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THE DYNAMICS OF THE EFFECT OF POTASSIUM ON FROG'S MUSCLE

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Potassium ion has very important actions on muscle (see Kahn & Sandow, 1952, for references). The large difference between its concentration inside and that outside the cell (combined with the low permeability of the cell membrane to sodium) is the source of the resting potential; this behaves more or less as a diffusion potential should when the external potassium concentration is varied (see, for example, Gerard & Jenerick, 1953).

If the external potassium concentration is raised above 8 mM from its usual value of 1–2 mM, potassium enters the cell against the concentration gradient (see Kahn & Sandow, 1952, p. 103). Under the same conditions the resting metabolic rate increases, reaching at about 20 mM a new high level which may be twenty times its normal value. In concentrations above about 9 mM the muscle becomes reversibly inexcitable (Overton, 1904; Dulière & Horton, 1929). This loss of ability to generate a propagated action potential occurs when the resting potential is reduced below a critical level of about 57 mV (Gerard & Jenerick, 1953). The muscle can, however, still contract locally in the region where a stimulus is applied. Raising the external potassium concentration has therefore been used, for example by Sten-Knudsen (1954), to block propagation in order to study local responses. It is quite likely that the two response peaks already reported in uterine muscle (Csapo, 1954; Csapo & Goodall, 1954) correspond to propagated and local responses respectively.

The loss of excitability is supposed to be effected at the surface of the fibres, so its time course should be determined by diffusion of potassium into the spaces between the muscle fibres. However, preliminary experiments by one of us (A. C. in collaboration with P. Nordquist), indicated that the time course was rather slow. This suggested that the loss of response might be associated with some factor other than loss of excitability, e.g. penetration of potassium

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ions inside the fibres, where they might have a direct action on the contractile actomyosin, which is known to be very sensitive to its ionic environment; and that his neglect of this factor might shed some doubt on Sten-Knudsen's results.

The following experiments were undertaken to find out whether diffusion could account for the observed time course of the potassium effect and to repeat on the sartorius Sten-Knudsen's experiments with longitudinal and transverse stimulation.

METHODS

All experiments were performed during the months of July and August, on sartorii from *Rana pipiens* bathed in fresh Ringer's fluid composed of NaCl 111 mM, KCl 1·34 mM, $CaCl_2$ 3·6 mM, $NaHCO_3$ 11·9 mM, phosphate buffer 1·7 mM, bubbled with 3% CO_2 in O_2, giving a pH of 7·2.

In some experiments it was inconvenient to provide 3% CO_2, so these were performed in areated phosphate Ringer's solution (NaCl 115·3 mM; KCl 2·0 mM; $CaCl_2$ 3·6 mM; phosphate buffer pH 7·4, 1 mM). There was no evidence that this made any difference to the results obtained. High-potassium Ringer's solution was made by adding an appropriate amount of 1 or 2% KCl to the muscle bath. The change in effective osmotic pressure which resulted (see Boyle & Conway, 1941) was always small.

The muscle was mounted horizontally in a square chamber of methacrylate polymer (Lucite) (5 × 5 × 2 cm deep) which had platinum plate electrodes covering two opposite internal walls. The electrodes and chamber were sufficiently large compared with the muscle for the electric field set up to be reasonably uniform and parallel; and the chamber could be rotated through 90° so that this field could be applied to the muscle transversely or longitudinally as required. The arrangements for fixing the muscle were made with as small a frontal area as possible in order that they should not 'shadow' the muscle. The pelvic end was held in a thin J-shaped glass hook with a slot about 1 mm wide and 6 mm long. The pelvic end of the muscle just fitted this slot and was prevented from pulling through by a small piece of its cartilaginous origin; the remainder of the pelvis was cut away. The tibial end of the muscle was tied to a thin and flat Lucite lever which transmitted the tension to a Grass strain gauge, no. FT10, whose output was recorded on paper by a Grass ink writer, type IIID (response flat to 70 c/s).

When required, the diaphasic action potential could be recorded by lowering the square chamber out of the way and raising an array of four platinum wires into contact with the muscle. The pair at the pelvic (nerve-free) end of the muscle were used for stimulating, the third was earthed and the fourth taken to the pre-amplifier. The wires were spaced 4, 15, 25 mm respectively from the one at the pelvic end. Square 0·5 msec pulses from a Grass stimulator type S4A were used in the routine tests for the presence of propagation.

The stimulus. Some preliminary experiments were made using a small muscle chamber and single square-pulse stimuli. The resulting twitch responses showed essentially the effect described by Kahn & Sandow (1952), that in Ringer's solution containing a raised concentration of potassium, the decline in response was preceded by a phase in which the twitch was augmented. Records of tetani under corresponding conditions show only a steady decline. This suggests that the potassium may be acting to prolong the active state temporarily in the same way as do some other ions (see Kahn & Sandow, 1950; Ritchie, 1954; Hill & Macpherson, 1954). In order to eliminate this variable all our experiments were made with tetanic stimulation. The stimulator used was that employed by Csapo (1954) and Csapo & Goodall (1954), which consists essentially of a variable auto-transformer supplied by the house mains and delivers sinusoidal current at 60 c/s. In some experiments we were able to use sine waves of other frequencies derived from a 10 W gramophone amplifier. Sinusoidal stimulation of nerve was used, for example, by Coppée (1934) and by Hill, Katz & Solandt (1936); and the mathematical theory involved was worked out by Hill (1935) as a special case of his accommodation theory.

Mains current was used as a stimulus for muscle in the pioneer experiments of Hartree & Hill (1921) and in those of Winton (1937); our experience is that 50 or 60 c/s current is a suitable and very convenient stimulus for frog's striated muscle. Maximal, fused and well-maintained tetani are obtained at all temperatures from 0 to 25° C and the muscles survive extremely well. The electrical power required to tetanize is about the same as that required when square pulses of duration 1 msec are used; it amounts to only about 3% of the heat rate produced by the muscle itself at 0° C. Each half-cycle sets off a propagated disturbance in the region which is temporarily acting as cathode. The resulting diphasic action potential is small and spread out, either because fibres at different distances from the electrodes are not fired off synchronously by the slowly rising current, or because individual fibres show multiple responses.

After propagation has been blocked by KCl, procaine, etc., alternating currents can still produce massive local responses (Katz & Lou, 1947), but for this purpose a high field strength is required (4–6 V/cm (r.m.s.) according to Sten-Knudsen, 1954 and private communication). Preliminary experiments showed that 1 V/cm (r.m.s.) would give maximal tetani in the conducting muscle (not curarized) without producing appreciable local response in the blocked one. With a stimulus of this strength, mechanical response and action potential disappear at the same moment during the course of potassium depolarization.

RESULTS

The dynamics of the action of potassium on the propagated response

When the potassium level in the Ringer's solution is less than about 9 mM, propagation is never completely blocked. (Kahn & Sandow (1952) also find a value between 7·2 and 9·8 mM.) Above 9 mM the block comes on more and more quickly the higher the potassium concentration, as shown in Fig. 1.

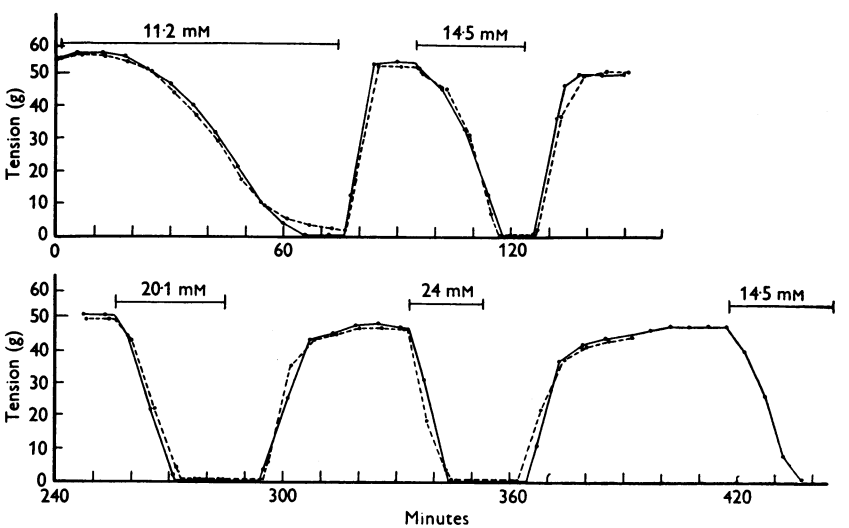

Fig. 1. The effect of various concentrations of potassium on a frog's sartorius (34 mm long, 93 mg) at 22·8° C. Ordinate, tetanic tension in grams; abscissa, time in min since beginning of experiment. Stimulus: 1 V/cm (r.m.s.), 60 c/s, 0·5 sec; full line, longitudinal, broken line, transverse. The same muscle was used throughout the experiment, but there was a break in recording from 170th to 240th minute.

The effect of potassium is almost completely reversible on replacing normal Ringer's solution so long as the muscle has not been fatigued by tetanizing it too frequently. In no case could we detect any contracture, though a tension of 1 g wt. could have been easily seen. This confirms previous findings (see Solandt, 1936; Sandow, 1955).

According to Gerard & Jenerick (1953), muscle fibres cease to conduct impulses when their resting potential falls below about 57 mV (summer frogs). The external potassium concentration required to depolarize the muscle to this extent varies with the calcium concentration. Their Fig. 1 shows the relation between membrane potential and potassium concentration for 1·3 mM and for 10·4 mM calcium. Interpolating logarithmically for the 3·6 mM in our Ringer's solution, 57 mV resting potential should be obtained for almost exactly 9 mM of potassium, thus checking with our and with Sandow's observations of mechanical response. Moreover, under normal conditions the change in membrane potential in superficial fibres follows the change in potassium concentration without appreciable delay (see Gerard & Jenerick, 1953, p. 83; Sandow & Mandel, 1951, p. 283); this is to be expected if the membrane potential is simply a diffusion potential. When the potassium concentration around any given fibre is less than 9 mM, impulses can be conducted normally, leading to twitches or tetanic responses. When the potassium concentration surrounding the fibre is greater than this, only local responses can be obtained.

The simplest hypothesis to account for the curves in Fig. 1 is that the potassium simply acts at the surface of the muscle fibres; and that the dynamics of its effect are determined by diffusion of potassium into and out of the muscle interspaces. The fractional tension developed by the muscle would then indicate in what fraction of the muscle the potassium concentration was still below the critical level of 9 mM. This hypothesis can be tested by finding whether the eight experimental curves of onset and recovery shown in Fig. 1 correspond with a theoretical calculation based on diffusion.

The theoretical calculation uses the equation given by Hill (1928) for diffusion into an infinite plane sheet. This represents the flat sartorius closely enough for practical purposes. If the concentration of any substance in the fluid bathing the muscle is suddenly changed from zero to y_0, the concentration in the muscle interspaces will rise eventually to the same value. At any given time t after the sudden change, the fractional concentration y/y_0 at a depth x below the surface of the muscle is given by

$$y/y_0 = 1 - (4/\pi)\left[e^{-k\pi^2 t/4b^2} \sin(\pi x/2b) + \tfrac{1}{3} e^{-9k\pi^2 t/4b^2} \sin(3\pi x/2b) + \text{etc.}\right], \quad (1)$$

where k is the diffusion constant, $2b$ the thickness of the muscle. Fortunately the series is rapidly convergent, and except at the very beginning it is safe to neglect all terms except the first.

Then, rearranging equation (1),

$$(k\pi^2/4b^2)t = \log_e [(4/\pi)\sin(\pi x/2b)/(1-y/y_0)]. \quad (2)$$

For each curve of Fig. 1 let us measure the time, $T_{\frac{1}{2}}$ min, for the tension to fall to 50 % of its original value (onset) or to rise to 50 % of its final value (recovery). At each such point half the muscle must have more than 9 mM and the other half less than 9 mM in its interspaces; i.e. when $t = T_{\frac{1}{2}}$, $x = \frac{1}{2}b$ if y/y_0 corresponds to 9 mM. The appropriate values of y/y_0 may be easily calculated in each case from the experimental conditions. Let the potassium content of the test solution $= M$ mM. The potassium content of our normal Ringer's solution was 1·3 mM. Then, during onset of effect,

$$y = 9 - 1{\cdot}3, \quad y_0 = M - 1{\cdot}3;$$

and during recovery

$$y = M - 9, \quad y_0 = M - 1{\cdot}3;$$

from which it is clear that if one plots experimental values of $T_{\frac{1}{2}}$ (abscissae) against $\log_e [0{\cdot}900/(1-y/y_0)]$ (ordinates), one should obtain a straight line through the origin whose slope $= k\pi^2/4b^2$.

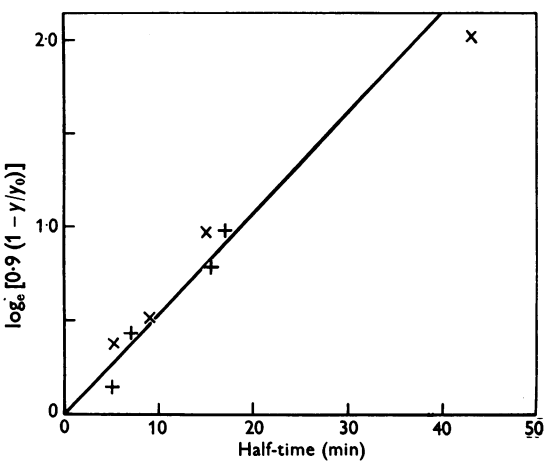

Fig. 2. Abscissa, experimental values of $T_{\frac{1}{2}}$ in min, taken from Fig. 1; ordinate, $\log_e [0{\cdot}9/(1-y/y_0)]$ calculated from the experimental conditions, as described in text; from the data of Fig. 1, omitting the final treatment with 14·5 mM-K. ×, onset; + recovery.

Fig. 2 shows that the experimental values do fit in reasonably well with the hypothesis. The slope of the line is 0·054 min^{-1}, and taking $2b = 0{\cdot}08$ cm, this gives $k = 0{\cdot}58 \times 10^{-6}$ cm^2 sec^{-1}.

The upright and diagonal crosses in Fig. 2 are fairly evenly distributed about the line. This provides further confirmation that 9 mM is the critical potassium concentration, for an error in this choice would have shifted the onset and recovery points in opposite directions. Kahn & Sandow (1952) have made

a somewhat similar calculation, but it was concerned only with the *mean* potassium level inside the muscle, while it is in reality the *distribution* of potassium levels which determines how large a fraction of the muscle is still active. This may in part account for their conclusion (their p. 109) that changes in the mechanical response could not result from the changes in resting potential.

The value for the diffusion constant of potassium in the muscle interspaces which is indicated by this calculation is very small, $0{\cdot}58 \times 10^{-6}$ cm^2 sec^{-1}, compared with the value $3{\cdot}5$–$4{\cdot}3 \times 10^{-6}$ cm^2 sec^{-1} calculated by Hill & Macpherson (1954) from Harris's (1952) measurements of radio-potassium efflux; and this higher value is supported by its similarity to the diffusion constant of iodide and sodium (Harris & Burn, 1949; Keynes, 1954) and by a geometrical argument (Hill & Macpherson, 1954). Sandow & Mandel (1951) give values $1{\cdot}0$–$1{\cdot}7 \times 10^{-6}$ cm^2 sec^{-1}, based on a study of the kinetics of depolarization by potassium. Their method of calculation involved only the mean potassium concentration, which sheds some doubt on the accuracy of their estimate.

How is this discrepancy to be explained? Consider what happens if potassium enters the fibres. It can only do so from the neighbouring interspaces. Thus part of the potassium which enters any given interspace will not serve to raise the potassium concentration there. This will reduce the effective diffusion constant; for

$$k = [\text{local rate of increase of concentration, } \mathrm{d}y/\mathrm{d}t]/[\mathrm{d}^2y/\mathrm{d}x^2]$$

at any given depth x. Our test solutions all contained more potassium than the maintenance level (about 8 mM, Kahn & Sandow, 1952), so potassium must have entered the fibres and thus reduced the apparent diffusion constant. In order to calculate how large this reduction may be it is necessary to know the relation between potassium entry and local potassium concentration. According to Boyle & Conway (1941, Fig. 5) the relation is simply that if the external potassium concentration is raised by Δy, the internal potassium concentration also increases by Δy. If this condition applies also in our muscles, and is followed with reasonable speed, the effect will be to reduce the effective diffusion constant to the fraction.

$$(\text{volume of interspace})/(\text{total volume of muscle}) \simeq 0{\cdot}15.$$

This agrees remarkably well with the ratio of our diffusion constant to Hill & Macpherson's mean value, i.e. $0{\cdot}58/3{\cdot}9 = 0{\cdot}149$. The closeness of this agreement must, of course, be largely fortuitous; for many uncertainties are involved in the calculation. However, it shows that the experimental facts are quite compatible with the view that potassium is acting at the surface of the fibres and that the dynamics of its action are determined by diffusion through the interspaces, the diffusion itself being slowed down by entry of potassium into

the fibres. Even if the potassium moves instantly between interspace and fibre, the movement of potassium into, or out of, the whole muscle will still be quite slow; with an effective diffusion constant of $0{\cdot}58 \times 10^{-6}$ the 'time constant' of potassium movement will be 15·6 min (half-time 9·2 min; the function is not exactly exponential). These times can be most easily calculated from Hill's (1928) Fig. 5.

The theory outlined above can then be further tested by showing experimentally whether potassium does in fact move into the whole muscle with this predicted time course, in sufficient amount to raise the concentration in the whole muscle by Δy when the external concentration is increased by Δy. Any additional slow potassium entry will not upset the theory because it will not appreciably alter the potassium concentration in the interspaces, i.e. at the fibre surface.

Experiments with radioactive tracers are often somewhat difficult to interpret because one cannot easily separate the effect of net movement of potassium from that of mixing between intra- and extracellular potassium. Harris's (1953) experiments on the sartorius showed that there was a phase of potassium entry with a time constant (his a) of about 20 min, but that the amount of potassium entering (his A) was only about half that required for our theory (2–3 times the amount in the extracellular space instead of about 6 times). Carey & Conway (1954) have also found a phase of potassium entry with a time constant of 12–20 min, during which the count ratio (counting rate per gram muscle/counting rate per gram solution) rises to 0·6–1·3 (see their figs. 3, 4 and 5A, which relate to chloride-Ringer's solution). This is in better accord with our theory, which predicts a count ratio for this phase of 1·0.

The movement of potassium in muscle thus appears to follow three fairly distinct phases:

(1) Movement into or out of the interspaces, with a time constant of about 2 min. This can only be observed if media with high (e.g. 120 mM) potassium are used to reduce the effective participation of intracellular potassium (Harris, 1952); and it leads to the 'interspace' diffusion constant (4×10^{-6} cm^2 sec^{-1}) discussed above.

(2) In ordinary chloride media, initial entry into the whole muscle is much slower, with a time constant of about 15 min. This, we believe, is associated with rapid net entry of potassium into the cells under the conditions of our experiments, and leads to a reduction of the effective diffusion constant.

(3) Finally there is a slow phase of mixing between intra- and extra-muscular potassium with a time constant that varies between 2 and 17 hr. It is noteworthy that in Keynes's (1954) measurements on the small toe muscle only the third phase is to be seen, presumably because in this preparation the second phase is too quick for observation.

Local responses

When the potassium concentration in any given region is greater than 9 mM, the muscle fibres in that region can no longer propagate impulses and show twitch responses. However, a fibre can still respond by a local contraction if its membrane is further depolarized by applying a negative electric pulse (see e.g. Gerard & Jenerick, 1953). A similar depolarization can be produced by applying alternating current (Katz & Lou, 1947; Sten-Knudsen, 1954). The advantage of using alternating current is that there is no electrolysis (which damages the muscle), and quite reproducible results are obtained with field strengths up to 6 V/cm (r.m.s.). By using massive electrodes, a large fraction of the muscle can be activated at once in this way, with tension development amounting to 80% of the tetanic tension (Sten-Knudsen, 1954), in the extensor

digiti IV of the frog. In later experiments (private communication, 1955), he gets as much as 92% under optimal conditions (1000 c/s, 6 V/cm (r.m.s.).) This large tension development shows that there can have been no substantial interference with the contractile actomyosin inside the fibres.

Our experiments on sartorius give roughly the same result as Sten-Knudsen's. We too find, Fig. 3, that there is an optimal frequency of about 1000 c/s and that a transverse field is much more effective than a longitudinal one, especially if the frequency is optimal. Unfortunately we were unable for technical reasons to produce a field strength greater than 4 V/cm (r.m.s.), so our maximal tension is only 76% of the tetanic tension. Sten-Knudsen finds a similar value (72%) with this field strength, so the difference between the muscles in their bulk and in the diameter of their fibres cannot much alter their sensitivity.

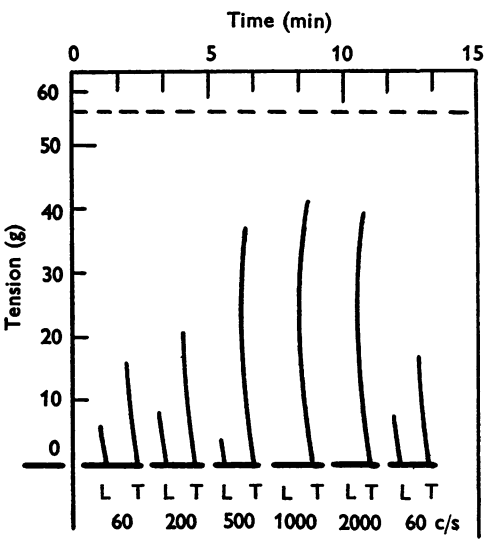

Fig. 3. Contractions of non-propagating frog's sartorius (80 mg, 30 mm long) at 25° C. Ordinate, tension developed, grams; abscissa, time, min. Stimulus, sinusoidal field, 4 V/cm (r.m.s.) between massive electrodes, 0·5 sec duration; frequency, 60, 200, 500, 1000, 2000, 60 c/s as indicated, applied first longitudinally (L), then transversely (T). Conduction blocked by long soaking in 11·2 mM potassium. The dotted line shows the tension developed by the same muscle in a maximal tetanus.

Dynamics of onset. With external potassium concentrations of 11–20 mM the size of the 'local' response to 6V/cm (r.m.s.) settles down to its final value fairly soon after the disappearance of the propagated response. This final value is not quite steady; it declines slowly, falling to 50% in about 3 hr. With more than 20 mM potassium in the Ringer's solution the decline is much more rapid, so that fairly soon no response at all can be obtained. With 20 mM potassium the membrane potential must be about 40 mV. The threshold for local responses increases with increasing depolarization (Gerard & Jenerick,

1953), so presumably 6 V/cm (r.m.s.) is about equal to the threshold when the membrane potential is 40 mV. The potassium concentration at which local contractions disappear is thus quite arbitrary. By using strong shocks Gerard & Jenerick (1953) were able to elicit responses even with a membrane potential of 20–30 mV (see also Katz & Lou, 1947).

The rate at which the local response disappears increases sharply as the potassium concentration is raised above 20 mM. This suggests that once again we are concerned with a threshold membrane effect whose time course is determined by diffusion. The very slow decline found with potassium concentrations between 11 and 20 mM can hardly be a diffusion effect. Possibly in this case the potassium which penetrates the fibres has a direct action on their contractile mechanism; an alternative suggested by Professor A. V. Hill is that under these conditions the resting metabolic rate might rise so much that the interior of the muscle became seriously anoxic.

'Local' contractions of the whole muscle deserve further study since they provide a way of studying contraction in the absence of an action potential. It would be particularly interesting to see in what way the duration of the active state is related to the duration of the stimulus.

Although a field of 6 V/cm (r.m.s.) does not of itself lead to irreversible reduction of the mechanical response, a muscle regularly stimulated in this way for some time seems to be in an altered condition. It does appear to be unduly sensitive to oxygen lack (Katz, personal communication, found this also, in 1947) and on replacing normal Ringer's solution recovery is delayed, particularly at low temperature.

The effect of temperature on the action of potassium

We have suggested above that potassium probably acts on the surface of the muscle fibre, and that the time course of its action is determined by diffusion through the interspaces combined with entry into the cells. We thought it might be interesting to study the time course of the action of potassium at low temperature, which was done by removing the whole apparatus to a cold room at 2° C. This procedure ensures that there is no risk of even a brief exposure to a higher temperature when the muscle is removed from its bath in order to test for the presence of an action potential. This is an important consideration, as will appear later.

The experiment was performed in exactly the same way as before, though mostly we confined our attention to the propagated response (1 V/cm (r.m.s.), 60 c/s) and to 24 mM potassium. The result is surprising, as shown in Fig. 4. The *onset* of effect is hardly altered, but *recovery* is very much slower than at the higher temperature. (Compare with the 24 mM curve in Fig. 1.) The size of the action potential runs roughly parallel with that of the mechanical response. If the muscle is left for a period to soak in the potassium-rich solution, there is an

almost equal period of delay before recovery begins (Fig. 4B). However, the *rate* of recovery is about the same as it was before.

If the temperature of the muscle is suddenly raised by refilling the electrode chamber with warm Ringer's solution, recovery is extremely rapid. If the temperature is lowered again, tension development falls but not to its original value, and it can be increased and decreased at will by altering the temperature; i.e. the tetanic tension has a fairly large temperature coefficient. This will be dealt with in more detail later: the important point here is that the change on first warming the muscle is of a different kind, reflecting a process which happens only once and is not reversed by lowering the temperature again. The effect is seen just as clearly in a muscle depolarized at room temperature and then transferred to cold normal Ringer's solution for recovery.

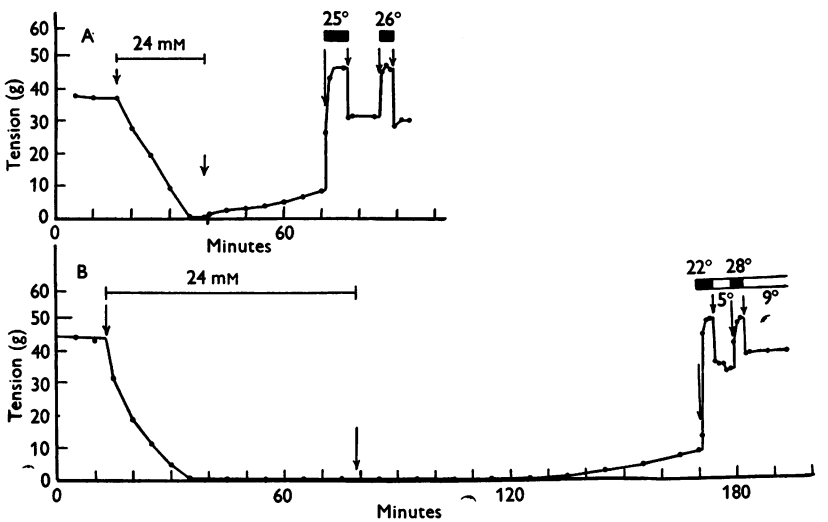

Fig. 4. Effect of 24 mM potassium on frog's sartorius at 2° C except where otherwise indicated. Ordinate, tension developed (grams); abscissa, time, min. Stimulus: 0·5 sec tetanus, 1 V/cm (r.m.s.) 60 c/s, longitudinal. A, short exposure: muscle 30 mm long, 75 mg; B, long exposure: muscle 34 mm long, 119 mg.

The extreme slowing at low temperature of recovery from the effect of potassium has nothing to do with the particular conditions of our experiment. The effect is shown equally well if the muscle is stimulated with single shocks only a little above the original threshold strength, or by the strong (6 V/cm (r.m.s.)) alternating field required to elicit local responses: and it has been found in *R. temporaria* in London (October and April) as well as in *R. pipiens* at Woods Hole (July and August). The result is not at all easy to explain:

(1) It cannot arise simply from the temperature coefficient of diffusion, for this is known to be small (Q_{10} approx. 1·3, Hill & Macpherson, 1954, p. 100) compared with that of the rate of recovery (Q_{10} approx. 3, calculated from

Figs. 1 and 4). Moreover, a slowing of diffusion at low temperature would affect onset and recovery almost equally.

(2) It might be that the potassium which enters the fibres from the potassium-rich solution can leave again only with difficulty if the temperature is low. This could account for the result if the high internal potassium interfered with the contractile process in some way. If it is true there should be only a slight efflux of potassium in the cold, and a sudden large increase when the cold muscle is warmed.

(3) Taking the opposite view, it might be that potassium leaks out of the muscle fibres very freely in the cold after they have been depolarized. This could maintain the potassium level in the interspaces at such a high level—in spite of the loss at the surface of the muscle—that contraction was prevented, as in the experiments of Dulière & Horton (1929). However, recovery on raising the temperature could hardly be as quick as is shown in Fig. 4 for even if potassium efflux from the fibres stopped instantly and completely, the excess potassium in the interspaces would take an appreciable time to diffuse away. The almost instantaneous recovery on raising the temperature suggests strongly that the potassium concentration in the interspaces is already the same as that of the normal Ringer's solution in the bath, unless there is a barrier to diffusion beyond the excitable membrane, similar to the one described by Frankenhaeuser & Hodgkin (1956) in the isolated squid axon. This could maintain a difference in potassium concentration between the excitable membrane and the interstitial fluid, but it would have to be temperature-sensitive.

(4) It might be that in spite of having a normal (or raised) ratio of (potassium inside):(potassium outside), the normal resting potential is not at once set up. This could happen if the membrane itself had to be reconstituted by some metabolic process after having been altered by depolarization. These possible explanations came to mind; but we may well not have thought of the correct one. The following experiments on ion movements and resting potentials shed some light on the question.

Potassium and sodium efflux. Measurements of ^{42}K efflux were made under conditions which imitated those of our other experiments.

Both sartorii were dissected from a frog (*R. temporaria*, November) and separated by splitting the pelvis carefully with a razor blade. The muscles were mounted separately on bent glass tubes by means of which they could be easily transferred from one test-tube to another. Both were soaked for 40 min at 20° C in Ringer's solution containing 15 mM-K, part of which was radioactive. They were then transferred to tubes containing normal Ringer's solution, in each of which they were left for 10 min. The efflux during each 10 min period was estimated by measuring the activity of the soaking fluid. The two muscles were treated in exactly the same way except that the right one was kept at 20° C and the left one at 0° C in a vacuum flask. At the end of the experiment the muscles were returned to radioactive 15 mM-K and soaked for 2½ hr; then the whole experiment was repeated except that now the left muscle was at 20° C, the right at 0° C.

The result is shown in Fig. 5. It is clear that the two muscles are behaving in roughly the same way and that the total amount of potassium efflux has not been much increased by the prolonged soaking in 15 mM potassium which preceded experiment B. Potassium is lost slightly more slowly from the cold muscle than from the warm one, the Q_{10} for the process being roughly 1·1 (mean 0–20° C). However, the efflux does not merely show a temperature coefficient; for when the cold muscle is warmed to 20° C its efflux is 25–50% greater than that of its companion, also at 20° C. Fig. 5B shows this effect even when the muscle is warmed up for a second time. This is a somewhat complicated result, but at least it is clear that hypotheses (2) and (3) above cannot be true—potassium leaves the cold muscle almost as easily as it leaves the warm one (2): potassium is not leaking continuously and excessively from the cold muscle (3).

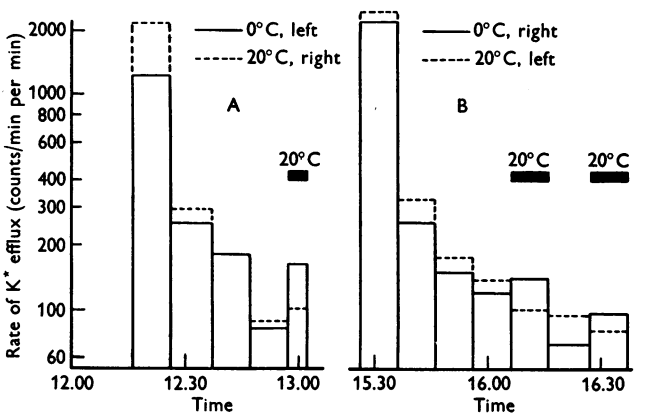

Fig. 5. Potassium efflux into Ringer's fluid from a pair of sartorii (30 mm, 90 mg each) previously soaked in 15 mM potassium. Ordinate, rate of efflux, counts/min per min, corrected for decay; abscissa, time. Solid lines, 0° C; broken lines, 20° C: A, left muscle cold, right muscle warm; B, right muscle cold, left muscle warm; the temperature of the cold muscle was raised to 20° C during the periods indicated.

A similar experiment was performed on a pair of muscles which had been soaked in Ringer's fluid containing ^{24}Na and 24 mM potassium. The sodium efflux was observed during recovery in normal Ringer's solution at 0° and 20° C. Once again there was a temperature coefficient for the efflux of about 1·1 but no sudden change on warming the cold muscle to parallel its sudden recovery of excitability.

Depolarization potentials. Hypothesis (4) proposes that recovery from potassium depolarization is slowed down at low temperature. We have investigated this question by measuring depolarization potentials on a whole sartorius employing the double Perspex chamber shown in Fig. 6 (inset). The partition, 3 mm thick, is pierced by a narrow slot through which the

muscle is drawn. Electrical leaks around the muscle were reduced to a minimum by careful application of petroleum jelly. Both pools originally contained Ringer's fluid, but in one of them (usually the pelvic) potassium-rich solution was substituted as required. The potential difference between the pools was measured with a Leeds and Northrup 7666 pH meter used as a millivoltmeter. The remarkable stability of this instrument permits one to estimate accurately to within a millivolt in spite of a rather close scale. Connexion was made to the chamber by Ag–AgCl electrodes through long Ringer-agar bridges so that the electrodes did not participate in the temperature changes imposed on the chamber. The experiments at low temperature were carried out in a cold room.

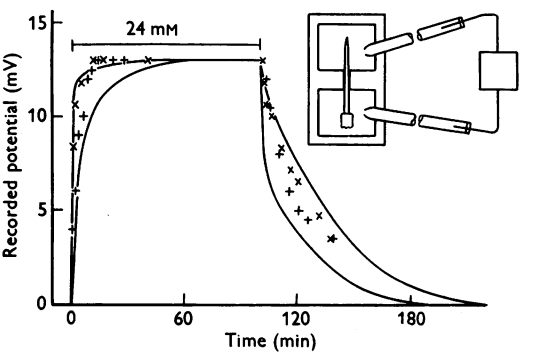

Fig. 6. Depolarization potentials in 24 mM potassium at 25° C. Ordinate, potential, mV; abscissa, time, min. Two independent experiments are indicated by upright and diagonal crosses. The full lines are calculated as described in the text for the mean of the whole muscle (slow rate of change) and for the outer one-fifth only (rapid rate of change). The experimental curves have been broken and shifted so that they coincide at the onset of recovery.
Inset, diagram of the experimental arrangement.

The result of two such experiments at 25° C is shown in Fig. 6. The curves are similar in shape, size and time scale to those of Sandow & Mandel (1951) and they show the same asymmetry between onset and recovery. We were interested in them for two reasons: (1) whether they could be accounted for theoretically by the theory put forward above, i.e. that potassium concentration in the interspaces determines the membrane potential but that its rate of change within the muscle is limited by the small effective diffusion constant; (2) if theoretical limits could be set to the experimental curves it would then be possible to decide whether repolarization was indeed delayed at low temperatures as suggested in hypothesis (4).

The theoretical calculation is made difficult by the fact that fibres inside the muscle are shunted to an unknown extent by the interspaces and by other fibres, which reduces their contribution to the measured potential. Sandow & Mandel (1951) have considered this problem but they give no details of their reasoning. Their own method of calculation consisted in finding the mean

potassium level throughout the muscle at any given moment, using the graph given by Hill (1928, Fig. 5), then converting this to millivolts depolarization. In order to understand what is going on it is better to calculate the depolarization potential at different depths in the muscle at each instant, then to combine these figures as a separate operation; for one can then vary this combining operation in accordance with a variety of hypotheses about the shunting situation in order to see which hypothesis fits best with the experimental facts.

The calculation follows the method given by Hill & Macpherson (1954) who divided the muscle thickness into ten equal zones. Their table 1 (p. 98) then gives the mean concentration in each zone at various times after changing the concentration at the surface. In the case of potassium one can then use the equation derived from Gerard & Jenerick (1953):

$$\text{membrane potential} = 93 - 43 \log (\text{mM-K}/1{\cdot}34)$$

to calculate the depolarization in each zone. The problem remains of combining these figures for the ten zones. Fig. 6 illustrates two of the possible ways of doing this which may be regarded as limiting cases: one line shows the mean for all zones, i.e. the assumption is made that all zones contribute equally; while the other line is calculated on the assumption that the measured potential is determined by the outermost zones (1 and 10) only. In both cases there is marked asymmetry between onset and recovery, similar to that seen experimentally. One could make the 'mean' curve fit the experimental curve of onset by increasing the diffusion constant about four times, but this asymmetry would then be largely lost.

This method of calculating sheds doubt on the accuracy of Sandow & Mandel's (1951) determination of the diffusion constant of potassium, for the answer obtained by their method depends entirely on what arbitrary assumption is made about shunting inside the muscle.

At low temperature the onset of depolarization is somewhat slowed—in Fig. 7 to about two-thirds for a temperature difference of 23° C. This corresponds to a Q_{10} of 1·2, which is in reasonable accord with the value 1·3 given by Hill & Macpherson (1954). However, recovery from depolarization is very much slower at the low temperature, corresponding to a Q_{10} of about 2·5. This is almost as large as the value ($Q_{10} = 3$) calculated above from tension records. If the recovering muscle is suddenly warmed, the potential drops suddenly. The subsequent recovery follows the same time course as though the process had been determined from the beginning by diffusion.

This result strongly supports the hypothesis (4) that at low temperature and after potassium depolarization the membrane potential does not immediately assume the value appropriate to the difference in potassium concentration across it. The membrane immediately regains this property on warming. Thereafter the potential is determined by diffusion into the interspaces.

The temperature coefficient of tetanic tension. It has been mentioned above in connexion with Fig. 4 that tetanic tension has a temperature coefficient (Q_{10} = 1·2–1·3) which is large compared with the value 1·1 which can be calculated from Hill's (1951) experiments over a similar temperature range. (The familiar Q_{10} notation is used here because it provides a convenient way of comparing results over slightly different temperature ranges. However, the temperature coefficient of tetanic tension is very different at different temperatures, so the mean Q_{10} values given here must be used with caution.) It seemed worth while to investigate the point further since there has been disagreement about it (Dr Albert Szent-Györgyi, personal communication).

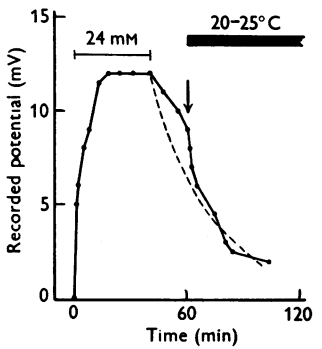

Fig. 7

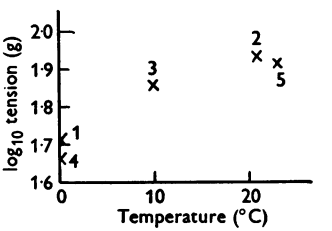

Fig. 8

Fig. 7. Depolarization potentials in 24 mM potassium at 2° C. Ordinate, potential, mV; abscissa, time, min. The full line is an experimental curve. Where indicated, the temperature was suddenly raised to 20–25° C. The broken line is the mean of the two experimental recovery curves of Fig. 6, corrected appropriately for temperature.

Fig. 8. The variation in tetanic tension with temperature (*R. pipiens*). Ordinate, $\log_{10}$ tetanic tension, grams. Abscissa, temperature, ° C. Crosses are experimental points. The numbers indicate the order in which the observations were made.

The experimental technique was made as much like Hill's as possible. The muscle was stimulated on a multi-electrode assembly (Hill, 1949) by supramaximal rectangular pulses which were altered so as to be optimal at each temperature. Thus at 0° C the muscle was given a 2 sec tetanus of 4 msec pulses at 25/sec. Both durations were halved, and the frequency doubled, for each 10° C rise in temperature.

The result is shown in Fig. 8 from which can be calculated a mean Q_{10} of about 1·3 for the temperature range 0–24° C considered by Hill. It appears therefore that there must be a genuine difference in this respect between American *R. pipiens* (July and August) and English *R. temporaria*. Hajdu & O'Sullivan (1951) have investigated the same question, using *R. pipiens*. Their curve is a different shape from Fig. 8 and hardly rose at all above 10° C though it was on linear co-ordinates. Possibly their stimulus frequency (32/sec) was not high enough at the higher temperatures.

DISCUSSION

The time course of the action of potassium in blocking the propagated action potential of frog's sartorius has been accounted for over quite a wide range of applied potassium concentrations by a theory based on the following three assumptions:

(1) That conduction in any given fibre is blocked when the potassium concentration around it reaches a threshold value, approximately 9 mM.

(2) That potassium ion diffuses into the interspaces with about the same diffusion constant (about 4×10^{-6} cm^2 sec^{-1}) as other univalent ions.

(3) That potassium ion enters the cell in such a way that there is a fixed difference between its concentration inside and outside the cell; and that this equilibrium condition is reached by each fibre fairly quickly.

The situation is thus one of diffusion with entry of the type dealt with by Harris & Burn (1949). However, we suppose the cell permeability to be very large (i.e. local equilibrium quickly reached). The observed slowness of the whole process is then explained quite satisfactorily by the way in which local potassium storage is known to vary with local potassium concentration.

Our experiments on the non-propagating muscle give essentially the same result as those of Sten-Knudsen (1954), i.e. that sinusoidal stimulation at 1000 c/s, 4 V/cm (r.m.s.) is much more effective with a transverse than with a longitudinal field. However, we were not able to examine this phenomenon over the full range of possible potassium concentrations, field strengths and frequencies, so one of us (A. C.) is continuing to work on this topic. Preliminary experiments by the other (D. R. W.) indicate that the mechanical properties of the muscle in massive 'local' contractions are similar to those of normal propagating muscle.

The delay in recovery from the effects of potassium which we observe at low temperature is particularly interesting because it cannot easily be explained. It is not certain whether it is the membrane potential or the action-potential-generating mechanism which fails to recover; the experiments on depolarization potentials suggest the former. However, further study of this phenomenon must yield interesting results; for in spite of having the proper ratio between potassium concentration outside and that inside the fibre, either (*a*) the appropriate resting potential is not set up; or (*b*) even though the appropriate resting potential is present, no propagated action potential can be generated.

The dramatic recovery which follows even a brief period of warming suggests that after depolarization the membrane has to be reconstituted by some metabolic process with a large temperature coefficient. Once it has been 'reconstituted' in this way the membrane is able to function in a perfectly satisfactory way, even at low temperature.

Our experiments have not extended over a long enough period for us to assess the effect of the seasonal variations in potassium sensitivity described by Solandt (1936) and Gerard & Jenerick (1953). However, further experiments on delayed recovery at low temperature (J. R. Hill and Wilkie, unpublished) indicate that there is a sudden change more or less coinciding with the breeding season in April. Immediately after this time a higher concentration of potassium must be used (25–30 mM instead of 15–25 mM) in order to elicit the effect. By the middle of June the muscles seem to have regained their former sensitivity. Far more striking is the effect of raising the potassium concentration in the recovery Ringer's solution to 6 mM. This prevents recovery indefinitely at 0° C, but even after many hours the muscle returns to full activity promptly on warming.

SUMMARY

1. The time course of the action of potassium in blocking propagation in frog's sartorius can be satisfactorily explained by diffusion into interspaces combined with entry of potassium into the cells.

2. The 'local' response elicited in the blocked muscle by an alternating field is greater for transverse than for longitudinal stimulation under the conditions of our experiments.

3. At low temperature recovery from the effects of potassium is very slow. A brief period of warming leads to sudden and dramatic recovery; after this the muscle functions normally, even at low temperature. Several possible explanations for this phenomenon are examined, leading to experiments on potassium efflux and depolarization potentials.

The authors are most grateful to Dr Albert Szent-Györgyi for his generous hospitality and for the benefit of discussions; they appreciate the interest and helpfulness of other members of the Institute for Muscle Research, and wish to thank Dr E. J. Harris for his help, especially with the isotope experiments.

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