PHYSIOLOGICAL REVIEWS
Vol. 74, No. 1, January 1994
Printed in U.S.A.

Cellular Mechanisms of Muscle Fatigue

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I. INTRODUCTION

A. Definition and Current Theories of Fatigue

Historically, muscle fatigue has been defined as the failure to maintain force output, leading to a reduced performance (18, 156, 433). More recently, Edwards (157) defined fatigue as "failure to maintain the required or expected power output." This definition recognizes that the ability to sustain a given work capacity without decrement requires the maintenance of both force and velocity. Furthermore, any factor that reduced the rate of force development (dP/dt) would contribute to fatigue by decreasing the percent of peak force obtained in the initial period (first few milliseconds) following muscle activation. As a result, the velocity and power achieved would also be compromised. Despite considerable research, the etiologies of muscle fatigue have yet to be clearly established. The problem is complex, since multiple factors are clearly involved; the relative importance of each is dependent on the fiber type composition of the contracting muscle(s), the intensity, type, and duration of the contractile activity, and the individuals degree of fitness (183). For example, fatigue experienced in high-intensity short-duration exercise is surely dependent on different factors from those precipitating fatigue in endurance activity. Similarly, fatigue during tasks involving heavily loaded contractions such as weight lifting will likely differ from that produced during relatively unloaded movement (running, swimming).

Bigland-Ritchie (47) identified the major potential sites of fatigue as *1*) excitatory input to higher motor

centers, *2*) excitatory drive to lower motor neurons, *3*) motor neuron excitability, *4*) neuromuscular transmission, *5*) sarcolemma excitability, *6*) excitation-contraction coupling, *7*) contractile mechanism, and *8*) metabolic energy supply and metabolite accumulation. Considerable controversy exists regarding the role of these sites, in particular, the relative importance of central and neuromuscular transmission (*sites 1-4*) versus peripheral (*sites 5-8*) mechanisms in the etiology of muscle fatigue (46, 47, 53, 156, 163, 270). This is not a new controversy. In the late 1800s, Lombard (318) observed the work capacity of finger muscles to be maintained in response to electrical stimulation but to show considerable fatigue in response to voluntary contractions. He concluded that the results supported a central mechanism of muscle fatigue. However, in 1901, Hough (250) argued that the electrical and volitional contractions did not recruit exactly the same muscle fibers, in part, because electrical stimulation activated only those fibers directly under the surface electrodes. Consequently, the volitional contractions produced considerably more work, which in turn elicited a higher rate of fatigue. The controversy (central vs. peripheral fatigue) was rekindled in the 1950s when Merton (346) hypothesized that fatigue could be explained entirely by alterations within the muscle, while Krnjevic and Miledi (295, 296) and later Stephens and Taylor (454) and others (221, 292, 367) emphasized the importance of the failure of neuromuscular transmission. Krnjevic and Miledi (295) suggested that transmission failure could result from inactivation of presynaptic intramuscular nerve endings and/or from an apparent decreased sensitivity of the postsynaptic membrane to acetylcholine. The latter would decrease the amplitude of the end plate potential and, coupled with an increased activation threshold, lead to a reduced sarcolemma activation rate (295). However, Brooks and Thies (69) found no evidence for a change in postsynaptic sensitivity to acetylcholine during stimulation at 20 pulses/s and concluded that although the end plate potential declined, it was probably not a major rate-limiting factor. Although failure in neuromuscular transmission has been observed, it is generally associated with unphysiologically high stimulation frequencies (196, 295, 298). Additionally, high-intensity exercise is frequently associated with a reduced neural drive and α-motor nerve activation frequency; however, rather than precipitate fatigue, this change is thought to protect against its development (49, 54). The preponderance of evidence suggests that the primary sites of fatigue lie within the muscle itself (156, 157, 183).

The focus of this review is on the cellular aspects of muscle fatigue, and thus emphasis is placed on the putative fatigue factors located distal to the neuromuscular junction. Reviews on muscle fatigue have been numerous, but most have concentrated on one aspect, such as alterations in excitation-contraction coupling (9, 48, 158, 490), the role of ionic changes (317, 428, 429, 438), disturbances in cell metabolism (157, 220, 229, 258, 282, 325, 406, 419, 463), or alterations in cell ultrastructure (12, 13, 16, 80, 95, 147, 191, 342). In this paper, I review all putative fatigue agents, discuss the relative importance of each, and consider possible interactions that might collectively contribute to fatigue. Although numerous mechanisms have been suggested as causative in muscle fatigue, most are related to alterations in excitation or cell metabolism. Figure 1 presents the cell sites most frequently linked to the etiology of skeletal muscle fatigue. *Sites 1-4* involve disturbances in excitation-contraction (E-C) coupling and involve changes in the amplitude and conduction of the sarcolemma and T tubular action potential (*sites 1* and *2*) and the T tubular dihydropyridine receptor (charge sensor) and the sarcoplasmic reticulum (SR) Ca^{2+} release channel (*sites 3* and *4*). *Sites 5-7* relate to metabolic factors affecting the SR, the thin filament regulatory proteins, and the cross bridge.

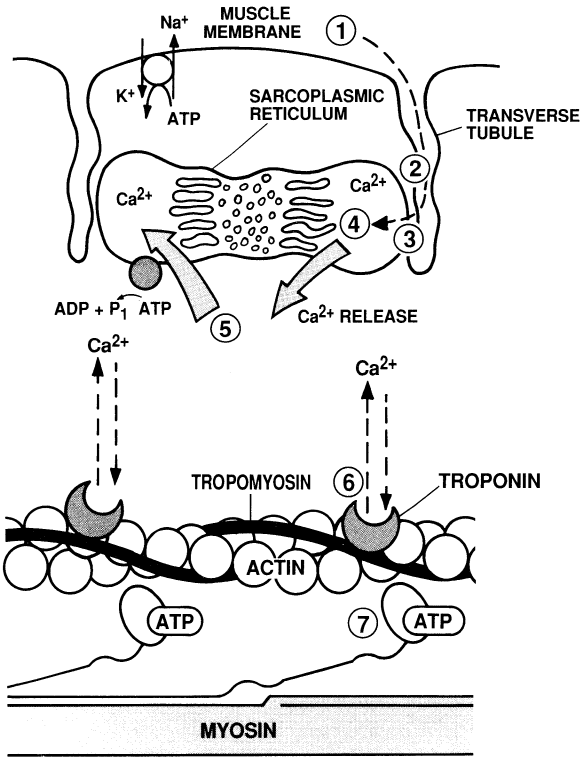

FIG. 1. Diagrammatic representation of major components of a muscle cell involved in excitation-contraction coupling. Numbers indicate possible sites of muscular fatigue during heavy exercise and include the following: *1*) surface membrane, *2*) T tubular charge movement, *3*) mechanisms coupling T tubular charge movement with sarcoplasmic reticulum (SR) Ca^{2+} release, *4*) SR Ca^{2+} release, *5*) SR Ca^{2+} reuptake, *6*) Ca^{2+} binding to troponin, and *7*) myosin binding to actin, ATP hydrolysis, and cross-bridge force development and cycle rate. [From Fitts and Metzger (184), with permission from S. Karger AG, Basel.]

B. Muscle Fiber Type Composition

Any discussion of skeletal muscle fatigue requires the consideration of the fiber type composition of the involved muscle or muscle group. Adult mammalian skeletal muscles contain at least three distinct fiber

types classified on the basis of their functional and metabolic properties as fast glycolytic (FG), fast oxidative glycolytic (FOG), and slow oxidative (SO). The FG and FOG fiber types are fast-twitch fibers characterized by high SR and myofibrillar adenosinetriphosphatase (ATPase) activities and correspondingly short isometric twitch durations and high maximal shortening velocities (V_o) (99, 390). In contrast, the SO fiber possesses low SR and myofibrillar ATPase activities, prolonged twitch duration, and low V_o compared with the fast-twitch fiber types (62, 68). Each fiber type contains a specific isozyme for the contractile protein myosin, and fibers are frequently identified on the basis of their histochemically determined myosin ATPase activity as type I, IIa, or IIb (62, 68). Recently, adult skeletal muscle has been shown to contain a fourth fiber type containing a specific myosin isozyme identified as IIx or IId (62). In addition to the different isozymes for myosin, the three main fiber types can be distinguished by specific isozymes for a number of muscle proteins and by their mitochondrial enzyme content. In the context of muscle fatigue, this latter property is particularly important. The FOG and SO fibers contain a high mitochondrial content and thus are relatively fatigue resistant compared with the FG fiber type. Consequently, the soleus and deep region of the gastrocnemius containing primarily SO and FOG fibers, respectively, are considerably more fatigue resistant than muscle or muscle regions containing primarily the FG fiber type. The mitochondrial concentration of the FOG and SO fiber type shows important species differences. In humans, pigs, and other large vertebrates, the SO fiber possesses the highest mitochondrial enzyme content, whereas in rodents, the mitochondrial content and oxidative capacity are highest in the FOG, intermediate in the SO, and lowest in the FG fiber type (21, 185).

Many studies evaluating the etiology of muscle fatigue have employed frog limb muscle (148, 152, 180, 182, 187, 305, 320, 474). For the most part, these muscles are fast twitch and contain a high glycolytic, but poor oxidative, capacity (467). Although the fast-twitch fibers of the frog show some diversity in regard to their myosin isozyme content, all appear to have V_o values at least as high as the mammalian FG fiber type (150, 152, 467).

C. *Exercise Intensity and Environmental Conditions*

The etiology of muscle fatigue is dependent on the exercise intensity and the environmental conditions. The factors eliciting fatigue during short-duration high-intensity exercise are clearly different from those involved during submaximal prolonged work. The former involves the recruitment of all three fiber types, a high contraction frequency, and a high degree of anaerobic metabolism (176, 206). As a consequence of the high activation frequency, disturbances in E-C coupling, such as a conduction block of the action potential or an inhibition in SR Ca^{2+} release, are more likely to occur. Additionally, the high level of anaerobic metabolism will lead to an increase in intracellular H^+ and P_i, factors known to inhibit peak force (140, 141, 167, 176, 231, 352, 379, 468). In contrast, during prolonged submaximal exercise, cell energy is derived primarily from aerobic metabolism and, consequently, the muscle lactate, H^+, and P_i contents remain relatively unchanged (35, 230, 256, 421). Although the causes of fatigue during prolonged exercise are not clearly understood, the depletion of muscle glycogen and, in some cases, low blood glucose appear to be important contributing factors (103, 113, 202, 420). The exercise frequency and the environmental conditions can play a role in determining not only the degree and cause, but also the time course of fatigue during prolonged exercise. For example, endurance exercise conducted twice daily can lead to a chronic reduction in the preexercise muscle glycogen content, thus facilitating the onset of glycogen depletion and fatigue during exercise (432). Work in a hot environment generally leads to a more rapid onset of fatigue due to either dehydration and/or muscle metabolic changes associated with a reduced muscle blood flow. The latter results from both a decreased blood volume due to body water loss and the increased skin blood flow mediated by the high body temperature (369, 432).

In this review, the alterations in contractile function directly responsible for the reduced work capacity of the fatigued muscle are described. Although fatigue elicited by short-duration, high-intensity, and prolonged exercise is mediated by distinctly different factors, the mechanical disturbances, such as a reduced capacity for peak force and power, are qualitatively the same. After the review of the muscle contractile changes, the potential cellular causes of fatigue are discussed. The reader should keep in mind that the relative importance of each factor, and the time course for the onset of fatigue will vary depending on the state of fitness of the exercising individual and the intensity and environmental conditions of the exercise (183).

II. MECHANICAL PROPERTIES

The mechanical properties of a single muscle cell are dependent on the fiber type, which in turn is dependent on the protein composition. Although all striated muscle fibers contain contractile and regulatory proteins, mitochondria, and SR, the adult fast type IIa, IIx, IIb, and slow type I fibers contain specific isozymes that are thought to be responsible for the fiber type-specific differences in contractile function (62, 465). For example, each fiber type contains a specific isozyme of the contractile protein myosin, the protein containing the ATPase thought to control V_o (62). Thus the fast type IIb and slow type I fibers contain the highest and lowest myofibrillar ATPase activity and fiber V_o, respectively (62, 465). The fast type IIb fiber shows the highest fatigability due to its low mitochondrial content, low efficiency, and dependence on anaerobic metabolism.

Each contractile property (fiber V_o, twitch duration, peak force) is dependent on specific cellular and

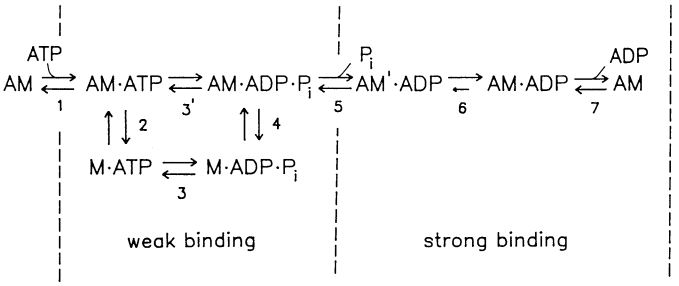

FIG. 2. Schematic model of actomyosin ATP hydrolysis reaction during contraction in skeletal muscle, where A is actin and M is heavy meromyosin or myosin S1. Scheme is adapted from current models of ATP hydrolysis. [From Metzger and Moss (355).]

molecular events associated with the cross-bridge cycle. The exact molecular events of the cross-bridge interaction responsible for tension development and sarcomere shortening are as yet unknown. Figure 2 presents a schematic model of the kinetics of the actomyosin ATP hydrolysis reaction (cross-bridge cycling) in skeletal muscle. The scheme was recently published by Metzger and Moss (355) and represents their adaptation of the current models of ATP hydrolysis, which in turn were derived from modifications of the model originally proposed by Lymn and Taylor (323). Force production depends on the binding of the myosin head (M) to actin (A). Inorganic phosphate release (Fig. 2, *step 5*) is thought to be coupled to the transition in actomyosin binding from a weakly bound low-force state (AM-ADP-P_i) to the strongly bound high-force state (AM'-ADP). This latter state is likely the dominant cross-bridge form during a peak isometric contraction (355). Brenner (65) and Metzger et al. (351) have suggested that this step is limiting in terms of the peak dP/dt. Peak rate of tension development can be measured experimentally by determining the rate of tension redevelopment following an imposed slack-unslack (shortening followed by reextension to the initial optimal length) in a fully activated fiber (67). Employing this technique, Metzger and Moss (356) recently showed a sevenfold higher rate constant of tension redevelopment (k_{tr}) in fast- compared with slow-twitch fibers. The k_{tr} has also been shown to be Ca^{2+} sensitive; thus the peak k_{tr} was reduced at suboptimal Ca^{2+} concentrations (66, 356). This Ca^{2+} sensitivity may result from a direct effect of Ca^{2+} on the forward apparent rate constant of *step 5* of the cross-bridge reaction scheme (Fig. 2).

The V_o in skeletal muscle is highly correlated with, and thought to be limited by, the rate of ATP hydrolysis by myosin (25). Maximum shortening velocity is obtained during maximal unloaded contractions where the requirement for the strongly bound high-force state of the cross bridge (Fig. 2) is low, and the overall cycle rate is maximal. In contrast to dP/dt, where the rate of cross-bridge transition from the weakly to the strongly bound state appears to be limiting, V_o is thought to be limited by the rate of cross-bridge dissociation. The rate-limiting step in cross-bridge detachment is unknown, but the possibilities include *steps 1, 2, 6,* and *7* of the scheme shown in Figure 2. Fiber V_o can be determined by the slack test method (150) or from extrapolation of the force-velocity relation to zero load (241). The slack test is the preferred method, since the force-velocity technique appears to underestimate V_o (280).

The ability of humans and other vertebrates to perform work is dependent on the capacity to displace a load; thus an important consideration is the power output of a muscle or muscle group. The power spectrum for a single fiber, a muscle, or a muscle group can be calculated from the force-velocity relationship. From the analysis of force-velocity curves, it is known that peak power output is obtained at intermediate velocities (133).

In muscle fatigue, force, V_o, and power are all depressed. These changes can be related to alterations in specific steps of the cross-bridge cycle (Fig. 2). In section II*A*, the fatigue-induced alterations in contractile function are reviewed, and the etiology of the observed changes is related to specific disturbances in the cross-bridge scheme (Fig. 2).

A. Isometric Contractile Properties

Long before the mechanism of muscle contraction was understood, and as early as the 1700s, it was recognized that muscles remained viable longer in oxygen than in air, hydrogen, or nitrogen (186). This fact is vividly demonstrated in a monograph published by Fletcher in 1902 (186) and reproduced in this review (Fig. 3). In this experiment, a pair of frog gastrocnemius muscles was arranged in duplicate chambers, and fatigue was induced by direct stimulation at 1 pulse/s. One muscle stimulated in nitrogen (Fig. 3*B*) showed a more rapid decline in peak force and slower relaxation compared with the other, which was stimulated in oxygen (Fig. 3*A*). The prolongation of relaxation with fatigue was more clearly observed in another figure published by Fletcher (186) and reproduced here (Fig. 4). In more recent times, the peak force response to a single stimulus, the isometric twitch (P_t), has frequently been used as an indicator of muscle fatigue (180). Twitch tension was thought to provide information about the number of active cross bridges. However, it is now generally recognized that other factors, such as the duration of the Ca^{2+} transient and/or muscle temperature, change with fatigue, producing independent effects on P_t (182). Nevertheless, as shown in Figures 3 and 4, peak twitch tension generally declines with fatigue (76, 148, 182, 350). In a recent study, peak twitch tension was reduced by 64%, and the contraction and one-half relaxation times (CT and 1/2RT) were 300 and 800% of the prefatigued values, respectively (467). The prolongation of relaxation has been observed during in vivo exercise in humans as well as in isolated muscles contracting in situ or in vitro (38, 47, 50, 75, 163, 327, 425). Although muscle temperature affects the twitch duration, muscle fatigue prolongs the CT and 1/2RT at all temperatures between 10 and 35°C (75, 76, 474). The initial slowing of relaxation occurs early in the fatigue process before the increase in

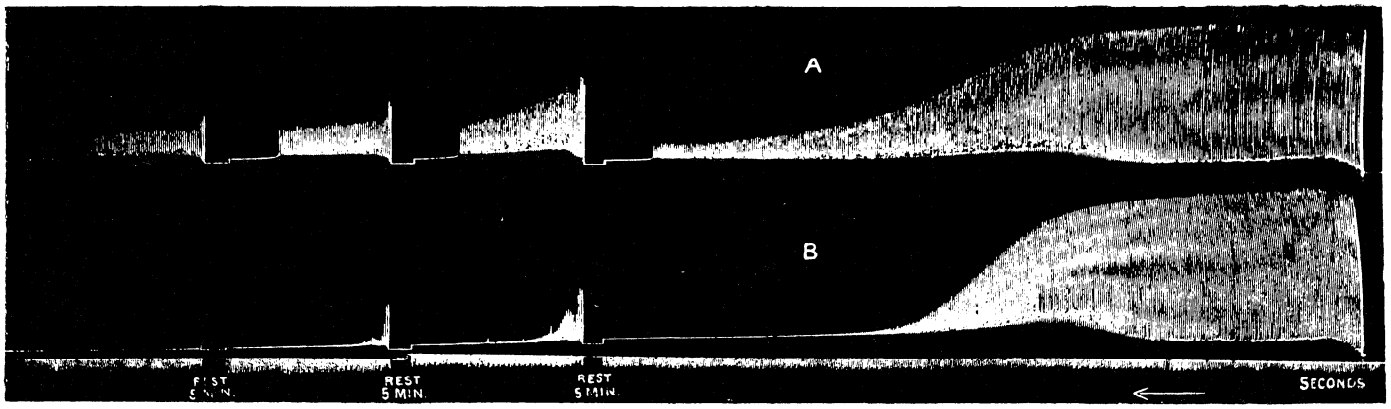

FIG. 3. Fatigue in a pair of gastrocnemius muscles (maximal break-shocks, ~1/s; load, 10 g; temperature, 18°C). *A*: exposed to oxygen. *B*: exposed to nitrogen. [From Fletcher (186).]

metabolic factors such as lactate or H^+ (240, 385). The peak rate of twitch tension development (P_t + dP/dt) becomes depressed during the development of fatigue (75, 182).

Peak tetanic tension (P_o) decreases with contractile activity (6, 182, 474), with shortening contractions at optimal length producing the largest decline (7, 119). The extent of the decline in P_o is generally used as an indicator of the severity of fatigue (156, 184). Figure 5 [reprinted from Thompson et al. (467)] shows a frog semitendinosus tetanic contraction (P_o) before and 10 s, 60 s, 5 min, and 20 min after a fatiguing protocol (1/s, 100-ms, 150-Hz trains for 5 min). Peak tetanic tension dropped to 9% of the prefatigued value, and the peak rate of tension development (+dP/dt) and relaxation (−dP/dt) also underwent a significant decline. The fall in −dP/dt is greater than that observed for +dP/dt (350). The reduced dP/dt ($g \cdot ms^{-1} \cdot cm^{-2}$) was largely due to the decreased force output. Thus, when the dP/dt data were corrected for the changes in tension [$dP/dt \times$ peak force $(P)^{-1}$], the observed decrease was attenuated (350). The recovery of both P_t and P_o following fatigue generally occurs in two phases (149, 182, 305, 327, 350, 364, 458): a rapid phase complete within 1 min, followed by a slow (30–60 min) recovery to the prefatigue tension (Fig. 5). However, under some conditions, recovery from fatigue in amphibian muscle is slowed by a period of reduced tension (486, 487). Westerblad and Lännergren (486, 487) have referred to this condition as postcontraction depression (PCD); the etiology of PCD is entirely unknown. However, it is apparent that it is not caused by sarcolemma inactivation, since the resting and action potentials were unaltered during PCD (306). Recently, Lännergren et al. (304) observed a similar, but somewhat different, phenomenon during recovery from fatigue following in situ stimulation of single motor units in the rat.

Edström and Kugelberg (153) and Burke and Levine (77) demonstrated that the properties of individual motor units of rats and cats, respectively, could be classified into three nonoverlapping groups on the basis of their contractile and histochemical properties. Two fast-contracting units were identified on the basis of their fatigability as fast-contracting fast-fatigue (FF) and fast-contracting fatigue-resistant (FR), while the third was a slow-contracting fatigue-resistant (S) unit (77). A given motor unit is thought to contain only one fiber type, and thus the FF, FR, and S units are frequently referred to as types IIb, IIa, and I motor units, respectively. Recently, a third fast-type motor unit thought to contain exclusively the type IIx fiber was identified (310). The type IIx units have contraction and relaxation times similar to those of the type IIa and IIb units but show an intermediate resistance to fatigue. This separation based on fatigability is clearly shown in Figure 6, reproduced from the paper of Larsson et al.

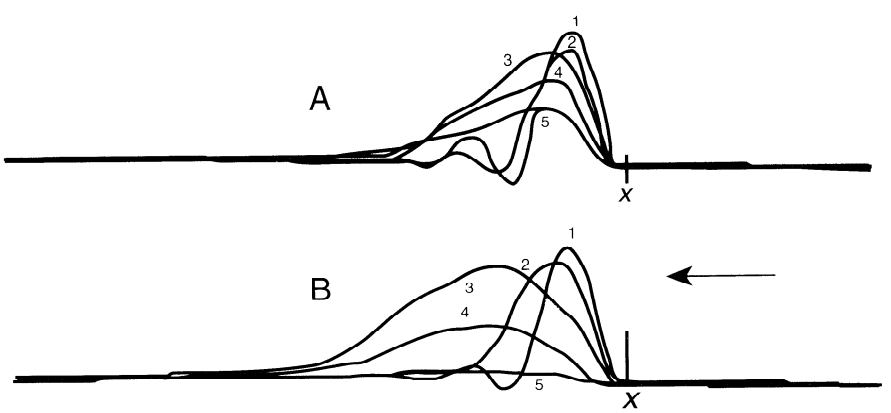

FIG. 4. Contractions of a pair of gastrocnemius muscles (break-shocks of same strength throughout; load, 10 g; temperature, 18.5°C). Stimulus was applied at X. *Contraction 1* was the first given. Between each 2 successive recorded contractions as numbered, 120 unrecorded contractions were given, a little slower than 1/s. *A*: exposed to oxygen. *B*: exposed to nitrogen. [From Fletcher (186).]

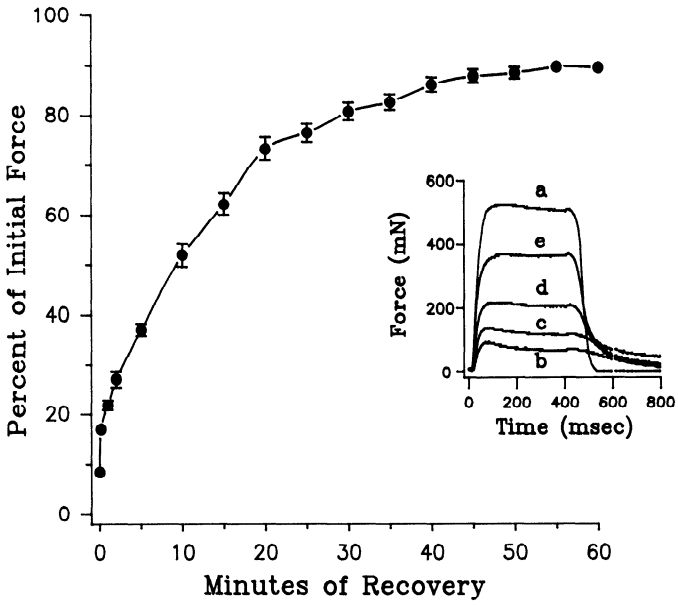

FIG. 5. Recovery of force production (percent of initial force) after stimulation. Values are means ± SE; n = 6–44 experiments. *Inset*: representative tetanus records at prefatigue (a) and at 10 s (b), 60 s (c), 5 min (d), and 20 min (e) of recovery. Peak tetanic tension returned to prefatigue value by 45 min. [From Thompson et al. (467).]

(310). The fatigue-resistant type I motor units are low-threshold units recruited during low-force contractions (162). The average discharge rates for the type I unit increase with increased force, but generally range between 10 and 20 Hz (61, 162). The higher threshold fast motor units are recruited during high-force contractions at discharge frequencies that vary with the effort from 25 Hz at 40% to 40 Hz at 80% of maximal voluntary contraction (134). Because of their high contraction and relaxation times, these fast units primarily display unfused contractions; however, discharge bursts as high as 60 Hz have been observed during maximal voluntary contractions (134). Consistent with whole muscle experiments, single motor units showed prolonged contraction and relaxation times, reduced rates of tension development and decline, and a lowered shortening speed following fatigue (78, 79, 218, 228, 298). These stimulation-induced changes in contractile function were most apparent in the fast type IIb motor units.

Isolated single fibers appear to fatigue less rapidly than whole muscles, but consistent with whole muscles show accelerated fatigue under anaerobic conditions (308). Recently, Lännergren and Westerblad (308) and Westerblad and Lännergren (488) showed the tetanic force of single mouse fibers to decline in three phases (Fig. 7). Phase 1 occurred in the initial 8–14 tetani (a to b, Fig. 7) and was followed by a comparatively long nearly steady tension generation (phase 2, b to c, Fig. 7), and finally by a rapid decline in tension (phase 3, c to d, Fig. 7). It is also apparent from Figure 7 that relaxation slowed with fatigue. The initial linear component of relaxation underwent the greatest prolongation, and the maximum reduction occurred at the end of phase 2 (Fig. 7).

The fatigue-induced prolongation of the twitch duration (Ca^{2+} transient) caused an increased fusion of tension at low frequencies and a leftward shift in the force-frequency curve (177, 350, 467). Consequently, the optimal stimulation frequency for peak force decreased as fatigue developed (269, 271, 350, 467).

Edwards and co-workers (108, 154), studying humans, and Jones et al. (272), evaluating animal as well as human muscle, observed that fatigue in response to low frequencies of stimulation persisted after force in response to high frequencies had fully recovered. Because skeletal muscle is generally activated by low-frequency (10–30 Hz) stimulation (61, 162), this selective effect could have important functional implications. Edwards et al. (154) referred to this selective effect as low-frequency fatigue and suggested that it was mediated by disturbances in E-C coupling. Reduction in force at low frequencies means that the force-frequency relationship is shifted to the right. This result is difficult to understand considering that muscle contractile duration is prolonged after fatigue (76, 467, 474), and thus force production at low frequencies should benefit from the resultant increase in fusion. Metzger and Fitts (350) reexamined the effect of high- and low-frequency stimulation on force recovery at both ends of the force-frequency relationship. They observed force to recover

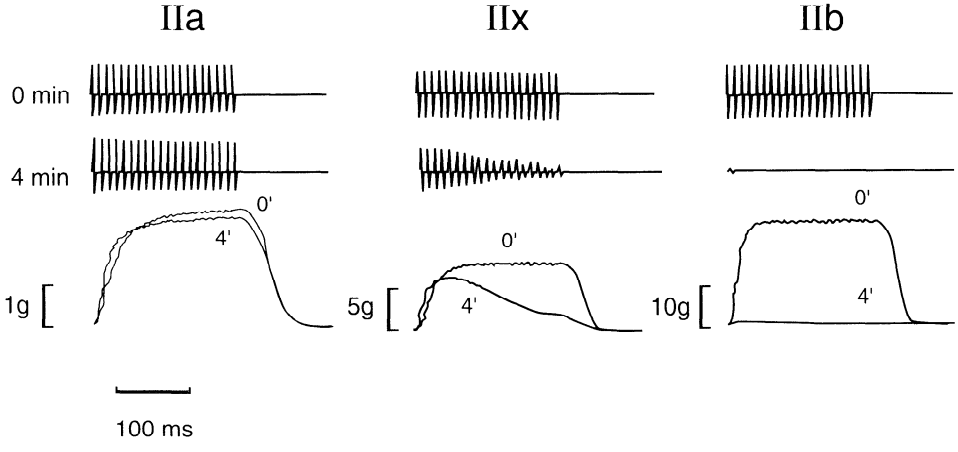

FIG. 6. Electromyographic and mechanical responses at 0 and 4 min during intermittent high-frequency stimulation (200-ms 100-Hz trains given twice per second) in type IIa, IIx, and IIb motor units. [From Larsson et al. (310).]

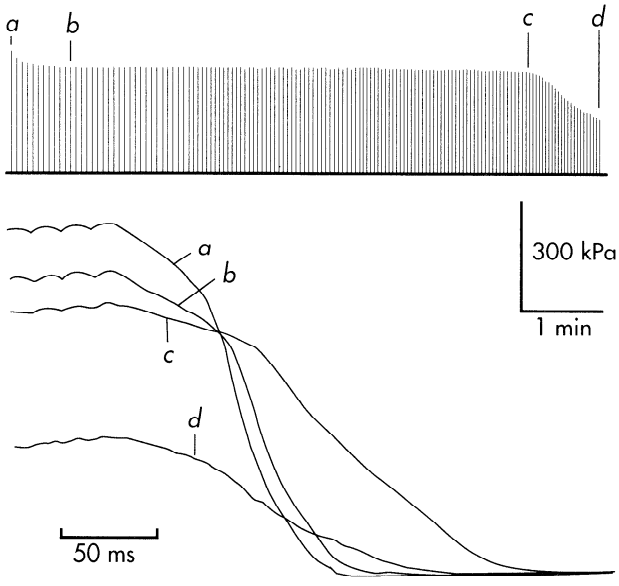

FIG. 7. Slowing of relaxation during fatiguing stimulation. *Top panel*: typical fatigue curve of a single fiber. Each tetanus appears as a vertical line. *Bottom panel*: relaxation of tetani indicated above fatigue curve (*a–d*). Tension bar refers to *top panel* only. [From Westerblad and Lännergren (488).]

more rapidly at low- compared with high-hertz stimulation. Furthermore, in the fatigued state, the muscle generated considerably more force during low (5 Hz)-compared with high (75 Hz)-frequency stimulation. Jones and co-workers (269, 271) and Bigland-Ritchie (47) observed a similar result, and they and others (221) found the activation frequency during the course of a sustained voluntary contraction to decline. Bigland-Ritchie and co-workers (49, 52, 495) suggested that the reduced neuronal firing rate was mediated via muscle afferents, thus taking advantage of the relatively higher forces at low frequencies as fatigue developed. Regardless of the stimulation frequency (low or high) employed to elicit fatigue, a prolonged twitch duration (which likely reflects a prolonged Ca^{2+} transient) accompanies muscle fatigue; thus one would expect the force-frequency relationship to undergo a leftward shift. It is not clear why Edwards et al. (154) found a rightward shift; however, their measurements were restricted to a few stimulation frequencies elicited after an hour or more of recovery. Consequently, the prolonged relaxation time associated with fatigue, and responsible for the leftward shift in the force-frequency relationship, may have recovered (350, 467). Alternately, low-frequency fatigue (LFF) may be a result of muscle damage. This possibility was first suggested by Jones (269), who hypothesized that mechanical damage to the SR might result in less Ca^{2+} release in response to each action potential. Consequently, low frequencies of stimulation would produce lower than normal intracellular Ca^{2+} and thus fatigue. At high frequencies of stimulation, enough Ca^{2+} would be released from the SR (perhaps from the undamaged vesicles) to reach near-normal tensions. This hypothesis is supported by the frequent association of deleterious structural changes following eccentric contractions (14, 338) and the observation that LFF is more pronounced following eccentric compared with isometric or concentric contractions (123, 274, 374, 377). Davies and White (123) studied the effect of box stepping and found considerably more LFF (based on 20/50-Hz force ratio) in the lead compared with the trail leg. The former conducts primarily eccentric work as it absorbs the weight of the body on return to the floor. The studies of Edwards et al. (154), Newham et al. (377), Jones et al. (274), and more recently, Binder-Macleod and McDermond (55) in which the force-frequency relationship in human muscle shifted to the right following fatigue all elicited contraction via percutaneous electrical stimulation. Thus, in addition to muscle damage, these data could be explained by a decreased membrane excitability postfatigue, such that the applied stimulation frequencies actually elicited considerably fewer muscle action potentials (22).

The decreased P_o suggests a reduction in the number of cross bridges in the strong binding state (Fig. 2). The reduced force could result from deleterious alterations in E-C coupling producing a reduced Ca^{2+} release from the SR, changes in the regulatory proteins, and/or direct effects acting at the cross bridge to limit the transition from the low- to the high-force state. If the dP/dt in an intact muscle is limited by the cross-bridge transition(s) rate (as it appears to be in fully activated single fibers), then the rate constant for cross-bridge binding may be reduced in the fatigued cell (*step 5*, Fig. 2).

B. Maximal Shortening Speed and Peak Power

Edman and Mattiazzi (152) observed a progressive decline in V_o with the development of fatigue. However, V_o did not significantly change until P_o had fallen by at least 10% of the prefatigued tension. In general, muscle fatigue is characterized by a greater fall in P_o than V_o (133, 152, 182, 467). Thompson et al. (467) recently showed the P_o and V_o of the frog semitendinosus to decline with electrical stimulation to 9 and 37% of the prefatigue value, respectively. Similar to peak force, Hatcher and Luff (228) observed V_o to recover from fatigue in an initial rapid phase followed by a slower phase. De Haan et al. (133) demonstrated that peak power is compromised even more than P_o or V_o. Additionally, in the fatigued muscle, the optimal velocity for peak power is reduced (133).

III. EXCITATION-CONTRACTION COUPLING

A. Sarcolemma Resting Potential

It has been suggested by some (317, 438) that alterations in sarcolemma function induce muscle fatigue by preventing cell activation. The general theory is that K^+

efflux and inhibition of the Na^+-K^+ pump (or its inability to keep pace with K^+ efflux and Na^+ influx) causes cell depolarization, a reduced action potential amplitude, and in some cells, complete inactivation. Edwards (156) suggests that cell depolarization would provide a safety mechanism to protect the cell against ATP depletion and Ca^{2+} accumulation. The uncoupling of activation at the first step in E-C coupling rather than latter steps, such as the cross-bridge cycle, would prevent activation of subsequent ATP-utilizing steps and increases in intracellular Ca^{2+}. The latter might activate Ca^{2+}-sensitive proteases and phospholipases, leading to the disruption of the sarcolemma and intracellular organelle (16). Lindinger and Sjøgaard (317) speculate that the membrane mechanism of fatigue would allow contractions at reduced rates and forces while preventing catastrophic changes in cellular homeostasis that might lead to cell damage. Although cell depolarization of 10–20 mV is commonly observed in fatigued muscle cells (226, 276, 305, 306, 466), it is not established that this change affects the propagation of the sarcolemma action potential into the T tubules or subsequent steps in E-C coupling. Lännergren and Westerblad (305) did observe a steep decline in tension once membrane potential fell below −60 mV. The evidence linking K^+ efflux and cell depolarization to muscle fatigue is reviewed in section III*A1*. Additionally, a number of detailed reviews on this topic have recently been published (317, 428, 429, 437, 438).

1. Potassium and cell depolarization

The resting membrane potential (V_m) of skeletal muscle is largely a K^+-dependent potential (248); thus any change in the K^+ conductance or concentration gradient across the sarcolemma will affect V_m. Since the work of Fenn and Cobb (168), it has been known that fatigue in both mammalian and frog muscle is associated with a loss of intracellular potassium ($[K^+]_i$) and a gain in Cl^-, Na^+, and water. The extent of the ion and water shifts is greater in fast- compared with slow-twitch muscle and increases with stimulation frequency (452). The work of Fenn and Cobb (168) and Hodgkin and Horowicz (248) suggested that the intracellular Na^+ increase with muscle activity was slightly larger than the K^+ loss. More recently, Juel (276) employed ion-sensitive microelectrodes to measure Na^+ and K^+. He observed a higher K^+ in the fast mouse extensor digitorum longus (EDL) (182 mM) compared with the slow soleus (168 mM), and the fast muscle showed a correspondingly more negative resting V_m. In contrast to the earlier reports (168, 248), Juel (276) and others (246, 314, 316, 435, 439) have found the K^+ loss to exceed the Na^+ gain during fatigue produced by the electrical stimulation. After 1 min of repetitive stimulation (400-ms 40-Hz trains, 1/s), the V_m had depolarized by 12 and 18 mV in the slow soleus and fast EDL, respectively (276). Juel (276) suggested that the exercise-induced decrease in $[K^+]_i$ could only account for approximately one-half of the observed depolarization and that the remaining depolarization must be due to an increased extracellular (interstitial) K^+ ($[K^+]_o$). Juel (276) and others (246, 247, 428, 429, 435) have observed an approximate doubling of $[K^+]_o$ following stimulation. The loss in $[K^+]_i$ was 30–40 mM following both prolonged submaximal and short-duration intense exercise (276, 435, 439). The latter showed a similar decline in $[K^+]_i$ despite less net K^+ loss due to a larger increase in intracellular water (435, 437, 439). The work of Creese et al. (116) suggests that cell depolarization with contractile activity is greater in centrally located fibers. This observation is not surprising in that increases in $[K^+]_o$ in the interstitium and T tubules would likely be higher in the core of the muscle. The amount of K^+ lost from muscle cells during contractile activity appears to exceed that explained by the K^+ efflux attributable to the sarcolemma action potentials (437). Consequently, it has been suggested that the V_m depolarization frequently observed in fatigued muscle cells results from the combined effects of a reduced $[K^+]_i$, elevated $[K^+]_o$, and an increased K^+ conductance (317, 437). The increased K^+ conductance could result from activation of the ATP-dependent and/or the Ca^{2+}-dependent K^+ channel (89, 173, 174). Recently, Davies et al. (125) employed patch-clamp methods to study the ATP-dependent K^+ channels (K_{ATP} channels) in frog skeletal muscle. At intracellular pH (pH_i) 7.2, the K_{ATP} channel showed little activity at 1 mM and was essentially inactive at 3 mM ATP; however, at pH_i 6.3, the K_{ATP} channel activity was detectable at both ATP concentrations. Thus the possibility exists that K^+ efflux increases as the pH_i falls during high-intensity exercise. This would contribute to V_m depolarization and to a reduced action potential amplitude, and perhaps depolarization block of the sarcolemma or T tubular action potential (87, 88, 125, 145, 437, 438). It is also possible that K^+ conductance increases during contractile activity by activation of Ca^{2+}-dependent K^+ channels (332, 437, 438). Fink and Lüettgau (174) reported a 100-fold increase in the K^+ conductance of metabolically poisoned and mechanically exhausted frog skeletal muscle fibers. The increased conductance was prevented by the electrophoretic injection of the Ca^{2+}-chelating agent H_2-ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid^{2-}. From these observations, Fink et al. (173) concluded that internal free Ca^{2+} promoted the activation of K^+ conductance in exhausted muscle fibers. The observations that extracellular markers were not found in the myoplasm, that the membrane capacitance was unaltered, and that the conductance was inhibited by K^+ channel blockers in the exhausted fibers (89, 172, 174) led Fink and Lüettgau (174) and Castle and Haylett (89) to conclude that the increased K^+ conductance occurred without any disruption in the structural integrity of either the surface or T tubular membranes. Fink et al. (173) and Lüettgau and Wettwer (322) suggested that the lack of metabolic energy (low ATP) increased the probability of Ca^{2+}-activated K^+ conductance. This suggestion was based on their observation that membrane conductance did not increase during caffeine contrac-

tures or electrical stimulation of normal (nonpoisoned) fibers. Because cell ATP rarely falls by >30%, even in highly fatigued muscle fibers (176), it seems unlikely that Ca^{2+}-activated K^+ channels contribute to cell depolarization under physiological conditions. More recently, Castle and Haylett (89) proposed that the increased K^+ conductance of electrically stimulated poisoned muscle fibers was predominantly due to activation of ATP-sensitive channels. This conclusion was based on the observation that specific blockers of ATP- but not Ca^+-sensitive K^+ channels inhibited the increased K^+ conductance. The apparent conflict between these results and those of Fink et al. (173) could be reconciled if metabolic exhaustion induced a Ca^{2+} dependence of the ATP-sensitive channel.

Hicks and McComas (237) have questioned the hypothesis that cell depolarization mediates fatigue. After in situ stimulation of the rat soleus (4 s at 20 Hz every 5 s for 5 min), they observed membrane hyperpolarization and an increased amplitude of the muscle fiber action potential. When ouabain was added to the bathing medium, the fibers no longer exhibited a hyperpolarization in the recovery period; this result supports the authors' conclusion that the hyperpolarization resulted from stimulation of the Na^+-K^+ pump. Hicks and McComas (237) suggest that the depolarization observed by others (225, 276) can be attributed to unphysiologically high stimulation frequencies and to the absence of protein in the bathing media, which they suggest may be necessary for the normal operation of the Na^+-K^+ pump. However, numerous human studies have observed an increase in plasma K^+ during exercise (83, 96, 278, 410, 428, 434–436). An increase in venous K^+ draining contracting muscles suggests that the $[K^+]_o$ must increase, and the increase is likely greatest close to the surface membrane (439). Van Beaumont et al. (472) showed that more than one-half of the exercise-induced increase in plasma K^+ could be attributed to hemoconcentration, which results from a shift of water from the plasma into the interstitial and intracellular compartments of contracting muscle (314, 440). Nevertheless, Sjøgaard et al. (439) and Sahlin and Broberg (410) have observed continuous release of K^+ during prolonged exercise. The latter group found the arteriovenous plasma K^+ concentration difference to become increasingly negative near exhaustion. The highest level of K^+ efflux was found during maximal exercise (439). Employing a K^+-sensitive electrode, Vyskocil et al. (477) observed $[K^+]_o$ to increase from 4.5 to 9.5 mM during maximal voluntary contractions in humans. These results have led some (315, 317, 437–439) to conclude that fatigue may be related to changes in the K^+ gradient across muscle cells and that the rapid recovery following brief rest periods reflects partial restoration of the resting membrane potential.

2. *Sodium-potassium pump*

According to the membrane hypothesis of muscle fatigue (317, 438), the sarcolemma Na^+-K^+ pump is unable to maintain the ionic gradients for K^+ and Na^+ essential for the maintenance of the V_m and cell excitability. Sjøgaard (438) suggests that the Na^+-K^+ pump may be limited by insufficient ATP. However, the Michaelis constant (K_m) for ATP is ~0.5 mM (428), and during contractile activity, cell ATP rarely falls below 3 mM (176). Some have suggested that cell ATP is compartmentalized (437); however, for this to be an important factor in skeletal muscle, the subsarcolemma region would have to contain considerably less ATP than the cell average. During exercise, the combined effects of contractile activity, catecholamine release, and increases in intracellular Na^+ concentration ($[Na^+]_i$) and $[K^+]_o$ stimulate the Na^+-K^+ pump. It is apparent from the increase in $[K^+]_o$ and $[Na^+]_i$ that the pump capacity is insufficient. The pump density may not be high enough to fully compensate for the ionic fluxes during the action potentials (428). This condition would be expected to be exacerbated in the T tubules, where the Na^+-K^+ pump density is lower than that of the sarcolemma membrane (428). Stimulation of the Na^+-K^+ pump by epinephrine has been shown to release the force-inhibiting effect of high $[K^+]_o$ (97). The $[K^+]_o$ content likely increases in the T tubular lumen, reaching the highest value in the fiber core. Thus inactivation due to depolarization is more likely to occur in the central core region.

Despite a net loss of 1,300–1,400 nmol $K^+ \cdot g\ muscle^{-1} \cdot min^{-1}$ during maximal exercise in humans (233, 439), full activity of the Na^+-K^+ pump was apparently not obtained (98). Due to a relatively low K_m for $[K^+]_o$ (>1 mM), the increase in K^+ efflux has little effect on the Na^+-K^+ pump activity (428). The increased $[Na^+]_i$ associated with muscle fatigue would stimulate the pump, but maximal activity may require 10 mM $[Na^+]_i$ (428). Medbo and Sejersted (345) showed that both the rise of plasma K^+ concentration during maximal exercise in humans and its decline during recovery followed exponential time courses with a half time of 25 s. Postexercise, the plasma K^+ content fell below the preexercise level by 0.5 mM, which suggests that the sensitivity of the pump was stimulated by the exercise (166). It is apparent from these results that fatigue resulting from cell depolarization (increased $[K^+]_o$) would rapidly reverse within a few minutes postexercise. Thus the membrane theory cannot explain the slowly recovering phase of muscle fatigue (183). Furthermore, the finding by Aljure and Borrero (6) studying toad muscle, and more recently by Fitch and McComas in humans (175), that greater fatigue occurred at optimal compared with short muscle lengths implicates factors other than the surface and T tubular membrane in the fatigue process.

B. *Sarcolemma Action Potential*

The question of whether muscle fatigue can be attributed to disturbances in membrane excitability (either sarcolemma or T tubular) has been studied and debated for years. In 1954, Merton (346) demonstrated that a maximal voluntary effort developed the same

tension in the human adductor pollicis muscle as a tetanic stimulation elicited through the ulnar nerve, and this relationship was maintained as fatigue developed. During extreme fatigue, the sarcolemma action potentials recorded from surface electrodes did not diminish in amplitude, leading Merton (346) to conclude that fatigue was not caused by neuromuscular junction or sarcolemma failure, but rather by events within the muscle cell. A similar conclusion was reached by Edwards and Lippold (155) following their observation of an increased electrical activity during a continuous isometric contraction of a given strength. As muscle fiber fatigue progressed, additional motor units were recruited to maintain the same voluntary tension. However, in the 1970s, a number of investigators observed a good correlation between reduction in the electromyogram (EMG) amplitude and muscle force (see Hultman and Sjöholm, Ref. 257), which led to the hypothesis that excitation failure caused fatigue. Subsequently, it was recognized that the EMG frequently increased early in the development of fatigue, and after fatigue, both the EMG and surface action potentials recovered rapidly (<1 min), while force remained depressed (54, 224, 257, 365, 366). Consistent with the membrane fatigue hypothesis, Sandercock et al. (423) found a high correlation between the amplitude and duration of single motor unit action potentials (MUAP) and tension during and after stimulation at high frequencies. However, a poor correlation was observed during low-frequency stimulation, and the variables were completely uncoupled following stimulation as the MUAP quickly recovered while force remained depressed. Hultman and Sjöholm (257) and Edwards (156) concluded that EMG measurements can be misleading as an index of muscle fatigue. Recently, Bevan et al. (43) observed single motor unit force to decline considerably faster than the EMG signal during a fatigue test. They concluded that at least the early phase of fatigue could not be caused by an altered surface membrane activation.

Brown and Burns (70) calculated that the average response frequency of cat muscle declined from 100 to 25/s after 1,000 stimuli of the nerve at 100/s. They concluded that although neuromuscular block occurred at frequencies of stimulation within the physiological range, the block was not responsible for the fall in tension. They reasoned that the prolonged twitch duration allowed the muscle to compensate for the loss of tension due to its failure to respond to every nerve impulse. Krnjevic and Miledi (295) observed a reduced sarcolemma action potential frequency with fatigue in rat skeletal muscle, and Lüettgau (320) found action potentials to drop out without effect on mechanical force when frog muscle fibers were stimulated at frequencies above 40 Hz. In fact, Jones et al. (271) and others (47, 350) have shown an increased force when the frequency of stimulation was reduced following high-frequency fatigue. A partial explanation for this observation [one eluded to by the early work of Brown and Burns (70)] is that the optimal stimulation frequency for force development decreases as muscle fatigue develops. Additionally, the degree of action potential dropout during high-frequency stimulation (100–150 Hz) can reach 50–70% (Ref. 320; and Balog, E. M., L. V. Thompson, and R. H. Fitts, unpublished observations). Thus, in fatigued muscle, the sarcolemma action potential frequency in response to 150 Hz of direct muscle stimulation averages 75 Hz and can be as low as 45 Hz (unpublished observations). At the maximal in vivo activation rate of ~50 Hz (134), the action potential dropout rate would be small and unlikely to fall below the optimal for force development (320). Bigland-Ritchie and co-workers (46, 49, 51) have shown fatigue in humans to be associated with a reduced motor nerve firing frequency. They suggested that the reduced frequency was mediated by a mechanism whereby the slowed contractile process somehow triggered a reflex inhibition of nerve activation. Rather than cause fatigue, the reduced frequency protected against fatigue by shifting activation to a lower more optimal rate.

Muscle fatigue is frequently associated with changes in the sarcolemma action potential characterized by a reduced amplitude, a prolonged duration, and an increased amplitude of the early negative afterpotential (31, 219, 225, 226, 269, 305, 306, 348). However, the question that remains unanswered is whether or not these changes compromise the muscle's ability to generate force. If the amplitude of the action potential was reduced enough, it would fail to initiate or at least reduce the T tubular charge movement, which in turn would inhibit SR Ca^{2+} channel opening and Ca^{2+} release (397, 398). The results of Benzanilla et al. (31) and Grabowski et al. (219) argue against this possibility. They found a reduction in the size of the spike amplitude, even to the point of no overshoot (0 mV), to have no effect on peak tension. Furthermore, Metzger and Fitts (348) showed that the muscles's peak force-generating capacity was significantly more depressed after high- compared with low-frequency stimulation, yet the action potential amplitudes were reduced to the same amount. The action potential recovered from fatiguing stimulation considerably faster than force (297, 348). Sandow (424) observed no fixed relationship between the size of the action potential and force output and concluded that a considerable "safety factor" exists regarding the extent of depolarization necessary for full activation.

As reviewed in this section, the membrane hypothesis directly links fatigue to the exercise-induced increase in $[K^+]_o$. The resulting sarcolemma and T tubular depolarization would increase the excitation threshold, decrease the action potential spike height and conduction velocity, and possibly result in complete inactivation (269). In support of this view, Jones (269) observed action potentials similar to those seen with high-frequency fatigue when $[K^+]_o$ was increased from 5 to 10 mM.

C. T Tubular System

Changes in the ionic environment of the cell would be the greatest in the depths of the T tubular system,

where diffusion limitations might exist. Hodgkin and Horowicz (249) were the first to show that repolarization of a muscle fiber following a change in the extracellular K^+ from high to low required seconds. They attributed the slow repolarization time course to the time required for T tubular K^+ to equilibrate with the $[K^+]_o$. This observation led to the suggestion that the late afterpotential following tetanic stimulation was caused by the accumulation of K^+ in the T tubular system (190). Conduction velocity has been reported to be slower within the T system compared with the fiber surface (213).

1. *Physiological role of the T tubular system*

Since the work of Costantin (109) and Huxley and co-workers (summarized in Ref. 261), it has been recognized that a primary role of the T tubular system is to allow the sarcolemma action potential to propagate into the core of the fiber via the T tubules. It has also been recognized for some time that a large percentage of the surface area of the T tubular system is in close proximity to the terminal cisternae of the SR (387). The degree of the T tubule-sarcoplasmic reticulum (T-SR) junctions varies, being more highly developed in fast- compared with slow-twitch fibers (388). The coupling mechanisms between the propagation of the action potential into the T tubules and the subsequent release of Ca^{2+} from the SR have yet to be completely worked out. However, since Schneider and Chandler (426) first observed a T tubular charge movement during cell activation, considerable progress has been made (72, 396, 398, 399). It is now generally believed that the T tubular action potential is sensed by an intramembranous T tubular protein [dihydropyridine (DHP) receptor], which during activation undergoes a voltage-driven conformational change (T tubular charge movement) that in turn somehow triggers Ca^{2+} release from adjacent SR Ca^{2+} channels (397–399). The T tubular voltage sensor of E-C coupling has an essential Ca^{2+} binding site on its extracellular (T tubular lumen) side (71, 72, 398, 399). Consequently, metal-free conditions suppress intramembranous T tubular charge and the amplitude of the Ca^{2+} transient (71, 72, 391, 398, 399).

2. *Activity-induced change in T tubular function*

Activity-induced changes in the ionic status of the T tubular lumen and intracellular fluid compartments could contribute to the development of muscular fatigue by directly reducing the extent of T tubular charge movement. This effect could occur independently of any change in the amplitude and/or conduction velocity of the T tubular action potential. While an elevated T tubular Ca^{2+} might mediate fatigue by blocking conduction of the action potential into the axial core of the fiber (253), low Ca^{2+} could directly reduce intramembranous T tubular charge movement (71, 391), leading to a reduced Ca^{2+} release and force production.

Howell and Snowdowne (254) observed a linear fall in peak tension as extracellular Ca^{2+} was increased from 1 to 20 mM. This treatment had no effect on the surface action potential amplitude, but the conduction velocity was slowed. These authors (254) and Howell and Oetliker (252) suggested that an activity-induced increase in T tubular Ca^{2+} might slow the T tubular action potential conduction velocity to the point of conduction block, producing incomplete activation of the axial core of the fiber. High Ca^{2+} altered the early afterpotential, the origin of which has been attributed to T tubular conduction events (190, 249, 252). Howell and co-workers (252, 253) also observed myofibril waviness in the axial core of fibers activated in 10 mM Ca^{2+}, and attributed this to T tubular action potential conduction failure. Recently, Garcia et al. (195) found a similar condition following electrical stimulation of single frog muscle fibers. The extent of wavy myofibril formation increased in parallel with the development of muscle fatigue, and caffeine contractures eliminated wavy myofibrils and restored tension. Previously, Taylor and Rüdel (461) observed wavy myofibrils in nonfatigued fibers at short sarcomere lengths; however, Garcia et al. (195) report that wavy myofibrils were never seen in prefatigued fibers. They concluded that fatigue was caused by either a failure of the tubular action potential or the conduction signal between the T tubular system and the terminal cisternae. González-Serratos et al. (214) and Somlyo et al. (443) found the preferential development of vacuoles in the T tubular system of fatigued muscle fibers, and with recovery the vacuoles disappeared. In their recent work, Garcia et al. (195) suggested that the activation failure may occur at the site of tubular vacuolation. Consistent with the theory of T tubular conduction block, Westerblad et al. (489) found high-frequency stimulation to produce a spatial gradient of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), with higher concentrations near the edges of the fiber. The authors (489) attributed the low Ca^{2+} in the fiber center to action potential conduction block in the T tubule network. To contribute significantly to the force decline, T tubular Ca^{2+} would have to approach 15 mM during contractile activity [see Fig. 2 of Howell and Snowdowne (254)]. Bianchi and Narayan (44) employed $^{45}Ca^{2+}$ to measure T tubular Ca^{2+}. They concluded that T tubular Ca^{2+} could reach 28 mM following as few as 120 twitches. However, they failed to consider the movement of Ca^{2+} out of the T tubules via diffusion either into the cell or intercellular space.

The extent to which T tubular Ca^{2+} or K^+ increases or Na^+ decreases or whether the T tubular ionic environment changes at all with the development of muscle fatigue is far from established. It could be argued that a modest increase in T tubular Ca^{2+} (increases of 5–10 mM) would serve to stabilize the DHP receptor, thus preserving charge movement and reducing the likelihood of fatigue (71, 72, 391, 399). In a classic paper, Lüettgau (319) demonstrated that the resting V_m was

depolarized and the peak K^+ contracture tension reduced in low extracellular Ca^{2+} (0.2 mM), and the latter was inhibited completely in zero extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$). Perhaps more importantly, he observed the threshold potential to be altered by $[Ca^{2+}]_o$. Low $[Ca^{2+}]_o$ shifted the activation threshold to the left, with weak contractions recorded at 10-20 mM $[K^+]_o$ or a V_m of −60 mV. Increasing extracellular calcium from the control value of 1.8 to 5 mM increased the threshold potential from −35 to −18 mV (319). Consistent with this observation, Shelvin (431) observed high external Ca^{2+} to shift the T tubular charge-voltage relationship to the right, such that the half-maximal charge was obtained at more depolarized potentials. Additionally, after a 190 mM KCl contracture, fibers in 5 mM $[Ca^{2+}]_o$ were able to reprime to a greater extent at a given level of depolarization. For example, in 1.8 and 5.0 mM $[Ca^{2+}]_o$, repriming reached 50% at −37 and −25 mV, respectively. The effects of extracellular calcium on the activation threshold were confirmed by Frankenhaeuser and Lännergren (188). Recent data from Rios and coworkers (391, 399, 400) demonstrate that high $[Ca^{2+}]_o$ would also increase the rate of repriming. Grabowski et al. (219) have shown that the activation threshold was shifted by ~20 mV more positive following fatigue. In addition, the peak contracture elicited by 190 mM KCl was reduced in the fatigued fibers (219). The rightward shift in the activation threshold in the fatigued fibers would be expected if T tubular $[Ca^{2+}]_o$ increased (71, 319, 431). However, if T tubular calcium does not exceed 5 mM, the threshold for activation would be reached by −20 mV and full activation obtained at 0 mV (212, 319, 431). Because the action potential spike height generally reaches 0 mV even in extreme fatigue (226, 305, 306, 348), this shift in the voltage-tension relationship should not contribute to fatigue. The high T tubular Ca^{2+} might help prevent fatigue both by stabilizing the resting V_m and decreasing the likelihood of inactivation that might occur with depolarization. Although the sarcolemma action potential spike height does not appear to decline enough to prevent activation, the possibility exists that the amplitude is considerably less in the depths of the T tubule, and/or that complete inactivation occurs in the core of the fiber.

Because the electrochemical gradient favors Ca^{2+} influx, and the T tubular calcium channels are opened by depolarization to 0 mV (27), the possibility exists that T tubular calcium drops with fatigue. Lüettgau et al. (321) employed the voltage-clamped technique to study the effects of zero extracellular calcium on the activation of frog single fibers. They showed a shift of the steady-state potential dependence of force inactivation to more negative potentials. Furthermore, prolonged depolarization has been shown to cause inactivation of T tubular charge movement (90). If T tubular depolarization develops with fatigue, it would be more likely to cause inactivation if T tubular Ca^{2+} was reduced (71, 321). Lüettgau et al. (321) suggested that $[Ca^{2+}]_o$ might stabilize the T tubular membrane and that depolarization might reduce the affinity of Ca^{2+} as "a force-controlling system in the T-tubular membrane." Rios and co-workers (71, 391, 396, 398) have identified this site as the DHP receptor or the charge sensor of the T tubular membrane. They demonstrated that charge movement and Ca^{2+} release were inhibited in zero extracellular calcium. In the experiments of Lännergren and Westerblad (305), V_m depolarized from −80 or −90 mV to −50 mV with fatigue. In normal Ca^{2+}, the effect on intramembranous T tubular charge movement would be negligible; however, in low Ca^{2+}, considerable inactivation of charge movement and Ca^{2+} release would exist (see Fig. 5, Refs. 71 and 73), and severe inhibition of force would be expected (see Fig. 2 in Ref. 321).

An important component in the regulation of T tubular Ca^{2+} would be the density and activity of the T tubular Ca^{2+}-ATPase pumps. Although the T tubular membranes of skeletal muscle are known to contain Ca^{2+}-ATPase pump sites (44, 45, 428), their activity during the development of muscle fatigue is unknown. The increase in resting $[Ca^{2+}]_i$ associated with fatigue (8) would be expected to activate the pump sites, but other factors, such as an increased intracellular H^+, might inhibit the Ca^{2+}-ATPase pumps.

D. T Tubule-Sarcoplasmic Reticulum Junction and Calcium Release From Terminal Cisternae

1. Physiological characteristics of T tubule-sarcoplasmic reticulum junction

In the past 5 years, considerable progress has been made in elucidating the structure and function of the T-SR junction (398). Since the work of Franzini-Armstrong and collaborators (170, 189), it has been recognized that the T tubular and the SR membranes are separated by 100 Å and that this gap is spanned by electron-dense protein structures called SR feet. Recently, these feet structures have been identified as the Ca^{2+} release channel of the SR, and the channel has now been cloned and sequenced (459). The channels are activated by ATP and micromolar Ca^{2+}, whereas high Ca^{2+} inhibits channel opening (262, 324). Structurally, the channels are tetramers aligned in two parallel rows (171, 302). Every other foot process is arranged directly opposite and likely touches an intramembranous T tubular protein, a tetramer identified as the charge sensor of E-C coupling (58, 398, 399). The exact mechanism of transduction across the T-SR junction has not yet been established. The morphological relationship between the SR Ca^{2+} release channel (ryanodine receptor) and the intramembranous T tubular charge sensor (DHP receptor) supports the hypothesis that the main mechanism involves mechanical or allosteric coupling (397, 399). Two additional mechanisms, Ca^{2+}-induced Ca^{2+} release and activation by inositol trisphosphate ($InsP_3$), are thought to play secondary or modulatory roles in regulating Ca^{2+} release in skeletal muscle (397, 398, 400). The observation that vertebrate skeletal muscle can

contract in the absence of external Ca^{2+} eliminates Ca^{2+} influx as a primary trigger in E-C coupling (11, 397). However, it has recently been suggested that Ca^{2+} released from the SR may activate the sensorless SR channels or those not facing T tubular charge sensors (398). The released Ca^{2+} may bind to the charge sensor as well, causing additional charge movement (Q_γ) and SR channel opening (118). The arguments for and against a role for $InsP_3$ in T-SR transmission in skeletal muscle has been reviewed by Hidalgo and Jaimovich (239) and Rios et al. (397). The major evidence against a primary role for $InsP_3$ is the observation that high sensitivity of the Ca^{2+} release channel to $InsP_3$ occurs only after inactivation of the voltage sensor by prolonged depolarization. This suggests that any effect of $InsP_3$ would be redundant (397).

2. *Activity-induced changes in calcium release*

Blinks et al. (57) and, more recently, Allen et al. (8), Westerblad et al. (489), Westerblad and Allen (483), and Gyorke (223) have demonstrated that the amplitude of the Ca^{2+} transient decreases as fatigue develops. The question remains whether this decrease is due to blockage of the T tubular action potential, a reduced intramembranous T tubular charge movement, an inhibition of the SR Ca^{2+} release channel, or depletion of releasable Ca^{2+} from the SR. Although the last possibility may contribute to the reduced Ca^{2+} transient during intense contractile activity, it seems unlikely in view of the observation of Eberstein and Sandow (149) to be wholly responsible. These investigators demonstrated that caffeine, a compound stimulating direct release of Ca^{2+} from the SR, reverses the tension loss of fatigued fibers. This observation has since been confirmed by Grabowski et al. (219), Vergara et al. (474), Nassar-Gentina et al. (372), Lännergren and Westerblad (307), Garcia et al. (195), and Westerblad and Allen (483). Furthermore, fatigued muscles exposed to high K^+ respond with high levels of Ca^{2+} release and force contractures (8, 219). If fatigue was caused by depletion of SR Ca^{2+}, caffeine and K^+ contractures would fail to produce significant tension. Additionally, electron-probe analysis also supports the theory that the SR Ca^{2+} stores were not depleted with stimulation to fatigue (214). Recently, Gyorke (223) observed no decline in T tubular charge movement despite a significant reduction in the amplitude of the Ca^{2+} transient during fatiguing stimulations of single frog fibers. He concluded that the reduced Ca^{2+} transient was caused by a direct inhibition of the SR Ca^{2+} release channel and not to disturbance in T tubular action potential or the DHP charge sensor.

The rate and extent of Ca^{2+} release from the SR could decline during contractile activity, even without an altered action potential or intramembranous T tubular charge movement, if either the coupling step between the T tubular and SR membrane or the release process itself were inhibited. Vergara et al. (473) and Volpe et al. (476) have suggested that the linking step may involve the second messenger $InsP_3$. As reviewed in section III*D*, it seems unlikely that $InsP_3$ plays a primary role in triggering Ca^{2+} release. Nevertheless, myoplasmic $InsP_3$ has been shown to increase rapidly with stimulation (380), and depletion of $InsP_3$ during contractile activity could facilitate the onset of fatigue.

The positive-feedback effects of Ca^{2+} on both the ryanodine and DHP receptors might be inhibited during the development of fatigue. For example, H^+, known to increase with intense contractile activity, might inhibit the release process. After reconstitution of the purified ryanodine receptor channel into a lipid bilayer, Ma et al. (324) studied the pH dependence of the release channel. Reducing pH on either side of the bilayer resulted in a decrease in the frequency and duration of channel openings. Open probability was maximum at pH 7.4 and decreased to almost null at pH 6.5. Rousseau and Pinkos (403) found acidification (pH 7.4 to 6.6) of the myoplasmic side of the SR lipid bilayers to decrease the open probability of the Ca^{2+} release channel, whereas acidification of the luminal side decreased channel conductance but not the open probability. They suggested that the rate of protonation might change the affinity of the Ca^{2+}-activating binding sites. Although it has not yet been studied, the possibility exists that an increased free H^+ inhibits Ca^{2+} binding to the myoplasmic surface of the T tubular charge sensor, thus reducing the Q_γ component of charge movement and the amplitude of the Ca^{2+} transient.

The large increase in twitch contraction and relaxation times (Fig. 4) associated with muscle fatigue can only be caused by a prolonged Ca^{2+} transient. Although the evidence reviewed above suggests that the releasable stores of SR Ca^{2+} are not depleted with the onset of fatigue, a redistribution of Ca^{2+} from the SR release site to the Ca^{2+}-binding protein parvalbumin and the SR pump must occur. This redistribution would decrease the driving force for Ca^{2+} release, producing a reduced rate of release. The prolonged Ca^{2+} transient can in part be explained by the redistribution placing a greater load on the reuptake process. Cannell (86) demonstrated that the rate of $[Ca^{2+}]_i$ decline following a tetanic contraction decreased as the tetanus duration increased. This result was attributed to the saturation of intracellular calcium buffers during the tetanus. Additionally, the SR Ca^{2+} pump rate may be slowed by elevated H^+ in fatigued muscle fibers (82, 371). Due to the rapid dissociation of Ca^{2+} from parvalbumin, any slowing of relaxation attributable to the saturation of the Ca^{2+}-binding sites of parvalbumin would be reversed within 1 min of recovery.

Allen et al. (8) employed the photoprotein aequorin to study the Ca^{2+} transient in *Xenopus laevis* fibers. They showed that the slowing of relaxation in the fatigued state was associated with a slowing of decline of the aequorin light signal. In both this study and that of Westerblad and Allen evaluating mouse muscle (483), the amplitude of the Ca^{2+} transient initially increased as force declined, after which both force and the Ca^{2+} signal declined. This result is shown in Figure 8 [redrawn from Westerblad and Allen (483)]. It is also ap-

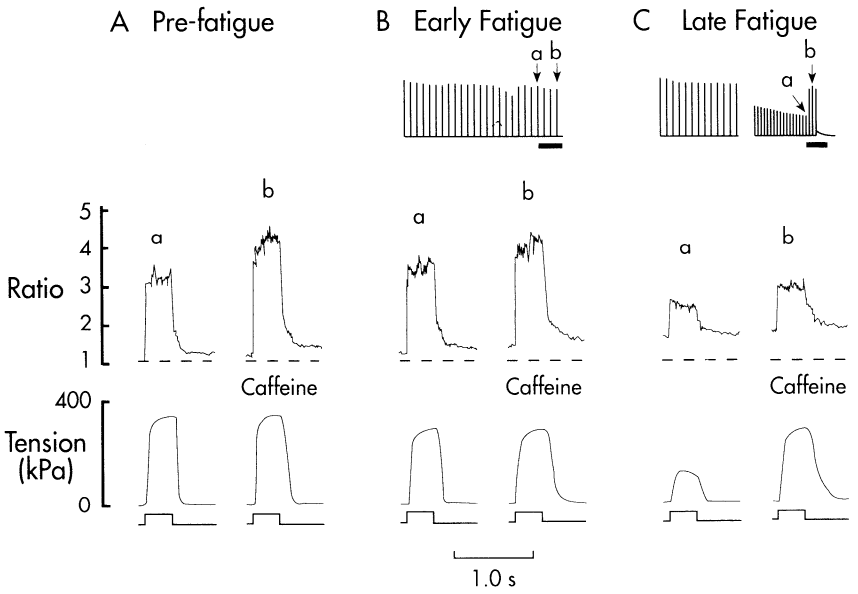

FIG. 8. Application of 10 mM caffeine in control (*A*) and during 2 successive fatigue runs (*B* and *C*). Bars below tension records in *top panels* indicate caffeine exposure during fatiguing stimulation; caffeine was applied after 22 fatiguing tetani (*B*) and when tetanic tension was depressed to 0.36 times peak tetanic tension by 187 tetani (*C*). Fluorescence ratio and tension records from tetani elicited before application of caffeine (*a*) and in presence of caffeine (*b*) are shown in *middle* and *bottom panels*. Dashed lines represent resting ratio in control; stimulation periods are displayed below tension records. Note that tetanic ratio increase induced by caffeine in late fatigue was accompanied by a substantially enhanced tension production, whereas tension was not markedly affected by increased ratios in the other 2 states. [From Westerblad and Allen (483). Reproduced from *The Journal of General Physiology*, 1991, vol. 98, p. 615–635, by copyright permission of the Rockefeller University Press.]

parent (Fig. 8) that the resting $[Ca^{2+}]_i$ rose continuously throughout the stimulation period. The authors (483) concluded from these results that fatigue was initially caused by metabolic factors (such as an increased H^+ or P_i) acting via a direct inhibition of the cross bridges. They attributed the early rise in tetanic $[Ca^{2+}]_i$ to a saturation of the myoplasmic Ca^{2+} buffers rather than to an increased Ca^{2+} release from the SR. As stimulation continued, a reduced Ca^{2+} release became quantitatively more important in the fatigue process (483). The observation that 10 mM caffeine had little effect early in fatigue but greatly increased both the $[Ca^{2+}]_i$ and force late in fatigue supports their contention that the depression of Ca^{2+} release and its contribution to fatigue develops after the initiation of fatigue by Ca^{2+}-independent factors acting directly at the cross bridge.

IV. LACTIC ACID, INTRACELLULAR PH, AND FATIGUE

A. Historical Perspective

The major source of acid production in skeletal muscle is the anaerobic production of lactic acid from glucose and glycogen (206, 282, 404, 405, 407, 408). At physiological pH, the acid produced primarily dissociates into lactate and H^+. A relationship between lactic acid production and muscle activity has long been recognized. In the article entitled "Lactate and phosphagen concentration in working muscle of man," Karlsson (282) states that "Berzelius wrote to Lehmann in 1841 stating that he had found lactic acid in the muscles of exhausted game." Writing in 1902, Fletcher (186) states, "In 1795 von Humboldt showed that a frog's muscle when excised kept its irritability longer in oxygen than in air or hydrogen." Fletcher and Hopkins (187) concluded that oxygen delayed, but did not prevent, the loss of irritability in frog muscles contracted in vitro. However, in the late 1800s, considerable controversy existed as to whether or not muscle contraction elicited lactate formation. Fletcher and Hopkins (187) concluded that the controversy was fueled by the improper determination of lactate in "fresh" unstimulated muscles, which led to high lactate in both the fresh and stimulated muscles. This 1907 publication entitled "Lactic acid in amphibian muscle" (187) is considered a classic, since it clearly established that lactic acid was produced during contractile activity and laid the foundation for the hypothesis that lactic acid caused muscle fatigue. The work established that resting muscles in oxygen produced little lactate, while under anaerobic conditions lactate production was high. With stimulation, Fletcher and Hopkins (187) observed lactate production to increase 10-fold, a finding that agrees amazingly well with more recent studies in which lactate was measured with enzymatic techniques (20, 34, 230, 281, 282, 421). The lactate content of stimulated muscle never exceeded 0.28%, which was considerably less than that obtained by chemical irritants. The authors (187) suggested that the lactic acid may self-limit by inducing fatigue in the stimulated muscle. This concept was supported by the observation of a relationship between both the development of and recovery from fatigue and the appearance and disappearance of lactic acid (32, 187, 282). During the 1920s, Meyerhof (see Karlsson, Ref. 282) observed a relationship between glycogen usage and lactate production in stimulated frog muscle, while Meyerhof and Lohmann (see Furusawa and Kerridge, Ref. 193) reported frog muscle pH to decline from 7.11 at rest to 6.31 after fatigue. In 1927, Furusawa and Kerridge (193) found a similar change in cat gastrocnemius muscle where, with stimulation, pH declined from 7.04 to 6.26. During this time, Hill and colleagues (161, 243, 244) recognized the importance of cell buffers in the handling of lactic acid and showed the relationship between exercise intensity, blood lactate, and oxygen debt in humans. In the 1930s and 1940s, it was recognized that after an ini-

tial increase, blood lactate decreased if the work was continued beyond 10 min and that muscle lactate must be higher than blood lactate (23, 282). It was clear by this time that the etiology of fatigue during prolonged endurance exercise was not caused by changes in cell lactate or pH, while the hypothesis that high-intensity exercise was limited by muscle lactate gained further support from the work of Asmussen and co-workers (19, 282). Following Bergström's 1962 paper (33) in which he introduced the needle biopsy technique to the study of exercise metabolism in humans, the relationship between muscle lactate and pH, and their role in limiting muscle and whole body performance, has been extensively studied (176, 202, 282, 406). In recent years, studies on living and skinned single fibers have provided important information on the cellular mechanisms of muscle fatigue and established that the relationship between lactic acid content and muscle fatigue is mediated indirectly through the associated decrease in cell pH (152, 176, 200, 352, 355). In sections IV*B* and IV*C*, I review selected results on the relationship between muscle lactate and exercise intensity, discuss the mechanisms of lactate transport, and describe in detail the role of cell pH in muscle fatigue.

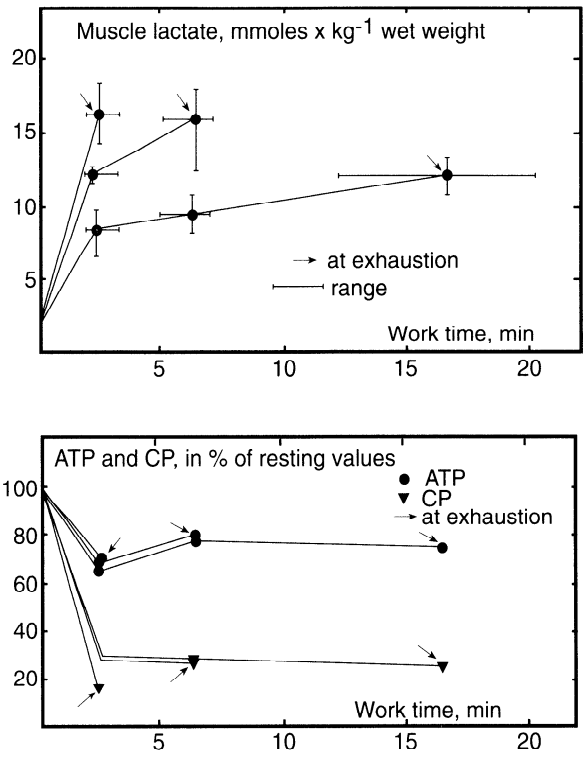

FIG. 9. Mean value and range for lactate (*top panel*) and ATP and phosphocreatine (CP) concentrations (*bottom panel*) in exercising muscle during and immediately after exhaustive exercise expressed as percent of resting value. [From Karlsson and Saltin (284).]

B. Muscle Lactate

1. Blood and muscle lactate and work capacity

At work loads above 50–60% of the maximal aerobic capacity, blood lactate concentration increases and generally averages 10–20 mM following short duration maximal exercise (230, 282, 405). Higher blood lactates are observed following maximal intermittent compared with continuous exercise, with the highest values associated with exercise protocols involving a large muscle mass (230, 282, 419). Osnes and Hermansen (384) observed blood lactates as high as 32 mM following intermittent 40- to 60-s maximal exercise bouts. Maximal muscle lactate is ~10 mM higher than blood lactate, and significant increases can occur within 10 s of the initiation of supramaximal exercise (135, 267, 281, 282). Karlsson and Saltin (286) found high muscle lactates to be consistently associated with exhaustion following repeated 1-min bouts of maximal bicycle exercise. The average muscle lactate following the first bout (23.2 mM) was not significantly different from the 22.5 mM obtained after the fifth bout. In contrast, ATP and phosphocreatine (PCr) showed progressive depletion with each bout of activity, while the subjects were equally exhausted after the first bout as after the fifth bout. In another study, Karlsson and Saltin (284) studied metabolic changes following three different work loads leading to exhaustion at 2, 6, and 16 min of bicycle exercise. Muscle lactate averaged 16.1 mM at the two highest loads but only 12.0 mM at the lowest load (Fig. 9). They concluded that fatigue at the two highest loads may have been caused by the high lactate, while fatigue at the lowest load was clearly dependent on other factors. Isometric contraction of the knee extensor muscles at 30 and 50% of maximal voluntary contraction (MVC) resulted in high lactates at fatigue, while contractions at lower (20% MVC) and higher (80% MVC) loads yielded lower lactates (283). These observations led Karlsson et al. (283) to conclude that lactate or the associated change in pH may have caused fatigue at the intermediate loads, but not at the higher or lower tensions. High muscle lactates have also been observed following heavy dynamic knee extension exercise. In one study, muscle lactate increased from 2 to 28 mM, and the net lactate release was 16.2 mmol/min at exhaustion (24). This produced a total lactate efflux of 14.8 mM or ~50% of the total accumulated in the muscle during the exercise period.

It is well known that fast muscles and fast single motor units fatigue more rapidly and to a greater extent than slow muscles and slow motor units (77, 153, 310). After both dynamic and static contractions to exhaustion in humans, the fast type II fiber contained higher lactate (25–27 mM) than the slow type I fiber (15.8 mM) (165), and a high correlation (r = 0.86) was observed between fatigability and percent fast type II fiber content (469). In skeletal muscles stimulated in vitro, the fast EDL fatigued faster and showed higher lactates and lower pH than the slow-twitch soleus (471). In both muscles, the lactate increase and pH decrease were both highly correlated with the decline in tension. In situ

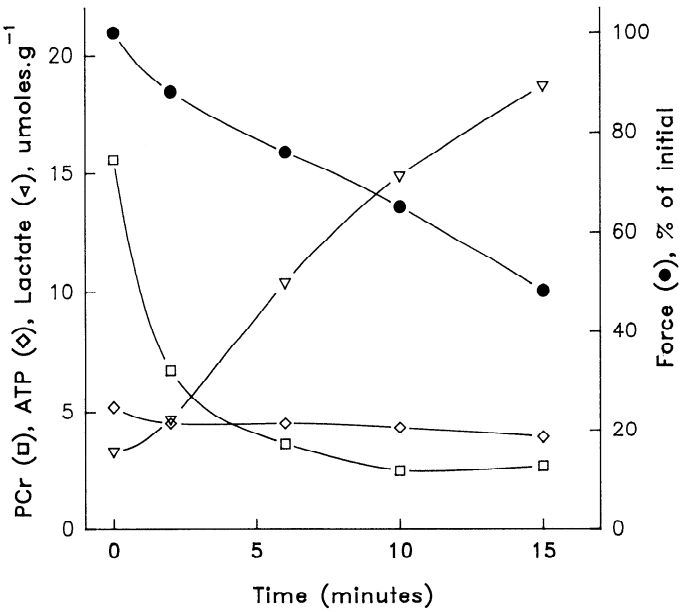

FIG. 10. Changes in peak train force (●), lactate (▽), ATP (◇), and phosphocreatine (PCr; □) concentrations during development of fatigue in isolated frog sartorius muscles stimulated at 30 single pulses/min under anaerobic conditions. All data except force were redrawn from Fitts and Holloszy (180). Force data were redrawn from Fitts and Holloszy (182). [From Fitts (176). Copyright (c) 1992 Wm. C. Brown Communications, Inc., Dubuque, IA. All rights reserved. Reprinted by special permission.]

stimulation via the motor nerves produced similar results (59). After 5 min of 100-Hz stimulation (200 ms each second), the EDL and soleus muscle lactates were 87 and 9.9 mmol/kg dry wt, respectively, while at 4 min, tension had decreased by 32% in the EDL and 14% in the soleus. Regardless of whether muscle fatigue is produced by in vitro or in situ stimulation of an isolated muscle or from in vivo exercise, if the work requires a high glycolytic rate, the decline in force will be highly correlated with an increase in muscle lactate. This point is clearly shown by the comparison of the data of Fitts and Holloszy (180) and Spriet et al. (451), presented here as Figures 10 and 11. The change in force (percent of initial), ATP, PCr, and lactate with electrical stimulation of the frog sartorius in vitro is shown in Figure 10, and the anterolateral aspect of the human thigh in vivo is shown in Figure 11. Although the time course of change (x-axis) is faster in the in vivo human experiment, this can be attributed to the higher stimulation frequencies employed (20 Hz vs. single pulse). Importantly, the relationship between the decline in force and the change in lactate is essentially identical in both experiments despite the vastly different stimulation conditions and muscles studied.

2. *Mechanisms of lactate efflux and the effects of extracellular pH*

It has long been realized that muscles perform better in high-bicarbonate solutions and that external pH can affect the functional capacity of limb skeletal muscle (32, 242, 275, 282). In high bicarbonate (25 meq/l), the fatigue-induced prolongation of relaxation following a tetanus is less and recovery of force is faster than muscles incubated in low bicarbonate (1 meq/l) (326, 328, 331). The faster recovery of force in the high bicarbonate was associated with a faster lactate efflux (326, 331). Despite the faster lactate efflux in high bicarbonate, the decline in force during stimulation was not affected by the external bicarbonate concentration. Whether or not a reduced extracellular bicarbonate affects the degree of fatigue seems to depend on the duration and intensity of the exercise. With high-intensity short-duration exercise, a reduced extracellular buffer had no effect; however, an increased fatigability was observed in heavy endurance exercise in humans and during prolonged in situ stimulation of the rat hindquarter (336, 449). The experiments of Mainwood and Worsley-Brown (330) demonstrated that the lactate efflux rate is dependent on both external pH and external buffer concentration. The efflux rate was reduced when either the pH was reduced at a constant buffer concentration or when the buffer concentration was decreased at a constant pH. The reduced efflux of both lactate and H^+ slowed or prevented the recovery of tension; in fact, Mainwood and colleagues (329, 330) observed a secondary fall in muscle tension under these conditions during the first 20 min poststimulation. Additionally, at low buffer concentrations (<10 mM), the lactate and H^+ efflux become uncoupled, and the lactate efflux exceeds that observed for H^+ (330). This result suggests that lactate does not efflux simply as the undissociated acid. Mainwood et al. (329) reviewed the possible mechanisms for lactate ef-

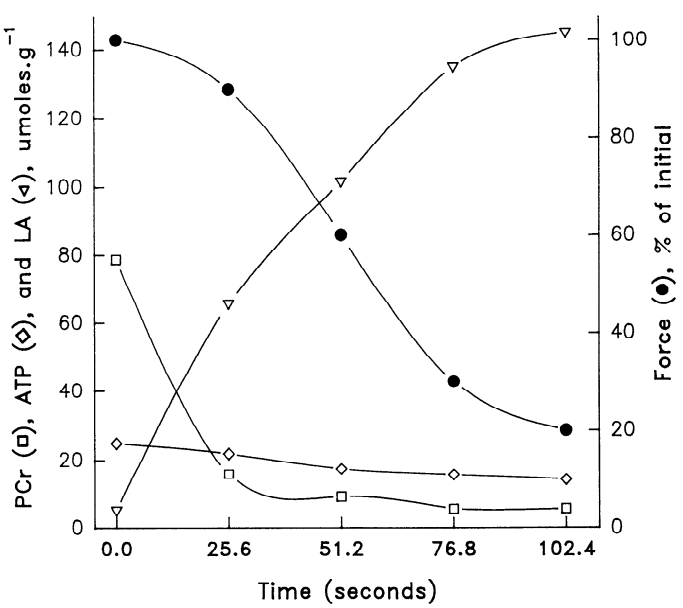

FIG. 11. Human quadriceps contractile force (●), lactate (LA; ▽), ATP (◇), and PCr (□) concentrations during intermittent electrical stimulation (20-Hz 1.6-s pulses separated by 1.6-s rest periods) with occluded circulation. Data redrawn from Spriet et al. (451). [From Fitts (176). Copyright (c) 1992 Wm. C. Brown Communications, Inc., Dubuque, IA. All rights reserved. Reprinted by special permission.]

flux and concluded that muscles lose lactate by three mechanisms: diffusion of the undissociated acid, ionic diffusion of lactate, and via a proton-linked carrier-mediated transport. A fourth mechanism, bicarbonate-linked anion exchange, is thought to be unimportant, since normal lactate efflux was observed in non-bicarbonate-buffered systems and in the presence of the anion-exchange inhibitor 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (277, 330). The existence of a proton-linked lactate carrier has been demonstrated in both mouse soleus and frog sartorius muscle. In both muscle types, the lactate efflux was partially inhibited by cinnamate, a known inhibitor of the monocarboxylate carrier (277, 335). In mouse soleus, cinnamate inhibited 80% of the lactate exchange, which suggests a dominant role for facilitated diffusion of lactate (279). Immediately after stimulation, when the cell pH is low, the contribution of the undissociated lactic acid to the total efflux would be somewhat higher than later in recovery when cell pH has returned to 7.0 (277). This would be particularly true following extremely heavy exercise in which high muscle lactate might saturate the carrier mechanism. Efflux of the undissociated acid and the facilitated diffusion of lactate via a proton-linked carrier would both be limited by the extracellular diffusion of protons, and thus affected by the extracellular buffer concentration. Mainwood et al. (329) also observed a buffer-independent lactate efflux that they attributed to free lactate ion efflux.

3. *Relationship between muscle lactate and cell pH*

With fatigue, the osmotic pressure within muscle rises, causing increased intracellular water and cell swelling (24, 34, 161, 409). The increased muscle water with exercise was caused by an increase in extra- as well as intracellular water content, and the intracellular water change was highly correlated with the increase in lactate (34, 409, 415). The lactate-induced fiber swelling would affect the lateral spacing of the contractile filament lattice, and this could reduce peak force. This effect is likely to be small, however, since skinned fibers, known to swell by ~20% compared with living fibers, showed only a small increase in P_o when cell width was compressed by dextran (199, 293, 353). High muscle lactate could significantly increase ionic strength, which in turn would depress peak force (215, 216). However, the observation of Chase and Kushmerick (91) that 50 mM lactate had no effect on the P_o of single rabbit psoas fibers provides direct evidence that the lactate ion does not directly induce fatigue in limb skeletal muscle. Sahlin and co-workers (409, 415) found a high correlation between the fall in muscle pH and the increase in lactate and pyruvate content following dynamic exercise in humans. It is now generally recognized that the high inverse correlation between lactate and force is, for the most part, dependent on the high correlation between lactate and free H^+, and that the force-depressing agent is H^+ and not lactate (351, 409).

C. *Hydrogen Ion and Muscle Fatigue*

The pH_i of both frog and mammalian skeletal muscle is ~7.0 (120, 234, 260, 351, 402, 415, 466, 494), and with high-intensity exercise, it falls to values as low as 6.2 (260, 351, 494). Representative values of pH_i in resting and fatigued muscles are shown in Table 1. Despite the employment of different techniques, the decline in pH_i with fatigue is amazingly similar across species lines. As reviewed in section IV*B*, the higher the exercise intensity the greater the decline in pH_i, with the highest fall observed in the FG fiber type. Exercise training has been shown to reduce the extent of lactate increase and pH_i decline during high-intensity exercise (230, 420, 421, 471). The reduced free H^+ concentration can be attributed to both a reduced H^+ production and an increased cell buffer capacity (408, 471). Whether determined in humans following dynamic exercise or in mammalian or frog muscle in vitro, the recovery of pH_i was exponential and showed complete recovery within 20–25 min (351, 415, 466). The pH_i recovery was considerably faster than lactate, which required from 30 to 40 min for full recovery (277, 468). The faster efflux of H^+ can be attributed to Na^+-H^+ and anion exchange systems, with the former being quantitatively more important (5, 277). In the presence of amiloride, a Na^+-H^+ exchange blocker, the time course for pH_i recovery following fatigue was doubled in mouse soleus muscle (277). A low external buffer concentration [1 mM 3-(*N*-morpholino)propanesulfonic acid] also more than doubled the time constant for the recovery of pH_i (277). Unfortunately, the effect of amiloride and low buffer concentration on the time course of tension recovery was not determined (277). Recently, Westerblad and Allen (484) found amiloride to have no effect on pH_i or the development of fatigue in mouse muscle, while the lactate transport inhibitor cinnamate caused a more rapid decline in both pH_i and tension. Clearly, under their experimental conditions, the lactate transporter was the most important proton-extruding mechanism. In frog muscle, both low external pH and low buffer concentration are known to delay the recovery of tension following fatigue (328, 330). The extent to which this inhibition was caused by a delayed recovery in pH_i is unclear. The recovery of tension was unaffected by amiloride and 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid either alone or in combination, and this observation led Mainwood et al. (328) to conclude that low extracellular pH may directly inhibit force. Alternatively, the H^+ exchange inhibitors may have had little effect on the time course of H^+ efflux. This possibility is supported by the observation that the H^+-lactate coupled mechanisms of exchange (which are known to be reduced by either low external pH or buffer content) appear quantitatively more important in frog muscle (1). The net proton load during fatigue is generally less than the lactate load due to the hydrolysis of PCr, a proton-utilizing reaction (329). Additionally, in the first few minutes of recovery, pH_i continues to fall in large part due to the resynthesis of PCr (351). The maximal rate of proton generation exceeds

TABLE 1. *Skeletal muscle pH*

Method	Rest Value	Fatigue Value	Reference Number
Muscle homogenate	6.92 ± 0.03	6.41 ± 0.04	Hermansen and Osnes (234)
Calculated from HCO_3^- + P_{CO_2}	7.04 ± 0.05	6.37 ± 0.11	Sahlin et al. (409)
Microelectrode	7.06 ± 0.04	6.33	Metzger and Fitts (349)
Microelectrode	7.00 ± 0.02	6.42 ± 0.12	Thompson et al. (466)
DMO method	7.06 ± 0.02		Roos and Boron (402)
NMR method	6.99 ± 0.04	6.17 ± 0.33	Wilson et al. (494)

Values are means ± SE, except for Sahlin et al. (409), where ±SD values are listed. DMO, 5,5-dimethyl-2,4-oxazolidinedione; NMR, nuclear magnetic resonance.

the peak efflux rate by a factor of 12, which demonstrates the rate-limiting nature of the proton efflux mechanisms (328).

1. Intracellular pH and force

As reviewed earlier, tension following fatigue produced by in vitro stimulation generally recovers in two phases: a rapid increase complete within 2 min and representing ~25% of the total recovery, followed by a slow exponential rise reaching the prefatigued value by 40 min (350, 467). The rapid initial phase of force recovery is clearly independent of H^+ as cell pH_i is falling (264, 349); however, as can be seen from Figure 12, the second slow phase shows a highly significant correlation with pH_i. This relationship has been observed in both mammalian and frog skeletal muscle and supports the hypothesis that fatigue is in part caused by the elevated H^+ (351, 466). These results conflict with the findings of Westerblad and Lännergren (487) who found pH_i to always normalize well before force during recovery from fatigue in *Xenopus* muscle fibers. A possible explanation for this discrepancy is that the *Xenopus* fibers frequently showed postcontractile depression, a delayed force suppression during the recovery period. The etiology of this phenomenon is unknown but is likely independent of pH_i. Recently, Westerblad and Allen (484) reported only a slight (0.063 units) decline in pH_i despite a 30% loss of tension in stimulated single mouse fibers. However, pH_i was determined with the fluorescent indicator 2′,7′-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF). The possibility exists that BCECF failed to accurately monitor pH_i in the fatigued cells. It is clear from the mean resting pH_i value (7.33) that the technique underestimated the free H^+ activity.

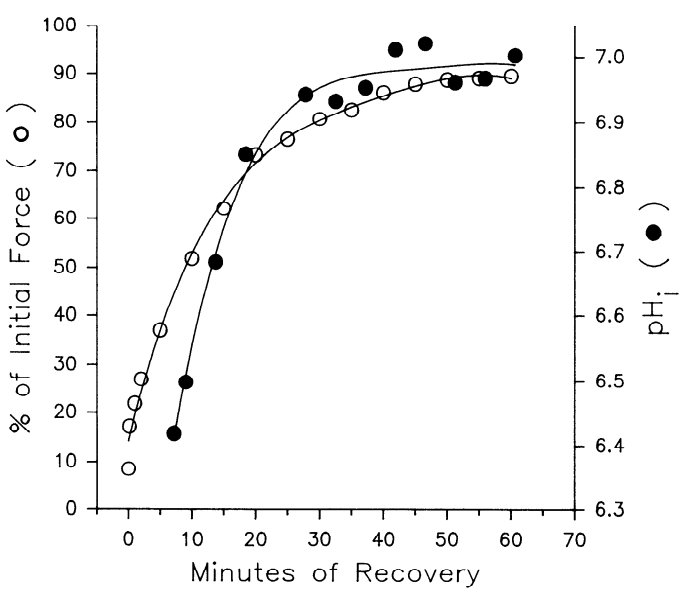

FIG. 12. Recovery of peak force, expressed as percent of initial force and intracellular pH (pH_i). Values are means, with best-fit lines drawn through points. [From Thompson et al. (466).]

Although a consensus exists that cell pH_i declines with high-intensity exercise, some question whether the pH_i change is causative in fatigue (328, 394, 413, 484, 487). However, skinned fiber studies have definitively showed acidosis to depress tension in skeletal as well as cardiac muscle (60, 140, 141, 167, 347, 349, 352). Decreasing pH from 7.0 to 6.2 not only reduced the maximal tension in the presence of optimal free Ca^{2+}, but also increased the free Ca^{2+} required for the initiation of contraction (activation threshold). The force-pCa curve shifted to the right such that higher free Ca^{2+} was required to reach a given tension (60, 140, 167). Fast-twitch fibers were more sensitive to the acidotic depression of tension than were the slow muscle fibers (91, 140, 352). This fact is clearly seen in Figure 13, reprinted from the results of Metzger and Moss (352). Reducing pH from 7.0 to 6.2 caused a greater decline in force in the fast-twitch vastus lateralis fiber than the slow-twitch soleus fiber. The observation that low pH still inhibits force at saturating levels of free Ca^{2+} suggests that the effect cannot be attributable to a simple H^+ interference of Ca^{2+} binding to troponin (167, 352). Fabiato and Fabiato (167) observed a 33% reduction in maximal rigor tension of the frog semitendinosus when pH was reduced from 7.0 to 6.2. A similar effect was observed by Metzger and Moss (354) studying mammalian fast and slow fibers. Both groups interpreted this as evidence for a direct effect of H^+ on the contractile proteins. It is currently thought that H^+ directly inhibits force by reducing the cross-bridge transition from the low- to the high-force state (*step 5*, Fig. 2). A direct effect of pH on the cross bridge could involve *1*) a reduction in the number of cross bridges and/or *2*) a reduction in the force per cross bridge. The number of attached cross bridges

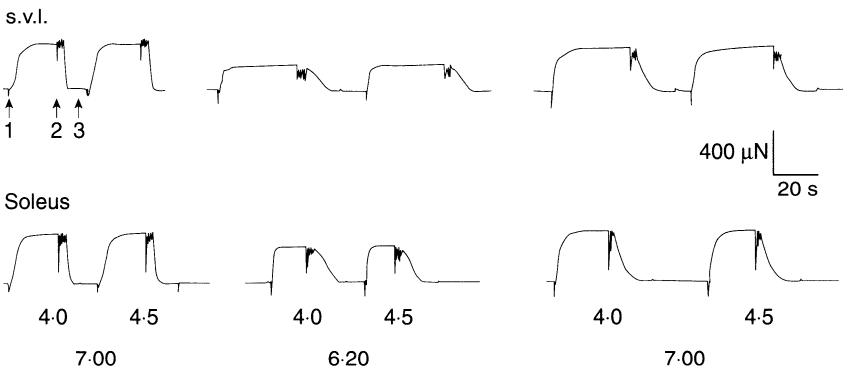

FIG. 13. Original slow time-base recordings of tension from superficial vastus lateralis (s.v.l.) (*top traces*) and soleus (*bottom traces*) skinned single fibers at maximal (pCa 4.5) and supramaximal (pCa 4.0) levels of Ca^{2+} activation at pH 7.00, pH 6.20, and return to pH 7.00. Fibers were activated at *time point 1*, slackened and subsequently relaxed at *2*, and reextended to original length at *3*. At pH 7.00, force developed by s.v.l. fiber was 420 μN at pCa 4.0 and 430 μN at pCa 4.5. At pH 6.20, force was reduced to 240 μN at pCa 4.0 and 250 μN at pCa 4.5; returning pH to 7.00 restored force production both at pCa 4.0 (420 μN) and at pCa 4.5 (410 μN). Force production by soleus fiber at pH 7.00 was 470 μN at pCa 4.0 and 480 μN at pCa 4.5. Lowering pH to 6.2 reduced force to 340 μN at pCa 4.0 and 350 μN at pCa 4.5. Force at each pCa was recovered completely when pH was returned to 7.00. [From Metzger and Moss (352).]

can be estimated from fiber stiffness measured during high-frequency length oscillations of a fully activated fiber (354). When pH_i was reduced with high CO_2, peak force decreased 24 and 30% for the mouse soleus and EDL muscles, respectively, but stiffness only decreased 9 and 14%, respectively (395). These results suggest that the primary effect of low pH is to reduce the force per cross bridge. More recently, Edman and Lou (151) established this to be true in living single fibers. During development of moderate levels of muscle fatigue (75% of initial force), they observed only a 9% reduction in fiber stiffness. In nonfatigued fibers, stiffness reached its peak before force, and after fatigue, this difference was magnified as the rate of rise of force ($+dP/dt$) was markedly depressed while the rate of stiffness development remained unchanged. This result implies that fatigue altered the rate of transition from the cross-bridge attached state (reflected by stiffness) to the high-force state of the cross bridge (*step 5*, Fig. 2). Intracellular acidification by high CO_2 altered cell function in a manner similar to that observed with fatigue. However, for a given fall in tension, stiffness fell less with acidosis compared with fatigue. Unlike fatigue, the rate of rise of stiffness showed a progressive decline as acidosis developed. This result suggests that other factors unique to fatigue (such as P_i or the amplitude of the Ca^{2+} transient) modified the pH effect on the cross-bridge kinetics. In skinned fiber studies, Metzger and Moss (354) showed low pH to decrease the number of cross bridges in fast- but not slow-twitch fibers. Additionally, they observed a high H^+ to reduce the force per cross bridge in both slow- and fast-twitch fibers.

In addition to the decline in tension, fatigue has long been known to be associated with a reduced $+dP/dt$ (186). Part of the decline in $+dP/dt$ can be explained by the decrease in the number of active cross bridges acting in parallel caused by incomplete activation (inhibition of E-C coupling) or direct inhibition of the cross bridge. Consequently, when postfatigue $+dP/dt$ data were corrected for the fall in force ($+dP/dt \times P_o^{-1}$), the observed decrease in $+dP/dt$ was attenuated (350). If the peak $+dP/dt$ in an intact muscle is limited by the cross-bridge transition rate from the weakly bound low-force state to the strongly bound high-force state or *step 5* in Figure 2 (as it appears to be in fully activated single fibers), then the rate constant for cross-bridge binding may be reduced in the fatigued cell. Alternatively, the rate-limiting step in the rate of force development in intact muscles may be limited by other factors (such as the rate of Ca^{2+} release), or the limiting step may switch with fatigue from cross-bridge binding to Ca^{2+} release. This could occur if the E-C coupling process was altered such that fewer SR Ca^{2+} release channels were opened (324, 403). Regardless of what limits $+dP/dt$, several lines of evidence suggest that its decline with fatigue is at least partially caused by the development of low pH_i. We have recently observed a high correlation between $+dP/dt$ and pH_i during the recovery from fatigue (466). Furthermore, Edman and associates (151, 152) found a similar fall in peak force and $+dP/dt$ with fatigue and acidosis induced by CO_2 exposure in single living fibers. Finally, at suboptimal Ca^{2+} concentrations (pCa >5.0), Metzger and Moss (355) showed low pH to inhibit the k_{tr} in both slow- and fast-twitch single skinned fibers. These authors (355) observed no effect of low pH on the k_{tr} during maximal Ca^{2+} activation (pCa = 4.5). However, because the amplitude of the intracellular Ca^{2+} transient is known to be reduced with fatigue (reviewed previously), the pH effect on the k_{tr} could be of physiological importance.

Some controversy exists as to whether or not acidic pH directly inhibits Ca^{2+} binding to troponin C. Blanchard et al. (56) concluded that lowering pH from 7.0 to 6.2 reduced the affinity of Ca^{2+} binding to troponin, while others (382) have found no direct effects of pH on the affinity of Ca^{2+}-binding sites on troponin. The tension-pCa curve is less steep in slow- compared with fast-twitch fibers, a phenomenon attributed to greater cooperativity in the Ca^{2+} activation of tension in the fast fiber type (64, 352). Low pH_i appears to affect the positive cooperativity in fiber type-specific ways, with slow and fast fibers showing an increased and decreased cooperativity, respectively (352). However, in both slow- and fast-twitch fibers, decreasing pH from 7.0 to 6.2 in-

creased the free Ca^{2+} required for the initiation of tension development (threshold activation) and for half-maximal tension (352). These alterations in the tension-pCa relationship are likely caused by a combination of factors induced by low pH to include a direct inhibition of Ca^{2+} binding to troponin, reduced cooperativity between adjacent regulatory subunits, and a reduced cooperativity caused by fewer active cross bridges (352, 355).

The data reviewed above are convincing in demonstrating that pH declines with the development of fatigue during high-intensity contractile activity and that an acidic pH directly inhibits force, $+dP/dt$, and shifts the tension-pCa relation to the right. All of these factors would be expected to contribute to the fatigability of limb muscle contracting in vivo. Nevertheless, controversy still exists regarding whether or not the decline in pH_i is an important fatigue factor. The major arguments against a role for H^+ in the etiology of fatigue are the observations that *1*) pH_i recovers at a different rate than tension following fatigue, and *2*) reduction in pH_i to levels observed in fatigued fibers by CO_2 exposure yields an inhibition of force considerably less than that observed in fatigue. Westerblad and Lännergren (487) argue that intracellular acidification is unlikely to be a major cause of fatigue, since they observed a large variation in pH_i in fibers fatigued to the same standardized tension loss and pH_i recovered considerably faster than tension. Metzger and Fitts (349) and Thompson et al. (466) also observed pH_i to recover before tension, but nonetheless, the correlation between pH_i and force was high. These authors interpreted the data to imply that although pH_i was an important fatigue agent, other factors (such as P_i, alterations in the free energy of hydrolysis, and disturbances in E-C coupling) must also be involved. After a 50-s isometric contraction of the knee extensors at 66% MVC in humans, Sahlin and Ren (418) observed force to recover in 2 min, while they calculated (based on muscle lactate) that pH_i remained low. They concluded that the high intracellular concentration of H^+ did not limit the capacity to generate force in vivo. Under the experimental conditions employed, only a modest level of fatigue was obtained (decrease from 66 to 50% MVC), and the rapid recovery of force suggests that the fatigue was elicited by alterations in E-C coupling. It is not clear why the low pH_i did not inhibit force in this experiment, but perhaps the pH_i obtained was not as low as calculated.

Nuclear magnetic resonance (NMR) technology has shown a good correlation between the decline in pH_i and force during either in situ or in vivo contractile activity in slow- as well as fast-twitch skeletal muscles (2, 363, 494). However, Adams et al. (2) found no significant effect of hypercapnia (70% CO_2) on P_o in either slow or fast muscles of the cat. Although pH_i was as low as that obtained with electrical stimulation, the Ca^{2+} distribution within the fibers was entirely different. Large changes in pH_i would have less effect with Ca^{2+} release, and thus the amplitude of the Ca^{2+} transient is high, as it would be in the hypercapnic condition. Furthermore, none of the other factors thought to be involved in muscle fatigue or any H^+ interactions with such factors was likely present in the hypercapnic condition. As the authors (2) point out, they have not examined the effects of hypercapnic acidosis on force during fatiguing stimulation. Under these conditions, Ca^{2+} release would be reduced, and the effects of H^+ on the regulatory protein troponin and cross-bridge kinetics (reviewed previously) would become magnified. In contrast to the NMR results, Sahlin et al. (412) found high CO_2 to decrease pH_i (0.34 units) and force (55% of initial), approximately the same amount as electrical stimulation. Additionally, Hultman et al. (255) reported a 75-s contraction of the quadriceps femoris in humans to produce a greater decline in cell pH (6.54 vs. 6.70) and force (44.6 vs. 55.4% of initial) after induced metabolic acidosis compared with control conditions. The work of Mainwood and Renaud (328) clearly points out the quantitative difference in the effect of pH_i on muscle force when produced by hypercapnia versus contractile activity. With equal proton loads, tension declined by 30 and 70% following hypercapnia and electrical stimulation, respectively. Wilson et al. (494) conducted an experiment in which 2 min of submaximal exercise preceded maximal wrist flexion exercise in humans. The submaximal bout reduced pH_i, and thus during the subsequent maximal exercise, a given percent decline in force was associated with a lower pH_i than observed in a control maximal exercise bout. From this and the hypercapnic experiments, one cannot conclude that H^+ has no direct effect on muscle fatigue, but only that the magnitude of the effect is dependent on other factors. In the case of the hypercapnic experiments, a particularly important factor is the amplitude of the Ca^{2+} transient, which is surely higher than that observed in fatigued fibers.

2. pH and fiber maximal shortening velocity

Edman and Mattiazzi (152) demonstrated a significant decrease in V_o of fatigued frog skeletal muscle fibers; however, V_o never declined until force fell by at least 10%. They suggested that the decline in V_o was mediated by an increased myoplasmic H^+ concentration, since unstimulated fibers incubated in high P_{CO_2} showed a similar reduction in force and V_o. The fibers with the highest initial velocity (presumably fast twitch) showed the greatest drop in V_o. Skinned fiber experiments have shown acid pH to inhibit V_o in fast and slow rabbit and rat fibers (91, 105, 352). In the experiments of Metzger and Moss (352), the inhibitory effect of H^+ was greater in fast- than slow-twitch fibers, an observation consistent with that of Edman and Mattiazzi (152) in living single fibers. The latter investigators reported a large fiber-to-fiber variation in the extent of V_o inhibition with fatigue. In fact, Fitts and Holloszy, utilizing the frog sartorius (182) and rat soleus (181) muscles, showed the V_o calculated from force-velocity relationship to be resistant to fatigue. Recently, Thompson et al. (467) found the frog semitendinosus V_o

to fall significantly from a prefatigued value of 6.7 to 2.5 muscle lengths/s at fatigue. Peak tension fell to 8% of its initial value, while in the earlier studies in which V_0 was unaltered, P_0 decreased only 52 and 30%, respectively (181, 182). Maximal shortening velocity is proportional to the myofibrillar ATPase activity (25). An increased H^+ inhibits both V_0 and ATPase, presumably by a direct effect on the ATPase, thus slowing the cross-bridge cycle rate (Fig. 2). Apparently, the activity of the myofibrillar ATPase is unaltered until force falls by at least 10%; however, the exact relationship between force, V_0, and ATPase during fatigue seems dependent on the fiber type and activation frequency.

3. *pH, isometric relaxation rate, and sarcoplasmic reticulum adenosinetriphosphatase*

A prolonged relaxation time and Ca^{2+} transient is a consistent feature of fatigued muscle, and the slowed relaxation has frequently been attributed to an increased H^+ (84, 121, 122, 413, 414). The observation of a significant correlation between pH_i and 1/2RT during the recovery from fatigue supports this hypothesis (466). Relaxation from a peak contraction shows an initial linear phase followed by a nonlinear, almost exponential, decline to the resting tension (122, 488). The major prolongation of relaxation occurs in the initial linear phase (122, 488). During contractile activity, Ca^{2+} is redistributed from the SR release site to the Ca^{2+} binding protein parvalbumin and the SR pump, therefore placing a greater load on the reuptake process. With contractile activity, parvalbumin becomes rapidly saturated and thus likely does not contribute to the slowing of relaxation beyond the first few seconds of contractile activity (41, 86, 488). Both fatigue and acid pH produced by high CO_2 slow relaxation; however, the latter is not accompanied by a slowed Ca^{2+} transient (8). Westerblad and Lännergren (488) hypothesized that the inhibition of relaxation with fatigue was due to the combined effect of an altered cross-bridge kinetics and an impaired ability of the SR to resequester Ca^{2+}. Because acidic pH did not alter the Ca^{2+} transient, they concluded that the pH effect was mediated through a direct effect on cross-bridge kinetics. Considerable evidence exists to suggest that a reduced pH_i is in part responsible for the prolongation of relaxation associated with fatigue. Fibers showing the largest decline in pH_i consistently show the greatest prolongation of relaxation, and lactacidosis prolongs relaxation (329, 484). Iodoacetic acid-poisoned muscles underwent a 50% fall in tension with stimulation, but no change in pH_i or relaxation time (405, 414). Although these data are consistent with a role for H^+ in the prolongation of relaxation, it is clear that additional factors are involved. Cady et al. (84) observed a differential effect of muscle fatigue on the relaxation rate of the first dorsal interosseous in control and myophosphorylase-deficient (MPD) subjects. For the control subjects, pH_i fell to 6.5, and the rate constant for relaxation was reduced to 24% of its initial value. In contrast, the MPD subjects showed no significant change in pH_i; however, the relaxation rate declined, although less than that observed in the control subjects. Additionally, the relaxation rate recovered considerably faster in the MPD than the control subjects. Furthermore, in the first few minutes after fatigue, the relaxation rate increased while pH_i remained acidic, and later in the recovery period, pH_i returned to the control value at a time when relaxation was only partially recovered (84, 466).

Skinned fiber experiments of Fabiato and Fabiato (167) and Lamb et al. (303) showed acidic pH to reduce the extent of Ca^{2+} reloading into the SR, while the latter group concluded that Ca^{2+} release was not inhibited. This conclusion was based on the observation that the force decreased in acidic pH the same regardless of whether contraction was induced by depolarization or direct Ca^{2+} activation. The observation that acidic pH inhibits Ca^{2+} reuptake is not consistent with the hypothesis of Westerblad and Allen (484) that the pH-dependent effect on relaxation primarily acts on the cross-bridge cycle. Considerable evidence exists to suggest that H^+ compromises the functional capacity of the SR pump protein. Sarcoplasmic reticulum ATPase and Ca^{2+} reuptake are known to be depressed following high-intensity exercise (82). The inhibition could in part be mediated by H^+, since the SR ATPase has an optimal pH of 7.0 and its activity decreases with lowered pH (263, 333). Additionally, acidic pH has been shown to depress SR Ca^{2+} reuptake, presumably by inhibiting both the formation and cleavage of the phosphorylated enzyme intermediate (263).

4. *pH and glycolysis*

A number of observations suggest that low pH inhibits glycolysis. Hill found that lactate formation during muscle stimulation stopped when pH_i dropped to 6.3 (242). Additionally, Hermansen and Osnes (234) measured the pH of muscle homogenates and observed no change during a 60-s measurement period for the most acidic homogenates of fatigued muscle; the pH values of the homogenates from resting muscle fell markedly, owing to significant glycolysis during the measurement period. A decrease in cell pH could inhibit both glycogenolysis and glycolysis by inhibiting phosphorylase and phosphofructokinase (92, 294, 470). In fact, a number of studies have demonstrated the glycogenolytic rate to decline with fatigue during high-intensity exercise (337, 448). The important question is whether the reduced glycogenolytic rate contributes to fatigue by limiting ATP production or results in response to a reduced ATP requirement as fatigue develops. Although it has been suggested that H^+ inhibition of glycolysis may be a limiting factor for performance (159, 409), this seems unlikely in that cell ATP rarely falls below 70% of the prefatigued value during intense exercise (176, 468). It seems likely that the inhibitory effects of H^+ are countered by increases in AMP, IMP, and P_i, known activators of phosphorylase and phosphofructokinase (92, 392,

393, 447). The high correlation between glycogenolysis and the ATP turnover rate during the development of fatigue supports the hypothesis that the former declines in response to the reduced energy requirement of the fatigued muscle.

V. INORGANIC PHOSPHATE AND MUSCLE FATIGUE

A. *Inorganic Phosphate Concentration in Skeletal Muscle*

The P_i concentration in skeletal muscle appears quite variable, with published values in nonfatigued muscle ranging from 3 to 41 mmol/kg dry wt (40, 128, 363, 468, 493, 494). The values on the low end of the range have all been estimated or determined by ^{31}P-NMR technology. Meyer et al. (359) suggested that the chemical analysis of P_i overestimates the free P_i, since it measures total P_i, a portion of which exists intracellularly in the bound state. A second potential problem is that the chemical method may produce high results due to hydrolysis of PCr during the freezing and extraction procedure (357, 359). Both chemical and NMR analyses demonstrate higher P_i content in slow- compared with fast-twitch muscles (301, 357). For example, Kushmerick et al. (301) compared both techniques and observed a P_i content for the soleus and biceps of 9 and 6 μmol/g (chemical analysis) and 5.8 and 0.9 μmol/g (NMR), respectively. The difference between the techniques is clearly greater in fast muscle, where the NMR P_i peak is sometimes difficult to detect (Fig. 3 in Ref. 357). One explanation for the discrepancy is the presence of a NMR-invisible pool of P_i. Kushmerick and Meyer (300) believe this to be unlikely due to the stoichiometric changes observed between P_i and PCr both during the development of and the recovery from fatigue (10, 363, 468).

B. *Inorganic Phosphate and Muscle Fatigue*

1. *Inorganic phosphate and muscle force*

With contractile activity, P_i increases stoichiometrically with the decrease in PCr, and both show a significant correlation with the development of fatigue (117, 126–128, 363, 482). In contrast to H^+, where a high cell-buffering capacity exists, the handling of P_i appears to depend primarily on a mitochondrial membrane carrier protein for the transport of the P_i into the mitochondria. The carrier appears to be specific for the divalent anion (HPO_4^{2-}) form (264). After fatigue, both Pi and PCr recover with a similar time course, which is generally related to the recovery in P_o (363, 468). However, the relationship between P_i and force is not always coupled. Miller and colleagues (363, 482) observed P_i to increase more rapidly than the decline in the peak force of MVC, and after 2 min of contraction to plateau while the MVC continued to fall. Dawson (126) observed a high correlation between the decline in force and the increase in the diproteinated ($H_2PO_4^-$) form of P_i in frog skeletal muscle, and Wilson, McCully, and co-workers (339, 340, 494) reported a similar response in human muscle when the exercise consisted of 1 s MVC every 5 s. However, when a ramp exercise protocol was employed, no correlation was observed between $H_2PO_4^-$ and muscle fatigue (339, 340). Nevertheless, the data from living and skinned fiber experiments generally support the hypothesis that an increased P_i contributes to fatigue. High P_i has been shown to inhibit the maximal force of skinned fibers isolated from both skeletal (105, 107, 287, 288, 334, 379, 455, 457) and cardiac muscle (235, 291). Skinned fiber studies demonstrate the inhibition of force to be particularly sensitive to increases in P_i between 1 and 15 mM (105–107), while NMR analysis of muscles contracting in vivo or in vitro shows little effect on force until P_i exceeds 20 mM (2, 85, 128, 340). The reason for this discrepancy is not readily apparent. In a recent study, Thompson and Fitts (468) evaluated the relationship between P_i and force during the recovery from fatigue in the frog semitendinosus. These authors determined P_i chemically and found 49 and 103 mmol/kg dry wt P_i (~10 and 20 mM P_i wet wt) to be associated with a P_o of 50 and 37% of the prefatigued value, respectively. The latter value was obtained at 5 min of recovery when pH_i was 6.5. This result shows considerable agreement with the skinned fiber data of Cooke and Pate (106) who observed a relative tension of 30% with fiber activation in 20 mM P_i and pH 6.5. When the entire recovery period was considered, Thompson and Fitts (468) observed a significant inverse correlation between both the diprotonated and total P_i and force; however, during the second slow phase of recovery, only the $H_2PO_4^-$ form was significantly correlated with force. The effect of P_i on tension is greater in fast- than slow-twitch fibers. Stienen et al. (457) found 15 mM P_i to reduce force to 58 and 78% of the control situation in single skinned fibers isolated from the fast psoas and slow soleus, respectively.

Controversy exists regarding the active species of P_i in the etiology of fatigue (362, 386, 493). Wilkie (493) plotted the data of Dawson et al. (128) as total P_i and the $H_2PO_4^-$ form versus fatigue and observed a simple inverse linear relation between the latter and force with zero force attained at a $H_2PO_4^-$ concentration of ~20 mmol/kg wet wt. From this observation, Wilkie (493) and Dawson et al. (131) hypothesized that the diprotonated form of P_i was causative in fatigue. This hypothesis was supported by the data of Nosek et al. (379), Miller et al. (363), and Weiner et al. (482), all of whom observed a similar inverse linear relation between $H_2PO_4^-$ and force. Figure 14 shows the force inhibition induced by acidic pH (6.0) and the additional effect of 30 mM P_i in skinned rabbit psoas fibers. Nosek et al. (379) reported skinned fiber force to approach zero at 30–35 mM $H_2PO_4^-$, which agrees well with the relationship between diprotonated P_i and force in human skeletal muscle (363, 482). In contrast, Pate and Cooke (107, 386), and Millar

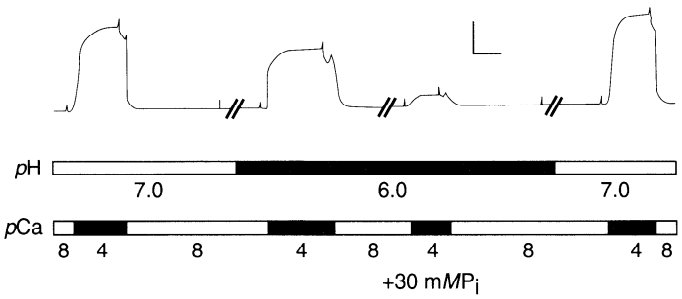

FIG. 14. Effect of P_i and pH on maximal Ca^{2+}-activated force, pCa 4.0, of skinned muscle fiber. Typical record of effect of pH (6.0) and P_i (30 mM) on maximal force of skinned psoas fiber is shown. Calibration bars show 50 mg and 10 s, and spikes on record are solution exchange artifacts. For concision, record shown is not continuous but has been truncated at interrupted lines. [From Nosek et al. (379), copyright 1987 by the AAAS.]

and Homsher (362) found the decline in force to be linearly related to the logarithm of the average P_i concentration. The former authors (107, 386) found the slope of the relative tension versus log $[P_i]$ to be the same at pH 7 and 6.2. This result confirmed the earlier finding of Chase and Kushmerick (91) who reported the relative force at 15 mM P_i (normalized to that at 1 mM P_i) to be the same at pH 7.1 and 6.0. This observation led Chase and Kushmerick (91) to reject the hypothesis of Dawson et al. (128) and Wilkie (493) that $H_2PO_4^-$ is the primary causative agent in muscle fatigue. Pate and Cooke (386) argue that if tension depends on the logarithm of P_i concentration, then the question cannot be answered by determining the relationship between P_i (total or the $H_2PO_4^-$ form) and force, since both forms will increase by the same factor as fatigue develops. Recently, Nosek et al. (381) reevaluated the effect of P_i on skinned fiber force and concluded that the diprotonated form correlated with force in the fast-twitch psoas, but not in slow-twitch or cardiac fibers. This result agreed with the finding of Kentish (291), who found total but not the diprotonated form of P_i to be correlated with reduced force in cardiac muscle.

An important problem is understanding the mechanism by which high P_i inhibits force. The P_i release step of the cross-bridge cycle (*step 5*, Fig. 2) is associated with a large free energy reduction, and thus this step is generally believed to be the "power stroke" that results in force generation (290, 386). Hibberd et al. (236) observed 10 mM P_i in the presence of Ca^{2+} to increase the rate of force redevelopment, while reducing the steady-state tension following the release of ATP from a caged precursor compound in single skinned rabbit psoas fibers. During the steady-state phase of contraction, P_i decreased tension more than stiffness (236, 288, 334). In experiments in the absence of Ca^{2+}, Hibberd et al. (236) observed high P_i to increase the rate of final relaxation from rigor upon the release of caged ATP. These observations led the authors (236) to hypothesize that P_i release was closely associated with the power stroke and that an increased P_i concentration increased the rate constant for tension redevelopment by increasing the rate of reversal of *reaction 5* in Figure 2. Force declined due to a redistribution of cross bridges from the strong to the weak binding state (Fig. 2). The observation that high P_i did not affect rigor tension suggests that P_i had no direct effect on strong cross-bridge binding (236). A second possibility is that P_i acts to accelerate cross-bridge detachment by increasing the rate of the forward *reactions 6* and *7* (Fig. 2). Hibberd et al. (236) suggest that this is unlikely, since ATP hydrolysis would increase markedly and, in fact, high P_i has been shown to decrease fiber ATPase activity (288).

High P_i has been shown to shift the force-pCa relationship to the right and increase the Hill coefficient or slope (n) (63, 362, 481). Millar and Homsher (362) found P_o, pK, and n to all vary linearly with log $[P_i]$. Inorganic phosphate had its greatest effect on tension as Ca^{2+} was reduced. This has functional importance, since the amplitude of the Ca^{2+} transient is known to be depressed in the fatigued muscle cells (8). A P_i-induced decrease in strongly bound cross bridges would reduce thin filament activation accenting the decline in tension, particularly at suboptimal Ca^{2+} (481). This effect would explain the right shift in the force-pCa curve and reduced pK in the absence of a direct effect of P_i on calcium binding to troponin (334).

2. *Inorganic phosphate and relaxation time*

During the development of muscle fatigue, the relaxation time increases, and as discussed in section III*D*, this change is associated with a prolongation in the Ca^{2+} transient. Bergstrom and Hultman (40) demonstrated that altered relaxation time was highly correlated with $H_2PO_4^-$ both during the development of and recovery from fatigue. It is not known if the correlation is causative or at what site(s) P_i could affect relaxation. High P_i could act to reduce the rate of Ca^{2+} reuptake into the SR, thus slowing the Ca^{2+} transient and relaxation time. Increasing P_i would reduce the free energy change associated with ATP hydrolysis, which in turn could affect Ca^{2+} uptake (130). Dawson et al. (130) reported the free-energy change with fatigue to be small but correlated with the relaxation rate constant.

3. *Inorganic phosphate and maximal shortening velocity*

Maximal shortening velocity decreases with fatigue. As reviewed in section IV*C*, this change appears to be in part caused by an increased intracellular H^+ concentration (152). In skinned fiber experiments, Cooke and Pate (106), Cooke et al. (105), and Pate and Cooke (386) found increases in P_i up to 20 mM to have no effect on V_0. An increased P_i has been shown to depress fiber ATPase activity, but not as much as tension (105, 288). Consequently, the tension cost (ATPase-to-tension ratio) increased with increasing P_i. Kawai et al. (288) suggest that the decreased hydrolysis rate at high P_i concentrations was not caused by a reduced cross-bridge

cycle rate, but simply reflected the mobilization of fewer cross bridges. Pate and Cooke (386) observed P_i in excess of 10 mM to depress V_o, but only at low ATP concentrations. They proposed that P_i competitively inhibited the binding of MgATP to the myosin nucleotide site at the end of the cross-bridge power stroke, which prevented the dissociation of myosin from actin and slowed fiber velocity. However, even in cases of extreme fatigue, cell ATP rarely falls by >30% of its prefatigued value. Consequently, it seems unlikely that high P_i contributes to the fatigue-induced decline in V_o. Dawson et al. (131) and Wilke (493) hypothesized that rather than acting directly on the cross bridges, the deleterious effects of low pH_i might be mediated by producing an inhibitory high concentration of $H_2PO_4^-$. Because high H^+, but not P_i, depresses V_o, this hypothesis seems unlikely.

VI. HIGH-ENERGY PHOSPHATES AND MUSCLE FATIGUE

A. Cell Concentrations

Fast-twitch muscles contain a significantly higher content of both ATP and PCr than do slow-twitch muscles (176). Fast muscles average ~27 and 90 mmol/kg dry wt ATP and PCr, compared with 19 and 58 mmol/kg dry wt, respectively, for slow muscles (176). To avoid fatigue, adequate tissue ATP levels must be maintained, because this substrate supplies the immediate source of energy for force generation by the myosin cross bridges. Adenosine 5′-triphosphate is also needed in the functioning of the Na^+-K^+ pump, which is essential in the maintenance of a normal sarcolemma and T tubular action potential. Additionally, ATP is a substrate of the SR ATPase and thus is required in the process of Ca^{2+} reuptake by the SR. As discussed previously, a disturbance in any of these processes could lead to muscle fatigue.

B. Alterations With High-Intensity Exercise

With the onset of high-intensity exercise, PCr shows a rapid decline reaching 5–10% of the prework value within 30 s (176, 451). This decrease in PCr occurs in all muscles (fast and slow) and all species studied (176, 180, 337, 451, 464). The decrease is generally somewhat larger in fast- compared with slow-twitch muscle (176), which likely reflects the some three- to fourfold higher ATP utilization rate in fast skeletal muscle (446). Additionally, at high speeds of locomotion, the slow fibers may become functionally unloaded and/or develop low tensions (due to slow activation and low dP/dt), either of which might lower the energy costs (146). The fiber type difference in PCr decline was perhaps best demonstrated in the study of Ivy et al. (265), who studied single slow- and fast-twitch fibers isolated from the vastus lateralis muscle of humans at exhaustion and found a considerably greater fall in the PCr content of the fast fiber type. In contrast to PCr, ATP shows only modest declines, rarely falling below 60–70% of the preexercise content even in conditions yielding extensive fatigue (176, 180, 283, 442, 451).

The changes in creatine (Cr) and P_i with contractile activity show an inverse correlation with PCr (129, 130, 176, 300, 414). This observation is not surprising in that PCr participates exclusively in the creatine kinase (CK) reaction

$$PCr + ADP + H^+ \rightarrow Cr + ATP \quad (1)$$

which, in turn, is driven by cell utilization of ATP

$$ATP + H_2O \rightarrow ADP + P_i + H^+ + \text{energy} \quad (2)$$

A second reaction important in the maintenance of cell ATP in intense contractile activity is the adenylate kinase reaction

$$2\ ADP \rightarrow ATP + AMP \quad (3)$$

With high-intensity exercise, increased H^+ and ADP concentrations serve to drive the CK reaction, and in the case of ADP, the adenylate kinase reaction producing ATP + Cr and ATP + AMP, respectively. Collectively, these reactions maintain a high ATP and low ADP, which helps to maintain a high free energy of ATP hydrolysis (130, 411, 463).

C. Relationship of Adenosine 5′-Triphosphate and Phosphocreatine to Muscle Fatigue

1. Cell adenosine 5′-triphosphate as a limiting factor

An important question in the study of muscle fatigue is whether or not cell ATP declines to a critical level, such that the force-generating capacity and/or cycle rate of the cross bridge is directly compromised (183, 433). The overriding evidence suggests that it does not, that fatigue produced by other factors reduces the ATP utilization rate before ATP becomes limiting (39, 184). This statement is supported by a number of observations. Perhaps most compelling is that the ATP concentration in skeletal muscle rarely drops below 60–70% of the preexercise level even in cases of extensive fatigue (176, 180, 245, 265, 300, 331, 372, 373, 442, 468). In fact, the ATP content of slow-twitch muscle may actually increase at fatigue (245, 492). The only hypothesis consistent with these data and a role for ATP in limiting performance is that 70–80% of the cytoplasmic ATP is unavailable to the cross bridges. This seems unlikely in that Nassar-Gentina et al. (372) demonstrated that fatigued frog semitendinosus muscles, containing 70% of their initial ATP, could upon caffeine activation generate considerable extra tension, and further deplete >50% of the remaining ATP. Furthermore, when glycol-

ysis is blocked by iodoacetate, skeletal muscle hydrolyzes up to 75% of the cell ATP (74, 144). Additionally, no correlation exists between ATP and force in frog (180), rat (471), or human (39) skeletal muscle, and Karlsson and Saltin (284) observed the same depletion of ATP in the first few minutes of work whether the load was exhaustive or not (Fig. 9). If ATP became limiting at the cross bridge, resting muscle tension would increase as rigor bridges developed. Yet resting tension does not change in skeletal muscles fatigued in vitro, and Spriet et al. (450) found no occurrences of rigor despite one of the largest decreases in muscle ATP (average 57% with a low of 33% of resting content) concentration reported in healthy men.

Sahlin et al. (417) and Dawson et al. (130) suggested that ATP could limit performance without depletion due to a decrease in the free energy of ATP hydrolysis. The free energy available in the cytoplasm (G') is calculated by

$$G' = G^0 - RT \ln [ATP]/[ADP][P_i] \quad (4)$$

where G^0 is the standard Gibbs free energy change, R is the ideal gas constant, and T is absolute temperature. Because ADP and P_i increase as fatigue develops, the available free energy will decrease even if ATP stays constant. After maximal bicycle exercise in humans, the free energy of ATP hydrolysis decreased from 54 to 50 kJ (417). Sahlin et al. (417) suggested that the actual decrease might have been considerably higher as the value was calculated from an ATP/ADP in biopsy samples obtained 4–6 s after exercise (417). In the experiments of Dawson et al. (130), the fall in free energy was small but paralleled the increase in muscle relaxation rate, leading them to conclude that the active process of Ca^{2+} uptake by the SR might be compromised by the reduced free energy. However, they found no correlation between the fall in force and the free energy change. Additionally, Taylor et al. (460) found the free energy of ATP hydrolysis to be similar in lightly exercised and exhausted human muscle. Both Sahlin et al. (417) and Terjung et al. (463) proposed that an exercise-induced activation of AMP deaminase limited the rise in ADP and thus helped to prevent a decline in available free energy.

Recently, Godt and Nosek (201) employed the skinned fiber preparation to evaluate how activity-induced changes in the high-energy phosphates, ADP, and AMP might affect the maximal Ca^{2+}-activated force (P_o). They observed no significant change in P_o as ATP was varied over the range known to exist during high-intensity exercise. In fact, P_o actually increased when ATP was reduced to 1 mM. Increases in ADP and AMP both increased P_o. When the affinity for ATP hydrolysis was reduced to that calculated to exist in fatigue (by adding ADP, AMP, and creatine), they observed no effect on P_o. This suggests that a reduced affinity during exercise may reduce peak tension but that this effect would be countered by the increased ADP.

Numerous skinned fiber studies have evaluated the effect of ATP on P_o with the general conclusion that P_o is elicited between 15 and 100 μM ATP (104, 169, 197, 198, 299). Additionally, low ATP shifts the pCa-force relationship to the left such that less Ca^{2+} is required for a given level of cross-bridge activation (63, 198). This could be important in a fatigued cell, where the amplitude of the Ca^{2+} transient is known to be reduced (8). Because ATP rarely falls by more than 30% of the ~5 mM resting value, it seems inconceivable that ATP depletion could be causative in fatigue (176, 468). Nevertheless, it has been suggested that with the depletion of PCr, muscle fatigue could result from the inability to maintain an ATP production rate sufficient for the energy demand (259, 406, 413). Recently, Westerblad and Allen (485) calculated cell ATP (based on increases in intracellular Mg^{2+}) to decline to 30% of the prefatigued value in stimulated fibers. In a second publication (484), they proposed that the decline in ATP would be more likely to reach limiting levels in single cells fatigued in vitro where potentially deleterious changes in the extracellular fluid, such as an increased K^+, are avoided. The results of Nagesser et al. (370) do not support this hypothesis. They observed PCr to decline to undetectable levels when force reached 0.6 P_o and ATP concentrations as low as 25% of the rested fiber value when force reached 0.2 P_o. After stimulation, ATP recovered while force remained depressed, and thus the authors (370) concluded that the reduced energy stores could not explain the depressed force. Bergström and Hultman (39) observed fatigue to develop significantly faster during short- compared with long-duration contractions despite the same total activation time. The short-duration protocol elicited a higher ATP utilization rate, and the authors (39) concluded that the capability to resynthesize ATP had not reached its limit.

A decline in ATP is more likely to inhibit fiber V_o than force. Fast-fiber V_o showed a hyperbolic dependence on MgATP concentration, with a K_m of ~0.5 mM (169, 456). Slow fibers showed an even lower K_m of <0.2 mM (456). Consequently, ATP would have to fall below 1 mM before it would significantly inhibit V_o of either slow- or fast-twitch fibers (104, 169, 456). Glyn and Sleep (197) also observed fiber ATPase to display a hyperbolic relation with MgATP concentration. The ATP required for maximal fiber ATPase was somewhat higher than that for peak force, but considerably less than the ~2 mM required for fiber V_o. Edwards et al. (160) observed a significant relationship between the decline in ATP and the slowing of relaxation in fatigued mouse soleus. The substrate K_m values for both the cross-bridge dissociation and the SR Ca^{2+} pump are in the micromolar range, and thus the slowed relaxation cannot be mediated by substrate limitations.

Sahlin (406) and de Haan (132) have suggested that fatigue may be caused by degradation products of ATP rather than low ATP per se. The former author (406) proposed that fatigue during high-intensity exercise was mediated by transient and local increases in ADP. However, Godt and Nosek (201) found 0.7 mM ADP to

increase P_o and Ca^{2+} sensitivity, and Cooke and Pate (106) and Kawai and Halvorson (289) concluded ADP had no effect on P_o. De Haan and co-workers (132, 491) have suggested that either IMP or some compound formed during the conversion of ATP to IMP induced fatigue. Sahlin and Broberg (406, 411) showed IMP to increase at exhaustion in human muscle, and Meyer and Terjung (361) observed an IMP increase following in situ stimulation in fast- but not slow-twitch rat skeletal muscle. To date, there is no evidence that an increased IMP concentration mediates fatigue. With the metabolism of AMP to IMP, ammonia (NH_3) is also produced. Meyer et al. (358) have demonstrated high-intensity exercise to increase skeletal muscle and blood NH_3, with the highest increase observed in fast-twitch muscle. High NH_3 is known to be toxic and thus could participate in fatigue. However, to date, there is no convincing evidence that NH_3 is a fatigue agent (368).

2. *Cell phosphocreatine as a limiting factor*

The relationship between force and PCr during and following contractile activity appears to be dependent on the type of exercise (dynamic or isometric) and the relative intensity of the work (227, 300). Spande and Schottelius (444) and Sahlin et al. (413) reported force and PCr to decline in parallel during electrical stimulation of mouse and rat soleus muscles, respectively. The former concluded that the depletion of PCr caused fatigue, while the latter believed the decline in tension resulted from the inability to regenerate ATP at a sufficient rate following the depletion of PCr. However, the usual pattern is for PCr to decrease considerably faster than tension (180, 364, 451). This pattern is shown for the frog sartorius and human quadriceps in Figures 10 and 11, respectively. It is apparent from the results of Harris et al. (227) and Kushmerick and Meyer (300) that PCr recovery following contractile activity occurs in two phases. In human muscle, the initial fast phase shows a half time of 20–30 s, which is followed by a slower phase requiring 20 or more minutes for full recovery (227). The second phase of PCr recovery is somewhat slower following isometric compared with dynamic exercise, and although the general pattern of recovery is the same, both phases are slower when measured in vitro compared with in vivo (180, 227, 451). The second slow phase of recovery shows a high inverse correlation with lactate, and it was theorized by Harris et al. (227) that the associated H^+ altered the equilibrium state of the CK reaction, thus slowing PCr recovery. This could also explain the slower recovery following isometric exercise, a condition likely to produce higher muscle lactate and lower pH compared with dynamic exercise (92). Recently, Thompson and Fitts (468) observed the PCr of single frog semitendinosus fibers to show a high correlation with P_o ($r = 0.99$) during recovery from fatigue. Because the fatigued cell ATP averaged 33 mmol/kg dry wt, it is unlikely that inadequate energy supply was a causative factor in fatigue. These authors (468) also observed a tight fit between the recovery of PCr and H^+ concentration during recovery from fatigue and suggested that the correlation between PCr and P_o could be attributed to the fatigue-inducing effects of H^+. Although pH_i certainly affects the slow phase of PCr recovery, the initial rapid phase appears to be independent of pH. In fact, during the rapid phase, pH continues to fall due to the H^+ produced with PCr resynthesis (349). The fast phase of PCr recovery appears to mirror the fast component of O_2 debt repayment, suggesting that this phase may be more dependent on ATP supplied by oxidative metabolism (227, 332). Kushmerick and Meyer (300) observed a considerably more rapid PCr recovery following electrical stimulation of the rat triceps surea when utilizing ^{31}P-NMR compared with chemical analysis. They reported a recovery time constant of 30 s with ^{31}P-NMR analysis, which was some fivefold faster than the recovery of pH_i. A possibility exists that the ^{31}P-NMR analysis lacks the sensitivity to detect the slow phase of PCr recovery.

It seems unlikely that PCr limits force production. The only possible mechanism implicating PCr would involve a reduced ATP resynthesis rate once PCr fell below some critical concentration. For the reasons stated above, this possibility seems unlikely. It has been suggested that PCr is essential for the transport of energy from the mitochondria to the various ATPase sites in the cytosol, a concept termed the phosphocreatine shuttle (42). The primary premise of this theory is that ATP is compartmentalized, with the major pool (~80%) confined to the mitochondrial fraction of the cell, and that PCr and Cr function as a shuttle for the transport of high-energy phosphates. Proponents of the PCr shuttle theory have failed to demonstrate that ATP is actually compartmentalized and that PCr and Cr function as essential carriers (361). However, it is undisputed that PCr functions as an important energy store acting to buffer cell ATP and facilitate its diffusion from the mitochondria to the myofibrils. Even in highly fatigued fibers, ATP concentration is over 100-fold higher than the micromolar amounts required for peak force (176, 468). Thus, for compartmentalization to be a factor, one would have to hypothesize that 99% of the cell ATP was unavailable to the cross bridges.

In summary, PCr can only limit performance if its depletion reduces ATP resynthesis, such that low ATP limits force production. The data reviewed here suggest this is unlikely. With high-intensity exercise, PCr declines faster than force, and ATP remains relatively high even at exhaustion. The correlation between PCr and force during recovery is likely not causative, but rather related to the effect of H^+, which inhibits force and PCr resynthesis.

VII. BLOOD GLUCOSE AND MUSCLE GLYCOGEN

A. *Historical Perspective*

In the early 1900s, it was suggested that the rate of carbohydrate utilization increased with the intensity of the work (183, 401). This belief was based on the observation that the respiratory exchange ratio (RQ) increased from a resting value of 0.75 to 0.95 during heavy

exercise. Studies in the 1920s showed that blood hypoglycemia was frequently associated with exhaustion during prolonged exercise. For example, Levine et al. (311) evaluated a group of runners participating in the Boston marathon and found blood glucose to increase in the first few miles, fall back to normal, then in some, but not all, runners to decline below normal between the 14th and 18th mile. The decline in blood glucose during the latter stages of the race was associated with weakness and fatigue. In the following year, blood glucose was maintained normal by ingesting carbohydrates during the run (217). This dietary supplement reduced weakness and fatigue and resulted in improved running performances.

In 1932, Dill et al. (136) observed that the length of time a given rate of energy output could be maintained diminished as the environmental temperature increased. They hypothesized that if the environmental temperature was low and water was provided, then prolonged submaximal exercise was limited by the availability of carbohydrates. To test this hypothesis, they exercised dogs on a treadmill after either a 36-h fast or a heavy carbohydrate meal. In the latter case, glucose in the form of candy was supplied at regular intervals throughout the run. Without the carbohydrate meal and supplementations, the longest run was 6.5 h, at which time the dog showed complete exhaustion. In contrast, the same dog fed carbohydrates ran for 13 h without showing signs of fatigue.

In 1939, Christensen and Hansen (94) conducted a series of laboratory experiments on human subjects in which they evaluated the relative importance of fat and carbohydrate fuels during prolonged exercise. They observed that a high-fat diet markedly impaired performance, while glucose feedings during exercise improved performance and prevented hypoglycemia. After glucose ingestion, they observed only a small increase in RQ, which suggested to them that the main beneficial effect was the prevention of low blood glucose and the symptoms of neuroglucopenia. Importantly, Christensen and Hansen (94) found glucose ingestion 2.5 h before exercise to produce exercise hypoglycemia and a reduced work capacity.

It was clear from these early studies that carbohydrate supplementation delayed the onset of fatigue during prolonged endurance exercise. However, the mechanism of the protective effect was unknown. Carbohydrate supplementation could improve performance by preventing low blood sugar and the development of neuroglucopenia or, alternatively, the important factor could be the continued high supply of a carbohydrate fuel (113). The latter would be particularly important in the final stages of prolonged exercise when muscle glycogen stores were low or depleted. Over the past 25 years, this question has been extensively studied, and the preponderance of evidence supports the notion that carbohydrate ingestion delays fatigue by maintaining a high-carbohydrate fuel source in the form of blood glucose (102, 113). This conclusion necessitates the hypothesis that carbohydrate oxidation is essential to the maintenance of prolonged exercise at moderate to high intensities [65–90% of ones maximal oxygen uptake ($\dot{V}O_{2\,max}$)]. To date, a cellular explanation for an obligatory oxidation of carbohydrates has not been established. The possibilities include the following: *1*) high muscle oxidation rates cannot be maintained without a carbohydrate fuel source, *2*) carbohydrates supply critical metabolic intermediates such that carbohydrate depletion reduces the oxidation rate of available fats and proteins, or *3*) carbohydrate oxidation is not obligatory, but rather carbohydrate depletion is correlated with (and perhaps causative of) changes in other cellular events that in turn elicit fatigue. Little direct support exists for any of these possibilities. The strongest evidence linking any of these possibilities to muscle fatigue is the consistent observation that fatigue during endurance exercise is associated with glycogen depletion (3, 36, 37, 232).

B. Muscle Glycogen

The rate of muscle glycogen utilization is dependent on the intensity of the work and increases from 0.3 to 3.4 glucose units $\cdot kg^{-1} \cdot min^{-1}$ as the relative work load increases from 25 to 100% of $\dot{V}O_{2\,max}$ (420). At work intensities <60% $\dot{V}O_{2\,max}$, muscle glycogen remains high as free fatty acids (FFA) provide the primary substrate (420). Consequently, fatigue resulting from prolonged work at low exercise intensities cannot be attributed to muscle glycogen depletion. In such cases, the inability to continue work may result from dehydration, hyperthermia, or simple boredom (136, 369, 432). Although muscle glycogen is rapidly metabolized at work loads >90% $\dot{V}O_{2\,max}$, fatigue and the inability to continue work develop within minutes while muscle glycogen remains high (420) (see sections II–VI for causative factors of fatigue during high-intensity exercise). At work loads between 65 and 85% $\dot{V}O_{2\,max}$, muscle fatigue is highly correlated with muscle glycogen depletion. This fact was clearly established by a series of papers published in the late 1960s and early 1970s (3, 36, 37, 232, 285, 420). Bergström et al. (36) demonstrated that resting muscle glycogen and one's exercise duration could be increased by a diet rich in carbohydrates. Furthermore, at exhaustion, the blood glucose was higher than that observed following a protein or mixed diet. Bergström and Hultman (37) found the highest glycogen utilization rate in the initial phases of exercise, and along with Ahlborg et al. (3) found glucose administration to have little or no effect on the muscle glycogen depletion rate. These authors (3, 37) concluded that not only did muscle glycogen provide the primary energy source during moderate to heavy exercise, but that muscle glycogen was an obligatory substrate and thus its depletion induced muscle fatigue. This hypothesis was supported by the work of Karlsson and Saltin (285), who examined the performance of 10 subjects participating in a 30-km run with and without a high-carbohydrate diet. The high-carbohydrate diet increased resting muscle glycogen and was correlated with improved performance times. The authors (285) noted that in all subjects running speed declined when the glycogen content of the lateral quadriceps muscle was reduced to 3–5 g/kg wet muscle wt. Additional evidence linking muscle glycogen depletion

with fatigue was the observation of Saltin and Karlsson (420) that regular endurance exercise training reduced the rate of muscle glycogen depletion and delayed the onset of fatigue. In a subsequent study, Fitts et al. (178) found the glycogen-sparing effect and improved endurance of exercised trained rats to be highly correlated with an increased muscle respiratory capacity.

From 1970 to 1975, a number of studies evaluated the question of whether or not glycogen depletion (and presumably muscle fatigue) showed fiber type-specific differences. During prolonged exercise of moderate intensity in humans, Gollnick and co-workers (203, 207, 210, 211) and Costill et al. (111) both observed a preferential depletion of glycogen from the slow type I fiber type. Gollnick and co-workers (203, 207) noted that the slow fibers were the first to become depleted, but in the latter phases of exercise, the fast-twitch fibers were also depleted. In these studies, no attempt was made to separate the fast fibers into type IIa and IIb populations. However, in horses (313) and rats (15), glycogen depletion occurred in the fast type IIa and slow type I fiber types at all exercise intensities, while in the fast type IIb fibers, glycogen stores were not altered except during the final stages of prolonged exercise or at high exercise speeds. With high-intensity bicycle exercise in humans (exercise loads = 150% $\dot{V}O_{2\,max}$), the fast type IIb fibers were the first to demonstrate significant glycogen utilization (204). However, as discussed above, fatigue in this type of exercise results from other factors before limiting levels of muscle glycogen are reached. The rate of glycogen depletion for a given muscle is directly dependent on the type and intensity of the exercise, which relates to the degree of recruitment of a given muscle or muscle fiber type for a particular exercise task. For example, prolonged running on level terrain depletes muscle glycogen in calf muscles more than the quadriceps, while bicycle exercise results in greater quadriceps muscle glycogen depletion than running (112, 164, 432).

From the data reviewed here, it can be concluded that glycogen depletion can only be causative in fatigue during either prolonged endurance exercise requiring between 65 and at most 90% of one's $\dot{V}O_{2\,max}$, or possibly during repeated high-intensity exercise bouts. In the former, the slow type I and fast type IIa fibers would be the first affected, while in the latter the fast type IIb fibers would be recruited and show the highest rate of glycogen depletion. If each high-intensity bout was maintained to exhaustion, the causative factor in fatigue in the initial bouts could not be glycogen depletion. Åstrand et al. (20) employed intense exercise of the arms and legs to produce fatigue within 5 min, but glycogen was depleted by <50%. Additionally, Cheetham et al. (93) used intense exercise to cause extremely high rates of glycogen degradation and fatigue in 30 s, yet total glycogen concentration was only reduced by 25%. However, with multiple bouts, a critical low muscle glycogen concentration could conceivably be reached (101).

C. Blood Glucose

In the early 1970s, considerable debate centered on the relative importance of blood glucose as a substrate during endurance exercise. Wahren and co-workers (479, 480) and Saltin et al. (422) demonstrated glucose uptake and metabolism by contracting skeletal muscle to increase with the duration and intensity of the exercise. During prolonged exercise, blood glucose utilization can account for up to 25% of the total energy output, and this percentage generally increases late in exercise as muscle glycogen depletes (422). However, in some cases, a decline in splanchnic glucose production late in prolonged exercise leads to hypoglycemia and a reduced glucose uptake and oxidation (4, 113). If blood glucose is maintained high by ingesting carbohydrate throughout exercise, then a distinct shift in carbohydrate energy occurs from predominantly muscle glycogen in the first hour to 100% blood glucose after 3.5 h (Fig. 15). In the 1980s, Coyle and colleagues (100, 114, 115) conducted a series of studies evaluating the importance of maintaining blood glucose in endurance exercise. Coyle (113) recently reviewed this work, and the major findings are shown in Figure 16. The experiment consisted of bicycle exercise at 74% $\dot{V}O_{2\,max}$ with and without (placebo) carbohydrate feedings. In the placebo condition, blood glucose declined to 2.5 mM, and the percent of carbohydrate oxidation was significantly reduced at fatigue. In contrast, carbohydrate feedings maintained both plasma glucose and the carbohydrate oxidation rate. In agreement with earlier reports (3, 37), the feedings had no effect on the rate of muscle glycogen depletion. The additional hour of exercise occurred with little or no further decline in muscle glycogen concentration (Fig. 16*C*). Coyle (113) interpreted these observations by stating "the lowering of blood glucose during the latter stages of prolonged strenuous exercise plays a major role in the development of muscular fatigue by not allowing leg glucose uptake to increase sufficiently to offset reduced muscle glycogen availability." Fatigue under these conditions is clearly preceded by a decline in carbohydrate oxidation which is preceded by a decline in

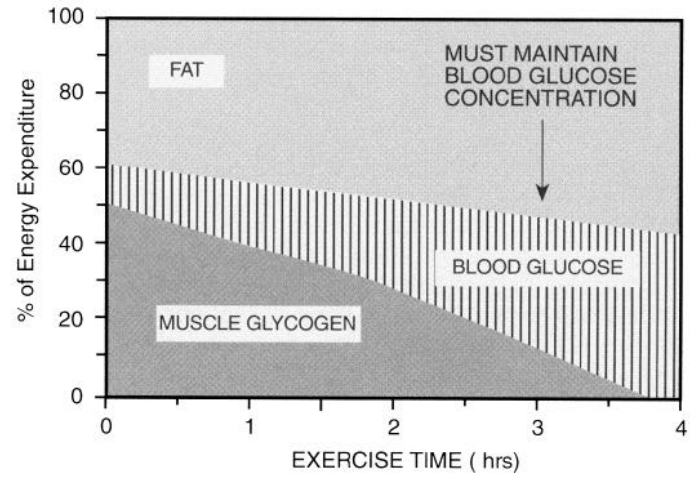

FIG. 15. Various sources of energy during prolonged exercise at 70% of maximum oxygen consumption. Note that blood glucose becomes predominant source of carbohydrate energy during latter stages of exercise; thus it is important to maintain blood glucose concentration by eating carbohydrates. [From Coyle (113).]

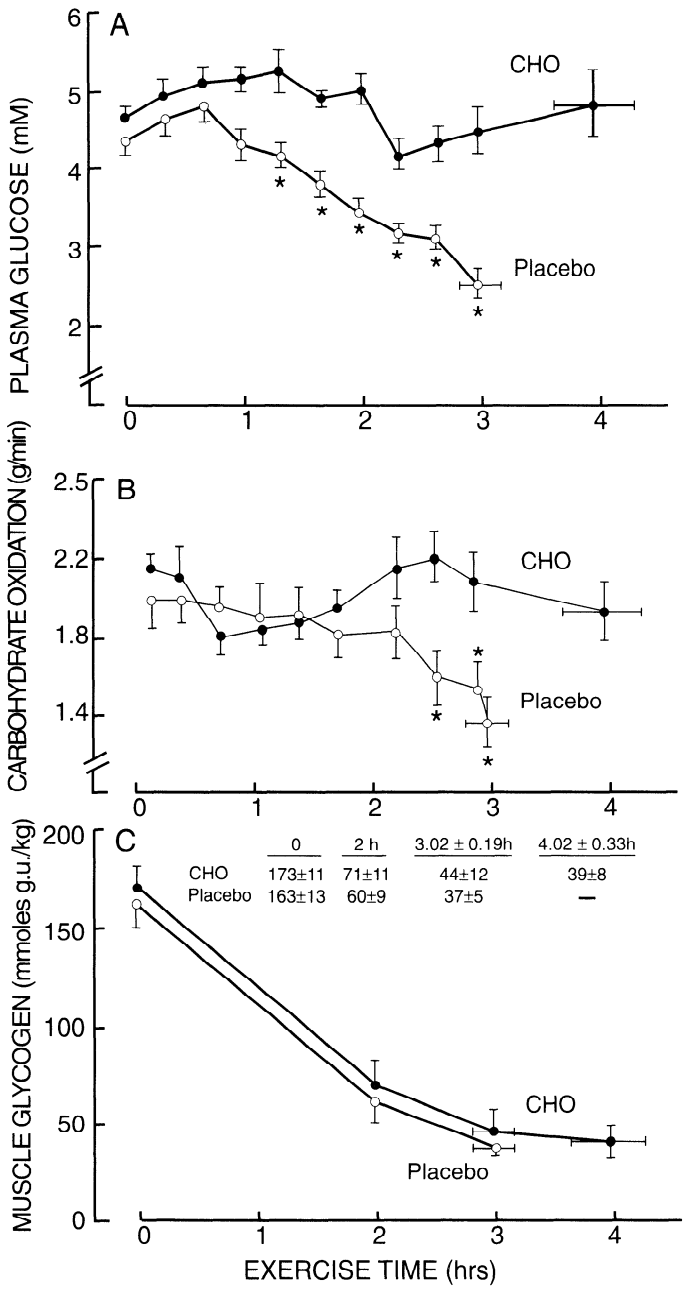

FIG. 16. Responses during exercise with placebo solution and when carbohydrate (CHO) was ingested every 20 min. *A*: plasma glucose responses. *B*: rate of carbohydrate oxidation estimated from O_2 uptake and respiratory exchange ratio. *C*: glycogen concentration, reported both graphically and numerically, measured in vastus lateralis muscle. * Placebo significantly lower than carbohydrate ($P < 0.05$). [From Coyle et al. (114).]

plasma glucose to approximately 2.5–3.0 mM. It is not clear what induced fatigue in the carbohydrate-fed group, since plasma glucose and carbohydrate oxidation remained unchanged throughout exercise (Fig. 16).

D. Mechanisms Linking Reduced Carbohydrate Oxidation With Fatigue

The primary evidence supporting a mechanistic role for a reduced carbohydrate oxidation rate in the etiology of fatigue during prolonged exercise is the highly reproducible observation that fatigue coincides with muscle glycogen depletion and, in some cases, blood hypoglycemia (3, 36, 37, 113, 232). Factors that reduce the rate of glycogen depletion by increasing FFA oxidation, such as heparin administration or endurance exercise training, delay the onset of fatigue during prolonged exercise (110, 238, 420). Additionally, the inhibition of FFA mobilization reduced work capacity (389). These observations led to the hypothesis that the maintenance of prolonged exercise at moderate to high intensities (65–90% of $\dot{V}O_{2\,max}$) required a finite level of carbohydrate oxidation. With the development of muscle glycogen depletion and plasma hypoglycemia, carbohydrate oxidation fell below the required threshold level, leading to muscle fatigue and the inability to continue exercise. The exact mechanisms of the protective effect of carbohydrate oxidation in the prevention of fatigue are unknown. After muscle and liver glycogen depletion and the onset of low blood glucose, the contracting muscle cell must depend on the uptake and oxidation of FFA. The translocation of FFA from the cytosol to the mitochondria may be rate limiting, producing a reduced tissue $\dot{V}O_2$ and cell energy supply. Additionally, the energy derived from the oxidation of fats per liter of oxygen consumed is less than that obtained from carbohydrate oxidation. However, this difference is small and would not explain the complete exhaustion frequently associated with prolonged exercise. Another possibility is that a certain level of carbohydrate oxidation is required for either the optimal production of NADH and electron transport or the maintenance of fat oxidation. The possibility exists that intermediates of the tricarboxylic acid cycle (TCA) may become limiting without sufficient carbohydrate oxidation. This possibility has recently been supported by the observations of Sahlin et al. (416) and Spencer et al. (445). The former found the sum of TCA intermediates to first increase then decline during cycle exercise to fatigue, while the latter observed carbohydrate feedings to reduce the fall in TCA intermediates. However, no evidence exists that the decline in TCA intermediates actually limited tissue oxidation. Recently, it was suggested that an increased metabolism of branched-chain amino acids following glycogen depletion could drain the TCA intermediate 2-oxoglutarate, which participates in the aminotransferase reaction (478). This possibility seems unlikely in that TCA intermediates are metabolic products of both valine and isoleucine metabolism.

It seems unlikely that muscle glycogen depletion, low blood glucose, and the resultant decline in carbohydrate oxidation are an exclusive fatigue factor during prolonged exercise. In the carbohydrate feeding experiments of Coyle and colleagues (100, 113), the group receiving carbohydrate supplements exercised an hour longer, but still fatigued despite a maintained normal blood glucose and carbohydrate oxidation rate. Furthermore, if glycogen depletion directly elicits fatigue by reducing energy production, cell ATP should decline, and in general, this has not been observed. A possibility exists that muscle glycogen depletion is causative in fa-

tigue via a mechanism independent of its role in energy production. For example, glycogen depletion may trigger functional changes in the SR or activation of lysosomal enzymes that in turn cause deleterious alterations in contractile function. Finally, in some cases, muscle glycogen depletion may be correlated with, but not causative in, fatigue. In these situations, other factors, such as structural changes in cell organelle, may be involved (179, 222).

VIII. ULTRASTRUCTURAL CHANGES AND THE RELATIONSHIP BETWEEN MUSCLE FATIGUE AND MUSCLE INJURY

A. Relationship Between Muscle Injury and Muscle Fatigue

In 1902, Hough (251) made an important distinction between muscle fatigue and soreness resulting from injury when he stated "when an untrained muscle makes a series of contractions against a strong spring, a soreness frequently results which cannot be regarded as a phenomenon of pure fatigue." Furthermore, he described two types of soreness: one occurring during maintained contractions and closely associated with fatigue, and a second type that generally occurred after exercise and persisted for a prolonged period of time. He believed that the former was a result of metabolic waste products in which the soreness was elicited by tissue swelling or a direct chemical stimulation of muscle afferents. This type of soreness was rapidly reversed following exercise. In contrast, the latter postexercise type of soreness was associated with rhythmic contractions with its degree dependent on the strength and suddenness of the muscular activity. Hough (251) suggested that this type of soreness "has its origin in some sort of rupture within the muscle itself"; in other words, that it was a direct result of muscle injury. From a mechanistic point of view, it is important to delineate between muscle fatigue elicited by the various factors described in this review and muscle injury. However, to the extent that the latter occurs during exercise, both would reduce peak power and be deleterious to human performance. Consequently, it is important to determine the extent to which muscle damage occurs during as opposed to following a given exercise bout. Asmussen (17) evaluated postexercise muscle soreness following a step exercise in which one leg was used to step onto a 50-cm stool (an activity involving concentric shortening of the quadriceps or positive work) and the other for descent (eccentric lengthening of the quadriceps or negative work). The activity was continued until fatigue set in, which always occurred in the leg performing the positive work. In contrast, muscle soreness 1 and 2 days postexercise was considerably more apparent in the muscles conducting the eccentric action. Since this observation, a number of researchers in different laboratories have provided ultrastructural evidence that eccentric, but not concentric or isometric contractions, can induce muscle injury (14, 191, 340, 377). These reports have clearly shown damage to be apparent immediately postexercise, which suggests that the injury contributed to the reduced muscle power and physical work capacity and thus to the sensation of fatigue and the inability to continue the exercise task. The observation that isometric strength was lowest immediately after eccentric exercise provides additional evidence that the injury developed during the exercise and contributed to the fatigue process. It is also apparent from the published literature that the degree of muscle injury becomes progressively worse in the first few days after intense eccentric exercise (12, 14). This observation explains why performance can remain depressed for days after exercise even though all the known fatigue agents have long since recovered.

In recent years, there have been a number of reviews in which the main focus has been the relationship between muscle injury and performance (12, 16, 95, 147, 191, 453). It is clear that injury can occur during exercise and thus participate in the development of fatigue. An important question is at what time in the exercise injury develops, and is it restricted to the late phase of prolonged exercise or can it develop early in the exercise task? Although the majority of studies demonstrating muscle injury evaluated prolonged endurance exercise, it is clear that it can be induced during high-intensity short-duration exercise (80, 191). The development of muscle injury appears to be related not so much to the duration of the work but to the nature of the contractions. Employing an in vitro mouse model, McCully and Faulkner (342) reported that the extent of muscle injury was related to the degree of force developed during lengthening contractions and not to the velocity of lengthening. They hypothesized that the majority of muscle injury occurred in the first 5 min of contractile activity, since beyond this time fatigue had reduced the fiber force to levels below the threshold required for the initiation of injury. Lieber et al. (312) provided additional evidence that at least during high-intensity exercise muscle damage occurs early in the contraction period. In addition to force, studies by Newham et al. (375) and Jones et al. (274) provide evidence that the degree of muscle injury increases at long fiber lengths. It may be that the mechanism of muscle injury and thus the time required for its development is dependent on the type of exercise. With high-intensity exercise, the high-force contractions initiate injury early in the exercise period. In this type of exercise, the causative event is the mechanical disruption of the sarcomeres and perhaps the sarcolemma membrane (191, 312, 338, 342). The latter would lead to an influx of Ca^{2+} and the activation of cell proteases and phospholipase activity, which would further contribute to fiber degradation (16, 146, 273). In contrast, the contractions associated with prolonged endurance activity are less forceful and, unless they contain a large eccentric component (such as downhill running), less likely to cause a direct mechanical disruption of the muscle cell. In this type of activity, the possibility

exists that the injury process develops late in the exercise period. One possibility is that the exercise-induced inhibition of SR function (reviewed in sect. VIII*D*) leads to a buildup in cytosolic Ca^{2+}, which then initiates the cell degradative processes described above (16). As reviewed in section VII*B*, fatigue during prolonged endurance exercise is generally associated with muscle glycogen depletion. An important unanswered question is whether or not glycogen depletion somehow mediates (and hence is a prerequisite for) the disruption of intracellular organelles such as the SR.

Some controversy exists as to whether or not muscle injury preferentially affects a particular fiber type (147). The answer may be that the type of exercise dictates whether fast- or slow-twitch fibers are most affected. With endurance exercise comprising a high eccentric component such as downhill running, slow fibers are preferentially recruited, and consequently, cell damage, when it occurs, appears to be restricted to the slow fiber population (14, 383). On the other hand, during high-intensity exercise, fast as well as slow fibers are recruited, and in this case, fast-twitch fibers appear more susceptible to injury (192, 312, 441).

Armstrong and co-workers (12, 16) have reviewed the mechanisms of exercise-induced muscle fiber injury. The two most mentioned theories are as follows: *1*) injury is induced as a direct effect of forceful eccentric contractions, and *2*) injury results from the toxic effects of metabolic waste products. As reviewed above, considerable support exists for the former. The latter seems unlikely in that muscle injury occurs most frequently during eccentric contractions, which are metabolically less demanding than concentric contractions (12, 16, 376). According to Armstrong and colleagues (16, 142), a central element to the induction of fiber injury is an increase in intracellular Ca^{2+}. Duan et al. (142) observed an inverse relationship between mitochondrial Ca^{2+} (marker of cell Ca^{2+}) and the number of intact fibers per square millimeter. Furthermore, muscle damage was attenuated by the intraperitoneal injection of the Ca^{2+} chelator ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid. The slow Ca^{2+} channel blocker verapamil reduced mitochondrial Ca^{2+} and fiber injury in the slow soleus but had no effect on the other muscles tested (142). Jones et al. (273) found that reducing extracellular Ca^{2+} decreased fiber injury in the mouse soleus contracting in vitro. These data provide support for the idea that at least in slow muscle an increased influx of Ca^{2+} may trigger the injury process. Duncan (146) employed the skinned fiber preparation to demonstrate that intracellular Ca^{2+} can trigger muscle injury and that the effective concentration was 10^{-7} to 10^{-5} M, which is within the range obtained during E-C coupling. Although considerable evidence exists linking high intracellular Ca^{2+} to the injury process (16), the exact mechanisms of how Ca^{2+} triggers cell injury remain unresolved. Although Ca^{2+}-activated proteases have been shown to degrade myofibrillar components, specific inhibitors of these enzymes have not proven effective in preventing Ca^{2+}-induced muscle damage (16). Jackson et al. (266) tested a variety of protease inhibitors and found none to be effective in preventing enzyme efflux from muscle following exhaustive exercise. In contrast, certain inhibitors of phospholipase A were effective in reducing enzyme efflux. Consequently, these authors (266) concluded that Ca^{2+} activation of phospholipase A could initiate muscle injury by degrading the sarcolemma as well as internal membrane structures. Other factors that could be involved in cell injury include an increased lysosomal enzyme activity and elevated free radicals (16, 147). The former seems unlikely in that 2 h of endurance exercise in rats produced no change in lysosomal enzyme activity (427, 475), and in mice exhausted by running, lysosomal enzyme activity did not increase until the second day postexercise (475). Davies et al. (124) reported a two- to threefold increase in rat muscle free radicals following exhaustive running. These authors (124) suggested that the increase in free radicals was gradual and likely dependent on the duration of the work. They hypothesized that the decline in mitochondrial respiratory control and the loss of SR and endoplasmic reticulum integrity resulted from an increased lipid peroxidation caused by the increased free radicals. Additional evidence that free radicals may be involved in the fatigue process or muscle injury was provided by Barclay and Hansel (26). They observed the addition of free radicals to increase fatigue, while blocking free radical production or increasing the content of scavengers reduced fatigue in mouse and canine muscle preparations. Questions still remain about the source and time course (during or after exercise) of the free radical production (268). Barclay and Hansel (26) hypothesized that free radical production may be accented during high-intensity exercise with periods of relative ischemia. The observation that blockers and scavengers appear to act as much, or perhaps more, during the recovery period than during contraction led these authors to suggest that the role of free radicals in fatigue may be especially important during exercise involving alternate periods of high-intensity exercise and rest. Free radical scavengers have also been shown to reduce the extent of muscle damage 3 days postexercise (496).

In sections VIII*B* and VIII*D*, the contribution of ultrastructural and functional changes in mitochondria, T tubules, SR, and myofibrils to the fatigue process is considered.

B. Mitochondria

Some controversy exists as to whether or not ultrastructural and/or functional changes in muscle mitochondria contribute to muscle fatigue. In 1969, Gollnick and King (208) reported marked swelling of mitochondria isolated from rats run to exhaustion and suggested that if the swelling occurred during exercise it could uncouple oxidative phosphorylation. However, in 1972, Terjung et al. (462) observed mitochondrial structure to be unchanged by exercise to exhaustion, and in 1974,

Gale (194) reported that the mitochondrial alterations observed by Gollnick and King (208) resulted from an artifact of fixation. Since these studies, the observation of swollen mitochondria following fatiguing exercise has been confirmed by Nimmo and Snow (378) and, more recently, by McCutcheon et al. (344).

Terjung et al. (462) found exercise to exhaustion to have no effect on the capacity of rat mitochondria to oxidize substrate and generate ATP. However, in a series of papers, Dohm and co-workers (137–139) reported muscle mitochondrial function to be compromised following prolonged endurance exercise in rats. In the quadriceps muscle, the oxidation rate of palmitate, pyruvate-malate, and succinate was depressed following almost 5 h of running in exercised-trained rats. In untrained rats, fatigue occurred considerably sooner (a little over 2 h of running), but curiously, only palmitate and not pyruvate-malate or succinate oxidation was depressed (139). Additionally, at exhaustion, the activities of NAD-specific isocitrate dehydrogenase, succinate dehydrogenase, and cytochrome oxidase were decreased in the gastrocnemius of the trained but not the untrained rats (138). Following the exhaustive exercise, mitochondrial yield from both the gastrocnemius (137) and quadriceps (139) of the trained rats was reduced. The mitochondrial function of the untrained rats was relatively unaltered at fatigue. Although it is not known whether or not the observed changes in mitochondrial function contributed to fatigue in the trained rats, it is clear that the untrained rats fatigued for other reasons before mitochondrial changes could develop. Perhaps the mitochondrial alterations require either a high exercise intensity or prolonged duration to manifest. The former possibility was recently evaluated by Gollnick et al. (205), who studied the effects of five high-intensity 600-m bouts on horse mitochondria. The time to complete the runs increased from 39.6 to 47.7 s, and muscle respiratory capacity declined by >20% after the first bout and was 45% of the control value after the fifth run. Currently, it is unknown the extent to which alterations in mitochondrial structure affect the in vivo muscle respiratory rate, whole body oxygen consumption, or physical work capacity. Gollnick et al. (205) suggest that the reduced functional capacity of mitochondria could mediate the reduction in whole body maximal oxygen uptake sometimes observed following fatiguing exercise. On the other hand, reductions in postexercise $\dot{V}O_{2\,max}$ could be explained by other factors, such as the reduced force-generating capacity of the fatigued muscle.

C. Myofibrils

Ultrastructural evidence of myofibril damage during exercise, particularly work involving a high eccentric component, has been observed in several laboratories (14, 29, 191, 376, 383, 441). The most commonly observed alterations include Z-line streaming (wavy Z-lines), A-band lesions, and lengthened and nonuniform sarcomeres. Although the lesions within a fiber can be extensive, the number of damaged fibers within a muscle following exhausting exercise is generally <10% (147, 376). Despite the ultrastructural evidence of sarcomere damage, the functional properties of the contractile proteins appear to be relatively resistant to fatigue. Fitts et al. (179) found a prolonged swim to have no effect on the myofibril Mg^{2+}-ATPase activity of either fast or slow rat hindlimb muscle. The V_{max} from the force-velocity relationship of the slow soleus showed no significant change despite a 26% decline in peak tension. The fast-twitch EDL did undergo a significant 34% decline in V_{max}, but this muscle exhibited extreme fatigue with the P_o falling by >70%. In this case, the fatigued (inactive) fibers might have provided a significant internal drag during the unloaded and lightly loaded contractions. These results imply that the activity of the myofibril ATPase and its functional correlate V_{max} are relatively resistant to alteration during prolonged exercise. This observation contrasts to high-intensity exercise, where fiber V_{max} and ATPase may be inhibited by an elevated free H^+ (reviewed in sect. IV). Further evidence for the relative resistive nature of the contractile protein to fatigue comes from the work of Seraydarian et al. (430) who observed no change in the myosin ATPase activity in fatigued frog muscle. Furthermore, Belcastro et al. (29) reported endurance treadmill running to have no effect on myofibrillar protein yield or the ATPase activity of rat muscle despite the appearance of wavy Z-lines.

D. T Tubules and Sarcoplasmic Reticulum

In 1978, Gonzáles-Serratos et al. (214) observed vacuolation in single fatigued frog fibers. They attributed the vacuolation to swollen T tubules that disappeared as force increased during recovery. More recently, similar vacuoles were observed by Lännergren et al. (309), but they attributed their appearance to damaged mitochondria. Swollen SR vesicles and a reduced Ca^{2+} SR ATPase have been observed following exhaustive endurance exercise (28–30, 81, 344). Fitts et al. (179) found a prolonged swim to have no effect on the SR Ca^{2+}-stimulated ATPase activity in rat muscle, but the SR concentration per gram muscle was reduced in the slow soleus. Despite the unaltered ATPase activity, Ca^{2+} uptake by the SR vesicles was depressed in the slow soleus and fast-twitch red region of the vastus lateralis. A decreased Ca^{2+} uptake with no change in the SR ATPase activity suggests either an uncoupling of the transport or a leaky membrane allowing Ca^{2+} flux back into the intracellular fluid. Recently, Byrd et al. (81) showed that with endurance exercise the initial rate of Ca^{2+} uptake in both muscle homogenates and isolated SR fractions declined at ~45 min, while the maximal SR uptake capacity was lower only after exhaustive exercise. As with the earlier study of Fitts et al. (179), the SR from fast white muscle was unaffected by exhaustive endurance exercise. Recently, high-intensity exercise has also been shown to deleteriously affect SR function. Byrd et al. (82) ob-

served maximal exercise to decrease the SR ATPase and the initial and maximal rate of SR Ca^{2+} uptake in gluteal muscle of thoroughbred horses. Additionally, Gollnick et al. (209) recently found the Ca^{2+} uptake of SR in human muscle to be reduced following ~3 min of high-frequency one-leg kicking. With recovery, the SR function returned to within 10% of the control value. It is likely that the reduced functional capacity of the SR following both short high-intensity and prolonged exercise contributes to the slowed twitch duration and reduced force characteristic of fatigued muscle. As described earlier, the reduced SR Ca^{2+} uptake capacity would cause Ca^{2+} to be redistributed from the SR release site to the cytosol.

IX. SUMMARY AND CONCLUSIONS

Fatigue, defined as the failure to maintain the required or expected power output, is a complex problem, since multiple factors are clearly involved, with the relative importance of each dependent on the fiber type composition of the contracting muscles(s), and the intensity, type, and duration of the contractile activity. The primary sites of fatigue appear to be within the muscle cell itself and for the most part do not involve the central nervous system or the neuromuscular junction. The major hypotheses of fatigue center on disturbances in the surface membrane, E-C coupling, or metabolic events. The cell sites most frequently linked to the etiology of skeletal muscle fatigue are shown in Figure 1.

Skeletal muscles are composed of at least four distinct fiber types (3 fast twitch and 1 slow twitch), with the slow type I and fast type IIa fibers containing the highest mitochondrial content and fatigue resistance. Despite fiber type differences in the degree of fatigability, the contractile properties undergo characteristic changes with the development of fatigue that can be observed in whole muscles, single motor units, and single fibers. The P_o declines, and the contraction and relaxation times are prolonged. Additionally, there is a decrease in the peak rate of tension development and decline and a reduced V_o. Changes in V_o are more resistant to fatigue than P_o and are not observed until P_o has declined by at least 10% of its initial prefatigued value. However, the reduced peak power by which fatigue is defined results from both a reduction in V_o and P_o. In the absence of muscle fiber damage, the prolonged relaxation time associated with fatigue causes the force-frequency curve to shift to the left, such that peak tensions are obtained at lower frequencies of stimulation. In a mechanism not clearly understood, the central nervous system senses this condition and reduces the α-motor nerve activation frequency as fatigue develops. In some cases, selective LFF develops that displaces the force-frequency curve to the right. Although not proven, it appears likely that this condition is associated with and likely caused by muscle injury, such that the SR releases less Ca^{2+} at low frequencies of activation. Alternatively, LFF could result from a reduced membrane excitability, such that the sarcolemma action potential frequency is considerably less than the stimulation frequency.

Fatigue-induced disturbances in E-C coupling could be mediated by an altered sarcolemma or T tubular excitability, a depressed T tubular charge sensor, inhibition of the SR Ca^{2+} release channel, or an uncoupling of the T tubular charge sensor and the SR Ca^{2+} release channel. The membrane hypothesis of muscle fatigue states that the sarcolemma Na^+-K^+ pump is unable to maintain the ionic gradients for K^+ and Na^+ essential for the maintenance of the V_m and cell excitability. The increased $[K^+]_o$, decreased $[K^+]_i$, and elevated K^+ conductance are all thought to contribute to the depolarized V_m frequently observed in fatigued muscle cells. The greatest depolarization occurs in centrally located fibers, and the effect is likely to be largest in T tubular regions located furthest from the sarcolemma. Consequently, the interior regions of the T tubules may depolarize enough to produce block of the action potential, which in turn would produce inactivation of the centrally located myofibrils. It seems unlikely that fatigue could be mediated by a simple drop in the amplitude of the action potential, since the reduced spike height is insufficient to elicit inactivation. Furthermore, changes in the action potential amplitude do not show a good temporal relationship with the change in force. Because the ionic gradients are rapidly reestablished following exercise, it is clear that the membrane hypothesis cannot contribute to the slow recovery phase of muscle fatigue.

Wavy or inactive myofibrils in the fiber core and spatial gradients in Ca^{2+} with the highest concentrations near the edges of the fiber have both been observed in fatigued cells. These observations support the hypothesis that fatigue in certain situations is mediated by a block in E-C coupling. Although the site(s) of the block has not yet been established, the available evidence suggests that the most likely mechanism involves direct inhibition of the SR Ca^{2+} release channel. Some have suggested that an activity-induced increase in T tubular Ca^{2+} might produce an action potential block. The activation threshold does shift to more positive potentials in fatigued cells; however, with modest increases in $[Ca^{2+}]_o$ to 5–10 mM, full activation would still be reached by 0 mV. In fact, an increased $[Ca^{2+}]_o$ may protect against fatigue by preventing inactivation of the T tubular charge sensor that might result from cell depolarization with normal or low extracellular Ca^{2+}. A universal finding is that the amplitude of the Ca^{2+} transient decreases as fatigue develops. Recently, it has been shown that this reduction can occur in the absence of any change in the T tubular charge movement. This observation suggests that the reduced Ca^{2+} transient was caused by a direct inhibition of the SR release channel. The mechanism of this inhibition is unknown, but it could involve an increase in cell H^+, since this ion has been shown to inhibit the release channel in bilayers. Additionally, a redistribution of Ca^{2+} from the SR to the cytosol with contractile activity would reduce the Ca^{2+}

concentration gradient and thus the amplitude of the Ca^{2+} transient.

A large body of evidence suggests that a component of muscle fatigue is mediated by metabolic factors. Since the work of Fletcher and Hopkins (187), it has been known that muscle contraction is associated with the production of lactic acid. It became clear in the 1930s that increases in blood lactate occurred with moderate- to high-intensity, but not prolonged, endurance exercise. More recently, it has been recognized that the highest lactate production occurs in the fast-twitch type IIb muscle fibers performing intermittent maximal exercise. A high inverse correlation between lactate and force during contractile activity has been observed both in vitro and in vivo. However, it is currently thought that the fatigue is mediated not by lactate, but by the increased free H^+ associated with lactic acid production. The direct application of lactate to a muscle cell elicits only a small decline in force, while an increased H^+ produces large effects, with the greatest inhibition of force seen in the fast type IIb fiber type. Although some question the importance on H^+ in the fatigue process, the supportive evidence is convincing. In addition to the skinned fiber experiments demonstrating a direct inhibitor effect of H^+ on force, a high correlation between force and pH has been observed during the recovery from fatigue in both respiratory and limb skeletal muscle. It is clear that pH can only explain a portion of the fatigue effect, since the rapid phase of recovery following fatigue occurs at a time when cell pH is still declining. An increased pH_i is an attractive fatigue candidate because it can act at multiple sites within the cell. Low pH has been shown to reduce the force per cross bridge in all fiber types and the number of bridges in the high force state in fast but not slow fibers. Additionally, an increased H^+ shifts the force-pCa relation to the right such that less force is developed at a given Ca^{2+} concentration. This effect is likely mediated by a combination of factors including a H^+ inhibition of Ca^{2+} binding to troponin C and a reduced thin filament cooperativity caused by the decline in the number of active cross bridges. The right shift in the force-pCa relation could become important because the amplitude of the Ca^{2+} transient declines with fatigue. The decreased peak dP/dt and V_o observed in fatigued fibers could also be in part mediated by the increased H^+ because low pH has been shown to inhibit the rate constant of cross-bridge transition from the weakly bound (low force) to the strongly bound (high force) state and the myofibrillar ATPase activity. Finally, high H^+ has been shown to inhibit the SR Ca^{2+} release channel as well as the SR Ca^{2+} reuptake process; the latter likely contributes to the slowing of relaxation associated with muscle fatigue. It is important to realize that the magnitude of the H^+ effects will depend on the cells contractile history and, in particular, the amplitude of the Ca^{2+} transient. Consequently, one would not expect to see acidic pH produced by CO_2 exposure to cause as large a fall in force as that elicited by stimulation, because the amplitude of the Ca^{2+} transient would be considerable larger in the former.

During intense contractile activity intracellular P_i increases, and this increase is thought to contribute to the development of fatigue. High P_i has been shown to inhibit the maximal force of skinned fibers with the greatest effect observed in fast-twitch fibers. Additionally, a high inverse correlation exists between P_i and force during the recovery from fatigue. Controversy exists regarding whether total or the diprotonated form of P_i is the active species in the etiology of fatigue. The mechanism appears to involve a direct effect of P_i inhibiting the transition of the cross bridge from the low to the high force state. A P_i-induced decrease in strongly bound cross bridges would reduce thin filament activation accenting the decline in tension, particularly at suboptimal Ca^{2+}. Inorganic phosphate may contribute to the slowing of relaxation by reducing the rate of SR Ca^{2+} uptake, but it does not appear to contribute to the fatigue-induced decline in V_o.

Considerable debate has centered on the question of whether or not depletion of high-energy phosphates (ATP and PCr) participates in the fatigue process. The overriding evidence suggests that they do not, that fatigue produced by other factors reduces the ATP utilization rate before ATP becomes limiting. The most compelling support for this conclusion is that cell ATP rarely falls below 70% of the preexercise level, even in cases of extensive fatigue. The possibility exists that a drop in the free energy of ATP hydrolysis could contribute to fatigue, but at most this appears to play a minor role. Some have suggested that ATP is compartmentilized, such that when PCr falls below some critical level, the rate of ATP resynthesis at the cross bridge becomes limiting. However, even in highly fatigued fibers, ATP concentration is >100-fold higher than the micromolar amounts required for peak force. Thus, for compartmentalization to be a factor, one would have to hypothesize that 99% of the cell ATP was unavailable to the cross bridges.

At work loads between 65 and 85% $\dot{V}O_{2\,max}$, muscle fatigue generally results after 2–3 h of exercise, and the inability to continue work is highly correlated with the depletion of muscle glycogen. The depletion is primarily confined to the slow type I and fast type IIa fiber types. It has been known for some time that carbohydrate supplementation during prolonged exercise can delay the onset of fatigue. The mechanism of this protective effect has been extensively studied, and the preponderance of evidence supports the notion that carbohydrate ingestion delays fatigue by maintaining a high-carbohydrate fuel source in the form of blood glucose. When blood glucose is maintained, one observes a shift in carbohydrate source from muscle glycogen to blood glucose following the first hour of endurance exercise. The exact mechanisms of the protective effect of carbohydrate oxidation in the prevention of fatigue are unknown. It seems unlikely that muscle glycogen depletion, low blood glucose, and the resultant decline in carbohydrate

oxidation is an exclusive fatigue factor during prolonged exercise. A possibility exists that muscle glycogen depletion is causative in fatigue via a mechanism independent of its role in energy production. Glycogen depletion may trigger functional changes in the SR or other cell organelles. Alternatively, in some cases muscle glycogen depletion may be correlated with, but not causative in, fatigue.

In 1902, Hough (251) made an important distinction between muscle fatigue and muscle injury. The latter can contribute to a reduced power output and thus muscle fatigue, but only if the injury occurs during the exercise period, unless of course the exercise involves repeated bouts of work, in which case postexercise injury could limit the performance of the subsequent exercise periods. Considerable muscle injury has been shown to occur postexercise, especially following forceful eccentric contractions. Immediate postexercise ultrastructural analysis suggests that injury can occur during exercise, with the most commonly observed changes involving sarcomere disruptions and swollen SR and mitochondrial vesicles. The extent to which these ultrastructural changes participate in the development of fatigue is unknown. The cause of cell injury is not established, but an increase in cytosolic Ca^{2+} may be involved. Despite the appearance of some disrupted sarcomeres, myofibrils isolated from fatigued muscle show essentially normal function; however, depressed SR function has been observed following both prolonged endurance and high-intensity exercise.

In conclusion, it should be clear from this review that the etiology of muscle fatigue is complex, likely involving multiple factors acting at numerous cellular sites. The relative importance of each fatigue agent is clearly dependent on the type of exercise and the fiber composition of the muscles involved. It appears that alterations in E-C coupling and metabolic factors participate in the fatigue process regardless of the intensity or duration of the exercise. However, during short-duration high-intensity exercise, the disruption in E-C coupling is likely mediated by ionic disturbances (such as high $[K^+]_o$ or intracellular H^+) that are rapidly reversed upon cessation of exercise, whereas during prolonged exercise, structural damage to the SR may compromise its ability to regulate Ca^{2+}. From a metabolic standpoint, it's clear that fatigue resulting from high-intensity exercise is in part caused by an increased intracellular H^+ and P_i acting at the cross bridge, and in the case of H^+, at other sites particularly the SR and the regulatory proteins. Muscle glycogen depletion is highly correlated with and undoubtably contributes to fatigue during endurance exercise. Although considerable progress has been made in understanding the etiology of skeletal muscle fatigue, a number of important unanswered questions remain. For example, it will be important to determine why carbohydrate metabolism appears to be obligatory to the maintenance of prolonged exercise and to establish the cellular mechanisms of the observed alterations in E-C coupling. Finally, it will be important to establish the relative importance of each fatigue agent and to devise countermeasures for the prevention of fatigue.

I thank Barbara DeNoyer for help in the preparation of this manuscript and my current and former graduate students and postdoctoral fellows for helpful suggestions. I particularly thank Ed Balog, LaDora Thompson, and Jeff Widrick for their reading of an earlier version of this manuscript. I thank my wife Mary Ellen and children, Ryan, Eric, Lara, and Paul, for support during the writing of this manuscript.

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