Metabolic Correlates of Fatigue and of Recovery from Fatigue in Single Frog Muscle Fibers

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ABSTRACT Fatigue and recovery from fatigue were related to metabolism in single fibers of the frog semitendinosus muscle. The fibers were held at a sarcomere length of 2.3 μm in oxygenated Ringer solution at 15°C and were stimulated for up to 150 s by a schedule of 10-s, 20-Hz tetanic trains that were interrupted by 1-s rest periods, after which they were rapidly frozen for biochemical analysis. Two kinds of fatigue were produced in relation to stimulus duration. A rapidly reversed fatigue occurred with stimulation for under 40 s and was evidenced by a decline in tetanic tension that could be overcome by 1 s of rest. A prolonged fatigue was caused by stimulation for 100–150 s. It was evidenced during stimulation by a fall in tetanic tension that could not be overcome by 1 s of rest, and after stimulation by a reduction, lasting for up to 82 min, in the peak tension of a 200-ms test tetanus. Fiber phosphocreatine (PCr) fell logarithmically in relation to stimulus duration, from a mean of 121 ± 8 nmol/mg protein (SEM, n = 12) to 10% of this value after 150 s of stimulation. PCr returned to normal levels after 90–120 min of rest. Stimulation for 150 s did not significantly affect fiber glycogen and reduced fiber ATP by at most 15%. It is suggested that the prolonged fatigue caused by 100–150 s of tetanic stimulation was caused by long-lasting failure of excitation-contraction coupling, as it was not accompanied by depletion of energy stores in the form of ATP. One possibility is that H^+ accumulated in fatigued fibers so as to interfere with the action of Ca^{2+} in the coupling process.

INTRODUCTION

The mechanical output of frog whole muscles and of single fibers falls because of fatigue when they are stimulated directly at low frequencies to produce twitches or at high frequencies to produce a tetanus (Asmussen, 1934; Ramsey, 1953; Maréchal and Mommaerts, 1963; Eberstein and Sandow, 1963; Vergara and Rapoport, 1974). Prolonged stimulation can markedly reduce muscle glycogen (Kugelberg and Edström, 1968; Bergström et al., 1971) and phospho-

creatine (PCr) (Carlson et al., 1967; Spande and Schottelius, 1970; Canfield et al., 1973) and, if muscles are poisoned, can reduce ATP as well (Cain and Davies, 1962; Dydyńska and Wilkie, 1966; Murphy, 1966). ATP is the immediate energy source for contraction (Cain and Davies, 1962; Portzehl et al., 1969; Weber and Murray, 1973), Ca^{2+} uptake by the sarcoplasmic reticulum (Ebashi and Endo, 1968) and for ion transport by the fiber membrane and for calcium transport at mitochondria (Cross et al., 1965; Lehninger, 1970).

During stimulation in vitro of the frog sartorius or mouse soleus muscle, tension falls in proportion to the decline of muscle PCr (Carlson et al., 1967; Spande and Schottelius, 1970). Insofar as PCr is in rapid chemical equilibrium with ATP via the creatine kinase reaction (Lohmann, 1934; Canfield and Maréchal, 1973; Canfield et al., 1973), this finding has suggested that fatigue is due to depletion of available energy resources. The conclusion appears further supported by a direct relation between muscle twitch tension and ATP content in muscles that are stimulated after creatine kinase has been inhibited (Cain and Davies, 1962; Dydyńska and Wilkie, 1966; Murphy, 1966).

On the other hand, exhaustive exercise in man produces fatigue despite high residual levels of glycogen and ATP, suggesting in this case that fatigue is due to intracellular sequestration of ATP and its unavailability to support contraction (Bergström et al., 1971). However, sequestration could not account for fatigue caused by repetitive stimulation in vitro of single frog muscle fibers, as these fibers when fatigued produce reversible contractures in response to caffeine or KCl (Eberstein and Sandow, 1963); ATP is required for their relaxation (Weber and Murray, 1973). The single fiber data suggest therefore that fatigue can be due to failure of excitation-contraction coupling, in relation to an insufficient release of Ca^{2+} from available intracellular stores or because of Ca^{2+} sequestration by mitrochondria (Eberstein and Sandow, 1963; Gonzales-Serratos et al., 1973).

It was thought useful to explore further whether ATP exhaustion or failure of excitation-contraction coupling accounts for fatigue in single fibers, by relating fatigue and recovery from fatigue to intracellular concentrations of glycogen, PCr, and ATP. Single fiber studies have the advantage over whole muscle studies because in whole muscle it is not known how tension parameters change with stimulation, as some fibers may fatigue faster than others (Länner-gren and Smith, 1966). Average measurements of metabolites are made on a heterogeneous fiber population in whole muscles, and intracellular fiber kinetics are masked by rate-limiting intercellular diffusion of O_2, ions and substrates for metabolism (Hill, 1965; Kushmerick and Paul, 1976 *a*). Fatigue of single fibers due to prolonged stimulation has been described in part (Ramsey, 1953; Eberstein and Sandow, 1963; Vergara and Rapoport, 1974; Vergara et al., 1977), and the relation of fatigue to fiber metabolism has been presented in abstract form (Nassar-Gentina et al., 1976).

METHODS

Single fibers were dissected at room temperature from the dorsal head of the semitendinosus muscle of female *R. pipiens* and were mounted in a Plexiglas chamber containing flowing, oxygenated Ringer solution at 15°C (Vergara and Rapoport, 1974; Vergara et

al., 1977). The Ringer solution (pH 7.0 to 7.2) contained the following millimolar concentrations of solutes: NaCl, 115; KCl, 2.5; $CaCl_2$, 1.8; Na_2HPO_4, 2.15; NaH_2PO_4, 0.85; tubocurarine hydrochloride, 0.01. Solution osmolality was 0.23 as determined by freezing point depression.

The large pale fibers that were chosen for this study had a mean cross sectional area of 5,266 ± 331 μm^2 (SEM, $n = 43$). Such fibers from the toad semitendinosus muscle contain higher concentrations of glycolytic than of oxidative enzymes, and lose tension rapidly when stimulated at a frequency of 35 Hz (Lännergren and Smith, 1966). Pale fibers from the frog semitendinosus rapidly fail as well when stimulated at this frequency.[1]

As previously described (Vergara et al., 1977), one tendon of the fiber was tied to the lever arm of a RCA 5734 tension transducer (RCA Solid States, Sommerville, N.J.), and the tendon at the other end was held by a small forceps. The output of the transducer was recorded on a paper chart and displayed on an oscilloscope face. The fiber was fixed at a sarcomere length of 2.3 μm, as measured with a laser beam, and was stimulated transversely with 0.3-ms differentiated square pulses through extracellular Pt/Pt-black electrodes. The stimulus schedule consisted of 10-s tetanic trains at 20 Hz, interrupted by 1-s rest periods. Total stimulus time was 30–150 s. Fiber tension was measured before and after this schedule by periodically delivering a single stimulus, followed after 1 s by a 200-ms test tetanic stimulus at 20 Hz (four pulses in 200 ms).

Biochemical Analysis

Whole semitendinosus muscles were isolated by dissection and plunged into liquid N_2 with rapid stirring. Portions of frozen tissue (35–100 mg) were removed and weighed at −30°C in acryostat, and then were homogenized with 10 vol of 0.03 N HCl at 0°C. Protein was precipitated with 0.1 vol of 3 N perchloric acid, the extract was centrifuged and the supernatant fluid was removed and neutralized with 3 M $KHCO_3$, which precipitated as $KClO_4$. (This extraction procedure does not affect the chemical stability of PCr (Lowry et al., 1964)). The neutral extract was removed from the $KClO_4$ and stored at −70°C until analyzed. The pellet was dissolved in 1 ml of 1 N NaOH for protein analysis.

When physiological studies with a single fiber were completed, the fiber was immersed in liquid N_2 within 6 s, cut from its mounting and transferred to a glass test tube (4.6 × 30 mm) in liquid N_2. The tube was stored at −70°C for up to 1 mo. In one set of experiments (Table I C, below), fibers were frozen within 1 s rather than within 6 s.

The tube containing the frozen fiber was transferred to a plastic holder in a dry ice-ethanol bath at −35°C, and subsequent manipulations were performed under a low-power dissecting microscope (×3–5). The fiber was extracted by adding 250 μl of ice-cold 0.3 N perchloric acid containing 1 mM EGTA (ethyleneglycol-bis-[β-amino-ethyl ether] *N,N'*-tetraacetic acid). After the solution froze, the tube was transferred to an ice bath at 0°C, the contents were allowed to thaw over a 15-min period and then were mixed by shaking the tube. This procedure provided equilibration of the perchloric acid with tissue water at the low temperatures, prevented metabolism in the tissue, and insured extraction of soluble tissue components (Lowry et al., 1964). The fiber settled to the bottom of the tube, and a 100-μl sample was removed and neutralized with 30 μl of 2 M $KHCO_3$. The neutral extract was used for the determination of PCr and ATP.

200 μl of 1 N NaOH were added to the remaining solution plus fiber in the test tube for determining glycogen and protein. The tube was heated for 10 min at 70°C to solubilize the protein, which was determined by a modification of the method of Lowry et al. (1951) on 40 μl of the sample in a total volume of 400 μl. Samples were placed in microcuvettes and read at a wave length of 750 nm in a spectrophotometer (model 240, Gilford Instrument Laboratories, Inc., Oberlin, Ohio).

All of the metabolites were measured by adaptations of enzymatic analytic techniques

which measured the fluorescence of NADH or NADPH. Glycogen was assayed with amylo-α1, 4-α-1, 6-glucosidase by the method of Passonneau and Lauderdale (1974). ATP, PCr, and lactate were measured by methods of Lowry and Passonneau (1972), noting special precautions necessary to measure 0.1 nmol. Lactate could be measured in whole muscle but not in single fibers, which were contaminated during the dissection procedure.

RESULTS

Metabolic Profile in Single Fiber and Whole Muscle

Table I lists mean concentrations of ATP, PCr, and glycogen in unstimulated, whole semitendinosus muscles (A, control), and in unstimulated, single fibers (B, control) that were obtained from the same batch of frogs. Respective mean

TABLE I

METABOLITE CONCENTRATIONS IN VARIOUS MUSCLE PREPARATIONS

Group	Tetanus duration (20 Hz)	Time after tetanus	No. of experiments	ATP	PCr	Glycogen (as glucosyl units)
	s				*nmol/mg protein*	
Whole muscles						
A	0 (Control)		10	28.1±0.9*	119.1±3.9	259±33
Single fibers						
B	0 (Control)	–	12	25.4±1.1	120.6±7.5	328±37
	150	6 s	7	21.9±0.7†	13.1±2.1†	304±39
	100	90 min	3	20.9±1.9	121.1±15.2	355±45
	150	120 min	6	23.6±1.2	124.5±7.9	409±58
C	0 (Control)	–	6	16.1±1.3	105.9±5.7	323±43
	150	1 s	9	13.8±1.0	11.2±4.1†	341±43

(A) Unstimulated, control semitendinosus whole muscle. (B) Control and stimulated single fibers, with 6-s freezing time, from same batch of frogs as (A). (C) Control and stimulated single fibers, from another batch of frogs, with 1-s freezing time.

* Mean ± SEM.

† Differs from control, $P < 0.05$.

metabolite concentrations did not differ significantly ($P > 0.05$), which demonstrates that the microanalytical procedures as applied to single fibers gave results that were comparable to the analytical methods that were employed with the larger tissue samples. Lactate could not be determined in single fibers (see Methods); in eight whole muscles, however, mean lactate concentration was 7.9 ± 1.5 nmol/mg protein SEM.

Mean protein content of whole semitendinosus muscle was found to equal 17.8% of tissue wet weight, as compared to a net dry weight equal to 18.4–21.1% of wet weight (Moran, 1929). On the basis of the protein measurement, metabolite concentrations in Table I can be converted to units of micromoles per gram wet weight by dividing by 5.6. Calculated concentrations for unstimulated whole semitendinosus muscle (Table I A, control) did not differ from

published values for frog skeletal muscle, which are, per gram wet weight, ~100 μmol of glycogen (as glucosyl units), 20–30 μmol PCR, and 2.5–5 μmol of ATP (Wilkie, in Lüttgau, 1965; Carlson et al., 1967; Canfield and Maréchal, 1973; Mainwood et al., 1972; Mommaerts, in Dydyńska and Wilkie, 1966; Dydyńska and Wilkie, 1966). Although control ATP concentrations in single fibers differed by a factor of 1.5 between batches (Table I B, control and I C, control), possibly because of seasonal or nutritional effects (cf. Conway, 1946), both means were within the normal range for whole frog sartorius. PCr/ATP ratios were 4.7 and 6.6 for the two control batches of fibers (Table I B and C) and 4.2 for the whole semitendinosus muscle (Table I A). In the frog sartorius, the ratio has been reported at 7.74 (Canfield et al., 1973), 7.2 (Dydyńska and Wilkie, 1966), and 5.8 (Mainwood et al., 1972).

Fatigue and Recovery

Fig. 1 illustrates mechanical responses of a single fiber to a schedule of 15 10-s periods of stimulation at 20 Hz that were interrupted by 1-s rest periods. Total stimulation time equaled 150 s. Twitches (*a*) and 200-ms test tetani (four stimuli in 200 ms) (*b*) were elicited every 10 s both before and after the prolonged stimulus schedule.

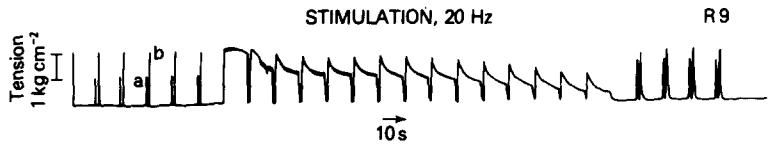

FIGURE 1. Effect of prolonged tetanic stimulation on fiber tension. The fiber was stimulated at 20 Hz for 10-s periods which were separated by 1-s interruptions. Total stimulus time was 150 s. (*a*) Twitches and (*b*) 200-ms tetani (20 Hz) were evoked before and after stimulation.

Fiber tension remained constant in the first 9 s of tetanic stimulation and then declined (Fig. 1). In a total of 19 fibers that were stimulated tetanically, tetanic tension remained constant for a mean duration of 22 ± 1.7 s SEM before declining. Even when tension declined after 22 s of stimulation in these fibers, 1-s rests allowed complete recovery to the initial tetanic tension for up to 41.8 ± 5.4 s SEM of tetanic stimulation. After nine 10-s stimulus periods (total stimulation time = 90 s), 1-s rest periods allowed recovery of tension to 90 ± 1.5% SEM of the initial value.

Fig. 2 *a* illustrates the time-courses of twitch tension and of the maximum tension of a 200-ms tetanus, after stimulation of a single fiber for a total of 150 s at 20 Hz according to the schedule of Fig. 1. Twitch tension remained elevated for 25 min because of posttetanic potentiation before declining below the prestimulation base line due to the influence of fatigue. Twitch duration (not illustrated in Fig. 2, but see Fig. 1) remained prolonged for 45 min.

Maximum tension of the 200-ms test tetanus, which was less influenced than was twitch tension by the time-course of the active state (Ritchie and Wilkie,

1955; Close and Hoh, 1968; Connolly et al., 1971), was used as a measure of posttetanic fatigue. It did not decline after 30 s of tetanic stimulation (cf. Vergara et al., 1977), but did fall below prestimulation control after stimulation for 100–150 s, as illustrated in Fig. 2 *a*. Furthermore, the peak tension of the 200-ms test tetanus did not reach a minimum immediately after stimulation ceased, but only after 15 min of recovery, and returned to a stable control base line after 80 min of recovery.

Fig. 2 *b* summarizes mean minimal and maximal tensions of twitches and 200-ms test tetani after a schedule of 100 or 150 s of tetanic stimulation. This schedule produced a proportionately greater decline in the maximum tension

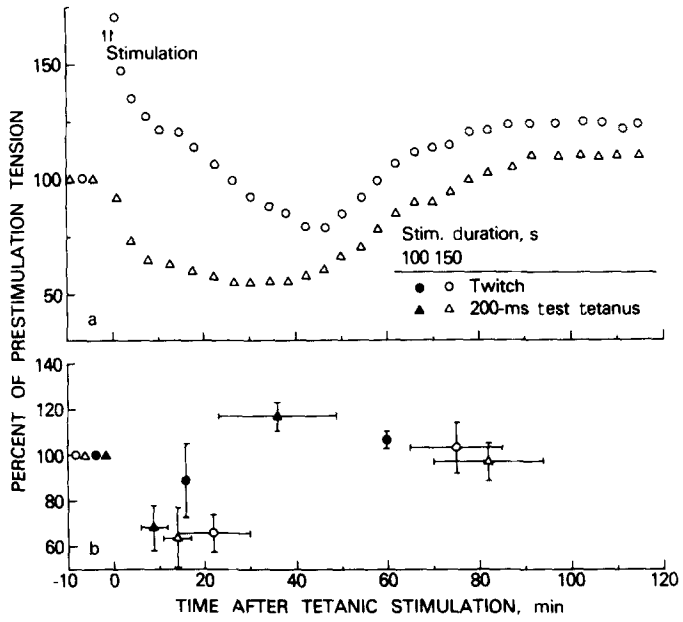

FIGURE 2. (*a*) Twitch tension (○) and maximal tension of 200-ms test tetanus (△) after stimulation of a fiber for 150 s at 20 Hz. Prestimulation twitch tension was 1.22 kg·cm^{-2}, and prestimulation 200-ms test tetanus tension was 2.2 kg·cm^{-2}. (*b*) Mean minimal and maximal tensions of twitch and 200-ms test tetanus after 100 s (●, ▲; 3 fibers) or 150 s (○, △; 6 fibers) of prolonged stimulation at 20 Hz. SEM's are given.

of the 200-ms test tetanus than in twitch tension, when tensions were compared in individual fibers ($P < 0.05$). The lesser decline of twitch tension probably was due to a prolonged active state caused by tetanic stimulation (Ritchie and Wilkie, 1955; Close and Hoh, 1968; Connolly et al., 1971). Posttetanic fatigue, as measured by the decline in peak tension of the 200-ms test tetanus below the peak tension before prolonged stimulation, lasted 36 ± 13 min SEM after 100 s of stimulation and 82 ± 12 min SEM after 150 s of stimulation.

Metabolic Profile in Stimulated Fibers

A fiber that was used as a control for metabolic studies (Table I B and C) first was stimulated 5–10 times over a 20-min period to establish that the twitch was stable and that the fiber was not damaged. Damaged fibers were discarded. Ten twitches would reduce control PCr concentration by at most 0.6 nmol/mg protein even in the absence of metabolic recovery (Carlson et al., 1967). A fiber that was analyzed after tetanic stimulation first was stimulated 5–10 times over 20 min with twitches followed by 200-ms test tetani, to determine its viability.

Tetanic stimulation for up to 150 s (not counting up to 2 s of stimulation by test 200-ms tetani) did not significantly influence fiber glycogen, but produced a logarithmic decline in fiber PCr in proportion to duration of stimulus train, with a halftime of 49.5 s (Fig. 3, Table I B). The logarithmic relation agrees with findings for whole sartorius at 0°C (Kushmerick and Paul, 1976 *b*), and reflects a proportional relation between PCr split and the tension-time integral of single fibers during a tetanus.[1]

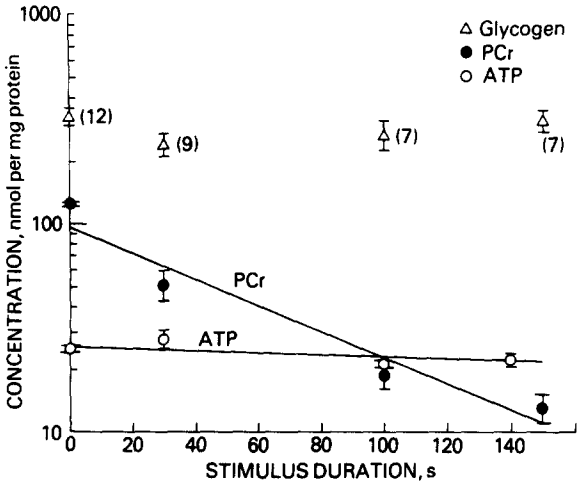

FIGURE 3. Fiber concentrations of ATP, PCr, and glycogen as a function of duration of tetanic stimulation. Points are means ± SEM. Glycogen did not change with stimulus time t (s) ($P > 0.05$), whereas PCr and ATP concentrations fell significantly ($P < 0.05$) as follows: ln PCr = 4.57 − 0.14t and ln ATP = 3.26 − 0.014t.

Final tetanic tension after 30, 100, or 150 s of stimulation could be related to fiber PCr at the end of a tetanus. As shown by Fig. 4, tetanic tension remained at its initial height so long as PCr remained at 50% or more of control concentration, fell below this height when PCr fell below 50% of control and virtually disappeared when PCr was about 10% of control. PCr returned to a control concentration in fibers that were allowed to recover for 90–120 min after

[1] Nassar-Gentina, V., J. V. Passonneau, and S. I. Rapoport. Unpublished data.

100–150 s of a tetanic stimulus schedule (Table I B). We did not, however, determine the relation between rate of recovery of the 200-ms test tetanus and rate of PCr resynthesis.

Despite the marked decline in fiber PCr caused by stimulation for up to 150 s, ATP concentration did not fall by more than 15% (Table I B and C). It remained possible that ATP was resynthesized in the 6 s taken to freeze the fibers (Table I B), so we examined fibers that were frozen within 1 s by pouring liquid N_2 on them while they were removed from the bath at the end of a 150-s stimulus train. This procedure should have reduced freezing time of a fiber, with a diameter of 100 μm, to < 0.25 s after exposure to liquid N_2 (Ferrendelli et al., 1972).

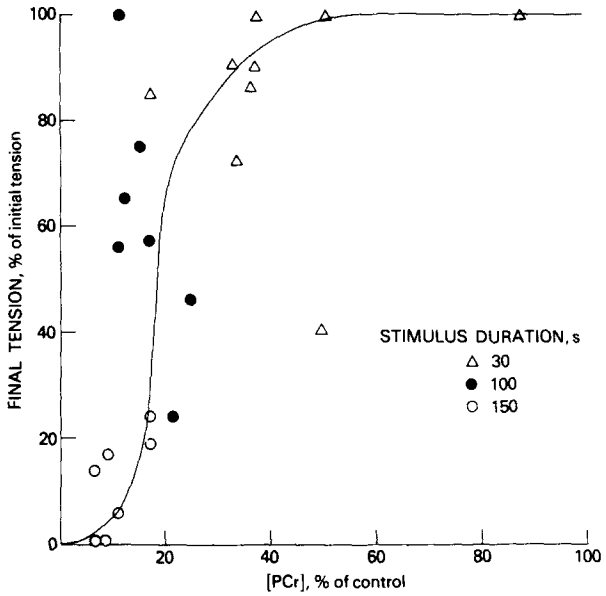

FIGURE 4. Relation of tetanic tension to fiber PCr at the end of 30–150 s of stimulation at 20 Hz. The curve was drawn by eye.

As illustrated by Table I C, fibers that were stimulated for 150 s and then frozen within 1 s had overall metabolite concentrations that were about the same as in fibers frozen within 6 s. Glycogen and ATP concentrations did not differ significantly from control concentrations ($P > 0.05$), and PCr was reduced by 89%.

DISCUSSION

This study presents for the first time quantitative measurements of metabolites in unstimulated muscle fibers, in fibers that are stimulated tetanically for up to 150 s and in fibers that are allowed to recover from such stimulation. Unstimulated single fibers have the same glycogen, PCr, and ATP concentrations as do

whole semitendinosus muscles, which indicates that the dissection procedure does not alter the metabolic profile of these fibers.

Tetanization for up to 150 s does not significantly reduce fiber glycogen and does not reduce ATP by >15% despite a large decline in PCr. PCr falls logarithmically in relation to stimulus duration; after 30 s of stimulation at 20 Hz, PCr is ~50% of control concentration, and after 150 s it is ~10% (Fig. 3, Table I). The rate of decline is slower in single fibers than in whole frog sartorius muscles at 20°C that are stimulated at 50 Hz, and in which 50% of PCr is consumed within 12 s (Canfield et al., 1973). A higher rate of PCr consumption at 20°C than at 15°C (in these experiments) can be accounted for by a Q_{10} of 3.1 for the ratio of energy consumption to the time-tension integral during a tetanus (Feng, 1931; Aubert, 1956).

Continued tetanic stimulation appears to produce two types of fatigue, depending on stimulus duration: (*a*) a rapidly reversible fatigue in the first 40 s of stimulation, from which the fiber can recover in a 1-s rest period and which is not followed by a decline below control of the peak tension of the 200-ms test tetanus and (*b*) a long-term fatigue that occurs after >40 s of stimulation and is manifested as a reduction in tension that cannot be reversed within 1 s of rest and which is followed by a reduced maximum tension of the 200-ms test tetanus, lasting for up to 82 min.

Data are insufficient to decide which of several possible mechanisms accounts for the early, reversible fall in tetanic tension during continued stimulation. There could be, for example, a transient depletion of ATP at a critical site; the maximum rate of ATP synthesis from PCr, 120 μmol ATP/g·s, is potentially rapid enough to allow recovery of ATP within 1 s (Kuby et al., 1974). Alternatively, a transient reduction in tetanic tension could be related to slowing of an excitation-contraction step involving Ca^{2+} (several steps can recover in seconds) (Connolly et al., 1971; Taylor et al., 1975; Winegrad, 1970), or to transient failure of the action potential to activate myofibrils within the center of the fiber (Edman and Kiessling, 1971). The action potential probably does not fail completely, as it is reported to change slightly if at all at stimulation frequencies below 25 Hz (Lüttgau, 1965; Lännergren and Smith, 1966; Hanson and Persson, 1971).

The long-lasting fatigue that follows stimulation for 40–150 s is accompanied by a fall in PCr by as much as 90%, although ATP does not fall by >15%. Evidence suggests that ATP is not sequestered or unavailable for contraction in these fatigued fibers (cf. Bergström et al., 1971), because measured concentrations of the substrates of the combined glyceraldehyde-3 phosphate dehydrogenase-3 phosphoglycerate kinase and creatine kinase reactions suggest that ADP rather than ATP is either sequestered in an intracellular compartment or somehow unavailable (Canfield and Maréchal, 1973).[2] In addition, fatigued single fibers produce reversible maximal contractures that consume residual ATP in response to caffeine (Eberstein and Sandow, 1963; Nassar-Gentina et al., 1977).

On the basis of the ability of fatigued fibers to produce caffeine and KCl

[2] Veech, R. L. Personal communication.

contractures, Eberstein and Sandow (1963) suggested that fatigue is caused by a failure of excitation-contraction coupling in relation to a deficient role of Ca^{2+} in the coupling process. As posttetanic potentiation occurs in fatigued fibers (Fig. 2), their myoplasmic Ca^{2+} would appear to be elevated rather than decreased (Connolly et al., 1971; Ritchie and Wilkie, 1955; Close and Hoh, 1968). It is possible, therefore, that an additional substance accumulates in fatigued fibers so as to interfere with the action of Ca^{2+} in excitation-contraction coupling. If such accumulation does occur, Figs. 3 and 4 suggest that it becomes sufficient to produce fatigue only after stimulation has progressed for longer than 40 s and after more than 60 nmol/mg protein of PCr are consumed (as ATP). The requirement that a threshold quantity of material accumulate before coupling is interfered with also could explain why twitch tension falls in proportion to the decline in ATP concentrations in whole muscles in which creatine kinase is inhibited (Dydyńska and Wilkie, 1966; Cain and Davies, 1962; Murphy, 1966). These muscles consume at most ~25 nmol/mg protein of high-energy phosphate (cf. Table I) before contraction is lost.

We speculate that H^+ (in the form of lactate) accumulates during prolonged stimulation of unpoisoned single fibers, and eventually interferes with the role of Ca^{2+} in excitation-contraction coupling either by elevating the Ca^{2+} concentration that is required to activate myofibrillar ATPase during stimulation, or by reducing the amount of Ca^{2+} that is released by the sarcoplasmic reticulum (Portzehl et al., 1969; Fuchs et al., 1970; Nakamaru and Schwartz, 1972). The latter mechanism might account for a reduced light response of aequorin in fiber bundles that are repeatedly stimulated (Taylor et al., 1975). The suggested inhibition by H^+ of excitation-contraction coupling remains to be directly tested.

Indirect evidence suggests that H^+ does accumulate in single fibers during prolonged stimulation. Glycolysis is augmented in such fibers, as shown by an increased intracellular concentration of glucose-6-phosphate.[1] Furthermore, the decline in the PCr/ATP ratio in fatigued fibers, from a mean of 4.2 to 6.6 to a mean of 0.6 to 0.81 (Table I), could be related to an increased intracellular H^+. Although the fact often is ignored (Canfield and Maréchal, 1973), H^+ drives the creatine kinase reaction to maintain a high ATP level when PCr is low (Lohmann, 1934; Noda et al., 1954; Collins et al., 1970). Equilibrium concentrations of other components of the reaction must be measured, however, before it can be concluded that the low PCr/ATP ratio in fatigued fibers is due to H^+ accumulation.

In whole muscle, the prolonged stimulation that produces fatigue also augments lactate and presumably H^+ (Berg, 1911; Mainwood and Lucier, 1972; Mainwood et al., 1972). Indirect dye measurements suggest that pH may increase after 40–50 twitches (MacDonald and Jöbsis, 1976), but nuclear magnetic resonance studies indicate that the rise is by, at most, 0.01 pH unit and that, after tetanization at 50 Hz for 25 s, there is a fall within 20 min of intracellular pH by as much as 0.5 U (Dawson et al., 1977). In frog sartorius, furthermore, lactate is augmented 10-fold by stimulation to fatigue, and continues to increase for the 15–20 min after such stimulation (Mainwood et al., 1972). Similar post-stimulatory increases of lactate and H^+, were they to occur in single fibers, might account for the observed time-course of the maximum

tension of the 200-ms test tetanus (Fig. 2). The H^+ hypothesis for fatigue, which was proposed by Hill and Kupalov (1929), also is consistent with the observed correlation between rate of recovery from fatigue after tetanization of whole muscle and rate of recovery of normal lactate concentration. Recovery from fatigue does not correlate, on the other hand, with the rate of PCr resynthesis (Mainwood et al., 1972; Fitts and Holloszy, 1976).

In normal muscle, the appearance of fatigue before ATP depletion may be a protective mechanism, inasmuch as ATP exhaustion can produce rigor, irreversible contracture, and cell death (Hsu, 1950; Weber and Murray, 1973). "Power failure" before energy depletion has been reported also in response to hypoxia at the peripheral nerve, superior cervical ganglion, and at cardiac tissue. In the heart, it has been ascribed to H^+ accumulation (Stewart et al., 1965; Härkonen et al., 1971; Williamson et al., 1976).

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