

# High Potassium and Low Sodium Contractures in Sheep Cardiac Muscle

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**ABSTRACT** Contractures develop in sheep atrial trabeculae if Tyrode's solution is rapidly replaced by a solution containing elevated potassium, reduced sodium, or both. Two phases of the contracture can be identified on the basis of differences in physiological behavior: a rapid and transient phase that predominates during the first few seconds of the contracture, and a slowly developed phase that is responsible for the steady level of tension reached later in the contracture. The transient phase is particularly prominent if the muscle is stimulated rapidly before the contracture, and reduced or absent if the muscle is not stimulated or if calcium is not present before the contracture. Recovery of the transient phase after a contracture parallels the recovery of twitches. This transient phase appears to reflect the depolarization-induced release of activator (calcium) from an internal store, possibly the same store that is involved in the normal contraction. The slowly developed tension is dependent on the contracture solution used, and is decreased if the calcium concentration is reduced or if the sodium concentration is increased. It does not depend on conditions before the contracture and does not require time to recover. This phase of the contracture may be due to entry of calcium from the extracellular solution.

## INTRODUCTION

Two general techniques have been used to study the voltage control of cardiac contraction. Contractures produced by depolarization of the membrane with high levels of extracellular potassium have been used to study the dependence of tension on voltage in the frog heart (Niedergerke, 1956; Lüttgau and Niedergerke, 1958; Lamb and McGuigan, 1966), and voltage-clamp methods have been employed by others (Fozzard and Hellam, 1968; Morad and Trautwein, 1968; Beeler and Reuter, 1970 *b*; Gibbons and Fozzard, 1971), to study contraction in mammalian ventricular muscle. Several differences are apparent between the contracture responses of amphibian heart to potassium depolarization and the contractile responses of mammalian ventricle to voltage-clamp depolarization. Potassium contractures typically have a slow onset,

and tension may be maintained for several minutes if the sodium in the contracture solution is reduced (Lüttgau and Niedergerke, 1958). Mammalian ventricle, on the other hand, gives a twitch in response to a voltage-clamp depolarization (Fozzard and Hellam, 1968; Morad and Trautwein, 1968; Beeler and Reuter, 1970 *b*; Gibbons and Fozzard, 1971). The twitch has a time-course similar to that of a contraction produced by an action potential, and does not resemble a potassium contracture. Under some voltage-clamp conditions, the twitch is followed by a steady or slowly rising tension (Morad and Trautwein, 1968; McGuigan, 1968), but it is not clear if this slow tension is physiologically equivalent to the contracture obtained in the frog heart.

It would be useful to know if the apparently different results obtained from mammalian and amphibian heart reflect species differences or differences in the way cardiac muscle responds to the two methods of depolarization. One approach to the problem is to apply both techniques to both types of tissue. Voltage-clamp methods are now being used to examine contraction in frog heart (Morad et al., 1970), but there have been few attempts to use the potassium contracture technique on mammalian heart. We undertook this investigation to determine whether, and under what conditions, potassium contractures could be obtained in sheep cardiac muscle, and to characterize the contractures for comparison with similar experiments on amphibian heart and with the voltage-clamp results obtained in mammalian heart.

In early reports of "potassium contractures" in mammalian heart (Ueno et al., 1965; Wollert, 1966; Lee et al., 1966), osmolarity of the contracture solution was kept normal by replacing all the sodium chloride in the solution with potassium chloride. A reduction in the external sodium of this magnitude can produce a contracture in frog ventricle whether or not potassium is added, so it was not clear if the responses reported in the mammal were due to the potassium depolarization or to the reduction in sodium. Since the work reported here was started, there have been two reports of experiments specifically designed to study mammalian contractures. Morad (1969) has found that contractures of cat myocardium are very small or absent unless the animals are treated with reserpine or the effects of catecholamines are blocked with propranolol. Scholz (1969 *a, b*) has obtained slowly developing contractures from mammalian atrial and ventricular muscle, but again the external sodium chloride either was completely replaced by potassium chloride or was not kept constant as the potassium concentration was increased.

Our experiments indicate that reproducible potassium contractures can be obtained from sheep cardiac muscle. Under the conditions we used, atrial trabeculae gave more consistent results than did ventricular trabeculae. In general, atrial contractures behaved much like contractures of frog ventricle. Three phases of the contractile response were apparent—a transient tension related to depolarization and to conditions before the contracture, a slowly

rising tension related to the sodium and calcium concentrations in the bathing solution, and occasionally a twitch as the contracture solution first reached the muscle. There is some similarity in the behavior of the transient phase of the contracture and that of the twitch response to an action potential or voltage clamp, but it seems likely that contractures and twitches represent slightly different aspects of the control of contraction.

#### METHODS

Sheep hearts obtained from a nearby slaughterhouse were transported to the laboratory in cold Tyrode's solution. Free running trabecular muscles were dissected from the hearts within  $\frac{1}{2}$  hr of death and kept in oxygenated Tyrode's solution at room temperature if not used immediately. The best preparations were found in the right auricle or in the right ventricle beneath the tricuspid valve. The diameters of the muscles used ranged from 0.15 to 0.5 mm.

The arrangement of the photoelectric force transducer (Hellam and Podolsky, 1969; Gibbons and Fozzard, 1971) and muscle chamber is illustrated in Fig. 1. The muscles were fastened to the transducer by tying each end of the muscle to connectors made by twisting together two strands of fine stainless steel suture wire. The connectors were threaded through small tubes on the movable and mechanical ground arms of the transducer and then bent to hold the preparations in place (inset, Fig. 1). The preparations were stretched to 140% of slack length by means of a worm gear that moved the mechanical ground arm. They were then centered in the chamber between platinum wire stimulating electrodes.

The chamber was made from a short length of acrylic tubing (Plexiglas, Rohm and Haas Co., Philadelphia, Pa.), with a 3 mm inside diameter. The top of the tubing was machined away to expose the part of the chamber where the muscle was located. If the mechanical ground arm was inset into a groove in the top of the chamber (inset, Fig. 1), surface tension kept the solution from overflowing. The chamber was wider and deeper at the downstream end to reduce the solution velocity near the movable arm of the transducer. Spent solution was removed by suction.

There were two channels for solution flow through the solution change valve. The solution that flowed through one channel was directed to the chamber; the solution flowing through the other was directed to a waste bottle. As the valve was turned, the solution originally flowing to waste was diverted to the chamber. The solution flowing through the chamber was simultaneously switched to waste. Overlapping the channels in this way kept the rate of solution flow through the chamber constant, and greatly reduced mechanical artifacts during solution changes. Dead space was minimized by positioning the valve close to the muscle. Solution flow rate during exposure to a test solution was usually 1.25–1.5 ml/sec, corresponding to a linear solution velocity of 1.8–2.1 cm/sec in the chamber. A linear solution velocity in this range should change the solution in the region occupied by a 5 mm long muscle in about 250 msec. The actual solution change took longer than this because the design of the valve and the presence of some unavoidable dead space allowed some mixing of solutions as the valve was turned. A test of the rate of solution change was made by measuring the change in conductance in the part of the chamber usu-

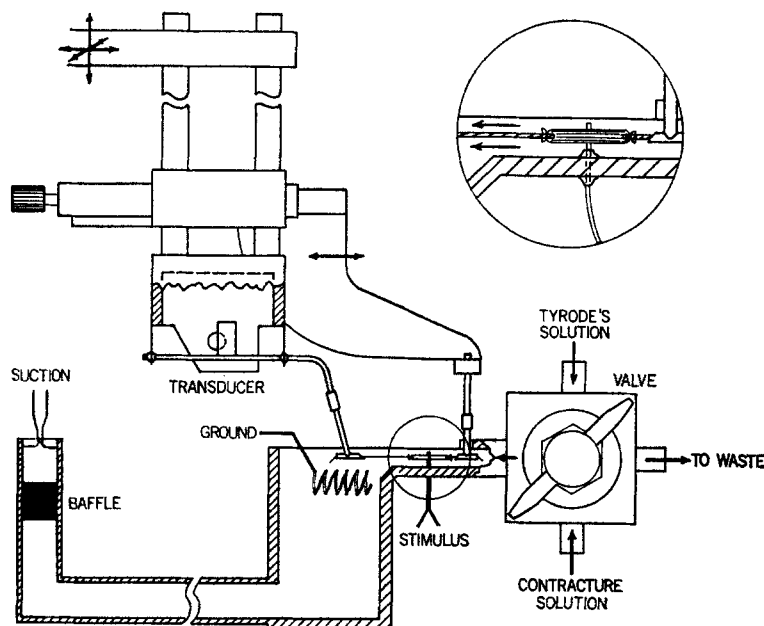


FIGURE 1. Physical arrangement of muscle chamber, transducer, and solution change valve. The valve allowed the contracture solution to flow to waste before a contracture and was designed so that solution flow was not interrupted during solution changes. The valve was turned by hand with the handle shown. Stops on the back side of the valve (not shown) stopped the valve rotation when the proper positions were reached. The body of the valve was made of stainless steel, the rotor of inert plastic (Kel-F 81, 3M Company, Chemical Products Div., St. Paul, Minn.). The chamber was made of acrylic plastic (Plexiglas, Rohm and Haas Co.). The *inset* shows in larger scale the position of the muscle and the method of attaching it to the transducer. Only one of the two platinum external stimulating electrodes is shown in the inset. The transducer has been described previously (see text for references).

ally occupied by the muscle, as we changed from one solution to another with a slightly different conductivity. This crude test indicated that the solution change was complete in about 400 msec.

Transmembrane potentials were measured using glass micropipette electrodes filled with 3 M KCl. Electrode resistance was 8–12 M $\Omega$ . Investigations of the depolarizing effects of the contracture solutions were made in atrial and ventricular muscles in separate experiments from those in which contractures were measured.

The Tyrode's solution contained (in millimoles/liter): NaCl, 137; KCl, 5.37; MgCl<sub>2</sub>, 1.05; NaHCO<sub>3</sub>, 13.5; NaH<sub>2</sub>PO<sub>4</sub>, 2.4; CaCl<sub>2</sub>, 2.7; dextrose, 11.1. The contents of the contracture solutions are listed in Table I. The use of potassium chloride to depolarize muscle requires a choice between decreasing the sodium chloride to keep solutions isotonic or working with hypertonic solutions. We chose to use hypertonic solutions for most experiments so that we could vary sodium and potassium

concentrations independently. Osmolarity of solutions was checked in every case by freezing point depression using a Fiske osmometer (Fiske Associates, Inc., Uxbridge, Mass.). Calcium chloride was added to or removed from solutions where noted, without correction for the small changes in osmolarity. The solutions were saturated with a gas mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub> to achieve a pH of 7.2–7.4. Experiments were performed at temperatures between 30° and 37°C, and care was taken to insure less than 0.2°C variation during an experiment.

The muscles were stimulated with square pulses delivered to extracellular platinum wire electrodes through a stimulus isolation unit. Pulse duration was between 2 and 6 msec (usually 2 msec) and stimulus voltage was generally 20–50% above

TABLE I  
CONTRACTURE SOLUTIONS

Solution	Sodium concentration	Potassium concentration	Sucrose concentration	Relative tonicity
	<i>mM</i>	<i>mM</i>	<i>mM</i>	
Tyrode's	153	5.4	0	1.0
1	50	5.4	147	1.0
2	50	108	0	1.0
3	50	5.4	380	1.7
4	50	260	0	1.7
5	102	5.4	306	1.7
6	102	207	0	1.7
7	153	5.4	232	1.7
8	153	155	0	1.7
9*	50	108	232	1.7

Sodium and potassium were added as NaCl and KCl in all experiments except that shown in Fig. 8. In addition to the above, the solutions each contained: MgCl<sub>2</sub>, 1.05 mM; NaHCO<sub>3</sub>, 13.5 mM; NaH<sub>2</sub>PO<sub>4</sub>, 2.4 mM; dextrose, 11.1 mM; CaCl<sub>2</sub>, 2.7 mM (unless stated otherwise).

\* This solution could also have been obtained by mixing solutions 3 and 4. It has been given a separate number for ease of reference only.

threshold. Excessive stimulus current can cause release of endogenous catecholamines from cardiac tissue (Jewell and Blinks, 1968), and such release would be expected to have important effects on contracture responses (Kavalier and Morad, 1966; Morad, 1969). In the voltage and duration ranges we used for stimulation, increasing the duration or voltage of the stimuli did not change contraction strength; decreasing either parameter either had no effect or caused the muscle to stop contracting. In fact, deliberate attempts to see effects of stimulus-induced catecholamine release were unsuccessful, apparently because the stimulator (Tektronix 161, Tektronix, Inc., Beaverton, Ore.) would not deliver enough current.

After the muscle was in place, the experimental procedure was to superfuse for some time with Tyrode's solution while stimulating the muscle, usually at 1/sec. Exposures to test solutions were started when contraction height was steady. 1–2 min before a contracture, the flow of contracture solution through the valve to waste

(Fig. 1) was increased in order to eliminate gas bubbles and to insure that freshly oxygenated solution from the reservoir was used during the contracture. The rate of flow of Tyrode's solution through the chamber was increased until it was the same as that of the contracture solution. External stimulation was stopped 5–6 sec before the solution change, so as not to distort the onset of tension with stimulated contractions. Then the muscle was superfused with the test solution, usually for 30 sec. After return to Tyrode's solution the stimulator was turned on when the muscle had relaxed, and the flow of Tyrode's solution was reduced to 10–15 ml/min. An interval of 20 min was usually allowed for recovery before exposure to the next test solution, during which time external stimulation was maintained. This time interval was sufficient for recovery of both twitches and contractures.

The transducer output was displayed on an oscilloscope with the horizontal sweep stopped and a continuous record was made with a kymograph camera. Individual contractures were also displayed on a storage oscilloscope and photographed. Experimental records used as illustrations were photographically processed and color reversed, but they were not otherwise retouched.

## RESULTS

**GENERAL CHARACTERISTICS** A representative contracture is shown in Fig. 2 *a*. It is the contractile response of an atrial muscle to a hypertonic contracture solution containing 108 mM potassium and 50 mM sodium (solution 9, Table I). After the valve was turned, a lag was usually seen before tension began to change. This latent period depended on the experimental conditions, but for the sort of contracture shown the latency ranged from 0.8 to 1.5 sec. Tension rose to a peak value with a time to half maximum of 4–6 sec. After peaking, the tension either remained constant or came gradually to a fairly stable plateau level. This plateau was observed for as long as 2 min (see Fig. 11), and the sudden relaxation seen in skeletal muscles during maintained depolarization was never observed. On return to Tyrode's solution, a delay of approximately 0.8–1.5 sec was again seen before the muscle began to relax. After this brief delay the muscle relaxed rapidly; in the experiment illustrated the half-time of relaxation varied between 2.1 and 3.0 sec.

Sometimes a brief initial twitch was observed upon exposure to the contracture solution, as though an action potential had been stimulated by sudden potassium depolarization. In a few cases, this mechanism was supported by the transmembrane recording of an action potential upstroke associated with the initial twitch. The twitch was most often seen with ventricular muscles, but it was also common with atrial muscles.

Fig. 2 also illustrates contractures in a ventricular muscle and a Purkinje fiber. Ventricular preparations were, in general, less stable than atrial muscles and sometimes showed little, if any, steady tension even when twitch height was normal. The example shown (Fig. 2 *b*) begins with a twitch similar to that described in atrial muscles, and tension then rises slowly to a maximum near

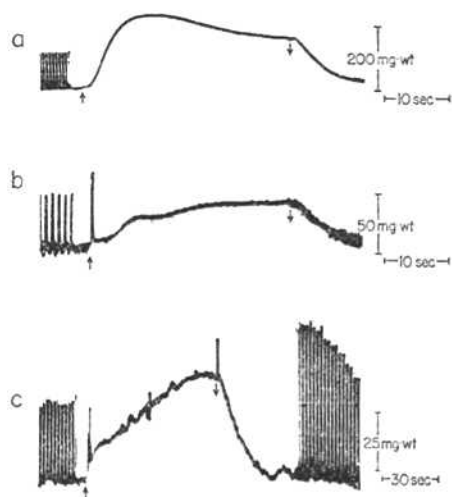


FIGURE 2

FIGURE 2. Representative contracture responses. (a) is the response of an atrial trabecular muscle to a hypertonic contracture solution containing 108 mM potassium and 50 mM sodium (solution 9, Table I). In this and in all subsequent contracture records, the upward arrow indicates the change to contracture solution and the downward arrow indicates the return to Tyrode's solution. Stimulation rate was 2.0/sec, temperature 36.5°C, muscle diameter 400  $\mu$ . (b) is the response of a ventricular trabeculum to a hypertonic contracture solution containing 183 mM potassium and 50 mM sodium (mixture of solutions 3 and 4). Stimulation rate was 1.0/sec, temperature 34°C, muscle diameter 600  $\mu$ . (c) is a contracture of a dog Purkinje fiber (false tendon) in response to a hypertonic solution containing 260 mM potassium and 50 mM sodium (solution 4). Stimulation rate was 0.5/sec, temperature 32.5°C, external fiber diameter 500  $\mu$ .

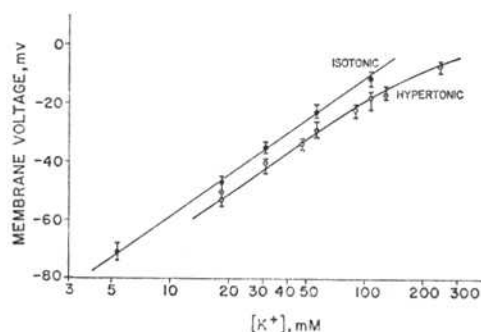


FIGURE 3

FIGURE 3. Influence of potassium concentration and osmolarity on transmembrane voltage of atrial trabeculae. The results of measurements of membrane potential are plotted as the average values obtained in isotonic solution (closed circles) and hypertonic solution (open circles). The bracketed bar indicates the range of membrane potential values obtained, with a minimum of four measurements in each of four fibers in each solution. Mixtures of solutions 1 and 2 were used for the isotonic observations, and mixtures of solutions 3 and 4 were used for the hypertonic observations. All solutions therefore contained 50 mM sodium. The slope of the curve in isotonic solution was 46 mv per 10-fold change in the concentration of potassium; the slope of the linear part of the curve in hypertonic solution was 44 mv per 10-fold change in potassium concentration. See the text for further description of the experimental technique.

the end of the 30 sec exposure to the contracture solution. There is a hump in the tension record early in the contracture suggesting the possibility that a mixture of effects may have combined to give the response. Under some circumstances it was possible to demonstrate similar behavior in the contractures of atrial muscles.

The sheep Purkinje fiber did not contract strongly enough to permit study under rapid flow conditions. The illustration (Fig. 2 c) is of an experiment on a

dog Purkinje fiber. In that experiment the gain of the tension recording was much higher than that in the other illustrations, giving a very noisy record, but the contracture seemed to resemble qualitatively those found in atrial and ventricular muscles.

When stimulation was resumed after a contracture, the first contractions obtained were often considerably larger than the steady-state twitches before the contracture. This occurred in atrial, ventricular, and Purkinje fiber preparations. The effect in atrial tissue is seen particularly clearly in Figs. 7, 8, 12, and 13. The effect looked different depending on when stimulation was resumed and on whether or not there was any spontaneous activity on returning to Tyrode's solution. Because of this variability, we did not attempt a systematic study of this phenomenon.

#### *Control Observations*

**INFLUENCE OF POTASSIUM CONCENTRATION AND OSMOLARITY ON TRANSMEMBRANE POTENTIAL** In four atrial trabeculae systematic measurements of membrane potentials were made by multiple insertions and withdrawals of the micropipette electrode after a 10 min equilibration period in the test solutions. A transmembrane potential value was accepted if withdrawal of the electrode gave a voltage change that did not differ more than 1 mv from that found on insertion. Fig. 3 illustrates the effect of varying the concentration of potassium when the sodium concentration was kept at 50 mM and the osmolality was kept normal by adding sucrose (mixtures of solutions 1 and 2, Table I). The mean voltage and the range of values obtained are given at each potassium concentration tested. The muscles depolarized as expected, but between 5.4 and 108 mM outside potassium the slope of the relation between membrane voltage and potassium was only 46 mv per 10-fold change in the potassium concentration. Hypertonic contracture solutions containing 50 mM sodium (mixtures of solutions 3 and 4, Table I) caused a hyperpolarization at each potassium concentration, as one would expect if the hypertonic solution caused the muscle to lose water. At the highest concentrations of potassium, the relationship between voltage and potassium clearly departed from linearity, but it would be expected that potassium would enter the muscles under these conditions. Transmembrane voltages in hypertonic contracture solutions containing normal sodium (mixtures of solutions 7 and 8, Table I) were examined at potassium concentrations of 31 mM, 56 mM, and 109 mM. The voltages observed fell within the range of values found in hypertonic solutions containing 50 mM sodium.

Alteration of the calcium concentration in the range of 0–5.4 mM did not change the relationship between potassium and the membrane potential when the measurements were made after brief exposure to test solutions. A similar relationship of membrane voltage to external potassium concentration was



found for ventricular muscle, except that the resting potential was usually more negative and the slope of the relationship between membrane voltage and outside potassium concentration in the isotonic solutions was 56 mv per 10-fold change in potassium.

A note of caution is necessary regarding the application of these voltage values to the contracture experiments. Although the voltage values were remarkably consistent, the conditions used were necessarily different from those during the experiments in which tension was recorded. We tried repeatedly to record voltages and tensions simultaneously, but the fast solution flow and the development of tension usually dislodged the microelectrode at some point during the contracture. We cannot, therefore, be certain that the voltages achieved during the short exposures to contracture solutions were identical to those illustrated in Fig. 3.

**REPRODUCIBILITY AND EFFECT OF FLOW RATE** The rate of development of contractures in frog ventricle is strikingly dependent on the rate at which Ringer's solution is replaced by contracture solution (Lamb and McGuigan, 1966). The rate at which solutions were changed in our system depended, of course, on the rate of solution flow through the chamber. Fig. 4 illustrates the results of an experiment in which we examined the effect of flow rate on atrial contractures. The contracture solution contained 108 mM potassium, 50 mM sodium, and 232 mM sucrose (solution 9, Table I). The contracture did not change when we reduced the flow of contracture solution from 1.7 ml/sec to 0.9 ml/sec, but a further reduction of flow rate to 0.6 ml/sec (third trace, Fig. 4) clearly reduced the rate of tension development. The rate of flow used in our experiments was usually 1.25–1.5 ml/sec. At these flow rates, contractures were quite reproducible; the fourth contracture shown in Fig. 4 was obtained at a flow rate of 1.5 ml/sec, and it is almost identical to the first record obtained 100 min earlier.

The first contracture in Fig. 4 was about the same height as the twitches obtained at a stimulation rate of 1.0/sec. This was usually true when the muscle was first mounted in the chamber. In many experiments the twitch size gradually decreased with time while the contractures continued to be reproducible. This can be seen clearly in the experiment illustrated in Fig. 4; 100 min after the first contracture, the twitches were much smaller than the contracture. This behavior is similar to that reported by Lamb and McGuigan (1966) for frog ventricle.

Almost all preparations that gave good twitch responses also gave good contractures. Occasionally, however, a freshly isolated muscle would show good twitch responses and quite small contractures, and in one or two experiments a muscle that had previously given good contracture responses suddenly ceased to do so. The latter problem we assumed to be due to deterioration of

the preparation, but we were unable to find any reasonable explanation for the failure of a few fresh preparations to give contractures of reasonable size.

**EFFECTS OF HYPERTONICITY AND CHLORIDE ON CONTRACTURES** Since hypertonic solutions influence twitch size in guinea pig atria (Little and Sleator, 1969) and contracture tension in the frog heart (Lamb and McGuigan, 1966), we felt it necessary to determine the effect of hypertonicity in these experi-

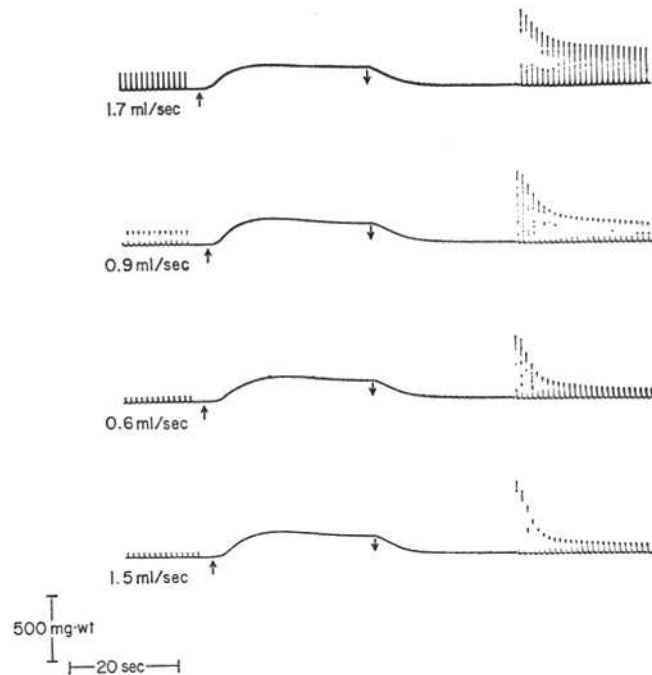


FIGURE 4. Effect of rate of flow on contractures in atrial muscle. Four contractures in response to a hypertonic solution containing 108 mM potassium and 50 mM sodium (solution 9) are shown. The rate of solution flow through the chamber is indicated below each record. The records were obtained in the order shown. Stimulation rate was 1.0/sec, temperature 36°C, muscle diameter 325  $\mu$ .

ments. A useful comparison between hypertonic and equivalent isotonic solutions could be made only in 50 mM sodium solutions. When muscles were exposed to hypertonic contracture solutions (Fig. 5 *a*) the maximal contracture tension was somewhat higher, usually about 20%, than the contracture tension obtained in an otherwise identical isotonic solution (Fig. 5 *b*). A small contracture response developed if muscles were exposed to solutions containing 50 mM sodium and normal potassium (see below and Fig. 13), and hypertonicity also caused a small increase in the size of such low sodium responses (Fig. 5, *c* and *d*). Except for the increase in tension, the time-course and be-

havior of the contractures did not seem to be altered by the hypertonic solutions.

Chloride permeability is not usually very large in heart muscle (Hutter and Noble, 1961; Fozzard and Sleator, 1967), although under certain conditions a substantial chloride current can be observed (Dudel et al., 1967). The use of potassium chloride in the contracture solutions resulted in a change in KCl product. To be sure that the increased KCl product did not influence our contracture results, the experiment in Fig. 6 was performed, comparing the contracture obtained with an altered KCl product (Fig. 6 *a*) to that obtained

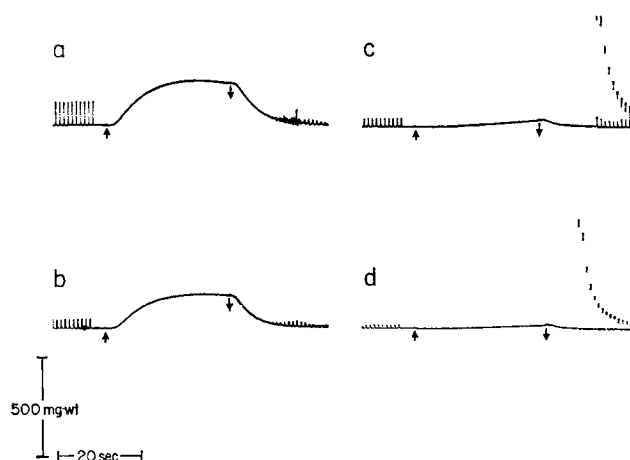


FIGURE 5. Effect of hypertonicity on contractures in atrial muscle. (*a*) and (*b*) are contractures in response to solutions containing 108 mM potassium and 50 mM sodium. The solution used in (*a*) was made hypertonic by the addition of 232 mM sucrose (solution 9); that used in (*b*) was isotonic with Tyrode's solution (solution 2). (*c*) and (*d*) are responses to solutions containing normal (5.4 mM) potassium and 50 mM sodium. The solution used in (*c*) was hypertonic (solution 3); that used in (*d*) was isotonic with Tyrode's solution (solution 1). Stimulation rate was 1.0/sec, temperature 36°C, muscle diameter 475  $\mu$ .

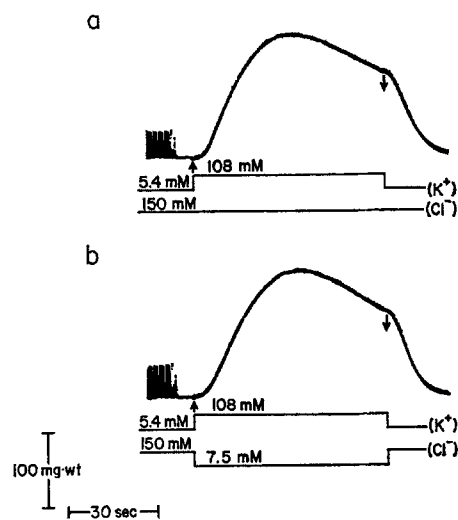
when chloride was replaced with acetylglycinate to maintain a constant KCl product (Fig. 6 *b*). There was no detectable effect of chloride concentration under these conditions.

#### *Effects of Altering Conditions Before Contractures*

The conditions imposed on muscles before exposure to contracture solutions can have a substantial effect on the contracture response. In particular, Hodgkin and Horowitz (1960) demonstrated the importance of recovery time in frog skeletal muscles, and Niedergerke (1956) pointed out that the size and shape of potassium contractures of frog ventricle depend on the stimulation rate before the contracture. The influence of conditions preceding the contracture were examined in the following experiments.

**INTERVAL BETWEEN CONTRACTURES** Atrial trabeculae required a 15–20 min period of recovery after a contracture before another exposure to the contracture solution would produce the same response. Fig. 7 illustrates the contracture responses to shorter recovery periods in 2.7 and 5.4 mM calcium. Short recovery intervals markedly decreased the initial rate of tension development but the final level of tension reached changed very little.

The time-course of recovery of twitches after a contracture can also be seen in Fig. 7. Immediately after the contracture, twitches were often larger than



**FIGURE 6.** Effect of maintaining a constant  $[K] \times [Cl]$  product during contractures. Both contractures were obtained in response to isotonic contracture solutions containing 108 mM potassium and 50 mM sodium (solution 2 and solution 2 modified as described below). During the contracture in (a), the product  $[K] \times [Cl]$  was allowed to change as usual. In the contracture solution used for (b), all but 7.5 mM of the chloride was replaced by acetylglucinate so the product  $[K] \times [Cl]$  did not change during the contracture period. Stimulation rate was 1.0/sec, temperature 30°C, muscle diameter 300  $\mu$ .

the control twitches before the contracture. Over the next 3–4 min, twitch height declined to a minimum. It then recovered gradually for 10–15 min and was almost always at a steady level 20 min after a contracture.

Recovery of twitches and recovery of the initial rate of contracture development appeared to follow a similar time-course, with the slowest rate of development of contracture tension being obtained when twitch height was small. Moreover, in the experiment illustrated in Fig. 7, twitches were still declining 1 min after a contracture, and the contracture (9) obtained at this time developed somewhat more rapidly than those obtained after 2 or 5 min of recovery. In the same experiment, we examined recovery in 5.4 mM calcium.

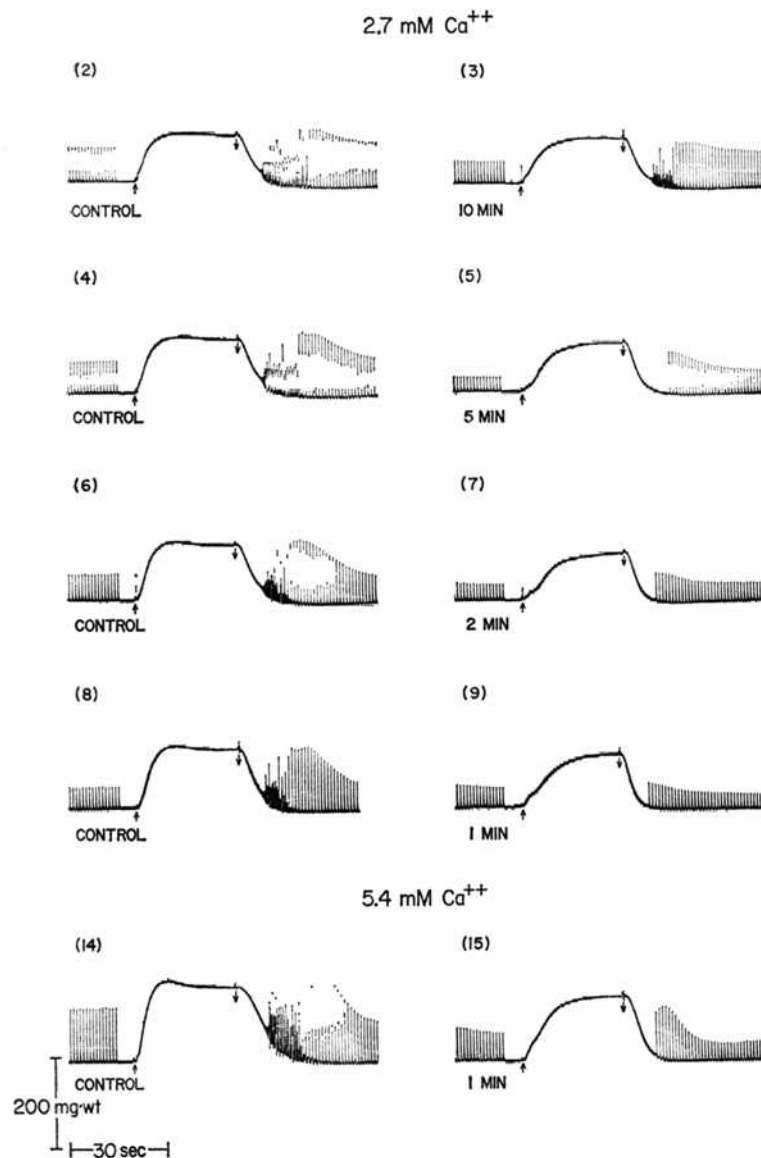


FIGURE 7. Influence of recovery time on contracture responses in atrial muscle. Eight consecutive contractures are shown in the upper part of the figure; they were obtained in response to a hypertonic contracture solution containing 108 mM potassium and 50 mM sodium (solution 9). Both the contracture solution and the Tyrode's solution contained 2.7 mM calcium. The small number in the upper left of each record indicates the order in which the record was obtained (the first is not shown). The interval between contractures was alternated between 20 min and 10-1 min. The contractures on the left (designated as *controls*) are those obtained at the end of the 20-min intervals. The responses on the right followed the control contractures at the intervals shown below the records. After the ninth contracture, the calcium concentration in the Tyrode's and contracture solutions was increased to 5.4 mM, and recovery in the higher calcium was tested at 10-, 5-, 1-, and 2-min intervals. Only the control and the contracture after the 1 min interval are shown. Stimulation rate was 1.0/sec, temperature 35°C, muscle diameter 500  $\mu$ .

The rate of development of contractures was increased at all times tested, but the increased calcium had little if any effect on the time necessary for recovery of twitches and contractures or on the relation observed between twitch and contracture recovery. A control and the contracture obtained after 1 min of

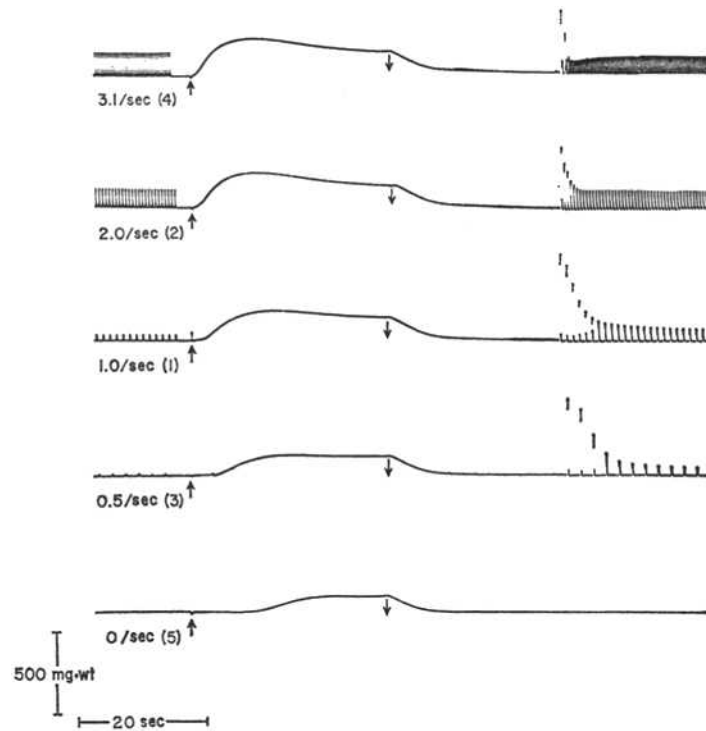


FIGURE 8. Influence of stimulation rate on contractures in atrial muscle. Each of the five contractures shown was obtained in response to a hypertonic contracture solution containing 108 mM potassium and 50 mM sodium (solution 9). The stimulation rate for the 15 min period before each contracture is indicated below each record. The number in parentheses following the stimulation rate indicates the order in which the observations were made. Temperature 36°C, muscle diameter 325  $\mu$ .

recovery in the higher calcium are shown in the bottom two traces of Fig. 7 (14 and 15).

**EFFECT OF PRIOR STIMULATION RATE** To see if changes similar to those described by Niedergerke (1956) occur in mammalian atrial muscle, we examined contractures elicited after steady stimulation at rates between 0 and 3.1/sec, as illustrated in Fig. 8. The same hypertonic contracture solution (solution 9, Table I) was used for each contracture.

The effect of stimulation rate on twitch size can be seen before each contracture. Peak twitch tension increased as frequency was increased from 0.5 to

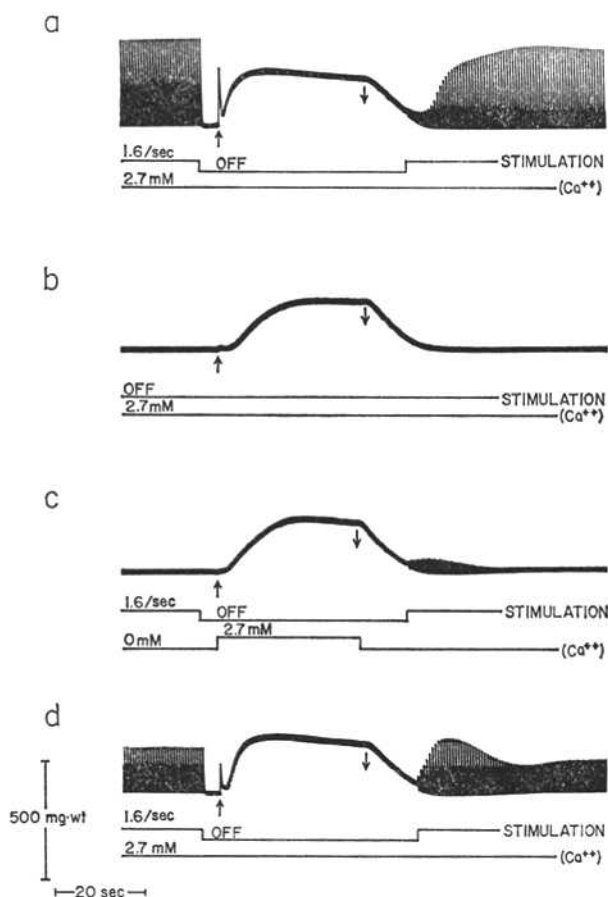


FIGURE 9. Influence of prior conditions on contracture responses: comparison of absence of stimulation with absence of calcium. Each of the four contractures shown was obtained in response to a hypertonic contracture solution containing 183 mM potassium and 50 mM sodium (mixture of solutions 3 and 4). The calcium concentration in the contracture solution was 2.7 mM. The contractures were obtained in the order presented, but they were not obtained consecutively. (a) and (d) were controls; before each, the muscle had been stimulated at 1.6/sec in Tyrode's solution. The stimulation was shut off 6 min before contracture (b), but conditions were otherwise the same as for the controls. Stimulation before contracture (c) followed the same pattern used for the controls, but 13 min before the contracture the solution bathing the preparation was changed to Tyrode's solution without calcium. Temperature 30°C, muscle diameter 300  $\mu$  (same experiment as in Fig. 10).

3.1/sec, as it does in other atrial muscle preparations (Kruta, 1937; Koch-Weser and Blinks, 1963). The rate at which contracture tension developed was strikingly dependent on the prior stimulation rate. When there was no stimulation before the contractures, there was a long delay before tension began to develop, and the rate of development was slow. Similar, but less striking, changes occurred in other experiments (see, for example, Fig. 9). As the

stimulation rate before the contracture was increased, the contractures developed faster, and peak tension became greater and occurred sooner. At the end of 30 sec, however, the tension was essentially the same regardless of the prior stimulation rate.

**EFFECTS OF CALCIUM LACK BEFORE CONTRACTURES** The changes in the time-course and peak tension of contractures seen as we changed the stimulation rate suggest that the tension developed early in the contracture can be suppressed or enhanced by conditions that have little effect on the tension developed late in the contracture. A similar pattern of changes also occurred in the experiment on recovery of the contracture (Fig. 7); short recovery times decreased the rate of contracture development while changing the final tension level very little. In both experiments there appeared to be a parallel between the size of twitches before the contracture and the rate of development of contracture tension.

We investigated this further in the experiment illustrated in Fig. 9, comparing conditions in which muscles were not stimulated before the contracture with conditions in which the preparation was stimulated at 1.6/sec, but in Tyrode's solution with no added calcium. The contracture solution used was hypertonic, and contained 183 mM potassium, 50 mM sodium, and 2.7 mM calcium (mixture of solutions 3 and 4, Table I).

Traces (a) and (d) of Fig. 9 are control contractures with prior stimulation at 1.6/sec. Fig. 9 b shows a contracture following a 6 min period without stimulation. As in Fig. 8, the rate of development of tension was less than that of the control and the early peak of the contracture disappeared. Interestingly, the initial twitch seen in the controls also disappeared. 13 min before the contracture shown in Fig. 9 c, the Tyrode's solution was changed to one without calcium, but stimulation was maintained at 1.6/sec as in (a) and (d). The contracture was virtually identical to that obtained following exposure to normal calcium in the absence of stimulation, with no initial twitch, slow development of tension, and the same final tension level.

#### *Effects of Altering Conditions during Contractures*

In the preceding experiments, recovery time, stimulation rate, and calcium concentration were each altered before contractures, but the same contracture solution was used throughout each experiment. There were dramatic alterations in the tension developed early in the contracture and in the rate of development of contractures, but the tension level reached at the end of the contracture was essentially unchanged during each experiment. This pattern of changes suggested to us that contractures could be regarded as the result of a relatively rapid, but transient, tension response—strongly dependent on conditions existing before the contracture—added to a more slowly developed



tension responsible for the level reached later in the contracture. Presumably, the slowly developed response would depend on the solution used to obtain the contracture rather than on conditions before the contracture. When the muscle is not stimulated before a contracture, when it is stimulated but calcium is not present, or when it is given insufficient time to recover from a previous contracture, the transient tension response is suppressed and the contracture obtained reflects primarily the slowly developed tension. The existence and approximate size of the transient response is inferred from the difference between the control contractures and those in which only the slowly developed tension is seen. The behavior of contractures and the suggested division of the contracture into "transient" and "slowly developed" components was further examined by varying the calcium, potassium, and sodium concentrations of contracture solutions.

#### EFFECTS OF ALTERING CALCIUM CONCENTRATION DURING CONTRACTURES

In another set of observations made during the same experiment shown in Fig. 9, we changed the calcium concentration during contractures (Fig. 10). The calcium concentration in the Tyrode's solution was 2.7 mM before each contracture. The contracture solution was the same as that used in Fig. 9, except that calcium concentrations of 0 mM, 2.7 mM, and 5.4 mM were used.

Before the contractures shown in panels (a), (b), and (c) of Fig. 10, the muscle was stimulated at 1.6/sec. During the control contracture (Fig. 10 a) the calcium concentration was 2.7 mM. During the contracture in Fig. 10 b, the contracture solution contained no added calcium. The initial rate of tension development was similar to that of the control, the peak tension was slightly less, and after the peak of the contracture, tension declined much more rapidly than in the presence of calcium. When a calcium concentration of 5.4 mM was used during the contracture (Fig. 10 c), the level of tension reached during the contracture was slightly greater than the control. The initial twitch in 5.4 mM calcium was larger and relaxed more completely than it did in the first two contractures, but the initial twitch was too variable for us to see if this occurred consistently. Once the contracture began, the initial rate of tension development was almost exactly the same as in the contractures in lower calcium.

In the contractures shown in panels (d), (e), and (f) of Fig. 10, calcium was varied in the same way as in the first three records, but the muscle was not stimulated before the contracture. Presumably, this should suppress the transient response and show more clearly effects of calcium on the slowly developed tension. The contracture in 5.4 mM calcium (Fig. 10 f) differed little, if any, from the control in Fig. 10 d. However, if no calcium was added to the contracture solution (Fig. 10 e), the rate of tension development was markedly less than that of the control and tension was small and declining slowly at the end of 30 sec.

Since altering the concentration of calcium during contractures changed the sustained tension level, we arranged an experiment to test more directly the effect of external calcium concentration on this phase of the contracture. Fig. 11 *a* is a record of a contracture in response to a hypertonic solution containing 108 mM potassium and 50 mM sodium (solution 9, Table I). The contracture

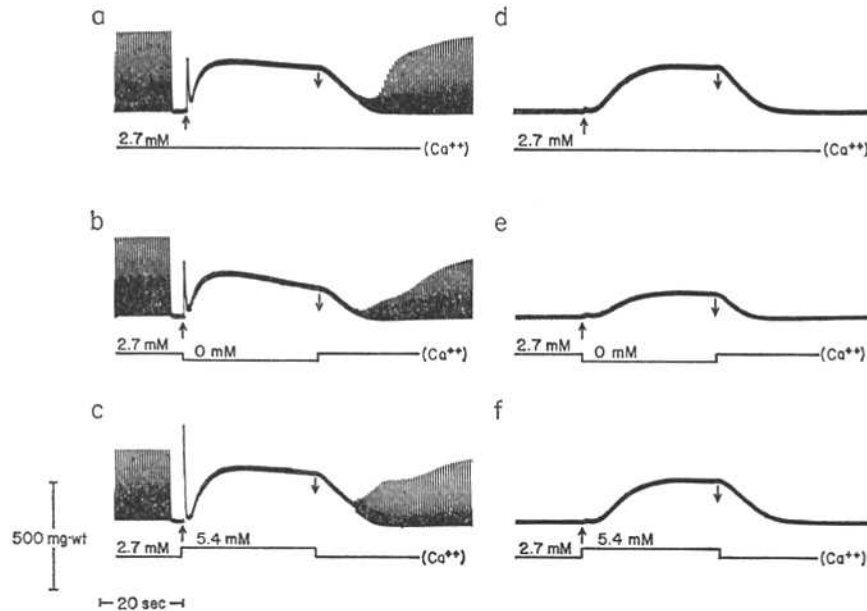


FIGURE 10. Effect of different calcium concentrations in the contracture solutions in the presence and in the absence of prior stimulation. All contractures were obtained in response to a hypertonic solution containing 183 mM potassium and 50 mM sodium (mixture of solutions 3 and 4). During most of the 20 min recovery period between contractures, the preparation was stimulated at a constant rate of 1.0/sec. 2 min before each of the contractures (*a*), (*b*), and (*c*), the stimulation rate was increased to 1.6/sec. 6 min before each of the contractures (*d*), (*e*), and (*f*), the stimulation was shut off. The calcium concentration in the contracture solution used for contractures (*a*) and (*d*) was 2.7 mM; no calcium was added to the contracture solution used for (*b*) and (*e*); and there was 5.4 mM calcium in the contracture solution used for contractures (*c*) and (*f*). The Tyrode's solution bathing the preparation before and after each contracture contained 2.7 mM calcium. Temperature 30°C, muscle diameter 300  $\mu$ .

was maintained for 135 sec, and 40–60 min was allowed for recovery between contractures. When the contracture was maintained for this length of time, the over-all shape of the response was itself suggestive of a transient tension added to a slowly developed tension, in that there was an early peak, a partial relaxation, and then a slow development of tension to a stable level. In Fig. 11 *b*, after the slow secondary increase in tension had started, the contracture solution was abruptly changed to one with no added calcium and tension promptly

began to decline. After 45 sec of superfusion with 0 mM calcium, the calcium concentration was returned to 2.7 mM, and tension increased. At the end of the contracture, tension was essentially the same as at the end of the control contracture.

**EFFECT OF POTASSIUM CONCENTRATION ON CONTRACTURES** Fig. 12 illustrates the effect of increasing the concentration of potassium in hypertonic contracture solutions containing 50 mM sodium (mixtures of solutions 3 and 4, Table I). The calcium concentration in the Tyrode's solution and in the con-

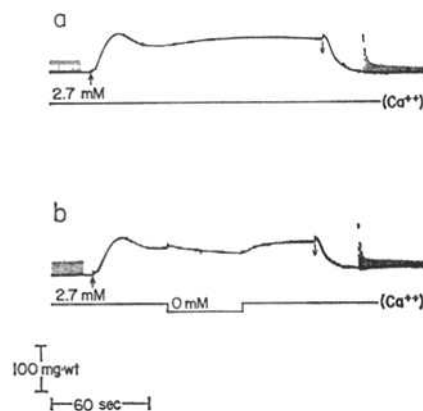


FIGURE 11. Effect of altered calcium concentration on the steady level of contracture tension. The contracture in (a) was obtained upon exposure to a hypertonic contracture solution containing 50 mM sodium, 108 mM potassium, and 2.7 mM calcium (solution 9). The same solution was used for the contracture in (b), but after the end of the transient tension response, the contracture solution was changed to an identical solution without added calcium. This solution flowed during the time shown by the break in the line indicating calcium concentration, and the original contracture solution was then re-admitted to the chamber. As in the other figures, the arrows indicate the beginning of exposure to contracture solution and the return to Tyrode's solution. Stimulation rate was 1.0/sec, temperature 36°C, muscle diameter 350  $\mu$ .

tracture solutions was 2.7 mM in the contractures on the left and 6.3 mM in those on the right.

The twitches at a stimulus rate of 1.0/sec were larger in the higher calcium, as expected. At 5.4 mM potassium, a small contracture developed in the low sodium solution, and the size of this response was slightly greater in the higher calcium. At 18 mM potassium, the contractures increased in size, and again contracture tension was slightly greater in the higher calcium. As the potassium concentration was increased, contracture tension increased and there was little difference in the peak tensions in the two calcium concentrations, although contractures consistently developed faster in 6.3 mM calcium. If only peak tensions are considered, the results in 6.3 mM calcium indicate a slight

shift in the relation between contracture tension and voltage toward more negative voltages. Comparing only peak tensions, however, obscures the fact that as the potassium concentration was made greater than 56 mM, the changes

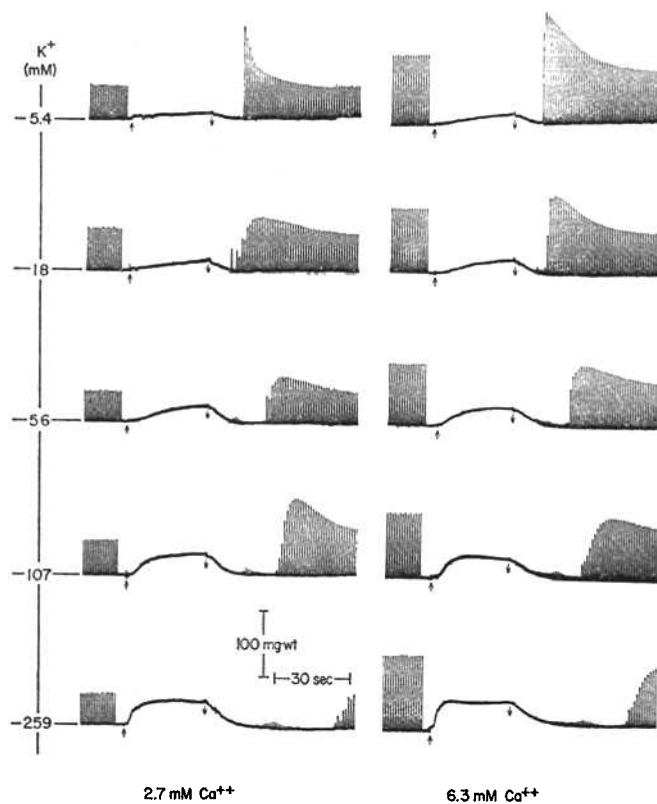


FIGURE 12. Effects of increased calcium on contractures. The contractures on the left were responses to hypertonic contracture solutions with 50 mM sodium and various potassium concentrations (mixtures of solutions 3 and 4). The calcium concentration was 2.7 mM in both the Tyrode's and contracture solutions. The contractures on the right were obtained during exposure to solutions that were the same except that the calcium concentration was 6.3 mM in the Tyrode's solution and in the contracture solution. The scale on the left indicates the potassium concentration in the contracture solutions used to obtain each pair of records. The responses on the left were recorded first, those on the right were obtained after the increase in calcium concentration. Stimulation rate was 1.0/sec, temperature 30°C, muscle diameter 375  $\mu$ .

in the rate of development of tension were greater than the changes in the peak tension. This observation is also demonstrated in the experiment illustrated in Fig. 13.

**EFFECT OF SODIUM CONCENTRATION ON CONTRACTURES** The relation between contracture tension and membrane voltage in frog ventricle depends

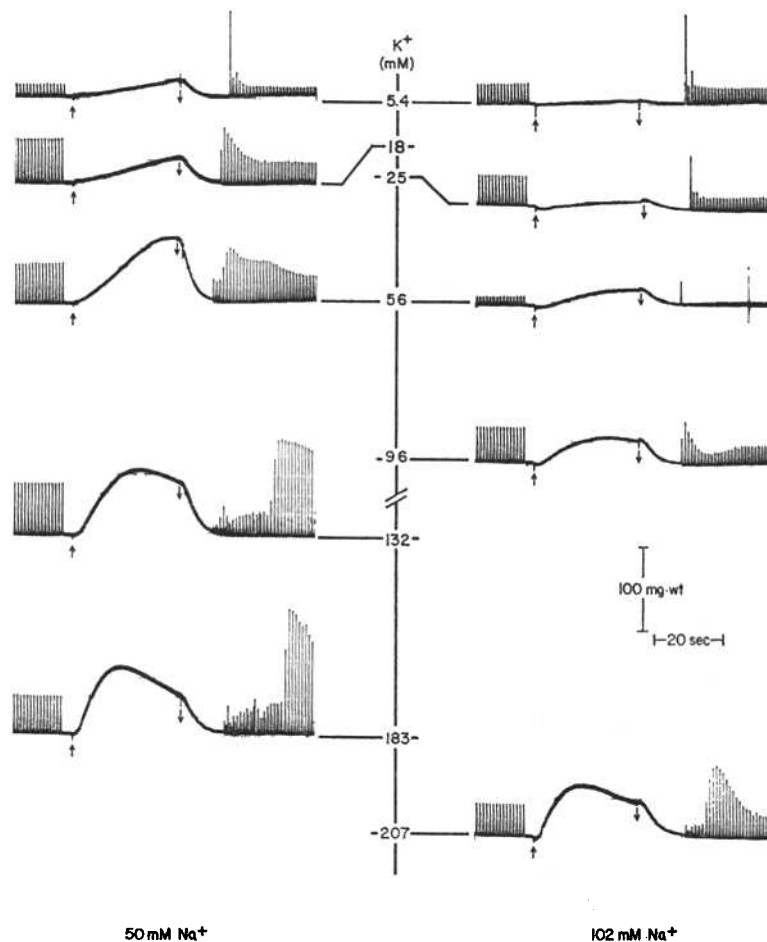


FIGURE 13. Comparison of contractures obtained at different sodium concentrations. The records on the left were obtained in response to hypertonic contracture solutions containing 50 mM sodium (mixtures of solutions 3 and 4). Those on the right were obtained in response to hypertonic contracture solutions containing 102 mM sodium (mixtures of solutions 5 and 6). The ordinate in the center indicates the potassium concentration used for each response. No particular pattern was followed in exposing the muscle to different potassium or sodium concentrations; twitch height was smaller later in the experiment than in the records obtained earlier. Tyrode's solution with normal sodium bathed the muscle before and after each contracture. Stimulation rate was 1.0/sec, temperature 30°C, muscle diameter 300  $\mu$ .

on the sodium concentration as well as on the calcium concentration (Lüttgau and Niedergerke, 1958; Lamb and McGuigan, 1966). Fig. 13 shows a sequence of contractures in response to different potassium concentrations at sodium concentrations of 50 and 102 mM; all contracture solutions were hypertonic (mixtures of solutions 3 and 4, and of solutions 5 and 6, Table I). The

slow contracture seen at normal (5.4 mM) potassium is much smaller in 102 mM sodium. In 50 mM sodium, as in Fig. 12, peak tension increased little at potassium concentrations greater than 56 mM, but the shape of the contracture continued to change dramatically as the potassium concentration was made larger. In 102 mM sodium, both the peak tension and the rate of tension development increased up to the highest potassium concentration that we could test.

The results of a similar experiment, in which contractures were obtained both in normal (153 mM) and in 50 mM sodium are presented in Fig. 14. Peak

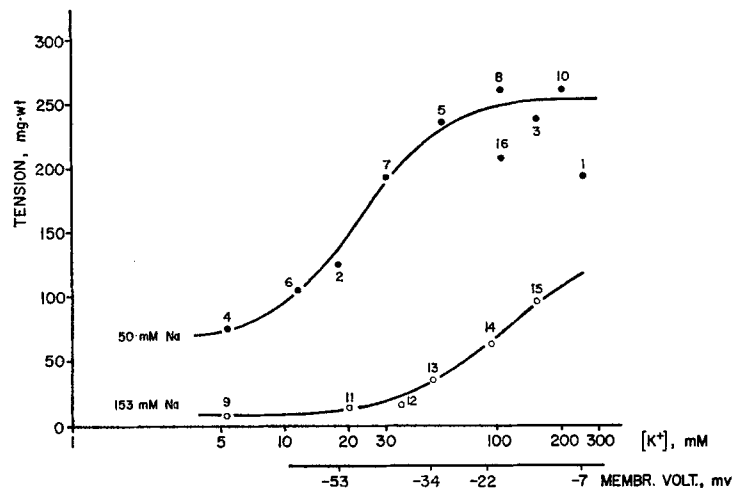


FIGURE 14. Relation between peak contracture size and potassium concentration. Peak contracture tensions recorded during an experiment similar to that illustrated in Fig. 13 are plotted as a function of potassium concentration. The sodium concentration in the contracture solution was either 50 mM (mixtures of solutions 3 and 4) or 153 mM (mixtures of solutions 7 and 8). The small numbers next to the data points indicate the order in which the observations were made. The voltage values on the lower abscissa were obtained from the data shown in Fig. 3. Stimulation rate before contracture was 1.0/sec, temperature 32.5°C, muscle diameter 350  $\mu$ .

contracture tensions are plotted as a function of external potassium concentration. An approximate scale of membrane voltages is given, taken from the data of Fig. 3. The contracture records underwent a pattern of changes similar to those seen in Fig. 13 as the potassium was increased. The curve for 50 mM sodium shows no clear threshold for tension development, since there was a slow contracture at this sodium concentration even when the potassium concentration was not increased. A clear tension threshold was seen in 153 mM sodium. Peak contracture height was still increasing at the highest potassium concentration examined (155 mM), a typical finding in normal sodium.

Several experiments were performed in 50 mM sodium. The results of these

experiments are summarized in Table II in which the potassium concentration necessary for half-maximal tension is given. For comparison, the measurements obtained in four experiments on ventricular trabeculae are also included. The potassium concentration that gave half-maximal tension in atrial muscle averaged 28.5 mM. In spite of the different shape of ventricular contractures, the same average value was obtained in this tissue.

The dependence of the potassium-tension curve in atrial muscle on extracellular sodium is illustrated by the potassium concentration for half-maximal tension found for various sodium concentrations. In addition to the average value of 28.5 mM K<sup>+</sup> at 50 mM Na<sup>+</sup> given in Table II, we obtained the following values at other sodium concentrations: 10 mM K<sup>+</sup> in 16 mM Na<sup>+</sup>; 51 mM K<sup>+</sup> in 76 mM Na<sup>+</sup>; 84 mM K<sup>+</sup> in 101 mM Na<sup>+</sup>; 102 mM K<sup>+</sup> in 153 mM Na<sup>+</sup>.

TABLE II  
POTASSIUM CONCENTRATIONS FOR HALF-MAXIMAL  
CONTRACTURE

	Atrium	Ventricle
	mM	mM
	42	26
	20	18
	20	34
	32	36
Average:	28.5	28.5

Atrial and ventricular muscles were exposed to contracture solutions containing 50 mM sodium, 2.7 mM calcium, and varying concentrations of potassium.

At the higher sodium concentrations, as noted above, a stable maximal value of contracture tension was usually not reached, and the largest contracture obtained was used to calculate the potassium concentration for "half-maximal" tension in these experiments. The figures clearly show the direction of the sodium concentration effect, but the actual values given above should be interpreted cautiously.

#### DISCUSSION

Contractures develop in sheep cardiac muscle in response to elevation of external potassium concentration or reduction in external sodium concentration. These contractures are reproducible in shape and magnitude for several hours after isolation of the muscle, provided the solution surrounding the muscle is changed quickly and sufficient time is allowed for recovery between contractures.

Although solution changes in the chamber were rapid, the ionic concentra-

tions in the extracellular space would be expected to change considerably more slowly than the external solution. Part of the time required for tension development must, therefore, have been related to diffusion in the unstirred extracellular compartment. It is difficult to assess the importance of the diffusion delay on contracture responses. Microelectrode recordings during contractures were very difficult, and, because the voltage of superficial cells is measured, do not give an accurate indication of how quickly cells deep within the muscle are depolarized. We did not find any clear dependency of the rate of development of contracture tension on muscle diameter, as one might have expected. This lack of correlation and the changes seen in the rate of contracture development when conditions before the contracture were altered suggests that other factors were more important than diffusion in determining the rate of contracture development.

*Division of the Contracture into Slow and Transient Phases* There is considerable evidence in these experiments to support the idea that two phases of the contracture, in addition to the initial twitch, can be separated on the basis of different physiological behavior. The ventricular contracture had a triphasic appearance, which was also seen, although less clearly, in some atrial contractures (e.g., Fig. 11). After short recovery intervals, in the absence of prior stimulation, or after stimulation in zero calcium, contractures developed much more slowly than the controls, but the same final tension was reached at the end of the contracture. Increasing the rate of stimulation before the contracture caused contracture tension to develop more rapidly and peak tension was reached earlier in the contracture. Varying the concentration of calcium during the contracture changed the final level of tension reached but did not affect the rate of tension development early in the contracture.

The increases in peak contracture tension seen as the potassium concentration was systematically increased and the effects of calcium and sodium on the relation between tension and potassium are similar to the results in frog heart (Lüttgau and Niedergerke, 1958), and, to the extent that the techniques are comparable, similar to the results in guinea pig atrium (Scholz, 1969 *a*). The changing shape of the contracture as the potassium concentration is increased may be another manifestation of the composite nature of the contracture, suggesting that the transient and slow phases depend differently on membrane voltage. Other conclusions about the different behaviors of the transient and slow phases also seem possible on the basis of our experiments.

*Slow Phase of Contracture Tension* Slowly rising contracture tensions were seen when the sodium concentration was lowered in the contracture solution even when the membrane potential was not altered by changing the potassium concentration. The magnitude of the slowly rising tension, as estimated by the tension level reached late in the contracture, increased as the potassium con-



centration was increased. At a given potassium concentration, the slowly developed tension increased if the sodium concentration in the contracture solution was reduced and decreased if the calcium concentration was reduced. The latter effect is clearly demonstrated by the experiment shown in Fig. 11. These results are generally similar to those demonstrated in frog ventricle by Niedergerke (1956) and by Lüttgau and Niedergerke (1958). The slowly developed tension did not require the presence of calcium before the exposure to contracture solution, and recovery of the ability to generate these slow tensions in our experiments was so rapid that it might be more accurate to say that no recovery time is necessary.

Niedergerke (1963 *b*) has shown that isotopically labeled calcium enters frog heart muscle during a contracture, depending strongly on the sodium and calcium concentrations outside the muscle. The relationships between calcium and sodium fluxes have been carefully studied in heart muscle by Reuter and Seitz (1968) and in squid axon by Baker et al. (1969). They find a complex interdependence of fluxes of the two ions, such that a reduction in external sodium concentration results in an increased influx of calcium. It seems possible that under the conditions of our experiments sufficient calcium enters the muscles to generate a steady contracture tension. This entry of calcium also might increase the store of calcium that can be released by a subsequent depolarization, so that after exposure to a contracture solution contractions in response to action potentials might be expected to be enhanced. Wood et al. (1969) and Antoni et al. (1969) reached a similar conclusion based on their experiments using electrically induced depolarization.

Our potassium contracture data and those of Scholz (1969 *a, b*) suggest that the slow tension responses in mammalian atrial and ventricular tissue are not identical. A slowly developed and maintained tension is also seen in response to voltage-clamp depolarizations of sheep ventricular muscle (Morad and Trautwein, 1968; Morad et al., 1968; McGuigan, 1968) but Beeler and Reuter (1970 *b*) did not see such a tension response in voltage-clamp experiments in dog ventricular trabeculae. At the present time, there is insufficient information about the behavior of slow tensions seen under voltage-clamp conditions to be sure that they are equivalent to the slow tensions seen during potassium contractures. If they are equivalent, the failure of Beeler and Reuter to obtain slow tension responses suggests that there may be a species difference as well as differences between atrial and ventricular muscle. Such species variation might help to explain some of the differences in voltage-clamp results from different laboratories, and the difficulties in obtaining reliable potassium contractures from some species.

*Transient Phase of Contracture Tension* The transient phase of contracture tension was strongly sensitive to the potassium concentration in the contracture solution. It was not dependent on the presence of calcium in the con-

tracture solution, but Figs. 13 and 14 indicate that it was increased if the sodium concentration in the bathing solution was reduced. When the calcium concentration or the rate of stimulation before contracture was changed, there were parallel changes in twitch size and the size of the subsequent transient contracture response. In particular, the transient response was greatly reduced or absent if prior stimulation was at a very low rate or if calcium was not present in the Tyrode's solution before the contracture.

Since this transient tension is dependent on the calcium concentration and the rate of stimulation before contracture, we suggest that it is produced by depolarization-induced release of an intracellular pool of calcium, possibly the same pool that governs the size of the normal twitch. Niedergerke (1963 *a*) has shown that stimulation results in an increased influx of calcium in the frog ventricle, and Winegrad and Shanes (1962) and Little and Sleator (1969) have shown a similar effect in guinea pig atria. Presumably stimulation in the presence of calcium allows loading of an internal store that can be used for activation of contraction when the muscle is depolarized, whether the depolarization is due to elevated potassium or due to stimulation of an action potential. The loading of such an internal store might occur via a voltage-dependent change in calcium conductance of the sort demonstrated by Beeler and Reuter (1970 *a*). The time-course of development or loss of the effect of prior stimulation on contractures was not studied systematically in these experiments, but the effect was manifest in a few minutes.

It is interesting to compare these transient contracture responses to twitches and to the tension responses to a voltage clamp. In mammalian ventricular tissue a voltage step produces a phasic contraction or twitch that resembles in most details the physiological contractile response to an action potential (Fozzard and Hellam, 1968; Morad and Trautwein, 1968; McGuigan, 1968; Beeler and Reuter, 1970 *b*; Gibbons and Fozzard, 1971). Several characteristics of the transient phase of the contracture resemble twitches. Both are strongly dependent on the prior stimulation rate and on the calcium concentration in the bathing solution, and the recovery of twitches and of the transient phase of contractures after a contracture each require many minutes and both follow approximately the same time-course.

Several differences between twitches and the transient phase of contractures can also be noted. The most dramatic is that the time-course of tension in response to an action potential or a voltage clamp is much faster than the transient part of the contracture. While both phenomena are voltage dependent, the relation between voltage and tension in the presence of a normal sodium concentration (Fig. 14) is shifted as much as 30 mv in the depolarizing direction from the relation found to hold for twitches in response to a voltage clamp (Fozzard and Hellam, 1968; Morad et al., 1968; Gibbons and Fozzard, 1971). Further, the twitches usually decline with time in the tissue bath, but

the transient phase of contractures does not. Finally, as reported by Kavalier and Morad (1966), the magnitudes of the twitches and the contractures respond differently to norepinephrine. Thus, if the same calcium store is involved in both types of contractile response, there may well be some difference in the mechanism by which depolarization induces release of calcium from the pool.

In summary, contractures in sheep cardiac muscle appear to have two primary components that can be separated by their different physiological behavior—a transient phase and a slowly developing steady phase. The latter appears to represent entry of calcium directly from the extracellular solution to produce contractile activation, and may be related to a calcium-sodium transport system. The transient tension appears to result from release of an intracellular calcium pool. This phase resembles in many respects the behavior of twitches, in that both are probably dependent on release of the same intracellular pool of calcium. However, the differences between these two events suggest that the control mechanisms responsible for the release of calcium are not identical.

We are indebted to Mr. Joel Eikenberry for his expert technical assistance during most of these experiments. H. A. Fozzard wishes to acknowledge the hospitality of Professor S. Weidmann and the University of Berne where many of the electrical measurements were made.

This research was supported by United States Public Health Service Grant HE 11665 and by Myocardial Infarction Research Unit Contract PH 43681334. The work was performed during Dr. Gibbons's term as a postdoctoral fellow on United States Public Health Service Fellowship HE 38359 and United States Public Health Service Training Grant HE 5673.

*Received for publication 13 May 1971.*

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