Filamentous Components—What Makes a Muscle Cell Look Like a Muscle Cell

MYOFIBRILS

Perhaps the most distinctive feature of the muscle cell is the ordered array of contractile filaments that are arranged throughout the cell (Figure 1.5; Table 1.2). There is a well-defined hierarchy of filament organization that proceeds from a large scale (on the order of microns, 10^{-6} m, abbreviated μm) to a small scale (on the order of angstroms, 10^{-10} m, abbreviated Å). The largest functional unit of contractile filaments is the myofibril (literally, "muscle thread"). Myofibrils are simply a string of sarcomeres arranged in series. Myofibrillar diameter is about 1 μm, which means that thousands of myofibrils can be packed into a single muscle fiber. One way in which a muscle fiber grows is to increase the number of myofibrils that it contains. Myofibrils are arranged in parallel (side by side) to make up the muscle fiber. However, their arrangements might not simply be like a bundle of spaghetti; there is some evidence that myofibrils within the fiber are arranged similar to the weave in a rope (Peachey and Eisenberg, 1978). (The functional consequence of this arrangement is that various myofibrils may not act completely independently during normal contraction.) Groups of muscle fibers are surrounded by a connective tissue sheath known as perimysium (literally, "around muscle") and arranged in bundles called fascicles. These fascicles are also bundled together, surrounded by more connective tissue (epimysium, literally, "on top of muscle") to form the whole muscle, which we can inspect visually.

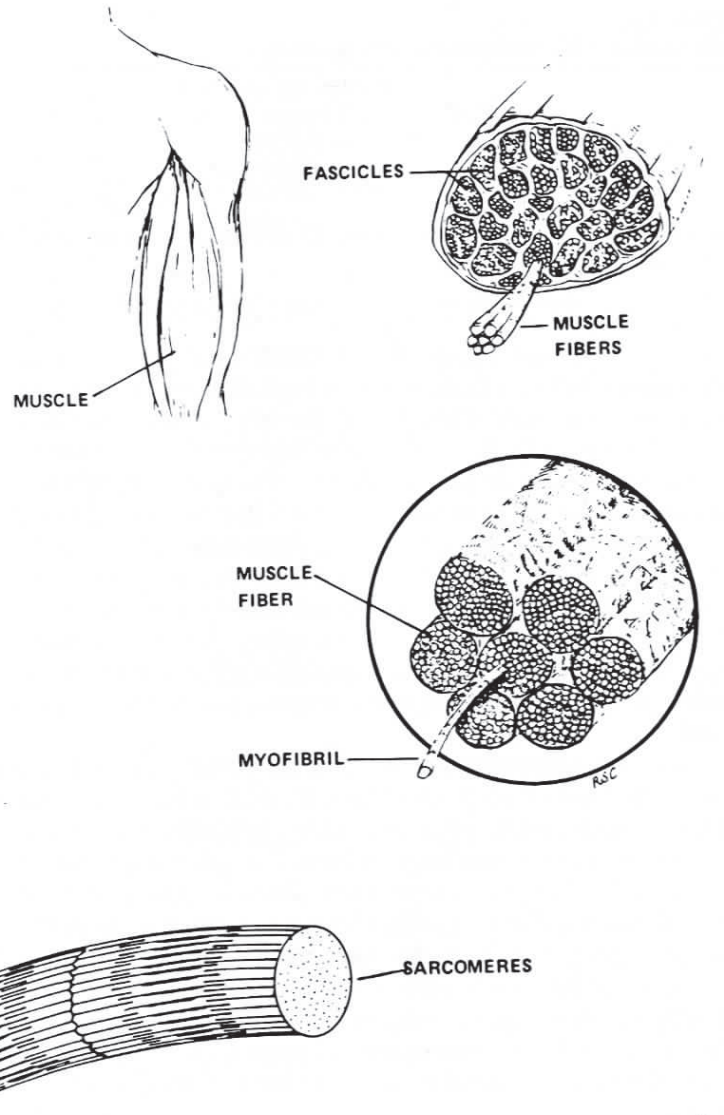


Figure 1.5. Structural hierarchy of skeletal muscle. Whole skeletal muscles are composed of numerous fascicles of muscle fibers. Muscle fibers are composed of myofibrils arranged in parallel. Myofibrils are composed of sarcomeres arranged in series. Sarcomeres are composed of interdigitating actin and myosin filaments.

Table 1.2.
Hierarchy of Skeletal Muscle Organization

Whole skeletal muscles
Muscle fascicles
Muscle fibers
Myofibrils
Sarcomeres
Myofilaments

SARCOMERES—FUNCTIONAL UNIT OF CONTRACTION

Myofibrils can also be subdivided into their component units known as sarcomeres (Figure 1.5), the functional unit of muscle contraction. A myofibril is therefore a number of sarcomeres (literally, "muscle segment") arranged in series (end-to-end). The total number of sarcomeres within a fiber depends on the muscle fiber length and diameter. Because of the series arrangements of sarcomeres within a myofibril, the total distance of myofibrillar shortening is equal to the sum of the individual shortening distances of the individual sarcomeres. This is why a whole muscle may shorten several centimeters even though each sarcomere can only shorten about 1 μm! It should also be stated that the number of sarcomeres in a mature muscle can change given the appropriate stimulus (Chapter 4, page 166). This means that muscle fibers have a great capacity for adaptation, which is the subject of the last half of this book.

Sarcomeres are composed of contractile filaments termed "myofilaments." Two major sets of contractile filaments exist in the sarcomere: One set is relatively thick, and the other set is relatively thin (Figure 1.6). These thick and thin filaments represent large polymers of the proteins myosin and actin, respectively. The myosin-containing filaments (thick filaments) and the actin-containing filaments (thin filaments) interdigitate to form a hexagonal lattice (Figure 1.6). It is the active interdigitation of these microscopic filaments that produces muscle shortening (Chapter 2, page 55). It is also this interdigitated pattern that gives the muscle its striated or striped appearance that is observable microscopically (Figure 1.7). In fact, another term for skeletal muscle is striated muscle, by virtue of the dark and light banding pattern.

Various regions of the sarcomere are named so that reference can be made to them (Figure 1.6). For example, the sarcomere region containing the myosin filaments is known as the A-band (A stands for "anisotropic," which is an optical term describing what this band does to incoming light). The region containing the actin filament is known as the I-band (I stands for "isotropic"). The region of the A-band where there is no actin-myosin overlap is called the

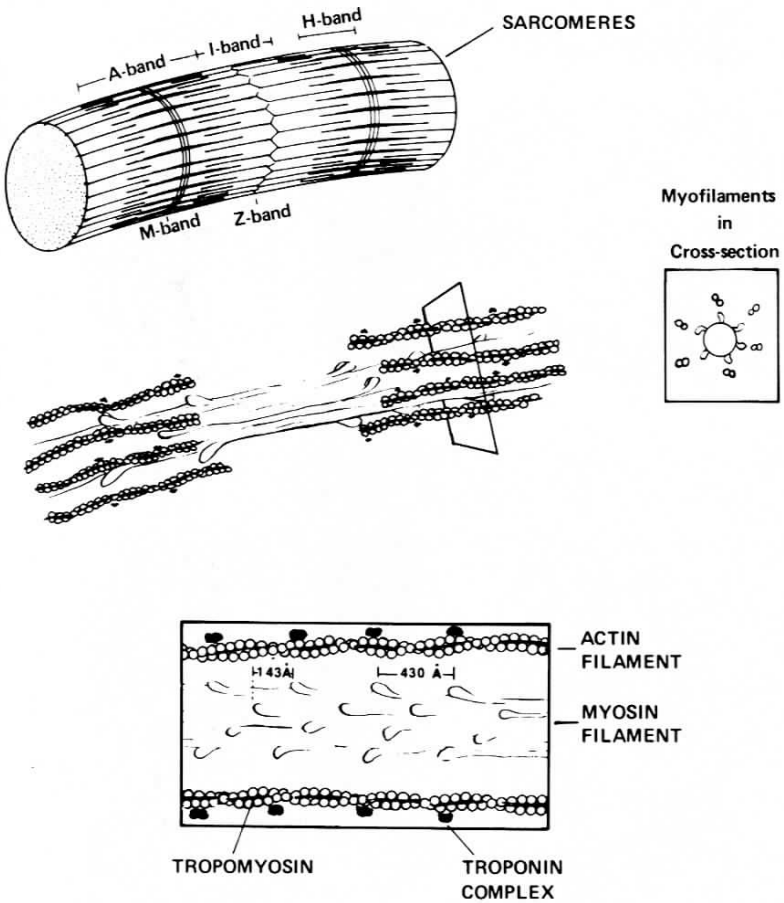
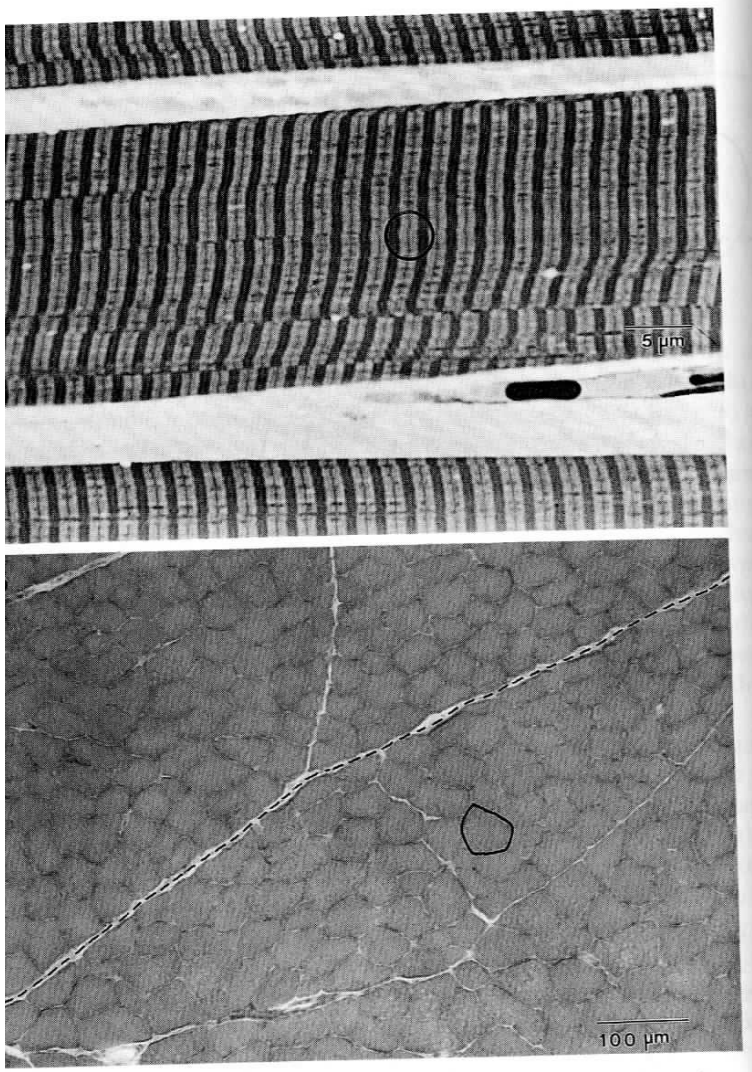


Figure 1.6. Hexagonal array of interdigitating myosin and actin filaments, which comprise the sarcomere. The myosin filament is composed of myosin molecules, and the actin filament is composed of actin monomers. Arranged at intervals along the actin filament are the regulatory proteins troponin and tropomyosin. See text for details.

H-zone (H stands for *belle,* which is German for "light"). The dark narrow line that bisects the I-band is the Z-band (Z stands for *zwitter,* which is German for "between"). Finally, the relatively dense structures noted in the center of the A-band are known as the M-band. The distance from one Z-band to the next is defined as the sarcomere length, which is an important variable relative to force generation (Chapter 2, page 55).



e 1.7. **A,** Longitudinal section of a tibialis anterior muscle biopsy specimen that was iically fixed, embedded in plastic, sectioned at 1 µm thickness, and stained with toluidine blue. lternating light and dark regions correspond to the sarcomere A- and I-bands. Several fibers are n in this section. Circled region enlarged in Figure 1.9. **B,** Cross-section of a vastus lateralis le biopsy specimen that was frozen, sectioned at 8 µm thickness, and stained with hematoxylin osin for inspection of normal fiber morphology. Note that, in cross-section, muscle appears to collection of densely packed polygonal fibers. Each muscle fiber is surrounded by a sheath of mysial connective tissue (shown for one fiber as a *solid line*), and collections of muscle fibers icles) are surrounded by more dense perimysial connective tissue (shown for one region as a *ed line*).

MYOSIN—HOME OF THE CROSS-BRIDGE

The myosin-containing filament is composed of myosin molecules that are relatively large proteins (about 470 kDaltons) known as the myosin heavy chain. This protein is one of the most widely studied proteins in all of biology since it is found in cells other than muscle cells (Squire, 1981). Myosin proteins are arranged in a so-called antiparallel fashion to made up the myosin filament (Figure 1.8). As individual myosin molecules pack together to form the thick filament, one molecule rotates about 60° relative to the molecules on either side (this arrangement varies slightly between vertebrate muscles). The myosin molecule has a long backbone and a region that extends from the backbone. Due to the antiparallel (tail-to-tail) arrangement of these molecules, as the filament is formed, it takes on a characteristic feathered appearance, with projections coming out at either end of the filament, but with the middle portion of the filament void of these projections. Because of the systematic packing of the myosin molecules into a filament, every 430 Å, the myosin molecules make a complete revolution (Figure 1.8), which means that myosin heads are spaced approximately 143 Å along the length of the myosin filament. The antiparallel arrangement of myosin actually gives the sarcomere symmetry down the middle so that each half-sarcomere is functionally identical and has a mirror image on the opposite side of the sarcomere.

Myosin was one of the first proteins to be isolated from muscle. It was found that when this molecule is placed in a mild protease (an enzyme that digests proteins, *e.g.,* chymopapain, the same enzyme that makes up meat tenderizer), the myosin molecule "falls apart" into two discrete components (Figure 1.8): the so-called light meromyosin (LMM) and heavy meromyosin (HMM), terms based on the observation that LMM has a formula weight of only 135 kDaltons while HMM weighs about 335 kDaltons. Further incubation of HMM in protease results in the production of two subfragments: subfragment 1 (S-1; molecular weight 115 kDaltons) and subfragment 2 (S-2; molecular weight 60 kDaltons). The reason that this sequential digestion of the myosin molecule furthered our understanding of muscle contraction was that each "piece" of the molecule could be tested individually to determine how myosin itself causes muscle contraction to occur. For example, it was determined that the protein's backbone is the LMM while the portion of the molecule projecting from the backbone is HMM. S-2 was shown to be the portion of HMM that projects out from the LMM backbone, while S-1 is the globular "head" of the projection. It is the combination of S-1 and S-2 that forms the well-known "cross-bridge," so central to the theory of force generation in muscle (see below). Also associated with the myosin heavy chain are two light chains (not shown in Figure 1.8). The precise function of these chains is not known. It is believed that they may provide structural support for the S-1 head. It is known,

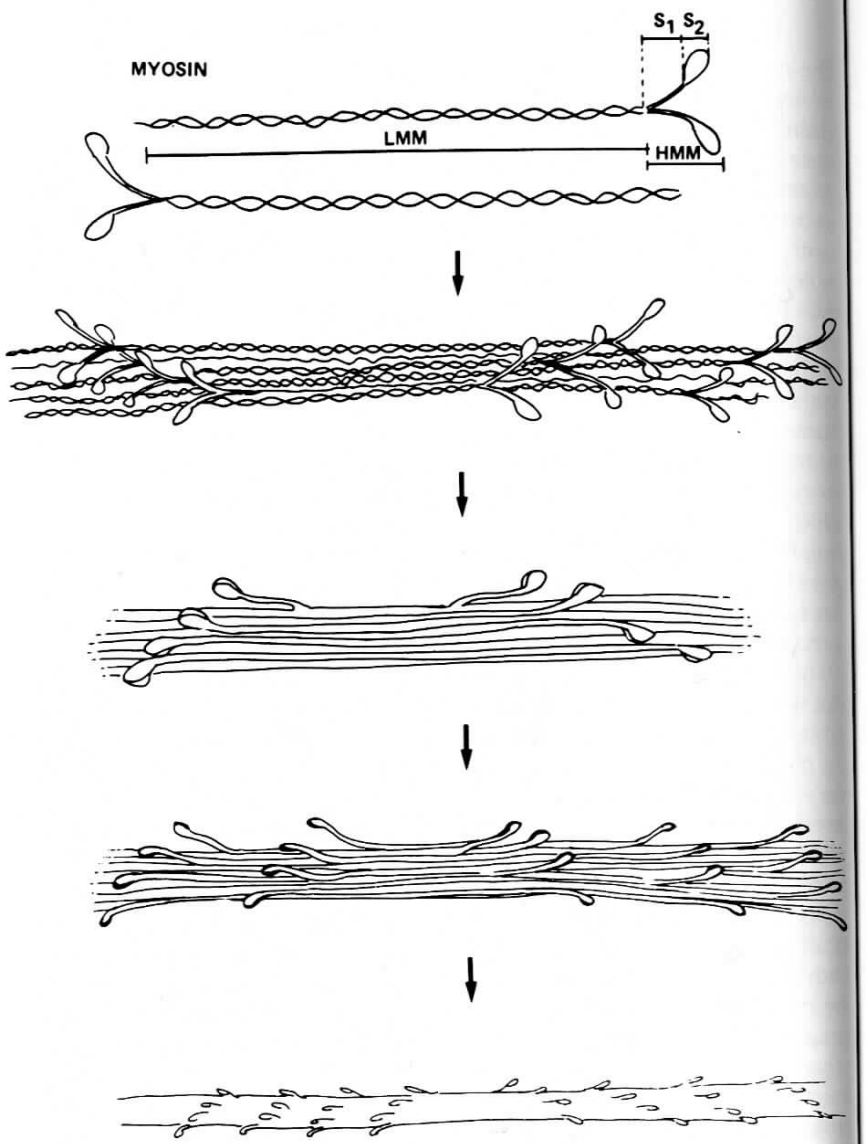


Figure 1.8. Schematic composition of the myosin filament. The myosin molecule can be digested enzymatically into its fragments. Subfragments one and two (S-1 and S-2) are composed of the globular head and neck regions of the cross-bridge, respectively. Together, S-1 and S-2 comprise the heavy meromyosin (HMM), while the remainder of the molecules comprise the light meromyosin (LMM). Myosin monomers polymerize into myosin filaments.

however, that various forms of the same light chain, so-called isoforms, exist in different muscles fibers. We will see in Chapter 4 that analysis of the myosin light chain isoform distribution provides insights into the nature of muscle adaptation (Chapter 4, page 163). Let's take a moment to state that, actually, isoforms of most of the sarcomere proteins exist. Myosin isoforms, however, have been most widely studied.

ACTIN—REGULATION OF CONTRACTION

The structure of the actin-containing filament is equally as elegant as that of myosin. While the myosin-containing filament *generates* tension during muscle contraction, the actin-containing filament *regulates* tension generation. (Not all muscle systems are regulated by the actin-containing filament. In several invertebrate systems, force regulation and generation are both performed on the myosin-containing filament [Ebashi *et al.,* 1980]. These so-called thick-filament-regulated systems are actually quite common. However, mammals use only thin-filament-based regulation.) The actin filament is composed of a long α-helical arrangement of actin monomers. In contrast to the myosin-containing filament, there is no directional symmetry to this helix (*i.e.,* the filament does not have a distinct middle section as does the myosin filament).

Actin is a ubiquitous protein found in virtually all cells as part of the intermediate filament network—part of the cell's cytoskeleton. (Interestingly, the discovery of actin filaments in cells other than muscle was first confirmed by "decorating" the actin-containing intermediate filaments with HMM! This was done by adding HMM to cytoskeletal cellular components and observing the feathered appearance of the HMM heads at regular intervals along the actin filaments.) Actin monomers are relatively small compared to myosin (only about 40 kDaltons) and are roughly spherical in shape. Thus as a result of their helical arrangement, a long groove is created along the filament's length (Figure 1.6). The regulatory protein, tropomyosin, fits nicely into this groove along the filament length. At intervals along the filament (approximately every seven actin monomers), the protein troponin is located. Troponin is the protein that is actually responsible for turning on contraction. Troponin (abbreviated Tn) is composed of three subunits: Tn-I,Tn-C, and Tn-T. The functions of these subunits will become more clear following discussion of the cross-bridge cycle (Chapter 2, page 52). Suffice it to say that Tn-T binds troponin to tropomyosin (hence "T"), Tn-C binds calcium during contraction (hence "C"), and Tn-I exerts an inhibitory influence on tropomyosin when calcium is not present (hence the "I").

In summary, contractile proteins within the muscle cells are arranged according to an elegant hierarchy. From the myosin molecule to the myofibril, structural hierarchy and organization are the rule, not the exception.

Membrane Components—A Vehicle for Excitation

In addition to the well-defined arrangement of force-generating components present in muscle cells, there exists an intricate system for activating these force generators. Recall that the skeletal muscle fiber is a highly differentiated cell that is specialized for producing force and movement. The membrane system present is actually a specially designed version of the membrane systems within normal cells. The two main components of this system are the transverse tubular system (T-system) and the SR.

TRANSVERSE TUBULAR SYSTEM AND SARCOPLASMIC RETICULUM

The T-system begins as invaginations of the surface membrane and is therefore physically contiguous with the sarcolemma. These invaginations extend transversely across the long axis of the muscle fiber (hence their name). The function of the T-system is to convey an activation signal to the myofibrils, which are themselves not in direct contact with the motoneuron (Chapter 2, page 51). The T-system thus acts as an electrical conduit for the nervous signal that reaches deep into the fiber. This provides a means for myofibrillar activation that is much faster than, say, diffusion of molecules from the cell surface. It is thought that the T-system is an electrically excitable membrane much like the sarcolemma and the membranes surrounding neurons.

The SR is a much more complex membrane system whose main function is to release and take up calcium during contraction and relaxation, respectively. As such, the SR envelops each myofibril to permit intimate contact between the activation and force-generation systems (Figure 1.4). The SR is also in contact with the T-system and therefore acts as the "middleman" in skeletal muscle activation and relaxation. Physical structures that link the T-system to the SR have been identified and termed the "junctional feet" of the SR (Peachey and Franzini-Armstrong, 1983).

The relative spatial arrangement of the T-system and SR gives rise to a characteristic pattern that is observable in a high magnification longitudinal section (a section parallel to the long axis) of a muscle cell (this is usually seen by chemically fixing the tissue, embedding it in plastic, and thin sectioning, staining, and viewing it under the electron microscope [*e.g.,* Figure 1.9]. Obviously, the true arrangement of the T-system and SR is a quite complex three-dimensional matrix. However, when one takes a slice through this matrix, a stereotypic arrangement of a T-tubule surrounded by two SR tubules is seen (Figure 1.4). This arrangement is so common that it has been named the "triad." Extending longitudinally from either side of the "triad" are membrane systems, which appear as flattened sacs. These represent longitudinal extensions of the SR that surround the myofibril. Remember, although these flattened sacs appear as a relatively minor area fraction in the longitudinal view, this is only because they have a complex three-dimensional arrangement that is not adequately represented in only two-dimensions. The various portions of the T-system and SR are so distinct that they can be physically isolated and experimentally manipulated using modern fractionation techniques now commonly used in cell biology.

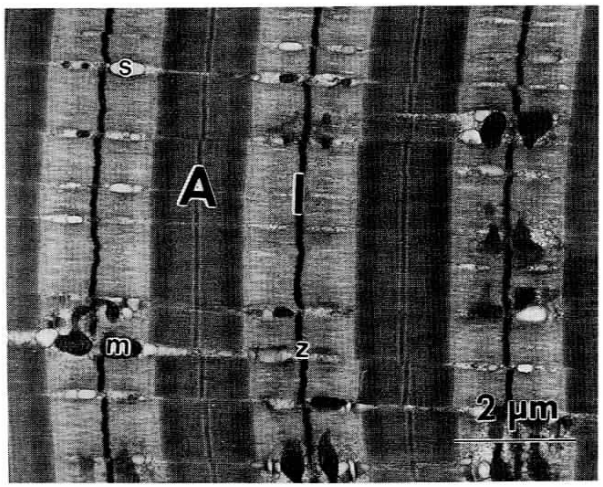


Figure 1.9. Electron micrograph of a rabbit tibialis anterior muscle. Note the much greater magnification of the A- and I-bands (compare to Figure 1.7**A**). Calibration bar corresponds to a distance of 2 μm. m, mitochondrion; z, Z-band; s, sarcoplasmic reticulum.

STUDIES OF MUSCLE FILAMENT AND SARCOMERE STRUCTURE

The uncanny degree of order that was just described lends muscle to investigation using a variety of tools. Some tools used to study muscle structure will now be presented, along with the evidence they provide for the description given above.

Light Microscopy

Numerous microscopic methods have been used to examine skeletal muscle structure. In fact, many of the modern-day electron and light microscopic techniques were pioneered by Hugh Huxley and Andrew Huxley, respectively (no relation). Obviously, these methods have provided unique insights into the structure of many other biologic tissues.

Early light microscopes did not provide a great deal of insight into the structure of the *living* muscle fiber. This was because, unstained, the muscle fiber appeared as a translucent cylinder. However, in the early 1950s Andrew Huxley developed an interference microscope that enhanced the contrast of different muscle fiber regions. Under the interference microscope, instead of appearing translucent, the muscle fiber took on a striped or striated appearance (Figure 1.7**A**). This striation pattern, which is so well-known today, resulted from the interdigitation of the sarcomere thick and thin filaments. Huxley and Niedergerke demonstrated that as muscle length changed, the striation pattern also changed (Huxley and Niedergerke, 1954). Thus in their interference microscope they knew that they had a tool that enabled investigation of contractile mechanisms on isolated, living muscle fibers.

Huxley and Niedergerke performed a series of experiments in which a muscle was caused to contract and was observed in the interference microscope. They observed that during muscle shortening, the width of the A-band remains constant while the I-band length changes. Based on what you know of the A and I bands, can you explain this observation? Huxley and Niedergerke hypothesized that this observation might result from interdigitation of filaments that maintained a constant length. The A-band stays a constant length since it represents the myosin filaments, which remain at a constant width. The I-band decreases in width since it represents the non-overlap region of the actin filament. I haven't detailed the experiments, but the highly technical nature of the microscopy along with the clever interpretation of the data (especially in light of the thinking at that time) are truly awe-inspiring.

Electron Microscopy

During a similar period, Hugh Huxley and his coworker, Jean Hanson, were developing preparative methods for the then-new electron microscope (EM; Figure 1.9). The advantage of the EM over the light microscope was its much greater magnification power (over 50,000X compared to the light microscope's 500X capability). Unfortunately, the material to be viewed under the EM could not be living—all of the water had to be extracted because the specimen was viewed in a vacuum chamber. Thus Huxley and Hanson developed methods for dehydrating, embedding, and sectioning skeletal muscles at various lengths (Huxley and Hanson, 1954). When sectioned longitudinally, the banding pattern described above was observed. Most scientists at the time thought that this banding pattern was simply an artifact of the rather harsh dehydration and embedding procedures. However, Huxley and Hanson succeeded in demonstrating that this almost crystalline array was truly representative of muscle. They then sectioned the muscle fiber transversely and demonstrated hexagonal lattices of myofilaments (Figure 1.6). The interesting point is that if they sectioned through the H-band, only a myosin filament lattice was observed. If they sectioned through the I-band, only a thin filament lattice was observed. However, if they sectioned through the overlap region of the A-band, an interdigitating hexagonal array of actin and myosin filaments was observed.

Huxley and Hanson's findings were complementary to Huxley and Niedergerke's and were equally as startling. Both groups of investigators independently proposed (and published the results side by side in the prestigious scientific journal *Nature*) that muscle contraction occurred by the relative sliding of the thick and thin filaments past one another. This became known as the "sliding filament hypothesis" and has since been elevated to the "sliding filament theory" as a plethora of data have been acquired that provide support. (However, not all scientists are sold on the sliding filament theory; see Pollack, 1983.)

X-ray Diffraction

A third tool for structural investigation of muscle that was highly influential in developing theories of muscle contraction is the method of X-ray diffraction. The principle of X-ray diffraction is similar to that observed in the ripple pond experiment from basic physics. If an X-ray beam is projected onto a living muscle, the X-rays are scattered by the tissue. If some of the scattering elements are arranged in a regular array (which the myofilaments are), an interference pattern results that represents constructive and destructive interference of the scattered X-rays. This diffraction pattern appears as a series of lines (the so-called layer lines), which can later be analyzed. The spacings between the layer lines can then be directly related to microscopic spacings in the sarcomere itself.

As with all diffraction methods, the size of the structures "seen" by the X-rays is close to the wavelength of the X-rays themselves. Since most X-rays fall in the Å range, X-ray diffraction resolves spacing between objects with molecular dimensions. Consider what some of these dimensions might represent in muscle. In a series of experimental studies, Hugh Huxley and colleagues measured the spacing between the actin and myosin filaments, the spacing between the various myosin heads (as we just saw, the 143 Å and 430 Å distances), and even the spacing between the monomers of the actin molecule in muscle at rest (Haselgrove, 1983). Sophisticated analysis of the intensities of these layer lines during passive muscle length change and active muscle contraction was consistent with muscle contraction occurring by the

relative sliding of filaments past one another and provided direct support for a portion of the myosin molecule extending out toward the actin filament during force generation! More recent studies have confirmed these earlier studies on a much faster time scale and with much better resolution (Huxley *et al.,* 1981).

Thus while X-ray diffraction may not be a technique with which you are familiar, in muscle structural studies it has provided some of the most direct, quantitative data available on normal muscle structure and muscle structural changes during contraction.

SATELLITE CELLS: RESERVES FOR INJURY AND REPAIR

An important but relatively rarely observed component of the skeletal muscle cell is the satellite cell. Maybe we shouldn't even include the satellite cells as part of the muscle cell since they are really distinct cellular entities with their own nuclei. These small cells are located beneath the fiber basal lamina (see page 3) and are approximately the same size as a muscle cell nucleus. While the satellite cell plays no known role in normal cell function, it has a central role to play in recovery of muscle fibers from injury. Satellite cells have the ability to differentiate into myoblasts and to form new muscle fibers. Clearly such an ability is central in formation of new muscle fibers following injury. This regeneration process will be discussed in Chapter 6 (page 261) as we consider muscle injury and recovery.

WHOLE SKELETAL MUSCLE STRUCTURE

Inspection of the body's numerous skeletal muscles reveals a number of common themes. Skeletal muscles attach to bones via connective tissue structures known as tendons. At times the amount of tendon is so small that the muscle fibers themselves appear to arise from bone. However, microscopic analysis of the muscle fiber end shows connective tissue such as tendon interposed between fibers and bones. A tendinous origin or insertion that is very broad and thin is termed an aponeurosis. At the gross anatomic level, each muscle has an origin (the proximal muscle end) and insertion (the distal muscle end, Table 1.1). Often the muscle origin is more broad than the insertion, in which the fibers converge onto a stout tendon (*e.g.,* m. soleus).

However, in spite of knowing a muscle's origin and insertion, it is not possible to simply describe a motion resulting from muscle contraction based only on this information. This is because muscles often cross more than one joint and therefore exert an influence at multiple location (inspection of Table 1.1 shows that most muscles cross multiple joints). For example, the rectus femoris crosses both the knee and hip joints. If the knee were fixed and the hip were free to move, rectus femoris contraction would cause hip flexion. Conversely, if the hip were fixed and the knee free to move, rectus femoris contraction would cause knee extension. Thus is the rectus femoris a hip flexor or a knee extensor or both? It is not possible to answer this question unambiguously without specifying a movement. For example, in standing from a squat, the rectus femoris is activated and generates tension, but clearly the knee and hip *both extend.* Therefore, anatomically the rectus is a hip flexor and knee extensor, but this is not always the case during the muscle's physiologic action. We must therefore resist the temptation to classify muscles in terms of anatomy. Instead, we can state that the rectus femoris always acts to generate a hip flexion moment and a knee extension moment (Zajac and Gordon, 1989). We will discuss this point further in analyzing biarticular muscle function in Chapter 3 (page 149).

Muscle Architecture—Fiber Arrangement Is Everything

Skeletal muscle is not only highly organized at the microscopic level; the *arrangement* of the muscle fibers at the macroscopic level also demonstrates a striking degree of organization. In making comparisons between various muscles, certain factors such as fiber type distribution are important, but there is no question that an important factor in determining a muscle's contractile properties is the muscle's architecture.

Skeletal muscle architecture is defined as "the arrangement of muscle fibers relative to the axis of force generation." While muscle fibers have a relatively consistent fiber diameter between muscles of different sizes, the *arrangement* of these fibers can be quite different. The various types of arrangement are as numerous as the muscles themselves, but for convenience we can discuss three types of fiber architecture.

Muscles with fibers that extend parallel to the muscle force-generating axis are termed parallel or longitudinally arranged muscles (Figure 1.10, page 38). While the fibers extend parallel to the force-generating axis, they never extend the entire muscle length (Tables 1.3 and 1.4). Muscles with fibers that are oriented at a single angle relative to the force generating axis are termed unipennate muscles (Figure 1.10, middle). The angle between the fiber and the force-generating axis generally varies from 0° to 30°. It is obvious when preparing muscle dissections that most muscles fall into the most general category, multipennate muscles—muscles composed of fibers that are oriented at several angles relative to the axis of force generation (Figure 1.10, right). As we will discuss in the next chapter, an understanding of muscle architecture is critical to understanding the various functional properties of different sized muscles.

Table 1.3.
Architectural Properties of the Human Hand, Arm, and Forearm[a,b]

Muscle	Muscle Mass (g)	Muscle Length (mm)	Fiber Length (mm)	Pennation Angle (°)	Cross-Sectional Area (cm^2)	FL/ML Ratio
			Extrinsic Muscles			
AbPL (n=9)	9.96±2.01	160.4±15.0	58.1±7.4	7.5±2.0	1.93±.59	.36±.05
BR (n=8)	16.6±2.8	175±8.3	121±8.3	2.4±.6	1.33±.22	.69±.062
EDC I (n=8)	3.05±.45	114±3.4	56.9±3.6	3.1±.5	.52±.08	.49±.024
EDC M (n=5)	6.13±1.2	112±4.7	58.8±3.5	3.2±1.0	1.02±.20	.50±.014
EDC R (n=7)	4.70±.75	125±10.7	51.2±1.8	3.2±.54	.86±.13	.42±.023
EDC S (n=6)	2.23±.32	121±8.0	52.9±5.2	2.4±.7	.40±.06	.43±.029
EDQ (n=7)	3.81±.70	152±9.2	55.3±3.7	2.6±.6	.64±.10	.36±.012
EIP (n=6)	2.86±.61	105±6.6	48.4±2.3	6.3±.8	.56±.11	.46±.023
EPB (n=9)	2.25±1.36	105.6±22.5	55.0±7.5	7.2±4.4	.47±.32	.54±.13
EPL (n=7)	4.54±.68	138±7.2	43.6±2.6	5.6±1.3	.98±.13	.31±.020
FDP I (n=9)	11.7±1.2	149±3.8	61.4±2.4	7.2±.7	1.77±.16	.41±.018
FDP M (n=9)	16.3±1.7	200±8.2	68.4±2.7	5.7±.3	2.23±.22	.34±.011
FDP R (n=9)	11.9±1.4	194±7.0	64.6±2.6	6.8±.5	1.72±.18	.33±.009
FDP S (n=9)	13.7±1.5	150±4.7	60.7±3.9	7.8±.9	2.20±.30	.40±.015
FDS I(C) (n=6)	12.4±2.1	207±10.7	67.6±2.8	5.7±.2	1.71±.28	.33±.025
FDS I(D) (n=9)	6.6±.8	119±6.1	37.9±3.0	6.7±.3	1.63±.22	.32±.013
FDS I(P) (n=6)	6.0±1.1	92.5±8.4	31.6±3.0	5.1±0.2	1.81±.83	.34±.022
FDS M (n=9)	16.3±2.2	183±11.5	60.8±3.9	6.9±.7	2.53±.34	.34±.014
FDS R (n=9)	10.2±1.1	155±7.7	60.1±2.7	4.3±.6	1.61±.18	.39±.023
FDS S (n=9)	1.8±.3	103±6.3	42.4±2.2	4.9±.7	0.40±.05	.42±.014
FPL (n=9)	10.0±1.1	168±10.0	45.1±2.1	6.9±.2	2.08±.22	.24±.010

Table 1.3.
Architectural Properties of the Human Hand, Arm, and Forearm[a,b]
(Continued)

Muscle	Muscle Mass (g)	Muscle Length (mm)	Fiber Length (mm)	Pennation Angle (°)	Cross-Sectional Area (cm^2)	FL/ML Ratio
PL (n=6)	3.78±.82	134±11.5	52.3±3.1	3.5±1.2	.69±.17	.40±.032
PQ (n=8)	5.21±1.0	39.3±2.3	23.3±2.0	9.9±.3	2.07±.33	.58±.021
PT (n=8)	15.9±1.7	130±4.7	36.4±1.3	9.6±.8	4.13±.52	.28±.012
			Intrinsic Muscles			
AbDM (n=9)	3.32±1.67	68.4±6.5	46.2±7.2	3.9±1.3	.89±.49	.68±.10
AbPB (n=9)	2.61±1.19	60.4±6.6	41.6±5.6	4.6±1.9	.68±.28	.69±.09
AddPol (n=9)	6.78±1.84	54.6±8.9	34.0±7.5	17.3±3.4	1.94±.39	.63±.15
DI I (n=9)	4.67±1.17	61.9±2.5	31.7±2.8	9.2±2.6	1.50±.40	.51±.05
DI II (n=9)	2.65±1.01	62.8±8.1	25.1±6.3	8.2±3.1	1.34±.77	.41±.13
DI III (n=9)	2.01±0.60	54.9±4.6	25.8±3.4	9.8±2.8	.95±.45	.47±.07
DI IV (n=9)	1.90±0.62	50.1±5.3	25.8±3.4	9.4±4.2	.91±.38	.52±.11
FDM (n=9)	1.54±.44	59.2±10.4	40.6±13.7	3.6±1.0	.54±.36	.67±.17
FPB (n=9)	2.58±.56	57.2±3.7	41.5±5.2	6.2±4.5	.66±.20	.73±.08
Lum I (n=9)	0.57±.019	64.9±10.0	55.4±10.2	1.2±.9	.112±.03	.85±.03
Lum II (n=9)	0.39±.22	61.2±17.8	55.5±17.7	1.6±1.3	.079±.04	.90±.05
Lum III (n=9)	0.37±.16	64.3±8.9	56.2±10.7	1.1±.8	.081±.04	.87±.07
Lum IV (n=9)	0.23±0.11	53.8±11.5	50.1±8.4	0.7±1.0	.063±.03	.90±.05
OpDM (n=9)	1.94±.98	47.2±3.6	19.5±4.1	7.7±2.9	1.10±.43	.41±.09
OpPol (n=9)	3.51±.89	55.5±5.0	35.5±5.1	4.9±2.5	1.02±.35	.64±.07
PI II (n=9)	1.56±.22	55.1±5.0	25.0±5.0	6.3±2.2	.75±.25	.45±.08

Table 1.3.
Architectural Properties of the Human Hand, Arm, and Forearm[a,b] *(Continued)*

Muscle	Muscle Mass (g)	Muscle Length (mm)	Fiber Length (mm)	Pennation Angle (°)	Cross-Sectional Area (cm^2)	FL/ML Ratio
PI III (n=9)	1.28±.28	48.2±2.9	26.0±4.3	7.7±3.9	.65±.26	.54±.08
PI IV (n=9)	1.19±.33	45.3±5.8	23.6±2.6	8.2±3.5	.61±.23	.52±.10

[a]Data on extrinsic muscles from Lieber *et al.*, 1990, 1991; data on intrinsic muscles from Jacobson MD. J Hand Surg, submitted, 1992.
[b]AbDM: abductor digiti minimi; AbPB: abductor pollicis brevis; AddPol: adductor pollicis; BR: brachioradialis; DI I to DI IV: dorsal interosseous muscles; EDC I, EDC M, EDC R, and EDC S: extensor digitorum communis to the index, middle, ring, and small fingers, respectively; EDQ: extensor digiti quinti; EIP: extensor indicis proprious; EPL: extensor pollicis longus; FDM: flexor digiti minimi; FDP I, FDP M, FDP R, and FDP S: flexor digitorum profundus muscles; FDS I, FDS M, FDS R, and FDS S: flexor digitorum superficialis muscles; FDS I (P) and FDS I (D): proximal and distal bellies of the FDS I; FDS I (C): the combined properties of the two bellies as if they were a single muscle; FPB: flexor pollicis brevis; FPL: flexor pollicis longus; L I to L IV: lumbrical muscles; OpDM: opponens digiti minimi; OpPol: opponens pollicis; PI I to PI IV: palmar interosseous muscles; PQ: pronator quadratus; PS: palmaris longus; PT: pronator teres.

Table 1.4.
Architectural Properties of Human Lower Limb[a,b]

Muscle	Muscle Mass (g)	Muscle Length (mm)	Fiber Length (mm)	Pennation Angle (°)	Cross-Sectional Area (cm^2)	FL/ML Ratio
AB (n=3)	43.8±8.4	156±12	103±6.4	0.0±0.0	4.7±1.0	.663±.036
AL (n=3)	63.5±16	229±12	108±2.0	6.0±1.0	6.8±1.9	.475±.023
AM (n=3)	229±32	305±12	115±7.9	0.0±0.0	18.2±2.3	.378±.013
BF_L (n=3)	128±28	342±14	85.3±5.0	0.0±0.0	12.8±2.8	.251±.022
BF_S (n=3)		271±11	139±3.5	23±0.9		.517±.032
EDL (n=3)	35.2±3.6	355±13	80.3±8.4	8.3±1.7	5.6±0.6	.226±.024
EHL (n=3)	12.9±1.6	273±2.4	87.0±8.0	6.0±1.0	1.8±0.2	.319±.030
FDL (n=3)	16.3±2.8	260±15	27.0±0.58	6.7±1.7	5.1±0.7	.104±.004
FHL (n=3)	21.5±3.3	222±5.0	34.0±1.5	10.0±2.9	5.3±0.6	.154±.010
GR (n=3)	35.3±7.4	335±20	277±12	3.3±1.7	1.8±0.3	.828±.017
LG (n=3)		217±11	50.7±5.6	8.3±1.7		.233±.016
MG (n=3)	150±14	248±9.9	35.3±2.0	16.7±4.4	32.4±3.1	.143±.010

Table 1.4.
Architectural Properties of Human Lower Limb[a,b] *(Continued)*

Muscle	Muscle Mass (g)	Muscle Length (mm)	Fiber Length (mm)	Pennation Angle (°)	Cross-Sectional Area (cm^2)	FL/ML Ratio
PB (n=3)	17.3±2.5	230±13	39.3±3.5	5.0±0.0	5.7±1.0	.170±.006
PEC (n=3)	26.4±6.0	123±4.5	104±1.2	0.0±0.0	2.9±0.6	.851±.040
PL (n=3)	41.5±8.5	286±17	38.7±3.2	10.0±0.0	12.3±2.9	.136±.010
PLT (n=3)	5.30±1.9	85.0±15	39.3±6.7	3.3±1.7	1.2±0.4	.467±.031
POP (n=2)	20.1±2.4	108±7.0	29.0±7.0	0.0±0.0	7.9±1.4	.265±.048
RF (n=3)	84.3±14	316±5.7	66.0±1.5	5.0±0.0	12.7±1.9	.209±.002
SAR (n=3)	61.7±14	503±27	455±19	0.0±0.0	1.7±0.3	.906±.017
SM (n=3)	108±13	262±1.5	62.7±4.7	15±2.9	16.9±1.5	.239±.017
SOL (n=2)	215 (n=1)	310±1.5	19.5±0.5	25±5.0	58.0 (n=1)	.063±.002
ST (n=2)	76.9±7.7	317±4	158±2.0	5.0±0.0	5.4±1.0	.498±0.0
TA (n=3)	65.7±10	298±12	77.3±7.8	5.0±0.0	9.9±1.5	.258±.015
TP (n=3)	53.5±7.3	254±26	24.0±4.0	11.7±1.7	20.8±3	.095±.015
VI (n=3)	160±59	329±15	68.3±4.8	3.3±1.7	22.3±8.7	.208±.007
VL (n=3)	220±56	324±14	65.7±0.88	5.0±0.0	30.6±6.5	.203±.007
VM (n=3)	175±41	335±15	70.3±3.3	5.0±0.0	21.1±4.3	.210±.005

[a]Data from Wickiewicz *et al.*, (1982).
[b]AB, adductor brevis; AL, adductor longus; AM, adductor magnus; BF_L, biceps femoris, long head; BF_S, biceps femoris, short head; EDL, extensor digitorum longus; EHL, extensor hallucis longus; FDL, flexor digitorum longus; GR, gracilis; FHL, flexor hallucis longus; LG, lateral gastrocnemius; MG, medial gastrocnemius; PEC, pectineus; PB, peroneus brevis; PL, peroneus longus; PLT, plantaris; POP, popliteus; RF, rectus femoris; SAR, sartorius; SM, semimembranosus; SOL, soleus; ST, semitendinosus; TA, tibialis anterior; TP, tibialis posterior; VI, vastus intermedius; VL, vastus lateralis; VM, vastus medialis.

Experimental Determination of Skeletal Muscle Architecture

Early studies of muscle architecture were pioneered by the anatomist Carl Gans (Gans, 1982). Gans and his colleagues developed precise methods for muscle architecture determination based on microdissection of whole muscles. These muscles were chemically fixed in order to maintain their

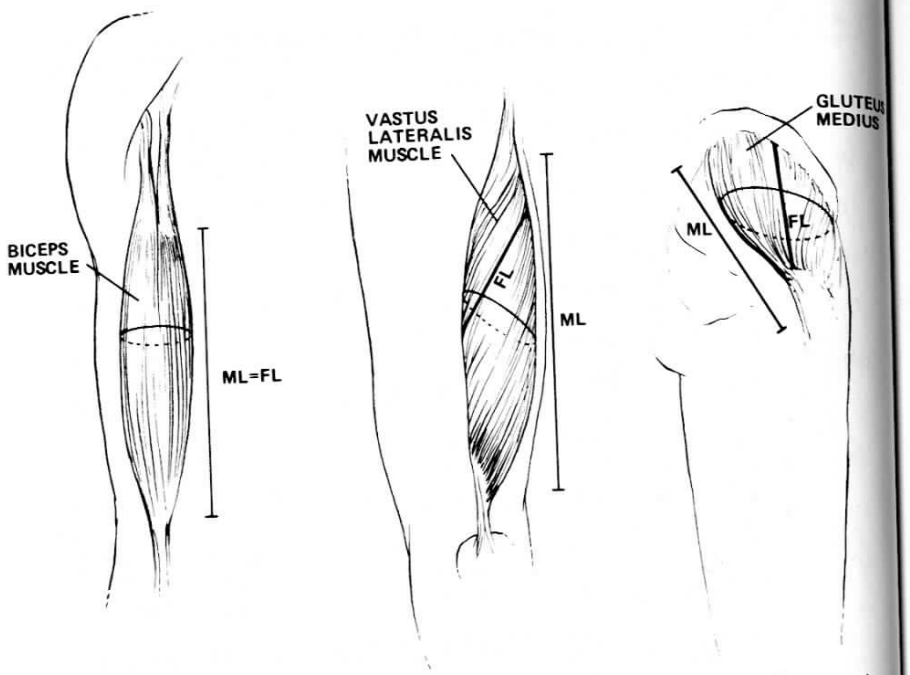


Figure 1.10. Generalized picture of muscle architectural types. Skeletal muscle fibers may be oriented along the muscle's force-generating axis (*left*), at a fixed angle relative to the force-generating axis (*middle*), or at multiple angles relative to the force-generating axis (*right*). Each of these represents an idealized view of muscle architecture and probably does not adequately describe any single muscle. ML, muscle length; FL, fiber length.

integrity during dissection. Ideally, the muscle was fixed while attached to the skeleton to roughly preserve its physiologic length. Following fixation, muscles were dissected from the skeleton, their mass determined, and their pennation angle (*i.e.*, the fiber angle relative to the force-generating axis) and muscle length were measured.

Currently, pennation angle is measured by determining the average pennation angle of fibers on the superficial muscle surface. While more sophisticated methods could be devised, it is doubtful they would provide a great deal more information. In measuring muscle length, it is important to note that muscle length is defined as the distance from the origin of the most proximal muscle fibers to the insertion of the most distal fibers. As mentioned above, muscle fiber length is *never* the same as the whole muscle length.

Muscle fiber length can only be determined by microdissection of individuals fibers from the fixed tissues. In general, unless investigators are explicit, when they refer to muscle fiber length, they are actually referring to muscle fiber *bundle* length. It is extremely difficult to isolate intact fibers, which run from origin to insertion, especially in mammalian tissue (Sacks and Roy, 1982; Loeb *et al.*, 1987; Ounjian *et al.*, 1991).

The final experimental step in performing architectural analysis is to determine the sarcomere length within the isolated bundles. This is necessary in order to compensate for differences in muscle lengths that occur during fixation. In other words, if we conclude that a muscle is "long," we must be sure that it is truly "long" and that is it was not simply fixed in a stretched position. Similarly, muscles measured to be "short" must be further investigated to ensure that they were not simply fixed at a short sarcomere length. In order to permit such conclusions, architectural measurements are always normalized or "adjusted" to a constant sarcomere length.

Having obtained muscle mass, fiber length, sarcomere length, muscle length, and pennation angle, a number of parameters can be calculated that summarize the muscle architecture. These parameters have a direct relation to the whole muscle's contractile properties.

Physiologic Cross-Sectional Area

Following measurement of the typical architectural parameters, the so-called physiologic cross-sectional area (PCSA) can be calculated. What is the significance of such a calculation? In short, the PCSA is directly proportional to the maximum tetanic tension of the muscle. This value is almost never the cross-sectional area of the muscle in any of the traditional anatomic planes, as would be obtained, for example, using a noninvasive imaging method such as magnetic resonance imaging (MRI) or computerized tomography (CT). Theoretically, PCSA represents the sum of the cross-sectional areas of all the muscle fibers within the muscle. It is calculated using Equation 1.1, which was pioneered by Carl Gans and verified experimentally by Roland Roy and Reggie Edgerton. In Equation 1.1, ρ represents muscle density (1.056 g/cm^3 for mammalian muscle) and θ represents surface pennation angle.

$$\text{PCSA}\,(\text{cm}^2) = \frac{\text{Muscle Mass}\,(\text{g}) \cdot \cos\theta}{\rho\,(\text{g/cm}^3) \cdot \text{Fiber Length}\,(\text{cm})} \qquad (1.1)$$

If we partition the equation into its components, the rationale for this expression becomes more clear. First, note that the muscle mass divided by density equals muscle volume (Equation 1.2). If the muscle were roughly cylindrical in shape, dividing volume by length (fiber length) would represent

the cylinder cross-sectional area (Equation 1.3). Since, in our example, fiber length does not equal cylinder length, the area is not an actual area; rather, it is a theoretical area that would be occupied by a cylinder with a length equal to that of the fibers. Now, since the fibers may be oriented at some angle relative to the axis of force generation, the cosine term must be included (Equation 1.4). For those of you with a background in physics, the basis for this term is obvious. Because this is a concept that will be useful to us at a later stage, consider the situation shown in Figure 1.10.

$$\text{Volume}\,(\text{cm}^3) = \frac{\text{Muscle Mass}\,(\text{g})}{\rho\,(\text{g/cm}^3)} \tag{1.2}$$

$$\text{CSA}\,(\text{cm}^2) = \frac{\text{Volume}\,(\text{cm}^3)}{\text{Fiber Length}\,(\text{cm})} \tag{1.3}$$

Suppose a muscle fiber pulls with x units of force at an angle θ relative to the muscle axis of force generation. Clearly, some of the force of the fiber will not be transmitted along the axis but will be lost. Thus only a *component* of muscle fiber force will actually be transmitted along the muscle axis. Noting the right triangle in Figure 1.11**A,** it can be seen that the component of muscle force transmitted will be x-cosθ, since $\cos\theta = \frac{F}{x}$, which will *always* be less than x since cosθ is always less than 1. In other words, pennation itself results in a loss of muscle force relative to a muscle with the same mass and fiber length but with zero pennation angle. Why would the system be designed this way, such that force was lost? Consider the alternative. If the pennation angle were zero, the absolute size of the muscle would prohibit placing it in many bodily locations due to the large number of fibers that would have a PCSA equal to an anatomic CSA in, say, the transverse plane (Figure 1.11**B**). Thus it appears that pennation is a space-saving strategy even though it costs a bit in force generation. Pennation angle (θ) does not appear to have a large detrimental influence on PCSA in spite of this argument. This is because, as you know, the cosine of 0° is 1 and the cosine of 30° (which would be a very large pennation angle and is rarely encountered) is 0.87 which represents only a 13% force loss for a huge increase in fiber packing ability.

$$\text{PCSA}\,(\text{cm}^2) = \text{CSA}\,(\text{cm}^2) \cdot \cos\theta \tag{1.4}$$

The usefulness of this equation was recently highlighted by Roy and Edgerton in an experimental comparison between the *estimated* maximum muscle tetanic tension (based on PCSA calculations) and *measured* maximum tetanic tension (measured using traditional physiologic testing techniques). These investigators found that the estimations and predictions agreed within experimental error (Powell *et al.,* 1984). The only exception to that conclusion was that the soleus muscle (a monoarticular plantar flexor of the deep calf) did not seem to agree. Interestingly, this was the only muscle tested that contained a large proportion of slow muscle fibers (see Chapter 2). These data may suggest that slow fibers generate less tension than fast fibers, but the jury is clearly not in in this regard.

It is important to highlight the observation that PCSA (and therefore maximum muscle tension) is *not* simply proportional to muscle mass (as is

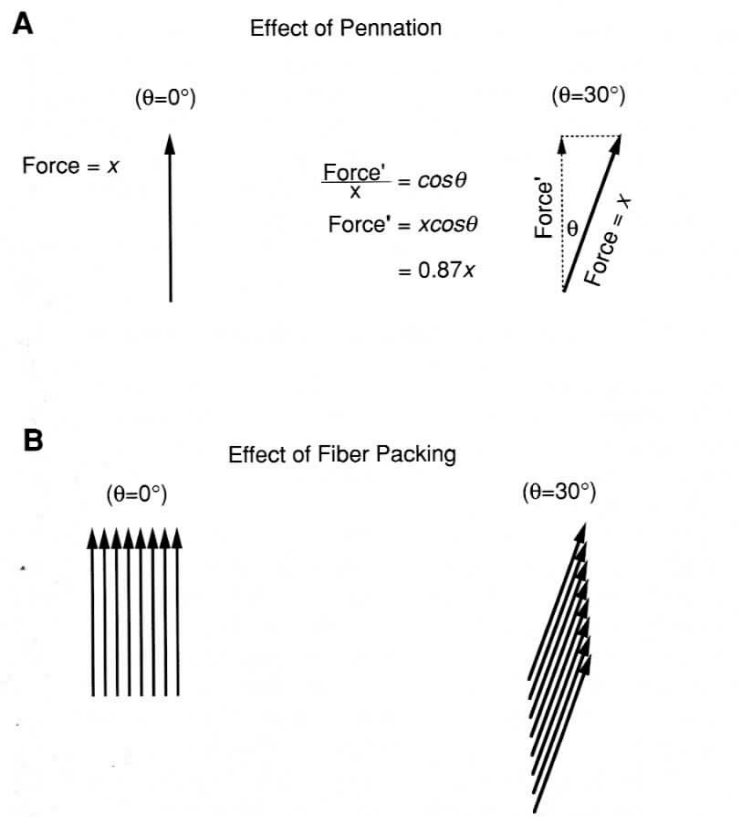


Figure 1.11. Schematic illustration of the effect of pennation. **A,** Muscle fibers oriented parallel to the axis of force generation transmit all of their force to the tendon. However, muscle fibers oriented at a 30° angle relative to the force-generating axis transmit only a portion of their force (cosine[30°] = 0.87, or 87%) to the tendon. **B,** While only about 90% of the muscle fiber force is transmitted to the tendon due to pennation, pennation itself permits packing of a large number of fibers into a smaller cross-sectional area.

clear from the equation). In other words, given information on muscle mass or on muscle mass change (say, due to immobilization or spinal cord injury), we can make *no* statement with respect to muscle force. This is another way of saying that while mass is proportional to the amount of contractile material in the muscle, the *arrangement* of that material is of critical importance. We should also state that in some of these pathologic conditions, mass may change due to noncontractile proteins (*e.g.,* increased connective tissue or inflammatory cells). In such cases, PCSA will not accurately predict tetanic tension.

ARCHITECTURE OF HUMAN SKELETAL MUSCLES

Experimental measurement of human muscle architecture has a great deal of importance not only in understanding normal muscle function but also in understanding muscle adaptation (as we shall see in Chapters 4–6). In fact, if you as a therapist know nothing else about a muscle, knowing its architecture may be *the* most important.

Several architectural investigations have been performed in human upper and lower limbs. Tables of the relevant architectural features are presented in Tables 1.3 and 1.4. These tables look intimidating at first but will serve you primarily as a reference. Take some time to look at the normal range of parameters seen. For example, notice that pennation angles normally range from about 0° to 30°. Thus as mentioned above, pennation probably has a relatively small influence on muscle PCSA (and function). Note also that the ratio of muscle fiber length to muscle length (FL/ML ratio) typically ranges from about 0.2 to about 0.6. In other words, even the most longitudinally oriented muscles have fibers that extend only about 60% of the muscle length. Finally, note that there is a very poor correlation between muscle mass and muscle PCSA. Again, mass gives *little* information that is relevant to function.

Muscles of the Lower Limb

While each muscle is unique in terms of its architecture, taken as functional groups (*e.g.,* hamstrings, quadriceps, dorsiflexors, plantar flexors), a number of generalizations can be made (Figure 1.12). In terms of architecture, the typical properties of the various groups can be articulated (compare properties shown in Table 1.4; Wickiewicz *et al.,* 1983). The quadriceps are characterized by their relatively high pennation angles, large PCSAs, and short fibers. In terms of design, these muscles appear suited for the generation of large forces. The hamstrings, on the other hand, by virtue of their relatively long fibers and intermediate PCSAs, appear to be designed for large excursions (because excursions are proportional to fiber length). The same

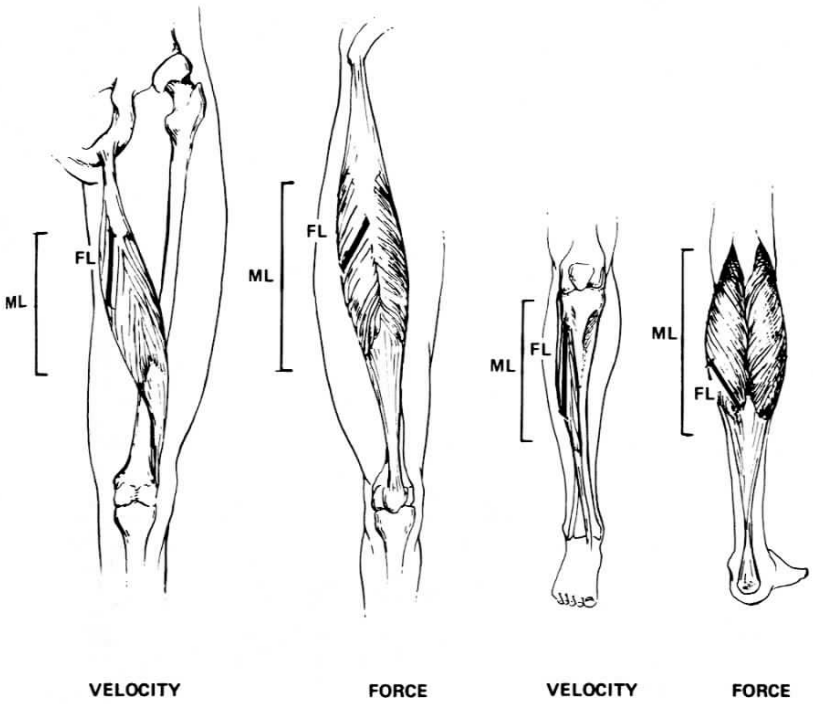


Figure 1.12. Schematic illustration of muscle architectural properties in the lower limb. Functionally, quadriceps and plantar flexors are designed for force production due to their low fiber length/muscle length ratios and large cross-sectional areas. Conversely, in general, hamstrings and dorsiflexors are designed for high excursions and velocity by nature of their high fiber length/muscle length ratios and long muscle fibers.

appears to be true of the plantar flexors and dorsiflexors, respectively. A very general (and a bit dangerous) conclusion might be that the antigravity extensors are more designed toward force production, while the flexors are more designed for high excursions. This generalization will break down upon close scrutiny but might provide a useful memory tool. You should be careful when considering architecture alone when trying to deduce function since normally muscles act via a moment arm to produce joint torque. Moment arms and strength will be discussed in Chapter 3 (page 121).

Probably the two most important muscle architecture parameters are muscle PCSA (which is proportion to maximum muscle force) and muscle fiber length (which is proportional to maximum muscle excursion). These

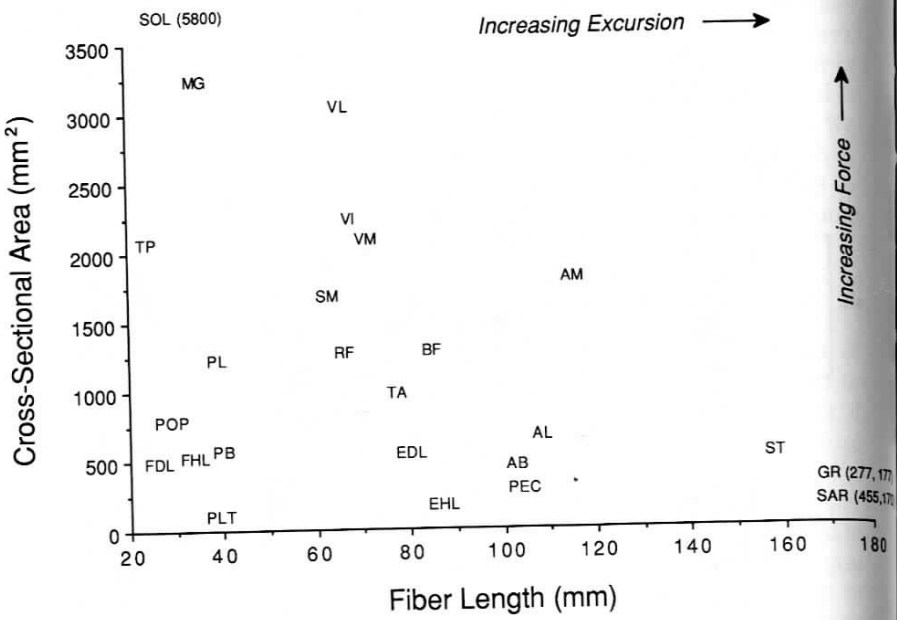


Figure 1.13. Scatter graph of fiber length and cross-sectional areas of muscles in the human lower limb (data from Wickiewicz *et al.*, 1983). Fiber length is proportional to muscle excursion, and cross-sectional area is proportional to maximum muscle force. Thus this graph can be used to compare the relative forces and excursions of leg muscles. See Table 1.4 for definitions of abbreviations.

two parameters are shown in graphical form for each muscle (Figures 1.13 and 1.14) and can be used to make general comparisons between muscles in terms of design. For example, note that the sartorius, semitendinosus, and gracilis muscles have extremely long fiber lengths and low PCSAs, which permit long excursions at low forces (Figure 1.13). At the other end of the spectrum is the soleus muscle, with its high PCSA and short fiber length, suitable for generating high forces with small excursions. Based on our understanding of the normal use of each of these muscles in the gait cycle (Chapter 3, page 143), these designs appear to be reasonable.

Muscles of the Upper Limb

In light of the specialization observed in the lower limb, the author and his colleagues were interested in understanding the architectural features of muscles in the human arm and forearm. While no such clear-cut generalizations could be made (as were made for the lower limb), it was possible to demonstrate the extreme degree of specialization present in many upper limb muscles (Lieber *et al.*, 1990 and 1991). The details of these results are presented in Table 1.3. Again, note the high degree of specialization "built into" each of these muscles by virtue of their design. For example, the superficial and deep digital flexors to each digit are very similar to one another but very different from the digital extensors (Figure 1.14). Again, this type of scatter plot can be used to compare functional properties between muscles of the forearm. Clearly, such differences could be considered in surgical and rehabilitative procedures involving the upper limb. One might expect that when a muscle is surgically transferred to perform the function of another muscle whose function has been lost, matching of architectural properties may prove beneficial. We will return to this topic in Chapter 4 (page 172).

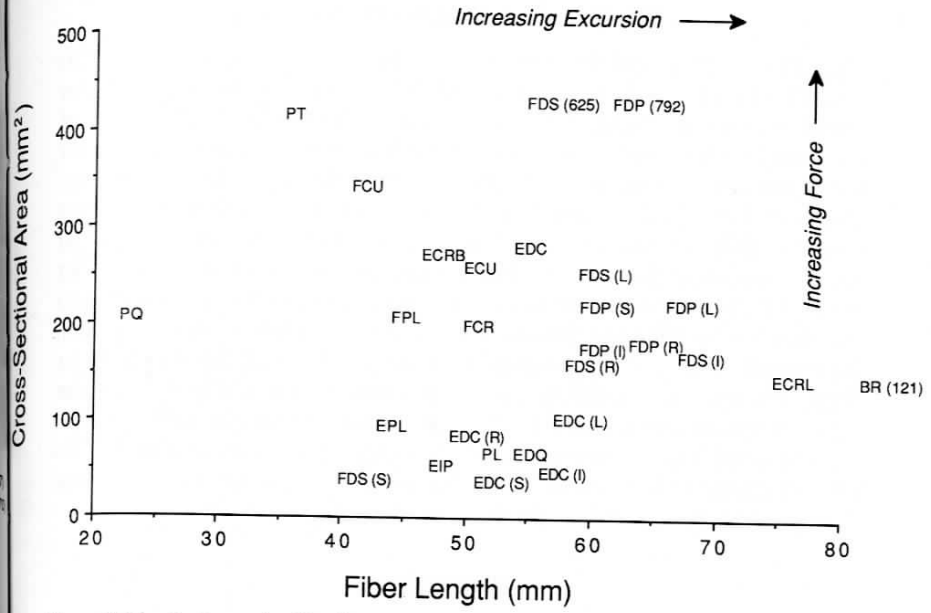


Figure 1.14. Scatter graph of the fiber length and cross-sectional areas of muscles in the human arm (data from Lieber *et al.*, 1990 and 1991). Fiber length is proportional to muscle excursion, and cross-sectional area is proportional to maximum muscle force. Thus this graph can be used to compare the relative forces and excursions of arm and forearm muscles. See Table 1.3 for definitions of abbreviations.

Significance of Muscle Architecture

After this relatively lengthy discussion of architecture, one might ask, "So what? What do we know now that we didn't know before?" My answer to that question is two-pronged. First, the fact that muscles that are composed of identical building blocks (sarcomeres) can have such dramatically different force-generating properties highlights the clever design of the human body. The body uses identical components and arranges them in different ways to construct different "motors," which produce movement. Second, by virtue of architectural specialization, it is clear that the neuromuscular system does not simply modify muscular force and excursion by changing the nervous input to the muscles. Muscles are *designed* for a specific function—large excursion, for example. The nervous system provides the signal for the muscle to "do its thing" but does not necessarily specify the details of that action. It is as if the nervous system acts as the central control while the muscle interprets the control signal into an external action by virtue of its intrinsic design. This elegant design is but one of many that we will encounter in our voyage through the neuromusculoskeletal system.

SUMMARY

Skeletal muscles arise by a unique developmental process that includes axonal outgrowth, myogenesis, neuromuscular junction formation, and synapse elimination. The fully differentiated muscle cell is uniquely suited to perform force generation and movement. A sterotypical view of the muscle fiber can be presented in which force-generating, force-regulating, and force-activating roles are assigned to various structures. The structural muscle hierarchy proceeds from the whole muscle all the way to the myofilaments. The arrangement of muscle fibers within whole muscles is known as muscle architecture and is an important factor in determining whole muscle properties. We will soon see that skeletal muscle fibers can be differentiated into various types, the details of which will be presented in the following chapter.

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Chapter

2

Skeletal Muscle Physiology

OVERVIEW

This chapter describes the way that muscle structures produce the desired function. Under the general classification of "physiology," the chapter has been divided into three parts. First, we discuss the activation and contraction sequence of muscle, along with some functional consequences of this scheme. Next, the two basic mechanical properties of muscle—the length-tension and force-velocity properties—are highlighted. The basis for all muscle contraction is presented as well as the details of the cross-bridge cycle. The manner in which architecture affects these mechanical properties is included. Finally, the topics of muscle fiber types and motor units are presented, which enables discussion of recruitment, locomotion, and fatigue.

INTRODUCTION

In this chapter, anatomy meets physiology. In other words, the rationale for the physical arrangement of the various muscle components will become apparent. An understanding of muscle physiology is predicated on a good understanding of muscle macro- and microanatomy. This chapter represents the payoff for having waded through the previous one. It should be noted that anatomic studies are rarely performed in isolation of physiologic studies and *vice versa*. In fact, anatomists routinely refer to physiologic data in describing the significance of their findings, and physiologists routinely refer to anatomic studies in proposing mechanisms for their observations. Thus the distinction between muscle anatomy and physiology is often one of orientation. Significant cross-referencing between muscle anatomic and physiologic studies will be required as we continue our discussion of skeletal muscle structure and function.

PART 1: FIBER ACTIVATION

EXCITATION-CONTRACTION COUPLING

Our discussion of skeletal muscle physiology begins with the process of muscle activation itself. It is well known that peripheral nerves innervate

skeletal muscles and that neural activation precedes muscle contraction. The precise process by which this neural activation signal culminates in muscle contraction is known as excitation-contraction coupling, or EC coupling (Figure 2.1). EC coupling is viewed as a sequence of events, each of which is necessary for contraction to occur. If any single step of EC coupling is impaired, muscle contraction does not occur normally. This impairment might be interpreted as muscle paralysis or fatigue. However, such a general classification is not useful unless the underlying cause is known (Ebashi *et al.*, 1980).

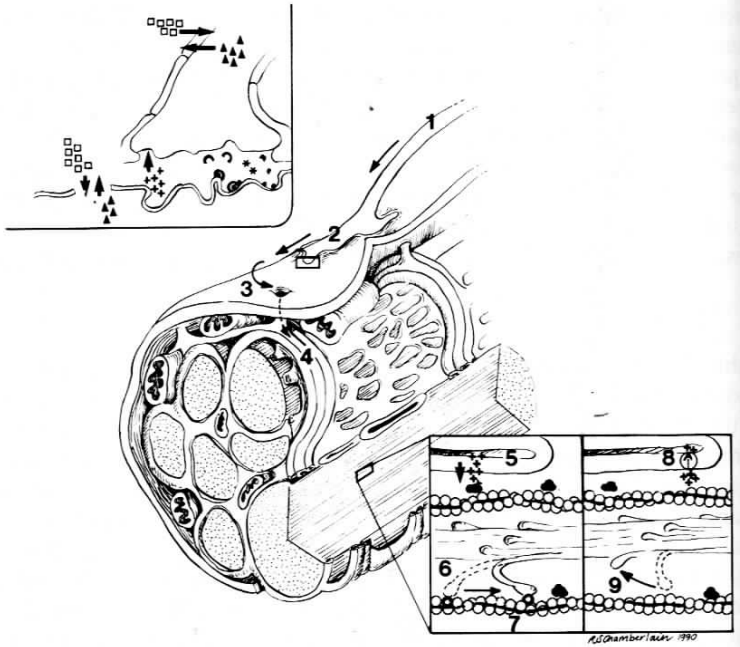

Figure 2.1. Sequence of events in excitation-contraction coupling of a nervous impulse to muscle contraction. **1,** Action potential conducted by nerve to muscle (squares represent Na^+ ions entering nerve, and triangles represent K^+ ions leaving nerve to conduct the action potential). **2,** Nervous impulse transmitted across neuromuscular junction to muscle fiber (crosses represent Ca^{++} ions entering nerve end, half-moons represent the neurotransmitter ACh, and asterisks represent the enzyme acetylcholinesterase degrading ACh). **3,** Action potential conducted along fiber surface. **4,** Action potential conducted deep into fiber via the T-system. **5,** Ca^{++} released from SR to activate actin filament. **6** and **7,** Cross-bridge produces force and filament sliding **8,** Ca^{++} pumped back into SR. **9,** Cross-bridge relax due to lack of Ca^{++} filament activation.

Action Potential—the First Step

The first step in the EC coupling chain is the generation of the peripheral nerve action potential. The action potential results from depolarization of the peripheral nerve axon that innervates the muscle. In addition to a signal from the CNS, the axon may be depolarized in a number of ways, including trauma to the peripheral nerve or application of an external electrical stimulating device. In any case, the resulting action potentials that propagate down the peripheral nerve are identical. The action potential arrives at the neuromuscular junction, the interface between muscle and nerve. The neuromuscular junction is itself a complex structure. The nerve ends in a small indentation on the muscle fiber surface, known as the synaptic cleft.

Acetylcholine Release—the Neurotransmitter

The end of the nerve contains packets of the neurotransmitter acetylcholine (ACh), which causes muscle fiber excitation. ACh is synthesized by the cell body of the motor nerve and transported down the axon where it is stored at nerve endings for later use. Following nerve depolarization, a quantum or unit of ACh is released into the small space between the muscle and nerve, the synaptic cleft (Figure 2.1). ACh then diffuses across the synaptic cleft and binds to the ACh receptor, which is integrated into the muscle membrane (refer to the discussion of synaptogenesis in Chapter 1, page 12). ACh binding results in depolarization of the muscle fiber sarcolemma and an action potential that propagates from the neuromuscular junction outward in all directions.

Transverse Tubular System and Sarcoplasmic Reticulum Involvement in Excitation-Contraction Coupling

At various intervals along the fiber surface, the action potential encounters invaginations of the sarcolemma that extend into the fiber—the transverse tubular system (T-system; Figure 2.1; Peachey and Franzini-Armstrong, 1983). The action potential is conducted deep into the fiber by the T-system. The interface between the "outside world" of the muscle fiber and the "inside world" of the contractile apparatus occurs at the next step where the T-system signals the sarcoplasmic reticulum (SR) to release calcium. The precise mechanism for this communication is not completely understood. However, it is believed that the SR feet, which anchor the terminal region of the SR to the T-system, are involved in some way. An important observation is that the lumen of the T-system contains *extracellular* fluid while the lumen of the SR contains *intracellular* fluid. Thus extracellular fluid is actually contained deep within the muscle fiber!

Calcium Release Results in Muscle Contraction

After the T-system signals the SR, the SR releases calcium ions in the region of the myofilaments (Figure 2.1). This release process is extremely fast. The calcium ions bind to troponin, the actin filament regulatory protein, which in turn releases the inhibition on the actin filament, permitting interaction with the myosin filament and resulting in cross-bridge cycling (see details below) *i.e.,* force generation.

Calcium Uptake Results in Muscle Relaxation

As long as neural impulses arrive at the neuromuscular junction and, therefore, calcium concentrations remain high in the region of the myofilaments, force generation continues. However, when the impulses cease, calcium is pumped back into the SR by the calcium-activated adenosine triphosphatase (ATPase) enzyme. The calcium-activated ATPase enzyme is an integral protein that is embedded in the bilayer of SR membrane. The mechanism of action of this enzyme has been thoroughly studied and is one of the best understood of the ion transport enzymes (Entman and Van Winkle, 1986). The calcium pumping process is energy dependent and requires ATP. When calcium levels in the region of the myofilaments drop below a critical level, thin filament inhibition again resumes, and actin-myosin interaction is prevented. This inhibition is manifest externally as muscle fiber relaxation.

We thus have the chain of events required for muscle contraction to occur following nerve depolarization:

1. Generation of the peripheral nerve action potential
2. Release of ACh from the nerve terminal
3. Binding of ACh to the muscle fiber ACh receptor
4. Depolarization of the sarcolemma after ACh receptor binding
5. Conduction of the action potential into the fiber by the T-system
6. Signaling of the SR by the T-system to release calcium
7. Binding of calcium to the regulatory protein troponin, permitting actin-myosin interaction
8. Force generation resulting from actin-myosin interaction
9. Pumping of calcium back into the SR when neural activation ceases, resulting in inhibition of actomyosin interaction and muscle relaxation

TEMPORAL SUMMATION

A well-known muscle contractile property follows directly from an understanding of the EC coupling sequence presented above. First, it should be obvious that the time required for activation, contraction, and then relaxation to occur is finite. That is, excitation (with accompanying calcium release) is relatively rapid (on the order of about 5 msec) while contraction and relaxation are relatively slow (on the order of about 100 msec). The mechanical consequence of the activation process (*i.e.,* the muscle twitch) lags far behind the activation process itself. For example, let us suppose that the entire EC coupling process requires 100 msec. If, after the first impulse, we deliver a second impulse before 100 msec have elapsed, the muscle will be signaled to contract before it has fully relaxed. In other words, the second impulse will be superimposed somewhat on part of the cycle initiated by the first one, resulting in summation. Because the two events have summated due to their relative temporal relationship, this process is referred to as temporal summation.

The physiologic effects of temporal summation are quite dramatic. One effect of the first stimulus is to cause the contracting sarcomeres to "stretch out" the passive structures that lie in series with them (*e.g.,* tendons or passive sarcomeres). When the second impulse "arrives at the scene," it is not required to stretch out any of these structures and causes a greater force to be generated at the ends of the muscle fiber. Thus two impulses that are delivered to a muscle fiber and separated by only about 50 msec result in more force than the same two pulses delivered to the muscle but separated by more time. If a "train" of such pulses (say, 50 pulses in a row) is delivered to the muscle, separated in time by different amounts, this results in a tetanic contraction, and the resulting force is quite different (Figure 2.2). Higher forces result when stimuli are delivered at higher frequencies since there is less time for relaxation (frequency = 1/interpulse interval; low intervals correspond to high frequencies). Notice, in Figure 2.2, that at relatively low frequencies (*e.g.,* 10 Hz), the contractile record almost completely relaxes between successive pulses. This is referred to as an *unfused contraction,* because it is still possible to distinguish individual contractile events within the force record. However, note that as stimulation frequency increases, the tetanic record becomes more fused, until at very high frequencies (*e.g.,* 100 Hz), the contractile record becomes a *fused contraction.* A fused tetanic contraction appears as such because the repeated calcium release onto the myofilaments is much faster than the rate at which the myofilaments can relax.

Rate Coding: The Physiologic Significance of Temporal Summation

The variation in force obtained by altering activation frequency is known as frequency or rate coding. Because muscle force varies as a function of activation frequency, this is one method the CNS can use to alter muscle force. If high forces are required at the periphery, the CNS can deliver high-frequency pulses. Conversely, if only low forces are required, the CNS can deliver low-frequency

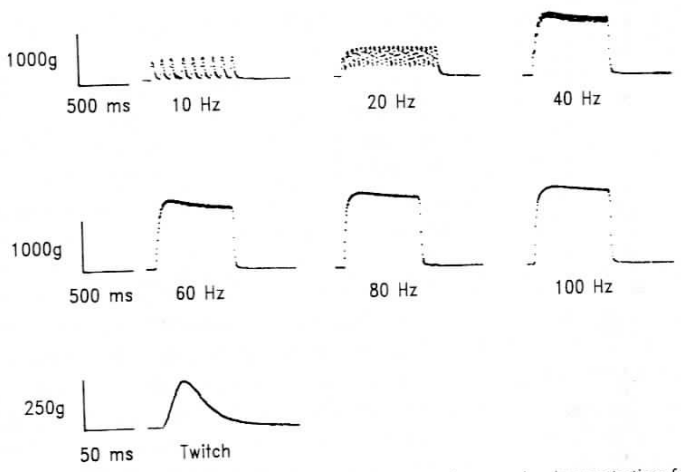

Figure 2.2. Contractile records from a rabbit tibialis anterior muscle demonstrating fusion of mechanical twitches as stimulation frequency increases (temporal summation). Note different tension calibration bars for tetani (upper panels) and twitch (lower panel). (From Lieber RL, Smith DE, Hargens AR. Real-time acquisition and data analysis of skeletal muscle contraction in a multi-user environment. Comp Prog Methods Biomed 1986;22:259–265.)

pulses. Of course, this type of effect is very difficult to demonstrate directly. In a very technical procedure involving single motor unit recording, ventral root recording, and muscle tension recording *in situ* during locomotion (as well as some fancy postexperimental data processing), Andy Hoffer and colleagues were able to demonstrate what they interpreted to be rate coding, which occurred during normal locomotion (Hoffer *et al.,* 1981 and 1987).

We shall see, however, that the control of muscle force by the CNS is very much more sophisticated than this. The area of study that includes the control of muscles by the nervous system is known as neuromotor control (Binder and Mendell, 1990), which will be discussed in the chapters to follow. Suffice it to say for the present that muscle and nerve properties are matched in a very sophisticated fashion in order to accomplish a particular task. Rate coding is only one of the methods by which this match is accomplished.

PART 2: SKELETAL MUSCLE MECHANICS

LENGTH-TENSION RELATIONSHIP: ISOMETRIC MUSCLE CONTRACTION

Since the late 1800s, it has been known that the force developed by a muscle during isometric contraction (*i.e.,* when the muscle is not allowed to shorten)

varies with its starting length (see review by Podolsky and Shoenberg, 1983). The isometric length-tension curve is generated by maximally stimulating a skeletal muscle at a variety of discrete lengths and measuring the tension generated at each length. When maximum tetanic tension at each length is plotted against length, a relationship such as that shown in Figure 2.3 is usually obtained. While a general description of this relationship was established early in the history of biologic science, the precise structural basis for the length-tension relationship in skeletal muscle was not elucidated until the sophisticated mechanical experiments of the early 1960s were performed. It was these experiments that defined the precise relationship between myofilament overlap and tension generation, which we refer to today as the length-tension relationship. In its most basic form, the length-tension relationship states that tension generation in skeletal muscle is a direct function of the magnitude of overlap between the actin and myosin filaments.

Sarcomere Length-Tension Relationship

In the late 1950s and early 1960s, Andrew Huxley, Albert Gordon, and Fred Julian, working in England (Gordon *et al.,* 1966), and Paul Edman, working in

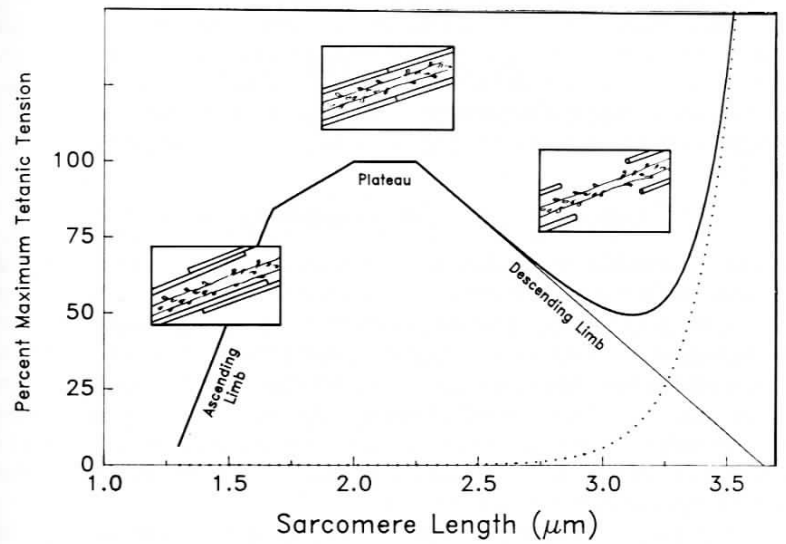

Figure 2.3. The sarcomere length-tension curve for frog skeletal muscle obtained using sequential isometric contractions in single muscle fibers. Insets show schematic arrangement of myofilaments in different regions of the length-tension curve. Dotted line represents passive muscle tension.

Sweden (Edman, 1966), defined what might be one of the most explicit structure-function relationships in all of biology. It was obvious to these investigators that in order to determine the detailed structural basis for the length-tension relationship, isolated, intact single skeletal muscle fibers would be required. The muscle used was that of the frog since a great deal was known at the time about its structure, and intact single fibers could be isolated. Picture this: The experiments I am about to describe were performed on tissue (single muscle cells) approximately 8 mm long and 75 μm in diameter! That's small!

Andrew Huxley (who by this time had already received the Nobel prize with Alan Hodgkin for determining the mechanism of the nerve action potential) invented a mechanical version of his voltage clamp apparatus that was used in the nerve studies. This apparatus was designed to keep a small segment of the fiber at a constant length (and therefore keep a region of the fiber at a constant sarcomere length). This enabled him to make a unique correlation between muscle tension and sarcomere length. It was much easier said than done because it turned out that 'isometric" force generation in the single fiber was anything but isometric! Sarcomeres in the end region of the fiber tended to stretch sarcomeres in the central region, and thus the apparatus Huxley developed was absolutely critical.

The results of the classic experiments by Gordon, Huxley, and Julian (1966) are summarized in Figure 2.3. In this figure, muscle relative tetanic tension (as a percentage of maximum) is plotted as a function of sarcomere length (in μm). This was one case where anatomy met physiology in dramatic fashion, because knowledge of the precise anatomic lengths of the myosin and actin filaments was crucial for understanding the basis of this relationship.

Descending Limb of the Length-Tension Curve

As a muscle was highly stretched by the investigators to a sarcomere length of 3.65 μm, the muscle developed no active force. Why did the muscle develop zero force at this length? The answer lay in the observation that, since the myosin filament is 1.65 μm long and the actin filament is 2.0 μm in length, at a sarcomere length of 3.65 μm, there is no overlap (interdigitation) between the actin and myosin filaments. Therefore, although the EC coupling process might *permit* actin-myosin interaction by removing the inhibition on the actin filament, because no myosin cross-bridges are in the vicinity of the actin active sites, no force generation can occur.

As the muscle was allowed to shorten, overlap between actin and myosin was possible, and the amount of force generated by the muscle increased as sarcomere length decreased. Increasing force with decreasing sarcomere length occurred until the muscle reached a sarcomere length of 2.2 μm. Why did tension slowly increase? Over the range of sarcomere lengths from 2.2–3.65 μm, as sarcomere length decreases, the number of cross-bridges between actin and myosin increases, resulting in increased force. This region of the length-tension curve is known as the descending limb.

Plateau Region of the Length-Tension Curve

As sarcomere length changed from 2.0 μm to 2.2 μm, muscle force remained constant. Again, this was a direct result of thick filament structure. Recall from Chapter 1 that the myosin filament is a polymeric arrangement of myosin molecules arranged in an antiparallel fashion. Because many myosin "backbones" (the light meromyosin portion of the myosin molecules) come together in the center of the myosin filament, there exists a bare region of the myosin molecule that is devoid of cross-bridges. You guessed it—the length of the bare region was 0.2 μm! Thus while sarcomere length shortening over the range 2.2–2.0 μm results in greater filament overlap, it does not result in increased force generation since no additional cross-bridge connections are made. The region of the length-tension curve over which length change results in no change in force is known as the plateau region. The maximum tetanic tension of the muscle in this region is abbreviated P_O. The length at which P_O is attained is known as optimal length (L_O).

Ascending Limb of the Length-Tension Curve

At a sarcomere length of 2.0 μm, notice that the actin filaments from one side of the sarcomere juxtapose the actin filaments from the opposite side of the sarcomere (Figure 2.3). It might be predicted that shortening past this point would be impossible. However, as sarcomere length decreases below the plateau region, actin filaments from one side of the sarcomere double overlap with the actin filaments on the opposite side of the sarcomere. That is, at these lengths, actin filaments overlap both with themselves and with the myosin filament. Under these double-overlap conditions, the actin filament from one side of the sarcomere interferes with cross-bridge formation on the other side of the sarcomere, and this results in decreased muscle force output. This occurs from 2.0–1.87 μm, and this region is known as the shallow ascending limb of the length-tension curve. The word "shallow" distinguishes it from the next portion of the length-tension curve, which is known as the steep ascending limb, because at these very short lengths, the myosin filament actually begins to interfere with shortening as it abuts the sarcomere Z-disk, reducing force precipitously.

An interesting observation relative to muscle force generation at short lengths was made in the late sixties by Rüdel and Taylor (1971). They

observed that when an intact muscle fiber was stimulated at very short sarcomere lengths (*i.e.,* sarcomere lengths on the ascending limb), electrical failure of the EC coupling apparatus occurred. This raised the question as to whether the decreased force at short sarcomere lengths was actually due to myofilament properties or was simply an electrical failure phenomenon. To address this question, Taylor and Rüdel (1970) ensured maximal single fiber activation by bathing the fiber in caffeine (which enhances calcium release from the SR) and obtained the same relationship as Gordon, Huxley, and Julian had obtained. Rick Moss repeated the experiment on small *pieces* of single muscle fibers, which were activated chemically using a calcium buffering system (Moss, 1979). Again, the same relationship was obtained. Thus while shortening deactivation as described by Rüdel and Taylor could occur, it did not seem to detract from the elegance and truth of the sarcomere length-tension relationship itself.

To summarize, the length-tension relationship states that muscle force varies as a function of sarcomere length (myofilament overlap). This is a physiologic property of the force-generating system and should not simply be viewed as an anatomic artifact. Recent experimental studies suggest that this length-tension relationship can be advantageous to the musculoskeletal torque-generating system, as will be described in Chapter 3.

Origin of the Passive Portion Length-Tension Curve

The solid line in Figure 2.3 represents the tension generated if a muscle is stretched to various lengths without stimulation. Note that near the optimal length, passive tension is almost zero. However, as the muscle is stretched to longer lengths, passive tension increases dramatically. These relatively long lengths can be attained physiologically, and therefore, passive tension can play a role in providing resistive force even in the absence of muscle activation. What is the origin of passive tension? Obviously, the structure(s) responsible for passive tension are outside of the cross-bridge itself since muscle activation is not required. Recent studies performed by Alan Magid have shown that the origin of passive muscle tension is actually *within* the myofibrils themselves. He demonstrated this by chemically stripping the sarcolemma from a single muscle fiber and measuring passive tension (Magid and Law, 1985). Interestingly, a new structural protein has also been identified, which may be the source of this passive tension. The very large protein, creatively named "titin," connects the thick myosin filaments end to end. This very large protein is also relatively fragile and thus has probably been missed in earlier studies because the laboratory techniques destroyed the protein. In addition to passively supporting the sarcomere, titin stabilizes the myosin lattice so that high muscle forces do not disrupt the orderly hexagonal array. If titin is selectively destroyed, normal muscle contraction causes significant myofibrillar disruption (Horowits and Podolsky, 1987).

Before leaving the length-tension relationship, let me present one caution: Never try to describe a shortening muscle using the length-tension relationship. In other words, looking at Figure 2.3, one might be tempted to predict that as a muscle shortens from a long length, force increases. However, one must remember that the length-tension relationship is strictly valid only for *isometric* contractions. Thus the curve represents the artificial connection of individual data points from isometric experiments. In order to describe *motion,* we will require an understanding of the force-velocity relationship, presented below.

FORCE-VELOCITY RELATIONSHIP: ISOTONIC MUSCLE CONTRACTION

Unlike the length-tension relationship, the force-velocity relationship does not have a precise, anatomically identifiable basis. The force-velocity relationship states that the force generated by a muscle is a function of its velocity. It can also be stated in the reverse, such that the velocity of muscle contraction is dependent on the force resisting the muscle. Historically, the force-velocity relationship was used to define the kinetic properties of the cross-bridges as well as the precise force-velocity relationship itself.

Experimental elucidation of the force-velocity relationship was first presented by A. V. Hill and Bernard Katz in their classic papers (Hill, 1938; Katz, 1939), but the current description of the force-velocity relationship has been ascribed to the physiologist A. V. Hill (see summary in Hill, 1970). Hill, in his decades of important muscle studies, generated an equation for the muscle force-velocity relationship that is still in use today. Interestingly, Andrew Huxley, in 1957, developed a theory of isotonic muscle contraction based on specific cross-bridge properties, which yielded the actual force-velocity relationship and explained the amount of energy used by a muscle during contraction at different velocities (Huxley, 1957; Hill, 1964). The beauty of this theory was its ability to explain both mechanical and energetic data.

Experimentally, the force-velocity relationship, like the length-tension relationship, is a curve that actually represents the results of many experiments plotted on the same graph. Experimentally, a muscle is stimulated maximally and allowed to shorten (or lengthen) against a constant load. The muscle velocity during shortening (or lengthening) is measured and then plotted against the resistive force. The general form of this relationship is plotted in Figure 2.4. On the horizontal axis we have plotted muscle velocity relative to maximum velocity (V_{max}) while on the vertical axis we have plotted muscle force relative to maximum force (P_O).

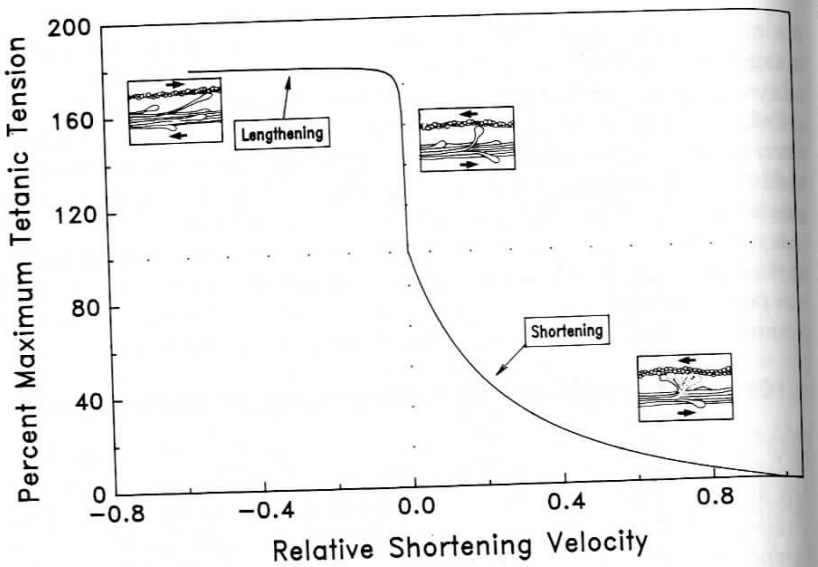

Figure 2.4. The muscle force-velocity curve for skeletal muscle obtained using sequential isotonic contractions in single fibers. *Insets* show schematic representation of cross-bridges. Note that force increases dramatically upon forced muscle lengthening.

Concentric Contractions—Muscle Actively Shortening

When a muscle is activated and required to lift a load that is less than its maximum tetanic tension, the muscle begins to shorten. Contractions that permit the muscle to shorten are known as concentric contractions. In concentric contractions, the force generated by the muscle is always less than the muscle's maximum (P_O). As the load the muscle is required to lift decreases, contraction velocity increases. This occurs until the muscle finally reaches its maximum contraction velocity, V_{max}. V_{max} is a parameter we can use to characterize muscle, which is related to both fiber type distribution and architecture. The mathematical form of the force-velocity relationship is a rectangular hyperbola and is given in Equation 2.1:

$$(P+a)\,v = b\,(P_O - P) \qquad (2.1)$$

where a and b are constants derived experimentally (usually about 0.25), P is muscle force, P_O is maximum tetanic tension, and v is muscle velocity. This equation can be used to determine the relative muscle force that occurs as a muscle is allowed to shorten. Some of these values are presented below in Table 2.1. It is important to note that the force-velocity relationship is a steep

rectangular hyperbola. In other words, force drops off rapidly as velocity increases. For example, in a muscle that is shortening at only 1% of its maximum contraction velocity (extremely slow), tension drops by 5% relative to maximum isometric tension. Similarly, as contraction velocity increases to only 10% maximum (easily attainable physiologically), muscle force drops by 35%! Note that even when muscle force is only 50% maximum, muscle velocity is only 17% V_{max}. The take-home lesson is that as a muscle is allowed to shorten, force drops precipitously.

What is the physiologic basis of the force-velocity relationship? It has been determined that the cross-bridges between actin and myosin attach at a certain rate and detach at a certain rate (see below). These rates are referred to as *rate constants*. At any point in time, the force generated by a muscle depends on the number of cross-bridges attached. Because it takes a certain amount of time for the cross-bridges to attach (based on the rate constant of attachment), as filaments slide past one another faster and faster (*i.e.,* as the muscle shortens with increasing velocity), force decreases due to the lower number of cross-bridges attached. Conversely, as the relative filament velocity decreases (*i.e.,* as muscle velocity decreases), more cross-bridges have time to attach and to generate force, and thus force increases. This discussion is not meant to be a definitive description of the basis for the force-velocity relationship, only to provide some insight as to how cross-bridge rate constants can affect muscle force generation as a function of velocity.

Eccentric Contractions—Muscle Actively Lengthening

As the load on the muscle increases, it reaches a point where the external load is greater than the load which the muscle itself can generate. Thus the muscle is activated, but it is forced to lengthen due to the high external load.

Table 2.1.
Relative Muscle Force at Various Muscle Velocities

Relative Force	Velocity
100% P_o	0% V_{max}
95% P_o	1% V_{max}
90% P_o	2.2% V_{max}
75% P_o	6.3% V_{max}
50% P_o	16.6% V_{max}
25% P_o	37.5% V_{max}
10% P_o	64.3% V_{max}
5% P_o	79.1% V_{max}
0% P_o	100% V_{max}

This is referred to as an eccentric contraction (please remember that contraction in this context does not necessarily imply shortening!). There are two main features to note regarding eccentric contractions. First, the absolute tensions are very high relative to the muscle's maximum tetanic tension generating capacity. Second, the absolute tension is relatively independent of lengthening velocity. This suggests that skeletal muscles are very resistant to lengthening, a property which we shall see comes in very handy for many normal movement patterns (Chapter 3).

Eccentric contractions are currently under study for three main reasons: First, much of a muscle's normal activity occurs while it is actively lengthening, so that eccentric contractions are physiologically common. Second, muscle injury and soreness are selectively associated with eccentric contraction. Finally, muscle strengthening is greatest using exercises that involve eccentric contractions. These phenomena will be elaborated upon in Chapters 4 and 6.

LENGTH-TENSION-VELOCITY RELATIONSHIP

From the preceding discussion, it is apparent that muscle force changes due to changing length and/or due to changing velocity. It should not be surprising, therefore, to suggest that when muscle length *and* muscle velocity change simultaneously, it is still possible to define the muscle force produced. It should also not be surprising that while the length-tension and force-velocity relationships are useful, such isometric and isotonic conditions are almost never encountered in daily activities. However, the length-tension experiment can be viewed simply as a series of length-force-velocity experiments performed at constant (zero) velocity. Similarly, the force-velocity relationship can be viewed as a series of length-force-velocity experiments performed at constant length (L_O). The point shared between the classic force-velocity and length-tension curves is the point of maximum isometric tension (L_O, at zero velocity, resulting in a tension of P_O). If both length and velocity simultaneously change, the result is the superposition of the two relationships.

The appearance of the length-tension-velocity relationship is shown in Figure 2.5. Don't let the three-dimensional nature of the relationship intimidate you. If the surface is viewed along one set of axes, it is simply a series of force-velocity curves at different lengths. When viewed along the other set of axes, it is simply a series of length-tension curves at different velocities. In this surface we have all possible combinations of muscle length and velocity and their resulting force. What can we conclude? For one thing, if muscle velocity is very high, force will be low no matter what the length. In other words, at high velocities, length is not very important. At low concentric

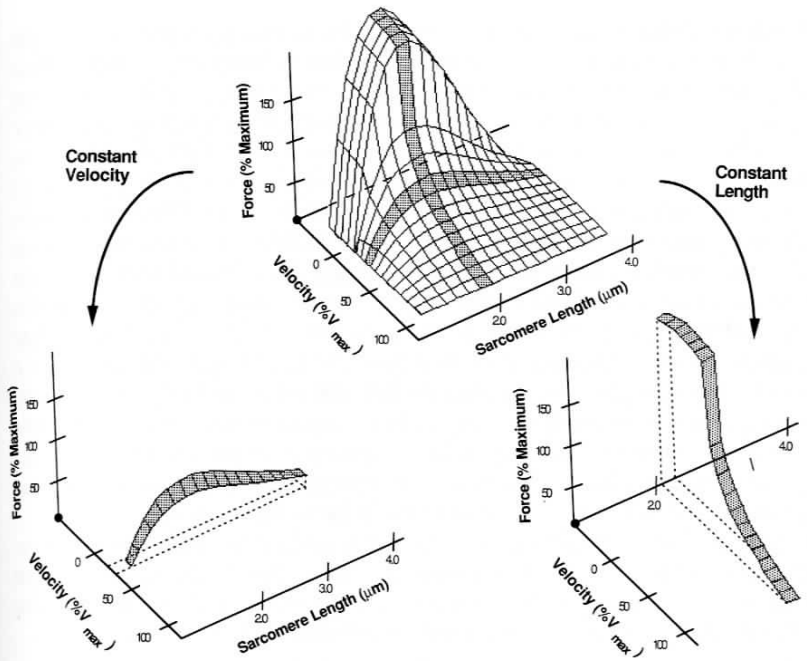

Figure 2.5. The hypothetical muscle length-force-velocity surface for skeletal muscle. Shaded regions represent a slice of the surface at either constant length or velocity. A slice of the surface at constant length is simply a force-velocity curve (compare with Figure 2.4). A slice of the surface at constant velocity is simply a length-tension curve (compare with Figure 2.3). (From Fridén J, Lieber RL. The structural and mechanical basis of exercise-induced muscle injury. Med Sci Sports Exerc, in press.

velocities, muscle length becomes an important force modulator. At eccentric velocities, again muscle velocity dominates length as the determinant of force. This relationship is of course important in neuromotor control as we attempt to understand how muscle actions can be responsible for external movements observed. We will have more to say on this topic in Chapters 3 and 4.

THE CROSS-BRIDGE CYCLE

We have alluded to cyclic interaction between actin and myosin in our structural discussion in Chapter 1 and in explaining the force-velocity curve above. How were such hypotheses generated? Much of our understanding of the mechanism of muscle contraction has come in large part from excellent biochemical studies performed from the 1950s to the mid-1970s (Webb and

Trentham, 1983). It was during this period that methods for isolating specific muscle proteins were developed as well as the methods for measuring their physicochemical and biochemical properties. For example, if a muscle was homogenized in a blender and mixed with a concentrated salt solution (ionic strength of about 600 μM, well-above that observed physiologically), the high-ionic-strength solution caused individual myosin molecules to let go of their ionic interactions with one another and become soluble. Then this soluble portion was removed from the rest of the muscle debris, the ionic strength was slowly lowered to physiologic levels (about 120 μM), and lo and behold, myosin and actin filaments reformed and, in fact, formed a solid precipitate complex (Szent-Györgyi, 1953). Simple addition of ATP rendered this precipitate perfectly clear (Maruyama and Gergely, 1962). Why? Obviously, ATP affected the relationship between actin and myosin. Biochemists believed that by performing experiments such as these, they could investigate different steps of the cyclic interaction between muscle proteins. These types of experiments are quite popular in recent muscle literature and have provided insights into muscle contraction as well as actin-myosin interaction in all eukaryotic cells.

In its simplest form, based on experiments such as those presented above, the cross-bridge cycle can be envisioned as actin (A) combining with myosin (M) and ATP to produce force, adenosine diphosphate (ADP), and inorganic phosphate, P_i. This can be represented as a chemical reaction in the form

$$A + M + ATP \rightarrow A + M + ADP + P_i + Force \qquad (2.2)$$

However, we also know that upon the death of a muscle, a rigor state is entered whereby actin and myosin interact to form a very stiff connection. This can be represented as

$$A + M \rightarrow A{\cdot}M \text{ "rigor" complex} \qquad (2.3)$$

If actin and myosin can interact by themselves, where does ATP come into the picture during contraction? As discussed in Chapter 1, the myosin molecule can be enzymatically split into its subfragments. Experiments that cleaved myosin into light meromyosin (LMM), subfragment 1 (S-1), and subfragment 2 (S-2) demonstrated that the myosin S-1 portion retained the ability to hydrolyze ATP into ADP and P_i. In other words,

$$M + ATP \rightarrow M + ADP + P_i \qquad (2.4)$$

It is now clear that ATP serves at least two functions in skeletal muscle systems: First, ATP disconnects actin from myosin, and second, ATP is hydrolyzed by the S-1 portion of the myosin molecule. Can you see the competition this sets up? In contracting skeletal muscle, ATP binds to the actin-myosin complex, causing actin and myosin to dissociate. When it does,

ATP is hydrolyzed by myosin into ADP and P_i, which then allows actin and myosin to reassociate! Thus Equation 2.2 (our simple cross-bridge cycle) can be combined with Equations 2.3 and 2.4 to yield the more detailed "two step" cross-bridge cycle shown below:

$$\begin{gathered} A + M \rightarrow A{\cdot}M \text{ "rigor" complex} \\ A{\cdot}M \text{ "rigor" complex} + ATP \rightarrow A + M + ADP + P_i + Force \end{gathered} \qquad (2.5)$$

This basic scheme has been expanded by many excellent works over the years, but the same basic idea remains: ATP is required to dissociate actin from myosin and is hydrolyzed by the S-1 portion of the myosin head. Several interesting experiments refined this concept to provide actual rate constants for the various reactions. For example, we now know that when myosin is alone in solution, it hydrolyzes ATP *very* slowly. Thus Equation 2.4 occurs only at a rate of about 0.1/second. However, an interesting observation is that when actin is added to a solution of S-1 and ATP, the previously slow hydrolysis rate increases about 200-fold! Thus actin acts as a catalyst for ATP hydrolysis by S-1. We can modify our ATP hydrolysis mechanism to include two paths for hydrolysis—a path with and a path without actin. The path without actin is shown is Equation 2.7 and the path with actin is shown is Equation 2.6:

$$A{\cdot}M + ATP \rightarrow A{\cdot}M{\cdot}ATP \rightarrow A + M + ADP + P_i \qquad \text{(fast) (2.6)}$$

$$M + ATP \rightarrow M{\cdot}ATP \rightarrow M + ADP + P_i \qquad \text{(slow) (2.7)}$$

It is also easy to interconnect these two schemes by adding a step whereby actin can dissociate from the A·M·ATP complex, and the scheme thus becomes

$$\begin{gathered} A{\cdot}M + ATP \rightarrow A{\cdot}M{\cdot}ATP \rightarrow A + M + ADP + P_i \qquad \text{(fast)} \\ \text{ACTIN DISSOCIATES } \downarrow\uparrow \text{ ACTIN ASSOCIATES} \qquad (2.8) \\ M + ATP \rightarrow M{\cdot}ATP \rightarrow M + ADP + P_i \qquad \text{(slow)} \end{gathered}$$

or, more simply,

$$\begin{gathered} A{\cdot}M + ATP \rightarrow A{\cdot}M{\cdot}ATP \rightarrow A + M + ADP + P_i \\ -A\downarrow\uparrow+A \qquad (2.9) \\ M + ATP \rightarrow M{\cdot}ATP \rightarrow M + ADP + P_i \end{gathered}$$

You can see that we have generated a reasonable approximation of cyclic interaction between actin, myosin, and ATP! The reactions with the fastest rate constants are shown in Equation 2.9 in boldface; they are the most likely to occur. This very simplified scheme actually explains a great deal of the experimental data and, conceptually, will allow you to understand many physiologic properties to be presented later in this chapter.

For those of you who are interested, there is an interesting caveat to the scheme of Equation 2.9. Experimental studies by Richard Lymn and Ed Taylor (1971) demonstrated that, after formation of the A·M·ATP complex, the actin quickly dissociates, so that ATP hydrolysis actually occurs with the actin and myosin filaments separated! After the hydrolysis, the actin quickly reassociates with the M·ADP·P_i complex to cause dissociation of the hydrolysis products. The rate-limiting step of the entire sequence is the release of the reaction products from myosin, so that actin increases the ATP hydrolysis rate by speeding the release of hydrolysis products from myosin. The ATP hydrolysis scheme is therefore more accurately represented as

$$\begin{array}{ccc} \mathrm{A{\cdot}M + ATP} \rightarrow \mathrm{A{\cdot}M{\cdot}ATP} & & \mathrm{A{\cdot}M{\cdot}ADP{\cdot}P_i} \rightarrow \mathrm{A + M + ADP + P_i} \\ \quad -\mathrm{A} \downarrow & & \uparrow +\mathrm{A} \end{array} \qquad (2.10)$$

$$\mathrm{M + ATP \rightarrow M{\cdot}ATP} \rightarrow \mathrm{M{\cdot}ADP{\cdot}P_i \rightarrow M + ADP + P_i}$$

with the normal route of hydrolysis (that is, the fastest rate constants) shown in boldface. This sequence of biochemical steps is referred to as the Lymn-Taylor actomyosin ATPase hydrolysis mechanism (Lymn and Taylor, 1971).

The relationship between the Lymn-Taylor kinetic scheme and the mechanical cross-bridge cycle is not fully known. However, Lymn and Taylor proposed that their biochemical data could be incorporated into a four-step cross-bridge cycle that could be envisioned thus (Figure 2.6):

1. The actin-myosin bridge very rapidly dissociates due to ATP binding.
2. The free myosin bridge moves into position to attach to actin, during which ATP is hydrolyzed.
3. The free myosin bridge along with its hydrolysis products rebinds to the actin filament.
4. The cross-bridge generates force, and actin displaces the reaction products (ADP and P_i) from the myosin cross-bridge. This is the rate-limiting step of contraction. The actin-myosin cross-bridge is now ready for the ATP binding of step 1.

It might be appreciated that confirmation of this mechanism would be very difficult indeed. In fact, a recent advance in biochemistry has allowed direct testing and manipulation of this scheme. The advance involves the development of "caged" compounds—compounds which are inactive in their caged form and become active when the cage is instantaneously removed by a pulse of high-energy laser light (McCray *et al.*, 1980). Using caged ATP, single muscle fibers have been subjected to experiments such as those described above and found to behave much as predicted based on the biochemical data (Goldman, 1987). These experiments, performed by Yale Goldman and his colleagues, are truly a case where biochemistry and physiology have met head-on.

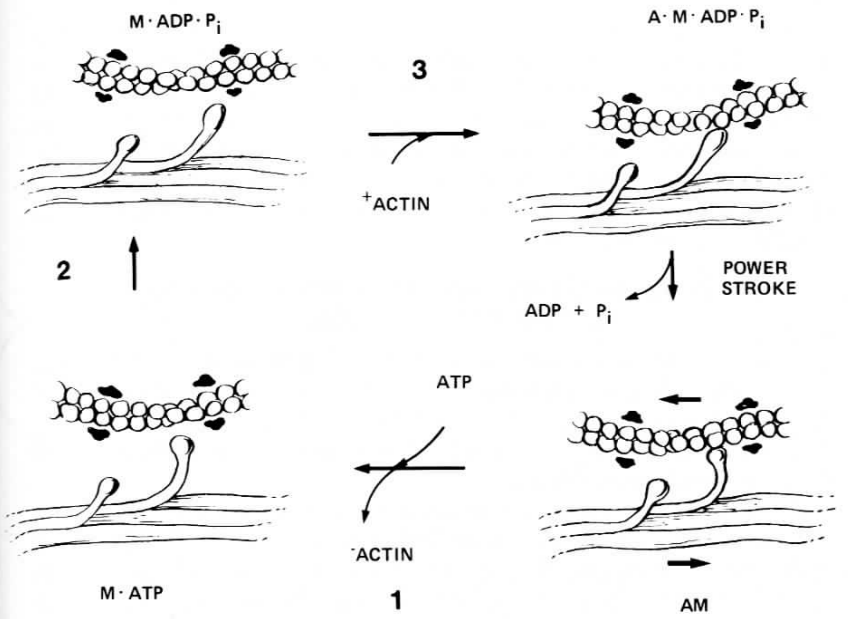

Figure 2.6. Schematic representation of the biochemical cross-bridge cycle along with the associated mechanical events. **1,** ATP binds to actin-myosin (AM) complex, displacing actin. **2,** ATP hydrolyzed on M·ATP complex. **3,** Actin reassociates with M·ADP·P_i complex. **4,** Attached cross-bridge generates force during power stroke. (After Lymn and Taylor, 1971.)

MECHANICAL PROPERTIES OF MUSCLES WITH DIFFERENT ARCHITECTURES

Congratulations! You have completed a complex discussion of two of the most important mechanical properties of skeletal muscle, as well as of the cross-bridge cycle. We are now in a position to further discuss the physiologic significance of muscle architecture, which was introduced in Chapter 1. We will see that even though we might understand the details of sarcomere structure and cross-bridge action, it is absolutely impossible to explain the force-generating property of a whole muscle without an understanding of muscle architecture. The important point to remember regarding muscle architecture is that *muscle force is proportional to physiologic cross-sectional area (PCSA), and muscle velocity is proportional to muscle fiber length.* We should note that by stating that velocity is proportional to fiber length, it is implicit that the total excursion (active range) of a muscle is also proportional to fiber length. Thus increasing fiber length results in both increased muscle

velocity and excursion. It is probably apparent to you, based on the discussion in Chapter 1, that neither fiber length nor PCSA can easily be deduced based on gross muscle inspection. The detailed methods of Chapter 1 are required for architectural determination. However, after you have determined these architectural properties, you are in a position to understand how much force a muscle generates and how fast it contracts (or how far it contracts). Let's look at two specific architectural examples and their impact on the length-tension and force-velocity relationships.

Comparison of Two Muscles with Different Physiologic Cross-Sectional Areas

Suppose that two muscles had identical fiber lengths and pennation angles, but one muscle had twice the mass (equivalent to saying that one muscle had twice the number of fibers and thus twice the PCSA). What would be the difference in their mechanical properties? How would the length-tension and force-velocity curves be affected?

The schematic in Figure 2.7 demonstrates that the only effect is to increase maximum tetanic tension so that the length-tension curve has the same basic shape but is simply amplified upward in the case of the stronger muscle. Similarly, the force-velocity curve simply changes the location of P_O, but the curve retains the same basic shape. Note that if both curves are plotted on *relative* scales (*i.e.,* percent maximum tension instead of absolute tension), the two muscles of different architecture appear to have identical properties. This demonstrates that while architectural properties profoundly affect the extrinsic muscle properties (*i.e.,* the properties that vary with absolute muscle size, such as PCSA or mass), they have no affect on its intrinsic properties (*i.e.,* the properties that are independent of absolute muscle size, such as fiber length/muscle length ratio).

Comparison of Two Muscles with Different Fiber Lengths

Let us consider the effects of architecture using an example of two muscles with identical PCSAs and pennation angles but different fiber lengths. Before reading ahead, try to draw the appropriate length-tension and force-velocity curves.

As shown in Figure 2.7, the effect is to increase the muscle velocity (or, stated identically, to increase the muscle excursion). The peak absolute force of the length-tension curves is identical, but the absolute muscle active range is different. Did I say active range? That sounds a lot like active range of motion (ROM), a measurement that is extremely important in clinical evaluation. In fact, it is directly related to ROM. I will have more to say on this in Chapter 3. Suffice it to say that ROM is a direct result of muscle architecture and the joint properties on which the muscle acts.

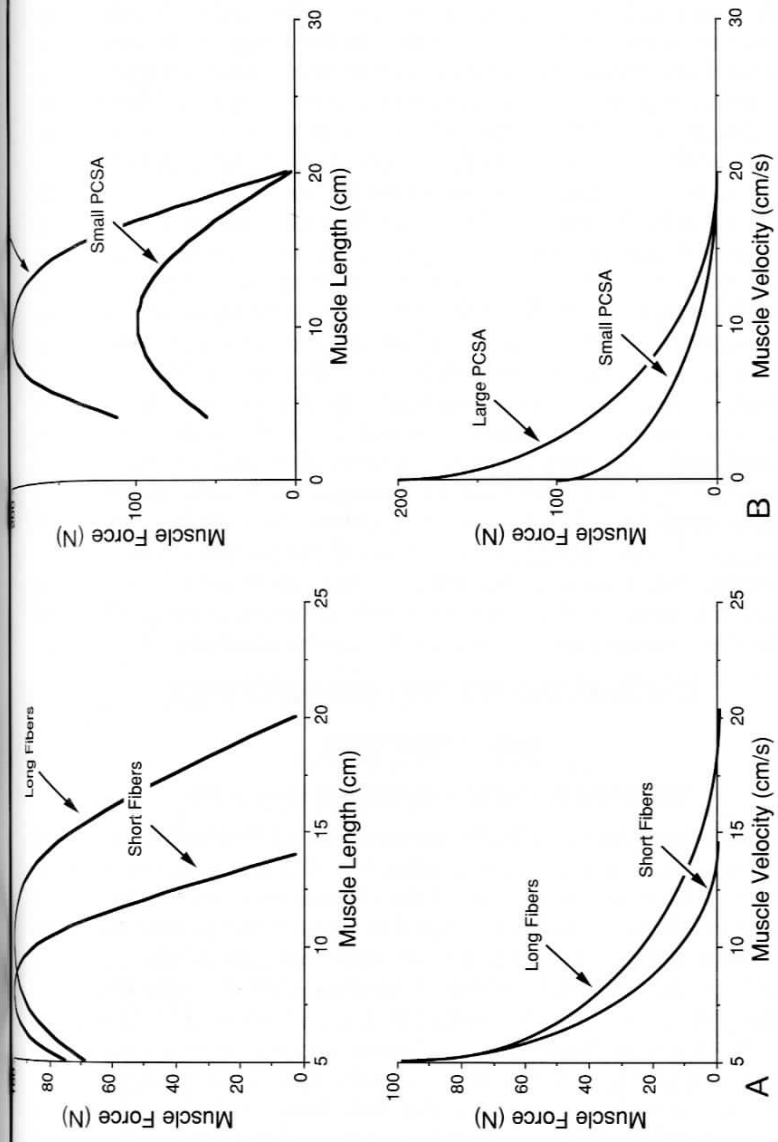

Figure 2.7. Schematic length-tension and force-velocity curves for muscles with different architectural properties. **A,** Length-tension and force-velocity curves for muscles with different fiber lengths but identical cross-sectional areas. **B,** Length-tension and force-velocity curves for muscles with identical fiber lengths but different cross-sectional areas.

For the same reason that fiber length increases the active muscle range of the length-tension relationship, it causes an increase in the muscle's absolute maximum contraction velocity (V_{max}). Again, while the fiber length increase causes an increase in these absolute properties, it has no effect on the intrinsic properties of the muscle. A similar exercise can be performed comparing muscles with different PCSAs and fiber lengths. Try predicting force-velocity and length-tension curves for the case where both architectural parameters are changed.

We have just seen vivid examples of the profound influence of muscle architecture on functional properties. You may want to return to the original discussion of architecture given in Chapter 1 and peruse Tables 1.3 and 1.4 in order to become more familiar with the architecture of several common muscles. Note that muscles with relatively long fibers (hamstrings, extensor carpi radialis longus, tibialis anterior) are muscles with high contraction velocities and large excursions. Conversely, muscles with large PCSAs (quadriceps, flexor carpi ulnaris) generate very large tensions. We now see that the architectural specialization observed in the numerous muscles has profound functional consequences. Muscles are able to perform a large range of tasks largely as a result of their intrinsic design rather than a specific set of command signals from the CNS. This design allows the CNS to act more as a coordinator of tasks rather than a definer of the particulars of the task. Interestingly, this is the same trend that is occurring in the microcomputer world regarding the tasks of computer central processing units (CPUs) and peripheral devices such as printers and video display terminals.