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POTASSIUM CONTRACTURES IN SINGLE MUSCLE FIBRES OF THE CRAYFISH

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SUMMARY

1. Contractures were evoked in isolated muscle fibres of the extensor carpopoditi muscle of the crayfish (*Astacus fluviatilis*) by increasing $[K]_o$ at constant $[Cl]_o$ or at constant $[K]_o[Cl]_o$ product.

2. The relation between tension and log $[K]_o$ is S-shaped with a less steep slope if $[K]_o$ was increased at constant $[Cl]_o$. This is due to a smaller drop in membrane potential for a given change in $[K]_o$ in the latter case.

3. The curves relating the tension to the membrane potential overlap in either case. In the linear part of the curve, the slope is around 0·3 kg $cm^{-2}mV^{-1}$.

4. The mechanical threshold of contracture is about -55 mV and mechanical saturation is at -20 mV.

5. Fibres exert the greatest tension when stretched to $1{\cdot}25l_o$ (8 kg/cm^2), where l_o is the length at which the fibres are just taut in the solution. Tension falls on either side of this optimal length. Tension vanishes when the fibre is stretched to $1{\cdot}95l_o$.

6. Sarcomere length at optimal fibre length is $10{\cdot}5 \pm 0{\cdot}3\mu$. The A band is $3{\cdot}95 \pm 0{\cdot}8\mu$ long and does not alter during stretch.

7. The crayfish muscle fibres were of the phasic type, since they relaxed spontaneously at maintained high $[K]_o$.

8. At $[K]_o$ near saturation point, contractures attain the maximum tension in $5 \pm 1{\cdot}3$ sec and the time to half decay is $8{\cdot}1 \pm 0{\cdot}5$ sec.

9. If the contracture is allowed to relax spontaneously, it is not possible to obtain initial tension until after 20–30 min. When the contracture is terminated by a return to low $[K]_o$ after reaching its maximum, but before spontaneous relaxation appears, the fibre is capable of repeatedly exerting the initial tension.

10. The rate of recovery after a spontaneously relaxed contracture depends on $[K]_o$ in the solution, in which the fibre lies before evoking the

test contracture. The relation of recovery upon log $[K]_o$ is S-shaped and the tension is the greater, the lower the $[K]_o$ in the solution in which recovery is taking place.

INTRODUCTION

The present report is concerned with the characteristics of contractures of isolated crayfish muscle fibres, evoked by a sudden increase of potassium ions in the external medium. The advantages of this method of depolarizing the membrane and activating the contractile apparatus became apparent from the very first time it was used on isolated phasic muscle fibres of the frog (Hodgkin & Horowicz, 1960). In the first place, membrane depolarization is defined better than in the case of natural activation by action potentials. Not less important is the fact that the depolarization involves practically the whole of the muscle fibre at once and uniformly. This makes it possible to study the tension independently as a function of the membrane potential and as a function of time. It was while studying the latter relation in the crayfish muscle fibre that some new data were obtained concerning the relaxation phase of the contracture.

However, there was yet another reason for extending the study of potassium contractures to another species and namely to isolated crayfish muscle fibre. The sarcomere of crayfish muscle fibres is several times longer than that in the frog twitch muscle fibres. This appeared to be suitable comparative material for correlating tension and length of the sarcomere since, according to the 'sliding theory' of muscle contraction (Huxley & Hanson, 1954; Huxley & Niedergerke, 1954), the maximum tension of the muscle fibre/unit cross-section is proportional to sarcomere length, the other quantities being constant (Huxley, 1957). In view of the innervation and the local character of the membrane and contractile responses, the potassium contracture represents the easiest approach to obtaining maximal tension in crayfish muscle fibres.

Some of these results have been published briefly elsewhere (Zachar & Zacharová, 1966).

METHODS

All experiments were performed on single muscle fibres isolated from the extensor carpopoditi muscle of the crayfish (*Astacus fluviatilis*). The dissection procedure and determination of maximal ($d_{max.}$) and minimal ($d_{min.}$) diameter of fibre was analogous to that described in previous reports (Zachar, Zacharová & Henček, 1964*a*; Zachar & Henček, 1965). The equivalent diameter, d_e, is defined by the relation $d_{max} \cdot d_{min.} = d_e^2$, since the fibres are elliptical in cross-section (Table 2).

The apparatus used for fixing the fibre for simultaneous registration of membrane potential changes and tension was also analogous to that used previously (Zachar *et al.* 1964*a*). This apparatus, similar in principle to that of Hodgkin & Horowicz (1960) made it possible to exchange the bathing solution around the fibre in a fraction of a second. The mean velocity of fluid flow in the canal in which the fibre was placed was 10–20 cm/sec. Because of the high absolute value, the exerted tension was not registered by the RCA 5734

transducer by connecting the tendon to the movable anode directly, but after reduction. The transducer characteristic was then linear up to 15 g.

The membrane potential was measured using a Ling–Gerard micro-electrode filled with 3 M-KCl. One micro-electrode of about 10 MΩ resistance was inserted into the fibre, another with a resistance of about 3 MΩ served as an indifferent electrode. The tip potential (Adrian, 1956) of these micro-electrodes was smaller than 4 mV. In order to prevent the micro-electrode from slipping out of the fibre during the contracture, it was made partially movable (Zacharová & Zachar, 1965) by shortening its body and fixing it to a rubber tube. Because the fibres are relatively large (Table 2), this ensured a successful insertion in 50 % of cases.

Sarcomere length. The length of the sarcomere was measured from microphotographs made with the use of a double microscope, constructed according to the Lau principle (Lau, 1960). The ground glass plate between the first (× 10) and second (× 6) objective lens rotated with the aid of compressed air. Using a × 10 eye-piece, it was possible to obtain × 600 magnification at identical distance of the first objective lens from the fibre as in a normal microscope at × 100 magnification. Even at × 1200 magnification it was still possible to work without immersion (first objective lens × 20). Microphotographs of the central part of the crayfish muscle fibre in van Harreveld (1936) solution, photographed with the double microscope are given in Plate 1.

Individual segments of the sarcomere are not so clearly delineated in *Astacus* as in frog muscle fibres, because the sarcomeres of adjacent myofibrils are not strictly in register and the fibres are thick. Furthermore, as shown recently (Brandt, Reuben, Girardier & Grundfest, 1965) by electron microscopic examination of *Procambarus* fibres, the thick filaments are irregularly staggered at the edge of the A band.

Sarcomere length was set at different lengths which were multiples (1, 1·25, 1·5, 1·75) of the initial length, l_o. The initial length is defined as the length of the fibre at which it is just taut in the solution. The majority of measurements were made at the length 1·25 l_o. Measurements of sarcomere length at different multiples of l_o were performed in order to ascertain which band is the one corresponding to the thick filament. Since the length of dark band did not change during stretch, it was identified as the A band, which was 3·95 μ long on the average (Table 2).

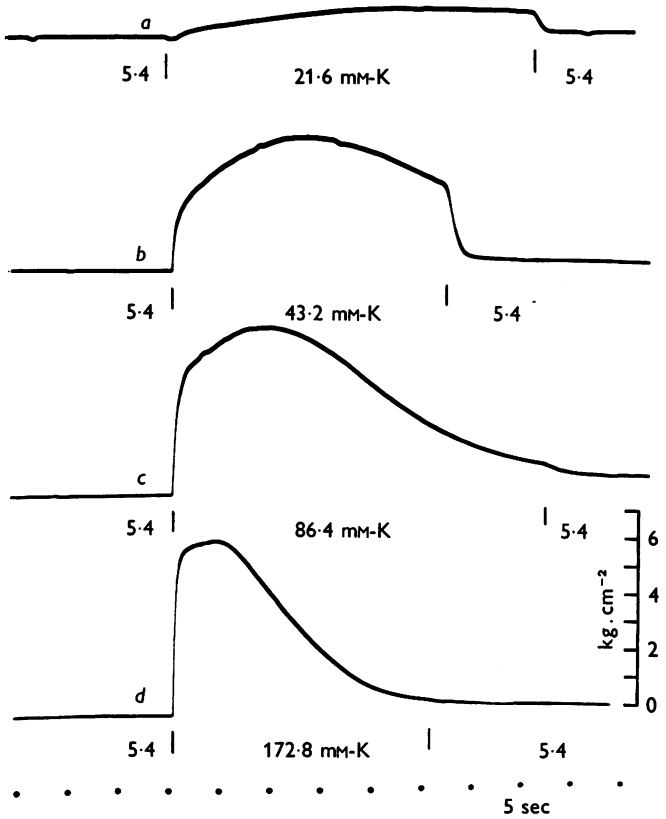
Solutions. Fibres were dissected and measured in van Harreveld (1936) solution of the following composition (in mM): Na^+, 208·4; K^+, 5·4; Ca^{2+}, 13·5; Mg^{2+}, 5·57; Cl^-, 249·5; HCO_3^-, 2·38. Tris-buffer was also used instead of the bicarbonate buffer; pH was 7·3–7·5. Contractures were evoked by raising external potassium concentration in the bathing solution (exchanged for $[Na]_o$). The concentration of chloride ions, $[Cl]_o$ was kept constant in one series, in another it was decreased by the same factor as $[K]_o$ was increased, in order to preserve the Donnan equilibrium ($[K]_o.[Cl]_o$ = constant). In the latter case, chloride was substituted for propionate, which does not interfere with the activity of calcium ions, as has been shown by Girardier, Reuben, Brandt & Grundfest (1963). The relative tonicity and ionic strength were the same in both cases as in van Harreveld solution. In a small number of experiments methylsulphate and sulphate were also used as substitutes for chloride ions. Experiments were performed at room temperature 18–23° C.

RESULTS

The time course of the potassium contracture

Contractures evoked by a sudden increase of $[K]_o$ at constant $[K]_o[Cl]_o$ product are shown in Text-fig. 1. The presence of spontaneous relaxation during maintained increased $[K]_o$ indicates that the fibres are of the phasic type.

Two distinct phases can be recognized in the course of tension development, a quick initial rise followed by a slower creep to the maximum. The ascending and descending phase of the contracture become shorter with increasing $[K]_o$ (Table 1). At saturation $[K]_o$ (173 mM-K; Text-fig. 2)



Text-fig. 1. Contractures in isolated crayfish muscle fibre evoked by sudden application of solutions with increased external potassium concentration (21·6–172·8 mM-K) at constant $[K]_o[Cl]_o$ product (Cl replaced by propionate). Contractures *a* and *b* (partly also *c*) were interrupted before complete spontaneous relaxation, by exchanging contracture solution for van Harreveld solution. Fibre 21*T*, diameter $d_e = 452\mu$, temperature 19° C. The fibre was stretched to $1{\cdot}25\, l_o$ (= 15·6 mm), sarcomere length $10{\cdot}8\mu$. Records taken in the order $a \rightarrow d$. The fibre was allowed to rest in van Harreveld solution for 40–50 min between contractures.

maximum tension is attained after an average of 5 sec ($t_{max.}$) and during relaxation tension drops to 50 % of the maximum (t_d) in 8 sec. During the initial fast phase of the ascent of contracture, tension reaches half the maximum value in 0·34 sec (t_a).

Contractures evoked by increased $[K]_o$ at constant external chloride follow a similar course. They differ from the above-mentioned ones in that at submaximal $[K]_o$, the second slower phase of the rise in tension represents a relatively larger proportion of the amplitude of contracture. This course can be explained if one takes into account the extremely steep curve of the relation between tension and the membrane potential and the slow creep of depolarization to the final value when the $[K]_o$ is increased at constant $[Cl]_o$ (Zachar, Zacharová & Henček, 1964*a*, *b*).

TABLE 1. The time characteristics of potassium contractures in single muscle fibres of the crayfish (*Astacus fluviatilis*)

(Contracture solution: $5{\cdot}4x$ mM-K and $(250/x)$ mM-Cl)

	$x = 8$	$x = 16$	$x = 32$
Half time of ascent, t_a*	$0{\cdot}82 \pm 0{\cdot}26$†	$0{\cdot}49 \pm 0{\cdot}12$	$0{\cdot}34 \pm 0{\cdot}10$
Half time of descent, t_d	$18{\cdot}96 \pm 1{\cdot}69$	$14{\cdot}08 \pm 1{\cdot}57$	$8{\cdot}09 \pm 0{\cdot}54$
Time to maximum, t_{max}	$15{\cdot}39 \pm 1{\cdot}98$	$11{\cdot}29 \pm 1{\cdot}69$	$5{\cdot}05 \pm 1{\cdot}27$

* The initial fast phase of ascent.
† Mean ± S.D. (seven fibres) in seconds.

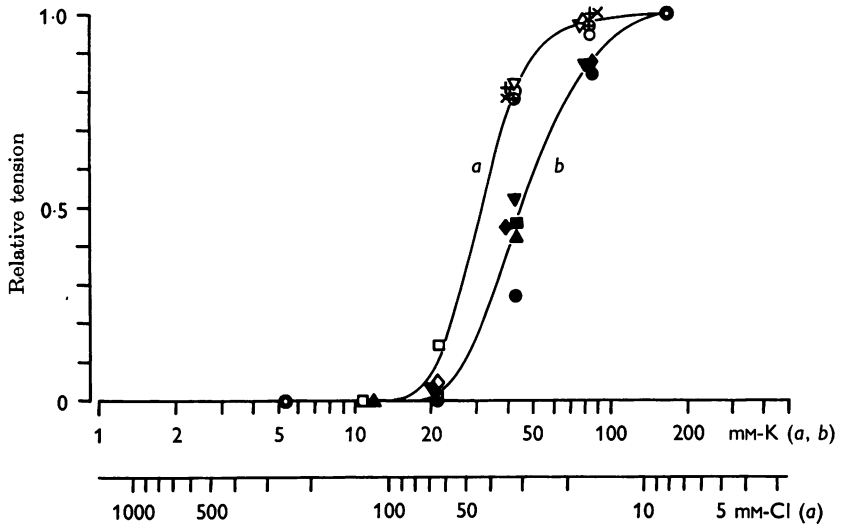
Contracture solutions contained varying amounts of sodium ions depending on the $[K]_o$. No attempt was made to replace them by an impermeable cation, since the regenerative action of sodium ions on membrane potential is so small in the muscle fibres studied (Fatt & Ginsborg, 1958) that it can be disregarded. Choline was not tried owing to its powerful electrogenic action in this type of muscle (Fatt & Katz, 1953). The records of depolarization by high K show, as demonstrated in subsequent sections, that a regenerative change in membrane potential is negligible.

The time course of the contracture in a crayfish muscle fibre differs from that of the isolated frog twitch muscle fibre (Hodgkin & Horowicz, 1960) especially in its relaxation phase. In the frog fibre, relaxation exhibits two distinct phases, namely a rapid decrease from the maximum to a plateau level from which, after several seconds, there is a steep return to initial tension, the half time of decay being distinctly shorter than in the spontaneously relaxing crayfish muscle fibre. At submaximal values of $[K]_o$ the crayfish fibres do not exhibit the latency and consequently the S-shaped increase of tension as is the case in phasic (Hodgkin & Horowicz, 1960 and tonic (Nasledov, Zachar & Zacharová, 1966) fibres of the frog.

The relation between potassium concentration and tension

The relation of peak tension to log $[K]_o$ is shown in Text-fig. 2. Amplitude of the contracture evoked by a 32-fold increase of $[K]_o$ was taken as 1. This relation is analogous to that described for isolated twitch (Hodgkin & Horowicz, 1960; Lüttgau, 1963) and tonic (Nasledov *et al.*

1966) frog muscle fibres. Contractures begin at about 20 mM-K and the increment in tension in response to further increases in $[K]_o$ is very steep. If $[K]_o$ is increased, however, at constant external chloride concentration (open symbols, five fibres), the increment is half as small as when $[K]_o$ is increased, at constant product $[K]_o[Cl]_o$ (filled circles, eight fibres). Differences in the slope can be explained by differences in the extent of depolarization using one or other method of increasing $[K]_o$, since the cell membrane of the muscle fibres studied is permeable to both K and Cl ions (Zachar *et al.* 1964*a*, *b*). This explanation is also supported by measurements of the relation between tension and the membrane potential in the following section (Text-fig. 4).

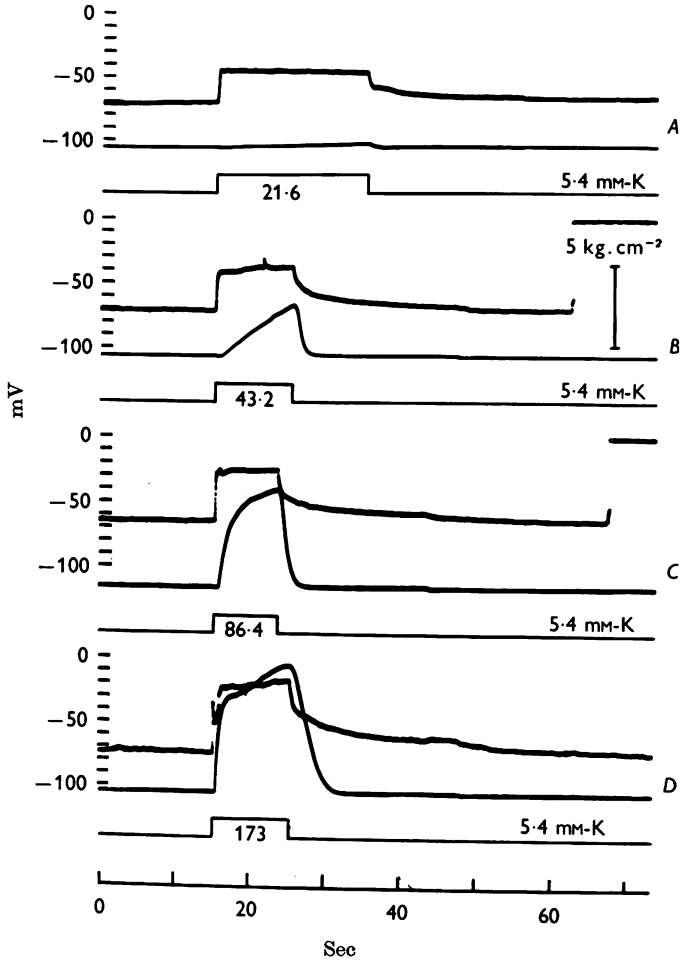


Text-fig. 2. The relation between contracture tension and logarithm of external potassium (*a*, *b*) and chloride (*a*) concentration. The maximum of tension at $[K]_o$ = 173 mM was taken as 1. Various symbols denote individual fibres. Open symbols (*a*): $[K]_o$ increased at constant $[K]_o[Cl]_o$ product (external Cl replaced by propionate). Filled symbols (*b*): $[K]_o$ increased at $[Cl]_o$ = const. and at $([Na]_o+[K]_o)$ = const.

The relation between membrane potential and tension

Text-figure 3 presents some simultaneous recordings of changes in membrane potential and of tension at 4-, 8-, 16- and 32-fold increases of $[K]_o$ at constant $[K]_o[Cl]_o$ product (chloride being replaced by propionate). Contracture began at the membrane potential of about -45 mV after application of a solution containing 21·6 mM-K and 62·5 mM-Cl. The mechanical saturation was reached when the fibre was depolarized to about -20 mV in 173 mM-K and 7·8 mM-Cl.

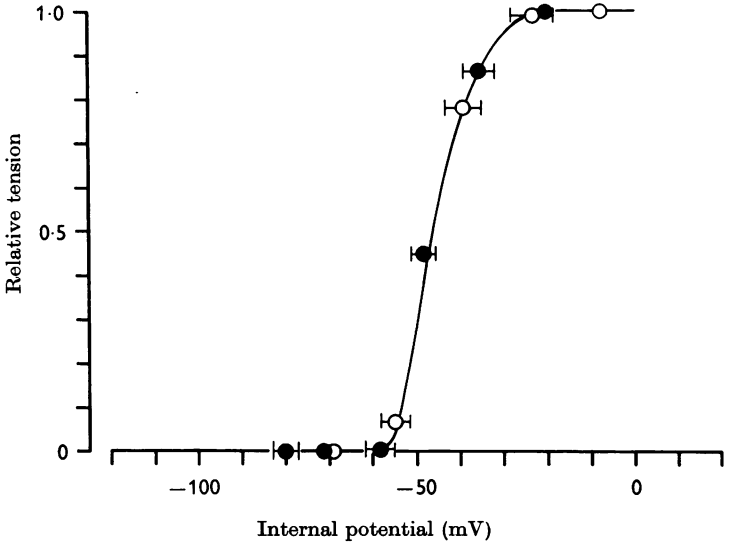
The relation between membrane potential and peak tension of contracture is demonstrated in Text-fig. 4. Open circles represent mean values of the membrane potential and of the tension of contractures evoked by



Text-fig. 3. Simultaneous recordings of the membrane potential (upper beam) and tension (lower beam) after sudden application of potassium concentrations varying between 21·6 and 173 mM at constant $[K]_o[Cl]_o$ product (Cl replaced by propionate). Fibre 25*B*, $d_e = 412\mu$, temperature 20° C. Fibre was stretched to $1{\cdot}25\ l_o$.

solutions with a constant $[K]_o[Cl]_o$ product; filled circles represent mean values obtained using solutions in which $[Cl]_o$ was kept constant. The mean values also include values of tension, where the membrane potential was not measured simultaneously and, vice versa, where the membrane poten-

tial was measured without recording the tension. The membrane potential was measured either by the procedure shown in Text-fig. 3, or by introducing the micro-electrode into the fibre during maximal tension or after spontaneous relaxation (Text-fig. 11). Value of the membrane potential was read off 15–30 sec after application of the contracture solution, i.e. after maximal tension had been reached, so that the drift of the membrane potential, demonstrated, e.g. in Text-fig. 3*B*, also contributed to this value. Simultaneous records of tension and membrane depolarization were evaluated, however, only if the tension generated attained a steady value in the course of recording.



Text-fig. 4. Relation between peak tension of contracture and membrane potential. ○ depolarization evoked by a sudden change of $[K]_o$ at constant $[K]_o[Cl]_o$ product (propionate substituted for Cl), ● depolarization caused by increased external potassium at constant external chloride. Horizontal bars indicate ± two S.E. at $n > 3$. Values of relative tension are mean values from the relation in Text-fig. 2.

It is evident from Text-fig. 4 that the tension amplitude depends on the membrane potential value, irrespective of the way in which membrane depolarization has been achieved. It then follows that a relation between tension and log $[K]_o$ which is steeper at constant $[K]_o[Cl]_o$ product than when $[K]_o$ is varied at constant $[Cl]_o$ (Text-fig. 2), can be fully explained by the steeper relation between the membrane potential and log $[K]_o$in the former case.

The contractile threshold, tension gradient and mechanical saturation

The contractile threshold, defined by the value of the membrane potential at which measurable tension is obtained, can be extrapolated from the relation of tension upon the membrane potential (Text-fig. 4). It corresponds to about -55 mV, when the resting potential is -80 mV.

After reaching the threshold, tension rises linearly with the membrane potential for practically 90% of the total exerted tension range. The tension gradient has a value 0·35 kg cm^{-2} mV^{-1} in the linear part of the relation, as can be found from Text-fig. 4 and using the absolute values of maximal tension (Table 2).

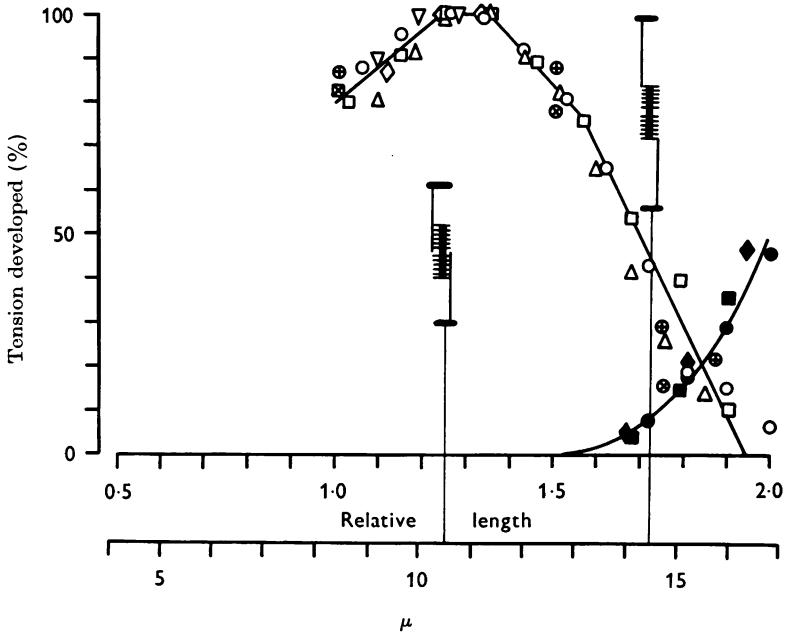
Mechanical saturation (Sandow, 1965), defined as the membrane potential at which tension attains maximal values, occurs when the membrane potential is around -20 mV. Mechanical activation thus takes place in the interval between -60 and -20 mV. About 80% of the activation occurs within about half this range, since the transition towards the threshold and to the mechanical saturation is more gradual.

This fact is evidently also associated with the time course of contractures at various $[K]_o$. When the membrane potential passes the contractile threshold, a change in membrane potential, which is small compared with the change from the resting potential, is then sufficient to cause an equal, or even greater, increase in tension. The closer the depolarization to mechanical saturation, the smaller is the increase in tension due to a given membrane potential change caused by the drift.

The relation between sarcomere length and tension

Potassium contractures were evoked with the fibre stretched to $1{\cdot}25\,l_o$. This length was chosen because at this extension one obtains an optimum of the curve relating the tension to the amount of extension of the fibre, as can be seen from Text-fig. 5. For determining this relation, solutions containing 173 mM-K and 7·8 mM-Cl were used; these represent the saturation $[K]_o$ (Text-fig. 2) for evoking tension. The tension attained at $1{\cdot}25\,l_o$ was taken as 1. Individual symbols designate mean tension values in different fibres. When the length–tension diagram was being determined, the amplitude at every new length of the fibre was tested by repeating the contracture at one of the preceding lengths, in order to eliminate changes in tension amplitude caused by other factors. The amplitude of the tension was completely reversible if the fibre was not stretched beyond the length of $1{\cdot}75$–$1{\cdot}80\,l_o$. The values of tension beyond this range were therefore determined only after the reversible range was tested by the above-mentioned procedure. The amplitudes of contractures beyond this range were, however, quite stable in spite of the decline in the tension

in the reversible range once the critical length was passed. They might indicate that the tension was generated in different parts of the fibre after stretching the fibre beyond the critical length.



Text-fig. 5. The length–tension diagram of isolated muscle fibres of the crayfish. Open symbols: maximal tension developed during potassium contractures (173 mM-K; 7·8 mM-Cl) cut short by re-introducing van Harreveld solution. Symbols with crosses refer to fibres allowed to relax spontaneously. Filled symbols: the resting tension about 10 min after slowly stretching the fibre. Symbols of the same shape refer to the same fibre. Both the active and passive tension are given in relative units taking the amplitude of contracture at $1{\cdot}25\,l_o$ as 100 %. The lines through the experimental points were drawn by eye. Upper horizontal scale: the length of fibres in fractions of the slack length ($l_o = 1{\cdot}0$). Lower scale: the sarcomere length. The inset diagrams indicate the assumed overlap between the thick and the thin filaments.

The length of the sarcomere was measured in each fibre at $1{\cdot}25l_o$ length and in five fibres also at three other lengths: l_o, $1{\cdot}25l_o$, $1{\cdot}75l_o$. The dispersion of values of sarcomere length measured at $1{\cdot}25l_o$ is relatively small (Table 2), so that the setting of l_o (see Methods) is reliable.

Sarcomere length in each of the five fibres studied was directly proportional to the extent of stretch and was in agreement, within a few percent, with the length of the sarcomere calculated from its length at $1{\cdot}25l_o$. It is thus simple to transform the abscissa in units of initial length to the abscissa in μ of sarcomere length (lower scale). Mean sarcomere

length at $1{\cdot}25l_o$ was taken as the basic value ($10{\cdot}5 \pm 0{\cdot}3$, mean $\pm$ S.D.; $n = 12$.

The shape of the curve relating the tension to the length of the fibre follows a similar course to that of isolated twitch muscle fibres of the frog, which were determined with the aid of tetanic stimulation of the fibre (Ramsey & Street, 1940). There is, however, a shift of the optimum to the right in the crayfish muscle ($1{\cdot}25l_o$) as compared with the optimum of the frog muscle (l_o). The initial length, l_o, was defined in both fibres in the same way. Tension falls to zero values in both the frog and the crayfish muscle fibres approximately at the same extension, if not at lower relative extension in the latter. This means that the decay in tension with stretching the fibre is steeper in crayfish muscle fibres. Contracture vanishes at the sarcomere length of about 16·3 μ.

TABLE 2. The maximum tension of potassium contractures in single muscle fibres of the crayfish (*Astacus fluviatilis*)

Row	Fibre	Maximal radius (μ)	Minimal radius (μ)	Fibre length (mm)	Sarcomere length (μ)	A band length (μ)	Contracture solution (mM-K)	Contracture solution (mM-Cl)	Tension (kg . cm^{-2})
1	20 W	228	113	13·5	10·9	4·4	173	250	7·02
2	21 A	266	213	14·7	10·1	3·8	173	250	7·07
3	21 B	203	138	14·7	—	—	173	250	7·33
4	21 C	269	212	14·6	—	—	173	250	5·86
5	21 E	240	215	17·5	—	—	173	250	5·67
6	21 S	221	170	15·7	10·6	4·0	173	7·8	6·78
7	21 T	234	218	15·6	10·8	4·3	173	7·8	6·25
8	21 U	281	182	14·4	10·5	4·0	173	7·8	6·99
9	21 W	216	178	14·6	10·4	3·7	173	7·8	8·41
10	21 X	203	159	12·5	10·4	3·7	173	7·8	7·43
11	21 Y	227	108	12·8	10·4	3·7	173	7·8	7·99
12	21 Z	195	142	12·8	10·8	4·1	173	7·8	6·50
13	22 A	228	110	15·0	10·0	—	173	7·8	11·56
14	22 B	202	179	17·3	11·0	—	173	7·8	9·25
15	22 E	186	164	14·2	10·4	3·8	173	7·8	8·39
16	22 H	266	190	13·7	—	—	173	7·8	8·29
17	22 I	229	124	14·2	—	—	173	7·8	10·85
Mean, all measurements		—	—	—	10·52	3·95	—	—	7·74
Mean, rows 1–5		—	—	—	—	—	—	—	6·59
Mean, rows 6–17		—	—	—	—	—	—	—	8·22

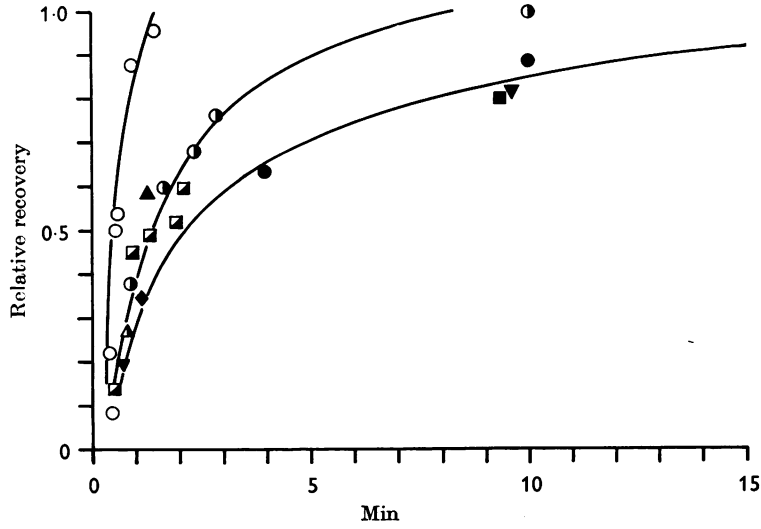
Temperature 18–23 °C. In contracture solutions with constant $[K]_o[Cl]_o$ product Cl was replaced by propionate.

The maximum tension of contracture

It follows from the relation in Text-figs. 2 and 5 that the contracture tension evoked by 173 mM-K at 10·5 μ sarcomere length may be considered maximal for the crayfish muscle fibre. Fibres exerted a mean tension of $7{\cdot}7 \pm 1{\cdot}6$ kg/cm^2 (mean $\pm$ S.D.; Table 2). The tension evoked by the 173 mM-K and 250 mM-Cl solution was lower (6·6 kg/cm^2—five fibres) than that evoked by the 173 mM-K and 7·8 mM-Cl solution ($[K]_o . [Cl]_o =$

constant), where fibres reached the maximal tension of 8·2 kg/cm^2 on the average (twelve fibres). This difference could be caused by the fact that depolarization of these fibres did not attain full mechanical saturation, as suggested by the relation in Text-fig. 2.

The fibres given in Table 2 represent less than a half of all the fibres investigated to which the 173 mM-K had been applied. The other fibres were either torn or torn off from their tendon insertion. This was caused rather by the rate of tension rise, which attains values around 10 kg cm^2 sec^{-1}, than by the tension itself, since the tension at the moment of tearing was smaller than the mean maximal tension of the fibres. Only a minor part of the fibres was torn several seconds after the summit of contracture was reached.



Text-fig. 6. The time course of recovery following the potassium contracture. Abscissa: interval between end of conditioning and beginning of test contracture. Ordinate: relative amplitude of test contracture as compared with the conditioning contracture. Same symbols denote values from the same fibre. Contractures were evoked by 173 mM-K and 7·8 mM-Cl (propionate substituted for Cl; filled symbols) or by 43·2 mM-K and 31·25 mM-Cl (Cl replaced with sulphate; other remaining symbols). Open circles: recovery (Text-fig. 7*B*) after partial spontaneous relaxation of the conditioning contracture. Half-filled symbols: contracture was allowed to relax almost completely (Text-fig. 7*C*). Contractures evoked by 173 mM-K were allowed to relax spontaneously.

The refractory period of potassium contractures

After a maximal contracture evoked by 173 mM-K and allowed to relax spontaneously, it is not possible to evoke another one of the same amplitude until after 20–30 min (Text-fig. 6). The maximal amplitude of

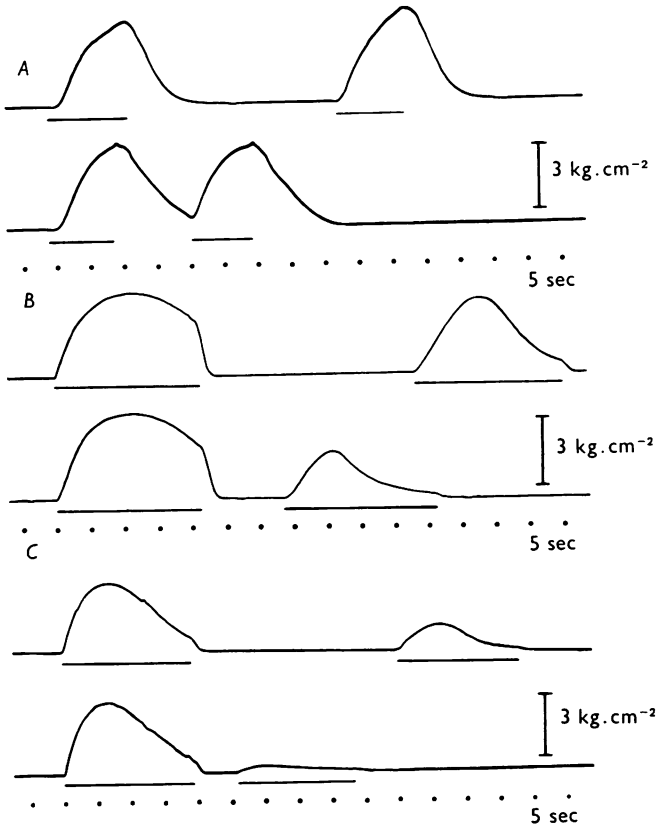
the test contracture after a shorter time interval is a non-linear function of the interval between the stimuli (line drawn between the filled symbols).

The above time course applies only if the conditioning contracture is allowed to relax spontaneously. If the contracture is terminated abruptly by exchanging the contracture solution for van Harreveld solution before the onset of spontaneous relaxation, the refractory period is absent, as can be seen in Text-fig. 7*A*. If the solutions are exchanged at the beginning of spontaneous relaxation, relative refractoriness is present, but the process of recovery is very rapid (Text-fig. 7*B* and open circles in Text-fig. 6). In the experiment demonstrated in Text-fig. 7*C*, the fibre relaxed spontaneously to 15% of maximal tension. Recovery was much slower than in the preceding case and comes close to that of the completely relaxed fibre, as can be seen in Text-fig. 6 (half-filled circles and squares, two fibres).

When studying the recovery demonstrated in Text-fig. 7*A–C*, the solution applied contained 43·2 mM-K with $[Cl]_o$ constant; here the danger of tearing the fibre is much less than at maximal stimuli, since the rate of rise of tension is smaller. Even if it was possible to explain the records in Text-fig. 7*A*, considered separately, by the fact that the stimuli were submaximal, it is not possible to explain in this way records in Text-fig. 7*B* and *C*.

That the relation between the rate of recovery and degree of relaxation is a real one is further confirmed by six experiments with the maximal $[K]_o$ concentration (173 mM-K and 7·8 mM-Cl) in which the contracture amplitude 10–60 sec after full spontaneous relaxation was compared with that following a conditioning contracture which was relaxed by a return to low $[K]_o$. In the first case the refractoriness is considerable (Text-fig. 8*B*), in the second case (Text-fig. 8*A*) the test contracture attains the amplitude of conditioning tension. The testing contracture is even higher than the conditioning contracture. Text-figure 8*C* shows that the contractures can be elicited several times in succession when the tension is terminated before the onset of spontaneous relaxation.

In two fibres we were able to record simultaneously the membrane potential changes and the tension output of the fibre. From the records shown on Text-fig. 9 it is evident that the reason for refractoriness does not lie in the membrane potential change, but rather in the process of excitation–contraction coupling, since the depolarization during the testing contracture attains values analogous to those during the conditioning contracture.



Text-fig. 7. The effect of extent of spontaneous relaxation of conditioning contracture on the rate of recovery, amplitude of the test contracture serving as indicator. Conditioning and test contractures were evoked by 43·2 mM-K and 31·25 mM-Cl (Cl replaced by sulphate). Time of application of contracture solution is represented by horizontal bar below tension record.

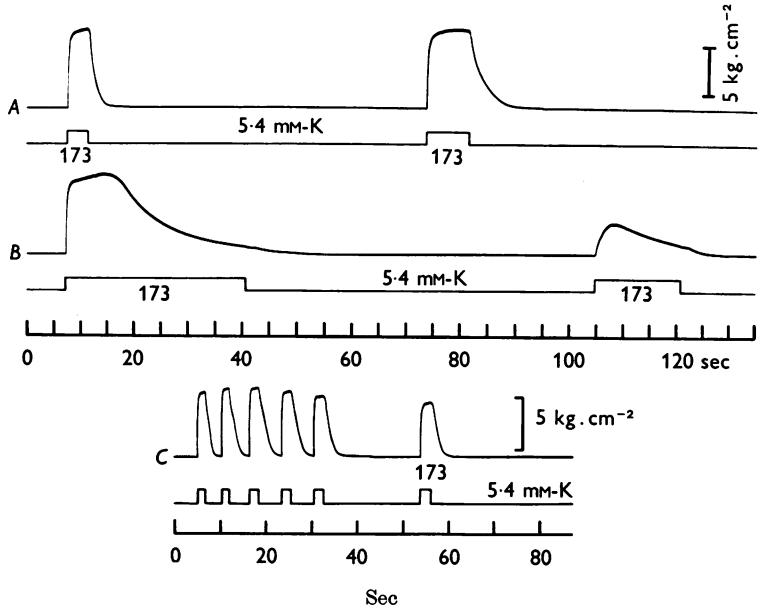
A, contracture was interrupted by exchanging contracture solution for van Harreveld solution before the onset of spontaneous relaxation. Refractoriness was absent. Fibre 9*Z*, $d_e = 472\mu$, temperature 21° C, length: 1·25 l_o.

B, conditioning contracture was terminated before tension had dropped to about half of the maximal value. Refractoriness was present. Fibre 4*V*, $d_e = 384\mu$, room temperature, length: 1·25 l_o. The whole course of recovery is graphically shown in Text-fig. 6 (hollow circles).

C, conditioning contracture was allowed to relax almost completely. Recovery is slower than in *B*. Fibre 5*D*, $d_e = 352\mu$, room temperature, length 1·25 l_o. The whole course of recovery is shown in Text-fig. 6 (half-filled squares).

Repriming of the contractile system

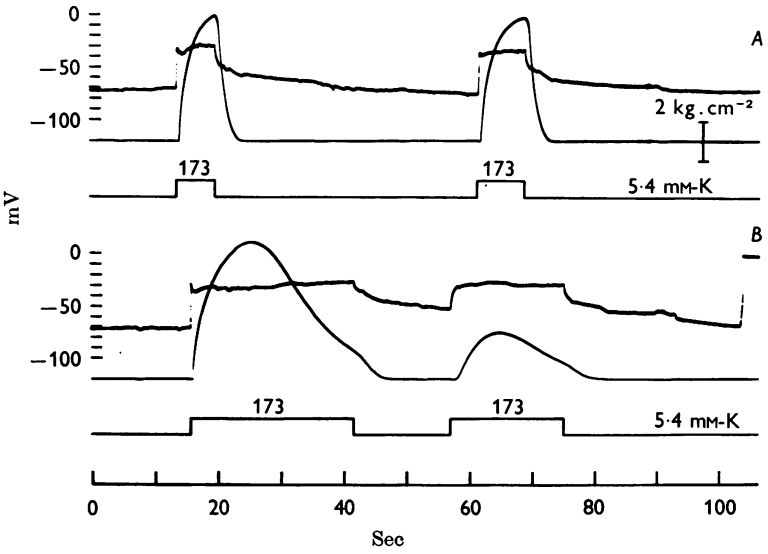
The recovery of the activational system of the contractile apparatus to resting conditions, when it is again capable of generating full tension, depends on $[K]_o$ and consequently on the membrane potential at which restitution takes place (Hodgkin & Horowicz, 1960).



Text-fig. 8. Effect of relaxing the conditioning contracture by a return to low $[K]_o$ (*A*), and of allowing it to relax spontaneously (*B*), on tension resulting from second application of 173 mM-K (7·8 mM-Cl). Note the absence of refractoriness in *A*. In *C* is shown a series of contractures cut short by a return to low $[K]_o$. *A*, *B*: fibre 23*B*, diameter 418 μ. Temperature: 20° C. *C*: fibre 23*I*, diameter 428 μ. Temperature: 21° C. The fibres were stretched to 1·25 times slack length, corresponding to sarcomere length of 10·5 μ.

When studying the relation between recovery and $[K]_o$ (Text-fig. 10), a fixed interval (10 min) was used between the conditioning and test contracture evoked by 173 mM-K and 7·8 mM-Cl. The contracture solution was applied for 30 sec, so that fibres relaxed spontaneously. Between each stimulus (contracture solutions), fibres were immersed in the testing solution containing 5·4 x mM-K and 250/x mM-Cl. Amplitude of the test contracture in van Harreveld solution ($x = 1$) was taken as 1. Other amplitudes at $x > 1$ are related to it. Between the experimental contracture pairs of fibres were immersed in van Harreveld solution for more than 30 min.

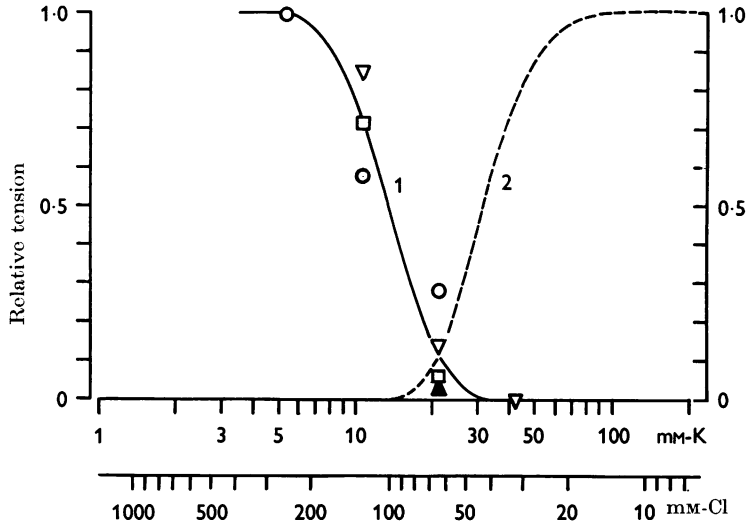
Amplitude of the test contracture decreases with increased potassium in the solution, in which the fibre is resting for 10 min between the stimuli. The decrease takes place along an S-shaped curve (continuous line), which is a mirror image of the relation between tension and log $[K]_o$ (interrupted line). The curves intersect at fourfold $[K]_o$, which represents a value, which is somewhat higher than the threshold concentration. Compared with the relation obtained from phasic fibres of the frog, where the curves intersect approximately in the middle (Hodgkin & Horowicz, 1960), the recovery curve of crayfish muscle fibres is shifted to the left, to



Text-fig. 9. Simultaneous records of membrane potential (upper trace) and tension (lower trace). In *A* the contractures were cut short by a return to van Harreveld solution before the onset of the spontaneous relaxation. The conditioning contracture in *B* was allowed to relax spontaneously. Contractures were evoked by 173 mM-K and 7·8 mM-Cl. Fibre 23 *V*; diameter 526 μ. Temperature: 22° C. The fibre was stretched to 1·25 l_o.

lower $[K]_o$. This shift, as compared with that in the frog, could be explained by a decrease in the membrane potential by about 13 mV during the 10 min interval. However, the fibres lay in solutions, the $[K]_o$ of which was increased while the Donnan equilibrium was preserved. Direct measurements of the membrane potential at $x = 4$ during the 10 min interval showed that the potential had only changed by about 2 mV or did not change at all. For a 5 min testing interval this is demonstrated on Text-fig. 11 for $x = 8(A)$ and $x = 2(B)$. The membrane potential just before the application of the test contracture solution has a value which

agrees with the Nernst equation (Zachar *et al.* 1964*a*), so that the cause of the shift must be sought elsewhere (see Discussion). The figure also shows that the membrane depolarization during the testing contracture attains the same value as during the conditioning contracture, indicating that the decrease in tension is due to the decoupling of the excitation–contraction link.



Text-fig. 10. The dependence of recovery upon log $[K]_o$ and $-\log [Cl]_o$ (curve 1). Interval between conditioning and test contracture was fixed (10 min—open symbols; 4 min—filled symbols). Conditioning contracture was allowed to relax spontaneously. Amplitude of the test contracture in van Harreveld solution was taken to be 1. Tension values are related to the latter, when restitution was taking place at high external potassium concentrations ($[K]_o[Cl]_o$ = const.). Contractures were evoked by 173 mM-K and 7·8 mM-Cl (propionate substituted for Cl). Curve 2: relation between tension amplitude and log $[K]_o$ and $-\log [Cl]_o$ (Text-fig. 2, curve *a*).

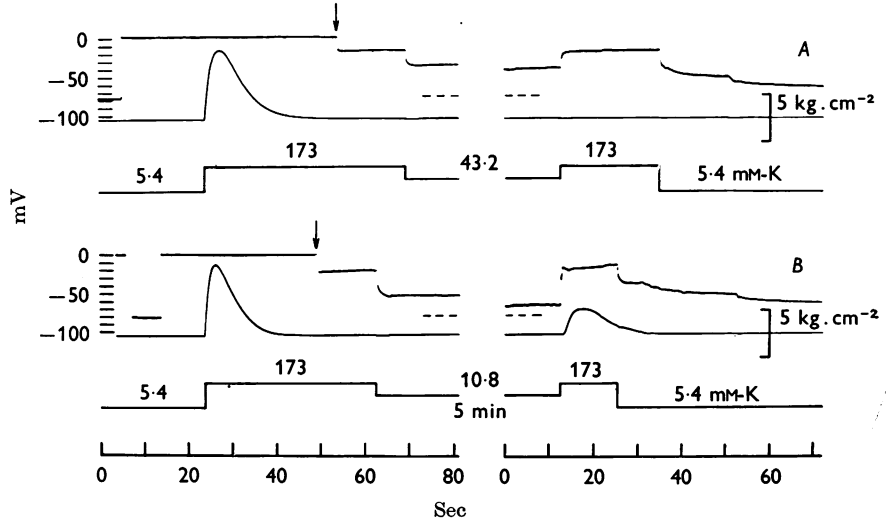
DISCUSSION

According to the sliding filament model the muscle tension/cm^2, P, is proportional to sarcomere length (s) and is given by the equation (Huxley, 1957, eqn. 6):

$$P = \frac{msk}{2l}\int_{-\infty}^{\infty} nx\,dx,$$

where m is the number of M sites on the myosin filament/c.c. of muscle, k is the stiffness in dyn.cm^{-1} of the elastic element, l is the separation of the A sites along the actin filament and n is the proportion of the sites, where M combines with A.

Sarcomere length of the crayfish muscle fibre is 10·5 μ at optimal fibre length, i.e. it is 5 times longer than the sarcomere length of the frog phasic fibre which is 2·1 μ at optimal length (Gordon, Huxley & Julian, 1964). If the other factors remain unchanged, crayfish muscle fibres should generate tension 5 times greater then the frog muscle fibre. According to Ramsey & Street (1940), frog fibres can exert a tension of 3·5 kg.cm^{-2} in response to isometric tetanic stimulation, so that during contracture



Text-fig. 11. Simultaneous record of the membrane potential (upper trace) and tension (lower trace) during the repriming of the contractile apparatus in 43·2 mM-K and 31·25 mM-Cl (*A*) and in 10·8 mM-K and 125 mM-Cl (*B*). Cl was replaced by propionate. Fibres were allowed to recover in the experimental solutions for about 5 min in both cases. (The gap in the recordings is 5 min.) The contracture solutions contained 173 mM-K and 7·8 mM-Cl. The micro-electrode was introduced into the fibre only after the spontaneous relaxation of the conditioning contracture (arrows) took place. The fibre rested in van Harreveld solution for 45 min between *A* and *B*. Fibre 23*Z*; diameter: 412μ. Temperature: 20° C. The fibre was stretched to 1·25 l_o.

a frog fibre would exert about 3·8 kg.cm^{-2}, since tension during a contracture is about 10 % higher than during a tetanus (Hodgkin & Horowicz, 1960). The mean value reported by these authors is similar (3·6 kg.cm^{-1}). Crayfish muscle fibres in a 173 mM-K and 7·8 mM-Cl solution exert 8·2 kg.cm^{-2} (Table 2), i.e. not 5 times but only twice as great as the frog muscle fibres. This could be explained on the assumption that also some other parameters controlling maximal tension (see the above equation), are not identical in the crayfish and frog fibres.

The length of the A band is 3·95 μ in the crayfish fibre (Table 2) and 1·6 μ in that of the frog. Optimal sarcomere length in the latter is 2·1 μ (Gordon *et al.* 1964) and 10·5 μ in the former (Table 2, Text-fig. 5). The ratio of the A band to the sarcomere length in the crayfish (0·38) is thus half that in the frog (0·76). This would seem to indicate that there are twice as many *M* sites/c.c. in the frog than in the crayfish. In this case the crayfish muscle fibre should exert tension only 2·5 times greater than the frog fibre, which comes near to the ratio of tension values actually obtained for the crayfish and frog muscle fibres (about 2). Better agreement could be found if the looser arrangement of myofibrils in crayfish fibres were taken into account, as this gives a smaller active cross-section of the fibre in the crayfish. This looser arrangement is evident at first sight from microphotographs of the compared fibres. Quantitative data from the aspect of comparative physiology concerning this 'dead' space are not, as yet, available.

It can be concluded from this comparison that the differences in tension between the two species can be explained on the basis of purely geometrical factors, without having to assume differences in the basic structure of those places where tension is generated. Other explanations are also possible. However, in order to be able to decide between the above simple explanation and other possibilities, it would be necessary to know more about the molecular architecture of the fibre under study.

The relatively shorter length of the A band as compared with the I band can also explain the length–tension diagram (Text-fig. 5), which differs from that of the frog by a steeper fall in tension after stretching the fibre above its optimal length. Gordon *et al.* (1964) demonstrated in the frog that at optimal length at which the fibre generates maximal tension, the whole thick filament is just overlapped by the thin filament. On applying greater stretch, tension of the fibre decreases proportionately to the overlap of the A band. If the fibre is stretched so that the A and I filaments are 'out of mesh', fibres cease to generate tension. Assuming that the sliding mechanism operates also in the crayfish muscle fibre, it can be expected that the A band, 3·95 μ long, is completely overlapped (see Text-fig. 5) by the thin filaments at the optimal length, which corresponds to 10·5 μ sarcomere length and consequently to double the length of the thin filament.

It follows from these data that the thick and thin filaments will be 'out of mesh' (Text-fig. 5, inset diagram on the right) at sarcomere length $10{\cdot}50 + 3{\cdot}95 = 14{\cdot}45\,\mu$, which corresponds to 171·4% extension of the fibre from its initial length l_o. The sarcomere length of the zero overlap happens to be the same as that representing the limit of the reversibility

range. This numerical coincidence is perhaps not fortuitous and might indicate that the original geometry of the overlap could not be renewed once the filaments were 'out of mesh'. The fact that fibres are still capable of generating tension even at this critical length can be explained by assuming that tension is generated in those parts of the fibre close to the insertion, where filaments still overlap, while central parts of the fibre are already 'out of mesh'. The value of the residual tension is close to that found on single twitch fibres of the frog (Carlsen, Knappeis & Buchthal, 1961) in tetani where the tendons were held similarly as in the present case. A better fit could be expected if the influence of the fibre at the ends on the length–tension diagram was avoided (Gordon, Huxley & Julian, 1963, 1964).

The observation that duration of the refractory phase of the contracture is dependent on the degree of relaxation is new and to a certain extent surprising, since in actual fact refractoriness is absent, if contracture is cut short after attaining the maximum, but before the onset of spontaneous relaxation. Recovery after complete spontaneous relaxation is very slow and its time course reminds one of the Michaelis–Menton relation between substrate concentration and enzyme activity. It is, therefore, the changes occurring in the muscle during spontaneous relaxation which are responsible for the appearance of refractoriness. The depolarization of the fibre during the refractory period attains the same value as during the conditioning contracture (Text-fig. 9). Thus the change in the tension most likely concerns the system which is involved in excitation–contraction coupling.

A likely explanation for the appearance of refractoriness or its absence in relation to the extent of spontaneous relaxation is in fact an extension of one of the two alternative possibilities suggested by Hodgkin & Horowicz (1960) for explaining the time course of the contracture. Suppose that the fall of membrane potential below a certain critical level initiates two processes: liberation of an activator and, with a certain delay, another inactivating process, by which the activator is destroyed. While those processes are a function of the membrane potential, resynthesis of the activator is dependent on metabolism.

It is possible to explain, using this hypothesis, both the dependence of recovery on the degree of relaxation and the time course of the contracture. When the contracture is interrupted before spontaneous relaxation sets in, the liberation of the activator is stopped because of membrane repolarization. The remaining activator is capable of being liberated even after early repetition of membrane depolarization (see Text-fig. 8*C*). When the contracture is allowed to relax spontaneously, the activator is broken down and must be resynthesized with the aid of metabolic pathways. The period of resynthesis represents the basis of refractoriness.

According to this hypothesis, spontaneous relaxation occurs when the activator is broken down below the level of mechanical saturation. When the amount of the activator liberated by depolarization is relatively large, or when the break-down process sets in late, the contracture will have a plateau, similar to that to be found in phasic muscle fibres of the frog (Hodgkin & Horowicz, 1960). The plateau in frog fibres, however, disappears when the contracture is evoked in the refractory period, which is comprehensible, since the amount of activator liberated is smaller. Absence of the plateau in fresh crayfish muscle fibres suggests that the quantity of activator liberated by maximal depolarization is only slightly greater than the saturation value, or that inactivation process sets in earlier.

The membrane potential affects, according to this view, only the liberation of the activator from its inactive form. Experiments with repriming of the contractile system showed, however, that the recovery depends on $[K]_o$ or on the membrane potential (Text-fig. 10). This is not necessarily in contradiction with the above hypothesis. The retarding influence of depolarization on the recovery can be explained by the fact that part of the activator resynthesized is liberated by maintained depolarization and part is broken down by the inactivation process. The actual extent of recovery would then be the result of these processes. It would thus follow from this that the recovery curve and the curve showing the relation between tension and log $[K]_o$ do not necessarily have to intersect in the middle, as could be expected if the amount of activator liberated as well as its recovery are governed directly by the membrane potential. It seems from the results of Curtis (1964) that also in twitch fibres of the frog these curves do not necessarily intersect in the middle.

The time when the inactivation process exceeds the process by which the activator is liberated can be estimated approximately from the records of contractures as the time at which the rapid initial phase of the contracture changes over into the slower ascending phase. The second slower phase could be caused by the action of this inactivation process itself. This phase is, however, also affected by drifting of the membrane potential especially when the contractures are evoked by increased external potassium concentrations, at constant $[Cl]_o$.

From the description of potassium contractures in isolated crayfish muscle fibres, it appears that they have several characteristics in common with contractures, which have been described in other isolated muscle fibres, whether phasic (Hodgkin & Horowicz, 1960) or tonic (Lüttgau, 1963; Nasledov, Zachar & Zacharová, 1966) muscle fibres, of the frog. It is interesting that the threshold membrane potential, V_t, of all these fibre types lies approximately at the same value of the potential difference

across the membrane, although the resting potential, V_r, is very different. In crayfish muscle fibres $V_r = -79$ mV and $V_t = -55$ mV. Phasic fibres of the frog are reported by Hodgkin & Horowicz (1960) to have $V_r = -92$ mV and $V_t = -54$ mV. In tonic frog fibres $V_r = -63$ mV (Kuffler & Vaughan Williams, 1953; Kiessling, 1960) and $V_t = -50$ mV (Nasledov *et al.* 1966). Thus, in order to attain threshold it is more essential to lower the membrane potential below a certain value than to obtain a certain depolarization from the resting potential. An attractive explanation is that the activator of contractures is the same in all cases and begins to be liberated when the membrane potential drops below a certain critical level. Differences in the rate of onset of spontaneous relaxation, or its absence (tonic fibres), could then depend on the stage of development of the process, which leads to the break-down of the activator.

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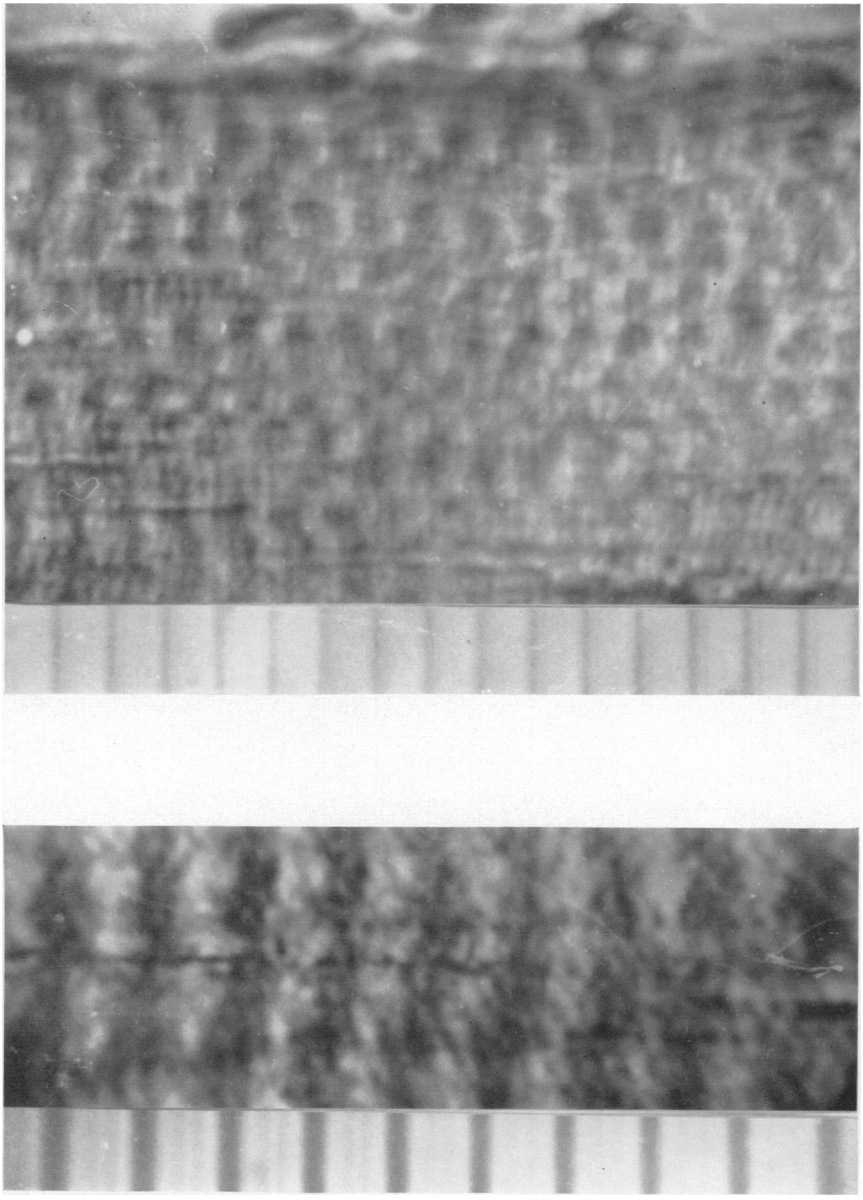
EXPLANATION OF PLATE

A microphotograph of a live, unstained crayfish muscle fibre in physiological saline, taken through a double microscope. Above: Total magnification ×600 (I objective: ×10, II objective: ×6, ocular: ×10). Edge of fibre. Below: total magnification ×1200 (×20, ×6, ×10). From central part of another fibre. Vertical strips: 10μ. Fibres were stretched to 1·25 of initial length.



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