bacterial cell wall, and two inner rings (S and M), associated with the cell membrane. A shaft runs through these rings and connects to the extracellular hook. The basal body is probably the "motor" that rotates the flagellum. The exact mechanism for flagellar rotation is not clear, but the energy is derived from an electrochemical proton gradient across the bacterial cell membrane, not from ATP hydrolysis. Proton flux through a protein pore may cause a mechanical movement that is transduced into rotation of the basal body rings or the shaft passing through the rings.

Microfilament Movement

The general principle of microfilament movement involves a sliding of adjacent filaments or microfilament and membrane structures, similar to that postulated for microtubule-based movement. A major difference between microfilament and microtubule movement is that microfilaments can only shorten; they cannot push (extend) in the manner that microtubules can.

There are many examples of cell motility due to microfilament (actin-myosin) interaction, but the best understood is the contraction of striated muscle cells because they have a highly-regular organization of microfilaments. Amoeboid cell motility, axonal movement of materials, and cell cleavage involve actin and myosin, but their mechanism is less clear. Actin and myosin have also been implicated in other aspects of cell mobility, such as the mitotic spindle, but their structural and functional roles are speculative at present.

Amoeboid Movement

Many protozoans (e.g., *Amoeba, Chaos*) and isolated cells of multicellular animals (e.g., fibroblasts, leukocytes, epithelial cells) are highly motile. Some examples of **amoeboid movement** involve obvious cytoplasmic streaming into finger-like extensions of the cell, called pseudopods or delicate cytoplasmic lamellae on the leading edge, although some small amoebas glide slowly over the substrate without cytoplasmic streaming (*Hyalodiscus*). Phagocytic leukocytes (white blood cells) have a clear ring of peripheral cytoplasm (it lacks subcellular organelles and membranes) (Figure 9–19). This clear zone contains all of the necessary structures for cell movement. It can detach itself from the rest of the cell if the leukocyte is heated to above its normal temperature and "walk away," leaving the rest of the cell rendered immobile!

Numerous models have been proposed to explain amoeboid motility but a likely general explanation is offered by an early theory that fluid cytoplasm (a "sol") has an inherent ability to stiffen (form a "gel"). "Gelled" cytoplasm is very rigid, able to withstand up to 1 kg cm^{-2} compression. Recent studies have suggested a mechanism for sol-gel transition that involves actin, myosin, and other related microfilament proteins that are present in amoeboid cells. The actin filaments form a rigid cytoskeleton.

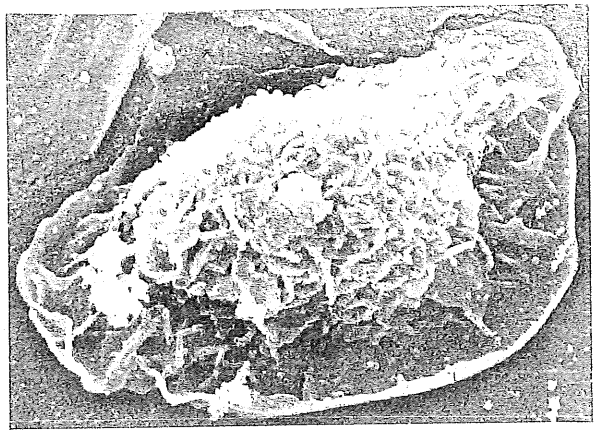
One model of epidermal cell movement (Bereiter-Hahn and Strohmeier 1986) combines features of a number of previous models for cell movement. A positive hydrostatic pressure is generated in the cell by contraction of fibrillar acto-myosin meshworks near the dorsal plasmalemma anterior to the nucleus and forms cytoplasmic extensions at weakenings of the plasmalemma. The edge of the lamella is a structurally weak point because it has a high radius of curvature and so "sol" cytoplasm is pushed into the extending leading edge of the lamella, and the meshwork is "gelled" by an influx of Ca^{2+} ions. A sliding movement of acto-myosin may provide the motile mechanism, but myosin does not seem to be required for movement in many cells.

Another model of movement by phagocytic leukocytes does not require a myosin-actin interaction (Stossel 1990). Solation of the actin cytoskeleton is stimulated at the cell surface, for example by the binding of an antibody to a receptor, and this elicits formation from phosphatidylinositol of a mobile C_6 messenger that liberates Ca^{2+} from intracellular storage vesicles. The Ca^{2+} ions activate gelsolin, which dismembers the actin cytoskeletal network (Figure 9–19Bi). The elevated local concentration of actin molecules causes an osmotic influx of water, which extends the cell membrane (Figure 9–19Bii). Finally, the cellular actin cytoskeleton is re-formed in the extended region. Actin/gelsolin molecules bind to polyphosphoinositols in the cell membrane, and this breaks up the actin-gelsolin complex, promoting resynthesis of the actin cytoskeletal network (Figure 9–19Biii). A similarly dynamic actin filament meshwork may be responsible for the rapid movement of amoeboid cells (Theriot and Mitchison, 1991).

start

Muscle

Muscle cells are specialized to actively shorten (contract). They are only able to actively shorten, and they elongate as a passive response to an external force provided, for example, by an antagonistic muscle or a hydrostatic force.



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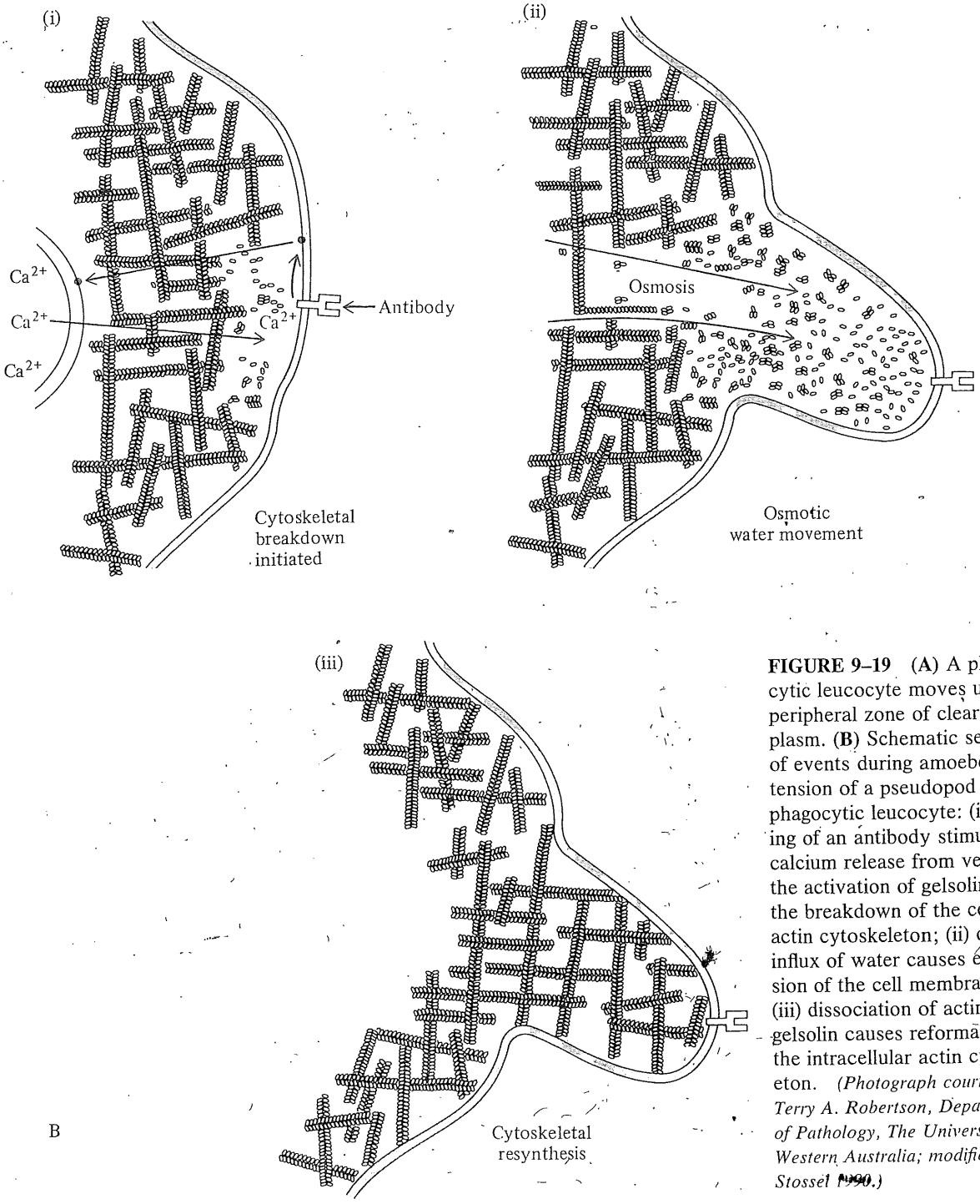

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FIGURE 9–19 **(A)** A phagocytic leucocyte moves using a peripheral zone of clear cytoplasm. **(B)** Schematic sequence of events during amoeboid extension of a pseudopod by a phagocytic leucocyte: (i) binding of an antibody stimulates calcium release from vesicles, the activation of gelsolin, and the breakdown of the cellular actin cytoskeleton; (ii) osmotic influx of water causes extension of the cell membrane; (iii) dissociation of actin-gelsolin causes reformation of the intracellular actin cytoskeleton. *(Photograph courtesy of Terry A. Robertson, Department of Pathology, The University of Western Australia; modified from Stossel 1990.)*

TABLE 9–2

Summary of major types of muscle
Skeletal muscle (striated)
Tonic
Phasic
Slow (slow contracting, slow fatiguing)
Fast: glycolytic (fast contracting, fast fatiguing); oxidative (fast contracting, fatigue resistant)
Asynchronous insect flight muscle
Cardiac muscle (striated)
Smooth muscle
Vertebrate
Unitary (visceral)
Multiunit (ciliary body, iris, pilomotor)
Invertebrate
Classical smooth muscle
Helical
Oblique
Paramyosin catch muscle

There are a variety of types of muscle cells (Table 9–2). Muscle cells can be divided into two general types based on their microscopical appearance: **striated muscle** and **smooth muscle**. Striated muscle can be further divided into skeletal and cardiac muscle. This categorization is based not on differences in basic contractile mechanism but on the degree of orderly arrangement of the actin and myosin contractile proteins. These microfilaments are highly ordered in striated muscle, and this confers a striking pattern of alternate dark and light striations. There are many other differences in the structure and physiology of skeletal, cardiac, and smooth muscle, including the nature of their innervative, the form of their action potentials, and their relation to the generation of a contractile force. The two types of vertebrate striated muscle, skeletal and cardiac muscle, differ more in their innervation pattern and mechanical and electrical properties than in basic ultrastructure.

Skeletal muscle is usually under voluntary control. Individual skeletal muscle cells, or fibers, are large (10 to 100 dia), long, multinucleate cells grouped into bundles (fascicles) that are visible to the naked eye. Individual fibers generally do not run the entire length of the muscle, although they may in some muscles that do not taper at the ends (e.g., frog sartorius muscle). Skeletal muscle can be further classified into types by innervation pattern, structure, and function. The skeletal muscle of vertebrates is generally organized into groups, termed a **motor unit** (Figure 9–20). Each muscle cell in a motor unit receives one or a few synaptic connections from a single motoneuron, whose soma is located in the spinal cord. An action potential is propagated over the surface of each muscle cell in response to an axonal action potential, and an "all-or-none" twitch is elicited.

Cardiac muscle cells differ somewhat in morphology from skeletal muscle cells (Figure 9–20). They are smaller, bifurcating cells that are uni- or binucleate. These cells are joined end to end with other cardiac muscle cells by specialized intercalated disks to form a 3-dimensional network. Cardiac muscle is under involuntary control. The cardiac action potential has an elongate plateau and the muscle contraction is of similar, long duration. Some cardiac muscle cells are specialized to conduct electrical depolarization, rather than to contract. Purkinje fibers and AV (atrioventricular) node fibers are both morphologically and physiologically specialized for conduction. Cardiac muscle has a longer contractile duration than skeletal muscle. Most cardiac muscle cells do not have any innervation but have an inherent rhythmicity of contraction. Each cardiac cell is electrically connected to adjacent cells, and they form an electrical syncytium so the entire cardiac muscle contracts as a unit. Some cardiac muscle cells are specialized as pacemaker cells; their inherent rhythmicity of electrical depolarization is normally faster than that of the other cardiac muscle cells, and so their rhythm determines the rate of contraction of the entire cardiac muscle system.

Nearly all invertebrates have striated muscle, although some have mainly nonstriated muscle (e.g., mollusks, annelids). The vertebrate pattern of striated skeletal muscle being voluntary and striated cardiac and smooth muscle being involuntary is not consistent among the invertebrates. For example, the alimentary canal muscles of arthropods are usually striated, and many locomotory muscles of annelids and cephalopods are smooth muscle.

Invertebrate striated muscle has relatively few, but large, fibers that are innervated by a small number of motoneurons. Muscle fibers are up to 1 to 2 mm diameter in barnacles and 4 mm in king crabs. An entire muscle may be innervated by as few as 2 motoneurons. The axons do not form discrete terminal synapses with end plates but release neurotransmitter at many contact points between the axon and muscle fibers. The arrangement of actin and myosin is essentially the same for invertebrate and vertebrate striated muscle, as are the changes in banding pattern that occurs during muscle contraction; the mechanism for contraction is also identical.

Smooth muscle cells lack the obvious transverse striations of striated muscle but they contain both

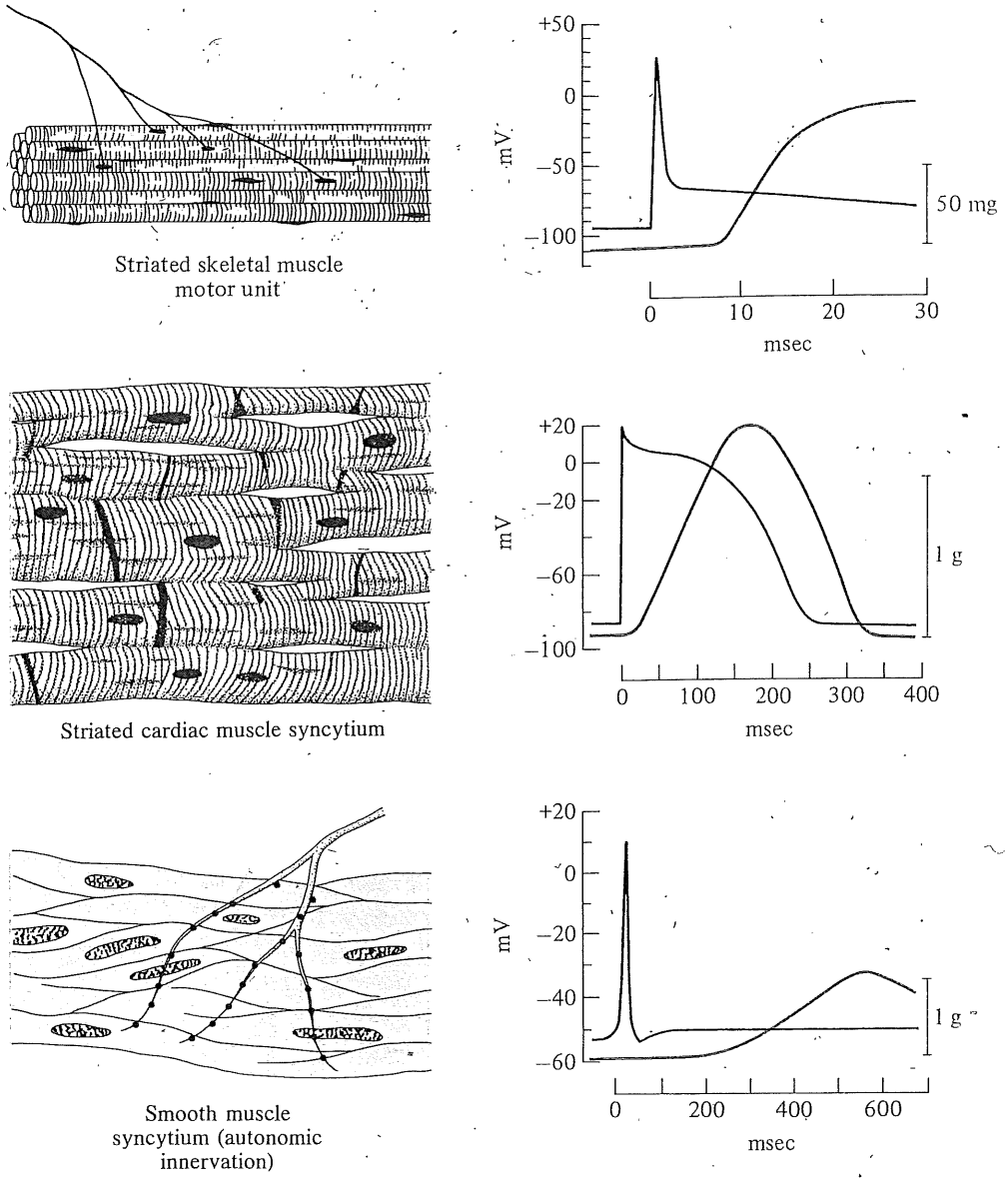


FIGURE 9–20 Major types of muscle: striated skeletal fibers, striated cardiac fibers, and smooth fibers showing details of action potential and time course of the muscle twitch.

actin and myosin. Typical smooth muscle cells, such as vertebrate visceral muscle, are small and spindle shaped with a single central nucleus (Figure 9–20). The cells may occur singly but they typically are associated with other smooth muscle cells with the thick central portion of one joined to the thin ends of adjacent smooth muscle cells. Vertebrate smooth muscle can be divided into functional subtypes: visceral (single unit, or unitary) smooth muscle and multiunit smooth muscle. **Visceral smooth muscle** cells generally are arranged as sheets, or bundles, of many cells that are electrically interconnected by gap junctions to form a single electrical syncytium (similar to cardiac muscle cells). These syncytial cells contract in unison, hence the arrangement is called single-unit smooth muscle. Electrical activity spreads as action potentials. These syncytia of smooth muscle cells have a diffuse innervation by varicosity synapses, synaptic terminals spread along autonomic nerves. They also respond to circulating hormones (e.g., epinephrine and norepinephrine), physical stretch, and temperature. In contrast, **multiunit smooth muscle** cells are organized as discrete fibers. Each cell is innervated by a single

nerve ending and operates independently of the others. Their sarcolemma generally does not support an action potential but relies on electrotonic propagation. The cell generally responds only to its neuronal innervation and not to other factors, such as circulating hormones, stretch, or temperature.

Striated Muscle. The most obvious characteristic of a striated muscle fiber is its regular transverse striations (Figure 9–21). The cytoplasmic matrix, or **sarcoplasm**, contains numerous long, cylindrical myofibrils (1 to 2 μ dia), which also are striated. Most of the sarcoplasm is myofibrils, although there also are organelles, e.g., mitochondria, nucleus, and deposits of glycogen and lipid. The myofibrils have alternating light and dark bands; because of their properties under polarized light, the light-staining bands are called I (isotropic) bands and the dark-staining bands are A (anisotropic) bands. A dark transverse line, the Z line, bisects each clear I band into two halves. The sarcomere is the portion of the myofibril between adjacent Z lines.

The sarcomere. The **sarcomere** is both the structural and functional contractile unit of striated muscle. It is a highly ordered array of thin actin filaments and thick myosin filaments (Figure 9–21). The actin molecules (about 5 nm dia, 1 μ long) are firmly attached to the Z line at each end of the sarcomere. Each actin filament appears to be connected to the Z line by four Z filaments that run through the Z line and connect to actin filaments of the adjacent sarcomere. The protein α-actinin is also present in the Z line, and binds the actins together. Adjacent myofibrils are interconnected at their Z lines by desmin and vimentin filaments. The thick myosin filaments (15 nm dia, 1.5 μ long) extend in parallel between the ends of the sarcomere, at about 45 nm apart. The myosin filaments are thicker in the middle and taper towards their ends. The myosin heads project transversely from the ends; they can form cross-bridge links to the actin filaments. Myosin filaments are held in their regular arrangement by slender cross connections at their centers (these form the M band).

The area of overlap of the actin and myosin in the center of the sarcomere forms the A (anisotropic) band. The H band, located in the middle of the A band, is the central region of the myosin where there is no overlap with actin. In the center of the H band is the M band, the central segment of the myosin that lacks cross-bridges.

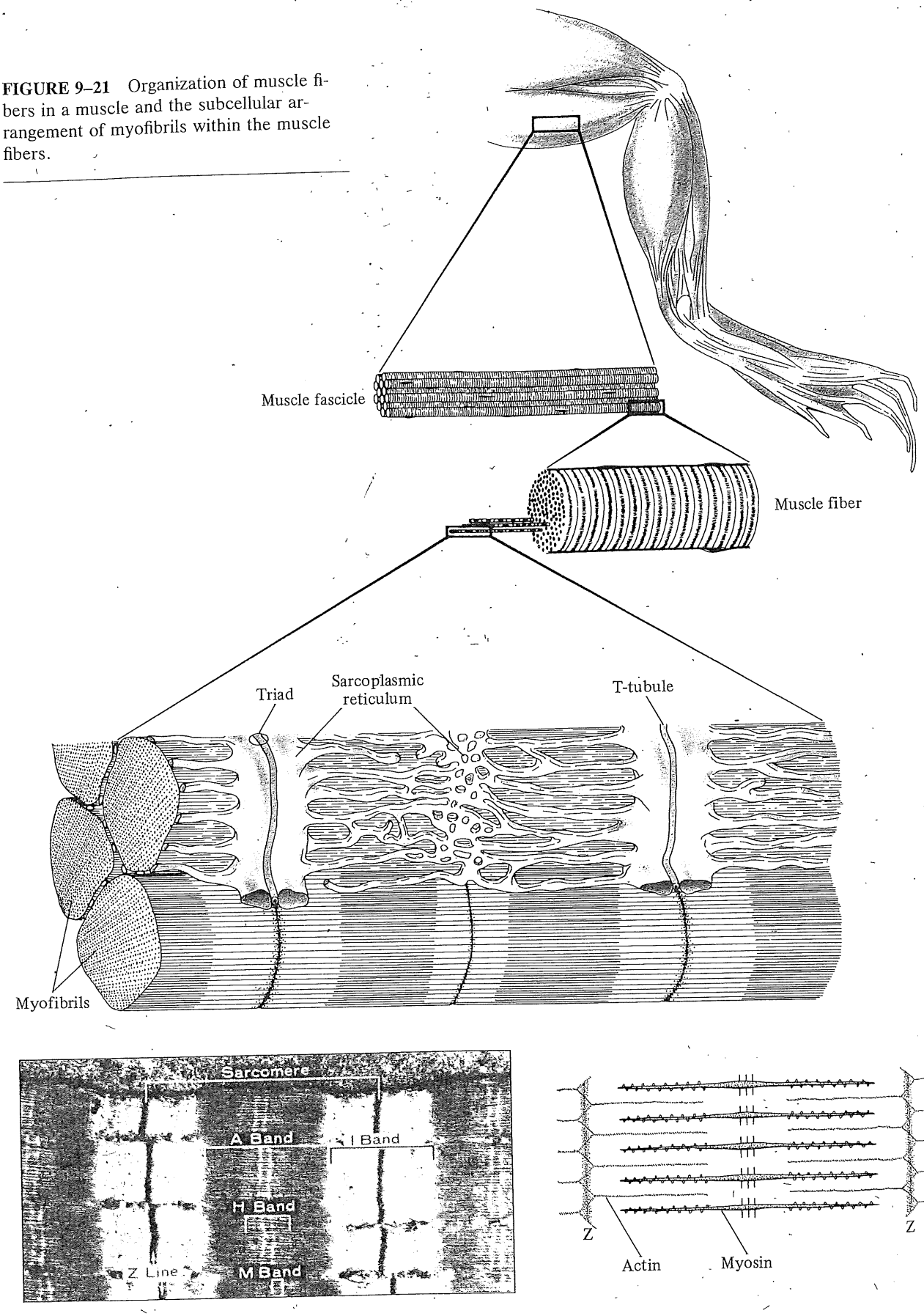
Striated muscle cells have an important membrane system, the **sarcotubules** and **sarcoplasmic reticulum** (Figure 9–21). These membrane structures are important in the transmission of electrical depolarization from the cell surface to the interior and with Ca^{2+} regulation of muscle contraction. Sarcotubules are long, thin, blind-ended membrane tubules that invaginate from the cell surface and branch and extend within the cell to form a ring of narrow (about 0.1 nm dia) tubules running around the perimeter of each sarcomere; these transverse sarcotubules are called **t-tubules**. The sarcoplasmic reticulum (SR) is an intracellular membrane system between adjacent t-tubules. It forms an irregular, hollow collar-shaped sheath around the sarcomeres. The SR of muscle cells is a special type of endoplasmic reticulum that lacks ribosomes. The terminal cisterna at each end of the SR is in intimate contact with a t-tubule. The SR of one sarcomere, the t-tubule, and the SR of the adjacent sarcomere are closely associated to form the **triad**. The SR normally sequesters Ca^{2+} and releases it to induce muscle contraction.

In amphibian skeletal muscle, each Z line has an adjacent t-tubule, hence each sarcomere has one triad. In mammalian skeletal muscle, the t-tubules are adjacent to the I through A band junction, hence each sarcomere has two triads. Mammalian cardiac muscle has one triad per sarcomere, at the Z line. The t-tubule and sarcoplasmic reticulum systems are less well developed in the cardiac muscle of lower vertebrates. For example, the amphibian cardiac muscle cell has no t-tubule system and only a poorly developed SR.

Sliding filament model. An early theory of muscle contraction proposed that specific muscle proteins shortened by some structural rearrangement. This theory was abandoned in favor of the **sliding filament model**, in part due to the microscopical evidence of sarcomere contraction and the dual myofilament nature (i.e., actin + myosin) of myofibrillar structure (Huxley and Niedergierke 1954; Huxley and Hanson 1954). The sliding filament model is the correct mechanism for muscle contraction, and the actin-myosin sliding filament model has provided inspiration for the examination and interpretation of movement systems in other cells (e.g., smooth muscle cells) and microtubular movement (e.g., cilia).

Sarcomere contraction is due to the sliding movement of adjacent thin actin filaments and thick myosin filaments. Neither the actin nor myosin filaments change length during sarcomere shortening but the Z lines move closer together (Figure 9–22A). The A band always has the same length, but the relative lengths of the I and H bands vary from short (contracted muscle cell) to long (extended

FIGURE 9–21 Organization of muscle fibers in a muscle and the subcellular arrangement of myofibrils within the muscle fibers.



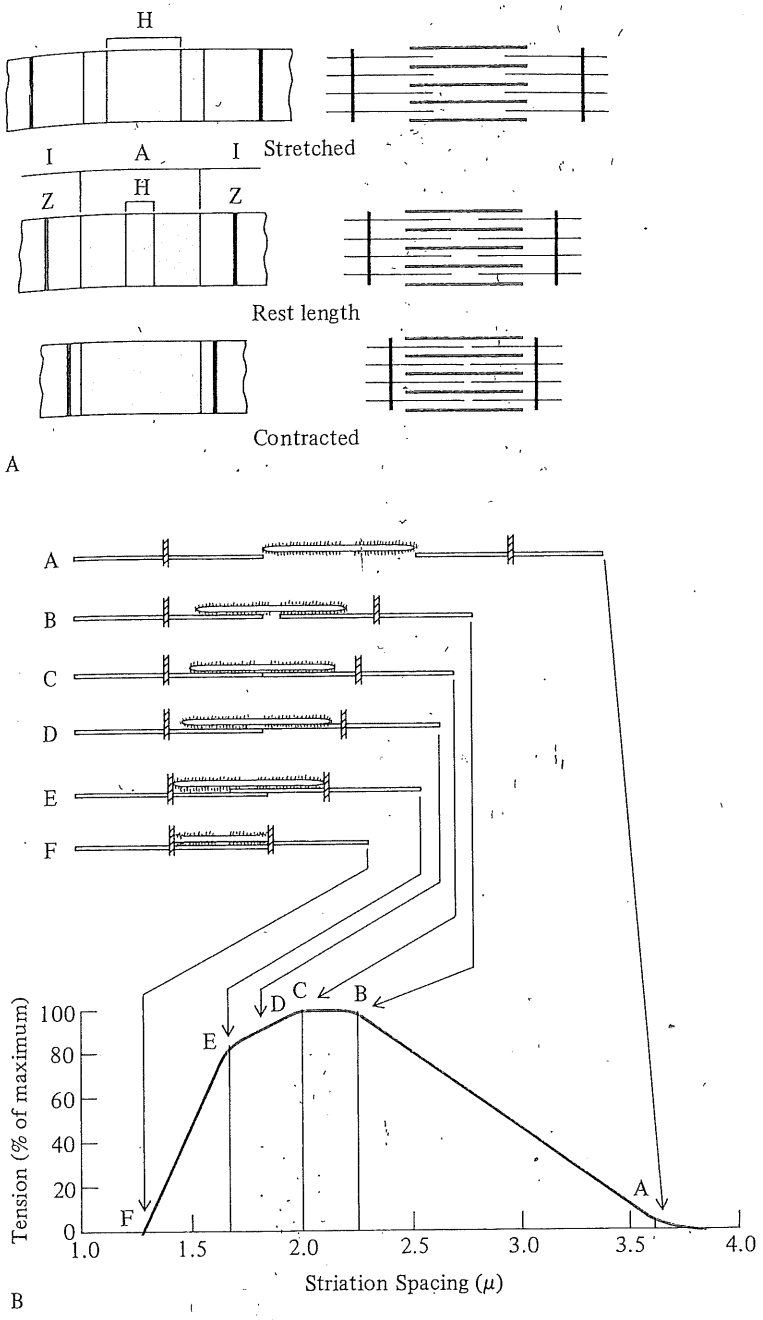


FIGURE 9–22 **(A)** Schematic representation of sarcomeric shortening showing the change in proportions of the I and A bands and the H zone as the Z lines move closer together. **(B)** Relationship between length of sarcomeres (indicated by striation spacing) and tension generated (as percentage of maximum tension). *(Modified from Gordon, Huxley, and Julian 1966.)*

muscle cell). The appearance of the sarcomere, and these changes that occur with contraction, are consistent with what we now understand of the molecular structure and function of actin and myosin. In fact, the histological appearance of sarcomeres provided much of the initial evidence for the sliding filament model of muscle contraction.

The sliding filament model predicted from microscopical evidence suggested that the molecular force generators (the "motors") were spaced along the thick filaments and moved the thin filaments by cyclical attachment of the thick to the thin filaments. The existence of such cross-bridges between myosin and actin was subsequently demonstrated by electron microscopy. Further studies have demonstrated cyclic positional changes of the cross-bridges that correspond to the relaxed and contracted (rigor) states. Part of the myosin head forms the cross-bridge with the actin binding site.

The sliding filament model predicts that (1) the number of cross-bridges varies with the length of the sarcomere; (2) the force generated by a sarcomere should be proportional to the number of cross-bridges, i.e., the length; (3) the myosin heads in the M band do not contact an actin filament and parts of the actin filament (in the I band) are not in contact

with myosin heads; and (4) no force can be generated when the sarcomere is so shortened that the ends of the myosin filaments abut against each Z line. Measurement of the relationship between force of contraction and sarcomere length has verified these predictions of the sliding filament model (Figure 9–22B).

Cross-bridge mechanics. The cross-bridges that form between the myosin head and actin convert ATP's chemical energy into mechanical work and provide the mechanism for filament sliding. The myosin head (HMM-S1) has ATPase activity. Pure myosin actually has a low ATPase activity, hydrolyzing about six substrate molecules (a Mg^{2+}-ATP complex) per minute. The rate constant k_1 varies with ATP concentration; it is about 10^{-6} M^{-1} sec^{-1}. k_2 is independent of ATP concentration, at about 100 sec^{-1}. The overall $\Delta G°$ is about -36.4 kJ $mole^{-1}$, i.e., is very favorable for ATP hydrolysis.

$$\text{HMM-S1} + \text{ATP} \underset{\substack{\text{(fast)}\\ \Delta G° = -66}}{\overset{k_1}{\rightarrow}} \text{S1.ATP*} \underset{\substack{\text{(fast)}\\ -16.4}}{\overset{k_2}{\rightarrow}} \text{S1*.ADP.Pi} \overset{k_3}{\underset{\substack{\text{slow}\\ +46 \text{ kJ mole}^{-1}}}{\rightarrow}} \text{S1} + \text{ADP} + \text{Pi} \qquad (9.1)$$

A mixture of pure F-actin and myosin (an actomyosin complex) has about 100 × the ATPase activity of pure myosin, i.e., actin activates the myosin ATPase. However, F-actin, which is associated with troponin and tropomyosin, does not stimulate the myosin ATPase. The inhibition by troponin and tropomyosin of myosin activation by actin is removed if Ca^{2+} is present at 10^{-7} to 10^{-6} M. The actions of actin, troponin/tropomyosin, and Ca^{2+} on myosin ATPase activity are not enzymatic effects, i.e., they are not cofactors. Rather, they are conformational regulators of the ability of myosin heads to bind to the actin binding site and to form cross-bridges.

The reaction of F-actin with myosin-ATPase is complex, but there is a favored cycle that includes ATP hydrolysis and attachment/detachment of the myosin head (Figure 9–23). A myosin head attaches to the actin filament, undergoes a conformational change (rotation) and slides the actin filament past the myosin, detaches from the actin filament, then rerotates to its original orientation so it can attach to another actin binding site. It is not clear whether both heads of each myosin molecule act independently of each other or whether they act in concert. One possible sequence of events for the myosin head rotation involves sequential attachment of four different binding sites of the myosin head (M_1, M_2, M_3, M_4); the myosin head rotates during the attachment and detachment of successive binding sites. Other models for myosin-actin sliding have the same overall effect.

Myosin head rotation is transduced to a longitudinal displacement of actin and myosin through the cross-bridge link between the myosin head and the

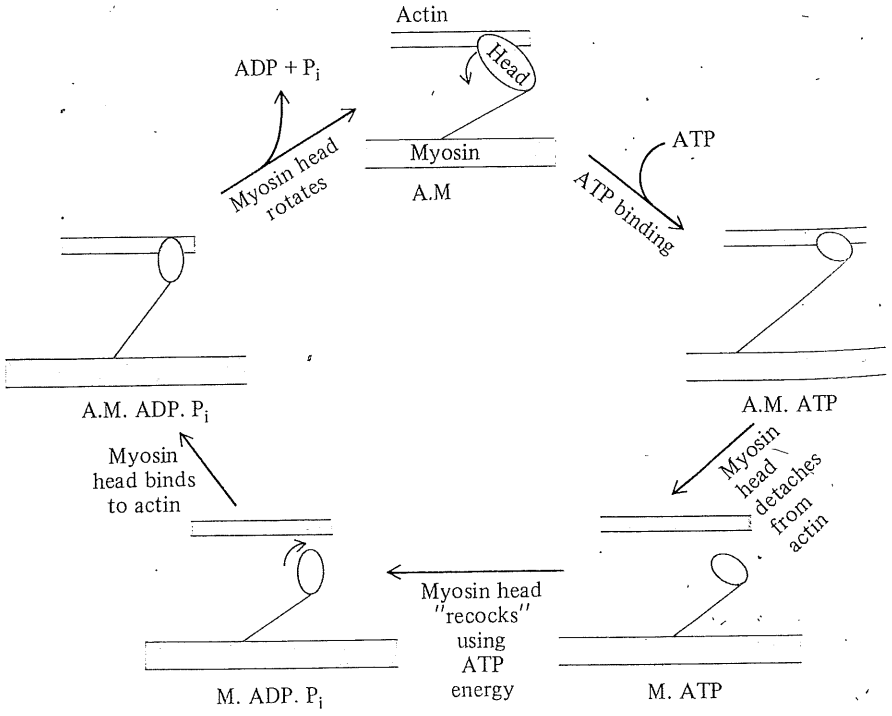


FIGURE 9–23 Dynamics of actin (A) and myosin head (M) resulting in net movement of actin and myosin filaments by cross-bridge formation, rotational movement of the myosin head, and cross-bridge detachment. The addition of ATP begins the cycle.

actin filament. The power stroke, i.e., myosin head rotation, does not require ATP hydrolysis although this is the step in the sliding filament cycle that performs useful work. The chemical energy that is converted to mechanical work was stored in the myosin head by prior ATP hydrolysis. ATP hydrolysis actually occurs during the step in the cycle when the myosin head detaches from the actin binding site and repositions in readiness for the next attachment to an actin binding site, i.e., ATP is hydrolyzed when the myosin "recocks" its position.

Tilting of the 19 nm-long myosin head would be expected to produce about 12 nm of actin-myosin filament sliding. However, recent estimates suggest that the actual filament sliding distance per ATP hydrolyzed is about 40 nm, or even more (Higuchi and Goldman, 1991). The 28 nm additional movement may be due to filament sliding while the cross-bridges bear a negative force, i.e., the myosin head is passively dragged 28 nm during filament sliding and active head rotation contributes a further 12 nm sliding. Alternatively, there may be multiple myosin head power strokes per ATP hydrolyzed.

Acto-myosin regulation. Calcium and magnesium ions are required for muscle contraction, and it was initially thought that both were enzymatic cofactors for ATPase activity. The Mg^{2+} does have this cofactor role; it forms an ATP-Mg^{2+} substrate for the myosin ATPase.

The regulatory role of Ca^{2+} is more complex than that of Mg^{2+}. Myosin head ATPase activity is Ca^{2+} dependent with little activity at a Ca^{2+} concentration of $<10^{-7}$ and maximal activity at concentrations $>10^{-5}$. For example, both mammalian and squid muscle show a superficially similar dependence of ATPase activity on Ca^{2+} concentration (Figure 9–24). Muscle cells have a total intracellular Ca^{2+} content equivalent to an average concentration of 1 to 5 10^{-3} M, but most of the intracellular Ca^{2+} is normally sequestered within vesicles and the normal cytoplasmic Ca^{2+} concentration is $<10^{-7}$ M. Consequently, there normally is no Ca^{2+} activation of actin-myosin and cross-bridge formation.

There are two different mechanisms for Ca^{2+} regulation of actin-myosin interaction. One mechanism depends on actin's capacity to bind to myosin heads, i.e., the myosin is **actin regulated**. The other mechanism is myosin-dependent control of its ATPase activity, i.e., the myosin is **myosin regulated**. Vertebrate striated muscle and the locomotor muscles of many invertebrates have only actin-dependent regulation, whereas myosin-dependent regulation is common in muscles of many other invertebrates (Figure 9–25). A number of the higher

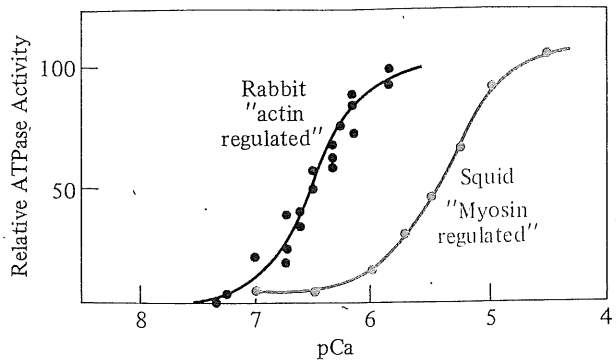


FIGURE 9–24 Mg-ATPase activity is dependent on the Ca^{2+} concentration ($pCa^{2+} = -\log_{10}[Ca^{2+}]$). For rabbit the actin is Ca^{2+} regulated, whereas for squid there is myosin regulation (and also some actin regulation). *(Modified from Bendall 1968; Konno, Arai, and Watanabe 1980.)*

invertebrate groups (e.g., annelids, mollusks, many arthropods) have myosin-dependent regulation. Single regulatory systems tend to be found in more primitive animals and double regulatory systems in higher invertebrates (Lehman and Szent-Gyorgyi 1975). Both the myosin and actin regulatory systems occur in primitive phyla, and so neither appears to be the "ancestral" regulatory system, although myosin regulation involves only one regulatory protein and actin regulation involves a number of regulatory proteins. Vertebrate smooth muscle has a different regulatory system, with both actin and myosin regulation by caldesmon (see below). Let us first examine actin-mediated regulation by Ca^{2+} in vertebrate striated muscle, since it is the sole regulatory mechanism.

The actin-mediated response to Ca^{2+} of vertebrate striated muscle requires the presence of tropomyosin and troponin in the F-actin filament (Figure 9–26A). **Tropomyosin** molecules are positioned end-to-end in the grooves of the double stranded F-actin with each tropomyosin traversing seven G-actin subunits. This position of the tropomyosin in the actin groove precludes the correct steric interaction of the myosin head with the G-actin binding site for myosin and prevents cross-bridge formation. Attached to one end of each tropomyosin is **troponin**, a complex globular protein. Troponin has three subunits: troponin C, which binds Ca^{2+}; troponin T, which binds to the tropomyosin; and troponin I, which inhibits the actin binding sites. Troponin C has four Ca^{2+} binding sites; two high-affinity sites bind Ca^{2+} or Mg^{2+} and two lower affinity sites bind only Ca^{2+}. The activating effect of Ca^{2+} on troponin

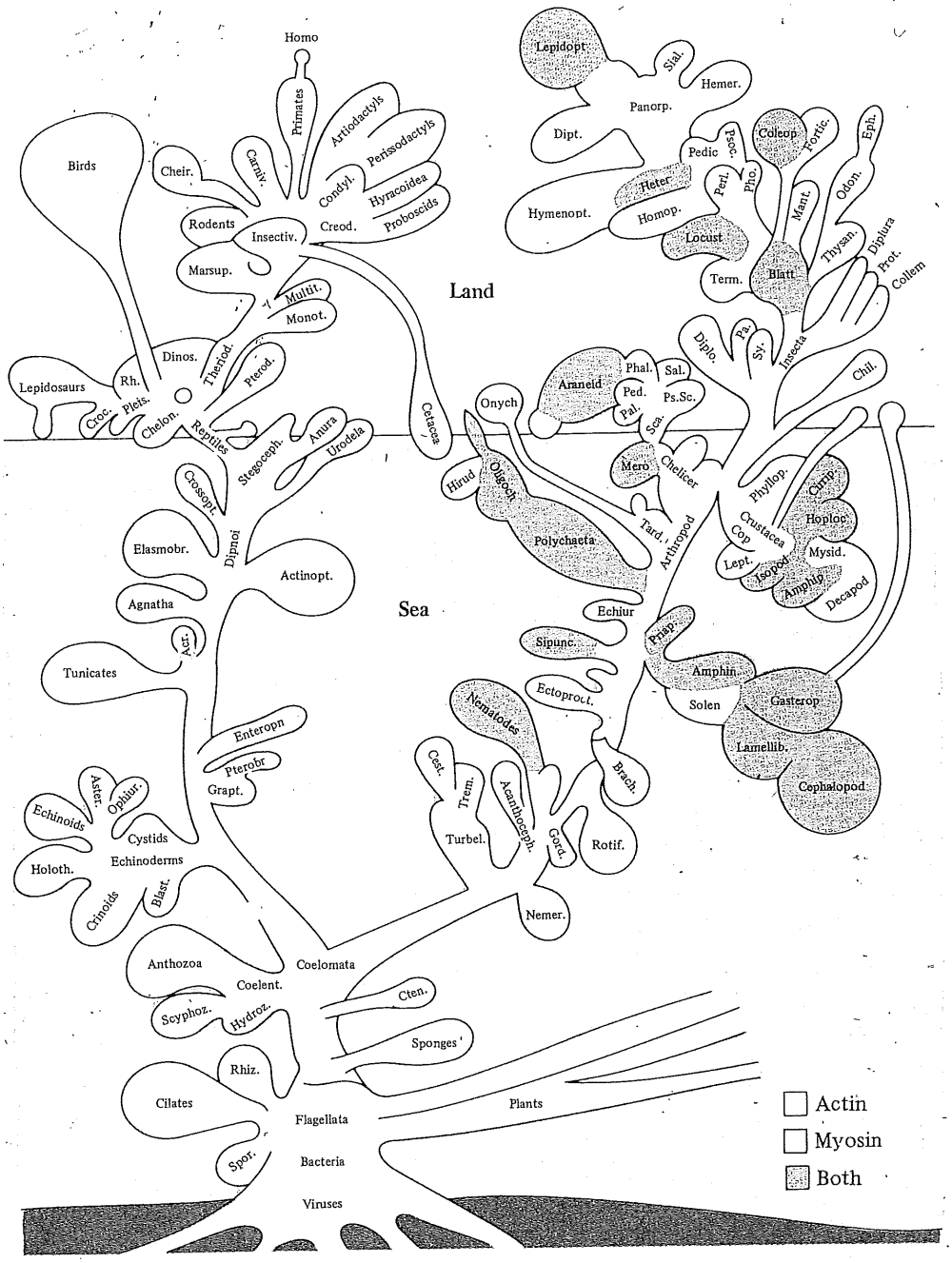
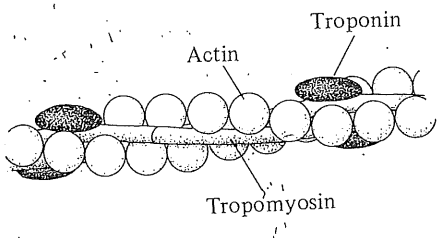


FIGURE 9–25 Phylogenetic distribution of myosin-linked (medium color), actin-linked (light color), or both types (dark color) of regulation of myosin Mg-ATPase in animals. Only regulation of vertebrate striated muscle and various locomotor muscles is included; vertebrate smooth muscle has a different regulatory system. *(Adapted from Lehman and Szent-Gyorgyi 1975; Lehman 1983.)*

C causes a change in the structure of troponin I. This involves movement of the tropomyosin so that the myosin heads can bind to the G-actin binding sites and form cross-bridges (Figure 9–26B).

Myosin control of muscle contraction is observed in some invertebrates (e.g., echinoderms, nemertines, brachiopods, mollusks, echiuroids). The myosin ATPase is directly activated by Ca^{2+}; it is not activated by pure actin. The Ca^{2+} binds to a small regulatory protein (MWt 1.7 10^4) that normally is tightly bound to the myosin. Removal of the Ca^{2+}-binding protein from myosin prevents ATPase activ-



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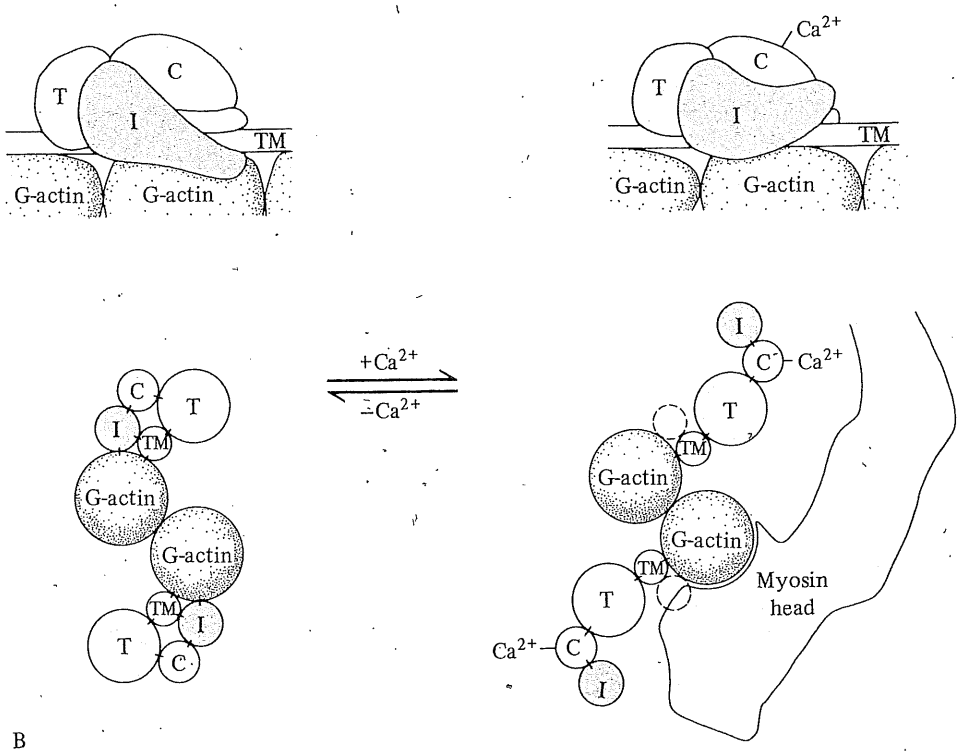

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FIGURE 9–26 **(A)** F-actin is a twisted bifilament of globular G-actin subunits with accessory proteins tropomyosin and troponin complex. **(B)** Representation of the structural rearrangement of troponin I in response to the binding of Ca^{2+} by troponin C, which results in the uncovering of the myosin head binding site on the G-actin subunit. *(Modified from Ohtsuki 1980; Gergely and Lewis 1980.)*

ity, even in the presence of excess Ca^{2+}, but replacement of the Ca^{2+}-binding protein restores the ATPase activity at high Ca^{2+}. There is one Ca^{2+}-binding site per myosin molecule (i.e., per two myosin heads) and it binds one or two Ca^{2+}. The myosin ATPase activity is minimal at the normal, low intracellular Ca^{2+} concentration ($<10^{-7}$ M) and is elevated at higher intracellular Ca^{2+} concentrations ($>10^{-7}$ M). This effect is superficially similar to the role of Ca^{2+} in an actin-activated system.

Myosin activity is regulated in the nematode *Caenorhabditis* by a very large muscle protein, twitchin (Benian et al 1989). Twitchin is a protein kinase enzyme that probably is associated with the myosin (it is located in the sarcomere A bands), whose function it regulates. *Caenorhabditis* muscle apparently also has both actin and myosin regulation by Ca^{2+}.

Excitation-contraction coupling. So far we have examined the molecular mechanisms for muscle contraction and the role of intracellular Ca^{2+} in initiating sliding filament movement, but how does synaptic depolarization of the muscle end plate elevate the intracellular Ca^{2+} concentration and initiate a muscle contraction? Stimulation of the presynaptic axon depolarizes the end plate, the intracellular Ca^{2+} concentration transiently increases, and the muscle cell develops tension (Figure 9–27). The sequence of events linking end plate depolarization and muscle contraction is **excitation-contraction coupling.**

The sarcolemmal membrane potential determines the force of contraction. Normally, an action potential is propagated over the sarcolemma, but some invertebrate muscle cell membranes do not support regenerative action potentials and only graded depolarizations spread over the sarcolemma. Localized depolarization of openings of the t-tubules at the sarcolemma surface elicits a localized sarcomeric contraction, but similar depolarizations at other sarcolemmal sites have no effect. The surface membrane depolarization moves by electrotonic spread

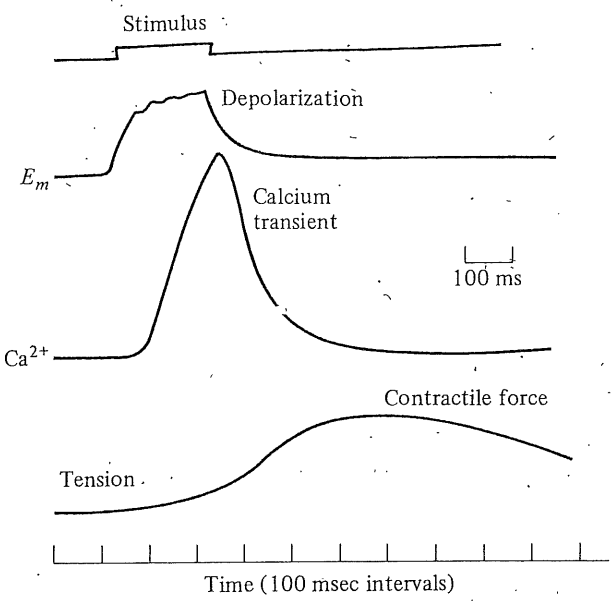


FIGURE 9–27 Relationship between intracellular Ca^{2+} concentration and muscle fiber tension in response to an electrical stimulus and membrane depolarization. *(Modified from Keynes and Aidley 1981.)*

down the t-tubules and releases Ca^{2+} from the adjacent sarcoplasmic reticulum. The elevated intracellular Ca^{2+} then elicits a localized sarcomeric contraction.

The t-tubules are in intimate contact with the sarcoplasmic reticulum membrane at the triads (Figure 9–28). There are specific t-tubular membrane proteins, arranged in tetrads, and also integral sarcoplasmic reticulum proteins. The sarcoplasmic reticulum tetrads are arranged in two parallel rows along the t-tubule, but only half contact t-tubular tetrads. Ca^{2+} efflux from the sarcoplasmic reticulum is mediated by a specific Ca^{2+} channel, the **"Ca^{2+}-release channel,"** that is distinct from the sarcoplasmic reticulum Ca^{2+} pump (Ca^{2+}-Mg^{2+}-ATPase), which sequesters Ca^{2+} within the sarcoplasmic reticulum.

It is not clear how depolarization of the t-tubule membrane transduces Ca^{2+} efflux from the sarcoplasmic reticulum. The signal transduction is thought to involve either a chemical messenger of unknown nature or mechanical coupling. Several recent (and not mutually exclusive) hypotheses for the transduction process include electrical depolarization of the sarcoplasmic reticular membrane; change in pH; Ca^{2+}-induced Ca^{2+} release; charge displacement and consequent mechanical movement of the feet bridging the t-tubule/sarcoplasmic membrane space; a chemical messenger, inositol 1,4,5 triphosphate (IP_3); and change in oxidation state of sulfydryl groups on the Ca^{2+}-release channel (Trimm, Salama, and Abramson 1986). The t-tubule protein tetrads may be voltage sensors, and depolarization of the t-tubule may induce a conformational change, thereby gating the abutting sarcoplasmic reticular foot to allow Ca^{2+} efflux from the sarcoplasmic reticulum. The sarcoplasmic reticular feet that do not contact t-tubular tetrads may have a different gating mechanism, perhaps Ca^{2+}, i.e., they may be Ca^{2+}-induced Ca^{2+} channels. IP_3 is a phospholipid metabolite that couples a variety of extracellular signals with a transient increase in intracellular Ca^{2+}, by stimulating Ca^{2+} release from intracellular stores or influx across the cell membrane. This occurs in brain neurons and hepatocytes, as well as probably muscle.

The details of excitation-contraction coupling are slightly different in cardiac muscle cells because there is a less extensive system of t-tubules and sarcoplasmic reticulum. In mammalian and avian heart muscle, some of the elevated intracellular Ca^{2+} concentration during an action potential is due to the influx of Ca^{2+} from the extracellular fluid through sarcolemmal Ca^{2+} channels during the plateau phase of the cardiac action potential. The influx of extracellular Ca^{2+} triggers further release of Ca^{2+} from the sarcoplasmic reticulum, i.e., there is Ca^{2+}-induced Ca^{2+} release. This is particularly important for mammalian atrial and Purkinje fibers and for avian cardiac fibers, since these cells lack functional t-tubules. Frog cardiac muscle has a sparse sarcoplasmic reticular system; most of the increase in intracellular Ca^{2+} in amphibian cardiac muscle is due to influx from the extracellular fluid.

Smooth Muscle Contraction. Smooth muscle cells contain numerous thin actin filaments that are generally oriented parallel to the long axis of the cell. Attached to actin filaments are dense bodies, which are scattered throughout the cytoplasm and at intervals along the inner surface of the sarcolemma. The thick myosin filaments of smooth muscle cells have all of their heads projecting in the same direction along their length (unlike the myosin of striated muscle cells).

Actin is the predominant intracellular protein filament of smooth muscle. The molar ratio of actin to myosin varies from 12:1 to 50:1 for smooth muscle (Figure 9–29A) compared to 4:1 in striated muscle. The actin is not homogeneously distributed throughout the cell, but is partitioned between two domains: the **acto-myosin domain** and the **filamin domain** (Sparrow 1988). The acto-myosin domain contains myosin, actin (plus tropomyosin), and caldesmon;

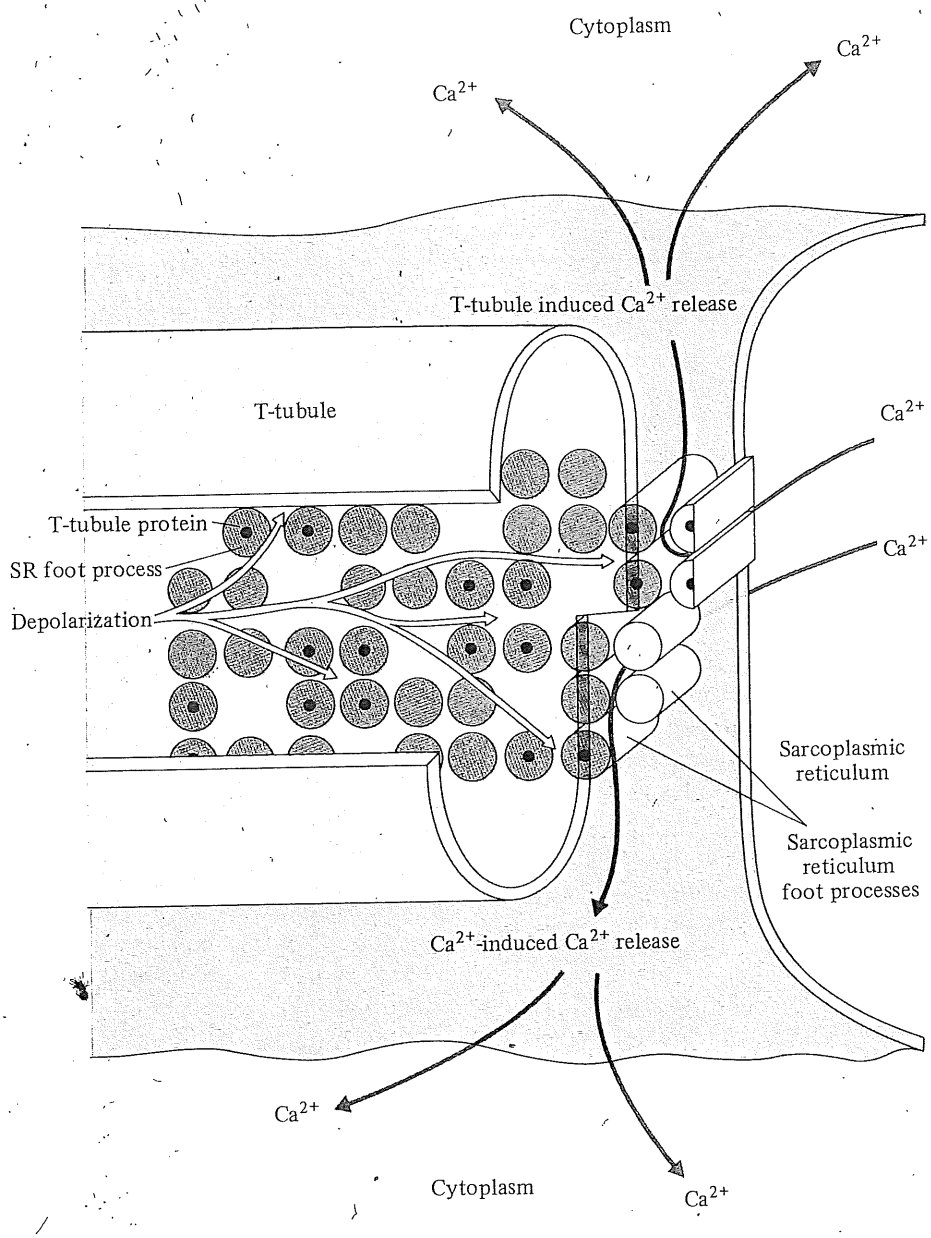
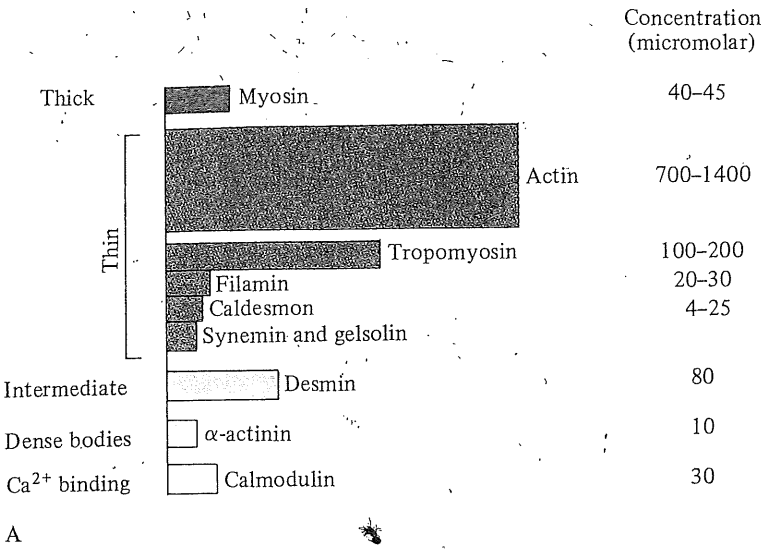
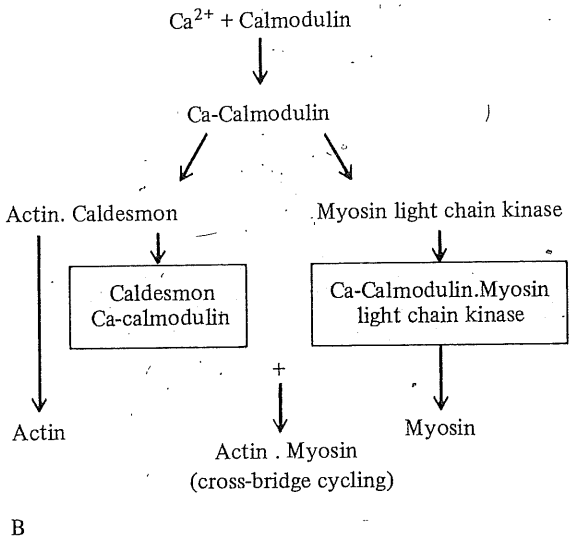


FIGURE 9–28 Schematic model for a possible mechanism of Ca^{2+} release from sarcoplasmic reticulum. Electrotonic depolarization spreads down t-tubules and activates voltage-dependent, t-tubular membrane proteins (solid). This includes a conformational change in underlying proteins of the sarcoplasmic reticulum membrane; units of four SR proteins (a SR "foot") allows Ca^{2+} to diffuse into the cytoplasm. The alternating SR feet without t-tubular, voltage-sensitive proteins may also undergo a conformational change, in response to the t-tubule-induced Ca^{2+} release, and allow further Ca^{2+} to diffuse from the SR into the cytoplasm, i.e., this is Ca^{2+}-induced Ca^{2+} release. *(Based in part on Rios and Pizaro 1988.)*

it is responsible for force development during contraction. The filamin domain contains filamin, actin (plus tropomyosin), desmin, and dense bodies (α-actinin); it is responsible for tonic maintenance of tension.

The sequence of events from sarcolemma depolarization to actin-myosin cross-bridge formation is generally similar for smooth muscle and striated muscle, although there are some differences. The intracellular Ca^{2+} concentration is the regulator





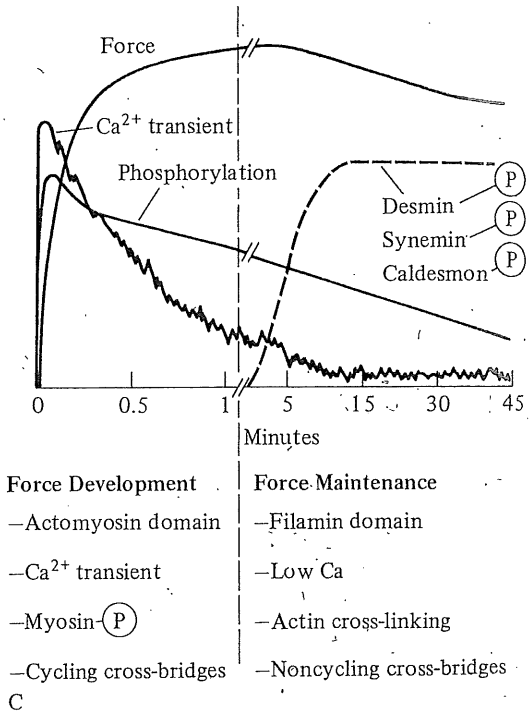


FIGURE 9–29 (**A**) Relative proportions of various contraction-related proteins in smooth muscle. (**B**) There is a dual calcium regulation of smooth muscle contraction by the effects of Ca^{2+}-calmodulin on the myosin (right) and actin (left). (**C**) Events during smooth muscle contraction initiated by a transient increase in intracellular Ca^{2+} concentration are an initial period of force development due to the acto-myosin domain (related to the Ca^{2+} transient, phosphorylation, and cross-bridge cycling) followed by a period of force maintenance due to the filamin domain (related to actin cross-linking and no cross-bridge cycling). *(From Sparrow 1988.)*

of actin-myosin cross-bridge formation in smooth muscle, as in striated muscle; it is increased from about 10^{-7} to 10^{-6} after stimulation. The rudimentary sarcoplasmic reticulum of smooth muscle is one source of the increased Ca^{2+} concentration. Intracellular Ca^{2+} is also elevated by the entry of extracellular Ca^{2+} into the smooth muscle cell through the sarcolemma.

The SR-sequestered Ca^{2+} is released by inositol 1,4,5 triphosphate (IP_3). The IP_3 initiates Ca^{2+} release from SR vesicles of mammalian smooth muscle but apparently not striated muscle vesicles (Ehrlich and Watras 1988). The IP_3-gated Ca^{2+} channel of smooth muscle vesicles is very different from the Ca^{2+}-activated Ca^{2+} channel of striated muscle.

The intracellular Ca^{2+}-binding protein of smooth muscle, **calmodulin**, has four Ca^{2+}-binding sites. The Ca^{2+}-calmodulin complex binds to a specific region of the myosin light chain kinase and exposes the catalytic binding site responsible for binding of myosin with ATP (Figure 9–29B right). The myosin binds to actin, cross-bridges form, the myosin heads rotate and detach, and ATP is hydrolyzed. This Ca^{2+}-regulated mechanism for smooth muscle contraction corresponds closely to the myosin-activation scheme for some striated muscles.

Smooth muscle also has another Ca^{2+}-regulatory scheme, at least *in vitro* (Figure 9–29B left). Smooth muscle actin is an F-actin double filament containing tropomyosin but has the protein caldesmon rather than troponin. Caldesmon normally binds strongly to the actin-tropomyosin complex and inhibits cross-bridge formation. The ratio of caldesmon:tropomyosin:G-actin subunits is 1:4:28. An increased intracellular Ca^{2+} forms a Ca^{2+}-calmodulin complex, which binds to caldesmon and weakens its binding to actin. This allows formation of myosin-actin cross-bridges.

Smooth muscle, unlike striated muscle, maintains tension even after the intracellular Ca^{2+} returns to the resting level (Figure 9–29C). The tension is maintained at a low energy cost, i.e., the rate of ATP hydrolysis is low. There may be some form of slow cross-bridge cycling or even formation of latched cross-bridges. Alternatively, the filamin domain, rather than the acto-myosin domain, may be responsible for the maintenance of tension. Proteins such as gelsolin may decrease the cytoskeletal rigidity during the Ca^{2+} transient of smooth muscle contraction, and the cytoskeleton is then rigidified after the Ca^{2+} transient. Phosphorylated proteins (desmin, synemin, and caldesmon) may maintain the resting tension; diacyl-glycerol (which is formed concurrently with IP_3 during the Ca^{2+} transient) stimulates their phosphorylation.

Many invertebrate smooth muscle cells resemble those of vertebrates, at least in general appearance, but **helical smooth muscle** and **paramyosin smooth muscle** differ in structure from typical smooth and striated muscle cells. The locomotory muscles of annelids and cephalopods have smooth muscle with helically arranged myofibrils. Some other mollusks and animals in other phyla (e.g., echinoderms, tunicates) also have helical smooth muscle but these have a "double-oblique" striation pattern due to the appearance of both aspects of the helix in the same focal plane. The adductor muscle of lamellibranch mollusks contains paramyosin smooth muscle cells. The adductor muscle closes the shell and maintains tension against a springy hinge; it can maintain tension for many hours at a very low metabolic cost. For example, an oyster adductor muscle can maintain 0.56 kg cm^{-2} for 20 to 30 days!

Properties of Muscle Contraction. The mechanical properties of muscle cells, as well as muscles, vary dramatically depending on the type of muscle and the metabolic capacity of the muscle cell. Vertebrate skeletal muscle has been studied in greatest detail with respect to its mechanical properties. We shall first concentrate on its general mechanical properties and then compare these properties with those of other types of skeletal muscle, cardiac muscle, and smooth muscle.

Threshold stimulation. Excitable cells, such as muscle and nerve, can be stimulated by an electrical impulse. The efficacy of the electrical impulse in eliciting a contraction is determined by both its magnitude and duration. A high-voltage impulse may be ineffective in eliciting a muscle contraction if it has a very short duration (i.e., subminimal duration), whereas a longer duration impulse may be equally ineffective if the voltage is too low (i.e., subthreshold voltage). The threshold voltage for very long duration impulses that elicits a response is the **rheobase**; the shortest duration that elicits a contraction of a rheobase-level stimulus is the **utilization time** (Figure 9–30). In practice, the utilization time is difficult to determine accurately, and so the minimum duration for a 2 × rheobase voltage (**chronaxie**) is used to indicate the "excitability" of the cell; a low chronaxie indicates a high excitability. Different types of muscle vary in rheobase and chronaxie; the five examples illustrated in Figure 9–30 are superimposed on the same curve by normalizing the voltage scale to rheobase and the duration scale to the chronaxie.

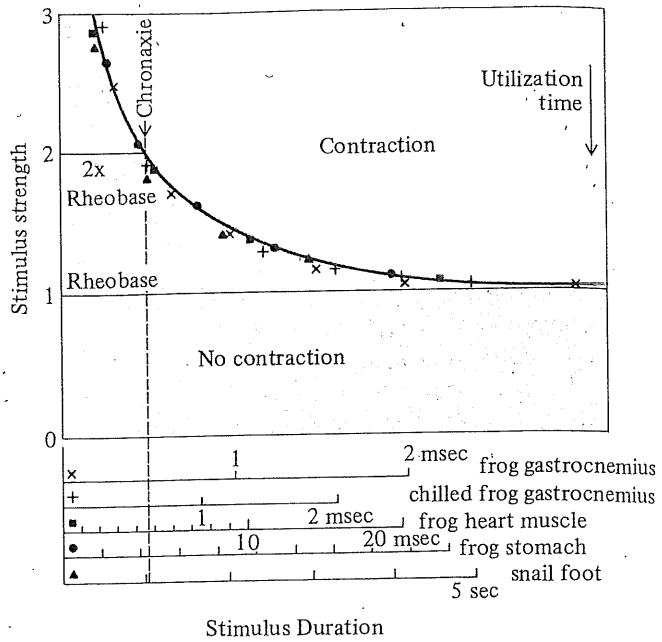


FIGURE 9–30 Relationship between duration of an electrical stimulus and the stimulus strength required to elicit a threshold response. Five strength-duration curves are superimposed by adjusting the time scale and using a relative strength scale (1 = threshold at infinite duration = rheobase). Chronaxie is the duration for 2 × rheobase strength.

Mechanical model. There are two different experimental types of muscle contraction: isometric and isotonic (Figure 9–31). In an isometric contraction, the muscle generates tension but the length of the muscle remains constant. In an isotonic contraction, the muscle shortens as it maintains a constant force. The muscles of an animal seldom have a pure isometric or isotonic contraction but contract in an intermediate fashion. Nevertheless, it is still convenient to study the properties of isolated muscles during isometric or isotonic contractions because this allows different aspects of muscle con-

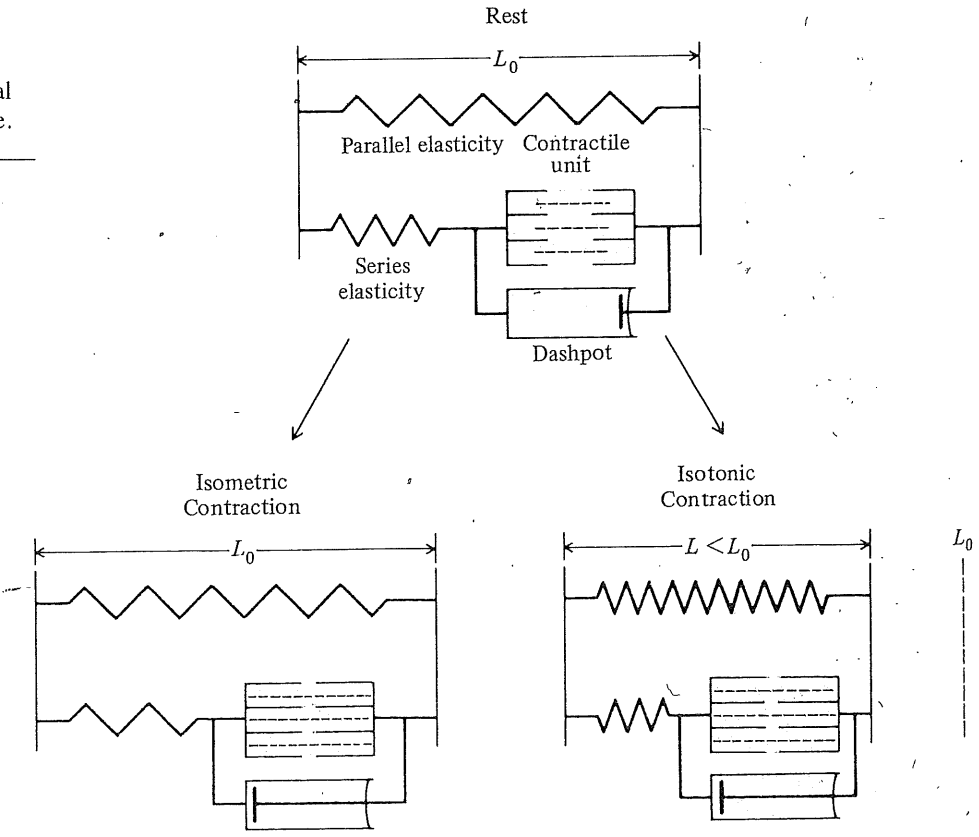


FIGURE 9–31 Mechanical analog of a skeletal muscle.

traction to be examined. Experimental results of force generated during isometric muscle contraction (F) are usually expressed as a ratio of the maximum force that can be generated by the muscle measured during continual maximal stimulation (F_0), i.e., F/F_0, or for isotonic experiments as a ratio of length (L) to the resting length (L_0) i.e., L/L_0.

The muscle cell contents must move as the cell contracts because the length of the cell decreases and its diameter increases. The viscous resistance to the shortening of muscle cells decreases the maximum force that they can generate. This viscous damping element of the cell is illustrated in Figure 9–31 as its mechanical analog, an oil pot damper, or dashpot. Another important mechanical property of muscle is its elasticity, i.e., parts of the muscle act as springs. A muscle contains two different types of "spring"; the **series elastic element** is a spring in series with the contractile sarcomere units and the **parallel elastic element** is a spring in parallel. The series elastic element consists of springy biological materials that are stretched as an immediate consequence of sarcomere shortening. The sarcomere Z band, the sarcolemma connections to myofibrils, and the tendons and other connective tissues that attach muscle cells to the bones act as series elastic elements. The parallel elastic element is muscle connective tissue and the sarcolemma; it is not influenced if the length of the muscle remains constant (i.e., in an isometric contraction). The properties of the elastic elements and viscous dashpot significantly determine the mechanical properties of a muscle.

An ideal spring has a linear relationship between tension and length; this is expressed by Hook's law

$$F = kL \tag{9.2a}$$

where F is the force exerted on the spring; L is the displacement; and k is a constant, the spring factor. A resting muscle is generally quite elastic, reflecting the flexibility of its parallel and series elastic elements as well as some stretch of the sarcomeres. Elastic tension is generated in an exponential manner as the muscle is passively stretched (Figure 9–32); the relationship between resting force F_r and stretch (L/L_0) is

$$F_r = ke^{c(L/L_0)} \tag{9.2b}$$

where c is an additional constant. Thus, resting muscles do not obey Hook's law. For example, the resting frog sartorius muscle (at 0° C) can be stretched to 1.1 to 1.2 × L_0 by small forces, but

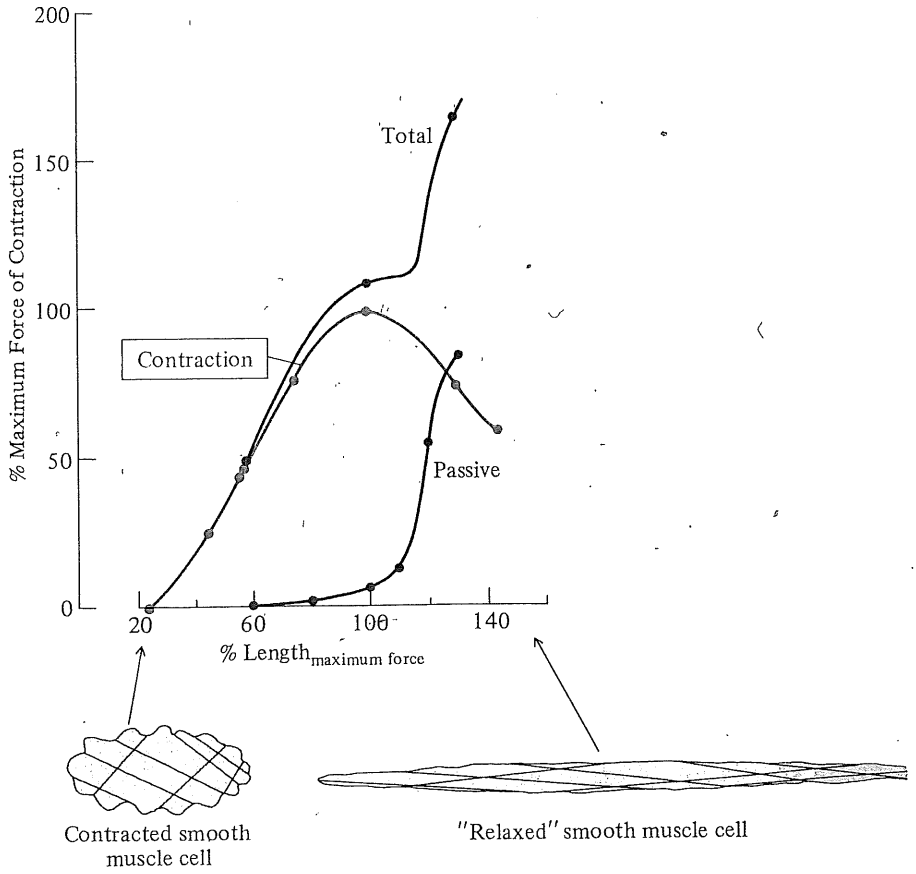


FIGURE 9–32 Passive tension, total tension (passive + contractile), and contractile tension of a smooth muscle cell as a function of initial length. *(Modified from Fay 1976.)*

requires a considerable force to be stretched to >1.4 L_0. A contracting muscle can generate a considerably greater force than at rest, up to a moderate degree of stretch, but the contraction force declines above a critical initial length, generally about 1.25 L_0. The difference between the active force and the resting force curves is the actual force generated by the contractile elements. This active force-generated curve has a strikingly similar shape to that derived for sarcomeres of contraction force as a function of sarcomere length (see Figure 9–22B, page 421). This similarity is to be expected because both reflect the basic contractile capacity of the sarcomeres.

There is a considerable variation in the passive length-tension curves for different muscles, depending on the particular sarcomeric organization (striated muscles) or acto-myosin dense bodies (smooth muscle) and the spring factor of the parallel and series elasticities. For example, insect flight muscles are very stiff compared to vertebrate striated muscle; the passive force increases dramatically with only short extension to lengths over the maximum *in vivo* length. This stiffness allows for considerable storage of elastic energy during the high-frequency wing beat cycle. Smooth muscle has a low spring factor and requires little force for extension to over 1.5 × the maximum *in vivo* length. The marked passive extensibility of smooth muscle cells is related to its absence of an organized sarcomeric structure and greater capacity for shape change. The maximal contraction force generally occurs at about the maximal *in vivo* length and is about 2 × that for maximal passive stretch (which occurs at much greater lengths).

Contraction time course. A single electrical stimulation of a muscle elicits a single contraction, or twitch (Figure 9–33). There is a brief (5 to 10 msec) **latency period** between the electrical stimulation and the first increase in tension. This corresponds to the time required for electrochemical coupling, Ca^{2+} binding to troponin, tropomyosin movement to allow myosin heads to cross-bridge with the G-actin binding sites, and stretch of the series elastic element. The maximum isometric tension occurs at about 150 msec after the stimulus, and the relaxation is complete after about 900 msec. The isometric contraction period is considerably shorter (150 msec) than the relaxation period (about 750 msec). For isotonic contractions, the time for maximum shortening is considerably longer than the time to maximum tension for an isometric contraction, and it is increased by elevated load.

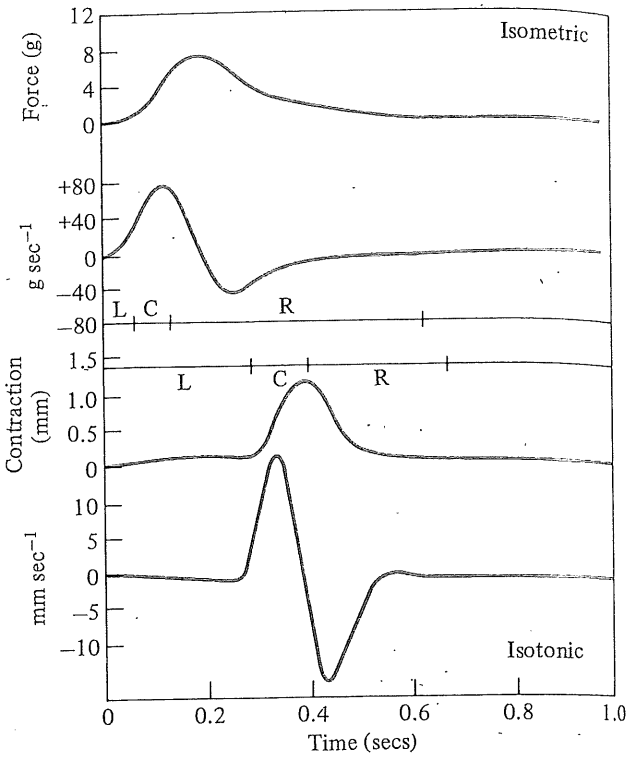


FIGURE 9–33 Single isometric and isotonic twitch for the gastrocnemius muscle of a marine toad *(Bufo marinus)* showing latency (L), contraction (C), and relaxation (R).

The contraction time varies markedly for different muscles. One functional classification of muscles is based on whether they are "fast" or "slow." The fastest mammalian muscle is the eye oculomotor muscle (5 to 6 msec contraction time). Some vertebrate muscles are extremely fast (e.g., the muscles of the puffer fish that are used for sound production; 1 to 1.5 msec). The soleus is an intermediate muscle (70 msec). The sloth claw retractor muscle is a slow muscle (150 to 300 msec). Many smooth muscles are very slow (e.g., gut, 100 to 300000 msec), although some are quite fast (e.g., trachea, 17 msec). Invertebrate muscles have a similar range in contraction time, from a few msec for fast striated muscles (e.g., cockroach coxal muscle) to 30000 msec (coelenterate smooth muscle). The tension generated by a muscle is generally related in an inverse fashion to the speed of contraction; fast muscles tend to have low tension development.

The rapid events at the beginning of a muscle contraction are quite complex. There is a very slight decrease in muscle tension after the stimulating action potential and towards the end of the latency

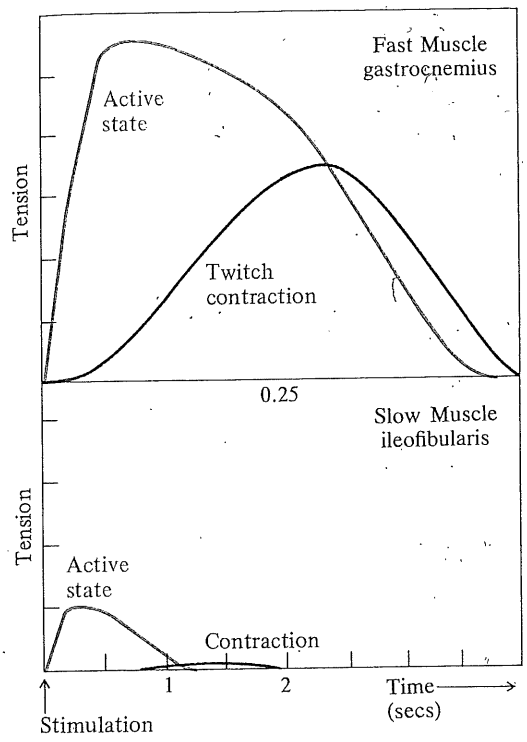


FIGURE 9–34 Active state, the actual contraction of sarcomere units, occurs rapidly after muscle stimulation; the mechanical development of tension occurs rapidly after muscle stimulation; the mechanical development of tension occurs more slowly, and achieves a lower maximal tension than does active state. The slower development, and lower maximal tension, is more pronounced for slow muscles (such as the frog ileofibularis and crayfish claw opener), than for fast muscle (such as the frog gastrocnemius and crayfish claw closing muscle).

period; this is **latency relaxation**. The tension rapidly returns to normal after the latency relaxation, then increases as the muscle begins to contract.

The period of force generation by a sarcomere is the **active state**. The intracellular Ca^{2+} is elevated, there is cross-bridge cycling, and ATP is hydrolyzed by the myosin ATPase during active state. In contrast, the cycle of tension generation by a single muscle twitch is considerably delayed compared to the active state of the sarcomeres, and the tension is considerably attenuated. The series elastic element of muscle is responsible for the delay and damping. Shortening of the sarcomeres first stretches the series elastic springs of the muscle. A high elasticity will greatly dampen the magnitude of a single twitch relative to maximal tetanic tension (which indicates the actual contraction force of the contractile units). For example, the ratio of single twitch tension to active state tension is about 0.2 for the frog sartorius muscle and 0.5 for the less elastic locust flight muscle; both have a considerable time delay to maximum twitch tension. The delay and attenuation are considerably greater in a slow muscle compared to a fast muscle. For example, the frog gastrocnemius muscle (a fast muscle) is still in active state at the end of its latency period and twitch tension is about 0.5 of active state tension (Figure 9–34). In contrast, a slow muscle (crayfish claw opener) has completed active state well before the end of the latency period and the twitch tension is much less than active state tension.

Muscle twitches are an "all-or-none" phenomenon. Successive twitches generate the same tension, as long as they are spaced at more than a minimal interval (Figure 9–35). Summation occurs if a second twitch is initiated while the muscle is still generating tension from the first twitch. The force of contraction is greater if the duration between successive stimuli is decreased. Both mechanical and contractile mechanisms contribute to summation. The first stimulus prestretches the elastic components of a muscle and allows a more rapid mechanical response to the second stimulus. An elevated intracellular Ca^{2+} due to the first stimulus will result in a higher Ca^{2+} in response to the second, hence a greater force of contraction.

A series of successive stimuli will cause continual summation of individually distinguishable twitches until a constant force of contraction is achieved

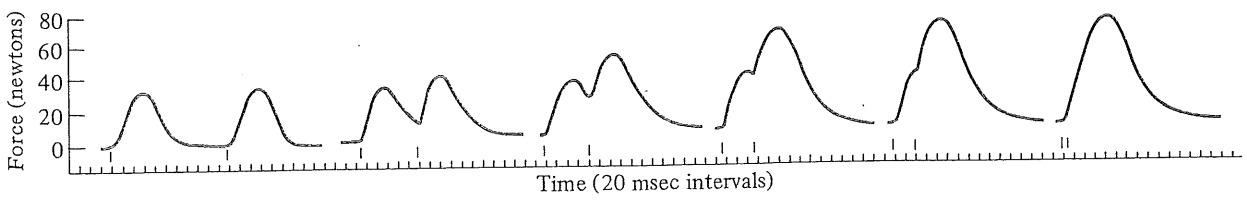


FIGURE 9–35 Mechanical summation of two muscle twitches by the cat gastrocnemius as a function of interval between double shocks. *(Modified from Cooper and Eccles 1930.)*

(although individual twitches can still be perceived); this is incomplete **tetany** (Figure 9–36). The individual contractions are no longer discernible and the muscle shows complete tetany if the frequency of stimulation is further increased to the fusion (critical) frequency. A fast muscle maintains discrete twitches at higher frequencies than does a slow muscle, i.e., the fast muscle has a higher fusion frequency.

Maximum force. The maximum force exerted by muscles varies widely, reflecting differences in their size rather than intrinsic properties. The maximum force expressed per cross-sectional area (F_{max}; N cm^{-2}) is relatively constant at about 10 to 30 N cm^{-2} for many vertebrate and invertebrate striated muscles (Table 9–3). Some muscles (e.g., mollusk catch muscles) can produce greater peak forces, over 100 N cm^{-2}. The remarkable tension that can be generated by these muscles is thought to be related to the presence of paramyosin, although paramyosin is not only found in molluskan catch muscle. It also occurs in byssal and pedal retractor muscles of bivalve mollusks, retractor muscles sipunculids and snails, the body wall of gordi worms, and the notochord of amphioxus. Paramy sin is also found in the striated and smooth musc of nematodes and various arthropods includi horseshoe crabs, spiders, scorpions, and insects.

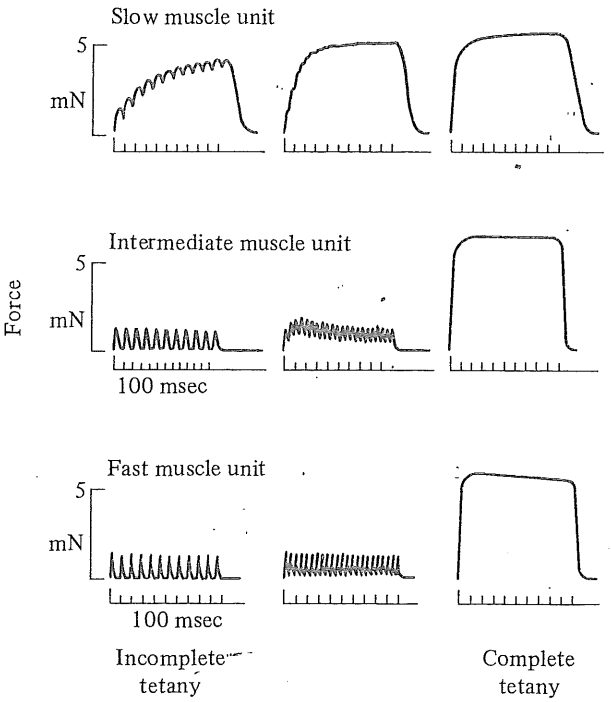


FIGURE 9–36 Isometric twitches and incomplete/complete tetany for a slow muscle unit and an intermediate muscle unit of a rat soleus muscle, and a fast muscle unit from a rat extensor digitorum longus. *(Modified from Close 1967.)*

Bivalve mollusks use one or more adduct muscles to close their shells against a springy elast hinge, and these adductor muscles can genera remarkably high peak tensions of 100 to 120 N cm⁻ The adductor muscles generally have a transluce (or colored or sometimes striated) phasic musc and a white, opaque tonic **catch muscle** (Hanson ar Lowy 1960). The catch muscles contain a lar amount of a special myofilament protein, paramy sin (Chantler 1983). The paramyosin molecules a rod-shaped, double α-helix coils of two polype tides. They form an organized array that acts as core which is covered by myosin molecules (perha organized as a single layer); the ratio of paramyosi to myosin can vary from 2:1 to 10:1 (by mass). Th myosin-paramyosin thick filaments are much longe and thicker than the typical myosin filament, bein 20 to 40 μ long and 60 to 80 nm diameter (cf. myosi of vertebrate striated muscle is 1.5 μ long and 1 nm dia).

The "catch" state is characterized by muscl rigidity unlike the flaccidity of the normal relaxe muscle. It was initially thought that catch did nc require metabolic energy expenditure, but ther actually is a low elevation of metabolism durin catch. For example, the resting metabolic rate c the anterior byssus retractor muscle of *Mytilus* i 75 μm O_2 g^{-1} min^{-1}, and in catch there is a 26 μr g^{-1} min^{-1} elevation in metabolic rate. In contrast phasic contractions (50 N cm^{-2}) elevate metaboli rate by up to 83 μm g^{-1} min^{-1}.

Although the catch mechanism is not fully under stood, the high force generated at low metaboli cost during catch appears to involve the stabilizatio of acto-myosin cross-bridges through some struc tural change of the paramyosin core of the myosi filaments, perhaps induced by paramyosin phospho rylation (Watanabe and Hartshorne 1990). Ther most likely is a slow cycling of the myosin-actii cross-bridges; normal muscle contraction involve a fast cross-bridge cycling. Stimulation of catcl muscle by cholinergic nerves increases the intracel lular Ca^{2+} and initiates a typical muscle contractior (Figure 9–37). There is a rapid cycling of myosin actin cross-bridges. Subsequent reduction of the intracellular Ca^{2+} concentration leads to catch in the absence of serotonin and to relaxation in the presence of serotonin. In the catch state, the muscle tension is maintained at a low metabolic rate. It is

TABLE 9–3

Maximum force developed by muscles (force per cross-sectional area). 1 N cm^{-2} = 0.10 kg cm^{-2}. *(Modified from Prosser 1973.)*

Animal	Muscle	Type	Maximum Force (N cm^{-2})
Rat	Heart	Cardiac	0.2
Lobster	Fast remotor	Striated	0.2
Toadfish	Sonic	Striated	1.0
Rabbit	Uterus	Smooth	1.3
Rat	Soleus	Striated	2.2
Cat	Papillary	Cardiac	7.8
Dog	Tracheal	Smooth	7.8
Cockroach	Coxal	Striated	7.8
Rabbit	Taenia coli	Smooth	8.7
Cat	Tenuissimus	Striated	13.7
Guinea pig	Taenia coli	Smooth	14.7
Sloth	Gastrocnemius	Striated	15.9
Rat	Gastrocnemius	Striated	17.6
Frog	Sartorius	Striated	19.6
Sloth	Diaphragm	Striated	20.6
Lobster	Slow remotor	Striated	27.4
Rat	Extensor digitorum	Striated	29.4
Oyster	Adductor	Catch	5.9 (tonic)
Oyster	Adductor	Catch	117.6 (peak)

not clear how catch is maintained; perhaps the large size and the high number of cross-bridges of the myosin/paramyosin complex confers the capacity to "catch." Stimulation of serotoninergic nerves releases serotonin and elevates the intracellular cAMP level. The high cAMP activates a protein kinase, which may phosphorylate paramyosin and myosin to induce relaxation.

Velocity, power, and energy. The average velocity (V) of contraction during an isotonic muscle twitch depends on the contraction time (Δt) and the distance shortened (ΔS); $V = \Delta S/\Delta t$. For an isometric contraction, ΔS is 0 so there is no velocity of shortening; however, the rate of change in tension ($\Delta F/\Delta t$) is an analogous measure of muscle contractile activity.

The velocity of shortening increases during an isotonic contraction to a maximum value about halfway through the contraction time, then declines to zero at the end of the contraction time. The velocity is negative during the relaxation time (i.e., the muscle lengthens rather than shortens) and is greatest about halfway through relaxation time. The maximum rate of shortening is generally higher than the maximum rate of lengthening because the contraction time is shorter than the relaxation time. The velocity of shortening depends on the load (F) against which the muscle has to work, i.e., the weight that the muscle has to lift (Figure 9–38). The velocity of shortening is less for greater forces. There is a hyperbolic relationship between force and and velocity (see Supplement 9–2, pages 445–447). The power (P) output of a muscle can be readily calculated from the $V - F$ diagram as force × velocity. It is zero at no velocity (isometric) and maximum velocity (isotonic, no load).

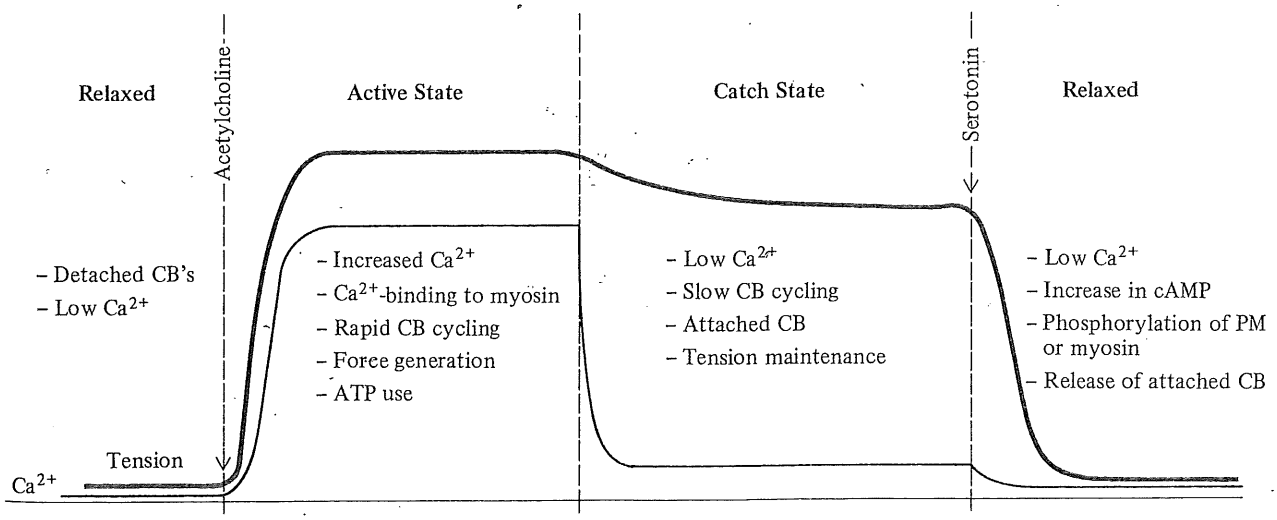


FIGURE 9–37 Possible model for contractile tension generation, "catch," and relaxation of catch muscle. *(Modified from Watanabe and Hartshorne 1990.)*

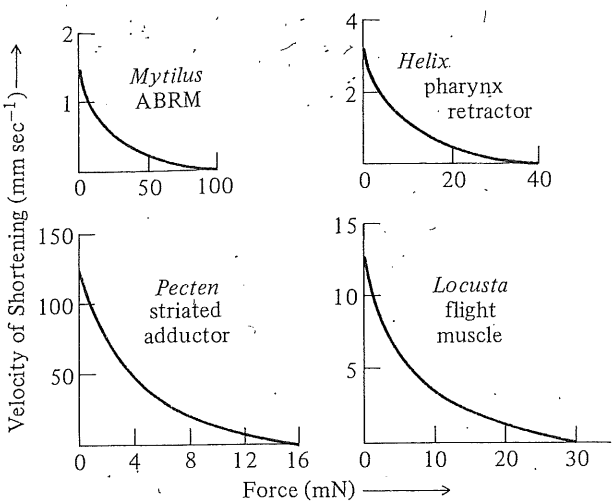


FIGURE 9–38 Hyperbolic force-velocity curves for a variety of vertebrate and invertebrate muscles: *Mytilus* anterior byssal retractor muscle, *Helix* pharynx retractor muscle, *Pecten* striated adductor muscle, and *Schistocerca* flight muscle. *(Modified from Hanson and Lowy 1960.)*

The energy expended by a muscle during a contraction can be measured by the heat produced or the depletion of high energy stores (e.g., ATP or creatine phosphate). Changes in the chemical energy balance of muscle cells (i.e., phosphagen levels, particularly creatine phosphate) can be related to total energy expenditure (work done + heat liberated). A value of 46.4 kJ mole^{-1} creatine phosphate is obtained whether the muscles contract isometrically (twitch or tetany) or isotonically, performing positive or negative work.

There is a complicated pattern of heat release by a muscle during and after a twitch. There is an initial, rapid release of heat soon after the initiation of a tetanic, isometric contraction even before tension is generated (Figure 9–39); this is the **activation heat** (H_a). The muscle soon reaches a steady-state rate of heat production, the **stable heat** (H_s). A third component of heat production, the **labile heat** (H_l), decays after about 3 to 4 msec. After the tetanic contraction ceases and the muscle relaxes, there is a slow but prolonged release of **recovery heat**, which can be approximately equal in magnitude to the total contraction heat. The activation heat may be due to Ca^{2+} release, Ca^{2+} binding to troponin, and rearrangement of the tropomyosin, as well as the internal work done in shortening the sarcomeres and taking up any slack in the series elasticity (there is some sarcomere shortening even if a muscle contraction is isometric). The stable (and perhaps labile) heat is the energy required to maintain steady-state tension. The recovery heat reflects the bioenergetic cost for removal of lactate that accumulated during the contraction.

The energy expenditure of a muscle is not equal to the mechanical work done, partly because of biochemical inefficiency (aerobic metabolism is only about 41% efficient; see Chapter 3) and partly for mechanical reasons. The **contractile mechanical efficiency** is the ratio of mechanical work done to energy expended for the contractile process. It is difficult to measure the contractile mechanical efficiency because many other energy-requiring processes occur simultaneously. The contractile mechanical efficiency is 0% for an isometric contraction and for an isotonic contraction against zero load and increases to a maximum at intermediate loads. Mammalian fast muscle (e.g., biceps brachii) has a maximum contractile mechanical efficiency of about 55% at a fast shortening velocity of about 5 muscle lengths sec^{-1} (Alexander and Goldspink 1977). A slow, phasic muscle, such as the soleus, has a maximal contractile efficiency of about 75% at a shortening velocity of about 1.5 muscle lengths sec^{-1}. Maximal contractile efficiencies of 75 to 80% have been measured for tortoise muscles and values up to 45% for frog and toad muscles. Thus, energy transduction by cross-bridges into mechanical work can be a highly efficient process. In contrast, the **overall mechanical efficiency** of muscle, the ratio of mechanical work done to total energy expenditure,

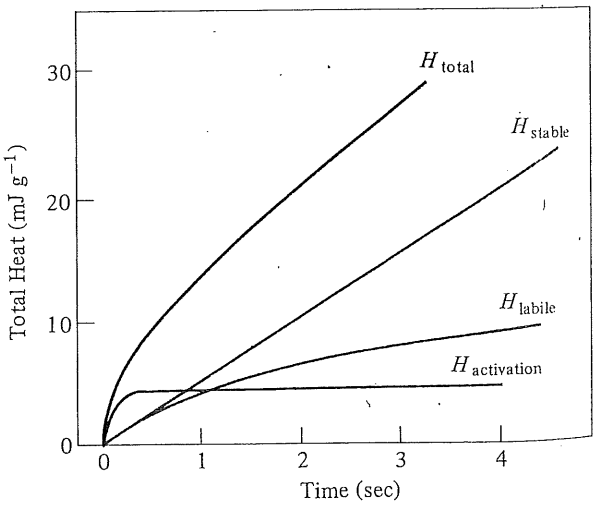


FIGURE 9–39 Total heat production by a toad muscle during an isometric contraction showing the various components: activation heat, stable heat, and labile heat. *(Modified from Carlson and Wilkie 1974.)*

is considerably lower because many processes other than cross-bridge cycling require energy expenditure. The maximal overall mechanical efficiency is generally 10 to 35%; e.g., 28% for a human pedaling a bicycle, 30% for *in vitro* muscle, 35% for tortoise muscle, and 10% for insect flight muscle.

Determinants of muscle properties. As we have seen, there are three general types of muscle: skeletal, cardiac, and smooth. However, there are many specific biochemical and mechanical differences for muscles even within one of these three general types. For example, vertebrate striated muscle fibers vary in their major structural/mechanical properties and are divided into tonic and phasic fibers. **Tonic fibers** have very slow conduction; they generally do not propagate a sarcolemmal action potential and require multiple stimulations to contract and can respond with a graded (not all-or-none) contraction. They normally are involved with maintaining posture rather than locomotory movement. **Phasic fibers** are either slow or fast fibers. Fast phasic fibers can be either oxidative or glycolytic. Slow phasic fibers (red muscle) contract relatively slowly (but faster than tonic fibers) and are slow to fatigue. Fast phasic glycolytic fibers (white muscle) are fast contracting and fatigue rapidly. Fast phasic oxidative fibers are fast contracting and fatigue more slowly than the fast phasic glycolytic fibers. There are numerous differences between these skeletal muscle fiber types in innervation pattern, electrical properties of the sarcolemma, enzymatic properties, metabolic capacity, and blood flow pattern (Table 9–4).

Differences in structure and organization of the contractile machinery can contribute to differences in mechanical properties of muscle cells, but the basis for many of the functional differences is the innervation pattern; the biochemical properties of muscle cells are determined by the innervation. The properties of fast muscle are induced by the nature of its innervating axon; "fast" axons innervate fast muscles, and "slow" axons innervate slow muscles. Denervation of a fast muscle and reinnervation by a slow axon will induce slow muscle properties.

Skeletal muscle fibers are organized as motor units, and all fibers of one motor unit are of the same type. A muscle will generally contain motor units of all types (Figure 9–40), although the proportions vary. For example, the locomotory muscles of mammals with rapid, sustained activity (e.g., wolves, dogs, ungulates) have a high percentage of fast oxidative fibers; the lion, in contrast, has fast glycolytic fibers. Fast-cruising fish (mackerel, tuna) have a high percentage of slow oxidative fibers, whereas stealthy, rapidly-striking fish (pike) have a high percentage of slow phasic fibers. The recruitment of different type units in a muscle is probably hierarchical, i.e., slow fibers are activated first, then fast oxidative fibers, then fast glycolytic fibers.

Invertebrate striated muscle differs from vertebrate striated muscle in fiber size and innervation pattern. It has relatively few, but large, fibers that are innervated by a small number of motoneurons;

TABLE 9–4

Types of phasic vertebrate striated muscle fiber and some of their important metabolic and biochemical properties. *(Modified from Goldspink 1977.)*

	Fast Phasic Glycolytic	Fast Phasic Oxidative	Slow Phasic Oxidative
Structure			
Mitochondrial content	Low	High	Intermediate
Z line	Narrow	Wide	Intermediate
Neuromuscular junction	Large, complex	Small, simple	Intermediate
Enzymes			
Oxidative activity	Low	High	Intermediate/high
Glycolytic activity	High	Low	Intermediate
Myofibrillar ATPase	High	High	Low
Glycogen content	Intermediate	High	Low
Myoglobin content	Low	High	High
Mechanical			
Contraction velocity	Fast	Fast/intermediate	Slow
Fatigue time	Very short	Fairly long	Long
Efficiency	Fairly high	?	High

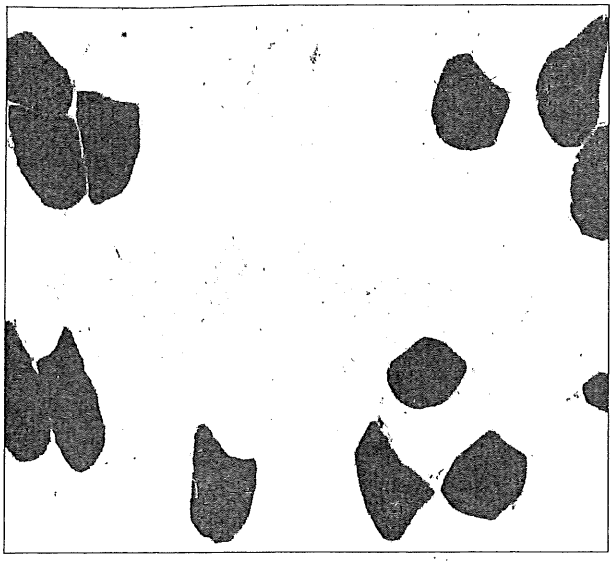


FIGURE 9–40 A human muscle illustrates the presence of different types of muscle fibers in a muscle. The different staining appearance of three fiber types is accomplished by special histochemical staining of ATPase. *(Courtesy of P. C. Withers and K. Cole.)*

an entire muscle may be innervated by as few as two motoneurons. The motoneurons are either excitatory or inhibitory (they are only excitatory in vertebrate muscle). There is considerable variation between muscles, even for different muscle cells in the same muscle in the innervation pattern. For example, there may be one inhibitory, one fast and one slow innervation; or two fast; or one inhibitory and one fast; or two slow; or two slow and one fast. One motoneuron may stimulate two different muscles, e.g., the opener and stretcher muscles of the crayfish claw share a single excitatory motoneuron; which muscle responds to nerve stimulation can depend on the frequency of stimulation. The closer, bender, and extensor muscles of the crab claw have one slow, one fast, and one inhibitory axon.

Electric Organs. Electric organs are muscle (or nerve) cells that are specialized for producing external electric fields. About eight different groups of fish have evolved electric organs (Table 9–5).

Strongly-electric fish can produce an external electric field of sufficient current and voltage to stun prey or deter predators, e.g., electric and torpedo rays can produce up to 60 V and 1 kW power, the electric catfish more than 300 V, the electric eel over 500 V. Strongly-electric fish tend to have simple, discrete monophasic impulses of high-power output (Figure 9–41A). **Weakly-electric** fish use their electric organ as an electrosensory system, for communication, and to locate objects. They have a lower voltage, current, and power discharge, which is either a "pulse type" with a rapid (5 to 120 msec) series of short pulses (0.5 to 2 msec duration) or a "wave type" of a continuous low frequency (Figure 9–41B), a constant high frequency (Figure 9–41C), or a variable frequency (Figure 9–41D).

The **electrocytes** are the cells of the electric organ that generate the current/voltage discharge. The electrocytes are modified muscle cells in all electric

TABLE 9–5

Types of electric fish, their distribution, and the nature of their electric discharge. *(Modified from Bennett 1971.)*

Common Name	Family	Distribution	Electric Organ Discharge
Skates	Rajidae	Marine	Weak pulse
Mormyrids	Mormyridae	Freshwater	Weak pulse
Gymnarchus	Gymnarchidae	Freshwater	Weak wave
Gymnotid eels	Gymnotidae, Sternopygidae, Rhamphichthyidae, Apteronotidae[1]	Freshwater	Weak pulse and wave
Stargazers	Uranoscopidae	Marine	Strong (5 V) pulse
Electric rays	Torpedinidae	Marine	Strong (60 V) pulse
Electric catfish	Malapteruridae	Freshwater	Strong (300 V) pulse
Electric eel	Electrophoridae	Freshwater	Strong (>500 V) pulse

[1] Neurogenic electric organ.

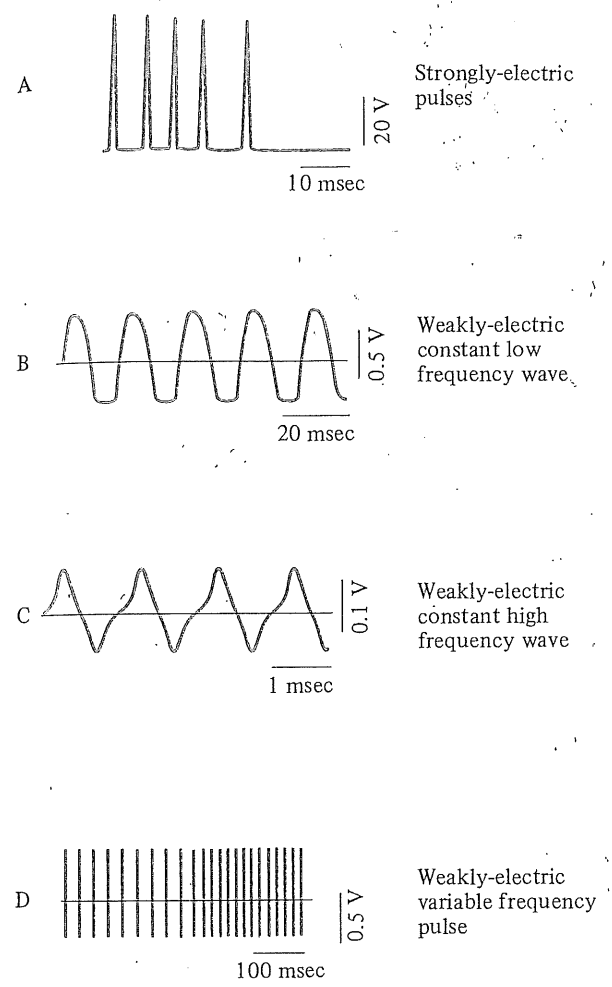


FIGURE 9–41 Pattern of electrical discharge by the electric organ of the following: **(A)** Pulses of the strongly-electric catfish *Malapterurus*. **(B)** Low-frequency, wave-type continuous discharge of a weakly-electric gymnotid, *Sternopygus*. **(C)** Constant, high-frequency wave discharge of the gymnotid, *Sternarchus*. **(D)** Variable frequency pulses of the gymnotid *Gymnotus*. *(Modified from Bennett 1969.)*

fish except apteronotids (sternarchids), which have electric organs formed by neurons. Myogenic electrocytes are often flattened, disk-shaped cells although some cells are cup shaped or have other complex shapes. Electrocytes produce electric fields using the same principles of bioelectricity already described for nerve and muscle cells, i.e., ion concentration gradients and selective ion permeability. A resting electrocyte has no external current flow (I_{ext}) or internal current flow (I_{int}) because the resting membrane potential of opposite sides of the electrocyte are equal but opposite in polarity (Figure 9–42). There is a constant, but low-current flow across each cell membrane (due mainly to K^+). The nerve innervating the electrocyte synapse depolarizes the adjacent cell membrane but not the opposite cell membrane. This creates an external electrical gradient and external current flow (I_{ext}) as well as an intracellular current flow (I_{int}). Some electrocytes have a more complex generation of their electric field, including a role of the noninnervated membrane and cell stalks. The high voltage and current discharge of strongly-electric organs is not due to an extraordinary membrane potential change, but to a combination of (1) the anatomical arrangement and synchronous activity of hundreds or even thousands of electrocytes to maximize the external field, (2) a low-electrical membrane resistance of electrocytes, and (3) accessory structures that channel external current flow.

The electric organs of the three marine groups of strongly-electric fish, electric rays (rajids), torpedo rays (torpedinids), and stargazers (*Astroscopus*; Figure 9–43) use the simple mechanism described above for electric organ discharge. The electric organ is densely innervated by numerous branches of the oculomotor nerve. The electrocytes are large, flattened cells horizontally arranged in about 150 to 1000 layers of cells that are stacked to form long columns. The dorsal, innervated membrane of the electrocyte depolarizes during organ discharge, but

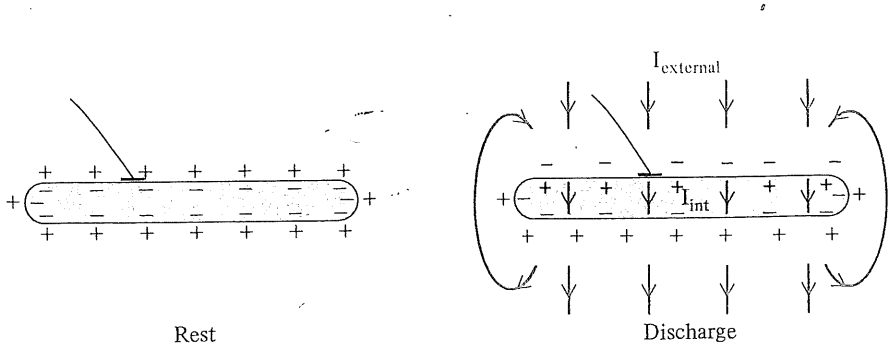


FIGURE 9–42 Representation of a simple type of electrocyte from an electric discharge organ showing the pattern of membrane potential at rest (left) and during discharge (right). During discharge, there is an intracellular current flow (I_{int}) and an extracellular current flow (I_{ext}).

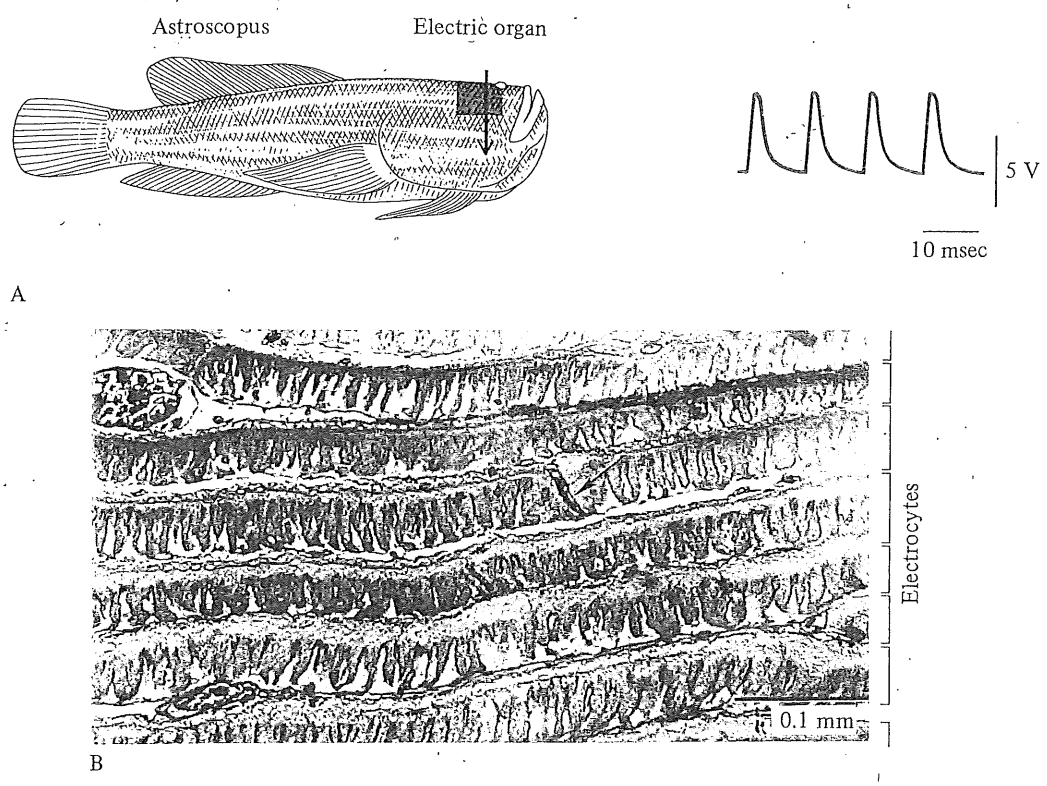


FIGURE 9–43 (**A**) Location of the electric organ and an electromicrograph of the stacked electrocytes of the electric organ for the strongly-electric stargazer *Astroscopus*. (**B**) The smooth dorsal membrane (top) is innervated; two innervating nerve bundles can be seen towards the left. Arrow indicates a probable junction between adjacent electrocytes. *(Modified from Bennett 1969.)*

the ventral membrane is not innervated and does not depolarize.

The strongly-electric silurid catfish *Malapterurus* has two electric organs, each containing millions of electrocytes. Each electrocyte is a flat disk with a central stalk that is innervated by a single giant neuron and axon. The stalk side of the electrocyte faces posteriorly. The electric organ discharge is a brief, 1 to 2 msec, high-voltage (up to 350 V) pulse. The depolarization of the posterior stalk face of the electrocytes is responsible for an initial small head positive potential, but the nonstalk (anterior) side of the electrocyte rapidly depolarizes and creates a strong negative anterior discharge.

The freshwater fish of tropical South America include the electric eel, the best-known strongly-electric fish, and a wide variety of weakly-electric fish. The electric eel *Electrophorus* emits monophasic pulses of either low amplitude (10 V) for electrosensory detection or high amplitude (100 to 500 V, depending on the length of the eel) for offensive and defensive actions. *Gymnotus* is a weakly-electric freshwater fish with a pulse discharge of about 0.5 V amplitude and 1 msec duration at about 50 sec^{-1}. The apteronotid (sternarchid) fishes, a group of about nine genera including *Apteronotus*, are also freshwater weakly-electric fish. They have the highest frequencies of diphasic wave discharge of all electric fish (700 to 1700 sec^{-1}). Their electrocytes are spinal neurons rather than modified muscle cells (see Supplement 9–3, pages 446–447). The South American weakly-electric rhamphichthyid fish are poorly known. The electric organ of *Gymnorhamphichthys* resembles that of sternopygids. It emits pulses at a fairly constant rate of 10 to 15 sec^{-1}.

The weakly-electric African fish *Gymnarchus* is closely related to the more numerous mormyrid fish but superficially resembles the South American gymnotids. Its electric organ discharge is a wave about 1 msec duration and 1 V amplitude; the discharge is head positive during pulses and head negative between pulses. The mormyrids are weakly-electric fish of tropical Africa. Their electrocytes discharge with irregular pulses (1 to 2 V, 0.5 to 1 msec; Figure 9–44A). The organ consists of four columns of electrocytes, each containing 100 to 200 cells. The electrocytes often have complex posterior stalks that fuse into a lower number of stalks before

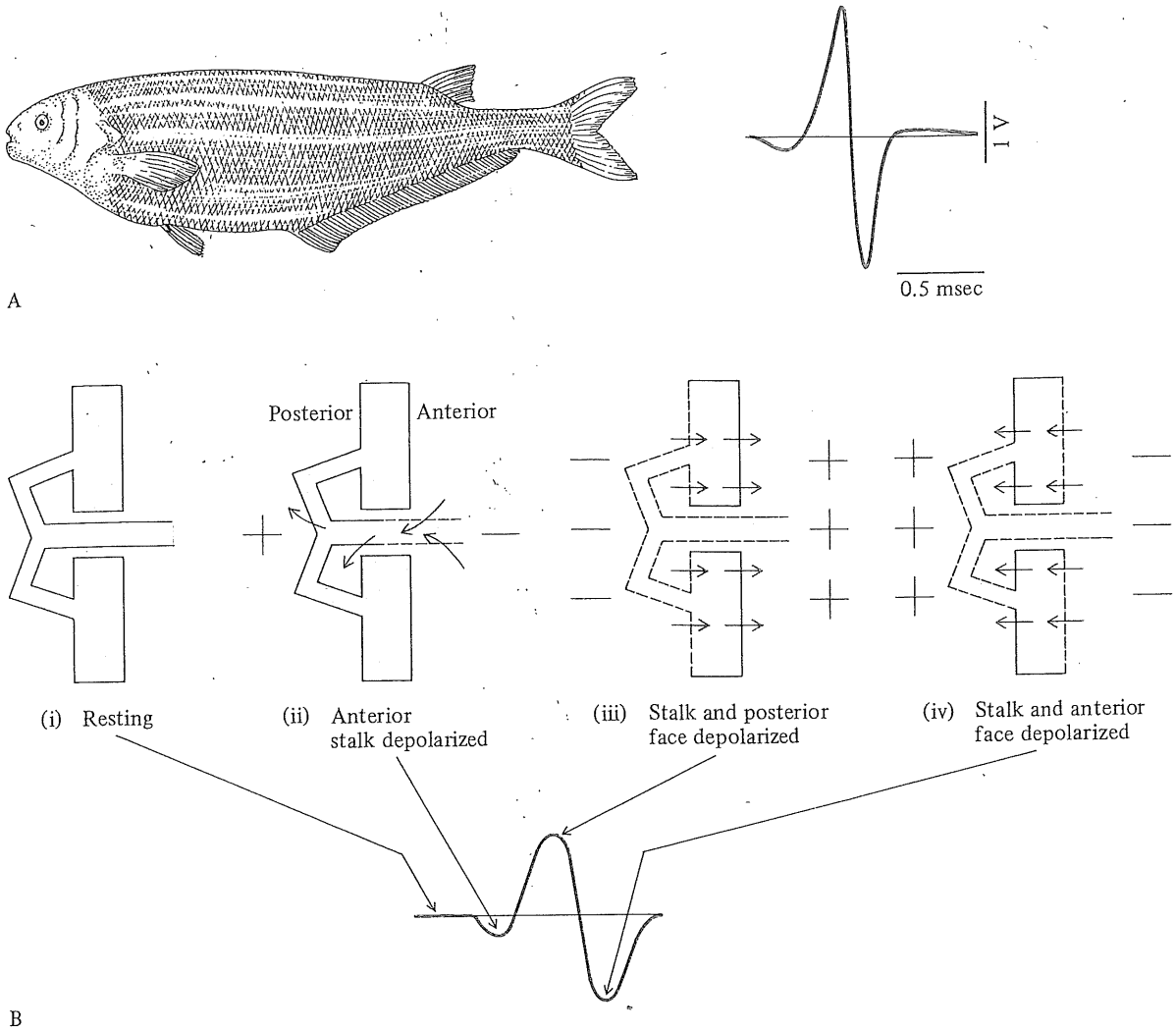


FIGURE 9–44 **(A)** The weakly-electric mormyrid fish *Hyperopisus* has a triphasic electric organ discharge. **(B)** The electric organ discharge has a triphasic discharge pattern due to a complex pattern of depolarization by the complicated, penetrating stalk and the anterior and posterior membranes of the electrocytes. *(Modified from Bennett 1969.)*

being innervated. In some species, the stalks pass anteriorly through holes in the electrocytes before being innervated. The form of the electric organ discharge depends on the extent of stalk penetration, varying from biphasic (no penetrating stalks) to triphasic (many penetrating stalks). In some species with many penetrating stalks, the head positive phase of electric discharge is caused by activity passing through the stalk from the anterior to the posterior face of the electrocyte (Figure 9–44B). Head positivity follows when the impulse from the stalk exits the posterior face; head negativity is later produced by excitation of the anterior face. Stalked electrocytes have independently evolved in South American and African weakly-electric fish.

The capacity of strongly-electric fish to emit high-voltage potentials depends on their ability to synchronously discharge many hundreds or even thousands of electrocytes. The control of electrocytes by the central nervous system involves two neurons in the first spinal segment, each of which controls all of the electrocytes on one side of the body. The two neurons are tightly electrically coupled to synchronize their outputs. The weakly-electric gymnotid and mormyrid fish have more complex central control because electric discharge is more continuous and requires a pacemaker to determine the characteristic frequency. In gymnotids, there are 30 to 200 pacemaker cells in the midline of the medulla that activate a relay center

of about 50 neurons, which in turn controls hundreds or thousands of spinal neurons to the electric organs. The pacemaker cells have an inherent rhythm of depolarization to threshold, firing, hyperpolarization, and then depolarization to threshold. In contrast, the relay neurons have stable resting membrane potentials and respond only to input from the pacemaker cells. The mormyrid fish have an even more complex neural control of their electric organ. Bilateral nuclei in the midbrain contain the pacemaker cells; one pacemaker is dominant, and the subordinate pacemaker nucleus is entrained to the rhythm of the dominant one. These pacemaker nuclei relay to a midline medullary nucleus thence to the spinal nerves. Mormyrids also have a connection between the sensory processing part of the brain and the pacemaker command nucleus that allows the sensory system to be prepared for when afferent information will be provided in response to pulses from the electric organ. The discharges can vary in frequency (audibilized pulses from a resting mormyrid sound like a geiger counter, with irregular electric organ discharges). Gymnotids lack this neural connection and have a constant wave discharge of the electric organ.

A further complication in ensuring synchronization of the numerous electrocytes is their variable distance from the medulla and spinal cord centers. Conduction time should be quicker to the more anterior electrocytes, hence they would be expected to discharge before more posterior electrocytes. This potential desynchronization can be minimized by either having a more tortuous pathway of axons to the anterior electrocytes or a lower conduction velocity to anterior electrocytes.

Summary

The cell cytoskeleton and mechanisms for cell movement depend on three general types of structural protein filaments: microtubules, microfilaments, and intermediate filaments. Microtubules are tubular fibers consisting of 13 protofilaments of α and β tubulin subunits. Microfilaments (actin and myosin) are helically twisted strands of subunits. Intermediate filaments (keratin, neurofilaments, desmin, vimentin) are complex arrangements of α-helical coiled protein subunits.

Microtubular movement can be due to tubular synthesis or breakdown, or enzymatic "motors" that move membrane particles along microtubules, or are responsible for the sliding of adjacent microtubules. Axoneme dynein is a "motor" ATPase responsible for the bending of cilia and flagella. These cell organelles beat with undulating waves (flagella) or a power stroke and recovery stroke (cilia). Th sliding filament model of ciliary and flagella beatin is supported by microscopical and biochemical evi dence.

Microfilament movement by actin and myosin i best understood for the sarcomeres of muscle cells but many other types of cell movement involv cytoplasmic actin and myosin, e.g., cleavage durin cell division, amoeboid movement. F-actin is filamentous polymer of globular G-actin subunits Myosin-1 is a rod-shaped molecule of two helicall twisted protein subunits, each with a globular hea region that has ATPase activity and binds to actin Myosin molecules are often aggregated to form thic myosin microfilaments (myosin-2). Microfilamen movement in striated muscle is a sliding of adjacen actin and myosin molecules by the repeated forma tion of cross-bridges between the myosin heads an the G-actin binding sites. The myosin head binds t the actin binding site and undergoes a conforma tional change and rotates, thereby sliding the acti past the myosin. Detachment of the myosin hea and ratcheting of the myosin head to its origina conformation requires ATP.

Control of actin-myosin interaction generally de pends on the presence of other proteins attached t actin (tropomyosin, troponin, calmodulin, twitchin and the regulation of the free intracellular Ca^{2+} concentration. In muscle, Ca^{2+} activates the actin myosin interaction by one (or both) of two genera mechanisms. In actin-activated systems, the Ca^{2+} binds to troponin; this induces a conformationa change in the tropomyosin and uncovers the G-acti binding sites, allowing the myosin heads to bind. I myosin-activated systems, the Ca^{2+} regulates th ATPase activity of the myosin by the mediation o a small regulatory protein bound to the myosin.

Excitation-contraction coupling of striated mus cle primarily involves the t-tubules and sarcoplasmi reticulum (SR). The SR sequesters Ca^{2+} and nor mally maintains a low cytoplasmic Ca^{2+} concentra tion ($<10^{-7}$ M). Electrotonic depolarization of th t-tubules induces Ca^{2+} release from the SR. Ther are several hypotheses for the coupling of t-tubula depolarization and Ca^{2+} release, including Ca^{2+} induced opening of SR Ca^{2+} channels. In smoot muscle, opening of the SR Ca^{2+} channels is due t inositol 1,4,5 triphosphate (IP_3), rather than bein Ca^{2+} induced.

A muscle maintains a constant length during a isometric contraction but shortens against a constan force in an isotonic contraction. The mechanica properties of muscle cells reflect their viscous resist ance and also the elastic properties of their cellula and subcellular materials. The series elastic elemen is the sum of all elastic elements in series with th

contractile units of the muscle cell. The parallel elastic element is the total effect of all elastic materials in parallel with the contractile units. Different muscles vary greatly in mechanical properties; some are very stiff and highly inelastic, e.g., insect flight muscle. Others are very stretchy, with a marked capacity for shape change, e.g., smooth muscle.

Single muscle twitches can summate if successive stimuli occur more frequently than a minimal interstimulus duration. Repetitive summation results in incomplete tetany or complete tetany if the stimuli occur so frequently that the muscle does not noticeably relax between stimuli. The maximum tension generated by tetanized muscles is generally 10 to 30 N cm^{-2}. Some muscles, such as bivalve mollusk catch muscle, can generate much higher peak tensions (up to 100 N cm^{-2}) and maintain tension at a low metabolic cost. There appears to be either a reduced cycle time for actin-myosin cross-bridges, or a complete latching of cross-bridges during "catch."

The velocity of shortening by a muscle is inversely related to the load. The power expenditure, calculated as force × velocity, is zero at no load (maximal velocity) and maximal load (no velocity), and is maximal at an intermediate velocity and load. There is a similar-shaped relationship between mechanical work done and velocity. The maximum contractile mechanical efficiency of muscle is high, at about 70 to 80% of the energy expended for contraction, but the overall mechanical efficiency for a muscle contraction is 20 to 30% of the total energy expenditure by a muscle.

Electric fish generate substantial electrical discharges for either intraspecific communication or electrolocation (weakly-electric fish) or for stunning prey and repelling predators (strongly-electric fish). The electric organ discharge is either a variable rate of pulses (strongly-electric and some weakly-electric fish) or a constant frequency wave discharge (some weakly-electric fish). The electrocyte cells of electric organs are generally modified muscle cells (but are modified neurons in apteronotid fish). The electric organ discharge is the summed depolarization of many (even thousands) of electrocytes arranged in series. Each electrocyte has an action potential-like discharge (about 90 mV), and the summed discharge can be a few volts or many volts. The discharge can be monophasic, biphasic, or triphasic, depending on the arrangement of electrocytes in the electric organ and their pattern of innervation and depolarization. Many electric fish have complex central nervous system involvement with the generation of electric organ discharges (pacemaker command centers) and the coordination of sensory reception with electric organ discharge (sensory gating).

Supplement 9–1

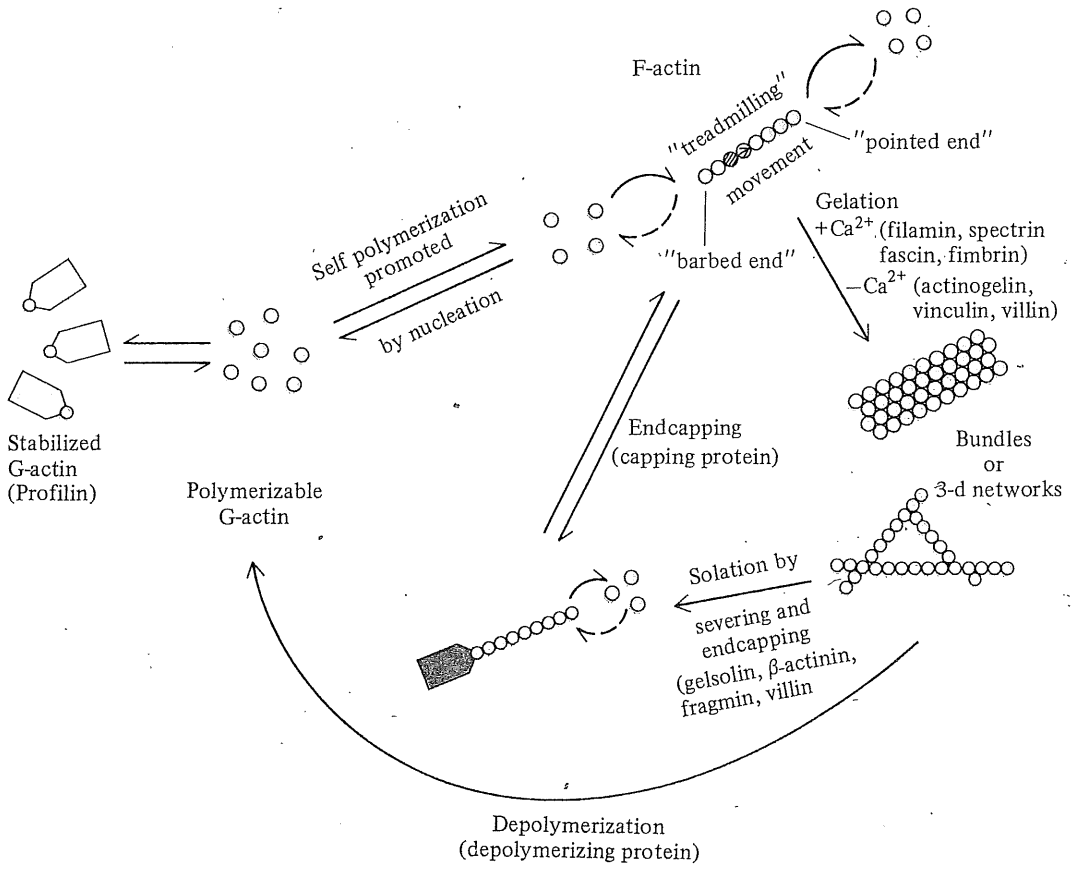
Dynamics of Actin

Actin is a ubiquitous intercellular microfilament that is involved in cell movement. Cytoplasmic actin appears to be in a dynamic state. Polymerization of G-actin into F-actin filaments occurs spontaneously at nucleation sites. Polymerization can be inhibited by proteins, called profilin. Self polymerization of G-actin forms filamentous strands of F-actin, each end of which may be in dynamic equilibrium between G- and F-actin. Different ends of F-actin may have differing rates of polymerization and depolymerization. For example, the so-called "barbed end" of F-actin may have a higher rate of depolymerization than polymerization; in this circumstance, the G-actin subunits "treadmill" from the pointed end, with a higher rate of polymerization than depolymerization, to the "barbed end," with a higher rate of depolymerization than polymerization.

F-actin can be organized into bundles or 3-dimensional networks by the process of gelation. A variety of proteins can cause gelation; some require Ca^{2+} ions (e.g., filamin, spectrin, fascin, fimbrin, nonmuscle α-actinin), whereas others do not (e.g., muscle α-actinin, actinogelin, vinculin, villin). Filamins are strand-shaped, actin-binding proteins of smooth muscle and other cells (e.g., blood cells: macrophages, neutrophils, platelets) that induce polymerization and cross-linking of actin. Spectrin is a similar protein of red blood cells; α-actinins are rod-shaped actin cross-linking proteins. In striated muscle, α-actinin is located in the Z bands of sarcomeres where the actins are interconnected. In nonmuscle cells, α-actinin is involved in binding actin to membrane surfaces possibly by cross-linking and spacing the actin filaments. Vinculin may also be involved with binding actin to membranes. Gelated actin can be depolymerized by "depolymerizing protein" or severed into smaller microfilaments by solation proteins (e.g., gelsolin, α-actinin, fragmin, villin). Actin fragments can be stabilized by capping proteins that bind to the end and prevent further polymerizing ("end-capping proteins"). Some properties of these various proteins involved with the dynamics of actin structure are summarized in the following table.

Actin-binding proteins (excluding those from muscle cells). *(See Weeds 1982.)*

Type	Ca^{2+} Sensitivity	Type	Ca^{2+} Sensitivi
Gelation Proteins		**Severing/Capping Proteins**	
Filamin	No	α-actinin	No
Spectrin	No	Gelsolin	Yes
Gelation and Bundling Proteins		Villin	Yes
α-actinin	Yes/No	Fragmin	Yes
Actinogelin	Yes	Capping Protein	No
Vinculin	Yes/No	Depolymerizing Protein	No
Bundling Proteins		**G-Actin Stabilizing Proteins**	
Fascin	No	Profilin	No
Fimbrin	No		



Dynamics of actin polymerization and depolymerization.

$$W = \int_{s0}^{s} \text{Force}.ds = F\Delta s$$

Hill's equation for the relationship between force and velocity can be rearranged to calculate the work done.

$$W = bFt(F_0 - F)/(F + a)$$

The maximum work can be shown to be done when

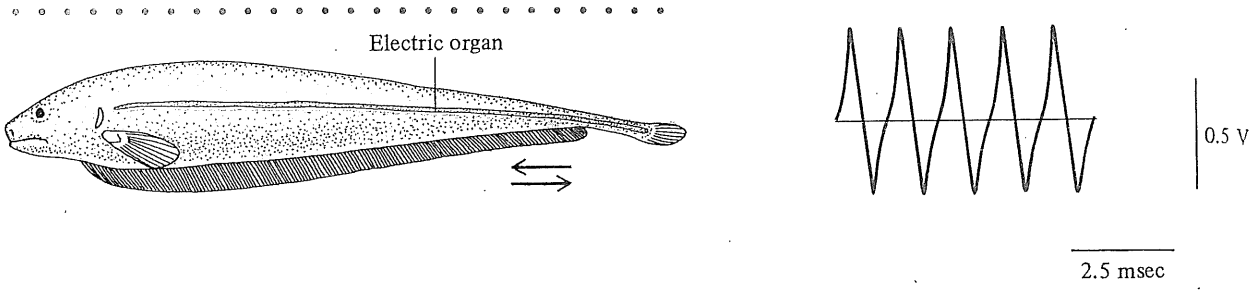
$$F = \sqrt{a(F_0 + a)} - a$$

Hence, mechanical work is maximal when $F \approx 0.31\ F_0$, since $a \approx 0.25\ F_0$.

Muscle efficiency, defined as 100 mechanical work/total energy released, increases from 0% at $F/F_0 = 0$ and 1, to a maximum at intermediate F. This efficiency curve has a very broad top, from about $F = 0.24$ to $0.7\ F_0$ for >90% of maximal efficiency. The maximum efficiency is about 25 to 30% for many muscles. (*See White 1977.*)

Supplement 9–3

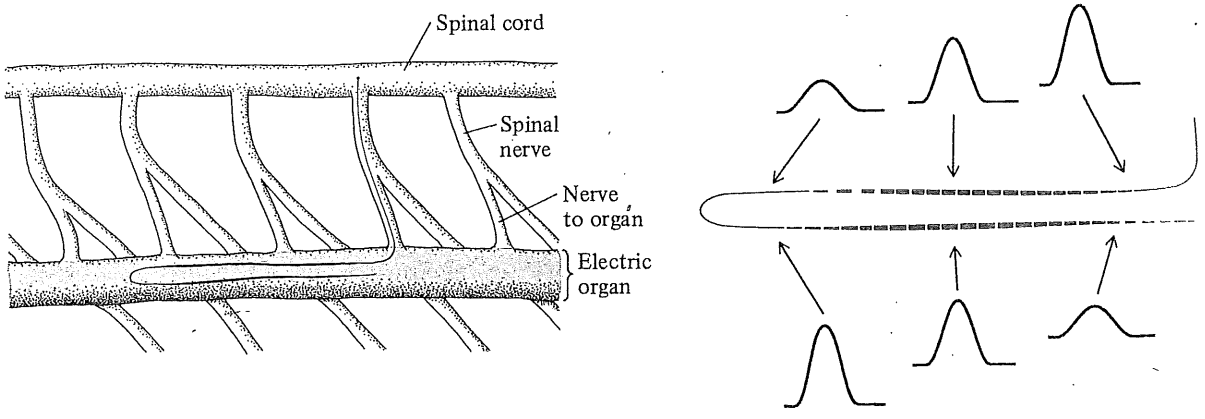
Neurogenic Weakly-Electric Fish



Location of the neurogenic electric organ and its discharge pattern for the weakly-electric fish *Apteronotus*. *(Modified from Bennett 1969.)*

The apteronotid (sternarchid) weakly-electric freshwater fishes of South America have neurogenic, not myogenic, electric organs and a continuous, wave-type electric organ discharge, e.g., *Apteronotus*. The discharge is a diphasic wave (initially head positive) with an exceptionally high frequency (700 to 1700 sec^{-1}, depending on the temperature and the species) for weakly-electric fish.

The electrocytes are highly modified spinal nerves. Spinal nerves enter the electric organ, run anteriorly for a few segments, then turn and run posteriorly to about



The electrocyte neurons pass from the spinal cord to the electric organ via spinal nerves and form a U-shaped terminus that produces the electric organ discharge (only one such electrocyte is shown). *(Modified from Bennett 1969.)*

the level at which they entered the electric organ. The myelinated spinal nerves are of normal size where they enter the electric organ (10 to 20 μ dia) but dilate to as much as 100 μ at the center of the anterior and posterior portions within the electric organ, then taper gradually until they terminate. Intracellular electrical recordings from the modified nerve fiber indicate a large amplitude action potential where the axon enters the electric organ but a smaller amplitude and delayed action potential as it travels anteriorly along the axon. The central dilated portion of the axon is presumably inexcitable and the depolarization spreads electrotonically. This part of the discharge cycle produces the head positive potential. The posteriorly running portion of the axon also conducts an impulse anteriorly; the magnitude is greater for the more anterior portions. Its impulse is delayed compared to that of the anteriorly running portion of the axon and is presumably caused by excitation from the anterior running segment of the axon. It generates the second part of the electric discharge, the head negative potential. Current runs posteriorly from the large anterior depolarization to the smaller posterior depolarization. (*Modified from Bennett 1971.*)

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Supplement 9–2

Muscle Force, Velocity, Power, and Work Done

An isotonically contracting muscle shortens against a load. The curvilinear relationship between load (F) and velocity (V) is closely fitted by a hyperbolic equation (developed by A. V. Hill),

$$(F + a)(V + b) = (F_0 + a)b$$

where F_0 is the maximum force developed by an isometric contraction, and a and b are constants. Hill's equation is a good fit for small loads but not for high loads because V is not constant throughout the muscle twitch. The constants a (with units of force, e.g., Newtons) and b (with units of velocity, i.e., m sec^{-1}) can be obtained by linearizing the relationship.

$$F = \frac{b(F_0 - F)}{V} - a$$

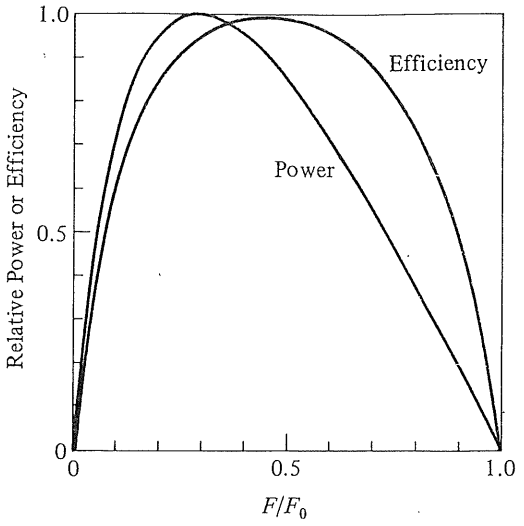
Graphing $(F_0 - F)/V$ as a function of F yields a straight line whose slope is b and whose intercept is $-a$. The values of a and b vary for different muscles; a is generally about 0.15 to $0.25F_0$ for vertebrate muscles.

A muscle will lengthen if the load applied to it exceeds its maximum capacity (F_0). Muscles that are mechanically stretched during a contraction will develop a tension greater than the isometric tension (see the extension of the force–velocity curve for frog sartorius muscle) and work is done on the muscle during its extension. The Hill equation is not a good fit for the lengthening curve.

The power (P) output of a muscle is equal to the force generated (F) × velocity (V); it can be readily calculated from the $V - F$ diagram. The load for maximum power output (F_{mp}) can be calculated from Hill's hyperbolic equation as follows.

$$F_{mp} = (a^2 + aF_0)^{1/2} - a$$

The value of F_{mp} is 0.29 F_0 if $a = 0.2F_0$.

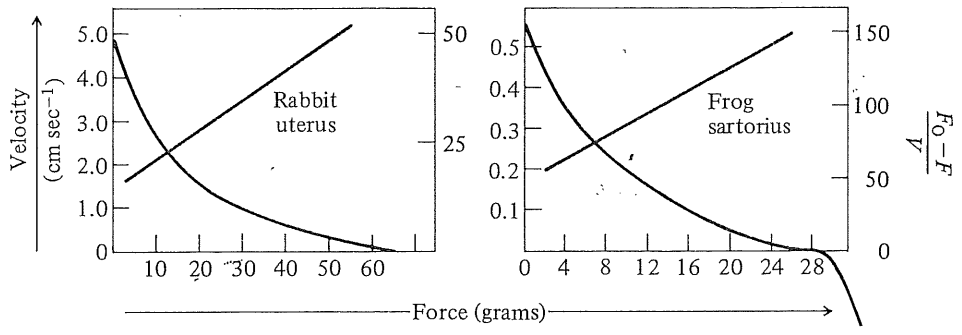


Relative power and efficiency of muscle contraction as a function of relative force of contraction (F/F_0).

The mechanical work (W) done by a muscle (or the work done on a muscle) is equal to the integral of power output over time.

$$W = \int_{t_0}^{t} \text{Power}.dt = P\Delta t$$

Alternatively, the work done can be calculated as the integral of force through a displacement, or force × velocity × time.



There is a hyperbolic relationship between the velocity (V) and force of muscle contraction (F) with a maximum contractile force (F_0) at zero velocity. There is a linear relationship between the relative force $(F_0 - F)/V$ and force. *(Modified from Csapo 1970.)*