a pressure change in the closed chamber which induces a voltage output across the crystal. A close correlation is reported between the volume decrease and the time course of the action potential. The change is very rapid and even in a fused tetanus a transient decrease occurs with each impulse.

The volume changes during activity have been re-investigated here for a number of reasons. A sensitive method has been developed whereby volume changes in a single sartorius muscle can be recorded throughout a single twitch; the sartorius muscle has parallel fibres and is a thin sheet of muscle, so that no internal pressure can develop. The very rapid volume change reported by Ernst *et al.* (1954) was coincident with the action potential. If it relates to muscle activity this must be the earliest mechanical event recorded in a twitch; or it could be associated with the changes at the membrane during the action potential. No further volume change was recorded between the successive transient changes, and no evidence was presented on the maintained volume decrease which accompanies a single twitch or the onset of a tetanus. Furthermore, the piezo crystal was connected directly to the input of a d.c. amplifier having a 2 MΩ input impedance. With a crystal having the sensitivity required for these measurements one would expect a decay time constant of the order of 10 msec for this circuit. This might account for the absence of other than short transient responses.

The method now available measures the volume change in terms of the movement of the surface of the liquid filling the muscle chamber. The movement produces a change in capacity between the bathing fluid and a wire in close proximity to the surface. Its response is rapid and is maintained if the change is maintained. Only changes associated with contraction have been studied, because these require a system with small inertia. For the slower changes in tetanus and recovery the earlier methods, in which a fine capillary tube was used, are adequate and possess much better long-term stability than the apparatus used in the present experiments.

METHODS

Sartorius muscles from the frog *Rana catesbiana* or *Rana pipiens* were dissected and allowed to equilibrate in oxygenated Ringer's solution for 15–30 min before any measurements of volume change were made. A limited number of experiments on gastrocnemii

were performed for comparison purposes. Both sartorius and gastrocnemius muscles were dissected with the muscle attached at the origin to a piece of bone. The muscles were mounted on a multi-electrode grid system of platinum wires, with the bone fastened in a suitable clamp and the tendon end tied to the other end of the grid. The grid wires were sealed at each end into a lucite frame continuous with the top cover of the flask (Fig. 1).

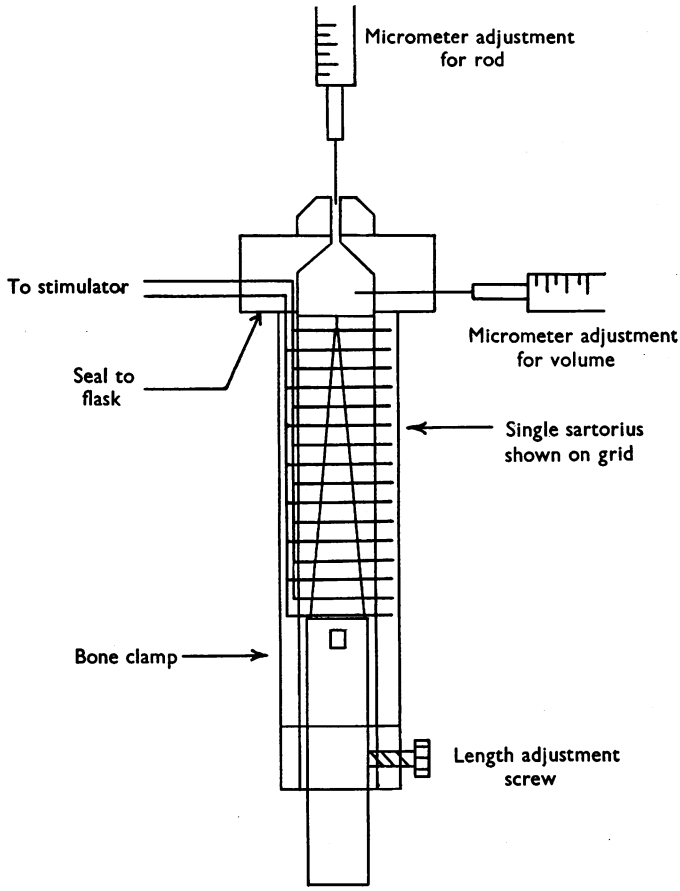

Fig. 1. Diagram of grid and cover assembly.

The electrode system consisted of a series of alternating anodes and cathodes, approximately 4 mm apart, along the entire length of the muscle. The muscle was stimulated in Ringer's solution with single rectangular pulses of 3 msec duration at a voltage adjusted for maximal response in each experiment. Provision was also made for recording the muscle action potential by means of two extra platinum wires shielded down to their points of contact with the muscle. This permitted muscle activity to be monitored in the absence of mechanical recording. The electrode systems were connected through the top cover of the flask. A hole in the top of this plastic assembly, 1 mm diameter, was used to expose the Ringer's solution and so to measure volume changes by measuring the movement of the surface of the solution. This top assembly was sealed to the top surface of a plastic tube,

ground flat and greased in order to obtain a tightly sealed chamber which when filled contained 3 ml. of fluid. The entire assembly was placed in a Dewar flask in order to assure thermal equilibrium. Experiments were made mainly at 20 and at 2° C, but also at some intermediate temperatures.

To record the volume changes both rapidly and accurately, a transducer mechanism was developed which can 'sense' a change in the level of a liquid surface relative to a wire set near it. The Ringer's solution was made to serve as the ground plate of a condenser while the wire placed near the liquid surface served as the 'live' plate. The capacitance formed by these two elements was coupled for frequency modulation of a 700 kilocycle oscillator. The signal was discriminated and amplified in a Dynagage detector and displayed on an oscilloscope.

The sensitivity of such a system is high, and the resolution for movement under the present conditions is 10^{-7} ml. The response of the system to a sudden mechanically induced volume change was 90 % complete in less than 2 msec.

The shape of the water meniscus and the method of setting the wire rod near it were both critical. After sealing the flask and filling it with Ringer's solution it was necessary to move the liquid level up to the middle of the measuring hole in the plastic-top assembly. Light mineral oil placed on top of this water surface enabled the water–oil interphase to be bulged upward by micrometer-controlled inward movement of a thin rod of pivot steel inserted through a grease seal into the chamber. A wire of diameter 0·05 cm, acting as the live plate of the capacitor, was lowered into the oil and the water surface was raised with the fine adjustment to within about 25 μ of the wire. Any movement of the meniscus was then recorded as a voltage change on an oscilloscope. The changes were calibrated by moving the micrometer-controlled rod through a measured linear displacement and recording the voltage change which resulted from the change in level of the meniscus. Sensitivity was a function of the distance between the meniscus and the rod but the same sensitivity was always used: the surface was always adjusted to give the same capacity between wire and liquid. The calibration referred only to the small range of movement used in these experiments, and the curve relating displacement and voltage change was linear over the limited range employed.

To correlate the volume changes with the other events of contraction it was necessary to record the tension changes with the muscle in the same condition as during the measurement of the volume changes. Tension and volume measurements could not be recorded at the same time, but tension was recorded as soon as possible after the volume measurement. For this the muscle was connected to the tension transducer by a steel wire which passed through the hole in the top assembly used for the capacity detector. An RCA transducer No. 5734 was used to record the tension changes during activity. The same transducer was used at much higher sensitivity to record changes early in the twitch. Latency relaxation was recorded in both the sartorius and gastrocnemius muscles for comparison with early volume changes. It was also taken as evidence that the muscles were in good physiological condition, for it was observed that the small transient tension drop decreases noticeably as the muscle deteriorates.

RESULTS

The results may best be considered in terms of the three components of the volume change which were observed—namely, an initial volume increase, a volume decrease and a return phase. In an isometric twitch at 20° C an increase in volume appears within 2–3 msec after the stimulus (Fig. 2*a*). At reference length (l_0) a peak is reached after about 6 msec and volume reaches a minimum at about 10 msec after the stimulus. At

2° C the events are all slower, the onset of the volume increase occurring after a latent period of 5 msec and a peak increase occurring after 10 msec. The volume decrease reaches its minimum after 35 msec (Fig. 3).

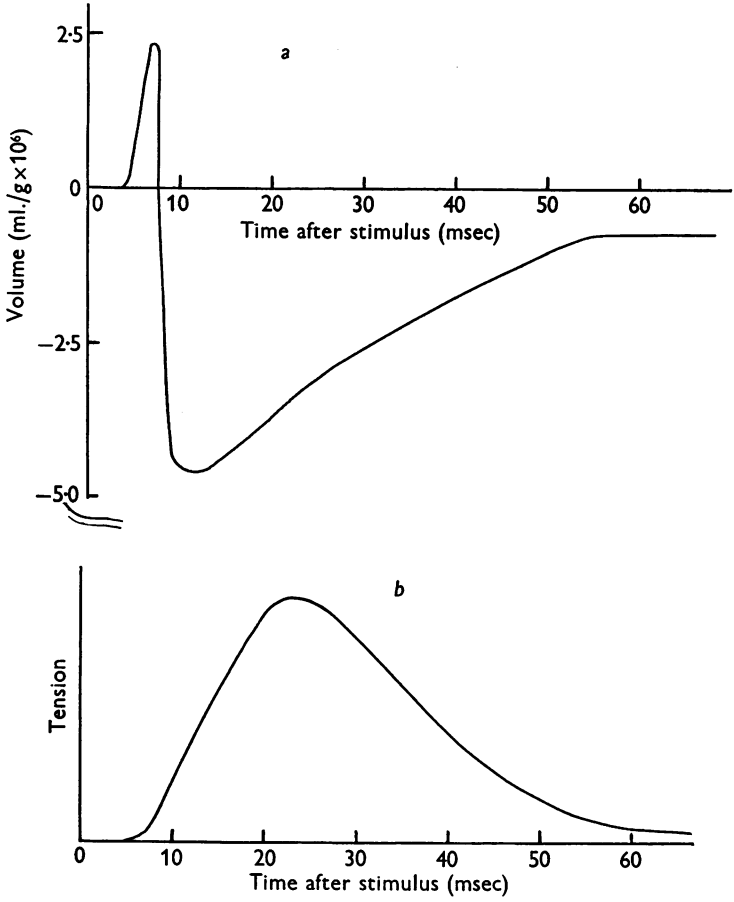

Fig. 2. *a*, Volume changes as a result of a single twitch of a frog sartorius muscle. The muscle was mounted isometrically at reference length. *b*, Tension change recorded from the same muscle as in *a*. Temperature 20° C.

The early volume increase varies with muscle length, getting smaller at lengths above the reference length l_0 (maximum in the body). The increase becomes negligible at about 1·25 l_0 in frog sartorius and at 1·35 l_0 in gastrocnemius muscle. At shorter lengths the rising phase of the volume increase follows the same initial slope (Fig. 4). When shortening is permitted the volume increase becomes larger and is greatest with unloaded shortening.

The temperature dependency of the volume increase was studied in sartorius and gastrocnemius muscles over a range 2–24° C. Over this

whole range the peak volume increase remains at about the same value while the duration of the change decreases with rise in temperature (Figs. 2 and 3). The existence of a volume increase below 4° C is an argument against the change being due to heating of the muscle or Ringer's solution; in this range heating would increase the water density and produce a volume decrease. Measurements confirming this were also made with dead muscles, inert material and with Ringer's solution alone. No change was

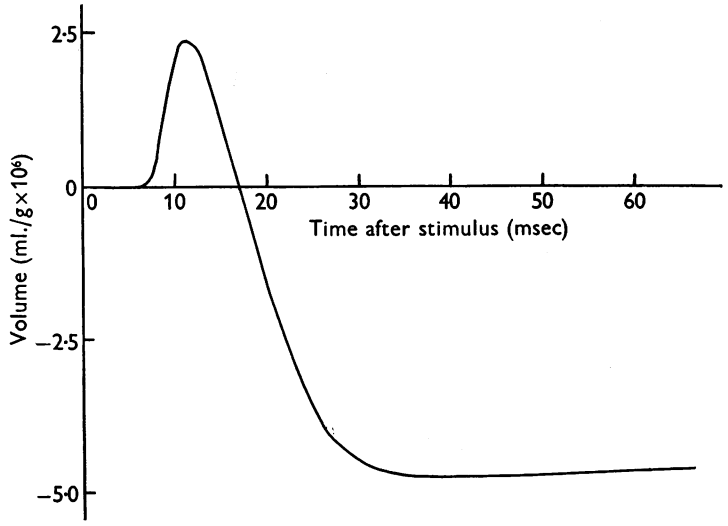

Fig. 3. Volume changes recorded from a frog sartorius muscle. The muscle was mounted isometrically at reference length. Temperature 2° C.

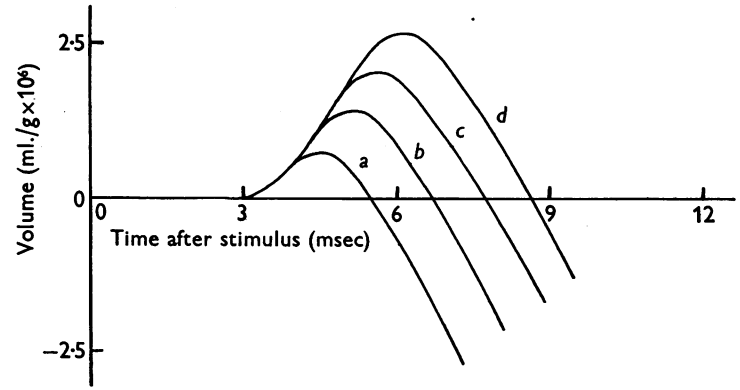

Fig. 4. Effect of change of initial length on the volume increase. *a* at l_0+4 mm, *b* at l_0+2 mm, *c* at l_0, and *d* at l_0-2 mm. The muscle was held isometrically at each length. Temperature 20° C.

detectable with the stimuli used in maximal twitches (although ten stimuli in rapid succession produced a noticeable heating effect).

The effects of a second stimulus on the volume change were also studied. If a second stimulus is applied within the latent period or during the volume increase, both the increase and the decrease are slightly augmented. If it is applied while the volume is decreasing, the second stimulus produces a repetition of the volume increase (Fig. 5) with the same latency as for the first stimulus. No appreciable effect on the magnitude of the decrease was noticeable. If the second stimulus arrived after the volume decrease has passed its peak value, a repeat increase and decrease in volume occurs.

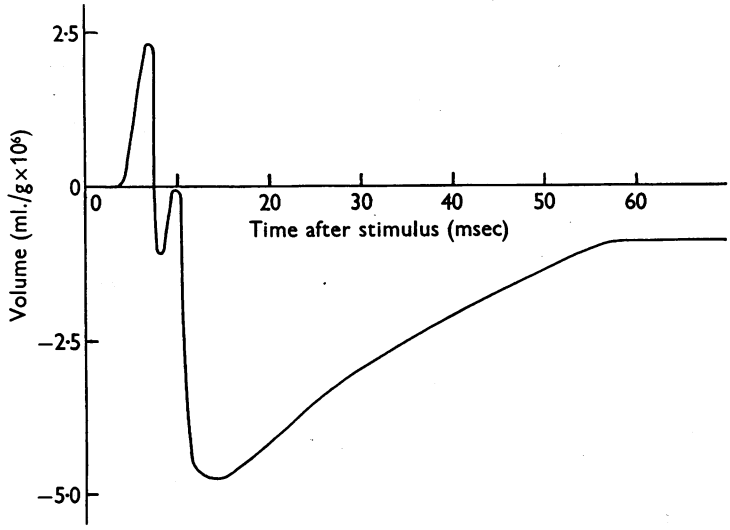

Fig. 5. Effect of multiple stimuli on the volume changes. Second stimulus was given 5 msec after the first. Muscle was held isometrically at reference length. Temperature 20° C.

After the volume has increased to a peak value it undergoes a decrease which steadily and rapidly moves to a maximal value. At 20° C this peak is reached by 10 msec after the stimulus (Fig. 2) compared with the peak tension at 25 msec. At 0° C the corresponding values are 35 msec for volume and 100 msec for the tension.

The return of volume after its decrease must be considered. The temperature-dependency of this phase is very pronounced. At 20° C the volume begins to return immediately after the peak, but about 20 % of the decrease remains at the end of the twitch. As the temperature is lowered this fraction increases until at 2° C there is no return of volume in the sartorius: the volume reaches its minimal value by 35 msec and remains there throughout the twitch (Fig. 3).

The volume increase during a twitch in the sartorius at 20° C and at reference length is about $2{\cdot}5 \times 10^{-6}$ ml./g. The volume increase in the gastrocnemius muscle is approximately the same. However, the magnitudes of the volume decrease are different in the two types of muscle. The average decrease in the sartorius muscle is $5{\cdot}0 \times 10^{-6}$ ml./g, but in the gastrocnemius muscle is much larger, approximately $1{\cdot}6 \times 10^{-5}$ ml./g. These values of the volume decrease are measured from the initial resting volume. It can be argued that the total volume decrease should be measured from the maximum of the volume increase, which would raise the magnitude of the decrease in both the sartorius and the gastrocnemius muscles by $2{\cdot}5 \times 10^{-6}$ ml./g. A more important difference between sartorius and gastrocnemius muscles can be seen in the low temperature studies. It can be seen from Fig. 3 that at 2° C the sartorius muscle shows no return from the volume decrease. This is not true of the gastrocnemius muscle, where by the end of the twitch some return of volume occurs even at low temperatures. At 2° C the return is about 1×10^{-5} ml./g, leaving a maximal net volume decrease of about $5\text{–}6 \times 10^{-6}$ ml./g. This corresponds to the maximal net decrease observed in the sartorius muscle. An explanation for the partial return of the volume in the gastrocnemius muscle even at low temperatures may be deduced from the work of Hill (1948), in which pressures of about 200 mm Hg were reported within a frog gastrocnemius muscle during contraction. Assuming the same compressibility for muscle as for water the following calculation can be made: at 4° C compressibility is about $5{\cdot}1 \times 10^{-5}$ ml./ml. × atm, so that a pressure of 200 mm of mercury would result in a volume decrease of about $1{\cdot}3 \times 10^{-5}$ ml./g. This is close to the transient component actually observed and is well within the experimental uncertainty involved in these measurements.

The volume changes described were studied only during the time of contraction of the muscle. Description of the volume decrease remaining at the end of a twitch often prompts a question as to whether the decrease persists. The change per twitch is, of course, extremely small, and will be reversed in the slower events of recovery. Such lasting decreases have already been reported by Meyerhof & Hartmann (1934). The time courses of the changes described here coincide with other known events of contraction. However, the early change described by Ernst *et al.* (1954) coincident with the action potential was never observed. This does not deny its existence because Ernst's apparatus may have been much more sensitive than that used here; but since no calibration was published it is impossible to be certain.

DISCUSSION

The time course of the early volume increase of muscle suggests that it reflects the processes linking excitation to contraction in the muscle. This coupling process appears to produce a rapid transient increase in volume, a drop in tension, called 'latency relaxation', of about 0·5–1·0 g/cm^2 (Abbott & Ritchie, 1951), and an increase in transparency of the muscle, all following about the same time course. These changes begin in the falling phase or just at the end of the action-potential spike. They appear well before any sign of positive tension develops in the muscle, before any movement has been reported and before any heat production above resting can be detected. The early changes of volume, tension and transparency all reach a peak at about the same time and are reversed as mechanical activity develops. Studies of the effect of resting muscle length on both volume (Fig. 4) and on latency relaxation (Abbott & Ritchie, 1951) suggest that two separate processes are superimposed. A first set of reactions, beginning soon after the action potential, produces the volume increase, tension drop and transparency increase. In the case of the experiment at 20° C shown in Fig. 4 the first sign of volume increase is at 3 msec. The second set of reactions linked with tension rise and volume decrease swamp the early increase. From the traces at different lengths in Fig. 4 it can be suggested that the actual volume decrease can be referred to a start at about 4 msec and follows a curve in general similar to the down-stroke of Fig. 4, curve *a*. If such a curve starting from zero at 4 msec is subtracted from curve *d* it will give an estimate of volume increase in the absence of the opposing reactions. Such a calculation shows a hypothetical curve for volume increase which is approximately exponential and reaches a plateau after about 10 msec with a volume increase twice the experimental peak value (i.e. about 5×10^{-6} ml./g). A similar curve can be deduced from latency relaxation measurements and a set of curves for frog sartorius muscle at 0° C can be seen in Fig. 3 of the paper by Abbott & Ritchie (1951).

After the volume increase event is completed, a second stimulus will induce a repetition of the increase. This again suggests that the coupling process is independent of, but precedes, the main contraction. It seemed likely that latency relaxation might also appear under the same conditions, were it not that the developed tension and rate of tension change at that point is far too great to allow the small tension decrease to be visible.

Carlson & Siger (1960) have reported the break-down of 0·29 μmole creatine phosphate/g muscle as a result of a single twitch. The experimental conditions were comparable to those used in the measurements of volume changes. Dephosphorylation of creatine phosphate yields the

volume decrease of approximately 11·5 ml./mole (Meyerhof, 1947). Therefore dephosphorylation of 0·29 μmole of creatine phosphate would yield a volume diminution of about 3×10^{-6} ml. Within the limits of accuracy of measurement this could account for the whole of the net or pressure-independent volume decrease which has been observed in both the sartorius and gastrocnemius muscles. This agreement in magnitude of volume changes between the experimentally measured amount and the amount calculated from creatine phosphate break-down is merely suggestive of a relationship between the two processes, and in no sense constitutes a proof that they are related.

The possibility of a relationship between volume decrease (or at least its development) and the active state of the muscle (Hill, 1953) is suggested by the time course of the events. Isotonic shortening in an unloaded frog sartorius muscle at 0° C begins about 20 msec after stimulation (Hill, 1949). It begins at maximal speed with multipoint stimulation, so that the muscle may be considered as fully active by that time. Ritchie (1954) found that in a twitch at 0° C the plateau of active state ended at about 25 msec after the stimulus. Sandow (1958) estimated that at 20° C the active state finishes its development 4·5 msec after the end of the latent period, which might indicate that at 0° C the active state would reach its full value about 30 msec after the stimulus (if one allows a Q_{10} for the active state process of 2·2 and a latency of about 8 msec). Jewell & Wilkie (1958), however, estimated, from tension release-recovery experiments, that the active state is not fully developed until about 60 msec after the stimulus. The volume decrease at 2° C reaches its peak at about 35 msec after the stimulus, well before the tension peak of the twitch, which occurs later than 100 msec. Thus it would seem that volume decrease is correlated with the active state rather than with developed tension. Further support is given by the result at 20° C, where peak of volume decrease occurs 15 msec before the tension peak; but here the picture is complicated by the fact that the volume decrease is partly reversed within the duration of the twitch.

Further information is given by the effects of multiple stimuli on the volume change. Between the times 10 and 35 msec after the first stimulus at 0° C a second stimulus will yield only an additional volume increase and not a further volume decrease. After this time, however, a second stimulus will result in both a volume increase and a volume decrease. It appears that when a stimulus is applied the excitation–contraction link is activated and is evidenced by a volume increase. The onset of the active state within the contractile system produces a volume decrease. This active state development, once initiated, proceeds to its full value. A second stimulus imposed during this rising phase of active state can reactivate the mem-

brane and the coupling system but cannot influence the rise of the active state. These findings strongly support the concept that in the frog sartorius muscle the full active state is reached in a twitch. They argue against the suggestion by Buchthal, Kaiser & Rosenfalk (1951) that the onset of activity is not maximal after one stimulus but that the onset is a function of stimulus frequency. On the other hand, they offer a method for study of the onset of the active state in other muscles, such as smooth phasic invertebrate muscles, where the smallness of the twitch and the very large effect of stimulus frequency on speed of shortening indicate that the active state is only partly developed by a single stimulus.

Consideration must be given to the effect of temperature on the return phase of the volume decrease. One implication of the results is that the decrease of volume in the muscle cannot be due entirely to a compression of its contents, since such a volume decrease would be expected to parallel closely the tension changes occurring within the muscle at any temperature. This 'return phase' is probably a reversal (or partial reversal) of the process which produces the volume decrease. The volume return could, however, be produced by some other process with a positive-volume change which has a high temperature coefficient. In either case the return phase is affected by temperature much more than are the other volume changes, and if this is representative of creatine phosphate resynthesis it supplies information on the temperature coefficient of this re-synthesis, a value not at present determined by other techniques.

The volume decreases reported in this paper agree in general time course and in magnitude with those reported by Meyerhof and his co-workers, as well as with other events of contraction. But no sign has been found of the very early volume decrements reported as coincident with the action potential by Ernst, who used a piezo crystal pressure transducer. In none of the available papers by Ernst on the piezo method has a calibration been found. Thus it may well be that Ernst has a sensitivity far greater than that available in the present method and is recording a much earlier change. The absence of the later changes recorded here and in Meyerhof's work may be the result of a short time constant in the crystal circuit of Ernst. Also, at the peak of a fused tetanus, where the majority of Ernst's records are reported, there may well be no change other than rapid membrane changes. Not many examples are available of the events at the onset of a contraction, but fig. D 14*a* on page 171 of the monograph by Ernst (1958) suggests that a transient response may occur then. Furthermore, this is quite different from the results in his fig. D 2 on page 161, which were obtained with the capillary method and which agree much more closely with the present results.

SUMMARY

1. A method has been described for the measurement of small, rapid volume changes occurring in a muscle during a twitch. Measurements have been made on single frog sartorius and gastrocnemius muscles.

2. In a muscle at 20° C a volume increase commences at between 2 and 3 msec after the stimulus and reaches a maximum at 6 msec. This is followed by a volume decrease which reaches its maximum at 10 msec after the stimulus. At 2° C latency is 5 msec, peak increase 10 msec and peak decrease 35 msec after the stimulus.

3. In sartorius muscle at reference length the transient increase is about $2{\cdot}5 \times 10^{-6}$ ml./g and the decrease during a twitch is about 5×10^{-6} ml./g.

4. At 20° C the volume decrease is 80% reversed by the end of the twitch, but at 2° C in the sartorius no return of volume is seen when tension has decayed completely.

5. It is suggested that the volume decrease parallels the rise of active state in the muscle.

6. A second stimulus applied during the phase of volume increase has little effect; applied during the falling phase of volume it induces a second volume increase; applied after the peak volume decrease it produces both changes again. This suggests that volume increase reflects the process linking excitation with contraction.

7. A portion of the volume decrease occurring in the gastrocnemius muscle has been correlated with the pressure developed inside the muscle during contraction.

This investigation was supported in part by a Post-Doctoral Fellowship HF-11803 from the National Heart Institute, Public Health Service, and Research Grant H-4019, Public Health Service, U.S.A.

REFERENCES

ABBOTT, B. C. & RITCHIE, J. M. (1951). Early tension relaxation during a muscle twitch. *J. Physiol.* **113**, 330–335.

BUCHTHAL, F., KAISER, E. & ROSENFALK, P. (1951). The rheology of the cross striated muscle fibre. *Biol. Medd., Kbh.*, **21**, 1–318.

CARLSON, F. D. & SIGER, A. (1960). The mechanochemistry of muscular contraction. I. The isometric twitch. *J. gen. Physiol.* **44**, 33–60.

ERNST, E. (1925). Untersuchungen über Muskelkontraktion. I. Volumänderung bei der Muskelkontraktion. *Pflüg. Arch. ges. Physiol.* **209**, 613–622.

ERNST, E. (1958). *Die Muskeltätigkeit*, 1st ed., p. 355. Budapest: Akadémiai Kiadó.

ERNST, E., TIGYI, J. & LÁSZLÓ, M. (1954). Volumverminderung und Aktionsstrom des Muskels. *Acta physiol. hung.* **6**, 171–180.

FISCHER, E. (1941). Changes during muscle contraction as related to the crystalline pattern. *Biol. Symp.* **3**, 211–236.

FULTON, J. F. (1926). *Muscular Contraction and the Reflex Control of Movement*, p. 644. Baltimore: Williams and Wilkins.

HILL, A. V. (1948). The pressure developed in muscle during contraction. *J. Physiol.* **107**, 518–526.

HILL, A. V. (1949). The onset of contraction. *Proc. Roy. Soc.* B, **136**, 242–254.

HILL, A. V. (1953). The plateau of full activity during a muscle twitch. *Proc. Roy. Soc.* B, **141**, 498–503.

JEWELL, B. R. & WILKIE, D. R. (1958). An analysis of the mechanical components in frog's striated muscle. *J. Physiol.* **143**, 515–540.

MEYERHOF, O. (1947). The main chemical phases of the recovery of muscle. *Ann. N.Y. Acad. Sci.* **47**, 815–834.

MEYERHOF, O. & HARTMANN, H. (1934). Über die Volumenschwankung bei der Muskelkontraktion. *Pflüg. Arch. ges. Physiol.* **234**, 722–729.

RITCHIE, J. M. (1954). The duration of the plateau of full activity in frog muscle. *J. Physiol.* **124**, 605–612.

SANDOW, A. (1958). A theory of active state mechanisms in isometric muscular contraction. *Science*, **127**, 760–762.