The Recovery of Contractile Ability Following a Contracture in Skeletal Muscle

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ABSTRACT When the potassium in Ringer's solution is increased above 25 mM, frog skeletal muscle contracts and then spontaneously relaxes. Elevated external potassium will not produce tension again until the stimulating potassium has been removed and a recovery process has taken place. The kinetics of this recovery reaction have been studied in toe muscles bathed in choline Ringer's solutions in which the levels of Ca and K were varied. In 2.5 mM K the recovery reaction appears to follow first order kinetics and the recovery is slowed by lowering the external Ca. In 1.8 mM Ca the recovery is complete in 60 sec.; in 0.2 mM Ca it is 70 per cent complete. When the external K is increased to 10, 15, or 20 mM, the recovery reaches a maximum at 60 sec. and then declines. As the external K is increased, the maximum recovery achieved declines. At K concentrations above 20 mM there is no recovery. When the external Ca is reduced to 0.2 mM, the area under the contracture tension curve is reduced by 40 ± 3 per cent (mean ± SEM). When 1.6 mM Ni is added to 0.2 mM Ca Ringer's, the contracture area is restored beyond the control value. This addition of the Ni to 0.2 mM Ca Ringer's does not, however, restore the recovery reaction to its rate in 1.8 mM Ca.

Treatment of frog skeletal muscle with an elevated potassium concentration (>24 mM) results in tension production followed by spontaneous relaxation. This relaxation occurs even though the high potassium stimulus is continued and is thought to be the result of a change in the intracellular system for the control of muscle contraction (Hodgkin and Horowicz, 1960 *b*). The muscle remains relaxed and insensitive to elevated external potassium until the stimulating potassium is removed and a recovery process has taken place. While the tension-producing phase of contracture has been studied for many years, an investigation of the recovery phase was initiated only recently (Hodgkin and Horowicz, 1960 *b*).

During the recovery phase, the muscle is converted from a state in which

tension is not set up by depolarization to a state in which tension *is* produced by depolarization. The manner in which the rate of this recovery reaction varies with certain concentrations of potassium, calcium, and nickel ions in the external solution is described in this paper.

METHODS

Material The frog (*Rana pipiens*) toe muscles, extensor longus digiti IV, used in this study had a diameter of 0.1 to 0.3 mm and were 10 to 13 mm long. They were dissected and mounted as previously described (Curtis, 1963).

Solutions The solutions used and their compositions (in millimoles per liter) are given in the following table. Potassium methyl sulfate has been abbreviated $KMeSO_4$. The pH of all solutions was between 7.1 and 7.2.

	Na^+	$Choline^+$	K^+	Ca^{++}	Cl^-	HPO_4^-	$H_2PO_4^-$	$MeSO_4^-$
Sodium Ringer's	125	0	2.5	1.8	126	2.1	0.9	0
Choline Ringer's	5	120	2.5	1.8	126	2.1	0.9	0
110 mM K	5	0	110	0	0	2.1	0.9	110

To obtain solutions of elevated K (2.5 to 110 mM) appropriate amounts of choline Ringer's and 110 mM K were mixed. Since the 110 mM K contained no Ca, this mixing diluted the Ca in the test solution. Control experiments showed, however, that restoration of the Ca concentration to the standard value did not alter the area under the contracture tension curve.

Choline Ringer's solutions of varied Ca concentration were made by adding different quantities of 0.1 M $CaCl_2$ and altering the amount of distilled water accordingly. To obtain a stable 18 mM Ca solution, 4 mM tris-malate buffer was substituted for phosphate buffer.

Choline solutions were made up fresh each day from a weighed sample of choline chloride, distilled water, stock solutions of 0.1 M KCl, 0.1 M $CaCl_2$, and phosphate buffer concentrated fifty times, pH 7.2.

The Muscle Chamber The muscle was mounted horizontally and stretched just beyond slack length in a chamber of 3 ml capacity. Solutions were run into the chamber through a wide bore stopcock and the fluid level was maintained by a suction outflow. Studies with dyes showed that the solution in the chamber could be completely changed in 3 sec. Isometric tension was measured with an RCA 5734 mechanotransducer connected in a conventional circuit.

General Procedure The muscle was dissected and mounted in sodium Ringer's. After 15 to 20 min. the muscle was stimulated electrically to give twitches and a tetanus. The muscle then equilibrated in choline Ringer's for 1 hour before experiments began. All the experiments were carried out at 18°C during the months May to October, 1962. Contractures were elicited every hour until the area under the contracture tension curve fell below 80 per cent of the area of the initial or control contracture, at which point the experiment was terminated.

The area under the contracture tension curve was used to quantitate contractures because the other convenient measure, maximum tension, did not describe many of

the observed changes in the contracture tension curve. The area under the contracture tension curve has been used previously (Hodgkin and Horowicz, 1960 *b*) to quantitate contractures. Whenever more than three contractures under identical conditions were done on a muscle, the standard deviation, s, of the area under the contracture tension curve was estimated from the range. In order to compare the data from different muscles, the mean area for each muscle was set at 100 per cent before s was calculated. A pooled s for all the contractures in normal Ca was 12 per cent (seventeen muscles). In 0.2 mM Ca, the pooled s was also 12 per cent (seven muscles).

RESULTS

The Effect of Low External Ca on the Contracture The effect of low external Ca upon the active phase of a contracture is shown in Fig. 1. All the contractures were induced by 110 mM $KMeSO_4$. Before the upper contracture was induced, the muscle rested for 30 min. in choline Ringer's with normal (1.8 mM) Ca; prior to the middle contracture, the muscle rested for 25 min. in normal Ca and for 5 min. in 0.2 mM Ca. Note that while the maximum tension is decreased only slightly in low Ca, the area under the contracture tension curve is markedly reduced.

The tetanus tension of the muscle shown in Fig. 1 was 0.67 gm and the contracture tension/tetanus tension ratio was 0.79. This ratio is typical of the tested muscles with a diameter of 200 to 300 μ. The average ratio was $0.81 \pm 0.05(\bar{x} \pm s/\sqrt{n})$. Muscles with a diameter of 150 to 200 μ gave an average ratio of 1.05 ± 0.06. Since the contracture tension/tetanus tension ratio of single fibers is 1.11 (Hodgkin and Horowicz, 1960 *b*), it is apparent that diffusion lags did not greatly influence the results obtained here with toe muscles. The average twitch tension-tetanus tension ratio was 0.36 ± 0.02.

The effect of varying the time in 0.2 mM Ca before inducing a contracture is shown in Fig. 2. The maximum effect is achieved within a brief equilibration period and thereafter the contracture area is constant with time. In nine muscles the mean contracture area $\pm$ one standard error after a 5 min. soak in 0.2 mM Ca was 40 ± 3 per cent of the control contracture in normal Ca. Lower Ca concentrations produce a progressive decrease of the area (Frank, 1960; Curtis, 1963). Furthermore there is no membrane potential decline in 0.2 mM Ca as there is in lower Ca concentrations (Curtis, 1963). As it was found that 0.2 mM Ca produced in the contracture tension curve a significant change which was constant with time, the effect of lowered external Ca upon the recovery reaction was studied in 0.2 mM Ca.

The Effect of Low Ca Upon the Rate of the Recovery Reaction

The series of events used to measure the recovery reaction is illustrated on the top line of Fig. 3 by a recovery in choline Ringer's with normal (1.8 mM) Ca. After the tension of the first contracture returned to zero, the high K was

flushed out by normal Ringer's (the recovery solution) and the muscle was allowed to recover for 50 sec. before the second contracture was induced. The area of the second contracture was 88 per cent of the area of the first; the per cent recovery was 88.

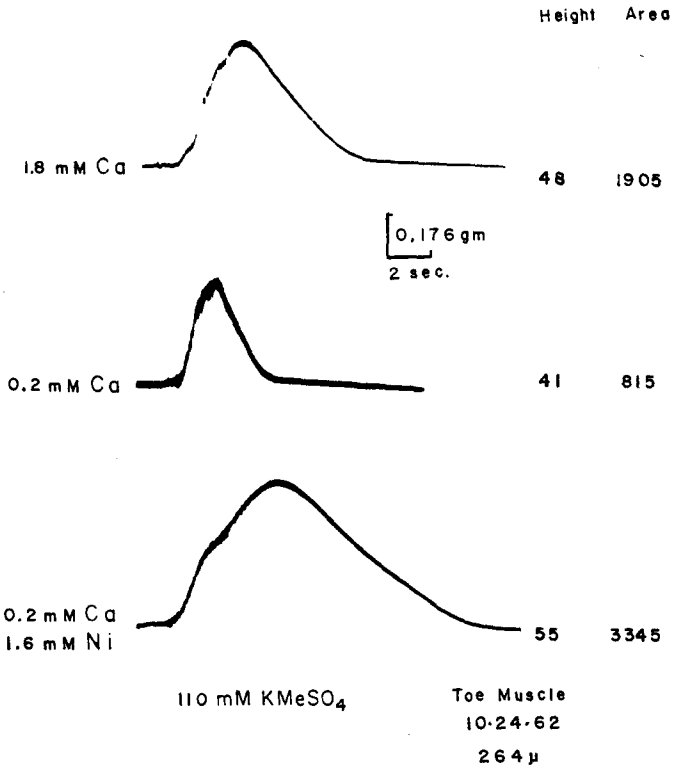

FIGURE 1. The effect of presoaking in 0.2 mM Ca or in 0.2 mM Ca plus 1.6 mM Ni upon the contractile response to 110 mM $KMeSO_4$. In each case the muscle was soaked in the test solution for 5 min. before the contracture was produced by high K. The high K was not replaced until after the tension returned to zero; the muscle relaxed spontaneously. The height (maximum tension) and area are given in arbitrary units. The effect of Ni will be considered later in the text.

The return of the tension to zero was judged by eye and therefore the time interval between the true return to zero and the introduction of the recovery solution must have varied somewhat from trial to trial. Controls in normal and in low Ca showed that variations (0 to 2 min.) in the time between relaxation and replacement of the high K solution did not affect the area of a second contracture induced 60 sec. after replacement.

The other two pairs of contractures in Fig. 3 were obtained in a similar manner but the time of exposure to the recovery solution was reduced. It

can be seen that shorter periods of exposure to the recovery solution result in less recovery.

These results along with those from twelve other muscles, all of which recovered in 1.8 mM Ca, have been pooled and are shown as the open circles of Fig. 4. The closed circles of Fig. 4 are the pooled results from ten muscles in which the external Ca was reduced to 0.2 mM for 5 min. previous to the first contracture and during recovery. Otherwise the format of the experiments was identical with that shown in Fig. 3.

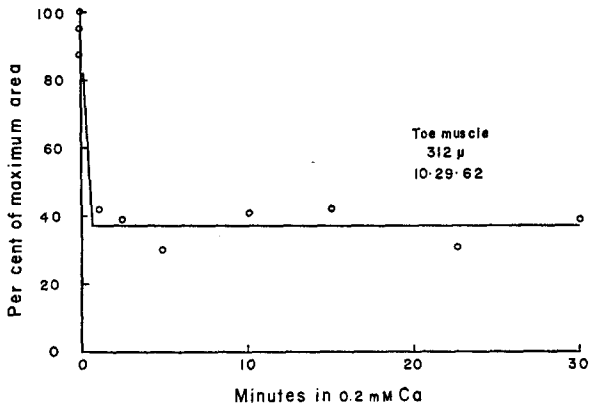

FIGURE 2. The effect of various presoak times in 0.2 mM Ca upon a subsequent contracture in 110 mM $KMeSO_4$. The area of the first contracture, with no low Ca soak, was taken as 100 per cent. The order in which the trials were done was: 0, 5, 1, 30, 0, 15, 2, 10, 22.5, 0 min. After each trial the muscle rested for 30 min. in normal choline Ringer's. The standard deviation, *s*, about the average (38 per cent) for these determinations is 12 per cent of the average. This is the same as the pooled standard deviation for all the determinations in 1.8 and 0.2 mM Ca.

The solid lines shown in Fig. 4 are single exponential curves with intercepts at 12 sec. and rate constants of 0.069 $sec.^{-1}$ for 1.8 mM Ca and 0.023 $sec.^{-1}$ for 0.2 mM Ca. The scatter in the data precludes a definite statement of the kinetics of the reactions at this time, although they suggest that the recovery reaction, in 2.5 mM K, follows first order kinetics. The recovery rate is significantly slower in 0.2 mM Ca. By an application of the sign test the probability that these two sets of points are the same curve is 1.5 per cent. Both the rate constants and the intercepts of the exponential curves in Fig. 4 were obtained from plots of log (100 − per cent recovery) *vs.* time, using a least squares fit.

If it is assumed that the recovery reaction follows first order kinetics, the 12 sec. intercept may be interpreted as a time delay before the recovery reaction begins. It will be shown (Fig. 5) that the rate of the recovery reaction is very slow in elevated K concentrations. Consequently, in Fig. 4 the 12 sec.

delay before the recovery reaction begins may be interpreted as the time required for the K in the extracellular spaces to return to normal from the 110 mM concentration of the stimulating solution. The time required for the K concentration to fall to 5 mM can be calculated from the following to be 15 sec.: (*a*) an apparent diffusion constant for K of 3.9×10^{-6} cm^{-2}/sec. in toe muscle extracellular space (Keynes, 1954), (*b*) the solution of the diffusion equation for a cylinder (Hill, 1928), and (*c*) an assumed diameter of 200 μ.

The Effect of High External K upon the Recovery Rate

In this series of experiments the Ca in the recovery solution was held constant and the K increased. In Fig. 5, note that when the K in the recovery solution is 10 mM or greater, the per cent recovery reaches a maximum at or near

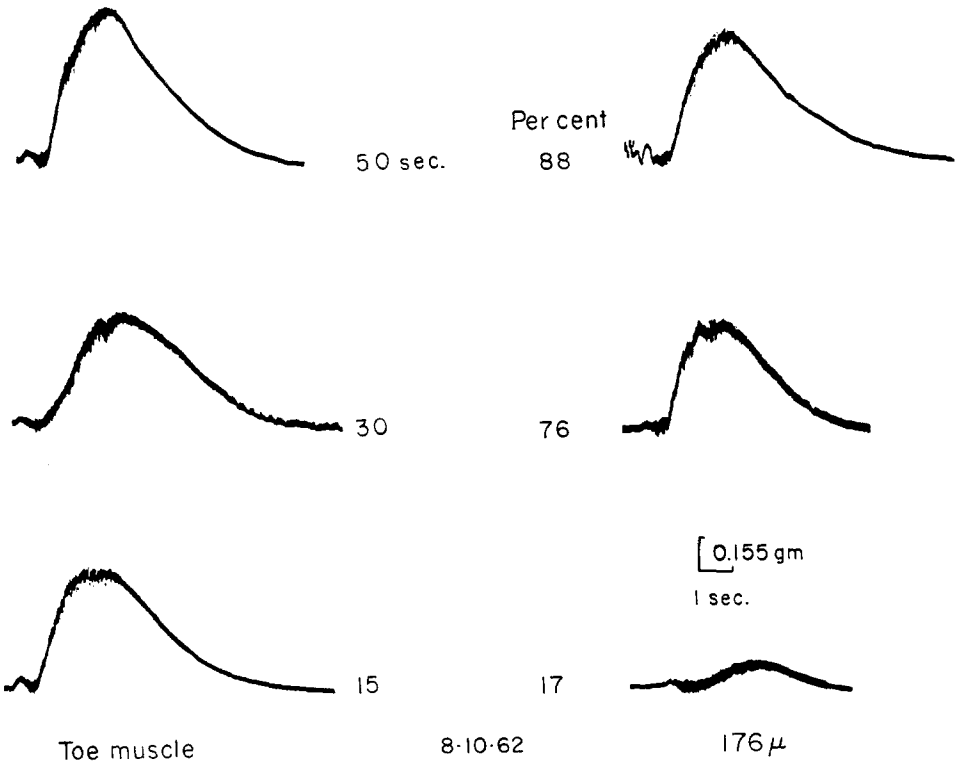

FIGURE 3. The effect of varying the time in the recovery solution upon the recovery of contractile ability achieved in normal (1.8 mM) Ca and normal (2.5 mM) K. After the muscle relaxed from the first contracture, the recovery solution was introduced into the muscle bath for the specified time interval and a second contracture was then induced by 110 mM $KMeSO_4$. The left hand column of numbers lists the recovery time in seconds and the right hand column lists the per cent recovery (the ratio of the area under the second contracture tension curve to the area of the first). The calibration scales apply to all records.

60 sec. As the K in the recovery solution is increased, the maximal recovery is decreased.

The Effect of Altering Both K and Ca upon the Recovery Rate

It is possible to characterize each of the curves in Fig. 5 by the maximal recovery achieved, which in this case is the recovery at 60 sec., and to plot

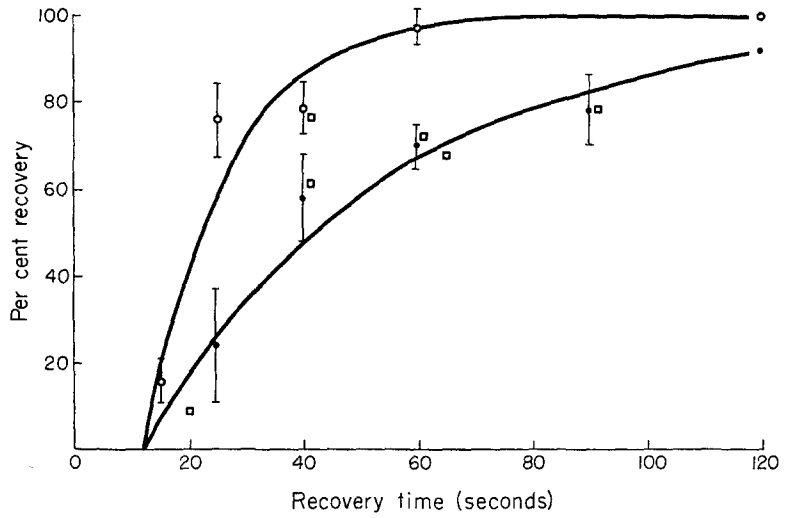

FIGURE 4. The effect of lowering the external Ca upon the rate of the recovery reaction Here the results from twenty-two muscles are grouped into the following time intervals: 10 to 20, 21 to 30, 31 to 50, 60, 90, and 120 sec. For those groups with a time range, the average is plotted at the midrange. The values for recovery in normal Ca (1.8 mM) are shown as open circles, the values for low Ca (0.2 mM) as closed circles. The curves are described in the text. The bars represent $\pm$ one standard error ($\pm s/\sqrt{n}$). In addition, the recovery in 0.2 mM Ca and 1.6 mM Ni was tested in two muscles and the individual determinations are shown by the squares. These data will be discussed later in the text.

recovery at 60 sec. *vs.* log K in the recovery solution. Such a plot is seen in Fig. 6. There are also three similar plots in this figure taken at three other Ca concentrations: 0.1, 0.2, and 18 mM. It should be emphasized that the choice of 60 sec. for the recovery time, although suggested by the curves in Fig. 5, is arbitrary. The data for 0.1 mM Ca are included even though the inhibition of the contracture tension curve is not constant with time. All the data for Fig. 6 were obtained by testing the recovery achieved after 60 sec. in recovery solutions of different K and Ca concentrations. Whenever the Ca concentration was altered, it was altered for 5 min. before the first contracture and

during the recovery period. The K was altered only during the recovery period.

It will be noted from Fig. 6 that the descending limbs of each of the curves are roughly parallel. This suggests that one of the effects of low external Ca is to shift the relation between per cent recovery and log K along the log K axis.

Hodgkin and Horowicz (1960 *b*) have published a curve for the relationship between per cent recovery and log K in *normal* Ca. The curve differs somewhat from the one shown in Fig. 6 in that the steep fall occurs between

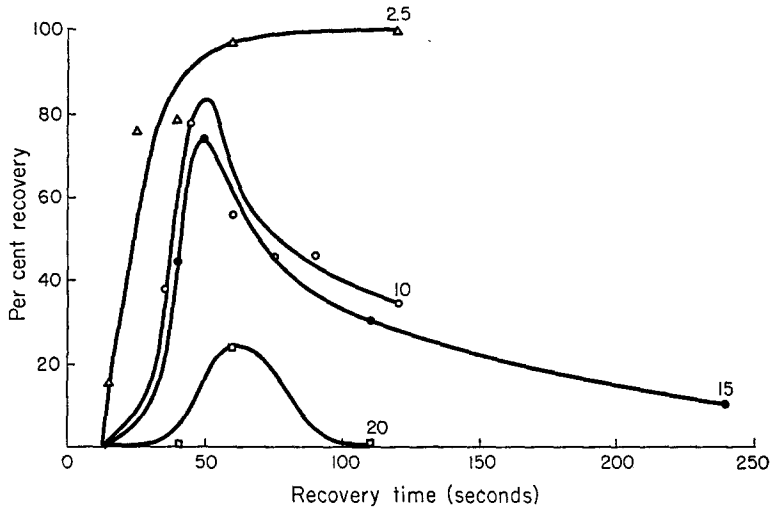

FIGURE 5. The kinetics of recovery with various K concentrations in the recovery solution. The values for 2.5 mM K are the average values shown in Fig. 4 for normal Ca. Each of the other curves is based on results from a single muscle recovering in a K concentration that is indicated by the number next to the curve. The Ca concentration is 1.8 mM throughout.

20 and 40 mM K. Their experiments were done with single fibers of the English frog *Rana temporaria* in sodium Ringer's and the contractures were induced with 95 mM K_2SO_4. Controls done on toe muscles of *R. pipiens* in these solutions showed no significant differences from the 1.8 mM Ca curve shown in Fig. 6. The explanation of the difference between the results presented here and the results of Hodgkin and Horowicz is not clear.

The Effect of the Substitution of Nickel for Calcium

Frank (1961) has shown that the addition of Ni to Ca-free Ringer's solution will restore the ability to produce tension in response to depolarization. The effect of the addition of 1.6 mM Ni to 0.2 mM Ca Ringer's upon single contractures is seen in Fig. 1. Thus the addition of another divalent ion can re-

verse the changes in the active phase of contracture brought on by lowering the Ca in the bathing solution. In contrast, the data of Fig. 4 show that the addition of Ni to the low Ca does not alter the rate of recovery of contractile ability. While the significance of this finding is not presently understood, it is interesting that certain functions of external Ca can be replaced by Ni while others cannot.

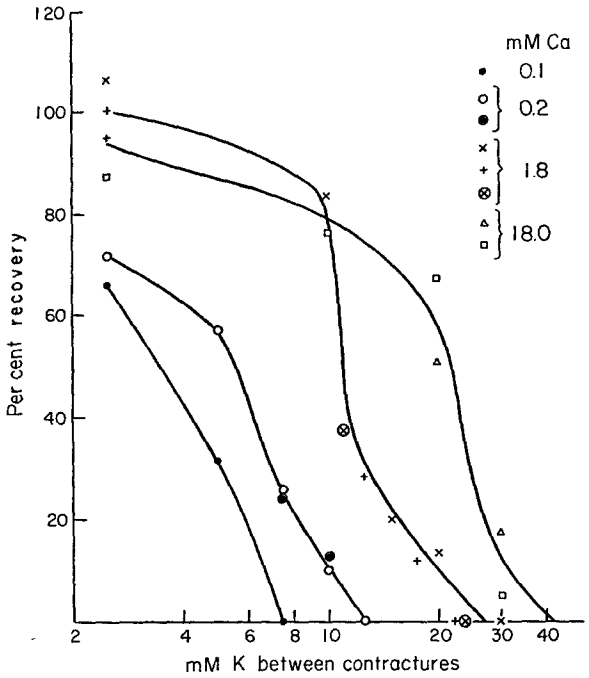

FIGURE 6. The results of a number of experiments in which the recovery after 60 sec. was tested in the indicated Ca and K concentrations. The K concentration is plotted on a log scale. The choice of 60 sec. is arbitrary but was suggested by the results in Fig. 5. Each type of symbol represents one muscle.

DISCUSSION

When a striated muscle is immersed in a high potassium solution, the membrane is depolarized and a contracture, followed by relaxation, occurs. This relaxation has been interpreted by Hodgkin and Horowicz (1960 *b*) as being produced by a change in the intracellular system which controls contraction rather than by a deficiency in the energy supply. The view that the energy supply is not the controlling factor has been confirmed by the experimental finding that a muscle which has relaxed in the presence of high K contains large quantities of creatine phosphate (Carlson and Edwards, personal communication).

Following relaxation, the muscle cannot be restimulated by high K until the stimulating K has been removed and a recovery period has elapsed. Hodgkin and Horowicz (1960 *b*) have postulated that the recovery time is used to reset the intracellular system which controls contraction. It might be argued that there is no intracellular mechanism to be "reset" and that the recovery period is only the result of a slow membrane repolarization. While membrane potential studies were not carried out on toe muscles, Hodgkin and Horowicz (1960 *a*) measured potential changes in single muscle fibers. They found that in chloride-containing solutions the repolarization in the transition 190 mM K → 2.5 mM K is nearly complete in 14 sec. (Fig. 6, p. 377) and that in the transition 80 mM K + 0 mM Cl → 2.5 mM K + 120 mM Cl the repolarization was half-complete in about 5 sec. (p. 383).

In the case of repolarization of the toe muscle, the flow of solution was considerably faster, but there was a diffusion lag of about 15 sec. before the change in concentration around the innermost fiber was largely completed. Consequently, it may have taken 20 to 30 sec. for the membrane potential of the innermost fibers to return to normal after the exterior solution had been changed. The lag before recovery began and the early phase of recovery in Fig. 4 were certainly influenced by these diffusion and repolarization lag times, yet it seems very unlikely that the entire 60 sec. course of recovery in ordinary Ringer's can be ascribed to these lags.

Hence the relaxation at the end of a contracture and the recovery from the relaxation would appear to be properties of the postulated intracellular system controlling contraction. The experiments reported here and in Curtis (1963) were undertaken to describe this intracellular system.

Considerable evidence has accumulated that contraction results only when Ca is bound by the actomyosin contractile system (Weber *et al.*, 1963). It is not, however, completely clear where this activator Ca comes from. One proposal (Bianchi and Shanes, 1959) states that activator Ca moves into the muscle from the extracellular spaces.

As Frank (1961) has noted, there are a number of objections to this view. First, as Hill (1949) pointed out, the interval between stimulation and contraction is too short to enable any substance liberated at the surface membrane to diffuse throughout the sarcoplasm. Furthermore, the fluxes of Ca which Bianchi and Shanes measured are much too small to activate the contractile proteins. Bianchi (1961) has also noted certain objections to this proposal.

Frank (1961) has instead proposed that the Ca which actually combines with the contractile proteins is always contained within the fiber. He suggests that a muscle in Ca-free solution is unable to contract in response to K depolarization because the chain of events between stimulation and contracture has been interrupted at or near either the surface membrane or the walls

of the transverse elements of the sarcoplasmic reticulum. The action of other divalent ions to restore K contractures in Ca-free Ringer's, even though they cannot directly activate the contractile proteins, also fits into this scheme.

A difficulty with the view that Ca-free Ringer's interrupts the chain of events between stimulation and contracture is that the passage of long hyperpolarizing current pulses will activate a muscle to contract in Ca-free solution (Curtis, 1963). Unless it is assumed that the hyperpolarization acts at a locus different from that of the depolarization, it must be assumed that the coupling system is intact but has been rendered inactive by the Ca-free solution.

This current-passing experiment supports the view that the Ca which combines with the actomyosin never leaves the fiber. If the activator Ca is stored in the transverse tubules of the sarcoplasmic reticulum as a negatively charged Ca complex (Hodgkin and Horowicz, 1960 *b*), a corollary to the above view is that the junction between the tubules and the extracellular space must be impermeable to this Ca complex in Ca-free solution.

Weber *et al.* (1963) propose that the contraction-relaxation cycle is the result of Ca movement within the fiber. These movements are presumably controlled by the membrane potential so that Ca is released to a free form when the membrane is depolarized. Upon depolarization by high K, it is released into the sarcoplasm and combined with actomyosin to initiate contraction (Weber *et al.*, 1963). When the initial K stimulus is maintained, contracture tension soon declines to zero. This suggests that the activator Ca has once again been separated from the contractile proteins but, since the maintained initial stimulus does not now release it, it is in a state different from that in the resting muscle. Since there is a considerable body of evidence that the activator Ca does not leave the muscle fiber, the activator Ca in the inactive form (such as it is at the end of a contracture) is presumably remade or transformed into an active form.

The recovery reaction described experimentally in this paper is thought to correspond to this reformation of the activator Ca. This recovery reaction has been shown to be dependent upon the external Ca concentration and to slow down as the external Ca concentration decreases (Figs. 4, 6).

Let it be assumed that the release and reformation of activator Ca are occurring continuously. It has been observed that the recovery reaction is slowed down in low Ca, and it may safely be assumed that the rate of reformation in Ca-free solution is reduced to this or a greater extent. This reduction would result in a piling up of the activator in an inactive form; the final result would be to render the muscle unable to respond to depolarization by high K. In other words, a muscle which will not contract in Ca-free solution may very well be in the same mechanically refractory state as is a muscle which has relaxed after a contracture.

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