

# Role of Magnesium, Potassium and Calcium in Normal Neuromuscular Function in Ruminants

by

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## Summary

The role of calcium, magnesium and potassium in normal neuromuscular function involves the regulation of membrane proteins (known as ionic channels, the electrical conduction of action potentials) the transmission of neural activity to skeletal muscle and finally the contraction and relaxation of skeletal muscle. The necessity for a concurrent control of energy metabolism to provide the energy needed for work performed is provided through the availability of magnesium for the activity of kinase enzymes, and the regulation of calcium through phosphorylation of enzyme systems to control energy availability for muscle contraction. These mechanisms are discussed, with an eye toward their involvement in neuromuscular abnormalities in disease.

## Introduction

Nerve and skeletal muscle cells are excitable cells, in that they produce on their surface membranes, all-or-none changes in electrical potentials which are conducted throughout the length of their electrically excitable membranes. Skeletal muscle cells also demonstrate the property of contractility, through which the work of postural support and locomotion is carried out. The activity of skeletal muscle is totally dependent upon activity in the nerves which innervate them, and therefore represents the neural output of the central nervous system for postural and locomotor control mechanisms. The neuromuscular junction, which transmits neural information to muscular activity is critical in this transfer of activity from nerve to muscle. Calcium, magnesium and potassium, as well as other ions (such as chloride, and sodium) play critical roles in the production and propagation of electrical activity in neurons and muscle cells, the transfer of neural activity to muscle activity, and the contractile activity of skeletal muscle. In the following discussion, the interrelationships of these mechanisms will be discussed.

Muscle and Nerve Excitability<sup>6,9,10,13,14,18,19,21</sup>

The excitability of nerve and skeletal muscle primarily reflects the physiologic capacities of the surface membranes of these cells. This organelle represents both a physical and a metabolic barrier to the diffusion of a large number of ionic substances and through this property, functionally separates two fluid environments of differing ionic composition. The extracellular fluid compartment characteristically contains a relatively high concentration of sodium and chloride ions

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and a low concentration of potassium and magnesium ions while the intracellular fluid compartment contains a relatively high concentration of potassium and magnesium ions and low concentrations of sodium and chloride ions. A major component of the intracellular magnesium ions are protein bound, and are thus not free to diffuse, while the sodium, chloride and potassium ions are relatively free to diffuse across the cell membrane. Calcium ions are high in concentration in the extracellular fluid and low in the intracellular fluid, but do not diffuse across the cell membrane because of membrane impermeability. Also present within the cytoplasm of the cells is a large concentration of organic anions (represented by proteins, purines and pyrimidines) which are virtually absent from the extracellular fluid. These ions, like calcium, cannot diffuse across the cell membrane. The ability of sodium, potassium and chloride to diffuse across the cell membrane, and their relationship to the intracellular fluid organic anions provide the basis for the property of excitability of nerves and skeletal muscle.

Sodium, potassium and chloride ions diffuse across the cell membrane through lipoprotein "channels," which provide a pathway for the water soluble ions to pass between intracellular and extracellular fluid compartments as dictated by their concentration gradients, and electrical potentials across the cell membrane. In resting nerve and muscle cells, potassium ions diffuse out of the cell more rapidly than either chloride or sodium ions diffuse in, due to differential permeabilities of their individual channels. Chloride ions can diffuse at a rate intermediate between that of sodium and potassium, and sodium ions diffuse very slowly into the cell.

As potassium diffuses out of the intracellular fluid compartment, leaving behind the organic anions, a membrane potential of as much as 100 mV can be produced, negative inside (due to the organic anions) and positive outside (due to the potassium ion diffusion). Because this is the major mechanism producing membrane potentials in resting cells, the resting membrane potential ( $E_m$ ) is considered to be a potassium ion diffusion potential (Note! It is diffusion, not the concentration gradient that produces the membrane potential). Factors which influence the rate of potassium ion diffusion, have a considerable influence on the resting excitability of nerve and muscle cells. These factors include, increased extracellular potassium ion concentration, decreased extracellular or intracellular potassium ion concentration (the latter is likely to occur with prolonged loss of potassium in the urine), or alterations in cell membrane permeability. The inward diffusion of sodium ions, even though slight, tends to reduce the membrane potential below that which would be produced if potassium ions were diffusion alone, so that the resting membrane potentials of nerve and skeletal muscle results in the summation of sodium and potassium ion diffusion. Chloride ions can diffuse through the cell membrane, but in the resting cell, the force driving chloride ions into the cell is exactly balanced by the membrane potential produced by sodium and potassium diffusion, which prevents inward diffusion. Chloride is therefore in equilibrium with the membrane potential, and does not contribute to the resting membrane potential.

The continual diffusion of sodium and potassium through the cell membranes of nerves and skeletal muscle is maintained through active transport mechanisms (the sodium-potassium pump) which transport sodium out of the cell and potassium into the cell. The inward transport of potassium requires only a small amount of metabolic energy, because inward movement of potassium is aided by the membrane potential. The outward transport of sodium, however, requires considerable metabolic energy as these ions must be transported against both their concentration gradients and against the extracellular positivity of the membrane potentials. The active transport mechanism for sodium and potassium derives its energy from the breakdown of adenosine triphosphate (ATP). The release of ATP energy is catalyzed by a membrane bound protein, closely related to the sodium-potassium pump, which is a sodium-potassium ion sensitive, magnesium dependent ATPase. A major proportion of the total metabolic energy consumed by nerve and muscle cells (estimates range from 25-35%) is utilized to maintain the concentration gradients of sodium and potassium ions.

Because the transport of sodium and potassium by these cells utilize such a high proportion of total metabolic energy, in deficiencies in energy metabolism, or in intracellular magnesium ion availability (a situation which often occurs in the pasturing of wheat), the primary clinical signs reflect deficiencies in the transport of these ions.

The ability to produce action potentials represents the property of excitability in nerve and skeletal muscle. Action potentials are non-decremental, propagated, all-or-none changes in the resting membrane potential, which is dependent upon a self-regenerating alteration in membrane permeability to sodium ions (known as sodium channel activation). In nerves, action potentials are produced by central nervous system mechanisms. In skeletal muscle, action potentials are normally generated by the action of acetylcholine upon specialized areas of the cell membrane comprising a portion of the neuromuscular junction. Acetylcholine is released from the nerve terminals of neuromuscular junctions when these terminals are depolarized by the arrival of an action potential in the nerve fiber. This depolarization induced release of acetylcholine is a calcium dependent mechanism. Depolarization of the nerve terminals causes calcium channels of the nerve surface membrane to be activated, resulting in an inward diffusion of calcium into the cytoplasm of the nerve terminal. An increase in cytoplasmic calcium ion concentration, causes nerve terminal vesicles (containing acetylcholine) to fuse with the nerve terminal membrane, and release their contents into the extracellular space between the nerve terminal and the endplate region of the muscle cell membrane.

The activation of calcium channels is inhibited by an extracellular fluid increase in magnesium ion concentration, but such concentrations do not occur without the intentional administration of magnesium salts.

The acetylcholine released from the nerve terminals attaches to the muscle endplate membrane and activates the sodium and potassium channels of this area, increasing the rate of diffusion of both sodium

and potassium. As in the resting state, potassium diffusion is nearly maximal, and sodium diffusion is relatively slow, the increase in diffusion rate of both ions, results in sodium ions exerting a greater effect. The membrane potential of the endplate, therefore, decreases (depolarizes) and causes a depolarization to spread over the surface of the muscle cell. When depolarization reaches a critical level (threshold membrane potential, usually 10 to 15 mV less than the resting potential), a spontaneous, complete (all-or-none) activation of sodium channels occurs, so that sodium diffusion further increases and results in a rapid depolarization of the membrane. This depolarization persists for only a short period of time (usually less than 1 msec.) because the activation of sodium channels is short lived, and followed immediately by inactivation to resting levels of permeability to sodium ions. During the time that sodium permeability is high, potassium permeability is also increased, and the increased rate of diffusion of potassium ions tends to limit the influence of sodium ion diffusion, but during a major part of the action potential, the membrane potential is dominated by sodium diffusion. As sodium diffusion rate diminishes, during inactivation of sodium ion channels, the high rate of potassium ion diffusion quickly restores the membrane potential back toward its resting values. Potassium permeability is regulated by the membrane potential, so that as the membrane potential is restored toward its normal value, the permeability of potassium channels and the membrane potential are restored to resting values. The action potential is conducted over the entire surface of the muscle cell membrane as action potentials in the vicinity of the neuromuscular junction results in depolarization of adjacent membranes to threshold.

#### Excitation-Contraction Coupling in Skeletal Muscle<sup>4,7,15,22</sup>

Associated with the depolarization and repolarization of the muscle cell membrane by action potentials, membranes directly associated with the muscle cell membrane, but which lie within the cell are similarly affected. The surface membrane invaginates into the intracellular compartment of the muscle cell to form an extensive transverse tubular membrane system (referred to as the T-tube system). In mammalian skeletal muscle, these invaginations occur in a segmental manner along the length of muscle cells, in register with the transverse striations of the cells known as the A-I junctions. Within the cell, the T-tube system makes contact with the endoplasmic reticulum (sarcoplasmic reticulum), which represents a second extensive intracellular membrane system. As the action potentials depolarize the transverse tubule system, calcium ions, which are bound to the lipoprotein membrane components of the tubule system, are released into the cytoplasm, increasing the intracellular free calcium ion concentration. The cytoplasmic calcium, released in this fashion reacts with surface lipoproteins of the sarcoplasmic reticulum, triggering a second release of large amounts of calcium, which in the resting state are stored within the cisternae of the sarcoplasmic reticulum, bound to a protein known as calsequestrin. The net effect of these activities is to increase the quantity of calcium within the cytosol of muscle cells.

Calcium within the cytosol of most cells, is rarely in the form of free calcium, but is predominantly bound to a cytosolic protein known as calmodulin. It is apparent that the calcium-calmodulin complex interacts with the sarcoplasmic reticulum membrane to trigger the release of calcium from calsequestrin. As this additional calcium enters the cytosol, it too is bound to calmodulin, which regulates its distribution within the cytosol, and the reaction of calcium with other muscle cell proteins which are involved in muscle contraction.

Muscle Contraction<sup>1,2,4,7,11,12,16,17,22,23</sup>

The most characteristic feature of skeletal muscle is the regular pattern of banding which occurs throughout the length of the muscle cells. The presence of such banding is responsible for classifying skeletal muscle as a type of striated muscle. With light microscopic examination, the banding is observed to represent regular alternating light and dark transverse bands known as I bands and A bands respectively. The I bands are interrupted in their midportions by a dense transverse line, the Z bands and the A bands are interrupted in their midportion by a lighter portion, the H bands. The segment of the muscle fiber which lies between adjacent Z bands represents the functional unit of skeletal muscle, the sarcomere. Each sarcomere is of similar intrinsic organization and chemical composition.

There are at least four proteins which form the banding in skeletal muscle, and form the basis for completing excitation-contraction coupling and the resulting muscle contraction. These proteins are myosin, actin, tropomyosin and troponin. Within the limits of the A band there are thick myofilaments, comprised predominantly of myosin. Myosin is a fibrous protein with a globular terminal, demonstrating a molecular weight of about 450,000. The fibrous portion is represented as an alpha helix, which cross-polymerizes with other fibrous segments of adjacent myosin molecules, thus forming the thick myofilament of skeletal muscle. The globular portion of the myosin molecules and the adjacent portion of the alpha helix does not cross-polymerize with other myosin molecules and thus protrudes from the surface of the thick myofilament. These globular protrusions contain an ATPase activity (which is functional in releasing energy for muscle contraction) and are capable of interacting with the adjacent actin molecules when the intracellular environment and physicochemical properties of actin molecules are altered.

Actin is a globular protein (G-actin) with a molecular weight of 47,000, which is polymerized in the cytoplasm of skeletal muscle to form a double stranded helix. The binding of globular actin to a nucleotide (ATP) and calcium is necessary for the maintenance of polymerization. Actin molecular strands are continuous through the Z lines into adjacent sarcomeres and thus link sarcomeres together. Actin is the major protein of thin myofilaments of skeletal muscle

and represents the principal protein of the I bands, but interdigitates between the thick myosin filaments of the A bands. The interdigitated ends of actin myofilaments do not extend to the center of the A bands and thus provide a central, lighter portion of the A band known as the H band.

Tropomyosin is a fibrous protein with a molecular weight of about 70,000, forming a double coiled helix, represented by two alpha helices wound around each other. This complex structure forms a stratum upon which the actin filaments are coiled. The close relationship between tropomyosin and actin in muscle at rest prevents interaction between actin and myosin. It appears that this inhibitory action is produced by a mechanism of steric hindrance.

Troponin is a globular protein with a molecular weight of about 80,000. It is comprised of three subunits known as troponin C, which has a high affinity for calcium; troponin T, which has an affinity for tropomyosin and attaches troponin to this molecule and troponin I which is inhibitory to the ATPase activity of the adjacent globular portion of the myosin molecules. Troponin is attached to the tropomyosin strands at intervals of about 410 Angstroms through its troponin T component. Thin filaments of striated muscle are therefore comprised of three proteins, tropomyosin, actin and troponin complexes, while thick filaments are comprised of myosin.

The sequence of events which begins with an increased intracellular concentration of calcium-calmodulin complexes and ends with muscle contraction is referred to as the sliding filament mechanism of muscle contraction. This mechanism results from an interaction between actin and myosin which results in the shortening of each sarcomere, with thin filaments sliding along the thick filaments, encroaching upon the H lines and pulling the Z bands toward each other.

Increased intracytoplasmic calcium calmodulin complexes result in the activation of protein kinase enzymes which phosphorylate troponin C, and results in the transfer of calcium from calmodulin to troponin C molecules to form calcium-troponin C complexes. The formation of calcium-troponin C complexes results in the alteration of the troponin I and troponin T components of troponin (and likely other phosphorylations), so that the troponin I inhibition of myosin ATPase and tropomyosin steric hindrance of actin and myosin interaction is removed. Thus energy is available for muscle contraction from the degradation of ATP, and actin and myosin complex formation results in muscle contraction.

The degree of contraction is related to the quantity of calcium available to form calcium-troponin complexes. The number of calcium-troponin complexes formed is directly proportional to the number and rate of actin and myosin interactions and the force of muscle contraction.

## Muscle Relaxation<sup>1,2,7,20,23</sup>

Muscle relaxation results from the reversal of the process of contraction and is the consequence of intracytoplasmic regulation of calcium ions. The calcium released in excitation-contraction coupling is rapidly rebound to the sarcoplasmic reticulum, and transported into the cisternae by calcium-sensitive ATPase dependent active transport mechanisms (Calcium pumps). Within the cisternae calcium is again rebound to calsequestrin. Calcium is also rebound to the transverse tubule systems and the surface membrane (probably through a mechanism which involves calmodulin), and is actively transported into the cisternae of the transverse tubules by calcium pumps. As the cytoplasmic store of calcium-calmodulin is reduced, calcium is released from calcium-troponin C complexes, restoring troponin C, troponin I and troponin T to their original state (most likely involving dephosphorylation reactions). These alterations in troponin result in the reestablishment of troponin I inhibition of myosin ATPase, and tropomyosin inhibition of interaction between actin and myosin, so that muscle relaxation occurs.

## Energy Metabolism in Skeletal Muscle<sup>3,5</sup>

The amount of ATP present in skeletal muscle to maintain the transmembrane distribution of diffusible ions and to be hydrolyzed by myosin ATPase to provide energy for muscular contraction determines both the state of excitability and contractility of muscle. The amount of ATP available is dependent upon the ability of muscle cells to form ATP directly from energy metabolism and from a storage form of energy as creatine phosphate. Creatine phosphate is formed through energy-producing metabolism in muscle from the transformation of ATP to ADP, releasing phosphate which is in turn bound to creatine without the loss of energy stored in the terminal phosphate bond of ATP. This reaction requires the presence of magnesium ions and a cytoplasmic enzyme, creatine phosphokinase (CPK).

The energy requirements of skeletal muscle cells differ in accordance with the conditions under which they are typically called upon to function. Three types of skeletal muscle cells have been identified on this basis: Type I (slow twitch-oxidative type, red muscle fibers or type B slow twitch fibers) depend primarily upon oxidative phosphorylation for energy. These muscle fibers contain numerous mitochondria and a lower concentration of myosin ATPase activity than other types of muscle cells. These muscle cells are found in muscles which are not called upon for strong muscular contraction and are therefore most numerous in muscles which function with the development of only slight strength. Type II cells (fast twitch-glycolytic, type A fast twitch or white muscle fibers) primarily depend upon substrate phosphorylation for their energy source. They contain higher concentrations of glycogen than type I cells and a greater concentration of myosin ATPase activity. A third type of skeletal muscle fiber known as type III is intermediate between types I and II. Type III fibers are classified as fast twitch-oxidative-glycolytic muscle cells.

It is evident from the above classifications that a single view of energy metabolism will not be adequate for all muscle cells, as white muscle cells depend primarily upon glycolysis for energy and operate at maximal contraction for only short periods of time before depleting this energy store, while red muscle fibers derive their energy primarily from the oxidation of fatty acids and glucose (as well as lactate, pyruvate, ketone bodies and amino acids) and are capable of maintaining sustained contractions without depletion of their energy source. In the latter type of muscle, glycolysis becomes an important source of energy only when oxygen is restricted or sustained muscle contraction restricts the blood flow through muscular tissue.

In red muscle, glucose metabolism comprises a major source of energy, as long as oxygen supply to the muscle is plentiful. Plasma glucose is transported into muscle cells through a carrier-mediated (facilitated transport) passive mechanism, which requires insulin for its activity. Insulin attaches to surface receptors of the muscle membrane and, through interaction with such receptors, alters the carrier for glucose to maximize its efficiency. As long as adequate insulin is present, transport of glucose across the sarcolemma does not limit the rate of glucose utilization in animals with normal blood glucose levels. Once glucose is inside the cell, it must be phosphorylated for further metabolism. This is accomplished through the action of hexokinase, which requires magnesium and ATP to produce glucose-6-phosphate (G-6-P). The activity of hexokinase is regulated by intracytoplasmic levels of G-6-P, thereby providing a control mechanism for the further transport of glucose into the cell. If adequate G-6-P is present within the cell to meet the metabolic needs, hexokinase inhibition prevents the phosphorylation of glucose and allows intracellular glucose concentrations to increase. This in turn decreases the rate at which facilitated transport can allow the passage of glucose into the cell.

Glucose-6-phosphate represents an important nodal point in the metabolism of glucose within muscle cells, as it may be converted by specific enzymes into glucose-1-phosphate (G-1-P) and fructose-6-phosphate (F-6-P). Conversion to G-1-P occurs through a reversible reaction catalyzed by phosphohexose isomerase and leads to glycogen formation. Conversion of G-6-P to F-6-P is catalyzed by the enzyme phosphohexose isomerase and leads to glycolysis to produce pyruvate. In some tissues G-6-P can be converted to 6-phosphogluconic acid to enter the pentose shunt. This metabolic pathway is absent in skeletal muscle due to the lack of the initiating enzyme, glucose-6-phosphate dehydrogenase.

#### Glycogen Metabolism

G-1-P is polymerized to glycogen through an intermediate reaction involving uridine triphosphate and catalyzed by the cytoplasmic enzyme,

glycogen transferase. This enzyme represents an avenue for control of glycogen synthesis, as it is transformed from a relatively inactive form (transferase I) to a more active form (transferase D) through the action of epinephrine (utilizing a cyclic AMP-dependent protein kinase mechanism) on the cell membrane surface. Transferase D is dependent upon the presence of G-6-P for its activity and is inhibited by ATP, so that variations of these substances in the cytoplasm regulate glycogen deposition.

A second enzyme system (which is dependent upon pyridoxal phosphate for its action) breaks down glycogen (glycogenolysis) so that it can be converted back to G-1-P. This system is represented by phosphorylase a and phosphorylase b. Phosphorylase b is considered to be inactive in resting muscle because it is inhibited by G-6-P and ATP. In circumstances in which the concentrations of these substances are low, as during periods of intense muscular activity or anoxia, phosphorylase b is quite effective as an enzyme. Phosphorylase b is converted to phosphorylase a by a specific kinase enzyme which is activated by the presence of calcium calmodulin complexes in the cytoplasm.

Phosphorylase a is not dependent upon G-6-P or ATP levels for its activity and thus can be activated prior to an energy deficit. Such activation is produced through the influence of epinephrine on a cyclic AMP mechanism and through an increase in cytoplasmic calcium, which results from action potentials on the sarcolemma of the cell. It is through the latter mechanism that glycogenolysis is regulated to produce adequate energy for muscle contraction. This mechanism for balancing energy metabolism from glycogen stores with the intensity of muscle contraction (as both are controlled by intracytoplasmic calcium concentration) is most significant in type II muscle fibers (fast twitch-glycolytic). There is some indication that phosphorylase activity is associated with intracytoplasmic magnesium levels. As a good deal of the magnesium of the cytoplasm is bound to ATP, the breakdown of ATP for muscle contraction results primarily in an increase in these ions in the cytoplasm, and secondarily in an enhancement of phosphorylase activity.

It should be noted that epinephrine affects both synthesis and breakdown of glycogen through a cyclic AMP mechanism. The net effect of epinephrine, however, is dependent upon the G-6-P levels of the cell. In circumstances of enhanced muscular activity, in which G-6-P levels are low, the influence of epinephrine is to turn off glycogen synthesis while promoting glycogen breakdown.

### Glycolysis

In the utilization of G-6-P for energy, its conversion to F-6-P by phosphohexose isomerase is critical to cellular metabolism of either glucose or glycogen for energy. This metabolic pathway produces pyruvate or lactate as its end-products, depending upon the state of

oxygenation of the tissues. A number of enzymes are used in this metabolism but the second reaction in the series, the conversion of F-6-P to fructose 1-6-diphosphate (F-1-6-DP) through the action of the enzyme phosphofructokinase, represents the major control point for the metabolic pathway. This enzyme is inhibited by increased cytoplasmic levels of ATP and of citric acid (both of which are products of aerobic metabolism of pyruvate) and is stimulated by ADP and AMP, thus providing a feedback control which regulates the production of pyruvate to be in accord with the utilization of energy and oxygen. As ATP levels and citric acid levels drop in response to enhanced energy utilization through muscular contraction or in response to tissue anoxia, an increasing amount of G-6-P is utilized by the glycolytic pathway to produce pyruvate for oxidative phosphorylation. Phosphofructokinase is an inducible enzyme, which means that the cytoplasmic concentration increases with continued use of the metabolic pathway. This may be an important mechanism in the conditioning of muscle for enhanced energy metabolism during training.

Glycolysis can proceed during periods of anoxia and provide three moles of ATP for each mole of glucose utilized. In anaerobic conditions, in which either the circulation to muscle is interrupted due to prolonged muscle contraction or in which the oxygen supply does not meet the energy requirements, pyruvate is converted to lactic acid. This reaction is produced through the mediation of the enzyme, lactic acid dehydrogenase. There are five forms (isozymes) of lactic acid dehydrogenase (LDH), the relative concentrations of which vary with different tissues, depending on the conditions under which they ordinarily function. Heart muscle, for example, contains an isozyme which is known as LDH-H<sub>4</sub> (the H indicating its dominance in heart). This isozyme is comprised of four sub-units of H (thus the subscript 4). This form of LDH operates only in tissues in which anaerobic conditions are not present during the normal circumstances of cell function. LDH-H<sub>4</sub> produces little lactic acid, as pyruvate which inhibits the enzyme is utilized through oxidative pathways to provide further energy for muscular activity. Because of the presence of this enzyme in heart muscle, lactic acid is not ordinarily formed, but is used as a substrate for energy metabolism in heart muscle. The isozyme of skeletal muscle is predominantly LDH-M<sub>4</sub> (the M indicating muscle and the subscript 4 indicating 4 units of the molecule), which is only weakly inhibited by pyruvate and converts large amounts of pyruvate to lactate at a rapid rate. This conversion is of importance in tissues such as skeletal muscle, which are at times required to function in periods of relative anaerobic states, as the conversion of pyruvate to lactic acid results in the oxidation of nicotinamide adenine dinucleotide (NAD) from its reduced form (NADH). NAD is necessary in the glycolytic pathway to serve as an electron receptor co-factor in the conversion of F-6-P to pyruvate. Thus, through the formation of lactic acid, the glycolytic pathway continues to function during anaerobic states.

The lactic acid formed during muscle contraction may diffuse into the extracellular fluid, enter the blood and be converted back to glucose by the liver. This process cannot occur in muscle, even at rest,

because muscle cells lack the enzyme, glucose-6-phosphatase, which is required to convert G-6-P to glucose. The glucose provided by the liver is available to serve as a source of muscle glucose. The mechanism whereby lactate, produced in muscle, is transported to the liver, where it is converted to glucose and transported back to muscle for utilization, is referred to as the Cori cycle.

Under aerobic conditions, lactic acid can be transformed back into pyruvate, which then undergoes oxidative degradation in the tricarboxylic acid cycle (TCA cycle). The conversion of lactic acid to pyruvate is catalyzed by LDH. As cytoplasmic concentrations of pyruvate are reduced by oxidative metabolism, the equilibrium is shifted from the production of lactic acid to the production of pyruvate. Under these conditions, lactate in the blood can be utilized for energy by skeletal muscle.

The enzymes which metabolize pyruvate in the TCA cycle are located within mitochondria. For pyruvate to be metabolized to CO<sub>2</sub> and water in the TCA cycle, it must enter the mitochondria. Pyruvate apparently accomplishes this through passive diffusion. Because there is a constant equilibrium between the pyruvate concentrations of the cytoplasm of the mitochondrial matrix, there is a dynamic relationship between intramitochondrial metabolism of pyruvate and its cytoplasmic concentration. Thus, mitochondrial metabolism of pyruvate determines whether or not lactic acid is allowed to accumulate or is converted to pyruvate for energy.

The first enzyme complex in the metabolism of pyruvate plays a key role in the regulation of glucose metabolism for oxidative energy. This enzyme complex, known as pyruvate decarboxylase, in the presence of Coenzyme A, thiamine pyrophosphate, lipoic acid and magnesium, converts pyruvate into acetyl-CoA, which is the entry substance for the TCA cycle. The conversion reduces NAD, which becomes available to the cytoplasmic compartment of the cell to allow continuation of glycolysis (thus replacing the function of pyruvate to lactate conversion). The enzyme complex, pyruvate decarboxylase, is inhibited by reduced NAD, acetyl-CoA (both of which are produced by the reaction) and by ATP (which is the end product of oxidation of pyruvate by the TCA cycle). There is, therefore, a fine-grained control of the entry of pyruvate into the TCA cycle for complete oxidation to CO<sub>2</sub> and water. Since acetyl-CoA is also produced as the end-product of beta oxidation of fatty acids within the mitochondria, fatty acid catabolism can effectively block the oxidative metabolism of pyruvate.

In situations in which dietary sources of glucose do not satisfy the metabolic demands of the animal, gluconeogenic substances are used to supply glucose for energy. A source of gluconeogenic substances is the amino acids derived from muscle protein. The release of amino acids from muscle is controlled by endocrine mechanisms which alter synthesis and degradation of protein. Insulin not only enhances uptake of glucose by muscle cells, but also stimulates the uptake of amino

acids. Glucocorticoids inhibit muscle membrane uptake of amino acids and incorporation into muscle protein, thus providing amino acids for hepatic gluconeogenesis (which is also stimulated by these hormones). The effect of glucocorticoids on muscle, however, overrides that on the liver and results in increased plasma levels of amino acid nitrogen. Growth hormone increases amino acid uptake and incorporation into muscle protein while decreasing plasma amino acid nitrogen.

The amino acid, alanine, appears to be more important than other amino acids taken up by muscle, as it serves as the basis for the so-called alanine cycle. The alanine cycle, like the Cori cycle, is functional in maintaining plasma glucose levels for adequate muscular energy formation through hepatic conversion (gluconeogenesis) of a substance produced in muscle. In this case, transaminase reactions result in the formation of alanine in muscle cells from pyruvate. Alanine diffuses out of the cell and is carried to the liver, where it is deaminated to pyruvate for conversion to glucose, which in turn becomes available to the muscle.

Besides contributing to energy metabolism, the alanine cycle also provides a protective mechanism for muscle. Muscle does not possess the enzymatic apparatus to synthesize urea; therefore, by releasing nitrogen as alanine, muscle is able to utilize amino acids as fuel without the risk of releasing large amounts of  $\text{NH}_3$  into the circulation.

Ketone bodies represent a significant source of energy for muscle cells, particularly in ruminants, colonic fermenters and cecal fermenters, in which volatile fatty acid metabolism often results in borderline ketotic states. The ketone bodies, acetoacetate, beta-hydroxy butyrate and acetone, are metabolized by way of acetyl-CoA and the TCA cycle. Because they generate acetyl-CoA, their metabolism results in depression of pyruvate metabolism and thus conservation of glucose as an energy source.

Magnesium ions, which are second in concentration of intracellular cations, being exceeded only by potassium, are indispensable for normal muscular function. Magnesium is required in all reactions involving ATP and other high-energy phosphate reactions. In these reactions, magnesium is bound to ATP and the complex serves as a substrate for energy metabolism. In the absence of adequate magnesium, the primary deficit can be related to this function in energy metabolism.

The role of selenium in muscle metabolism and its interrelationship with vitamin E metabolism has long been of concern to those who recognize the involvement of these agents in the production of muscle disorders when deficiencies occur. It has been clearly demonstrated that selenium is an essential element in the chemical structure of glutathione peroxidase, which prevents the accumulation of peroxides in the cytoplasm and organelles of muscle and other cell types. Peroxides are formed as a normal course of metabolism through the action of amino acid oxidases, xanthine oxidase and other similar systems of enzymes. Peroxides not only result in damage to the

sarcolemma resulting in alterations in membrane permeability and disturbing electrophysiologic mechanisms, but also disrupt the membranes of organelles within the cell (particularly mitochondria), which leads to disturbances of energy metabolism. Glutathione peroxidase is one of several peroxidase systems which prevent such as accumulation.

The interaction of vitamin E and selenium is believed to prevent lipid hydroperoxide formation, which is also destructive to cell membranes. Vitamin E is viewed to function in this regard primarily as a cellular "antioxidant." Lipid peroxides form most readily from unsaturated lipids and accumulate when these substances are being utilized as an energy source. The enhancement of lipid peroxidation has been demonstrated in skeletal muscle of aging animals; thus the requirement for peroxidase and antioxidant functions of selenium and vitamin E could be expected to increase with age.

Besides the production of peroxides as toxic byproducts of oxidative metabolism, the superoxide radical ( $O_2^-$ ) is an important agent of oxygen toxicity. All oxygen-metabolizing cells so far examined have been found to contain enzymes which catalyze the reaction  $2O_2 + 2H^+ \longrightarrow H_2O_2 + O_2$ . These copper, zinc metalloprotein enzymes are known as superoxide dismutases. The regulation of copper and zinc metabolism plays a critical role in the normal maintenance of this critical protective mechanism of muscle cells.

#### Conclusions

It is apparent from the preceding discussion that magnesium, calcium and potassium are involved in all stages of muscle activity, including the neural activity which activates skeletal muscle, the activation of skeletal muscle itself, the contractile and relation mechanisms of skeletal muscle, and muscle metabolism to provide the energy that is requisite for muscle contraction. The role of magnesium is primarily, but not limited to, its necessity for the activity of kinase enzymes which regulate the transfer of energy between metabolic molecules and finally to the mechanisms of muscle contraction and relaxation. Potassium plays a key role in the maintenance and regulation of nerve and muscle excitation, and may play important metabolic roles within the cytoplasm of muscle cells. Calcium, which regulates muscle contraction and relaxation, also regulates the activation of important enzymatic reactions, which regulate the energy available for muscle contraction, and also controls the intensity of muscle contraction. These calcium regulatory mechanisms operate through calcium carrier molecules within the cytoplasm of muscle cells, known as calmodulin. It is apparent that there are several calmodulins present in cells, the importance of different forms of calmodulin remains to be elucidated.

#### Areas of Needed Research

Virtually every aspect of neuromuscular function is in need of further investigation. The nature of surface membrane ionic channels,

and factors which regulate them is very limited, and today reside as models which are used to explain cause and effect interactions, without a clear insight to the molecular interactions involved. The means by which magnesium and calcium interaction occurs in the activation of surface membrane channels and in the control of energy releasing mechanisms within the cells contains many similar model systems of which details are lacking. These mechanisms are particularly important in understanding the close control which appears to be present between availability of energy and the energy needs of muscle contraction.

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