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THE EFFECT OF LOW TEMPERATURE ON THE EXCITATION-CONTRACTION COUPLING PHENOMENA OF FROG SINGLE MUSCLE FIBRES

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SUMMARY

1. Potassium contractures are affected by low temperature: the maximum contracture tension is diminished by about 15% at 3° C, while the response time course is greatly prolonged.

2. The contractile threshold for potassium contractures is lowered by about 10 mV at 3° C.

3. The fibre's membrane is depolarized by approximately the same amount when exposed to solutions with increased potassium concentrations at 20 or 3° C.

4. The repriming process, that is, the process by which the fibres recover their contractile ability following a potassium contracture, proceeds about six times slower at 3° C. This effect is not due to failure of the fibres to repolarize in the cold when transferred from a high potassium to a low potassium medium.

5. At low temperature repolarization occurs, even though it is somewhat slower. Following the solution change, from 190 mM potassium to a low potassium solution, the initial rate of repolarization is 8·5 mV/sec at 20° C, and 3·4 mV/sec at 3° C. This effect is not sufficient to account for the delay in the repriming process.

6. After a potassium contracture, recovery of the fibre's twitching ability at 3° C is also delayed. At a time when twitches have not yet been recovered, membrane potentials of -90 mV and almost normal action potentials can be recorded.

INTRODUCTION

The work of Hodgkin & Horowicz (1960*b*) on potassium contractures of single muscle fibres has provided considerable information on many of the

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phenomena involved in the excitation-contraction coupling process. Thus they clearly established the relationship between tension development and membrane potential, showed the membrane dependency of the repriming process, and explained the transient nature of these contractile responses in terms of a very simple model.

The present work describes in detail the effect of low temperature on potassium contractures and related phenomena. Although several authors had previously reported the effect of temperature changes on some of the phenomena involved in excitation contraction coupling, a systematic study of these effects had not been made. Furthermore, there appeared to be some discrepancies and uncertainties in the previous reports on the subject.

Luttgau & Oetliker (1968) did not observe any shift in the curve relating contracture tension to external potassium concentration in the cold, while González-Serratos (1965) has reported a shift toward the right, indicating an increased contractile threshold.

Work done with whole muscles by Erlij & van der Kloot (1964), and by Milligan & Edwards (1965) indicated that contractile repriming was delayed in the cold. While the work of these authors indicated the contrary, an earlier report by Grieve (1960) suggested that this effect could be due to failure of the muscle fibres to repolarize in the cold.

In the present work it has been found that at low temperature the curve relating tension to membrane potential is shifted toward the left indicating a lowering of the contractile threshold. It is also shown that the repriming delay, observed in the cold, cannot be attributed to failure of the fibres to repolarize, although in such conditions repolarization is somewhat slower. The results obtained in the present work also indicate the possibility of carrying out further experiments designed to study the factors that determine the time course of potassium contractures (Caputo, 1972). Partial reports of this work have been presented previously (Caputo, 1969; 1971).

METHODS

Single fibres dissected from the dorsal head of the semitendinosus muscle of *Rana pipiens* were used in all the experiments. The use of single fibres reduces diffusion delays.

The dissection was carried out under a microscope in normal Ringer solution to which tubocurarine at a concentration of 10^{-5} M had been added. The procedure was in most respects similar to that described elsewhere (Caputo, 1966; Caputo & Giménez, 1967). One departure from the standard procedure was that early in the dissection the muscle was split longitudinally in two or three portions and each of these was further used in the attempt to obtain a single fibre. When successful, this manoeuvre permitted one to obtain more than one fibre from a given muscle. After a single muscle fibre had been obtained, it was left to rest in the dissection dish for 30–60 min. During this interval, it was repeatedly tested for excitability. Only fibres which responded throughout with a brisk twitch to electrical stimulation were

mounted in the experimental chamber. The transfer of the fibres from the dissecting dish to the experimental chamber was made employing a plastic spoon which protected the fibre from being exposed to a liquid air interface.

The experimental chamber used in these experiments was basically similar to that used originally by Hodgkin & Horowicz (1959) and needs not to be described in detail. By turning of a multiple tap, one of six different solutions could be flushed into the chamber. The level of the fluid was maintained by suction at the other end of the chamber. With this set-up, solution changes could be accomplished in a fraction of a second as can be observed in some figures in the text in which the trace monitoring temperature of the bathing media can give a measure of the rapidity of the solution change.

The fibre rested in a groove in the centre of the chamber, with one tendon gripped in a clamp fixed in the bottom of the chamber and the other tied to a lever connected to the tension transducer.

In most of the cases, the temperature changes were made by flushing cold or warm solutions into the chamber. In this way the temperature could be changed as fast as the solution changes could be made. To achieve a rapid cooling to 3° C, solutions cooled in a refrigerator to 1° C, were flushed into the chamber. Before entering the chamber they circulated in an iced bath at 0° C. When the fibres were to be kept at low temperature for long periods of time (5–10 min), a thermoelectrical cooler mounted in thermal contact to the bottom of the groove where the fibres lie was used. Temperature was monitored via a thermistor mounted in the groove closely to the fibre.

The tension of the fibres was measured with a RCA 5734 transducer under isometric conditions. The transducer was connected to a standard bridge circuit and normally its output was displayed on the screen of an oscilloscope.

Conventional micro-electrodes filled with 3 M potassium and connected to the input stage of a high impedance amplifier were used. In most of the cases, to facilitate impalement of the fibres, a small lucite pedestal, covered with petrolum jelly, was positioned under the region of the fibre in which the impalement was made. The output of the amplifier was connected either to a cathode ray oscilloscope or to a pen recorder.

Most of the solutions used for these experiments were similar to those described by Hodgkin & Horowicz (1959). The composition of most of them is given in Table 1. In the experiments in which constant [K].[Cl] product solutions were used, these were prepared in the way described by Hodgkin & Horowicz.

TABLE 1. Composition of solutions (mg ion/l. solution)

Solution	K^+	Cl^+	Na^+	Ca^{2+}	HPO_4^{2-}	$H_2PO_4^-$	SO_4^{2-}
Normal Ringer	2·5	121	115	1·8	2·15	0·85	—
117·5 KCl	117·5	121	0	1·8	2·15	0·85	—
190 K	190	0	3	9	1·08	0·43	104
190 K low calcium	190	0	3	0·5	1·08	0·43	95·5
190 K-0 calcium	190	0	3	0	1·08	0·43	95

RESULTS

The effect of temperature on potassium contractures

The time course of a potassium contracture can be described in terms of a plateau during which the tension falls slowly, followed by a rapid exponential phase of relaxation (Hodgkin & Horowicz, 1960*b*). In the case of numerous fibres used in this work, the contracture plateau was much reduced or even absent with the relaxation phase following immediately after maximal tension had been attained. Fig. 1 shows that the time course of a potassium contracture can be greatly prolonged by lowering the temperature. This effect is due both to a prolongation of the plateau and to

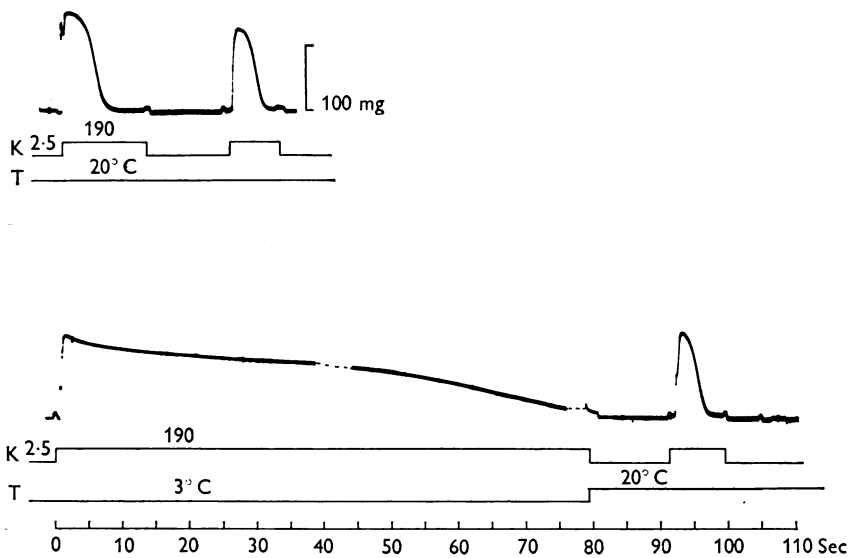

Fig. 1. Repriming after potassium contractures of short and long duration. In both cases, recovery was at 20° C. Fibre diameter: 70 μ. T, temp.

a marked decrease of the rate of the exponential relaxation phase. In the case of the fibres which at room temperature showed no plateau, the response in the cold showed a prolonged one. Fig. 1 shows also that repriming is not affected following a prolonged contracture at 3° C. The upper record shows two contractures induced with 190 mM potassium at room temperature. The fibre was allowed to recover for 10 sec in normal Ringer after the first contracture. After a long rest the fibre was cooled to 3° C and held at this temperature for 30 sec before exposure to a cold 190 mM potassium medium. This caused the long contracture shown in the lower record. When relaxation was complete the fibre was exposed to normal

Ringer at 20° C for 10 sec and then to a 190 mM potassium medium, also at 20° C; the repriming obtained in this case is similar to that obtained before, and it is thus clear that repriming is unaffected by the duration of the first contracture. For this particular fibre the peak contracture tension was reduced by 16 % at 3° C. The mean reduction at this temperature for twenty fibres was of 15 %.

The temperature effects on the peak tension, the plateau duration, and the relaxation rate are established very rapidly. In the case in which the fibres were not precooled but exposed directly to the cold contracture medium, the effects on the plateau and the relaxation phase could still be observed. In these cases, the peak tension was initially the same as that obtained at room temperature; however, it fell rapidly to the level of the plateau tension expected at low temperature. Long precooling periods (up to 30 min) did not have adverse effects on the fibres' behaviour. Independently of the precooling time the effects of low temperature were found to be rapidly abolished once the fibres were warmed up.

With respect to the contracture time course, it seemed of interest to calculate the Q_{10} of the relaxation phase at least for the case of one fibre. The values of tension, taken at different times during the contractures obtained with one fibre at different temperatures, were normalized with respect to the maximum values and plotted on semilogarithmic paper versus time.

The final part of the relaxation phase seemed to follow an exponential decay, in agreement with the results of Hodgkin & Horowicz (1960*b*). The rate constants for this late relaxation phase, obtained from these curves, were then plotted against the inverse of the absolute temperature. A straight line could be traced to obtain an approximate fit for all the points. Considering the slope of this line, a Q_{10} of 3·2 was obtained, corresponding to an activation energy of about 20 kcal/mole.

The area under the tension curve in an isometric contraction, that is, the time integral of force, is considered to be correlated with the energy utilization of the fibre during the contractile response (Jobsis & Duffield, 1967). During a contracture induced in the cold, one might think that the fibre energetic capability is taxed to its maximum. Hodgkin & Horowicz (1960*b*) showed that by using lower concentrations of potassium (35–50 mM), the time course of the contractures was prolonged. Fig. 2 will demonstrate that this is also the case for contractures induced at low temperature. Furthermore, Edwards & Carlson (1964) have shown that after immersion in solutions containing about 120 mM potassium at 20 or 3° C, the creatine phosphate and ATP content of frog sartorius muscles were not much reduced. From these experiments it can be concluded that the time course of contractures obtained at low temperature is not limited by exhaustion of energy stores.

The effect of temperature on the contractile threshold

The following experiment demonstrates that at low temperature the fibres start to develop tension when exposed to media with potassium concentrations lower than those necessary at room temperature. An example of this is shown in Fig. 2. The upper record shows a contracture obtained at 3° C with 117·5 mM potassium chloride. The middle record shows that

when exposed to 15 mM potassium at 20° C, the fibre responded with a very small response and rapidly relaxed; without further changes in the potassium concentration, the fibre was cooled down to 3° C and after a short delay it developed tension almost to its maximum value. The response so obtained was much more prolonged than that obtained with 117·5 mM potassium. In fact, after about 50 sec the fibre had not yet relaxed. At this time, the response was cut short by flushing normal Ringer

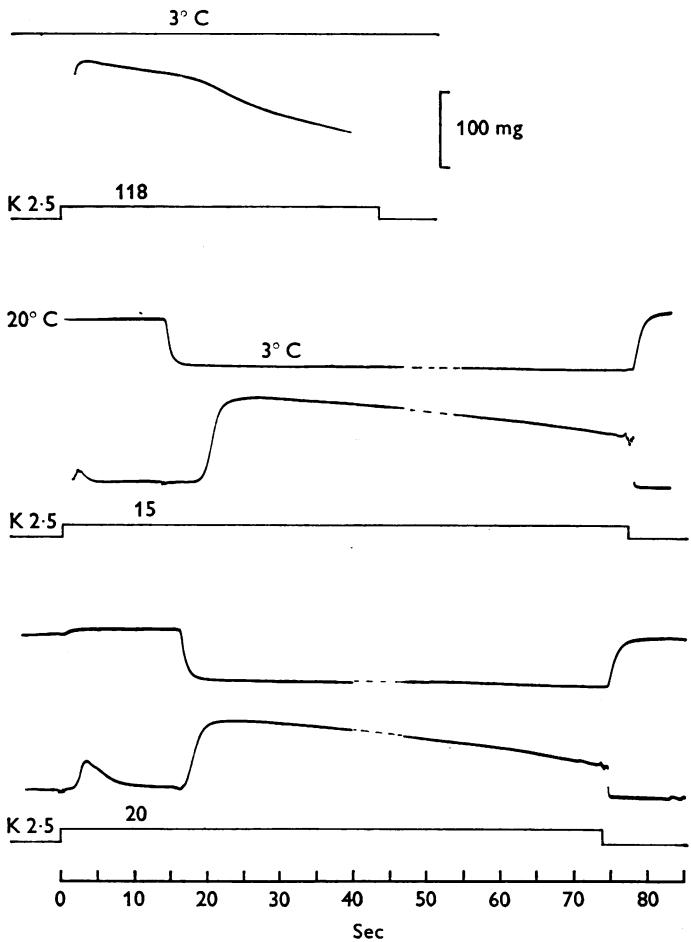

Fig. 2. Increased tension at low concentrations of potassium by cooling. The upper record was obtained with 117·5 mM potassium at 3° C after a 30 sec precooling period. In the lower two records, the temperature was decreased from 20 to 3° C, while the fibre was exposed to solutions containing 15 and 20 mM potassium, respectively. In each record, the upper trace shows the temperature of the bath measured near the fibre. Unretouched oscilloscope records. Fibre diameter: 70 μ.

into the chamber. The lower trace shows an experiment similar to the preceding one, carried out with a solution containing 20 mM potassium. In this case, the response obtained at 20° C was somewhat larger than in the previous case. On cooling, the fibre produced a second contracture that started with less delay than before, but in this case the tension produced was less. This effect can be explained in terms of the inactivation phenomenon described by Frankenhaeuser & Lannergren (1967).

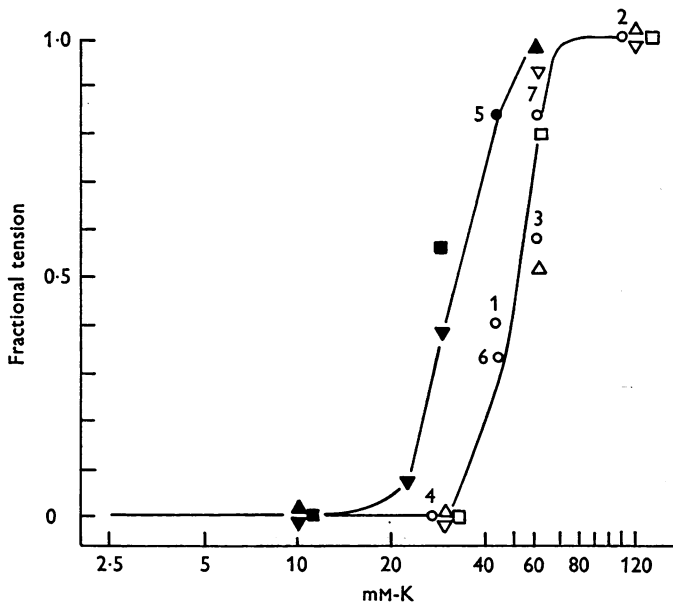

Fig. 3. Effect of temperature on the relation between fibre tension and the external potassium concentrations. The different symbols represent the results obtained with four different fibres. The filled symbols represent the results obtained at 7° C. The open circles represent the results obtained at room temperature (~23° C). Tension is represented as fraction of the maximal tension obtained at room temperature for each fibre. The number near the circles represent the order in which the responses were obtained for this particular fibre.

Hodgkin & Horowicz (1960*b*) have shown that the development of tension in muscle fibres is related to the logarithm of the external potassium concentration, by a step S-shaped curve, with tension starting at a determined potassium concentration. The relationship between peak tension development during contractures at 7° and 20° C by four single fibres, and the potassium concentration is shown in Fig. 3. The tension for each temperature is expressed as fraction of the maximum tension developed. For the experiments, shown in this Figure, the solution with high potassium

was prepared by substituting sodium for potassium. To avoid excessive loading of the fibres with chloride during the relatively long exposure during the cold contractures, these responses were cut short immediately after the peak tension had been attained by flushing the chamber with normal Ringer. It appears that lowering of the temperature causes a shift toward the left of the S-shaped curve, indicating a lowering of the contractile threshold.

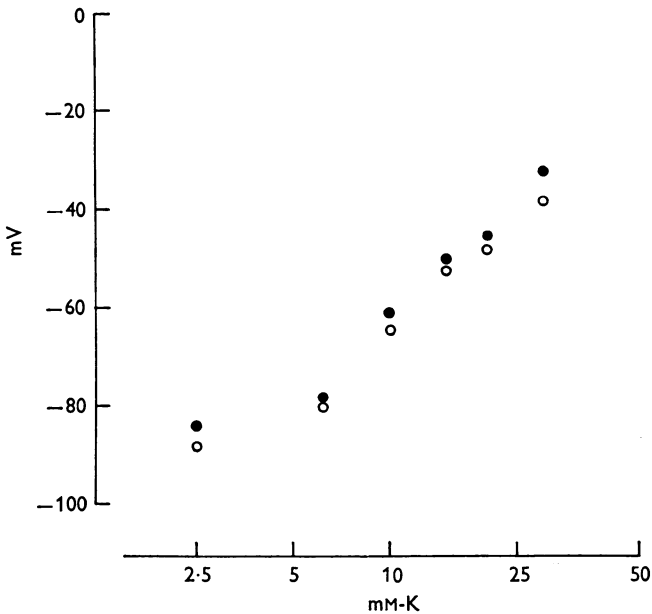

Fig. 4. Effect of temperature on the relationship between fibre membrane potential and external potassium concentration. The filled circles represent the results obtained at 3° C. The open circles represent the values at room temperature. In this Figure, each point is the mean value of several measurements.

The shift expressed in terms of the potassium concentration could, however, only be apparent and not reflect a true change in the contractile threshold of the fibres, if at low temperature the depolarization caused by a given potassium concentration differed from that obtained at room temperature. To test this possibility, experiments involving the measurements of the fibre membrane potential were carried out; the depolarizations caused by different potassium concentrations being measured at two temperatures, 20 or 3° C. The membrane potential of single fibres was initially measured in normal Ringer solution at room temperature, then the solution was substituted by one with a higher potassium content at the same temperature, or by a cold one containing different amounts of

potassium. Solutions containing 2·5, 6·3, 10, 15, 20 and 30 mM potassium were used. These solutions were prepared keeping the [K].[Cl] product constant to avoid loading the fibres with chloride (Hodgkin & Horowicz, 1959). In some cases, the micro-electrode was left in the fibre during the solution change. However, when the temperature was also changed, large junction potentials developed during the solution change. Therefore, it was preferred to withdraw the micro-electrode before the solution change, and then impale the fibre again after having balanced the zero base line for any junction potentials.

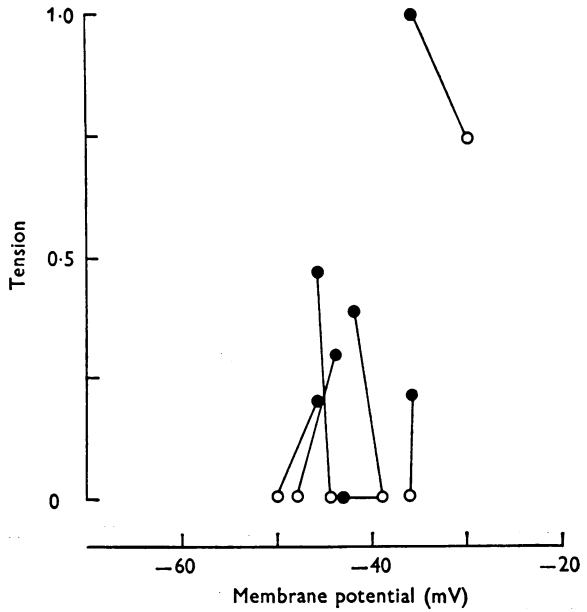

Fig. 5. Effect of temperature on the contractile threshold of single fibres. The filled and open circles represent the results obtained at 3° C and 20° C respectively. The points obtained from the same fibre are united by lines. For further details, see the text.

The results of these experiments are shown in Fig. 4. This Figure shows the relationship between the membrane potential of several fibres and the concentration of potassium in the bathing solution at two temperatures. In another set of experiments, the tension developed by the fibres was recorded continuously during the solution changes. In these cases, the membrane potential was measured immediately before and after the solution changes. The results obtained in this set of experiments are shown in Fig. 5, in which the relationship between the tension and membrane potential is obtained for several fibres at the two temperatures. The fibre tension is expressed as a fraction of the maximum tension, obtained with

higher potassium concentrations. These results show that lowering the temperature causes a real shift in the contractile threshold. For the fibres used in these experiments it appears that while at room temperature, the contractile threshold is higher than -35 mV; at 3° C depolarization of the fibres to -45 mV is sufficient for inducing a contractile response.

In this set of experiments the contractile threshold appears to be higher than that reported by other authors. The threshold for contraction reported by Hodgkin & Horowicz was -50 mV, corresponding to a potassium concentration of 25 mM. In other sets of experiments performed during this study, the value of the contractile threshold was somewhat lower than that shown for the experiments in Fig. 5. However, in every case, regardless of the initial value of the threshold, lowering of the temperature always caused a shift of the threshold toward lower values.

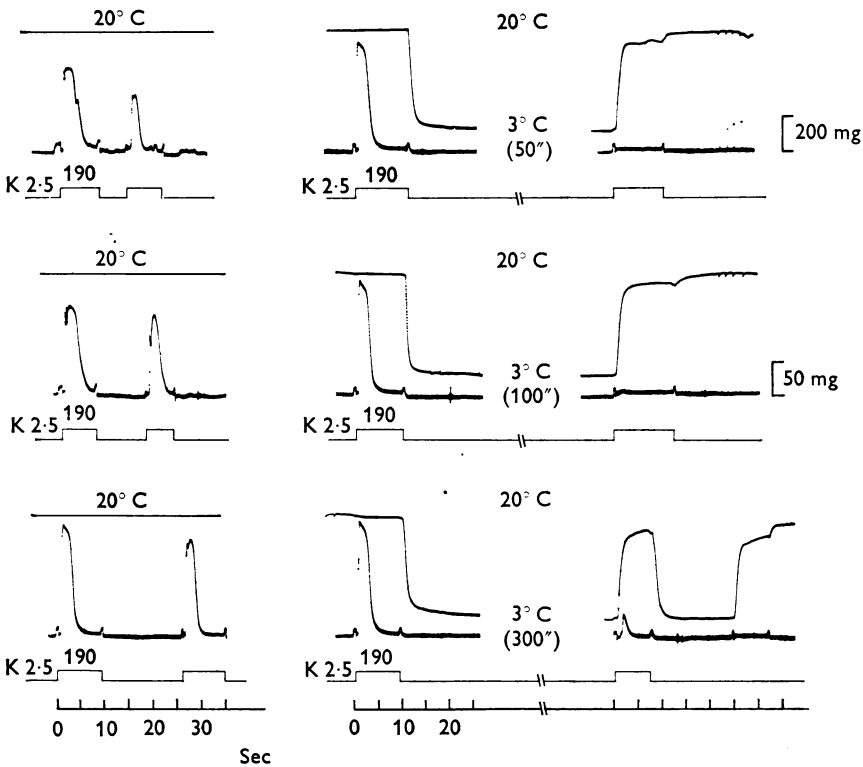

Fig. 6. Effect of temperature on the repriming of a single fibre. After the first contracture, the fibre was allowed to recover in normal Ringer either at 20° C (left side records) or at 3° C (right records). In this and in the following experiments which are similar (Figs. 6, 7, 8), the contracture medium was at room temperature and the recovery medium was either at room temperature or at low temperature. Fibre diameter: 63 μ.

The effect of temperature on the kinetics of repriming

Other authors have reported that contractile repriming is affected by low temperature. Fig. 6 shows how repriming is greatly retarded at low temperature. The left side of this Figure shows the kinetics of repriming at room temperature. For the particular fibre used in this experiment recovery for approximately 5, 10 and 15 sec in a medium containing 2·5 mM potassium gave reprimings to respectively 60, 80 and 90 % of the initial responses. The right side of this Figure shows repriming at 3° C. In this case, after 50 and 100 sec, no repriming had occurred, and after 300 sec there was about 20 % restoration.

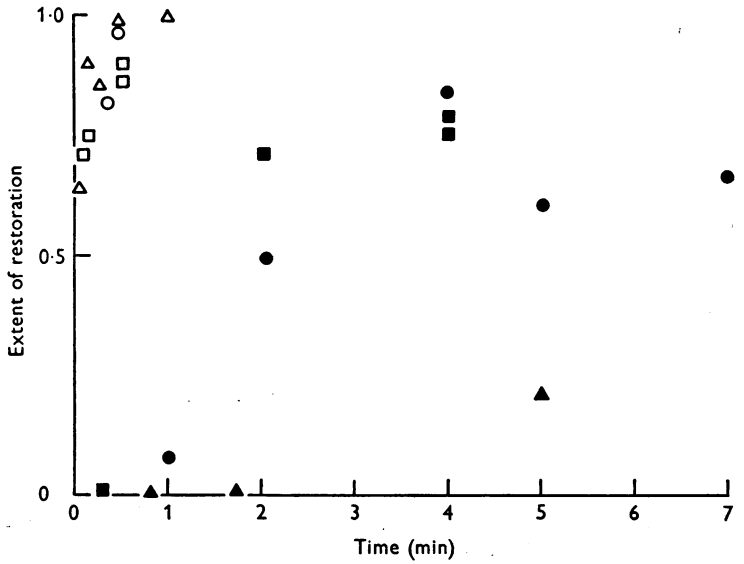

Fig. 7. Kinetics of repriming. The open and filled symbols represent results obtained at 20 or 3° C, respectively. Each symbol represents the results obtained with a different fibre. The ordinate gives the ratio (maximum tension in second contracture)/(maximum tension in first contracture), and the abscissa is the recovery interval.

Fig. 7 shows the time course of restoration for three fibres at either 22 or 3° C. In these experiments, the contractures were elicited with a medium containing 190 mM potassium. It appears that at low temperature the process of repriming is slowed down by about sixfold. After 5 min recovery was almost complete in the cold. These results in part confirm those obtained by Milligan & Edwards (1965), who used whole muscles. Fig. 8 shows the temperature dependence of the repriming process. In the experiments shown here the test for the second contracture was made after a recovery period of 30–90 sec. The plot gives on the ordinate the peak

tension of the second contracture divided by the peak tension of the first contracture. It can be observed that for temperatures above 12° C this recovery period was sufficient for almost complete repriming, while for temperatures below 10° C it did allow little or no recovery at all.

The slowness of repriming at low temperature could be due to failure of the fibre membrane to repolarize in cold normal Ringer. This explanation for this effect would be in agreement with the interpretation that Csapo & Wilkie (1956) gave to their results, obtained in somewhat different

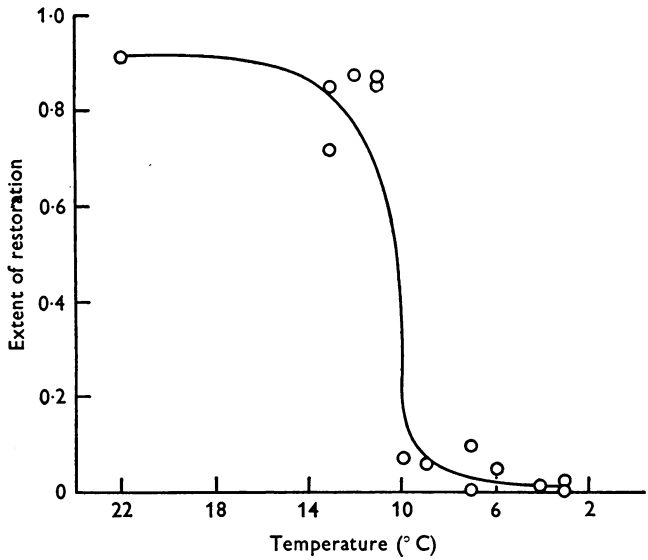

Fig. 8. Dependence of repriming on the temperature of the recovery medium. The results obtained from several fibres have been pooled in this Figure. Each point was obtained from an experiment of the type described in Fig. 6.

experimental conditions and with Grieve's (1960) report. The following experiments were carried out to test this point. The fibres were depolarized with solutions containing 190 mM potassium and then repolarized again with low potassium solutions. Such runs were carried out either at room temperature or at 3° C. Fig. 9 shows the results obtained with one fibre. At the beginning of the experiment, the resting membrane potential of this fibre was −88 mV. While still at room temperature, the fibre was exposed to 190 mM potassium, while the micro-electrode was not in the fibre. A strong contracture developed, and after it subsided a new impalement was made. The membrane potential was found to be −3 mV. With the micro-electrode still in the fibre, normal Ringer solution was flushed into the chamber, and the membrane repolarized at an approximately constant

rate to −78 mV in 7·2 sec, and then, with a roughly exponential time course, to −91 mV. The half time for repolarization was 4 sec. The lower part of this Figure shows a second run, carried out at 3 °C, twenty-five minutes after the upper one. At the beginning the fibre membrane potential was −95 mV and on cooling it dropped to −88 mV; a cold contracture was obtained when 190 K at 3 °C was flushed into the chamber.

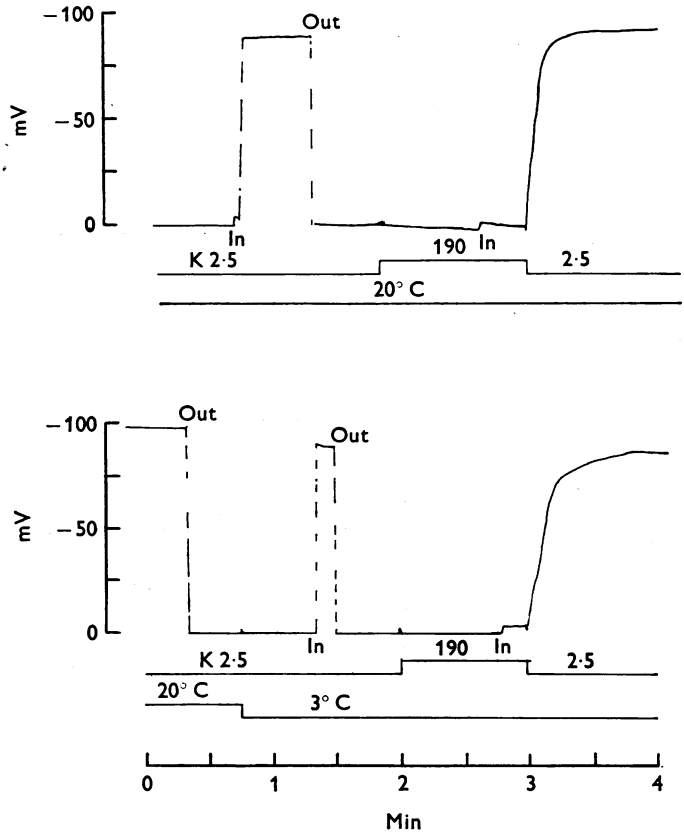

Fig. 9. Rate and extent of repolarization of a fibre at 20° C or 3° C. For further details see the test and Table 2.

After relaxation was completed the membrane potential was found to be −4 mV; on exposing the fibre to cold normal Ringer solution, it repolarized at an approximately linear rate to −65 mV, and then roughly exponentially to −82 mV. The half repolarization time for this case was of 7·2 seconds.

Several more experiments were carried out following the same procedure except that the repolarizing medium contained either a 2·5 mM potassium–120 mM potassium chloride or 5 mM potassium–0 chloride,

with sulphate substituted for chloride. In the 5 potassium-0 chloride experiments, a concentration of 5 mM was chosen because often, and especially at low temperatures, the fibres failed to repolarize in media containing lower potassium concentrations. The results of these experiments are summarized in Table 2. For the cases in which repolarization was achieved with a chloride medium, the rate of repolarization decreased from 8·5 to 3·4 mV/sec when the temperature was lowered, the half repolarization time being increased from 6·6 to 16 sec. In the case of sulphate repolarizing medium, the effect of temperature on the repolarization rate was much less. These results will be interpreted below; however, they make clear that the small delay in repolarization caused by lowering the temperature is not sufficient for explaining the repriming delay.

TABLE 2. Repolarization of muscle fibre membrane after exposure to 190 K solutions

Fibre	R.M.P. initial (mV)	T (° C)	R.M.P. in 190 K (mV)	Time in 190 K (sec)	Repolarizing solution	$t_{\frac{1}{2}}$ (sec)	V/t (linear) (mV/sec)	V_{Final} (mV)
7–13–1	−90	20	−1	120	2·5 K-120 Cl	12	5·4	−82
7–20–1	−80	20	+3	96	2·5 K-120 Cl	6	8·5	−75
7–20–2	−84	3	0	100	2·5 K-120 Cl	13	3	−78
7–20–2	−91	20	+1	76	2·5 K-120 Cl	7·2	5·6	−87
7–20–2	−86	3	−2	108	2·5 K-120 Cl	25	1·5	−65
7–21–1	−88	20	−3	66	2·5 K-120 Cl	4·2	11	−91
7–21–1	−88	3	−4	60	2·5 K-120 Cl	7·2	5·1	−82
7–22–1	−94	3	+1	96	2·5 K-120 Cl	10·8	3·8	−74
7–22–1	−85	20	+3	30	2·5 K-120 Cl	3·6	9	−83
7–23–1	−73	20	−4	59	5 K-0 Cl	14·4	2·5	−74
7–23–1	−61	3	+5	69	5 K-0 Cl	10·8	2·5	−53
7–23–2	−70	3	−5	300	5 K-0 Cl	6	4	−51
7–24–2	−87	20	+2	660	5 K-0 Cl	7·2	2·9	−68
7–24–2	−68	3	−3	270	5 K-0 Cl	6	2·4	−67
7–24–3	−67	20	−1	30	5 K-0 Cl	6·6	5	−81

In the present experiments, the exposure to the high potassium solutions lasted from 60 to 100 sec. It would be of interest to know the exposure duration to high potassium in the case of Grieve's experiments. In fact, a longer exposure to the high potassium solutions could be the underlying cause for the discrepancy of the results obtained. As will be shown in a following paper (Caputo, 1972), there are reasons to suspect that after a very short exposure (5–10 sec) to high potassium media, the fibre membranes repolarize more rapidly than after a 100 sec exposure.

When the fibres are exposed to a chloride-free high potassium solution, the membrane potential is much reduced. Under these conditions the fibre loses chloride, but

the amount lost is probably small during an exposure of less than 100 sec; on re-exposure of the fibres to the normal Ringer solution, the membrane potential value reached is determined by the equilibrium potentials for the potassium and the chloride ions and the membrane conductance to these ions. Hodgkin & Horowicz (1960*a*) found that repolarization in the absence of chloride proceeded at slower rate, a finding that has been confirmed here. In this case the repolarization of the fibre membrane depends on the rate at which potassium ions diffuse out of the transverse tubules. Therefore repolarization is diffusion limited and is not much affected by decreasing the temperature.

At room temperature the repolarization rate in the presence of chloride ions is greater than when these ions are missing. This reflects the fact that the membrane conductance to chloride is rather larger. In these conditions, the decrease in the initial rate of repolarization caused by lowering the temperature is indicative of a high Q_{10} for the membrane chloride conductance. Athough the present results do not give information on the temperature dependence of the potassium conductance, preliminary results obtained with radioactive tracer experiments have shown that the potassium conductance is also diminished at low temperature. Since the total membrane conductance of frog muscle fibres depends mainly on the potassium and chloride conductances one would expect it to be also temperature dependent. In agreement with this is the recent report of Nakajima & Hodgkin (1970), who found a Q_{10} value of 1·49 for the resting membrane conductance in normal Ringer.

Temperature also affects the relationship between the extent of recovery and the potassium concentration in the recovery medium, as shown in Fig. 10. In this experiment the fibre was allowed to recover in solutions with different potassium concentrations, at a temperature of either 22 or 3° C. The contracture inducing solution contained 190 mM potassium. After the first contracture, the recovery solution was flushed in the chamber and immediately after this, the membrane potential was measured. The electrode was left in the fibre for most of the recovery period, which lasted 60 sec for the runs at 22° C, and 300 sec for those at 3° C. Before exposing the fibre again to the 190 mM potassium solution, the micro-electrode was withdrawn. In this Figure the extent of recovery is expressed as the tension ratio of the second to the first contracture, and is plotted against the fibre membrane potential measured just before inducing the second contracture. From this experiment it appears that at low temperature it is necessary to repolarize the fibre to a higher membrane potential than is necessary at room temperature, if repriming is to occur. In these experiments the period of recovery at low temperature was 5 min. Curtis (1964), confirming findings of Hodgkin & Horowicz (1960*b*), has clearly shown that when muscles are allowed to recover after a first contracture in solutions containing 10–20 mM potassium at room temperature, repriming reached a maximum after 60 sec of recovery and tension of the second contracture declined rapidly as the recovery interval was increased beyond 60 sec. Since in the experiment of Fig. 10 the recovery at 3° C lasted 5 min, it was of interest to see whether under these conditions the recovery also had a transient character. Fig. 11 shows the kinetics of recovery for two fibres

at 3° C. After the first contracture, elicited by 95 mM potassium sulphate, the fibres were allowed to recover in a solution containing either 5 or 7·5 mM potassium. It appears that for these potassium concentrations at 3° C, the effect described by Curtis is not present. Hodgkin & Horowicz ascribed the effect to the increased metabolic expenditures that occur with increased potassium concentrations, between 10 and 30 mM. The absence of the effect in the present case could be due either to the fact that subthreshold concentrations of potassium were used, or to the lower temperature, or to a combination of both factors.

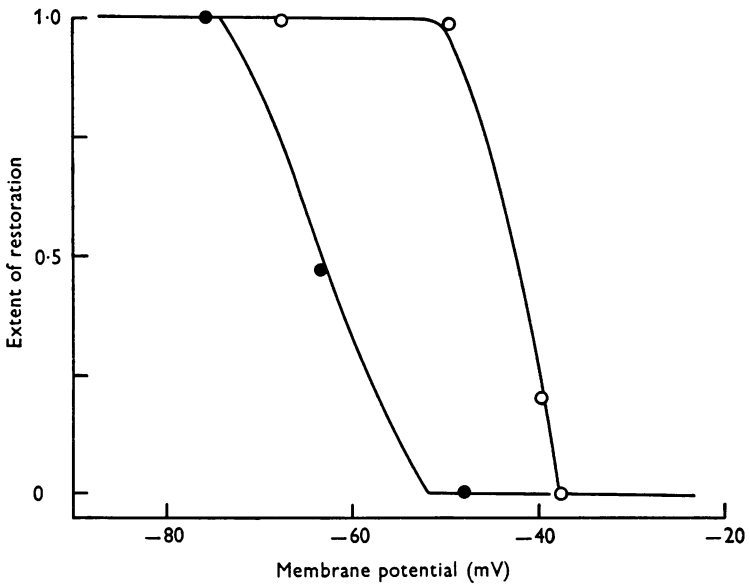

Fig. 10. Effect of temperature on the relationship between extent of re-priming and the fibre membrane potential. The filled circles represent the results obtained at 3° C, and the open circles were obtained at 20° C. The procedure was similar to that shown in Fig. 7. Membrane potential during recovery was monitored with a micro-electrode. The recovery periods at 20 and 3° C were 1 and 5 min, respectively.

Recovery of twitches after potassium contractures

After a potassium contracture, a short recovery period in low potassium solution is also necessary for the fibre to recover its ability to twitch. As shown by Hodgkin & Horowicz (1960*b*) the first twitches after the refractory period are rather small and increase progressively to their full size. Frankenhaeuser & Lannergren (1967) confirmed the suggestion of the former authors, that the small twitch amplitude during the early phase of recovery was due to a reduction of the action potential. It

appears then, that exposure to elevated potassium concentration affects the subsequent electrical and mechanical behaviour of the fibres. This effect was greatly enhanced at low temperature. In fact, in the course of this work, it was found that when the fibres were allowed to recover in the cold after potassium contractures, the recovery of their ability to twitch

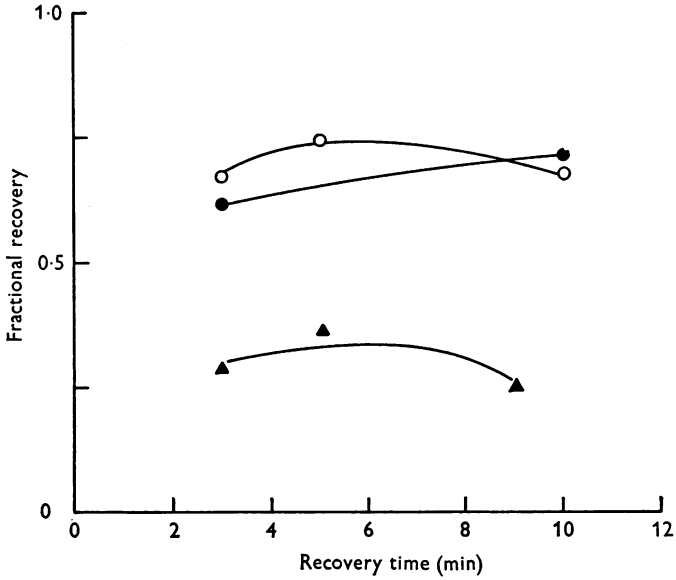

Fig. 11. Time course of repriming at low temperature in solutions with increased potassium concentrations. The two symbols represent results obtained with two different fibres. The open and filled symbols represent results obtained with recovery media containing 5 and 7·5 mM potassium respectively.

was also delayed, as it is shown in Fig. 12. However, it was found that the action potential recovered before the twitch as it is shown in Fig. 13. In these experiments immediately following relaxation from the contracture, a micro-electrode was inserted in the fibres for monitoring the presence of action potentials in response to stimulation. The four action potentials in Fig. 13 were obtained at the times marked with the arrows on the trace of Fig. 12. It appears that the two first action potentials were obtained when the fibre had not yet recovered its ability to twitch. It is interesting to notice that after exposure to a high potassium concentration, on returning to cold normal Ringer, the action potentials show at first a greatly slowed falling phase, and later they recover their normal shape. The shape of the action potentials obtained during the early stage of recovery, at a time when the twitches are still absent, suggest that the delayed rectifier conductance, which speeds repolarization, recovers very slowly from the

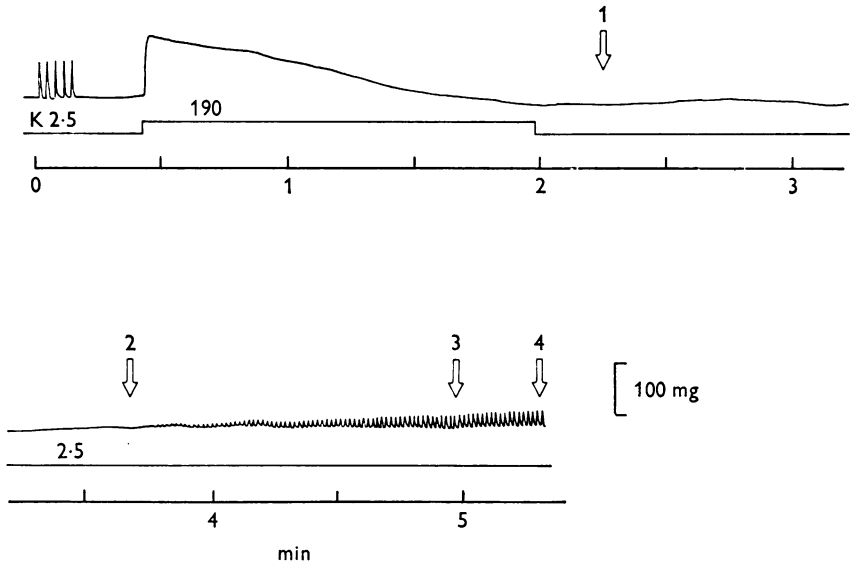

Fig. 12. Time course of recovery of twitches at 3° C after a potassium contracture. The numbered arrows mark the time at which the action potentials shown in Fig. 13 were recorded. Fibre diameter: 70 μ.

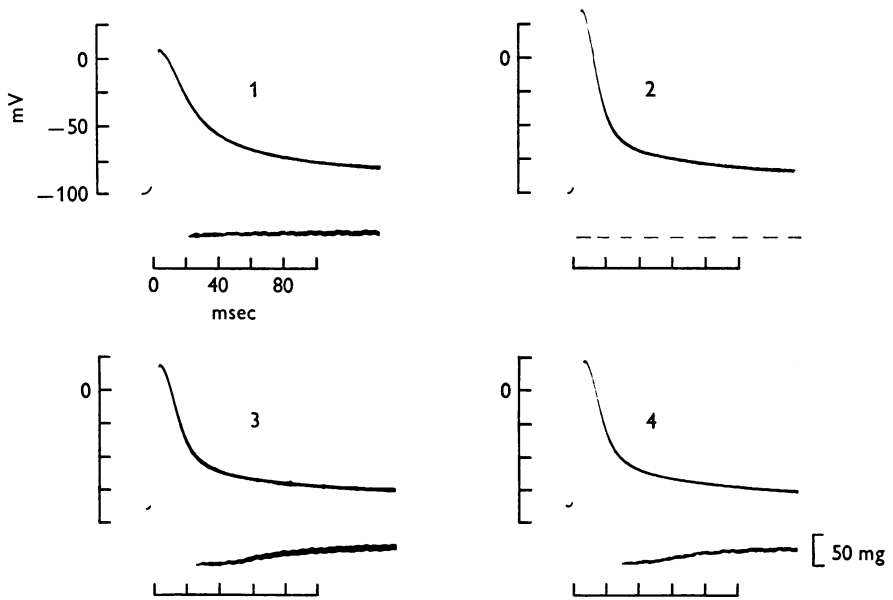

Fig. 13. Recovery of the action potentials and twitches at 3° C after a potassium contracture. See Fig. 12 and the test for further details.

inactivation caused by the high potassium solutions. Interestingly, the delayed rectifier conductance and the twitch seem to recover with about the same time course. There are other examples in the literature of similarities between these phenomena, e.g. threshold of contraction and the delayed rectifier, but these parallels seem to be coincidental (Adrian, Chandler & Hodgkin, 1969).

DISCUSSION

Low temperature greatly prolongs the time course of potassium contractures. This effect could be explained in terms of the high Q_{10} reported for the active uptake of calcium by the isolated vesicles of the sarcoplasmic reticulum; however, as will be shown in a next paper (Caputo, 1972) a slower release of calcium could also explain the effect. Hodgkin & Horowicz (1960*b*) assumed that during a contracture at 20° C, the contractile activator is released at a rate wastefully high. A slower release of the activator would allow a more efficient utilization of the calcium released. Some support for this idea derives from the recent work of Curtis (1970) who has measured the efflux of ^{45}Ca from the semitendinosus muscle of the frog. In normal Ringer solution, lowering the temperature to 1° C causes a marked decrease of the rate of calcium efflux; subsequent perfusion of the muscle with 117 mM potassium causes no increase in the efflux. This is in marked contrast with the results obtained at room temperature (Bianchi & Shanes, 1959).

The shift in the contractile threshold reported in this work is in the opposite direction of that reported by González-Serratos (1965). The reasons for this discrepancy are not clear, and certainly require further study. It is worth noticing, however, that González-Serratos fibres were held at a length below the normal slack length, while in the present work they were slightly stretched above slack length.

The effect reported here is compatible with the results of Sakai (1965) and of Luttgau & Oetliker (1968), concerning the increased sensitivity of the fibre to caffeine at low temperature. The shift in the threshold can be explained by assuming that calcium release starts at a membrane potential value which is nearer to the resting membrane potential. A similar mechanism of facilitated release of calcium in the cold has been considered by Ebashi & Endo (1968) to explain the results of Sakai (1965). It may be assumed that at room temperature the mechanism responsible for the reabsorption of calcium by the elements of the sarcoplasmic reticulum is sufficient to neutralize the release of small amounts of calcium in response to subthreshold depolarizations. At low temperature this mechanism might be affected and under these conditions

small quantity of calcium released by the sarcoplasmic reticulum might be effective in activating the contractile mechanism.

A marked slowing of the contractile repriming at low temperatures was previously reported by Erlij & van der Kloot (1964) and Milligan & Edwards (1965) for the case of whole muscles. The results presented here confirm and somewhat extend the findings of these authors. Several years ago, Csapo & Wilkie (1956) had reported that following exposure of sartorius muscles to high potassium recovery of the twitch was much slowed down at low temperature. This observation has also been confirmed here. Following a suggestion of Csapo & Wilkie, Grieve (1960) reported that the delayed recovery of the twitch at 2° C was a consequence of the delayed repolarization that he found to occur under his experimental conditions. The results presented here are in marked contrast with Csapo & Wilkie's explanation and with Grieve's results. In addition, it has been shown that after exposure to high potassium, when allowed to recover in cold normal solution, the fibre repolarize rather quickly, and, furthermore, they recover their action potentials before recovering their ability to twitch. Therefore, it is clear that low temperature slows down the repriming of the contractile ability, and that this effect is not due to failure of the membrane to repolarize. A tentative explanation for the delayed repriming at low temperature is provided by the results of Winegrad (1970). Using a radioautographic technique he has shown that after tetanic activity, calcium translocation in muscle fibres occurs with a time course that is comparable with that of the repriming process after potassium contractures. Furthermore, he also showed a marked temperature dependence for this intracellular translocation.

Curtis (1970) has recently reported that the calcium efflux is not significantly increased during a potassium contracture at 3° C, in spite of the longer duration of this response. Considering the Curtis and Winegrad results, it seems reasonable to argue that most of the contractile activator in a fibre is not lost during a contracture, but that some internal recycling occurs. This would confirm the view that calcium is more efficiently used during a prolonged contracture in the cold. It is possible, however, that extracellular calcium might contribute to the repriming process by making up for the fraction calcium that may be lost in the contractile cycle.

According to the facts mentioned above, repriming of the contractile ability can be considered as depending on at least two processes. The first could be related to the translocation of the calcium from the region near the contractile elements to the sarcoplasmic reticulum. This process of restoration of calcium in the terminal cisternae is temperature

dependent (Winegrad, 1970). Re-accumulation of calcium in the terminal cysternae is not a sufficient although it is a necessary condition for restoring the contractile ability. A second process, which is dependent on the fibre membrane potential must take place for achieving contractile repriming. The present work gives no information on the nature of this process or on its temperature dependence. It might be assumed that depolarization of the fibre membrane increases the conductance of the terminal cisternae membranes to calcium ions, allowing them to flow down their electrochemical gradient and into the sarcoplasm. This hypothetical calcium conductance of the membrane of the sarcoplasmic reticulum might be slowly inactivated with time, and it is conceivable that membrane repolarization is necessary for restoring its ability to be activated. This scheme, is of course, speculative, and will be dealt with in a later paper.

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REFERENCES

ADRIAN, R. H., CHANDLER, W. K. & HODGKIN, A. L. (1969). The kinetics of mechanical activation in frog muscle. *J. Physiol.* **204**, 207–230.

BIANCHI, C. P. & SHANES, A. M. (1959). Calcium influx in skeletal muscle at rest, during activity, and during potassium contracture. *J. gen. Physiol.* **42**, 803–815.

CAPUTO, C. (1966). Caffeine- and potassium-induced contractures of frog striated muscle fibres in hypertonic solutions. *J. gen. Physiol.* **50**, 129–139.

CAPUTO, C. (1969). Potassium contractures of single muscle fibres at low temperature. *Abstr. III int. Biophys. Congr.* II S.9.

CAPUTO, C. (1971). Time course of potassium contractures at low temperature. *Abstr. XXV int. physiol. Congr.*, **281**.

CAPUTO, C. (1972). The time course of potassium contractures of single muscle fibres. *J. Physiol.* **223**, 483–505.

CAPUTO, C. & GIMÉNEZ, M. (1967). Effects of external calcium deprivation on single muscle fibres. *J. gen. Physiol.* **50**, 2177–2195.

CSAPO, A. & WILKIE, D. R. (1956). The dynamics of the effect of potassium on frog's muscle. *J. Physiol.* **134**, 497–514.

CURTIS, B. A. (1964). The recovery of contractile ability following a contracture in skeletal muscle. *J. gen. Physiol.* **47**, 953–964.

CURTIS, B. A. (1970). Effect of cold on resting and stimulated ^{45}Ca efflux from skeletal muscle. *Fedn Proc.* **29**, 846.

EBASHI, S. & ENDO, M. (1968). Calcium ions and muscle contraction. *Prog. Biophys. molec. Biol.* **18**, 123–183.

EDWARDS, C. & CARLSON, F. D. (1964). Potassium contractures and creatine phosphate breakdown in frog muscle. *Biochim. biophys. Acta* **88**, 213–215.

ERLIJ, D. & VAN DER KLOOT, W. G. (1964). Effect of temperature on the recovery from K^+-contracture. *Physiologist*, **7**, 127.

FRANKENHAEUSER, B. & LANNERGREN, J. (1967). The effect of calcium on the mechanical response of single twitch muscle fibres of *Xenopus laevis*. *Acta physiol. scand.* **69**, 242–254.

GONZÁLEZ-SERRATOS, H. (1965). Differential shortening of myofibrils during contractures of single muscle fibres. *J. Physiol.* **179**, 12–14.

GRIEVE, D. W. (1960). The effect of low temperature upon the recovery of frog sartorius muscle from potassium depolarization. *J. Physiol.* **152**, 25–26*P*.

HODGKIN, A. L. & HOROWICZ, P. (1959). The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol.* **148**, 127–170.

HODGKIN, A. L. & HOROWICZ, P. (1960*a*). The effect of sudden changes in ionic concentrations on the membrane potential of single muscle fibres. *J. Physiol.* **153**, 370–385.

HODGKIN, A. L. & HOROWICZ, P. (1960*b*). Potassium contractures in single muscle fibres. *J. Physiol.* **153**, 386–403.

JOBSIS, F. F. & DUFFIELD, J. C. (1967). Oxidative and glycolytic recovery metabolism. *J. gen. Physiol.* **50**, 1009–1047.

LUTTGAU, H. C. & OETLIKER, H. (1968). The action of caffeine on the activation of the contractile mechanism in striated muscle fibres. *J. Physiol.* **194**, 51–74.

MILLIGAN, J. V. & EDWARDS, C. (1965). Some factors affecting the time course of the recovery of contracture ability following a potassium contracture in frog striated muscle. *J. gen. Physiol.* **48**, 975–983.

NAKAJIMA, S. & HODGKIN, A. L. (1970). Effect of diameter on the electrical constants of frog skeletal muscle fibres. *Nature, Lond.* **227**, 1053–1055.

SAKAI, T. (1965). The effects of temperature and caffeine on activation of the contractile mechanism in the striated muscle fibres. *Jikeikai Med. J.* **12**, 88–102.

WINEGRAD, S. (1970). The intracellular site of calcium activation of contraction in frog skeletal muscle. *J. gen. Physiol.* **55**, 77–88.