# Integration of ATP Production and Consumption

Peter J. Bechtel*
Philip M. Best

## Introduction

The objective of this presentation is to give a broad overview of two aspects of muscle contraction. First, we will examine how ATP levels are maintained in skeletal muscle and will focus on unique mechanisms in muscle that are used to maintain ATP levels. The second aspect of this presentation will deal with the consumption of ATP by crossbridge movement, the sodium-potassium pump of the plasma membrane and the calcium pump of the sarcoplasmic reticulum.

## High-Energy Phosphate Compounds in Skeletal Muscle

There are two major high-energy compounds that are associated with muscle contraction. They are ATP (adenosine triphosphate ) and PCr (creatine phosphate). The concentration of ATP, PCr and other compounds in a resting skeletal muscle is shown in Table I. During contraction, the content of PCr decreases as energy is utilized (Cain and Davies, 1962; Infante et al., 1965). The content of ATP in most resting vertebrate skeletal muscles is in the 5 to 8 m moles/$\ell$ range and is normally maintained at this level during contraction. The concentrations of ADP and AMP are approximately 1 and 0.2 m moles/$\ell$, respectively. The concentration of another high-energy compound, NAD, is approximately .5 - 1 m moles/$\ell$ in skeletal muscle.

**Table 1. Chemical Characteristics of Resting Skeletal Muscle**

| *Metabolites (M moles/$\ell$ muscle)* | *Extensor Digitorum Longus 20°C* | *Soleus 20°C* |
|---|---|---|
| ATP | 6 | 5 |
| $P_i$ | 9 | 7 |
| PCr | 24 | 13 |
| Total creatine | 30 | 20 |

Data taken from Kushmerick (1983).

*P. J. Bechtel, Department of Animal Sciences, University of Illinois, Urbana, IL 61801.

P. M. Best, Department of Physiology, University of Illinois, Urbana, IL 61801.

Reciprocal Meat Conference Proceedings, Volume 38, 1985.

### Creatine Phosphokinase Reaction

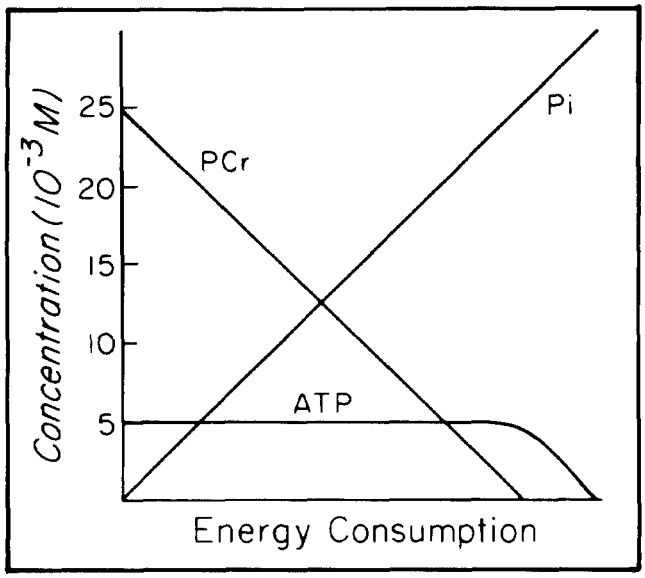
The typical muscle cell contains about 25 m moles/$\ell$ PCr which acts as a supply of high-energy phosphate from which ATP can be regenerated (Figure 1). The resynthesis of ATP from ADP and PCr is catalyzed by the enzyme ATP:creatine phosphokinase (CPK) according to the following reaction:

$$ADP + PCr \leftarrow\text{-}\rightarrow ATP + Cr + \alpha H+.$$

This reaction is nearly at equilibrium in muscle cells and has a high equilibrium constant (at least 20). The enzyme CPK is found on the M line of the myofibrils, in the cytoplasm and on the outer mitochondrial membrane. Both the forward and reverse rate constants are slightly greater than the ATP hydrolysis rate during contraction, suggesting that the creatine phosphokinase reaction is capable of acting as a high-energy phosphate buffer during muscle contraction.

Recently it has been suggested that PCr acts as a "shuttle" for the transport of high-energy phosphates between the mitochondria and the contractile proteins (Bessman and

Figure 1




Schematic representation of the use of energy-rich phosphate compounds during rapid energy consumption of skeletal muscle.

Geiger, 1981). This scheme supposes that ATP is localized at the myofibrils and in the mitochondria and that high-energy phosphate is transported between these two sites by PCr. However, Kushmerick and his co-workers (Meyer, Sweeney and Kushmerick, 1984) have argued that such "spatial" buffering need not depend on compartmentalization of substances within the cell. Rather a consideration of simple diffusion theory and coupled, near-equilibrium reactions suggest that the transport of high-energy phosphate by PCr results from the almost constant ATP concentration within the cell. A significant concentration gradient between the myofilaments and mitochondria exists only for PCr and not for ATP. Thus, high-energy phosphate will move in the form of PCr and not as ATP.

Several experimental observations are consistent with the buffering action of PCr. It is well known that inactivation of CPK by fluorodinitrobenzene (FDNB) causes a "rapid" fall in the muscle ATP levels during contraction (Murphy, 1966). However, when the creatine phosphokinase reaction is operable, ATP levels stay constant while the total PCr falls as a direct function of the stimulus duration (Nassar-Gentina et al., 1978). In frog muscle at 15°C, 30 seconds of contracture depletes PCr stores by 50% although tension is maintained. As PCr levels drop below the 50% level, tetanic tension also begins to drop.

When muscles contract at low temperature, ATP synthetic reactions (see below) may not play a significant role during short (20 to 30 second) intervals of sustained muscular activity. Thus, Curtin and Woledge (1979) found negligible resynthesis of ATP from reactions other than creatine phosphokinase following a 15-sec tetanus in frog muscle. Recovery processes involving ATP synthesis may normally occur during contraction in mammalian muscle in which more rapid ATP utilization occurs.

### Adenylate Kinase Reaction

ATP can also be regenerated from ADP via the following reaction which is catalyzed by ATP:AMP phosphoryltransferase (adenylate kinase, myokinase):

$$2\ ADP \leftarrow\!-\!\rightarrow ATP + AMP$$

The maximum velocity of this reaction is less than that of creatine phosphokinase but the reaction is thought to be at equilibrium in muscle. Thus, under conditions when ADP levels rise (i.e., when supplies of PCr are depleted), ATP can be generated from this pathway.

### Summary

The creatine phosphokinase and adenylate kinase reactions act as back-up mechanisms that allow muscle to function briefly under conditions where the rate of ATP utilization exceeds its production by glycolytic or oxidative mechanisms. Obviously, muscle tissue cannot function for prolonged periods under these conditions.

## ATP Synthesis

### Glycogenolysis

Glycogen is an ubiquitous carbohydrate in animals which can also be found in some plants and bacteria. In vertebrates, large concentrations of glycogen can be found in muscle and liver. The content of glycogen in skeletal muscle is approximately 0.7% but will vary with exercise and nutritional status. In liver, the content of glycogen is approximately 4% to 5% but fluctuates greatly depending on the nutritional status of the animal. Because there is more skeletal muscle than liver, the total amount of glycogen in skeletal muscle exceeds that in liver by about 3-fold. Liver glycogen is involved in blood glucose homeostatis, whereas muscle glycogen is used during muscle work.

**Table 2. Composition of a Glycogen Particle From Skeletal Muscle**

| | |
|---|---|
| Glycogen | 20 mg/ml |
| Phosphorylase | 70 μM |
| Phosphorylase kinase | 4 μM |
| Glycogen synthase | 4 μM |

From Preiss and Welsh (1981).

Glycogen is a branched polysaccharide of α-D-glucose units linked together through either 1-6 or 1-4 glycosidic bonds. The bush-like glycogen molecules have molecular weights in the millions. In skeletal muscle, glycogen forms subcellular particles designated as "glycogen particles." These small particles contain glycogen and high concentrations of the major enzymes that synthesize and break down glycogen (Table 2). Isolated glycogen particles contain 50% to 70% of the total cell phosphorylase, 70% to 90% of the glycogen synthase and 20% to 50% of the phosphorylase kinase (Preiss and Walsh, 1981).

Glycogen can be viewed as stored glucose that is rapidly mobilized and used to produce energy (see Table 4). Regulation of skeletal muscle glycogen metabolism has been one of the most extensively studied mammalian systems. All the major enzymes involved in glycogenolysis have been purified and the cascade system shown in Figure 2 shows how a number of different signals can be integrated to alter glycogen breakdown.

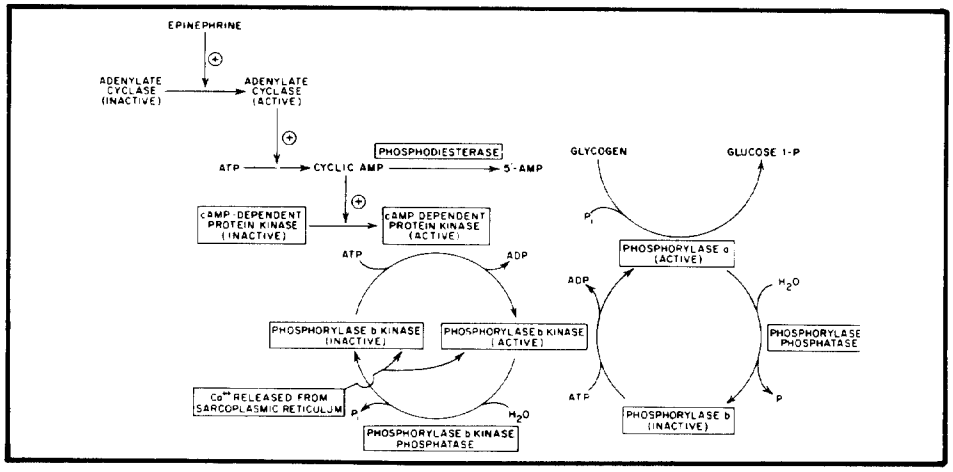
The key regulatory enzyme in this cascade is phosphorylase, which catalyzes the following reaction:

$$P_i + \text{glycogen (n-glucose units)} \leftarrow\!\text{-----}\!\rightarrow \text{G-1-P} + \text{glycogen (n-1 glucose units)}$$

Although the reaction is reversible, the low cellular concentration of G-1-P and high concentration of $P_i$ result in the reaction going from left to right. The enzyme from skeletal muscle has been extensively studied and its three-dimensional structure elucidated (Kasvinsky et al., 1978). The skeletal muscle enzyme binds much of the Vitamin B6 found in the body (Russell et al., 1985). As shown in Figure 2, phosphorylase can be in either an active (phosphorylase A) or inactive (phosphorylase B) form. There are a number of allosteric control mechanisms for phosphorylase and also covalent modification control with the enzymes phosphorylase kinase and phosphorylase phosphatase.

Activation of phosphorylase by the onset of contractile activity has been analyzed and appears to follow first-order kinetics and the extent of phosphorylase activation appears

Figure 2




Cascade mechanism of glycogen in skeletal muscle.

to reach a maximum as a function of stimulus duration (Kushmerick, 1983). During a series of twitches, there is a correlation of the extent of phosphorylase activation and total energy consumed (Mommaerts et al., 1975). Stull and Mayer (1971) have investigated the activation of phosphorylase in vivo.

Phosphorylase kinase is a very interesting enzyme found in large concentrations in skeletal muscle. The enzyme activity of phosphorylase kinase has an absolute requirement for calcium. In addition, the enzyme can be activated through covalent modification with the enzyme cAMP-dependent protein kinase as shown in the cascade system in Figure 2. Recently, the regulatory subunit of cAMP-dependent protein kinase was crystallized in our laboratories (Lee at al., 1985).

Skeletal muscle phosphorylase kinase is composed of four subunits with a stoichiometry of $\alpha 4$, $\beta 4$, $\gamma 4$, $\delta 4$ and a molecular weight of $1.36 \times 10^6$ daltons. The enzyme is found in unusually high concentrations in skeletal muscle (see reviews by Carlson, et al, 1979 and Preiss and Walsh, 1981). The calcium binding subunit of phosphorylase kinase is calmodulin (Cohen et al, 1978). The half-maximum concentration of calcium required to activate phosphorylase kinase is in the range of 0.1 to $3 \times 10^{-6}$ M calcium depending on the status of enzyme. During a contraction event, the concentration of calcium in the sarcoplasm can be increased 10 to 100 fold and this increased calcium concentration could activate phosphorylase kinase and thus activate glycogen breakdown. In an interesting experiment, Brostrum et al. (1971) were able to inhibit phosphorylase kinase by removal of calcium with a sarcoplasmic reticulum preparation. The effects of hormones such as epinephrine on skeletal muscle glycogen are thought to occur through the cAMP cascade mechanism while the effects of nerve action on muscle glycogen are thought to occur through calcium flux which operate the phosphorylase kinase.

Three ATP molecules are produced (net) from each glucose unit of glycogen as shown below.

$$3\ ADP + 3\ Pi + 1\ glucosyl\ unit \rightarrow 3\ ATP + 2\ lactate$$

Only 2 ATP are generated (net) from an extracellular glucose molecule because of the initial ATP required to phosphorylate glucose to glucose 6-phosphate after it enters the muscle cell.

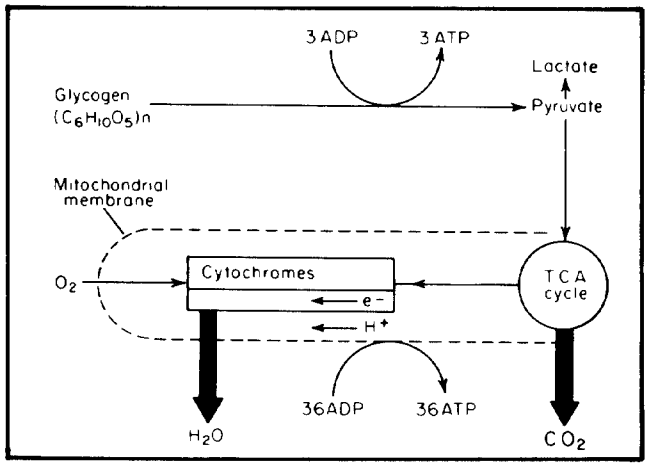
The importance of anaerobic glycolysis is to enable additional ATP to be produced under conditions where aerobic metabolism does not keep pace with ATP utilization. However, the buildup of lactic acid during anaerobic metabolism will inhibit glycolytic enzymes. The point at which exercise or muscle contraction leads to accumulation of lactic acid has been intensively investigated by exercise physiologists.

## Oxidative Metabolism

Although muscle can utilize both carbohydrates and lipids, most (90%) of its energy at rest is derived from circulating free fatty acids (Poland et al., 1977). As metabolic demand changes, carbohydrates are more readily available (glycogen breakdown and glucose transport). However, it should be remembered that lipid metabolism normally plays a dominant role in skeletal muscle ATP production. It is a well-established concept that oxidation of fat can provide essentially all the energy required by working muscle during light to moderate exercise. The intramuscular triglyceride stores can contribute between 50% and 75% of the free fatty acids needed during light exercise (Havel et al., 1964; Oscai et al., 1982).

Oxidative metabolism pathways are those of aerobic reactions taking place in the mitochondria which include utilization of pyruvic acid and free fatty acids (Figure 3). Reactions

Figure 3




Schematic diagram of the oxidative metabolism.

**Table 3. Metabolic State of Mitochondria**

| | *ADP* | *Respiration Rate* | *Rate Limiting* |
|---|---|---|---|
| Resting muscle | Low | Slow | ADP |
| Contracting muscle | High | Maximal | Respiration chain |

involve the reduction of oxygen, the oxidation of substrates and ATP generation. The utilization of oxygen has been examined during tetanic activity where a decrease in muscle capillary oxy-hemoglobin occurs within 200 m sec and a maximum response takes 1 sec.

The number of mitochondria varies greatly in muscles. Hoppeler et al. (1981) examined different skeletal muscles in wild animals and found the mitochondrial volume was 2% to 10% of the volume of the muscle. As a generality, there is a relationship between aerobic capacity, capillary density and mitochondria number. In resting muscle, the rate-limiting step in mitochondria is availability of substrates for ATP synthesis (Table 3). At the onset of contraction, the mitochondria are thought to shift to a different state in which the limitation is one of respiratory insufficiency.

The early events of increased respiratory activity are not likely to be limited by chemical diffusion and the rate of oxidative metabolism increases at least 20-fold between rest and exercise (Carlson and Wilkie, 1974). During or after rapid energy consumption, the concentration of ADP is thought to be the signal that maintains the rate of oxidative phosphorylation in the mitochondria until the PCr concentration has been restored.

In conclusion, the general scheme for energetics is as follows:

1. ATP is the primary source of chemical energy; however, the enzyme creatine phosphokinase must be inhibited in order to detect falling ATP levels.
2. The reaction catalyzed by creatine phosphokinase is such that utilization of ATP is observed as a net breakdown of PCr as follows:

$$\begin{array}{r} ATP \rightarrow ADP + Pi \\ ADP + PCr \longleftrightarrow Cr + ATP \\ \hline PCr \rightarrow Cr + Pi \end{array}$$

3. During brief contraction, the two important factors are depletion of PCr and subsequent recovery of $0_2$ consumption.

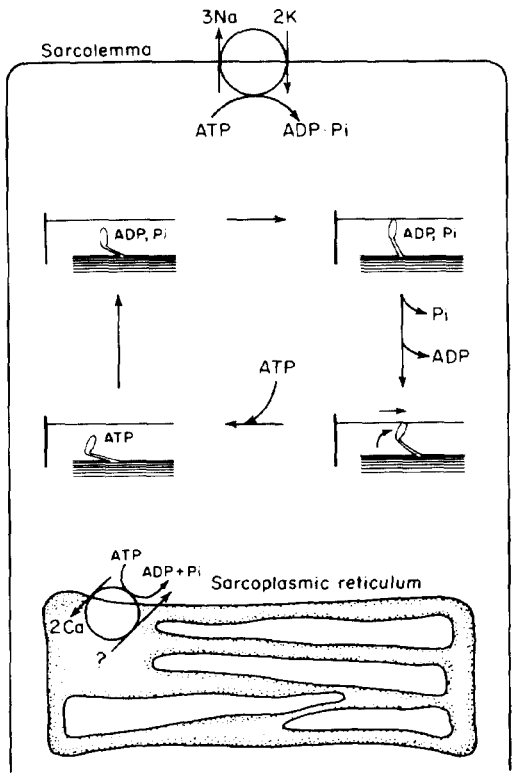
## Sites of ATP Utilization

The primary function of striated muscle is the transformation of chemical energy into mechanical work. The cyclical interaction of actin and myosin during the crossbridge cycle causes the hydrolysis of stoichiometric quantities of ATP, which is the immediate energy source utilized during tension development and shortening. Although this reaction accounts for the majority of the ATP split during a contraction, other energy-dependent processes also occur. In particular, ion pumps hydrolyze ATP in order to maintain the steady state distribution of sodium, potassium and calcium within the muscle cell. In the discussion that follows, three important hydrolytic reactions that occur during muscle contraction will be reviewed (see Figure 4 and Kushmerick, 1983 for further discussion).

### Actomyosin ATPase

According to the sliding filament theory of muscle contraction, shortening and tension development results from the relative movement or sliding of thick and thin filaments past one another. It is generally thought that this sliding action is caused by the cyclical attachment of crossbridges (myosin heads) which undergo some sort of conformational change when attached, resulting in filament motion or force generation. The energy driving the crossbridge cycle comes from the hydrolysis of ATP. Biochemical studies of isolated contractile proteins suggest that actin accelerates the ATPase activity of myosin by increasing the rate of release of the hydrolysis products from myosin. A particularly challenging problem has been the correlation of what is known about the actomyosin ATPase reaction with the hypothesized crossbridge cycle in which a myosin head binds to actin, undergoes a conformational change, unbinds and returns to its "resting" configuration. A highly simplified scheme is shown in Figure 4.

Figure 4




Schematic diagram depicting three sites of ATP utilization in skeletal muscle. The three sites are actomyosin ATPase, SR calcium pump and the Na/K ATPase.

**Table 4. Source of Energy Stored in a Fast-Twitch Muscle Fiber**

| *Stored energy* | *Pathway* | *Initial concentration m moles/ℓ* | *Number of twitches supported by energy source* | *Duration of isometric tetanic contraction supported by energy source (sec)* |
|---|---|---|---|---|
| ATP | ATP → ADP + Pi | 6 | 20 | 4 |
| PCr | PCr + ADP → ATP + Cr | 24 | 84 | 18 |
| Glycogen[a] | Glycolysis | 100 | 670 | 130 |
| Pyruvate from glycogen[a] | TCA and oxidative phosphorylation | 200 | 12,000 | 2,400 |

[a]Taken from Gordon (1982).

The amount of ATP hydrolyzed during a twitch by the contractile proteins is approximately 0.3 m moles/ℓ (Infante and Davies, 1962). During a tetanus, the use of high-energy phosphate compounds is highest in the first second of contraction and then declines (Homsher et al., 1975). Initial rates are on the order of 1 to 1.5 m moles/ℓ-sec (Dawson et al, 1977; Homsher et al., 1975). The rate of hydrolysis falls by at least one-third after the first second of a prolonged contracture (Homsher et al., 1975). These values are for fast twitch fibers of the frog measured at low (0° to 5°C) temperatures. Since the actomyosin ATPase rate of a fiber and the speed of its contraction are linearly related, the amount of ATP hydrolyzed during a twitch will vary depending on muscle type. Although this paper does not dwell on the different isozymes of myosin, it is common knowledge that purified myosin from fast-twitch (white) muscle has a higher ATPase rate than myosin from slow-twitch (red) muscle.

## Calcium Pump

The interaction of actin and myosin is regulated by calcium ion. At rest, the free (unbound) calcium ion concentration in the myofilament space is of the order of $10^{-7}$ moles/ℓ since most of the calcium is sequestered within the myoplasmic reticulum (SR). Upon stimulation, calcium is released into the sarcoplasm where its free concentration may reach $10^{-5}$ moles/ℓ thus activating the crossbridge cycle. Relaxation occurs as the released calcium is resequestered into the SR. Translocation of calcium from the myoplasm to the sarcoplasmic reticulum involves the movement of calcium against a large concentration gradient and thus is an energy-dependent process. Since essentially all the released calcium must be resequestered into the sarcoplasmic reticulum, it is possible to estimate the amount of ATP used in this process. Approximately 0.2 m moles/ℓ of calcium must be released into the sarcoplasm by the sarcoplasmic reticulum during a contraction if most of the calcium binding sites on the regulatory proteins (troponin) of the thin filament are to be saturated (Baylor et al., 1983). The stoichiometry of the sarcoplasmic reticulum Ca-ATPase is 1:2. Therefore, it takes about 0.1 m moles/ℓ of ATP to resequester the calcium released during a twitch. Interestingly, this is one-third of the amount of ATP needed to "drive" the actomyosin reaction. The rate of ATP hydrolysis by the calcium pump during a tetanus is unknown. Optical studies indicate that calcium release is maintained during a tetanus, although the rate may not be constant.

## Na/K ATPase

Under steady-state conditions, an electrical potential of about 90 mV (inside negative) exists across the surface membrane of all muscle cells. This potential results from the unequal distribution of ions (predominantly Na and K) across the cell surface. Since the cell is permeable to Na and K, the Na and K fluxes of a resting cell membrane are not zero and energy must be constantly expended to maintain these ion gradients. This is accomplished by an ion pump located in the sarcolemma which moves 3 Na out of and 2 K into the cell for every ATP molecule hydrolyzed. During activation of the muscle by the action potential, additional sodium ions move into and additional K ions move out of the cell. Thus, part of the ATP hydrolyzed during muscle contraction provides the energy needed to restore the original ionic conditions to the cell. Calculations based on electrophysiological parameters show that the number of ions that move during the action potential is very small. For every $cm^2$ of surface area, approximately $6 \times 10^{-12}$ moles of Na enter the cell. For a fiber 100 μ in diameter, this is equivalent to about $3 \times 10^{-6}$ moles/ℓ of Na ions. Since hydrolysis of one ATP molecule by the Na/K pump moves 3 Na ions, 1 μmole/ℓ of ATP is needed to remove the Na ions that enter during the action potential. This amount of ATP is inconsequential when compared to the amount utilized by the sarcoplasmic reticulum Ca pump (≈0.1 mM) and actomyosin ATPase (≈0.,3 mM) during a twitch.

## References


Baylor, S.M.; Chandler, W.K.; Marshall, M.W. 1983. Sarcoplasmic reticulum calcium release in frog skeletal muscle fibres estimated from Arsenazo III calcium transients. J. Physiol. 344:625.

Bessman, S.P.; Geiger, P.J. 1981. Transport of energy in muscle: The Phosphorylcreatine Shuttle. Science 211:448.

Brostrom, C.O.; Hunkeler, F.L.; Krebs, E.G. 1971. The regulation of skeletal muscle phosphorylase kinase by $Ca^{2+}$. J. Biol. Chem. 246:1961.

Cain, D.F.; Davies, R.E. 1962. Breakdown of adenosine triphosphate during a single contraction of working muscle. Biochem. Biophys. Res. Commun. 8:361.

Carlson, F.D.; Wilkie, D.R. 1974. Muscle Physiology, Prentice Hall, Inc. p. 100.

Carlson, G.M.; Bechtel, P.J.; Graves, D.J. 1979. Advances in Enzymology 50:41.

Cohen, P.; Burchell, A.; Foulkes, J.G.; Cohen, P.T.; Vanaman, T.C.; Nairn, A.C. 1978. Identification of the $Ca^{2+}$-dependent modulator protein as the fourth subunit of rabbit skeletal muscle phosphorylase kinase. FEBS Letters 92:287.

Curtin, N.A.; Woledge, R.C. 1979. Chemical change and energy production during contraction of frog muscle. How are their courses related. J. Physiol. 288:353.

Dawson, M.J.; Gadian, D.G.; Wilkie, D.R. 1977. Contraction and recovery of living muscles studied by $^{31}P$ nuclear magnetic resonance. J. Physiol. 267:703.

Gordon, A.M. 1982. Muscle. In: Physiology and Biophysics, Ed. Ruch and Patton, Vol. IV, Saunders Co., p. 208.

Havel, R.J.; Carlson, L.A.; Ekelund, L-G.; Holmgren, A. 1964. Turnover rate and oxidation of different free fatty acids in man during exercise. J. Appl. Physiol. 19:613.

Hoppeler, H.; Mathieu, D.; Krauer, R.; Cloasch, H.; Armstrong, R.B.; Weibel, E.R. 1981. Distribution of mitochondria and capillaries in various muscles. Respir. Physiol. 44:87.

Homsher, E.; Kean, C.J. 1978. Skeletal muscle energetics and metabolism. Ann. Rev. Physiol. 93.

Homsher, E.; Rall, J.A.; Walluer, A.; Ricchiut, N.U. 1975. Energy liberation and chemical change in frog skeletal muscle during single isometric tetanic contractions. J. Gen. Physiol. 65:1.

Infante, A.A.; Klaupiks, D.; Davies, R.E. 1965. Phosphorylcreatine consumption during single working contractions of isolated muscle. Biochim. Biophys. Acta 94:504.

Infante, A.A.; Davies, R.E. 1962. Adenosine triphosphate breakdown during a single isotonic twitch of frog sartorius muscle. Biochem. Biophys. Res. Comm. 9:410.

Kasvinsky, P.J.; Madsen, N.B.; Sygusch, J.; Fletterick, R.J. 1978. The regulation of glycogen phosphorylase A by nucleotide derivatives. J. Biol. Chem. 253:3343.

Kushmerick, M.J. 1983. Energetics of muscle contraction. Handbook of Physiology 10:189.

Lee, J.H.; Bechtel, P.J.; Phillips, G.N. 1985. Crystallization and preliminary X-ray investigation of the regulatory subunit of cAMP-dependent protein kinase. J. Biol. Chem. (In Press).

Meyer, R.A.; Sweeney, H.C.; Kushmerick, R. 1984. A simple analysis of the "phosphocreatine shuttle". Amer. J. Physiol. 246:C365.

Mommaerts, W.F.; Vegh, K.; Homsher, E. 1975. Activation of phosphorylase in frog muscle as determined by contractile activity. J. Gen. Physiol. 66:657.

Mommaerts, W.F.H.M. 1969. Energetics of muscular contraction. Physiol. Reviews 49:427.

Murphy, R.D. 1966. Correlations of ATP content with mechanism properties of metabolically inhibited muscle. Am J. Physiol. 211:1082.

Nassar-Gentina, V.; Passonneau, J.V.; Vergara, J.L.; Rapoport, S.I. 1978. Metabolic correlates of fatigue and of recovery from fatigue in single frog muscle fibers. J. Gen. Physiol. 72:593.

Oscai, L.; Caruso, R.A.; Wergeles, A.C. 1982. Lipoprotein lipase hydrolyzes endogenous triacyglycerols in muscle of exercised rats. J. Appl. Physiol. 52:1059.

Poland, J.L.; Hobart, D.J.; Payton, O.D. 1977. The Musculoskeletal System. Medical Examination Publishing Co., Inc., p. 60.

Preiss, J.; Walsh, D.A. 1981. The Comparative Biochemistry of Glycogen and Starch. In: Biology of Carbohydrates, Ed. V. Ginsburg, Vol. 1, p. 200.

Russell, L.E.; Bechtel, P.J.; Easter, R.A. 1985. Effect of deficient and excess dietary vitamin B-6 on amino transaminase and glycogen phosphorylase activity and pyridoxal phosphate content in two muscles from postpubertal gilts. J. Nutr. (In Press).

Stull, J.T.; Mayer, S.E. 1971. Regulation of phosphorylase activation in skeletal muscle in vivo. J. Biol. Chem. 246:5716.