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THE EFFECT OF METABOLIC INHIBITORS ON THE FATIGUE OF THE ACTION POTENTIAL IN SINGLE MUSCLE FIBRES

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Myelinated nerve fibres and squid nerve axons can carry action potentials at high frequencies for long periods of time. Muscle fibres, however, fail to respond to every stimulus after one to several seconds when stimulated at 50–100 shocks/sec. This is difficult to understand since the underlying ionic movements which generate action potentials are very similar in these tissues.

Bergmans (1959), who described the fatigue phenomenon in muscle fibres, pointed out that the relatively small change in internal concentrations of Na and K during activity could hardly account for this effect. He tentatively suggested that a reduction in energy resources during a tetanus causes fatigue. Experiments described in this paper, however, show that rather the opposite is true. Fibres whose energy resources had been exhausted by continuous stimulation or blocked by metabolic inhibitors and which were in contracture or unable to contract behaved like nerve fibres, i.e. they showed comparatively little fatigue. This observation suggests that processes connected with contraction and its activation cause the rapid decline in the height of the action potential.

The present paper gives a description of the fatigue phenomenon and the effect of metabolic inhibitors and hypertonic solutions on it. Single isolated fibres were used to avoid an extracellular accumulation of potassium which occurs in isolated bundles of fibres when they are stimulated at high frequencies.

Preliminary accounts of some of the experiments described here were given at meetings of the Physiological Society (Cook, Hodgkin & Lüttgau, 1963; Lüttgau, 1964).

METHODS

Dissection. Single fibres from semitendinosus and iliofibularis muscles of English frogs (*Rana temporaria*) were used throughout the investigations which took place from March to August 1963 and from May to August 1964. The frogs were stored in a refrigerator ($\sim$ 3° C) for up to 3 weeks without feeding. The muscles were dissected immediately after decapitation and kept in Ringer's solution (Adrian, 1956) at room temperature (17–22° C) between

½ and 5 hr before the isolation of a single fibre was attempted. The tonus bundle in iliofibularis muscles was carefully removed at the beginning of this operation. The isolated fibre was stretched to 1·25 times its slack length, the diameter was then estimated at 5–8 points along the fibre and the mean was taken. A loop of silver wire (diameter 100 μ) was tied to one tendon with a thinner silver wire (diameter 50 μ) to provide a connexion for a hook on the prolonged anode of the transducer. The fibre was then left for ½–1 hr in the dissection chamber, electrically tested for excitability and transferred to the experimental cell.

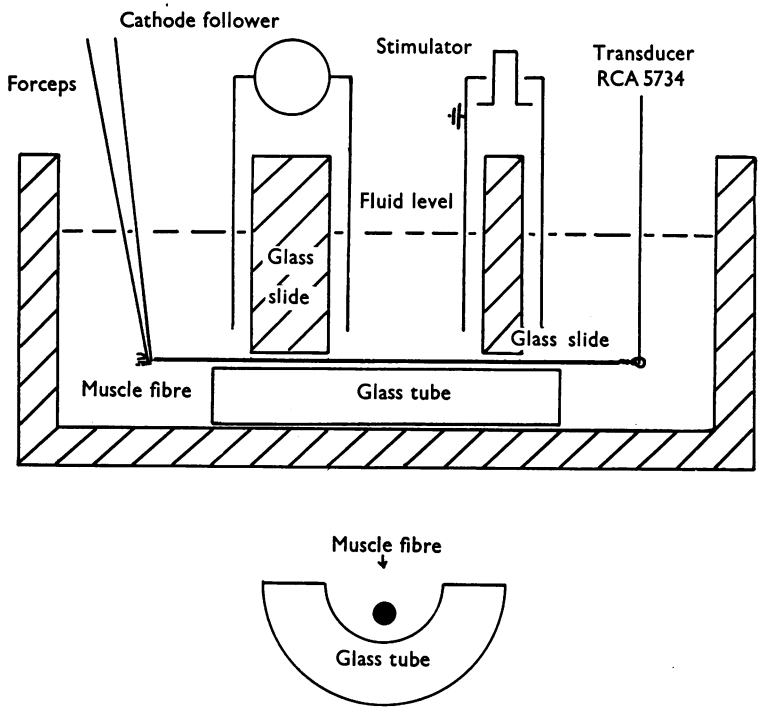

Fig. 1. Above: longitudinal section of experimental cell for recording action potentials with external electrodes. Below: cross-section through the bisected glass tube with a single muscle fibre in position.

Experimental cell for recording action potentials with external electrodes. Since internal glass micro-electrodes are rather unsuitable for recording action potentials in muscle fibres during a succession of tetani over a period of an hour or more, a method was developed of recording diphasic action potentials with external electrodes. For this purpose one half of a longitudinally bisected glass tube with an internal diameter of 700 μ and a length of 11 mm was mounted in a Perspex cell (Fig. 1) which was afterwards filled with Ringer's solution. One tendon of the isolated fibre was gripped by a pair of forceps and the other one was connected to the prolonged anode of the transducer (RCA 5734). Forceps and transducer were connected with a micro-manipulator and could be moved relative to each other to stretch the fibre to 1·2–1·4 times its slack length. A sarcomere distance of 2·8 μ was measured under these conditions (Lüttgau, 1963). The fibre was then brought into position in the groove of the bisected tube by horizontal and vertical movements of the whole unit consisting of forceps, fibre and transducer, and by rotary motion of the experimental cell. During this and the following operations the fibre was kept under observation through a binocular microscope to avoid any friction between it and the surroundings. Two glass slides were then

lowered into the Perspex cell. Their edges were covered with a thin layer of petroleum jelly and they were pressed into position by means of springs. The two slides were 5 mm apart and divided the cell into three compartments, causing a relatively high external resistance at two places on the fibre. This made it possible to stimulate the fibre electrically and to record a diphasic action potential. Platinum electrodes were used for both purposes (diameter 0·8 mm). The recording electrode in the central pool was insulated nearly up to its end; it could be moved along the slide with the help of a micro-manipulator and brought into a position where the stimulus artifact was smallest.

The absolute amplitude of the upstroke of the diphasic action potential will depend on the diameter of the fibre and the amount of petroleum jelly used; it varied from 0·5 to 10 mV and was usually several mV. Assuming a conduction velocity of 2 m/sec (Martin, 1954) and allowing 0·5 msec for the spike to rise from 20 to 100 % of crest (room temp., Nastuk & Hodgkin, 1950) one can calculate that with a slide of 3·2 mm thickness the upstroke of the recorded diphasic potential gives a rough measure of the absolute amplitude of the action potential. The slide which separated the stimulating electrodes was 1·6 mm thick. Some disadvantages of the method owing to the early after-potential are reported in connexion with Fig. 2. The strength of the stimuli was 1–4 times above the normal threshold.

Intracellular electrodes. All problems were first tackled with external electrodes as just described. To obtain further information about changes in resting potential and the shape and absolute height of action potentials several experiments were done with glass micro-electrodes of the Ling–Gerard type. Electrodes with a resistance of up to 20 MΩ and a tip-potential less than 10 mV were selected. The fibres were also placed in the groove of the glass tube (Fig. 1), but stretched up to 1·5 times their slack lengths to reduce movements during contractions. They were stimulated in the usual way. The rate of rise of the action potential was measured by electrical differentiation. In some experiments the membrane potential was altered by currents applied across the left slide (Fig. 1). The membrane potential was then recorded between an internal and an external glass micro-electrode placed closely together to reduce artifacts.

Solutions. Ringer's solution was the same as that used by Adrian (1956), and contained (mM): KCl 2·5; NaCl 115; $CaCl_2$ 1·8; Na_2HPO_4 2·15; NaH_2PO_4 0·85. The pH was measured with a glass electrode and ranged from 7·0 to 7·4.

Metabolic poisons. Iodoacetate (IAA; Hopkins & Williams): a 400 mM stock solution, neutralized with NaOH, was added to Ringer's solution. NaCN (British Drug Houses): a 20 mM stock solution of cyanide was made by adding 0·5 ml. M-NaCN and 3·5 ml. 4 % wt/vol HCN to unbuffered Ringer's solution to make 250 ml.

RESULTS

Action potentials and tension during a tetanus

External electrodes. Figure 2 shows action potentials and tension produced by trains of shocks at different frequencies, each train lasting for about 2 sec. The height of the action potential declined only slightly during stimulation at 12·5 and 25 shocks/sec. It fell to two thirds of its original value at 50/sec. At 100/sec monophasic potentials appeared after about 1 sec, during which time the potential height had declined to about 50 %, and 300–400 msec later the fibre responded only to every second stimulus. Evaluation of eight further experiments of this kind showed that in 'good' fibres up to 200 action potentials could be elicited at 100/sec before potentials dropped out or became monophasic. Very often, how-

ever, the number of potentials was much smaller, although the fibres survived for hours and showed no visible signs of damage.

The fusion of twitches was nearly complete at 25/sec. It became progressively smoother during the tetanus, a phenomenon which will be dealt with in a later section.

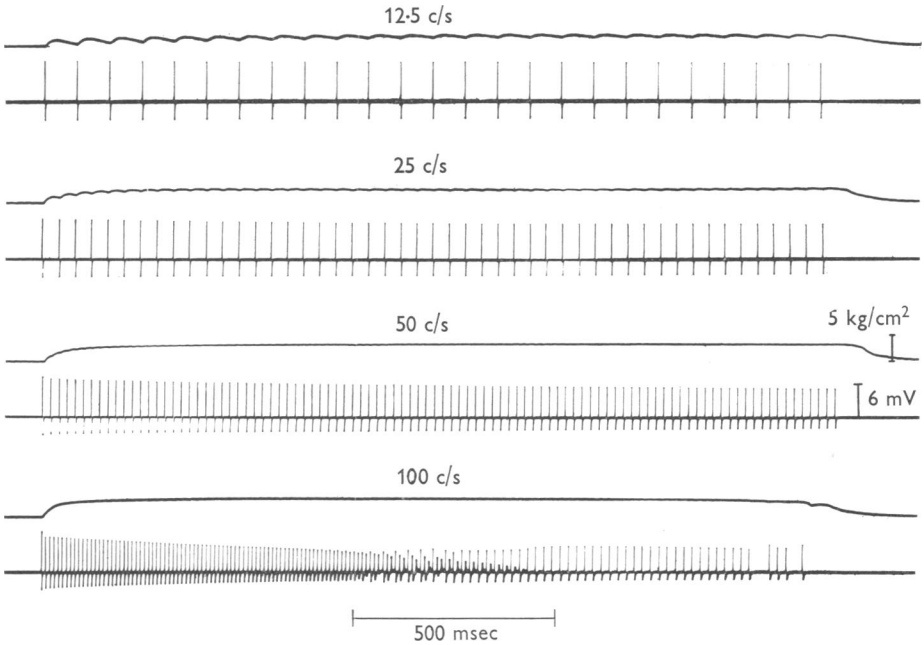

Fig. 2. Action potentials and tension at different stimulation frequencies. External electrodes. Fibre 22. The fibre had a diameter of 105 μ and was stretched to 1·35 times its slack length; temp. 21° C.

It is important to note that the fatigue of the action potential was not accompanied by a decrease in tension. As clearly demonstrated in Fig. 2, tension remained constant in a tetanus at 100/sec as long as the frequency of action potentials did not drop below the value which is necessary for a complete tetanus. It could be argued, however, that the frequency of stimulation was wastefully high since a complete tetanus is reached at 25–50 shocks/sec. To test this point a long-lasting train of shocks with a frequency of only 40/sec was applied to a fibre. During the tetanus (Fibre 32; 18° C) every second action potential became monophasic after about 2200 msec and dropped out completely after 5200 msec. At this time isometric tension was still maximal; but during the following seconds the tetanus became incomplete and tension dropped because the sequence of action potentials was then too low to maintain a complete tetanus.

In Fig. 2 a sharp drop in potential height can be seen between the first and the second potential at a frequency of 100/sec. This drop was not normally observed when internal electrodes were used, at least as long as the resting potential was high. It is, therefore, not due to refractoriness. The overshoot of the action potential recovers fully within 6–8 msec at 20° C (Buchthal & Engbaek, 1963). The reduction, which in different fibres amounted to about $\frac{1}{8}$–$\frac{1}{5}$ of the whole upstroke of the diphasic potential, is caused by the fact that with frequencies of 50/sec and higher the early after-potential lasted longer than the interval between stimuli. The potential, therefore, did not return to its original resting value and the external electrodes recorded a correspondingly smaller upstroke.

In one experiment tension and fatigue of the action potential were observed at frequencies ranging from 50 to 200 shocks/sec. The tetani always lasted for 540 msec. During this time the action potential declined to 81 and 62 % of the normal value when shocks at 50 and 158/sec were applied. At 200 shocks/sec the fibre failed to respond to every stimulus after 71 action potentials. The initial rate of rise in tension increased with frequency, though the time between the last action potential and the sharp drop in tension remained about 35 msec at all frequencies (temp. 20° C).

The effect of temperature on fatigue was investigated in two fibres. Four different frequencies were applied at room temperature and subsequently at a temperature below 10° C. Figure 3 shows the result from one of these fibres at 7·5° C. The tetani at room temperature (25° C) have not been included in the figure since they resembled those shown in Fig. 2. It can be seen that at 7·5° C shocks at 2 and 5/sec caused no fatigue during tetani of 7·5 sec duration. The decline in height at 10 shocks/sec was slightly less than that during the same time at 50/sec and 25° C. At 20 shocks/sec and 7·5° C the fibre failed to respond to every stimulus after 2550 msec as compared with 1020 at 25° C when 100 shocks/sec were applied. Thus, at 25° C the frequency must be 3–4 times higher than at 7·5° C to give the same rate of decline in the height of the action potential.

In the same experiment the decrease in temperature from 25 to 7·5° C prolonged the time during which twitch tension was half its maximal value from 64 to 317 msec (4·9 times) and the time from the last action potential of a train to the sharp drop in tension from 34 to 211 msec (6·2 times). At 7·5° C twitch fusion was nearly complete with 5 shocks/sec as compared with 25 shocks/sec at 25° C.

Internal electrodes. It was extremely difficult to get reliable records from contracting fibres which were stretched to 1·3–1·5 times their slack lengths. Electrodes either caused irreversible damage or were dislodged during contraction. The records which can be trusted most are those which were taken shortly after removal of NaCN (Fig. 10). The contractions were

still weak under these conditions and it was possible to keep the electrode inside for several tetani without causing visible damage to the fibre. When fibres were stretched to 1·8–2·2 times their slack lengths, contractions were no longer visible under the binocular microscope and the electrodes stayed inside. However, the fibres did not survive for long and there were also some indications that the fatigue effect was less in a fibre stretched beyond 1·6 times its slack length.

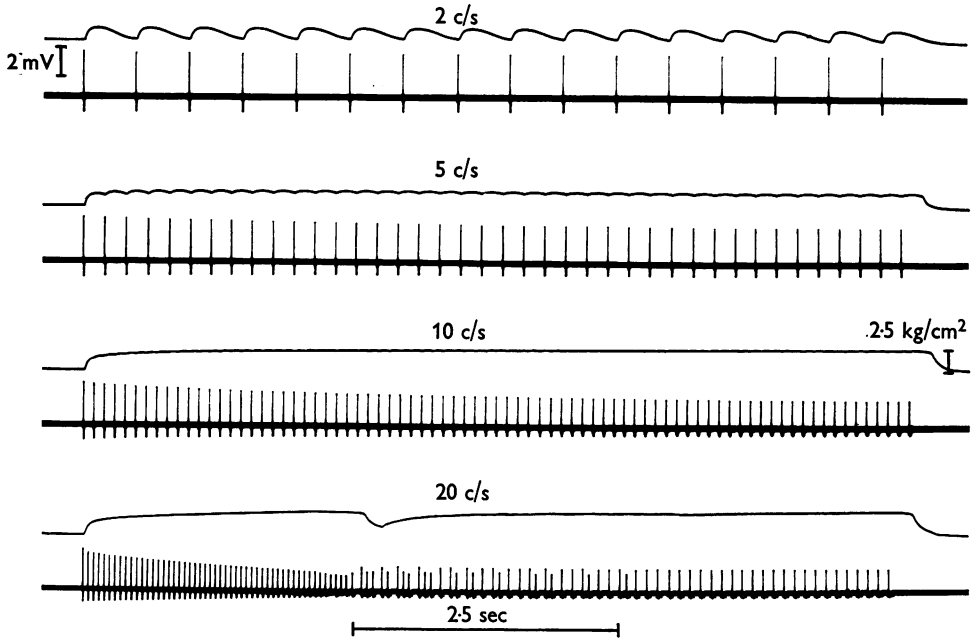

Fig. 3. Action potentials and tension at different stimulation frequencies. Low-temperature experiment. Fibre 29*b*. The fibre had a diameter of 82 μ and was stretched to 1·35 times its slack length; temp. 7·5° C.

Figure 4 shows action potentials during a 100/sec tetanus in a fibre which was stretched to 1·5 times its slack length. The resting potential was only -76 mV and during the tetanus the potential from which spikes started was -63 mV. The latter value remained nearly constant throughout the tetanus but the rate of rise of the action potential declined from 268 V/sec to about 50 V/sec. In addition a considerable prolongation of the falling phase of the spikes took place during the final part of the tetanus. This effect has already been described by Persson (1963).

In other experiments the membrane potential from which spikes started during a tetanus ranged from -50 to -70 mV. This potential did not change during the first 3–5 action potentials, which is in agreement with results described by Persson (1963). In fibres with high resting potentials,

however, it shifted by about 10 mV towards depolarization during the following 10–20 potentials (at 100/sec) and then remained constant until the end of the tetanus. In these cases the trains were followed by late after-potentials of the type described by Freygang, Goldstein & Hellam (1964).

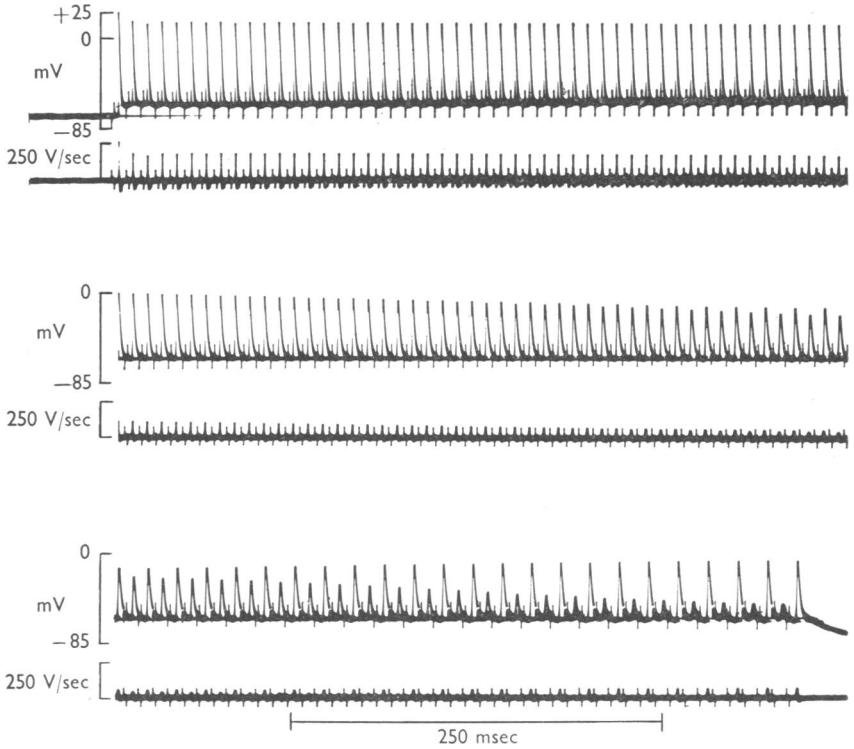

Fig. 4. Action potentials and differentiated action potentials during a tetanus at 100/sec. The tetanus lasted for 1950 msec; forty-nine action potentials were left out between the first and the second record. Internal electrodes. Fibre M14. The fibre had a diameter of 105 μ and was stretched to 1·5 times its slack length; temp. 20° C.

In five fibres a hyperpolarizing current of 100 msec duration was applied with external electrodes 1½ sec after the start of a tetanus at 100/sec. The current shifted the potential from which spikes started by about 15 mV so that in some cases the original resting potential was approached. (Stronger currents caused movements of the fibre and dislodged the electrode, probably due to a block of conduction under the cathode.) It was possible to restore the rate of rise of the fatigued fibre from its low value of 20 % to about 80 % of the original value. But even though the potential from which spikes started was 5–8 mV more negative during the application of the current than during the 2nd to 10th spike of the train, the height and the

rate of rise of the spikes were about equal during both periods of time or sometimes smaller during the time when the current was applied. This suggests that the S-shaped curve, relating rate of rise to membrane potential, shifts by 8–10 mV towards hyperpolarization when fatigue develops. The hyperpolarization did not remove the prolongation of the falling phase of action potentials in the fatigued state.

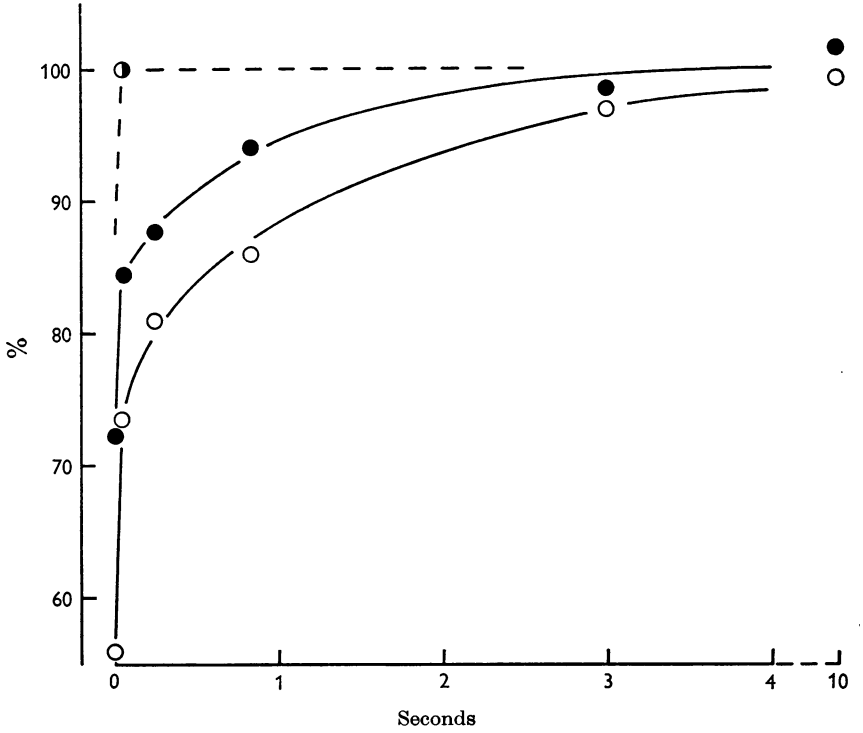

Fig. 5. Recovery of the height of the upstroke of the diphasic action potential after 1 impulse (◑) and after 42 (●) and 74 (○) impulses at 125/sec. Abscissa: time between the last action potential of the conditioning train and the test impulse. Ordinate: height of the test action potential as a percentage of the first action potential of the conditioning train. At zero time the height of the last action potential of the conditioning train was taken. Half-time of the slow exponential component: 950 (●) and 1060 (○) msec. Fibre 19. The fibre had a diameter of 85 μ and was stretched to 1·35 times its slack length; temp. 22·5° C.

Recovery of the action potential after a train of stimuli

Figure 5 gives the recovery curve of the diphasic action potential after a single impulse and after 42 and 74 impulses. It shows that after prolonged activity the first rapid recovery is followed by a slower recovery process. The half-time of this slow exponential component, estimated in fifteen fibres, was about 1 sec (range: 490–1750 msec; temp. 19–25° C). Condition-

ing trains of 10–74 potentials at 50–125/sec were employed. They caused a reduction in the height of the action potential to about 50–80 % of the original value. Temperature had little effect on the slow recovery process. In one experiment, for example, the half-time of recovery was 640 msec at 21° C and 940 msec at 9° C.

Time course of post-tetanic twitch facilitation

A conditioning tetanus not only affects subsequent action potentials but also the corresponding twitches. This finding was described by Guttman, Horton & Wilber (1937) and is known as post-tetanic twitch facilitation. Both effects might arise from the same reaction and it is, therefore, of some interest to investigate whether or not they disappear at the same speed.

In the experiment illustrated in Fig. 6 the conditioning train consisted of forty-seven action potentials at 77/sec. The time between the conditioning tetanus and the single test impulse and also the recovery of the diphasic action potential of the test impulse are given at the right of the superimposed twitches, taken before and shortly after the tetanus. The amount by which contraction was prolonged when twitch tension had reached half its normal value decreased to zero in a roughly exponential manner with a half-time of 520 msec. This decrease parallels the recovery of the diphasic action potential.

Another phenomenon which is probably related to post-tetanic facilitation is the fusion of contractions during a long-lasting tetanus. In one experiment the fibre was stimulated at 12·5/sec for 10 sec (temp. 19·5° C). During this time maximal tension of the incomplete tetanus nearly doubled but the difference between maximal tension at the top of the twitches and minimal tension at the bottom between two twitches declined to half owing to the increase in twitch duration. When the difference between maximal and minimal tension was plotted against the height of the upstroke of the corresponding action potentials a linear relation was obtained.

The effect of metabolic inhibitors

During a long-lasting experiment the surprising discovery was made that the fatigue of the action potential was less in an exhausted fibre, i.e. in a fibre which had produced contractions over a longer period of time. The fibre concerned had delivered about 2500 action potentials in response to single shocks or short tetani and had probably lost a good deal of its energy reserves while developing tension. Finally, it was stimulated at 50/sec for more than 13 sec as seen in Fig. 7; external electrodes were used. The maximal tetanus tension, which was about half of the tension developed by the fresh fibre during the first tetani after dissection, declined steadily to a small value. Since contractility recovered afterwards this

decline was probably caused by an insufficient supply of energy readily available for contraction or by a block in excitation–contraction coupling. The height of the action potential declined at the beginning of the tetanus, but as the tension diminished the height of the action potential increased

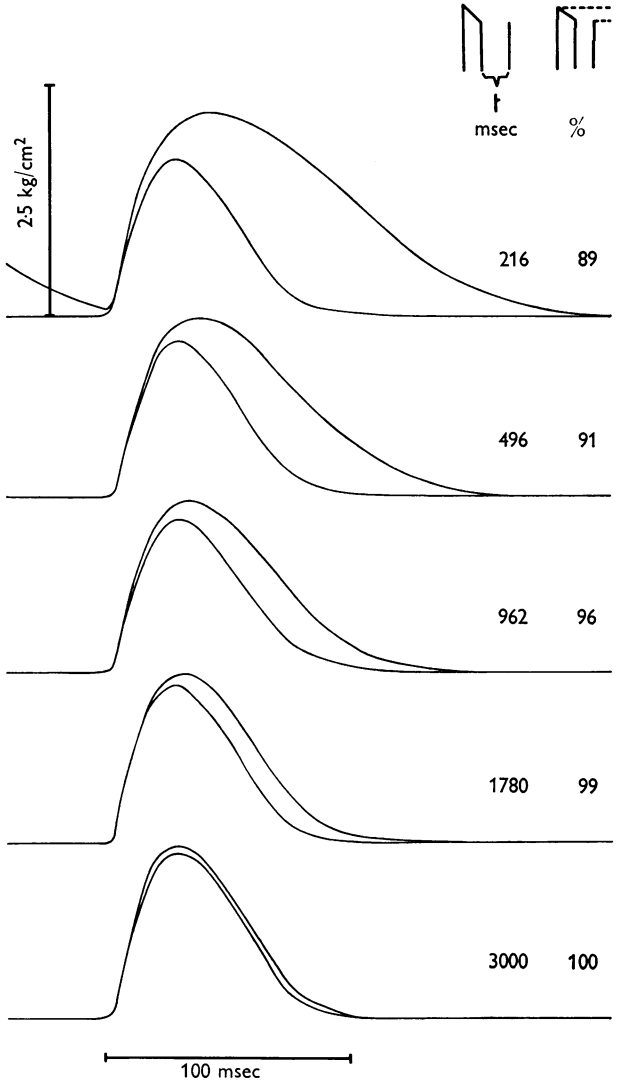

Fig. 6. Post-tetanic twitch facilitation. The figure shows twitches after a conditioning train superimposed on normal twitches taken after 10 min of rest. The conditioning tetanus consisted of forty-seven action potentials at 77/sec. The time between the last action potential of the tetanus and the test shock is given on the right together with the height of the test action potential as a percentage of the first action potential of the conditioning tetanus. Fibre 37, diameter 117 μ; the fibre was stretched to 1·35 times its slack length. External electrodes; temp. 20° C.

and approached temporarily the original value. After 677 action potentials —123 were omitted between the upper and the lower run in Fig. 7—the reduction in height was less than 20 %. So little fatigue was never observed when the tetanus tension remained maximal. Moreover, in a fully contracting fibre action potentials always failed in less than 5 sec after the onset of stimulation at a rate of 50 shocks/sec. The second increase in the height of the diphasic potential, however, probably does not reflect an

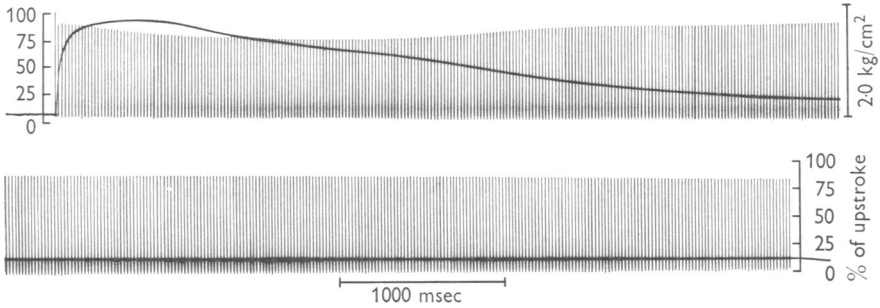

Fig. 7. Action potentials and tension of an exhausted fibre. Detailed description in text. Stimulation frequency 50/sec. In this and some further figures the lower part of the upstroke of the diphasic action potential is not shown. The height of the upstroke is given as a percentage of that of the first action potential of the first train in the figure. Fibre 39, diameter 135 μ; 100 % of upstroke = 4·2 mV. The fibre was stretched to 1·3 times its slack length; temp. 20° C.

increase in the crest of the action potential. Measurements with internal electrodes showed that in fibres which no longer contract spikes are followed by rather small early after-potentials. Thus, the second increase in the diphasic potential in Fig. 7 might be due to the disappearance of the early after-potential.

Five attempts were made to get isolated fibres into a similar state of exhaustion by stimulating them continuously at 2–4 shocks/sec. Microelectrodes were inserted when—after 3000 to 10,000 shocks—the height of twitches had declined to about one third of the original value. One of these fibres (No. 73; diameter 108 μ, temp. 19° C), which had been exhausted by stimulation at 2/sec for 24 min, was able to respond to 2590 shocks at 100/sec during which the peak of the action potential declined from + 29 to − 4 mV and the resting potential from − 88 to − 78 mV. Maximal tetanus tension was only 0·3 kg/cm²; it declined towards zero during the train (normal twitch tension: 1·1 kg/cm²). After 5 sec rest following the tetanus the overshoot was + 17 mV and the resting potential − 86 mV. Early after-potentials were rather small but not absent. The results were similar with a second fibre, whereas three other fibres deteriorated before the fatigue effect disappeared.

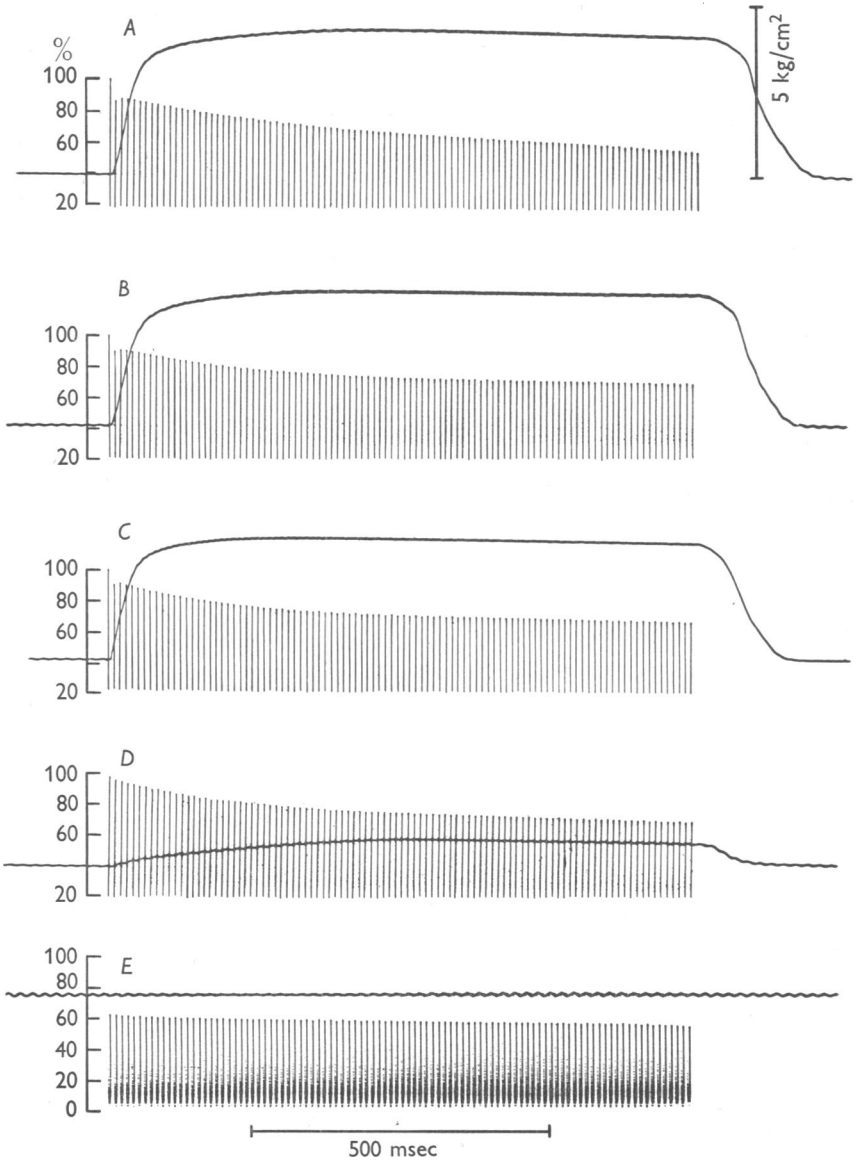

Fig. 8. Effect of metabolic poisons upon action-potential fatigue and isometric tension. *A*, Before, *B*, *C*, *D* and *E*, 3, 5, 7 and 10 min after poisoning with 1 mM cyanide and 2 mM-IAA. In *E* the fibre was in contracture, which amounted to about 1 kg/cm². (The change in the position of the tension trace related to the potential trace is of no significance.) The sensitivity of tension registration was doubled in record *E*; the small oscillations are artifacts. External electrodes; 100 % of upstroke = 4·4 mV. Fibre 41, diameter 95 μ. The fibre was stretched to 1·3 times its slack length. Stimulation frequency 100/sec; temp. 19·5° C.

These experiments suggested that contractile processes are related to the fatigue phenomenon and for this reason the following experiments with metabolic inhibitors were carried out.

NaCN and iodoacetate (IAA). This combination of poisons was chosen with the intention of blocking simultaneously both glycolysis and oxidative metabolism. Thirteen experiments were performed, eight with microelectrodes. The concentration of the poisons ranged from 0·5–1 mM for cyanide and from 0·5–5 mM for IAA. When stimulated at 1/sec—starting 5 min after poisoning—about 115 normal twitches could be obtained. Then tension declined steadily and after about fifteen further twitches fibres went into contracture.

Figure 8 illustrates an experiment with external electrodes and shows that the poisoned fibre, which was in contracture, was still capable of giving action potentials and that there was little fatigue of the electrical response. From this figure alone one might suppose that in a contracting fibre the action potential reaches a steady state at about 60 % of its original value—corresponding to the height in the poisoned fibre—and that this level remains constant during further stimulations. This is not the case. When stimulated at 100/sec unpoisoned contracting fibres failed to respond after ½–2 sec (Fig. 2), whereas fibres in contracture continued for much longer periods. Thus, an unpoisoned fibre which failed after 71 action potentials at 100/sec responded to each of 1181 shocks at 100/sec while in contracture after poisoning. The effect of cyanide plus IAA was normally irreversible and the fibres did not usually survive for more than an hour after going into contracture.

Intracellular recordings: The resting potential, measured after the fibres went into contracture, ranged from -75 to -97 mV. Early after-potentials were virtually absent—which is in agreement with Macfarlane & Meares' (1958) findings—and did not reappear when the membrane was hyperpolarized to -100 mV by externally applied currents. One fibre (No. 67, diameter 110μ; temp. 21° C) responded to 1900 shocks at 77/sec with propagated action potentials. During the first 1200 shocks the overshoot fell from $+37$ to $+21$ mV. Then the spike deteriorated faster and after 1900 shocks the peak reached only -20 mV. There was little recovery in the height of the spike afterwards. Late after-potentials were almost absent in exhausted fibres; also the lengthening of the falling phase of the action potential no longer occurred.

In these experiments part of the change in overshoot during the train of spikes is accounted for by the rise in sodium concentration per impulse and the equation

$$V = 58 \log \frac{[\mathrm{Na}]_o + b[\mathrm{K}]_o}{[\mathrm{Na}]_i + b[\mathrm{K}]_i},$$

where V is the overshoot in mV and b is the permeability ratio (P_{Na}/P_K) at the crest of the

spike (see Baker, Hodgkin & Shaw, 1962). Since $b \ll 1$ an approximate equation for the change in overshoot is

$$V'' - V' = 58 \log \frac{Y}{Y + \Delta[\mathrm{Na}]_i},$$

where V' is the initial and V'' the final overshoot; $\Delta[\mathrm{Na}]_i$ is the increase in internal sodium concentration which can be obtained from the number of impulses and the rise in sodium concentration per impulse (0·0077 mM, Hodgkin & Horowicz, 1959), and

$$Y = [\mathrm{Na}]_i' + b[\mathrm{K}]_i' = [\mathrm{Na}]_o \, 10^{-V'/58}.$$

After 1200 action potentials in fibre 67 a decrease in overshoot of 16 mV was measured against the calculation of 7 mV. In the case of fibre 73 (2590 spikes; p. 55) the calculated decrease was 11 mV which corresponds with the observed 12 mV difference in overshoot between the first spike and a spike after 5 sec of recovery following the train.

NaCN. The concentration of this poison, used to block oxidative metabolism, ranged from 0·5 to 4 mM. Twelve experiments were performed, three with micro-electrodes. The contractility of the poisoned fibre was exhausted either by continuous stimulation at 1/sec or by successive tetani of short duration. When stimulated at 1/sec the height of the twitch increased after about 300 stimuli to roughly twice its original value. This supernormal phase lasted for several hundred stimuli. Finally, after about 2000 shocks the twitch height returned to its original size and dropped to zero during a further 500–1500 contractions. After the complete failure of twitches the resting potential—measured in two fibres with micro-electrodes—was -70 and -79 mV.

The fatigue of action potentials in poisoned fibres is shown in Fig. 9 (external electrodes) and Fig. 10 (internal electrodes). The first train in Fig. 9 shows the normal situation with the unpoisoned fibre. After this tetanus the fibre was poisoned and stimulated to complete exhaustion. Subsequently the poison was removed and the recovery of contractility tested after 10 min (Fig. 9*B*). On replacement of the poison 7–9 trains were necessary before the fibre was again fully exhausted (Fig. 9*C*). One can assume that the early after-potential had disappeared because there was no drop in height between the first and the second action potential of the train. The last tetanus in Fig. 9 demonstrates the recovery of contractility and the reappearance of action-potential fatigue.

The main result from this experiment is that a poisoned fibre which has exhausted its supply of readily available energy shows only little sign of action-potential fatigue. The fatigue reappears together with contractility and the after-potential after removal of the poison.

The first record in the experiment with internal electrodes (Fig. 10) shows a train of action potentials after complete exhaustion of contractility with only little fatigue of the action potential during 108 shocks. In the second record, taken 15 min after removal of the poison, tension reached about 1 kg/cm^2. The height of the spikes fell rapidly, their falling

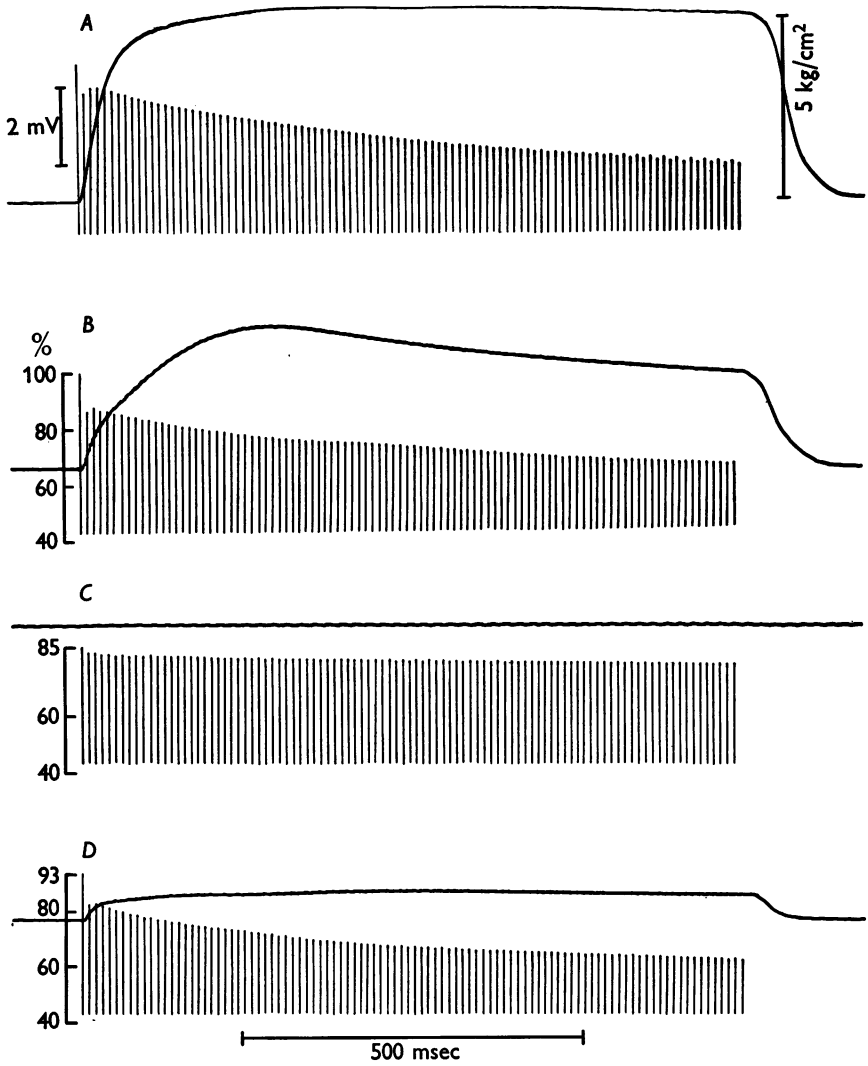

Fig. 9. Effect of 1 mM cyanide on action-potential fatigue and tension. *A*, Before poisoning. After this tetanus cyanide was added and the contractile mechanism exhausted by thirty-four tetani of identical duration and frequency within 30 min; *B*, after 10 min in Ringer's solution without poison; *C*, the 9th tetanus after the fibre had been in the poison again for 7 min; *D*, the first tetanus after recovery for 7 min in Ringer's solution. The relative position of the tension and potential traces in the different records is of no significance. The fibre was never in contracture. External electrodes, fibre 43, diameter 93 μ. The fibre was stretched to 1·4 times its slack length. Stimulation frequency 100/sec; temp. 20° C.

phase lengthened, and after about 50 potentials the fibre no longer responded to every stimulus. On replacement of the poison about 200 single shocks at 1/sec were needed to abolish contraction for the second time, and the third record shows again the absence of action-potential fatigue after abolition of contractility.

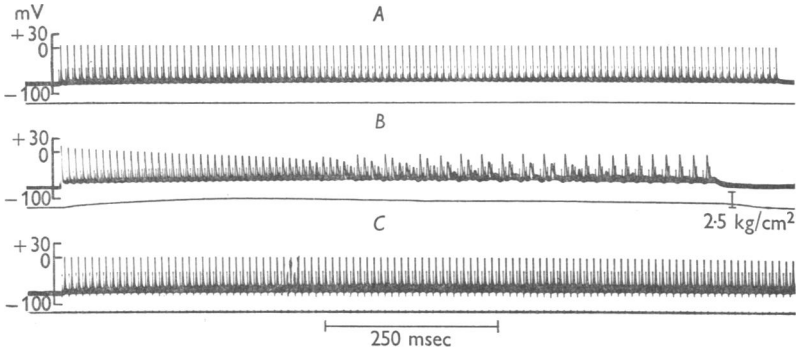

Fig. 10. Effect of NaCN on action-potential fatigue and isometric tension. Contractility had been exhausted in 4 mM cyanide with about 2400 shocks at a frequency of 1/sec before the experiment started. Tetani at 100/sec: *A*, in the exhausted state; *B*, after recovery for 15 min in Ringer's solution without poison; *C*, after replacement of the poison and 200 twitches at 1/sec to exhaust contractility. Internal electrodes. Fibre M 7. The fibre had a diameter of 75 μ and was stretched to 1·3 times its slack length; temp. 19° C.

Figure 11 shows results from the same fibre. The crest of the action potential and the membrane potential from which spikes started is given as a function of time after the start of a tetanus in the exhausted and in the contracting fibre. In the exhausted fibre (full circles) the crest of the action potential declined by only a few mV. The potential from which spikes started was about −73 mV. During the first 50 msec which followed the tetanus the membrane repolarized to −76 mV and after 4 min the potential was −78 mV as compared with −79 mV at the start. Thus, the early and the late after-potentials were much smaller than in a normal fibre. However, the magnitude of both after-potentials increased immediately after removal of the poison even though contractility was still low. The next tetanus (open circles) was elicited after the fibre had recovered for 10 min in Ringer's solution without poison. The resting potential was −83 mV before the train started. Immediately after the tetanus, which showed the usual rapid decline of the action potential, the membrane repolarized from −65 to −72 mV (early after-potential). After 4 min the value was −82 mV, i.e. the late after-potential amounted to 10–12 mV.

Two other experiments with micro-electrodes gave similar results. In one of these experiments 352 action potentials at 100/sec were produced,

during which the peak of the potentials fell by 12 mV and the resting potential shifted from −68 to −66 mV. The rate of rise of the action potential measured in poisoned fibres with resting potentials of −70 to −80 mV was similar to that measured in unpoisoned fibres with corresponding resting potentials.

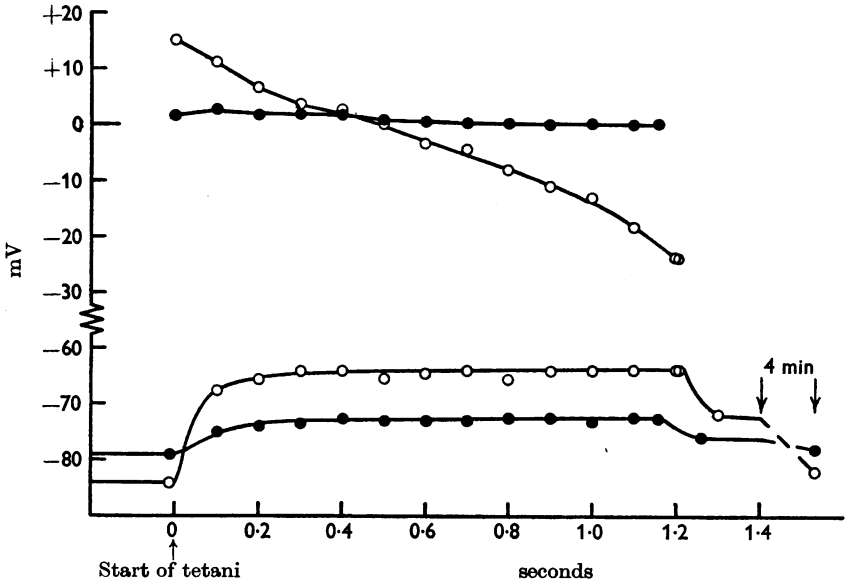

Fig. 11. Effect of NaCN on action-potential fatigue. Same fibre as in Fig. 10, but different tetani. Stimulation frequency 100/sec. Filled circles: after complete exhaustion of contractility in 4 mM cyanide. Open circles: after 10 min of recovery in Ringer's solution without poison. The tetani begin at zero time and last for 1160 and 1210 msec, respectively. The potential from which the action potentials started and the crest of every 10th and the first and the last action potential of each train have been traced, together with the membrane potential before and after the tetani.

Fibres in cyanide survived much better than in iodoacetate plus cyanide. One mechanically completely exhausted fibre fired 3505 propagated action potentials at a frequency of 25/sec without failing (2 mM cyanide, diameter 130μ, temp. 20° C).

The cyanide experiments so far described were carried out on fibres which were not in contracture even though the ability to contract had been completely exhausted. In two further experiments the tension–length curves were measured in isolated fibres before and after poisoning. In these experiments the fatigue of the action potential disappeared before there were measurable changes in the tension–length relation. However, when the fibres were left in the poison for longer periods of time after

exhaustion of contractility, they developed more tension than before poisoning when they were stretched and finally went into contracture.

Frog skeletal muscles contain, per gram, about 100 μmoles glucose as glycogen, 20–30 μmoles creatine phosphate (CP) and 2·5–5 μmoles ATP. Assuming that 3 mcal/g are necessary for a normal twitch then the store of ATP and CP allows about 100 twitches, and glycolysis about 2000 twitches if lactate is allowed to diffuse away. The number of twitches given by iodoacetate plus cyanide-poisoned fibres agrees reasonably with this calculation and so does the number after cyanide poisoning. Full oxidation of glucose would provide enough energy for 23000 twitches; this corresponds to 1 twitch/sec for more than 6 hr and in practice a single fibre normally becomes inexcitable for some other reason before this limit can be approached. (The data mentioned in this section were kindly given to me by Dr D. R. Wilkie of the Department of Physiology, University College London.)

The effect of hypertonic solutions on action-potential fatigue

When the external sodium chloride concentration is increased from 115 to 270 mM single twitches are completely suppressed while propagated action potentials still occur. During a tetanus the tension rises slowly after some delay and does not reach its maximum (Hodgkin & Horowicz, 1957). Since it is suggested in the discussion that contraction processes are involved in action-potential fatigue some experiments with hypertonic

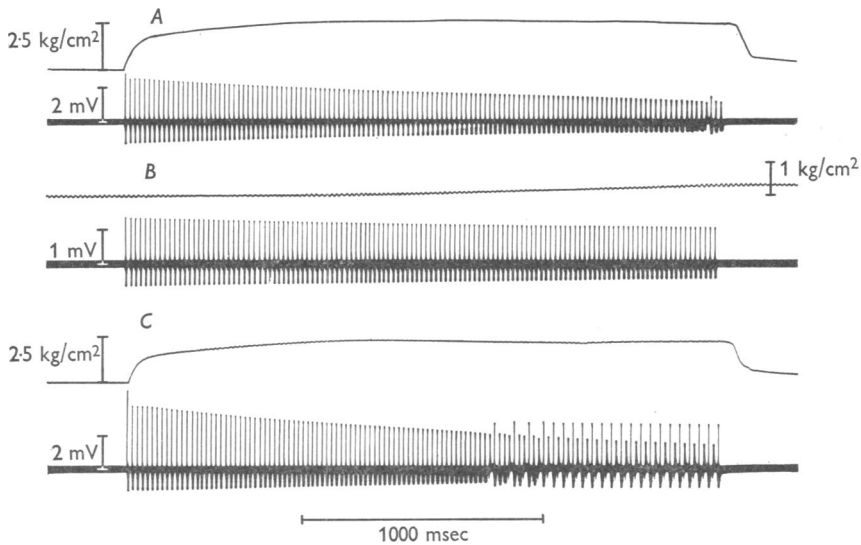

Fig. 12. The effect of hypertonic solutions on action-potential fatigue and tension. *A*, In Ringer's solution; *B*, after 15 min in Ringer's solution with 270 mM-NaCl instead of 115 mM; *C*, after recovery for 15 min in normal Ringer's solution. Stimulation frequency 50/sec. After the first record the external resistance was increased by putting a thicker sheet of petroleum jelly on the edge of the glass slide. External electrodes. Fibre 31. The fibre had a diameter of 105 μ and was stretched to 1·3 times its slack length; temp. 15° C.

solutions were performed to find out whether there is a difference in fatigue pattern.

Figure 12 shows one experiment out of three. Records *A* and *C* were control tetani before and after the fibre was immersed in Ringer's solution with 270 mM-NaCl instead of 115 mM. After 15 min in the hypertonic solution (record *B*) tension rose slowly after a delay of about 1000 msec. The height of the action potential remained nearly constant during several hundred msec and the subsequent rate of fall was much less than in the control records.

In another experiment 285 mM sucrose was added to Ringer's solution. This solution, too, caused a delay in rise of tension and less fatigue of the action potential compared with control tetani in Ringer's solution.

The effect of $[Ca]_o$

Replacement of external Ca by Mg for 110 min had no obvious effect on the fall of the action potential during a tetanus and on maximal tetanus tension. Similar results were obtained when Ca was increased for 10–15 min from 1 to 8 or 15 mM.

When caffeine in concentrations up to 0·75 mM was added to Ringer's solution an increase in twitch tension occurred. The rate of rise of tetanus tension increased and the time between the last action potential of a train and the sudden drop in tension became longer. Action-potential fatigue and maximal tetanus tension were not affected.

DISCUSSION

The present results agree with those of Ramsey & Street (1942) in showing that at high frequencies of stimulation the action potential of a single-muscle fibre fails before the mechanical response. At 20° C propagated action potentials at a frequency of 40/sec drop out after 2–3 sec, whereas maximal tension can be maintained for at least 5 sec. Whole muscles behave in a similar manner (see Wilska, 1939; Bergmans & Maréchal, 1960; Maréchal, 1960), so the results are not due to alterations in fibre characteristics during dissection.

The situation is rather different when frequencies below 10/sec are employed. Thus, Sandow & Eberstein (1963) stimulated a fibre at 1 shock/sec, and found that after 3000 shocks twitch tension had declined to 7 %, whereas the height of the action potential was still 75 % of the initial value. Similar results were obtained in the present study and may be attributed to some changes in the coupling process which has little effect on the spike.

The rapid decline of the action potential during a tetanus is not easy to understand. It cannot be due to accumulation of sodium or to loss of potassium, since the changes in concentration resulting from the ionic

movements during activity would only begin to have a perceptible effect after hundreds of impulses (Hodgkin & Horowicz, 1959, Table 8). The experiments in which fibres were subjected to continuous stimulation, to metabolic inhibitors, or hypertonic solutions provide an interesting clue. These results show that a fibre which no longer develops tension can carry up to several thousand impulses at a frequency of 100/sec without failing as against the 50–200 obtained when the muscle is generating tension. This suggests that muscular contraction liberates some substance or has some effect which reduces the action potential. When contractility is abolished the muscle fibre becomes more like a nerve and can carry long trains of impulses. The fatigue phenomenon is not completely absent in non-contracting fibres. The calculations on p. 57 show, however, that the slow decrease in overshoot during long trains at high frequencies can be explained at least partly by a reduction in the sodium equilibrium potential. It declines faster in muscle than, for example, in squid axons, because of the larger sodium influx per cm^2 and the smaller diameter of muscle fibres.

Lack of energy alone is known to influence membrane characteristics. Persson (1963) found a decrease in time constant of the membrane after poisoning with DNP. Preliminary experiments showed that this is also the case in iodoacetate-poisoned fibres. The absence of the early and late after-potential, which is characteristic for fibres in the exhausted state, might be partly due to a shunting effect by a high parallel membrane conductance. Consequently, an attractive possibility is to suppose that the decline of the action potential during a tetanus may be connected with the late after-potential. The two processes do not seem to have exactly the same time scale: Freygang *et al.* (1964) found 0·3 sec for the half-time of the late after-potential whereas the amplitude of the spike recovered with a half-time of about one second (Fig. 5). However, since Freygang *et al.* used the sartorius, whereas the present results were obtained on fibres from the semitendinosus and iliofibularis the apparent discrepancy may not be significant. On the other hand, several observations strongly suggest that the depolarization caused by the after-potential does not provide the whole explanation for action-potential fatigue:

(1) The decline of the action potential was accompanied by a marked prolongation of its falling phase. This effect was still present when the membrane was hyperpolarized by 15 mV.

(2) The fatigue effect was absent in non-contracting fibres even when the resting potential became as low as in the final phase of fatigue in contracting fibres.

(3) The 'build-up' of the late after-potential was largely complete before the development of the final phase of action-potential fatigue with the marked prolongation of the spike (Figs. 10 and 11).

(4) The experiments in which fibres were hyperpolarized during a tetanus can be explained by assuming that repetitive stimulation causes a shift in the S-shaped relation between the rate of rise and the 'resting' potential towards hyperpolarization. Thus, in addition to the depolarizing effect of the early and late after-potential, some changes in membrane characteristics appear to participate in action-potential fatigue.

Repetitive stimulation has two effects on twitch tension. Short full tetani are followed by larger twitches (post-tetanic enhancement), whereas a continuous stimulation at low frequencies causes a reduction in twitch height. Sandow & Eberstein (1964) found that in the latter case the contracture induced by potassium is of normal size and concluded that the decrease in twitch height is due to a failure in excitation–contraction coupling. The cause of post-tetanic enhancement is not yet fully understood (see Ramsey & Street, 1941, for references). It passes away exponentially with the same time constant as that with which the height of the action potential recovers. More direct experimental tests are needed to find out whether both phenomena originate from the same reaction.

It is interesting that a muscle fibre which has gone into contracture after poisoning with iodoacetate plus cyanide and stimulation with 130 shocks at 1/sec can still give long trains of action potentials. Since this procedure has been shown to break down all the creatine phosphate and ATP (Lundsgaard, 1930; Lohmann, 1934) it seems likely that in both muscle and nerve (Caldwell, 1960) energy-rich phosphate is not essential for the conduction of impulses and that ionic concentration gradients provide the immediate source of energy for the impulse.

SUMMARY

1. Previous work has shown that the height of the action potential in muscle fibres rapidly declines at stimulation frequencies between 50 and 125/sec. This effect, called the fatigue phenomenon, was investigated in more detail in isolated twitch muscle fibres of the frog. External electrodes as well as intracellular micro-electrodes were used.

2. When fibres were stimulated at frequencies above 40 shocks/sec action potentials dropped out without effect on the mechanical response. At 100/sec action potentials started to fail after 0·5–2 sec.

3. After a tetanus the recovery of the action potential showed a quick phase which was followed by a slow exponential component with a half-time of about 1 sec. Post-tetanic enhancement of twitches diminished at the same rate.

4. Fibres which had been stimulated at 2–4 shocks/sec until they responded to stimulation with small twitches showed little action-potential

fatigue when subjected to tetanic stimulation at 100 or 50 shocks/sec for up to 26 sec. During the tetanus tension decreased steadily.

5. Fibres in Ringer's solution containing NaCN and iodoacetate, or NaCN alone, whose capacity to contract had been exhausted showed also only little action-potential fatigue. The period before action potentials dropped out when fibres were stimulated at 100/sec became longer by up to 16 times when contractility had disappeared. The effect on contractility and action-potential fatigue was normally reversible with NaCN but not with iodoacetate.

6. Fibres which had gone into contracture after poisoning with iodoacetate plus cyanide were still able to carry up to 2000 action potentials.

7. Action-potential fatigue was less when contractility had been inhibited by hypertonic solutions.

8. Replacement of external calcium by magnesium or an increase in external calcium from 1 to 8 or 15 mM had no obvious effect on action-potential fatigue or tension during a tetanus.

9. It is suggested that the fatigue of the action potential is caused by reactions connected with contraction and its activation.

I wish to express my indebtedness to Professor A. L. Hodgkin for suggesting an investigation of the fatigue phenomenon and to him, Mr P. F. Baker and Dr W. K. Chandler for much helpful discussion. The equipment used in these investigations were designed and built by Mr R. H. Cook. My thanks are also due to the Deutsche Forschungsgemeinschaft and the Medical Research Council for financial support.

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