Contracture Coupling of Slow Striated Muscle in Non-Ionic Solutions and Replacement of Calcium, Sodium, and Potassium

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ABSTRACT The development of contracture related to changes of ionic environment (ionic contracture coupling) has been studied in the slowly responding fibers of frog skeletal muscle. When deprived of external ions for 30 minutes by use of solutions of sucrose, mannitol, or glucose, the slow skeletal muscle fibers, but not the fast, develop pronounced and easily reversible contractures. Partial replacement of the non-ionic substance with calcium or sodium reduces the development of the contractures but replacement by potassium does not. The concentration of calcium necessary to prevent contracture induced by a non-ionic solution is greater than that needed to maintain relaxation in ionic solutions. To suppress the non-ionic–induced contractures to the same extent as does calcium requires several fold higher concentrations of sodium. Two types of ionic contracture coupling occur in slow type striated muscle fibers: (*a*) a calcium deprivation type which develops maximally at full physiological concentration of external sodium, shows a flow rate dependency for the calcium-depriving fluid, and is lessened when the sodium concentration is decreased by replacement with sucrose; (*b*) a sodium deprivation type which occurs maximally without external sodium, is lessened by increasing the sodium concentration, and has no flow rate dependency for ion deprivation. Both types of contracture are largely prevented by the presence of sufficient calcium. There thus seem to be calcium- and sodium-linked processes at work in the ionic contracture coupling of slow striated muscle.

INTRODUCTION

The calcium ion is necessary for contraction of cardiac, skeletal, or smooth muscle (Ringer, 1883; Frank, 1960; Edman and Schild, 1962) and the movement of calcium has been correlated with development of tension (Sandow, 1952; Shanes, 1961). In the slow type striated muscle fibers of the frog the

deprivation of calcium by a rapidly flowing calcium-free solution induces a contracture-relaxation cycle. The contracture is unrelated to nerve activity and rapidly subsides with replacement of calcium (Irwin and Hein, 1963). After complete relaxation in a calcium-free solution, restoration of calcium does not induce contracture but the muscle again becomes excitable and regains its ability to contract. These findings relate the outward movement of calcium in a muscle to the onset of a contractile event. The frequent occurrence of contractures in frog muscles kept in non-ionic solutions is an earlier and still unexplained observation concerning contracture coupling (Fenn, 1931; Fleckenstein and Hertel, 1948; Vanremoortere, 1948; Swift *et al.*, 1960; Schaechtelin, 1961; Van der Kloot, 1961). Since placing muscles in non-ionic solutions removes calcium from the external medium, the question arose whether the development of tension is explainable solely by calcium loss or whether other processes are involved. The experiments reported here indicate that factors other than calcium loss are related to contracture coupling of slow striated muscle fibers occurring in non-ionic solutions.

METHOD

The experimental methods were similar to those used in a previous study of contracture during calcium deprivation (Irwin and Hein, 1963). The rectus abdominis and sartorius muscles, of male *Rana pipiens* obtained from Wisconsin, were used for measurement of tension. Before use the frogs were stored in the dark without food at 4°C, usually for several days but not longer than 2 months. The duration of storage did not appear to affect the results. The experimental procedures were performed at room temperature (21–24°C) in 5 ml baths. The muscles were bathed by fluid flowing continuously through the bath at 25 to 30 ml/min. while being aerated at 10 to 15 ml/min. with a mixture of 5 per cent CO_2 and 95 per cent O_2 or 100 per cent O_2; complete equilibration between the solution and the gas mixture was thus not obtained. In five control experiments 100 per cent O_2 was used as indicated in the text. The same bath inlets were used for ionic and non-ionic solutions. Since the non-ionic solutions have greater viscosity, the flow rates of these were slightly less. The muscles were not challenged with ACh, except in the reversibility experiments, and each muscle was kept in Ringer's solution about 30 minutes before use.

The Ringer's solutions were the same as those used by Swift *et al.* (1960) and contained 117.0 mM NaCl, 3.0 mM KCl, 4.0 mM $NaHCO_3$, and 2.7 mM $CaCl_2$. The sucrose solutions were 244.0 mM and are referred to as isotonic. To insure the absence of ionized calcium from some of the calcium-free solutions 0.1 mmole/liter of disodium ethylenediaminetetraacetate (EDTA) was added. The presence or absence of EDTA is indicated in the text and figures. The use of this small amount of EDTA was usually accompanied by an increased rate or magnitude of response but the differences were not considered of interpretive significance. Each experiment had its own control in that pairs of muscles from the same frog were used. In grouping of data the controls were from all experiments suitable to the condition under test. Thus in some experiments the number of control observations is appreciably greater

than the experimental ones and some control values apply to more than one experiment.

In the experiments relating rate of contracture to rate of flow the baths were emptied, filled with either calcium-free Ringer's or sucrose solution, emptied again, and then flushed at 25 to 30 ml/min. for 2 minutes before beginning the final flow rate. Tensions were recorded electronically with strain gauges, low drift chopper amplifiers, and direct pen writers. All chemicals, including sucrose, were of reagent grade and non-ionic and calcium-free solutions were made fresh daily.

RESULTS

Contracture in Isotonic Non-Ionic Solutions

The rectus abdominis muscles developed reversible contractures when the fluid flowing in the bath was changed from an isotonic Ringer's solution to an isotonic solution of sucrose, glucose, mannitol, or urea (Figs. 1 and 2). The contractures occurring in the sugar solutions can be attributed to tension development of the slow type fibers because under similar conditions sartorius muscles, which have few if any slow fibers, develop only small amounts of tension (Fig. 2). This probably explains why Fenn (1931), using many different kinds of muscles including the sartorius and rectus, observed contracture in some of the muscles and failed to see it in others. Fig. 2 shows that glucose, mannitol, and sucrose produced contractures in the rectus which were similar both in rate of development and magnitude. The use of urea was followed by an initial peaking of tension, a temporary relaxation, and then a later development of a greater maximal tension. The urea-induced contractures involve both types of fibers because sartorius muscles kept in isotonic urea solutions develop much tension during the latter part of the first 30 minutes in urea. Fig. 3 shows that rectus muscles develop more tension in sucrose than in a calcium-free Ringer's solution and that the sartorius muscles stay entirely relaxed in calcium-free Ringer's or in the same solution containing 4 mM EDTA. Other than the greater tension in rectus muscles and the occurrence of small amounts of tension in the sartorius muscles, the responses in sucrose appear similar to those brought on by a flowing calcium-free Ringer's solution. Both types of contracture in the rectus occur in the presence of procaine HCl (10^{-3} gm/ml). Furthermore, neuromuscular transmission in frog muscle fails within 30 minutes in isotonic sucrose solution (Koketsu and Nishi, 1959). It thus is unlikely that sucrose-induced contractures have a neural origin.

The reversible nature of the sucrose-induced contractures in rectus muscles is shown in Fig. 1. When the sucrose solution which induces contracture is replaced by Ringer's solution, the muscle relaxes, abruptly at first and later at a slower rate. After return to its original resting tension, or even slightly before, the muscle contracts fully in response to ACh, KCl, or to a calcium-free

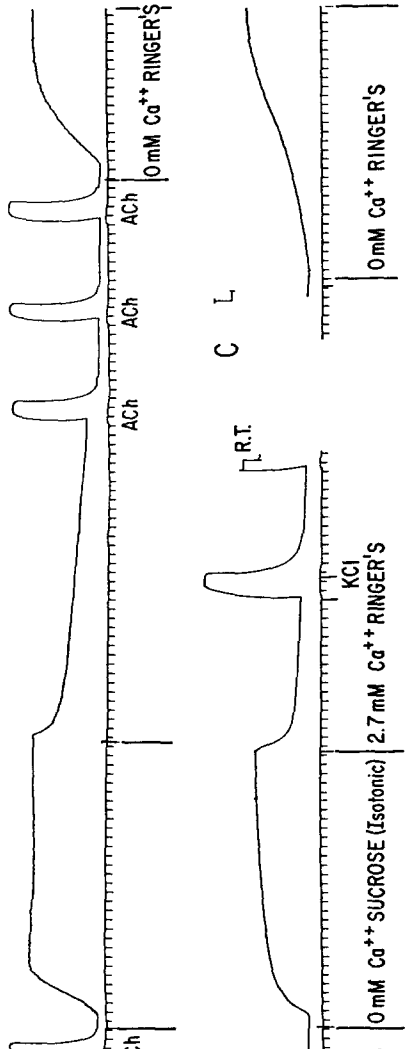

FIGURE 1. Sucrose-induced contractures and reversibility. Solution captions in B refer to both A and B. (A) ACh indicates 1 minute challenges with acetylcholine (2×10^{-5} gm/ml) in Ringer's solution. (B) KCl indicates challenges by Ringer's solution containing 40 mM KCl instead of NaCl. At R. T. the smaller vertical line was recorded when the link between the muscle and the gauge was cut and indicates the resting tension of the muscle at the end of the experiment. Initial resting tension was 1 gm. (C) A calcium deprivation contracture, induced by a calcium-free Ringer's solution, in a muscle which was previously treated exactly as the muscle shown in B. Notice that the responses to ACh and KCl are the same before and after the contracture induced by sucrose and that initial resting tension is regained. The ionic calcium deprivation contractures shown here are typical of those observed under similar conditions without a prior sucrose-induced contracture. Calibration marks refer to 1 gm and 1 min. Experiments were performed in October.

Ringer's solution. The slow muscle fibers thus remain fully excitable and responsive to depolarizing stimuli after completing a contracture-relaxation cycle. Reversibility is further emphasized by the fact that repeated challenging of the same muscle with isotonic sucrose initiates a similar contracture each time. The only difference is the slightly more rapid time course during later challenges. Although the stimulation of muscle to contract by a sucrose solu-

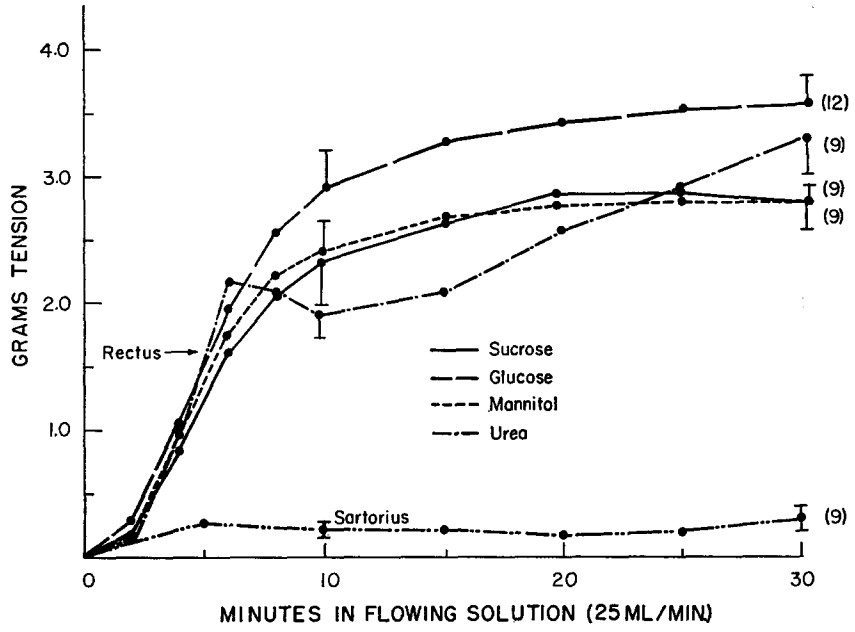

FIGURE 2. Contractures of frog muscle in isotonic non-ionic solutions. Data from the recti were obtained from December through February. The sartorii data are from nine muscles kept under various conditions (two in mannitol, three in glucose, and four in sucrose) and were obtained during April and May. EDTA (0.1 mmole/liter) was added to solutions used for sartorii but not for recti. Additional experiments using sartorii without EDTA were performed during July and gave similar data to those shown here. Figures in parentheses refer to number of muscles. Vertical lines indicate ± standard error of the means.

tion cannot be considered physiological, the complete reversibility indicates that the alteration which the sucrose solution produces may relate to a physiological process.

The Effect of Cations on the Development of Contractures Induced by Non-Ionic Solutions

CALCIUM

Since contractures in isotonic sucrose were similar to those in calcium-free ionic solutions, we determined whether the presence of calcium in the isotonic

sucrose would prevent or suppress the development of tension. Fig. 4 shows that 2.7 mM calcium, the concentration in the Ringer's solution, markedly reduced the contractures occurring in sucrose. Contractures taking place in mannitol or glucose were similarly reduced by the same concentration of calcium. 5.4 mM calcium, twice the amount in Ringer's solution, further limited the contracture development in sucrose solutions but did not completely abolish it. In the presence of 2.7 mM calcium most of the tension oc-

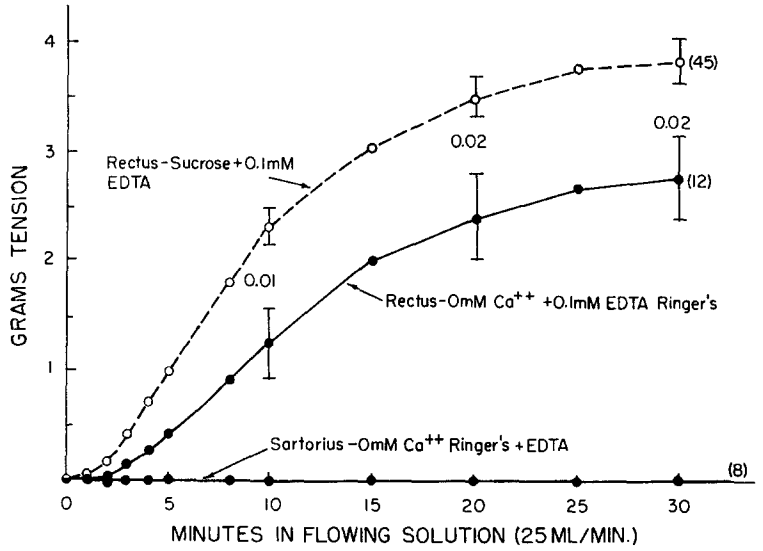

FIGURE 3. Comparison of contractures induced by isotonic sucrose and calcium-free Ringer's solutions. Figures in parentheses indicate number of muscles and those between lines indicate the level of statistical significance of the difference between the means at that point. Vertical bars indicate ± standard error of the means. Data showing lack of contracture in sartorii were obtained from six muscles kept in 4.05 mM EDTA in calcium-free Ringer's solution with the pH adjusted to 7.2 and two muscles with 0.1 mM EDTA in calcium-free Ringer's solution. Experiments were performed in April and May.

curred in the first 10 minutes. With 5.4 mM calcium the tension, although remaining small, steadily increased for the entire 30 minutes. Experiments which were continued longer than 30 minutes involve both types of fibers and are more complex.

SODIUM

Since the contracture in isotonic sucrose occurs while extracellular sodium ions are being depleted, experiments were performed using various amounts of sodium to replace some of the sucrose. The data shown in Fig. 5 indicate that as sodium substitutes for sucrose the contractures are reduced. The con-

centration of sodium necessary exceeds the concentration of calcium which prevents the contractures to the same extent. Sodium chloride (2.7 mM) in sucrose solution did not hinder the contractures to a statistically significant extent although the same amount of calcium markedly and significantly reduced them (Fig. 4). To reduce the sucrose-induced contracture to approximately the same degree as 2.7 to 5.4 mM calcium chloride, required 21.6 to 43.2 mM sodium chloride (Fig. 5). As sucrose was further replaced by sodium

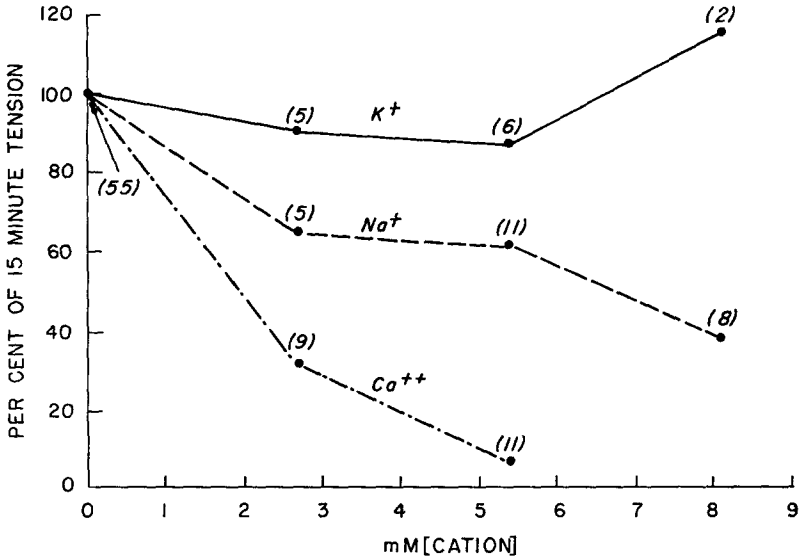

FIGURE 4. Calcium, sodium, and potassium and the development of sucrose-induced contractures in frog rectus muscles. Figures in parentheses indicate number of muscles. Fifty-five control muscles (sucrose only) developed an average of 3.72 gm tension. Twenty of these controls were for the calcium experiments (3.77 gm), eleven for potassium (3.65 gm), and 24 for sodium (3.71 gm). Values plotted are in per cent of the mean for the fifty-five grouped control muscles. All test solutions contained 0.1 mM EDTA. Experiments were performed in April and May.

chloride, the contracture again increased as the amount of sodium in the calcium-free Ringer's solution was approached. It can be seen from Fig. 5 that the substitution of about one-third of the sodium by sucrose in a calcium-free Ringer's solution attenuates the development of a calcium-free contracture. It was thus observed that partial replacement of sucrose by sodium chloride reduced tension of a sucrose-induced contracture and conversely, replacement of some sodium chloride by sucrose also reduced tension.

POTASSIUM

The replacement of some sucrose with either 2.7 or 5.4 mM potassium chloride did not significantly modify the sucrose contracture (Fig. 4). With 8.1 mM

potassium in sucrose the rate of contracture development increased and maximal contracture was reached sooner. This is in contrast to similar concentrations of either calcium chloride or sodium chloride which markedly decreased the contractures in sucrose. Concentrations of potassium higher than 8.1 mM were not used since potassium alone induces contracture in these muscles. In ionic calcium-free solutions without EDTA the absence of potassium lowered the mean contracture height but the difference was not statistically significant; this also has been observed using hypertonic solutions.

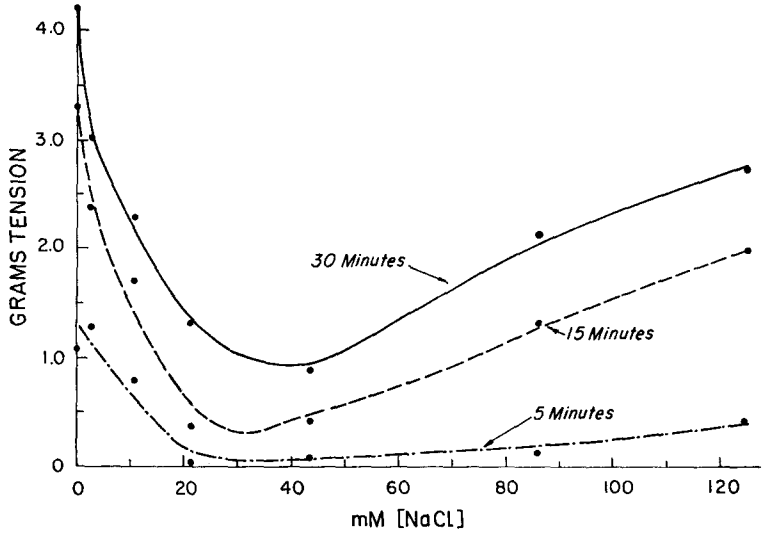

FIGURE 5. The suppressive effect of NaCl on sucrose-induced contractures in rectus muscles. The data points at 10.8, 43.2, 86.4, and 125 mM NaCl indicate tension measured during 5, 15, and 30 minutes in sucrose solution on at least twelve muscles. At 2.7 mM NaCl five muscles were used and at 21.6 mM six muscles were used. Solutions contained 0.1 mM EDTA. Experiments were performed in April and May.

It thus appears that potassium is not related to the development of contracture in either sucrose solutions or ionic solutions lacking calcium. The observations likewise indicate that the chloride ion is probably not involved with the inhibition of the contractures described here since only chloride salts were used in the sodium, potassium, or calcium replacement experiments. Kiessling (1960) and Schaechtelin (1961) found that arginine monohydrochloride can replace sodium chloride in suppression of a sucrose-induced contracture.

The Rate of Contracture Development in Sucrose or Calcium-Free Ringer's Solutions

The rate at which tension develops in a calcium-free ionic solution is at first dependent upon the flow rate of fluid over the muscle. Stopping the flow of

the fluid in the bath during the development of a contracture prevented further tension development and often induced relaxation. Muscles left in a stagnant calcium-free bath did not develop tension. When rectus muscles went into contracture in isotonic sucrose, the rates of tension development were independent of flow rates (Fig. 6). Each experiment of this kind demonstrated the rate dependency, or its lack, for the two different solutions. An experiment on four muscles using calcium-free Ringer's solution with 0.1 mM EDTA also demonstrated a flow rate dependency for ionic solutions; however,

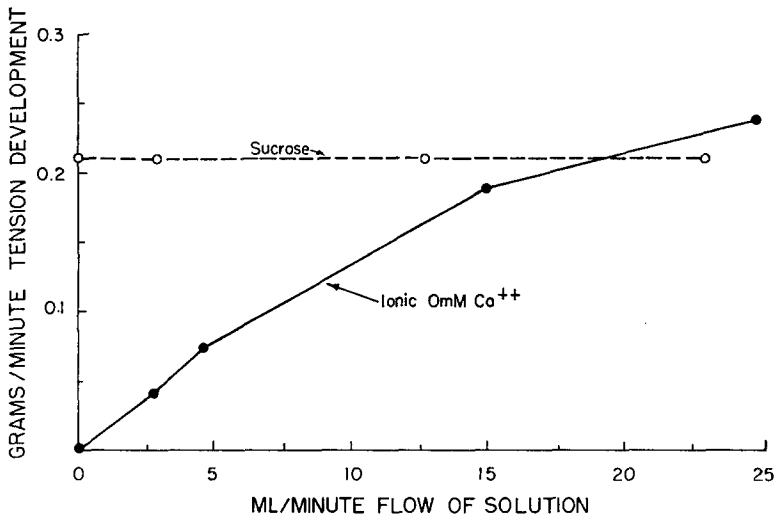

FIGURE 6. The effect of the flow rate of the bathing fluids, calcium-free Ringer's or isotonic sucrose, on contracture in the frog rectus. Plotted points indicate maximal rate of tension development for a given flow rate. EDTA was not added to the solutions. Experiments were performed in June.

the muscles did not stay relaxed in the stagnant bath with even this small amount of EDTA.

Sucrose-Induced Contractures and pH

Solutions of sucrose lack the buffering properties of Ringer's solutions. When aerated with 5 per cent CO_2 and 95 per cent O_2 they are more acidic than Ringer's solutions aerated the same way. Five muscles in sucrose solutions aerated with 95 per cent O_2 and 5 per cent CO_2 contracted to the same extent as their paired control kept in sucrose solutions with the pH adjusted to 7.2 and aerated with 100 per cent O_2. The lower pH values of the sucrose solutions thus do not appear to be related to the sucrose-induced contractures.

DISCUSSION

Because they occur in various non-ionic solutions the slow striated muscle contractures appear to be a general phenomenon related to a change in ionic environment rather than to the material used in maintaining osmolarity. The finding that the substitution of a small amount of calcium for sucrose partially suppressed the contractures suggests that lack or loss of calcium may have something to do with the origin of the contractures. Tension does not develop in muscles kept in ionic solutions in 1.0 mM calcium and as little as 0.3 mM calcium keeps some muscles relaxed (Kutscha and Pauschinger, 1961; Irwin and Hein, 1963). In sucrose solution about 25 per cent of control tension develops in the presence of 2.7 mM calcium. The concentrations of calcium needed to maintain relaxation in ionic and non-ionic solutions thus differ by several fold. This may indicate that in an ionic solution calcium could have mechanistically a different kind of action from calcium in a solution of sucrose. In ionic solutions in which calcium is effective at a lower concentration, it may act within the fiber to prevent contraction; that is, to maintain relaxation. In the sucrose solution with more calcium necessary, the calcium may be functioning indirectly by its well known membrane-stabilizing effect (Shanes, 1958), possibly by affecting the movement of other ions. The fact that enough calcium in the solution prevents most of the contracture, therefore, cannot be interpreted to indicate that calcium deprivation alone is entirely causative in the contractures which develop in isotonic sucrose solutions. A several fold difference in the suppressive action of calcium in the two different solutions does not seem to be accounted for by a possible decrease in the activity coefficient for calcium because of the sucrose solution. Another indication that lack of calcium alone does not adequately explain the origin of the contractures is the finding that the contractures decrease when sodium chloride replaces some of the sucrose. At the point of maximal suppression by sodium chloride the contracture tensions are less than the tensions which develop in calcium-free ionic solutions containing the full amount of sodium chloride. This indicates that at least certain of the processes responsible for contracture in sucrose are inhibited in some way by sodium chloride, but that in calcium-free ionic solutions they are not. The contractures induced by calcium deprivation in ionic solutions do appear to be somewhat dependent upon sodium chloride since those occurring in 86.4 mM NaCl with sucrose are considerably less than those in calcium-free Ringer's solution. There are thus at least two different mechanisms in contracture coupling in slow muscle, both affected by external sodium chloride, although in opposite directions. The mechanisms involved are still unclear since procaine does not affect the contractures but is known to interfere with sodium-carrying processes (Taylor, 1959).

From other work it appears that the origin of the sucrose-induced contracture does not depend on a depolarization of the membrane. The contracture accompanying depolarization by nerve stimulation in slow muscle fibers is well documented (Kuffler and Vaughan Williams, 1953; Burke and Ginsborg, 1956) and contracture follows the use of ACh or KCl. It is somewhat surprizing, therefore, to find that sucrose solutions produce hyperpolarization and contracture (Kiessling, 1960) in this type muscle and that the resting potential increases as sucrose replaces sodium. The fact that each muscle known to contain slow fibers also contains the fast type has so far precluded obtaining precise data from slow fibers which relate contracture development, membrane polarization, and ion fluxes. Data obtained from fast fibers are not pertinent because this type fiber spontaneously uncouples tension during continued depolarization.

In heart muscle Niedergerke and Luttgau (1957) and Niedergerke (1963) related tension development to $\frac{[Ca^{++}]}{[Na^{+}]^2}$. In the experiments reported here, tensions were greatest at 0 and 100 per cent of the physiological amount of external sodium chloride. Using data from this study and a previous one, and considering tension when both sodium and calcium were present, it has been calculated that the $\frac{[Ca^{++}]}{[Na^{+}]^2}$ relationship does not hold for the kind of tension development described here.

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