CHAPTER 6
Skeletal Muscle and Nerve-Muscle Junction

GROSS STRUCTURE AND FUNCTION.

Skeletal muscles are the major ending of the efferent branch of the central nervous system. We work our will upon the outside world through these muscles. Skeletal muscles occupy about 80 % of the total weight of the body. They use about 6 % of the resting oxygen consumption to maintain ionic gradients; after strenuous exercise they may use as much as 70 % of the oxygen consumption. Each anatomic muscle is delimited by strong fascial sheets and has a characteristic origin and insertion. The basic unit of the muscle is the muscle fiber or cell that runs from one end of the muscle to the other end and has a diameter of 50 to l00 μm.

Motor Units.

Anatomic muscles are subdivided into bundles of many fibers *(Fig. 6-1)*. Each muscle fiber is innervated by only one motor nerve fiber. Groups of muscle fibers are delimited functionally by their nervous innervation. As the *Fig. 6-1* shows, the motor nerve branches and innervates a number of muscle fibers. The muscle fibers that are innervated by a single motor nerve are called a motor unit or group. All these fibers act in the same manner since a single nerve controls them. The number of muscle fibers in a motor unit varies from 300 to 400 in the gastrocnemius (calf) muscle to 4 to 6 in the extraocular muscles. In general, the size of the muscle group is proportioned to the delicacy of the required movement. The extraocular muscles make very small, fine adjustments; the gastrocnemius muscle, coarse, powerful movements.

When the motor nerve is stimulated, an action potential travels down the axon until it reaches the end-plate region, where it releases a chemical transmitter, acetylcholine. The acetylcholine diffuses to a specialized portion of the muscle surface, the motor end plate, and initiates a second action potential on the surface membrane of the muscle fiber. We will discuss the motor endplate in greater detail later in the chapter.

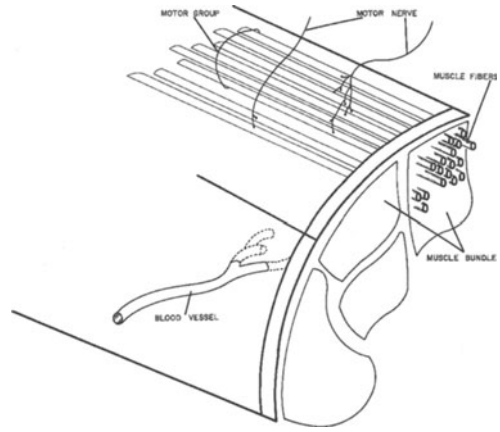

Figure 6-1. The organization of muscle fibers into structural units, muscle bundles and functional units, motor groups.

Contraction.

The action potential travels along the muscle surface from the end-plate region with a conduction velocity of about l meter/sec. The response to a single stimulus, either to the motor nerve or to the muscle surface, is called a twitch. Muscle activity usually occurs in response to a series of action potentials, a partial or complete tetanus as illustrated in *Figure 6-2B,C, and D.* It can be seen that increasing the frequency of stimulation to a muscle increases the tension generated. Since motor units are in parallel, their tension is additive. The total tension a muscle produces is primarily a function of the number of motor units activated.

Sarcomeres and Filaments. When we study the structure of the muscle fiber, the mechanism of contraction becomes clearer. *Figure 6-3, A-D* shows the structure of a 100-μm diameter muscle fiber and its component 1 μm myofibrils. The banded pattern (D) is clearly seen in the light microscope in either single living fibers or fixed and stained material. A dark A band alternates with a light I band. The I band is bisected by a thin, dark Z disk. The basic contractile unit is a length of myofibril from Z line to Z line

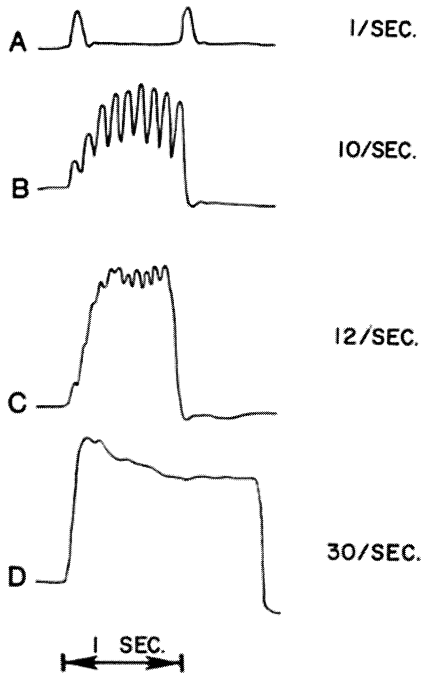

Figure 6-2 Isometric tension in response to stimuli of constant voltage and a varying frequency. Note that the total tension is greater when the frequency of stimulation is increased until a maximum (tetanus) tension is reached. Record from a human flexor carpi radialis muscle in situ. The subject's arm was held to a table with adhesive tape; stimulation was via a carbon electrode over the muscle mass in the upper forearm and a large ECG electrode at the wrist. The tension transducer was in contact with the styloid process on the wrist below the base of the thumb. Both wrist and finger flexors can be stimulated by this method.

called a sarcomere. After isolation, this unit, 1 μm in diameter and 2.5 μm long, will still contract.

At higher magnification, in the electron microscope *(Fig 6-3E)*, it is clear that the bulk of the muscle structure is made up of two types of filaments. The larger of these filaments, the thick filament, is 10 nm in diameter and 1.5 μm long and is located entirely within the A band. Indeed, all of the properties of the A band can be attributed to these filaments. The thin filaments are 4 nm in diameter and 1.0 μm long and run from the Z line various distances into the A band. During contraction the thin filaments are pulled past the thick filaments to reduce sarcomere length.

Excitation Contraction Coupling

Muscle activation begins when an action potential spreads over the surface and then into the depth of each fiber. Calcium released from intracellular structures allows the thick and thin filaments to interact, produce tension, and shorten. Contraction ceases when CA++ is transported into the same intracellular structures.

Reticular Structures. Skeletal muscle has an enlarged and specialized reticular network that is shown in *Figure 6-4.* It can be subdivided into two portions: the transverse tubules or T system and sarcoplasmic reticulum (SR). The T system is a tubular network that is continuous across the whole fiber and contains extracellular fluid. If a perfect cross section were cut across the fiber, the T system would look like a chicken wire fence with the fibrils running through the holes in the wire. The sarcoplasmic reticulum wraps around the myofibrils like the bun around a hot dog.

The (T) system conducts the surface action potential rapidly inward to initiate contraction. When small patches of the surface membrane are stimulated to induce local contraction, the sensitivity of an area depends on its location with respect to the T system. The most sensitive location varies; it is at the Z line in the frog and at the A-I junction in the lizard and many mammals (arrows, Fig. 6-4). Inward conduction is an active, Na+ dependent process in the tubular wall, probably much like the surface action potential. Depolarization of the transverse tubular system generates a charge = movement signal which precedes Ca^{++} release from the SR. This charge = movement signal has many similarities to the gating current of the axon.

The basic ionic mechanisms underlying the muscle action potential are quite similar to those in nerve, a regenerative increase in sodium permeability (to depolarize) which quickly inactivates, followed by an increase in potassium permeability (to repolarize). In skeletal muscle there is also a large, but unchanging, chloride permeability which participates in the repolarization phase. Chloride is in equilibrium across the membrane at a resting potential of -90 mV.

The surface area of the T system gives the 'surface' action potential a slow velocity (1 m/sec). Most of the potassium channels are in the T = tubule membrane so the potassium efflux

during the falling phase of the action potential is into the lumen of the T system. After several action potentials, K^+ builds up in the T lumen and begins to depolarize the fiber. In normal muscles this depolarization is not large and is buffered by the chloride conductance in the surface membrane.

Myotonia. In muscle fibers from myotonic goats the potassium buildup in the T tubules causes a significant depolarization and action potentials continue to fire after stimulation ceases. Much the same result is obtained when nor-

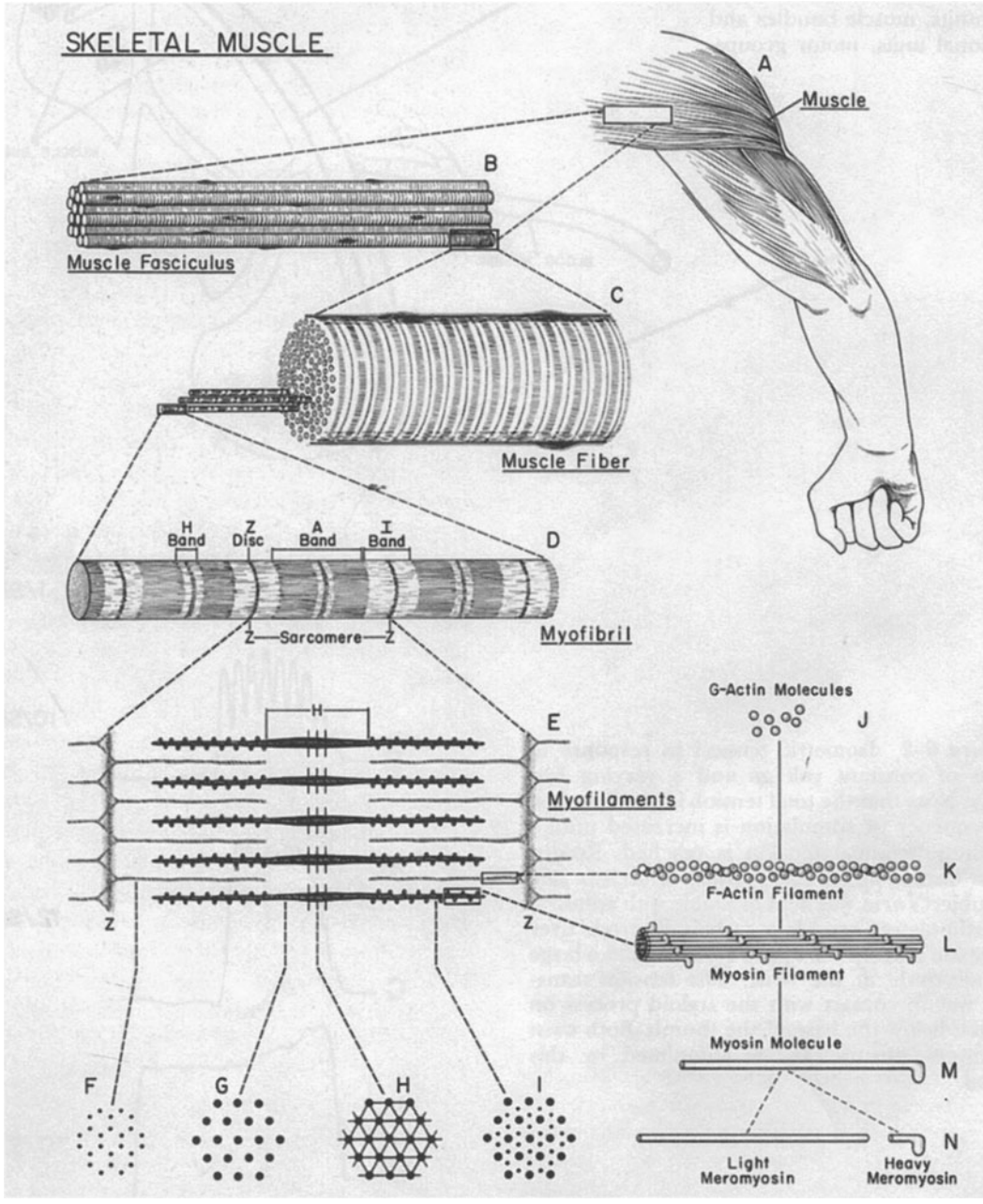

Figure 6-3 Diagram of the organization of skeletal muscle from the gross to the molecular level. F,G,H, and I are cross sections at the levels indicated. (Drawing by Sylvia Colard Keene from Bloom and Fawcett: A Textbook of Histology. Philadelphia, W.B. Saunders, 1968.)

mal fibers are placed in chloride-free solution.

T-SR Coupling.

The structure of the T system - terminal cisternae junction is shown in *Figure 6-5A*. The T tubule runs vertically in the center. The T system is ovoid and the portion adjacent to the terminal cisternae contains prominent particles.

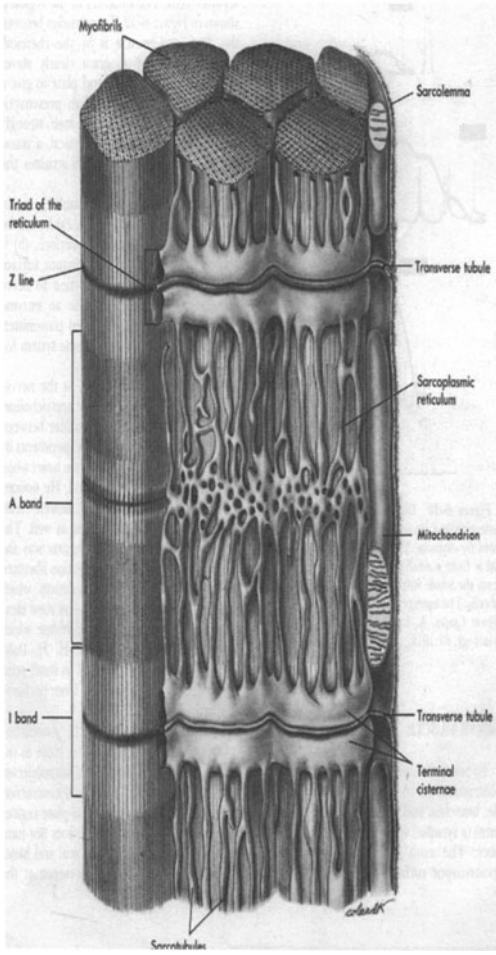

Figure 6-4 Schematic representation of the distribution of the sarcoplasmic reticulum around the myofibrils of skeletal muscle. The longitudinal sarcotubules are confluent with transverse elements called the terminal cisternae. A slender transverse tubule (T tubule) extending inward from the sarcolemma is flanked by two terminal cisternae to form the so-called triads of the reticulum. The location of these with respect to the cross-banded pattern of the myofibrils varies from species to species. In frog muscle, depicted here, the triads are at the Z line. In mammalian muscle there are two to each sarcomere, located at the A-I junctions. (Modified after L. Peachey, from Fawcett, D.W., and McNutt, S.: J. Cell Biol., 25:209, 1965. Drawn by Sylvia Colard Keene.)

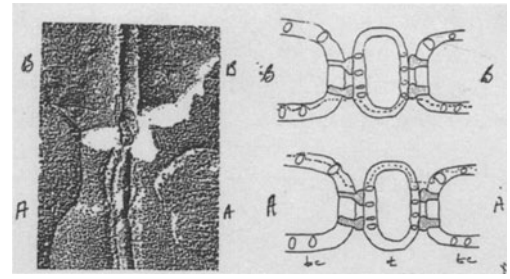

Figure 6-5. A. The T system - terminal cisternae junction. A freeze fracture study showing the T system running vertically flanked by terminal cisternae. B. A cross sectional drawing showing the fracture planes at AA and BB. Fracture lines follow the hydrophobic (center) line of the membrane. The foot processes of the SR are stippled. (Electron micrograph courtesy of Clara Franzini-Armstrong, Ph.D., University of Pennsylvania, Philadelphia, PA)

The terminal cisternae (tc) of the sarcoplasmic reticulum shows a rich array of structures which appear as either pits or particles depending upon the plane of fracture. At the T-tc junction are "feet" which create a distinct morphological gap. The terminal cisternae are not do not participate in any of the electrical events of the muscle fiber indicating there is no ionic connection between the two. The feet are the site of Ca^{++} release from the SR that initiates contraction. After Ca^{++} is released from the terminal cisternae, the Ca^{++} concentration in the sarcoplasm rapidly builds up as shown in Figure 6-6, well in advance of tension generation.

The ultimate result of the depolarization of the T system is Ca++ release from the terminal cisternae. How depolarization induced charge movement in the T system walls induces release of Ca^{++} from the SR is far from clear. One of the author's suspects there is a chemical transmission and Ca^{++} is the first messenger. Other authors suggest a molecular rod that spans the foot process that controls Ca release from the SR.

Dantroline sodium (Dantrium) is a muscle-relaxing drug that acts directly upon the muscle fiber. One-half hour after a clinical dose, the twitch tension is half it normal value; recovery occurs within 24 hours. Dantrolene sodium acts to reduce the amount of Ca^{++} released per

action potential, largely by blocking release of Ca^{++} from the terminal cisternae.

The large particles elsewhere in the SR membrane are the site where Ca++ from the sarcoplasm is taken back into the SR bringing about relaxation.

MOLECULAR ARCHITECTURE OF CONTRACTION

Muscle Proteins. Four major protein types have been extracted from skeletal muscle: actin, myosin, tropomyosin and troponin. The first two are the major protein constituents; the others are a relatively small fraction of the protein. Myosin has a molecular weight of 420 KD and contains two quite different regions; a long double helical tail and a dual headed structure containing both the ATPase and actin binding activities. Figure 6-3, L-N shows the packing of the myosin molecules within the thick filaments of the band. The myosin molecule can be readily divided into two subunits - heavy and light meromyosin. The heavy meromyosin retains the adenosinetriphosphatase (ATPase) and actin binding activity of the intact myosin. This fragment forms the bridges between this and the filaments. Light meromyosin rods aggregate to form a tension bearing rod, the thick filament.

Actin has a molecular weight of 60kD and readily forms long chains of a fibrous protein (Fig. 6-3,J-K). Two chains of F-actin, wound around each other, form the thin filaments along with tropomyosin and troponin. These two regulatory proteins alter the ATPase activity of the actin-myosin complex so that calcium ions are required for ATP breakdown and consequently for muscle activity.

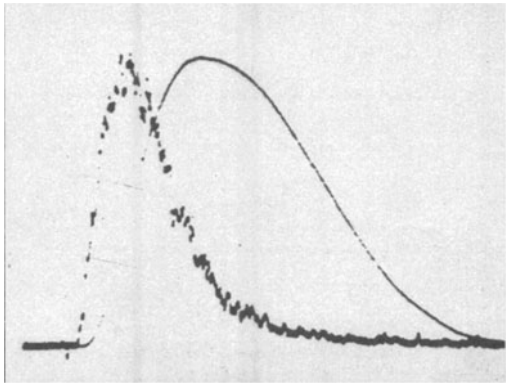

Figure 6-6. The broken line shows the average light emission from Ca++ sensitive protein aequorin inside a muscle fiber while the solid line is the twitch tension. Aequorin, a protein extracted from a luminescent jellyfish, emits light in proportion to Ca++ concentration. The light emission, hence the increasing Ca concentration within the fiber, proceeds the tension generation (solid line). From Blinks, JR, R. Rudel and SR Taylor, J. Physiol. 277. 291-323, 1978.

The immediate energy source for contraction is ATP and muscle converts chemical to mechanical energy with an efficiency of about 50%. Intact muscle contains relatively large quantities of creatine phosphate, an energy storage protein.

Ca^{++}-Troponin Interaction. Free Ca^{++} binds to troponin on the thin filaments causing the troponin-tropomyosin to 'roll-back' from the active site of actin. Actin and myosin can now interact, bridges can form and tension is generated. The interaction continues for as long as the Ca^{++} remains elevated. In the twitch shown in *Figure 6-6*, Ca^{++} is rapidly taken up by the sarcoplasmic reticulum and tension declines. During tetanic stimulation, Ca++ remains elevated. After the surface membrane and reticulum have been removed by chemical treatment, tension generation is related to Ca++ concentration *(Fig. 6-7)*. Tension is maintained for as long as Ca++ is present.

Filament Interaction.

A muscle fiber shortens when thin filaments slide past thick filaments. In this manner the distance between individual Z lines (sarcomere length) decreases. The decrease in muscle length is proportional to the product of the decrease per sarcomere and the number of sarcomeres per muscle.

Cross sections through the array of thick and thin filaments are shown in Figure 6-8, F-I. Each set of filaments is arranged in a basically hexagonal array. In the region of overlap, section I, each of the thin filaments has three thick filaments and each neighboring thick filament has six thin filaments. Contact between the filaments is made by cross bridges *(Fig. 6-8)*. These bridges stick out from the thick filaments and are arranged in a six-fold helix, like the treads on a spiral staircase. In this case, the stairs make a complete revolution in six steps.

Cross-Bridges.

It is the interaction between the two filament

arrays that produces tension; the bridges *(Fig. 6-8)* of the thick filaments "hook on" to and move the thin filaments. The three-dimensional structure of the heavy meromyosin (Fig 6-3) which contains both the actin and ATP binding sites has recently been worked out. There is a prominent cleft near the actin-binding site: when it is closed *(Fig 6-9A)*, actin-myosin binding is strong, when it is open (Fig 6-9B) binding is weak. A lateral pocket contains the ATP binding site; closing of the ATP binding pocket imparts a curvature to the head and a 5 nm movement of the actin binding site along the actin chain (Fig 6-9 B>C). Fig 6-9 shows a molecular model of bridge interaction consisting of actin-myosin dissociation with ATP binding (A>B), curving of the bridge with P hydrolysis to move the myosin head 5 nm along the actin chain (B>C), head reattachment (D) and finally the power stroke as ADP dissociates (D>E).

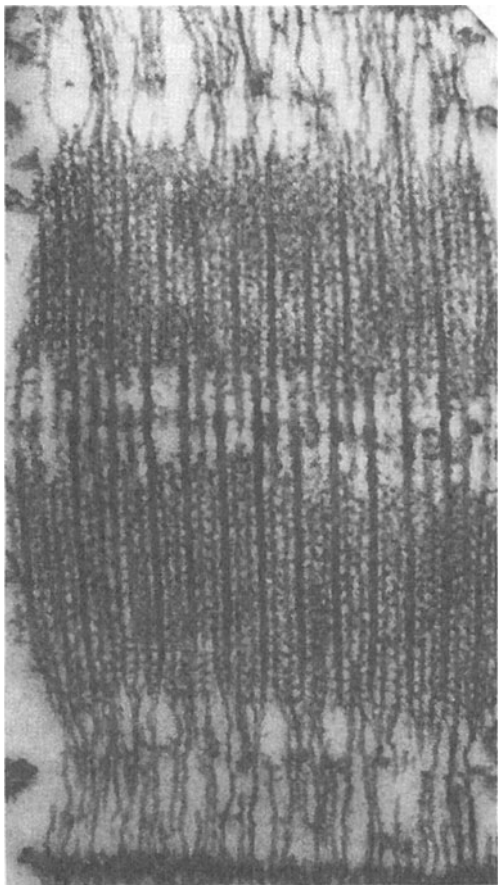

Figure 6-7. A view of the central region of the band at 600,000 x magnification. The projections from the thick filaments, the bridges, are the site of interaction between the thick and thin filaments. (From Huxley, H.E.: J. Biophys. Biochem. Cytol., 3:631, 1957.

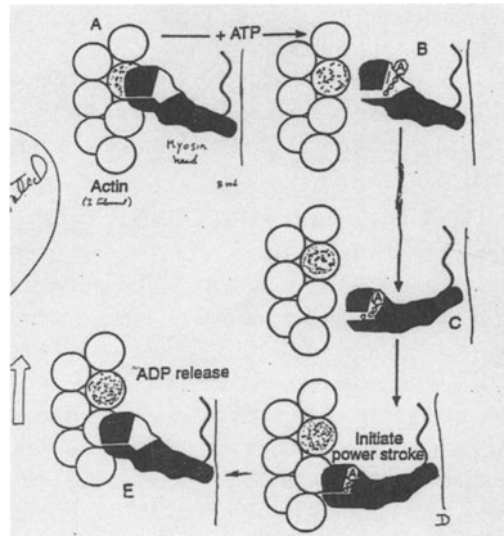

Figure 6-8. The power cycle of the bridge unit of myosin. In A the cleft between the two domains of the myosin head is closed and the myosin head is attached to an actin monomer (speckled for identification). The ATP binding pocket is open. As ATP begins binding, B, the cleft opens and the myosin head unbinds from actin. As ATP binding is completed and P hydrolysis occurs, the ATP pocket closes and the myosin head changes curvature, C, to move 5 nm along the actin chains, to bring it into alignment with the next actin monomer. As the P leaves, D, the cleft closes and the head attaches to the next actin monomer. As ADP is released, E, the pocket opens, the myosin head straightens forcing the actin chain up by 5 nm, the power stroke. Adapted from Rayment et al, Science 261:50-65, 1993.

Length - Tension: Relations. Tension production, then, should be related to the number of bridges connected to thin filaments, to the degree of overlap of actin and myosin filaments, and 2 to the sarcomere spacing. When resting muscle is fixed at various sarcomere spacing, the length of each set of filaments remains constant, as does their diameter *(Fig. 6-10)*. The amount of overlap between the thick and thin filaments varies in direct proportion to sarcomere length, but the A bandwidth remains constant during contraction. Experiments with contracting isolated fibrils also show the bandwidth with the I band decreases in width with decreasing sarcomere spacing. When the sarcomere spacing is just

greater than the sum of the lengths of the and I band filaments (3.6 μm) there should be no overlap, no bridge interaction, and consequently no tension production, Figure 6-9B. As the sar-

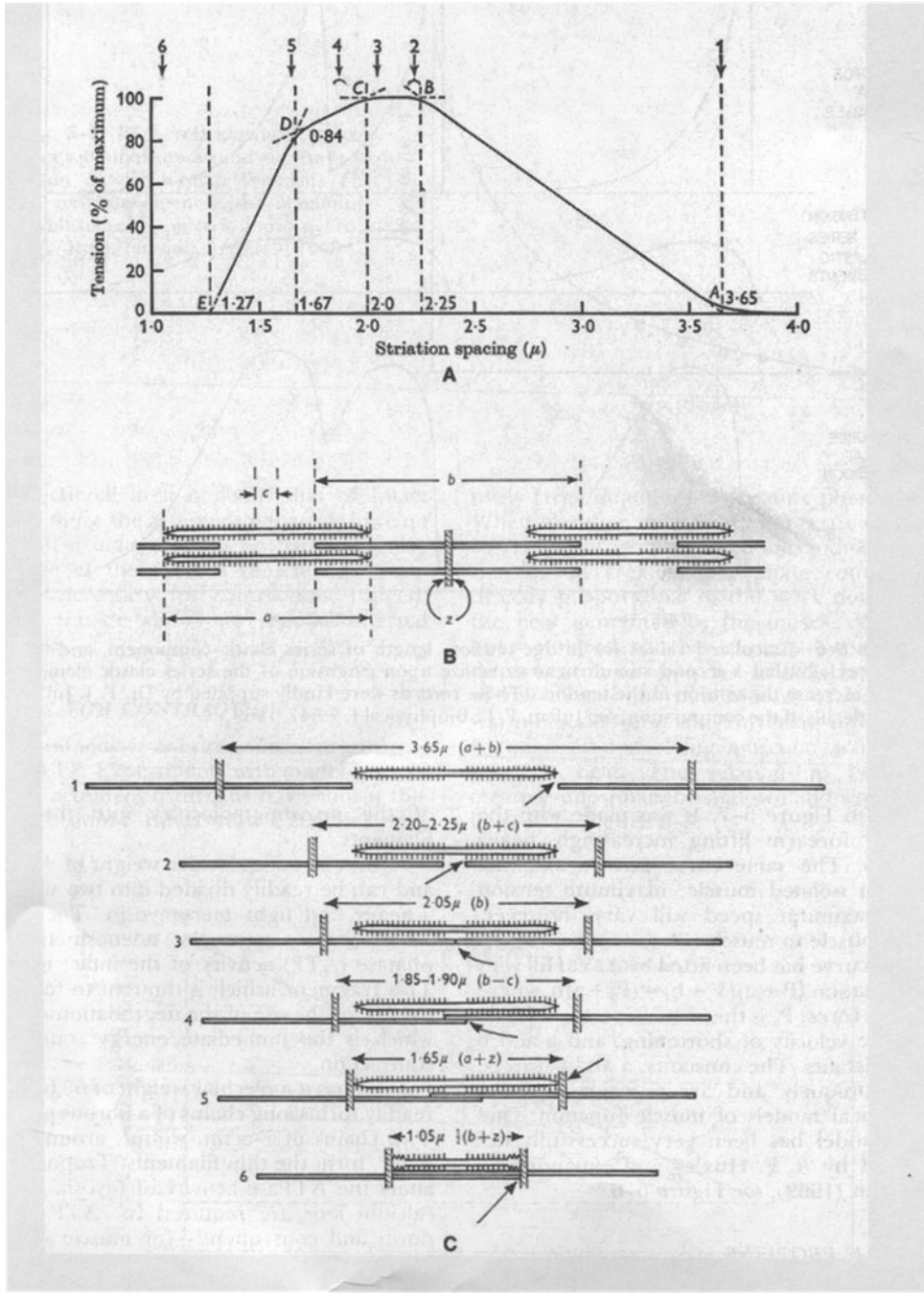

Figure 6-9. The length tension curve (A) and electron micrographs (B,C, and D) of skeletal muscle showing the overlap of the sliding filaments. When the filaments barely overlap (B), tension is low. When all bridges are attached (C), tension is maximum. When the thin filaments overlap (D), tension declines. (Data from AL Gordon, AF, Huxley and F.J. Julian. J. Physiol. 184:170-192, 1966. Electron micrographs courtesy of Brenda Eisenberg, Ph.D., University of Illinois College of Medicine, Chicago, IL.)

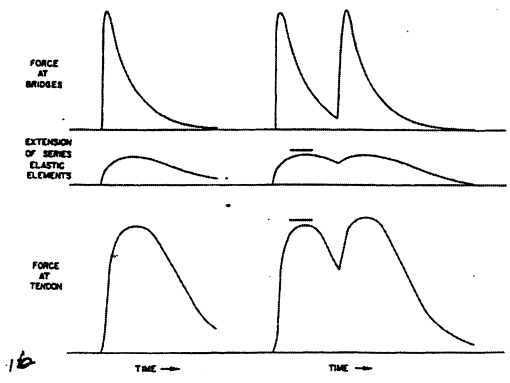

Figure 6-10. Calculated values for bridge tension, length of series elastic component, and tension. Note especially that a second stimulus can capitalize upon extension of the series elastic element and thereby increase the tension at the tendon. F.J. Julian, Harvard Medical School, kindly supplied (these records. For further details of the computation, see Julian, F.J.: Biophysical J. 9:547, 1969.)

comere spacing decreases, the number of bridges increases and so does the tension. The number of bridges and the tension increase until all of the bridges are attached. There are no bridges in the center of the band, as can be seen in Figure 6-8.

Further shortening gives no increase in tension since there is no more bridges to interact. In fact, further shortening leads to decreased tension since one thin filament must force its way past its opposite filament, probably disturbing the bridge filament interaction. Further shortening also requires compression of the band filaments and most of the tension that is produced goes to compress the thick filaments.

Force-velocity Relation.

We have concentrated so far on the generation of tension at constant length; to do useful work the muscle must shorten. When a muscle shortens against a very light load it shortens very quickly. As the load increases, the velocity

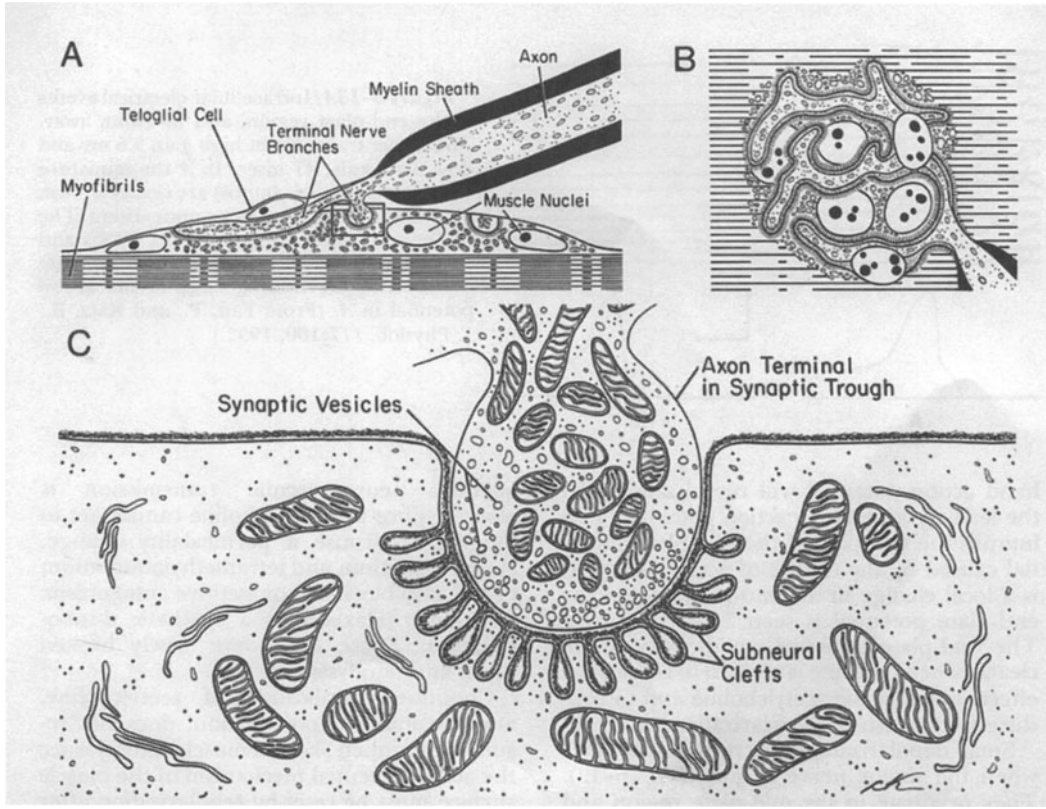

Figure 6-11. Schematic representations of the motor end plate as seen by light and electron microscopy. A, End plate as seen in histological sections in the long axis of the muscle fiber. B, As seen in surface view with the light microscope. C, As seen in an electron micrograph of an area such as that in the rectangle on A (After R. Couteaux. From Bloom and Fawcett: A Textbook of Histology. Philadelphia, W.B. Saunders, 1968.)

decreases. A force-velocity curve is shown in *Figure 6-10.* A human forearm was pulling against increasingly heavy weights. The same curve can be obtained with an isolated muscle; maximum tension and maximum speed will vary, however, from muscle to muscle.

The force-velocity curve is described by the equation

$(P+a)(V+b)=(P_o+a)b$, where P is the force, P_o is the isometric tetanic force, V is the velocity of shortening, and a and b are constants. The constants, a and b, can be fitted uniquely and are a major goal for theoretical models of muscle function.

Active State.

When the bridges from the thick filaments interact with the thin filaments, they develop tension 3 to 5 msec after the action potential runs along the surface. Tension cannot, however, be measured in the tendon for 10 to 20 msec following stimulation. What causes the delay? There is a great deal of elastic material in series with the tension-generating element, some indeed in the bridges themselves. The tension produced by the bridges must first stretch the elastic material before it can be transmitted to the tendon and the load. Consider for a moment a person pulling a stretchy nylon rope that is attached to a large rock. At first the rope stretches only after a delay does it transmit the full force of his pull. Remember that a pull of a given force will extend the rope to a characteristic length and no further, but the stretching will take time.

Let us return to the case of the muscle. Following a single stimulus, the bridges generate their maximum tension for a very short time *(Fig. 6-10).* This short phase corresponds to the active state in of A.V. Hills model. The tension is mainly expended in stretching the series elasticity; about one-half of the bridge tension appears as tendon tension. If a second stimulus follows soon after the first, the renewed bridge tension pulls on partially extended elastic elements and consequently must do less work on them to transmit tension to the tendon; more of the bridge tension is applied to the tendon. If a third stimulus follows the first two, the work the bridges do on the series elastic elements is further reduced; consequently, the work done and tension produced on the tendon is much greater during a tetanus (Fig. 6-10D) than during a twitch.

NERVE-MUSCLE JUNCTION

The Endplate

To return to the control of skeletal muscle contraction, the motor nerve enters the muscle, branches, and forms a very close junction, a synapse, with the center of each muscle fiber. The axon (presynaptic element) and muscle (postsynaptic element) remain two distinctly separate cells. The structure of the region is shown in Figure 6-11. Transmission between the axon and muscle is by means of the chemical, acetylcholine (ACh).

Acetylcholine. To be considered a transmitter, a substance must meet four criteria: (a) it must be effective at the postsynaptic surface, (b) it must be liberated by the presynaptic surface in response to and in proportion to nerve stimulation, (c) there must be an enzyme system for destroying or reabsorbing liberated transmitter, and (d) there must be an enzyme system for synthesizing the transmitter.

Each of these criteria has been met by acetylcholine at the nerve-muscle junction. The idea of a chemical transmitter between nerve and muscle came from experiments by Otto Loewi in 1921 that demonstrated the slowing of the heart when the vagus nerve was stimulated. He noticed that Ringer's solution that had dripped over a slowed heart caused a second heart to slow as well. The only link between the two hearts was the Ringer's solution. Vagal stimulation liberated acetylcholine into the solution that affected the second heart. Acetylcholine can be collected in from small veins in stimulated muscles.

Acetylcholine is effective in stimulating contraction if it is injected very close to the muscle. When acetylcholine is microinjected onto an excised muscle, it elicits contraction only when injected at the end-plate region. The rest of a normal muscle does not have ACh receptors. Both biochemical and histochemical studies show the presence of a cleaving enzyme, acetylcholine esterase at the myoneuronal junction. It is present in both the postsynaptic surfaces in the subneural folds and on the presynaptic side. It is

also present in fairly high concentration in the blood that explains the general ineffectiveness of acetylcholine when injected intra-arterially.

Another enzyme, choline acetylase, reconstitutes the acetylcholine, into the synaptic vesicles. The enzymes that produce the vesicles are produced in the nerve cell body; the number of vesicles decreases rapidly after section of the motor nerve. When an action potential enters the presynaptic terminal, the accompanying depolarization opens voltage gated Ca channels. Increasing intracellular Ca++ is essential for neurotransmitter release, catalyzing the fusion of synaptic vesicles with the surface membrane to release ACh into the synaptic cleft *(Fig 6-12)*.

Acetylcholine acts on the postsynaptic membrane and destroys its selective permeability to all small ions. The membrane has an equilibrium voltage of zero millivolts. This voltage is, of course, never reached, but would be if acetylcholine were continuously applied to the end plate. The high concentration of acetylcholine esterase prevents such an event. The depolarization brought about by the usual amount of acetylcholine causes a current to flow to the surrounding muscle fiber surface. As the current flows through the peripheral membrane, it depolarizes that membrane and sets up a propagated action potential. The end-plate region itself is not electrically excitable; current must flow to the surrounding area which area contains classical sodium channels that must be repolarized before it can fire again.

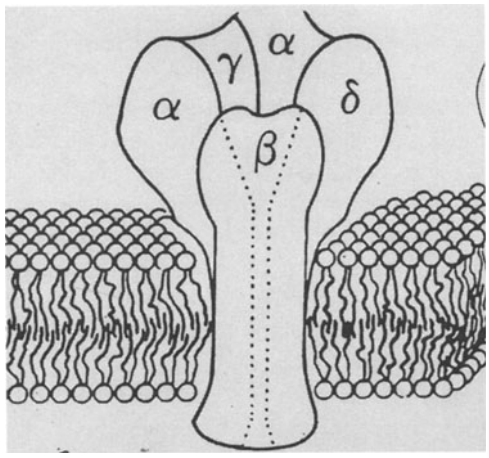

Figure 6-12. A cartoon of a single acetylcholine activated channel. Each channel has two ACh binding sites; one on the cytoplasmic side of each alpha subunit. Miles, K. and R.L. Huganir, Molecular Neurobiology 2: 91-124, 1988.

Acetylcholine Activated Channels. The receptor and channel are contained in a single transmembrane protein aggregate (Fig 6-12) made up of 5 subunits each of which is thought to contain three alpha helical columns with a 0.7 nm channel down the center. The receptors on the alpha subunits are some 6 nm from the gated ionic channel that is on the cytoplasmic end of the channel.

Single channel recordings *(Fig. 6-13)* show the channel to be either open or closed. ACh concentration alters the % time the channel is open. When a microelectrode is inserted into the end-plate region, the usual resting membrane potential is recorded. When the motor nerve is

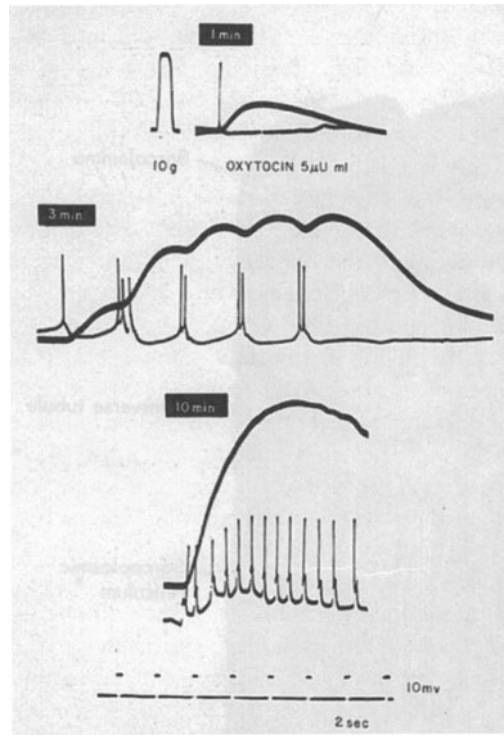

Figure 6-13. Intracellular electrical events at the end-plate region (A), and (B), 2 mm away. The upper traces are at high gain (3.6 mv) and slow time scale, (47 msec). In (A) the miniature end-plate potentials (minis) are clearly shown, whereas in (B) they are almost nonexistent. The two bottom traces are at low gain, (50 mv) and fast time scale, (2 msec). Note the end-plate potential at the leading edge of the action potential in A. (From Fatt, P., and Katz, G.: J. Physiol., 117:109, 1952.)

stimulated, the microelectrode records an action potential as shown in the lower traces of Figure 6-13. Careful examination of the left hand action potential will reveal a hump on the leading edge of the action potential. This hump is the part of the end-plate potential caused by the release of acetylcholine. It is a local change in the end-plate region; no end-plate potential is seen 2-mm away (B). The end-plate potential can be seen more clearly when curarine is applied to reduce the effectiveness of the acetylcholine and to produce a subthreshold depolarization.

Small depolarizations are recorded at times when the motor nerve is quiet . They originate in the end-plate region and can be recorded only very close to it. These miniature end-plate potentials ("minis") are quantized *(Fig 6-13)*. They represent the release of several vesicles of ACh. The amplitude of these potentials increases as more vesicles are released. Each pocket or vesicle releases some 10^{-17} moles of acetylcholine that results in a depolarization of 0.4 mV. An action potential arriving at the presynaptic terminal causes an increase in the rate of vesicle release so that about 100 vesicles discharge their acetylcholine over a very short interval; this amount of acetylcholine is sufficient to initiate an action potential on the muscle surface.

MECHANISMS OF DRUGS ACTING ON NERVE-MUSCLE JUNCTION

Drugs that Compete for Receptor Site. D-tubocurarine competes for the same postsynaptic site, as does acetylcholine; it binds more strongly to the site but does not cause a permeability change. Neuromuscular transmission is blocked because the acetylcholine cannot get to the sites. Gallamine (Flaxedil) is a synthetic d-tubocurarine. Several snake neurotoxins, including bungarotoxin, also bind very strongly and block the receptor. Hexamethonium and tetramethylammonium (TEA) also block by competitive antagonism. These drugs are slowly broken down and paralysis wears off.

Continued application of acetylcholine, and continued depolarization, does not result in continued skeletal muscle activity because the action potential mechanism must be reset after the first action potential. Any drug that produces a continual depolarization at the end plate produces a depolarization block. Nerve-muscle block and muscle relaxation should be achieved by infusing large quantities of acetylcholine but this method is very inefficient because the acetylcholine is broken down rapidly. Succinylcholine binds to the postsynaptic surface and causes a permeability change. Since it is only slowly broken down, it produces a long-lasting depolarization. It depolarizes the muscle membrane, which e contracts once and then relaxes. Decamethonium also acts in this manner.

Drugs that block the nerve-muscle junction are often used in conjunction with anesthetics to achieve muscle relaxation during surgery. It should always be borne in mind that the respiratory muscle would be blocked so that the patient must be artificially ventilated.

Drugs that prolong the action of acetylcholine. Blocking the esterase activity o that the action of the released acetylcholine is prolonged can also block the end plate. The anticholinesterase, such as neostigmine, edrophonium (Tensilon), eserine, and diisopropyl fluorophosphate (DFP), all combine with acetylcholine esterase and prevent it from cleaving acetylcholine, so ACh continues to depolarize the end-plate region and to block transmission. Many insecticides block acetylcholinesterase.

Agents that block the release of acetylcholine from the presynaptic terminal will be discussed below. The various disorders affecting the neuromuscular junction are discussed in great detail below.

EFFECTS OF MOTOR NERVE ON SKELETAL MUSCLE

From the discussion so far, one might conclude that the motor nerve supplies only the stimulus to contract; this is far from the case. When the motor nerve is cut, the muscle rapidly atrophies. Its volume and strength of contraction decrease – and all of the fibers decrease in size, as does the total muscle bulk. After three months, the muscle bulk may have decreased to as little as 25 % of its original bulk. This atrophy is not due to muscle disuse alone since disuse, such as that caused by immobilization, will cause muscle to atrophy to 3/4 of its original size in the same 3-month period. An intact motor nerve is necessary for continued survival of the muscle fiber. It

would appear that the continued release of acetylcholine, or possibly some other transmitter, from the nerve, is the agent responsible for this trophic influence.

Denervation Sensitivity. When the muscle is denervated, the entire surface of the muscle fiber slowly becomes sensitive to acetylcholine. After several weeks, application of ACh anywhere on the surface results in an action potential and contraction. Patients who have had a motor nerve severed show great sensitivity to injected ACh for this reason.

Reinnervation. If the cut ends of a motor nerve are rejoined, sprouts from the central end will grow down the tube left by the degenerated axon. If the distance between the cut and the muscle is short and the cut ends are well aligned, the nerve will make contact with the muscle. A new end plate will form, and muscle function will be restored. Passive exercise of the muscle during regrowth is helpful to prevent disuse atrophy and contractures.

Slow and Fast Muscles.

There are two sorts of skeletal muscle: red and white. The best known example of this difference is the white and dark meat of a chicken; the same qualitative differences appear in mammals.

Most muscles, particularly in mammals, are not pure red or white muscle but are made up of a mixture of fibers *(Fig 6-14)*. The fibers in red muscles are called Type I. They are rich in mitochondria but have a relatively low myosin ATPase activity. They generate ATP from glucose as it is used. Enzymes of oxidative metabolism cause the red color. Type II fibers found in white muscles have a very active myosin ATPase activity and are relatively lacking in mitochondria. Type II fibers which are responsive for quick, phasic contractions depend upon an anaerobic, glycolytic metabolism; they produce large amounts of lactic acid.

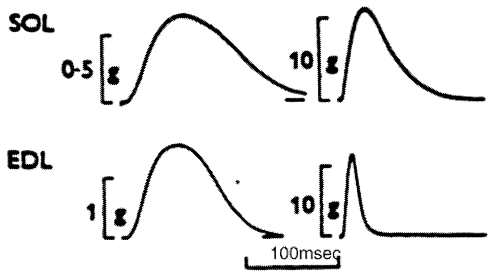

Figure 6-14. Twitch time in a fast, extensor digitorum longus (EDL) and a slow, soleus (SOL) muscle. At birth (left) the difference in twitch time is not striking, yet 5 weeks later (right) the difference is very pronounced. (From Close, R.: J. Physiol., 180:542, 1965.)

Red muscle is characterized by a long twitch time, and concomitantly a low frequency of stimulation will result in tetanus. These muscles are primarily antigravity or postural muscles; their movements are characterized by long-sustained contractions. The white muscles, on the other hand, have short twitch times and are used for quick phasic movements.

The speed of contraction is determined by the pattern of activity in the motor nerve. When nerves from fast and slow muscles are crossed, the fast muscle slows down and the slow muscle speeds up *(Fig 6-14)*. When a limb is immobilized, the activity in motor nerves to a slow muscle, which is usually intense, decreases. After several weeks the speed of contraction has increased markedly. It is not clear how the pattern of activity alters the biochemical control mechanisms and the contractile properties of a muscle. Some authors suggest a second transmitter released in very small quantities from motor nerves is responsible for modulating which proteins are expressed from the muscle genome.

DISEASES OF MUSCLE

Most classifications divide these disorders into two groups: inherited and acquired (Morgan & Hughes 1992).

Muscular Dystrophies

The major inherited disease is the muscular dystrophies that are characterized by a primary degeneration of skeletal muscle. The slow but progressive destruction of muscle results in a progressive weakness initially affecting the proximal muscles.

Duchenne's Muscular Dystrophy. The most common varieties of muscular dystrophy are X linked almost all cases occur in males (rarely females with Turner's syndrome or X-chromosome translocation may be affected). Duchenne originally described the most common type within this group in 1868. The typical patient with

Duchenne's muscular dystrophy (DMD) is a boy who has delayed walking. At some point between the ages of 2 and 5 years, the patient is noted to be clumsy and slow in exercises and games. It is soon evident that proximal pelvic girdle lower extremity weakness is present. In rising from the floor the patient uses his upper extremities and hands placed on the thighs to force the body in to an erect position (Gower's sign). Thus counteracting the weakness at hips and pelvis. At this point, marked enlargement of the calf muscles is present (pseudo hypertrophy). With progression of the disease, increasing atrophy of muscle and increasing weakness occurs. By age 12, the patient is confined to a wheel chair. Death occurs late in the teens or early twenties from respiratory complications and/or cardiac failure (heart muscle is involved). The blood creatine kinase level while the patient is still ambulatory is at least 40 times the upper limit of normal. Levels may be 300-400 times normal. Electromyogram (EMG) demonstrates myopathic features. The muscle biopsy taken early in the course of the disease before severe atrophy is present demonstrates characteristic myopathic features including: 1) wide spread necrosis and phagocytosis of muscle fibers 2) regeneration of muscle fibers, 3) marked variation in fiber size and 4) large rounded "hypercontracted hyalinized" fibers. *(Fig. 6-15).* In late stages replacement of muscle by proliferation of endomysial connective tissue and fat occurs.

DMD is the most common lethal, X linked disease with an estimated incidence of 1 in 4000 live male births (see Moser 1984). The maternal carrier can be identified based on family history and an elevated creatine kinase level in the carrier. A small % of carriers also has mild weakness or EMG or muscle biopsy changes. There is however a high frequency of isolated cases, 30-50% suggesting a high incidence of new mutants or mutant maternal carriers.

In families at risk, prenatal diagnosis is possible (Darras et al. 1987, and see below).

Becker's muscular dystrophy. Becker's muscular dystrophy described in 1955 is a less common (1 in 20,000 male births) and less severe form of x-linked disorders. Age of onset is later; and the rate of progression is slower. The patient is still ambulatory at age 15. Life expectancy is only slightly reduced. Creatine Kinase levels are markedly increased. The EMG demonstrates myopathic features. The muscle biopsy indicates features that are similar but less marked than those noted above.

Recent major advances have been made in our understanding of the genetics and molecular biology of these disorders (Arahata et al, 1989, Hoffman et al. 1988, see also review of Rowland 1988). The precise locus has been identified: the short arm of the X chromosome at the region designated as Xp 21. The specific affected gene has been isolated and characterized. DNA analysis has shown that both DMD and BMD affect the same gene (allelic). Portions of the coding sequence of the gene have been used to produce polyclonal antisera directed against the normal muscle protein produce of the normal gene. The specific protein, dystrophin, is a normal component of the plasma membrane, transverse tubule system of the normal muscle fiber. In patients with Duchenne's muscular dystrophy, the muscle contained less than <3% of the amounts of this protein found in control patients. In patients with Becker's muscular dystrophy, the dystrophin was normal but the size of the protein (molecular weight) was abnormal. Patients with an intermediate clinical course had results bridging these two disorders. Patients with other types of neuromuscular disorders had normal dystrophin.

Thus a defective gene at this specific site may result in a total defect in a failure to produce this muscle protein (DMD) in the production of a

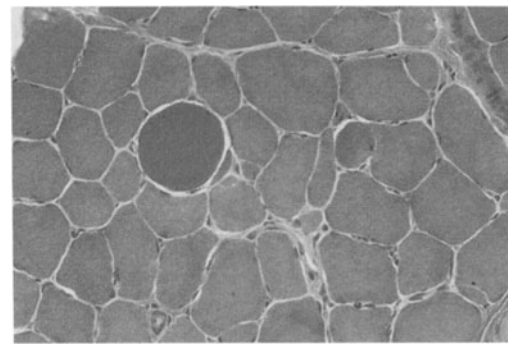

Figure 6-15. Muscle histopathology I: Duchenne's Muscular dystrophy Marked variation in fiber size is present with a large dense hypercontracted hyalinized fiber (Compare to 6-21 and 8-21). H&E x 63- Courtesy of Dr. Tom Smith, U.Mass Medical Center.

muscle protein of abnormal size (BMD) or in various combinations of these deficits. Specific therapy has not yet been achieved. Corticosteroids may produce minor improvement (Brown 1989).

The non X-linked muscular dystrophies are less common.

Facioscapulohumeral (FSH) dystrophy is an autosomal dominant with the gene linked to chromosome 40. The incidence is 0.5-5.0/100,000 persons. The phenotypic expression is variable. Some cases begin in childhood, and have a poor prognosis. Most begin in the late teens, or early twenties with a slow rate of progression. Often the specific age of onset is difficult to identify. The name of the disease reflects the prominent early features of bilateral facial weakness and proximal upper extremity involvement with weakness of shoulder abduction and winging of the scapula. Subsequently lower extremities are involved. In most cases little disability of significant degree occurs before the thirties or forties. Life span is relatively well preserved. Serum creatine kinase is borderline or only mildly elevated reflecting the slow rate of muscle breakdown. The EMG and muscle biopsy reflects the myopathic features.

Limb girdle dystrophies. The limb girdle dystrophies are heterogenous autosomal recessive disorders and some are autosomal dominants. Some begin in the upper extremities at shoulder and scapula, some in the lower extremes at pelvic girdle and knees. Progression is slow and severe disability usually not present until the thirties or even the fifties. Serum creatine kinase is moderately elevated. EMG demonstrates myopathic features; muscle biopsy demonstrates dystrophic features. From a clinical standpoint, there is considerable overlap with cases of indolent polymyositis, motor neuron disease (spinal muscular atrophy), endocrine myopathies, and carriers of the DMD gene with minor clinical manifestations.

The following case history illustrates this type of case:

Case History # 6-1.

This 21-year-old white male college graduate was referred for evaluation of bilateral leg weakness. The patient was delayed in walking until age 2. During grammar school, he was the slowest runner in the class and could not keep up with his peers. In high school he first noted minor weakness in climbing stairs. By college he had trouble in climbing one flight. By his senior year, he had difficulty descending stairs.

Past History and Family History not remarkable.

General Physical Examination: Unremarkable

Neurological Examination: The relevant findings included an absence of deep tendon reflexes at triceps and radial periosteal and trace reflexes at biceps. Proximal weakness was present with intact distal strength and normal muscle bulk.

Motor system:

At hip[1] strength was 3/5; at shoulders, 4/5. Gower's sign was present in attempting to stand from a recumbent position.

Gait was waddling - consistent with proximal weakness at hips.

Deep tendon reflexes[2] were absent at triceps and radial periosteal and trace at biceps. Quadriceps and Achilles reflexes were normal.

b. *Plantar responses* were flexor.

5) *Sensation*: All modalities were normal.

Lab:

1) *Erythrocyte sedimentation rate (ESR)*, thyroid studies, and electrolytes were all normal.

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[1]*The primary grading system for strength is that suggested by the Medical Research Council (MRC) during World War II: 0=no contractions; 1= flicker or trace of contraction; 2=active movement with gravity eliminated; 3=active movement against gravity; 4=active movement against gravity and resistance; 5=normal power. The scale is not a linear function- and because of the wide range included in grades 4 and 5 many examiners will grade 4(-), 4, 4(+) and 5(-).*

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[2]*Deep tendon reflexes are graded as follows: 0=absent; trace=minimally present; 1=hyperactive; 2=normal; 3= hyperactive-brisk; 4=hyperactive-unsustained clonus; 4+ sustained clonus. As above minor gradations maybe superimposed such as 2+, or 3+.*

2) *Muscle enzymes,* CK, SGOT, LDH and aldolase were all mildly elevated.

3) *Motor and sensory nerve conduction velocities* were all normal.

4) *EMG* - demonstrated myopathic features with decreased amplitude and duration of motor units with a full interference pattern on volitional effort.

5) *Muscle biopsy* (left deltoid) reported significant dystrophic features:

a. Marked variation in muscle fiber size in a random distribution.

b. Central position of subsarcolemmal nuclei.

c. Significant increase in endomysial connective tissue.

Subsequent Course.

Follow Up one year late indicated no progression. Two years later, minor progression was noted with a minor decrease in hand strength. Over the years slow progression occurred. The patient reported 17 years after his initial evaluation that he was still able to walk without assistance. Climbing stairs was a problem. Face and hands were not involved. Additional discussion of these less common dystrophies van be found in Padberg 1993.

Muscular dystrophies with predominant involvement of cranial nerves. Cranial nerves may be affected during the course of the more common muscular dystrophies. There have however been several families described with adult onset. Diseases beginning with predominant and relative selective involvement of cranial nerves - usually with autosomal dominant pattern of inheritance: chronic progressive ophthalmoplegia and oculopharyngeal dystrophy.

Myotonic Dystrophy: This is the most common inherited form of muscular dystrophy affecting adults. It is an autosomal dominant disorder with an estimated incidence of 1:8000 in which cranial nerve and distal limb involvement are prominent[3]. Multiple organ systems are involved with variable expression. The non muscular striated manifestations include cataracts, baldness, mental subnormality, gonadal atrophy, other endocrine disturbances, low plasma IgG, glucose intolerance, smooth muscle autonomic involvement and cardiac conduction defects. The latter may lead to syncope and sudden death. Although cases can present in the neonatal period, the most common ages of onset are late adolescence or early adult life.

The early symptoms relate to complaints of gait difficulty and clumsiness. At this point, the more specific diagnosis may not be apparent to the non-neurological observer. (The author once saw 6 patients in a 8-month period at an Army Basic Training base with this diagnosis, referred with the more general complaints). Examination however will demonstrate a distal limb weakness. Percussion of the thenar hand muscles or of the tongue will often demonstrate a myotonic reaction. (prolonged contraction delayed relaxation). Myotonia can also be demonstrated by asking the patient to squeeze the examiner fingers and to then rapidly open the hands. Myotonia of the eyelids may be noted in the continued retraction of eyelids and a lid lag after prolonged upgaze. With progression of the disease, a significant weakness and atrophy of muscles occur in muscles innervated by the facial, mandibular supplied muscles, and the accessory nerve to the sternocleidomastoid muscles occurs with the development of characteristic "myopathic facies." Most patients are disabled and unable to walk by the thirties or forties. Life expectancy is reduced because of cardiac and pulmonary complications.

The creatine kinase level is usually normal. The EMG demonstrates myopathic features plus the prolonged myotonic discharges. When audio amplified the EMG myotonic discharge sounds like a "dive bomber". The muscle biopsy shows characteristic features of chains of central nuclei, ringed fibers and selective atrophy of type 1 fibers.

Myotonic dystrophy should be easily distinguished from syndromes in which myotonic occurs in relative isolation: Myotonic congenita Thomsen's disease and an autosomal recessive

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[3]*Recent studies have demonstrated that the disorder is related to an increased number of cytosine-thymidine-guanine trinucleotide repeats in the region of the protein kinase gene located on the long arm of chromosome 19. A DNA probe that detects directly the mutation is available (Shelbourne et al 1993, Ptacek et al 1993).*

form (Becker). The myotonic syndromes are characterized by an abnormal increase in membrane excitability with persistent runs of action potential in the surface membrane. Abnormal low chloride conductance has been implicated in some types of myotonia: myotonia congenita in humans, congenital myotonia of the goat (due to mutagens) and myotonia following ingestion of aromatic carboxylic acids.

Subsequent K^+ accumulation in the transverse tubular lumen leads to excessive depolarization and the persistent firing of action potentials. Similar result occur when normal muscle fiber are placed in a chloride free solution

However, this mechanism is apparently not the explanation for the myotonia of myotonic dystrophy, where intracellular sodium concentration is elevated apparently because of abnormalities in the regulation of sodium channels. The underlying molecular basis of myotonic dystrophy has recently been discovered. An unstable DNA fragment occurs on chromosome 19q 13 related to an expanded CAG trinucleotide repeat at the end of a region encoding a protein kinase. The greater the number of repeats, the more sever the disease (Wang et. al 1994). Ptacek et provides a more complete review of the physiology of the myotonic disorders. al (1993).

Congenital myopathies.

(Refer to Fardeau 1982). These are relatively rare disorders of muscle which primarily presents with hypotonic weakness in childhood: "The floppy infant" and delay in motor developmental milestones. The specific name assigned to each of these syndromes is based on the unique features on muscle biopsy and the special histochemical stains. The first type described by Shy and Magee in 1956 is so named because type I fibers lack mitochondria in the central core which instead contains disorganized tightly packed myofilaments. In nemaline myopathy there are peripheral collections of rod shaped bodies - possibly derived from the Z bands. In centronuclearmyopathy there are chains of central muscles - surrounded by a zone devoid of myofibrillar - ATPase activity.

Metabolic Myopathies: (Refer to Rowland et al 1986 and DiMauro 1985)

Muscle contraction utilizes ATP (adenosome triphosphate) which is converted from ADP (Adenosine diphosphate) by the action of CK (Creatine kinase).

Moderate exercise is fueled by aerobic conditions with glycogen as the main fuel source. After 5-10 minutes blood glucose is utilized. Subsequently fatty acids are utilized. After 4 hours of exercise lipids and amino acids are utilized.

In high intensity exercise anaerobic glycogen breakdown and glycolysis generate additional fuel. At rest lipids are the predominant energy source.

The student is already aware that multiple enzymes are involved in the events of aerobic and anaerobic carbohydrate metabolism and in lipid fatty acid metabolism. The metabolic myopathies are relatively rare inborn errors of metabolism, which involve these enzymes. The specific defect may involve the lysosomal and cytosolic enzymes: The glycogen storage diseases - or the enzymes related to the mitochondrial respiratory chain for aerobic energy production: The mitochondrial myopathies.

Depending on the specific deficit - a) there may be multiple system involvement with persistent weakness, b) selective muscle involvement with persistent weakness or c) exercise intolerance with easy fatigue and muscle cramps but with little persistent weakness.

The first hereditary myopathy in which a specific enzyme defects was identified McArdle's Disease falls into this last category. The enzyme muscle phosphorylase is absent as demonstrated on special stains for this enzyme applied to the muscle biopsy. When the patient exercise glycogen can not be broken down to pyruvate and lactic acid and the expected rise in blood lactic acid in ischemic limb exercise fails to occur. As exercise continues, fatigue and painful cramps occur. If the patient persists, muscle breakdown will occur with a significant rise in blood and urine levels of myoglobin. Significant acute renal tubular impairment is a complication of any acute condition in which rapid breakdown of muscle occurs producing myoglobinuria.

Periodic Disorders of Muscles:
Familial Periodic Paralysis[4]

Other rare inherited (autosomal dominant) disorder of muscle that may also produce acute transient weakness. These are not related to energy metabolism but are characterized instead by episodic failure of muscle membrane excitability. The attacks are often associated with marked alterations of serum potassium. There are hypokalemic and hyperkalemia (and possibly normokalenic forms). In the hypokalemic form, at the onset of the attack a significant movement of potassium and sodium ions into skeletal muscle occurs with a decrease in serum potassium. Attacks may be induced by the administration of insulin and glucose or of sodium chloride, by alcohol, stress, cold exposure, or by rest after exercise. In the hyperkalemic form attacks may be precipitated by fasting, cold, stress or by rest after exercise. In all types mild persistent proximal weakness may develop later in the disease course. Muscle biopsy may demonstrate vascular changes and tubular aggregates.

In both forms the changes in serum levels of potassium are of such magnitude as to produce EKG changes. In the hypokalemic form serum K may be depressed to 2-3 mEg/Liter. In the hyperkalemic form the lead may be as high as 7-8 mEgl/Liter. (Normal 3.9-5.0 mEg/liter).

The following case history illustrates many of these points. **Case History 6-2.** Patient of Dr. Thomas Twitchell.

This 30-yr. old white housewife had the onset of episodes of night paralysis at age 9 years. These occurred several times the month but with the administration of potassium supplementation, attacks decreased to 1-2 times per year. At age 32, she remarried and was under increased stress. One year later she began to have frequent episodes of nighttime paralysis again. She could relate her attacks to high carbohydrate meals and cold water exposure as well as stress.

Shortly before the onset of these attacks she had noted a mild persistent proximal lower extremity weakness which was non-progressive but did result in difficulty in climbing steps.

Past History: - Negative
Family History :- Negative
General Physical Examination: - Negative
Neurological examination:.
1) *Mental status* - Normal
2) *Cranial Nerves* Normal
3) *Motor system*
a) No atrophy
b) Strength: Intact except: Hip flexors 4/5, Triceps 4/5
Deep tendon reflexes: present but relatively quiet at quadriceps and Achilles
5) *Plantar responses* flexor
6) *Sensation:* Normal
Laboratory data:
1) *Sed. rate and thyroid functions* normal
2) *Serum potassium on admission* 3.9 mEq/liter (normal 3.9-5.0 mEq/liter)
3) *Creatine phosphokinase* 12.8/12.0
4) *EMG* - Normal
5) *Nerve conduction studies* normal.
Additional studies

With a baseline potassium of 4.7 mEq/liters, the patient was given 38 grams of glucose solution by mouth plus 5% dextrose in water intravenously plus 15 units of regular insulin. Within 15 minutes after insulin administration, the patient had increased weakness in all four extremities. By 30 minutes, the potassium level had fallen to 2.1 mEq/liter. At 60 minutes, the patient had total paralysis of all four limbs. (Quadriplegia). and no deep tendon reflexes could be obtained. She was flaccid unable to lift her head from the bed. However, she was conscious she could speak, and breath and all cranial nerves were intact. EKG did show the typical features of low serum potassium (flat T. waves). She was given oral potassium and within 2 hours had returned to her baseline state. She was subsequently treated with potassium supplements plus acetazolamide a drug which affects sodium potassium transport. Studies by Tacek and associates 1994 have demonstrated a dihydropyridine receptor mutation produces a calcium channel disorder resulting in hypokalemias. A mutation in the sodium channel produces the hyperkalemic form of periodic paralysis.

These cases of primary periodic paralysis must be distinguished from the more common secondary varieties which occur in relationship to

[4]*The relationship of the periodic paralysis disorders to the myotonic disorders and the more specific genetics and physiology are discussed in Ptacek et al 1993.*

the electrolyte alterations of renal disease, adrenal cortical disease, effects of diuretics, cathartics and in gastrointestinal disorders with severe diarrhea.

Acquired Disorders of Muscle

The major categories are as follows:

1) *metabolic:* hypokalemic/hyperkalemic myoglobinuria and alcoholic. (see above). Drugs such as colchicine used in the treatment of gout may also produce a myopathy.

2) *Endocrine:* Hyperthyroid (Thyrotoxic myopathy) hypothyroid (Myxedema Myopathy) and corticosteroid. The corticosteroid myopathy occurs not only in patients with Cushing's disease but also in many patients receiving long term corticosteroid therapy. Typically the weakness begin in the pelvic girdle and proximal muscles of the lower extremities.

3) *Trauma:* Closed muscle compartment compression due to trauma or a deep unconscious state produced by drugs and alcohol may produce considerable rhabdomyolysis of an acute nature (see Owen et al 1979).

4) *Inflammatory.* (Refer to Dalakas 1991).

Inflammation of muscle may occur in a) viral disease such as influenza on Coxsackie, b) bacterial infections by staphylococcus aureus or c) in parasitic infections due to toxoplasmosis, cysticercosis, and trichinosis that are of the major importance in some areas of the world.

The major considerations in this section are those cases where the polymyositis occurs on autoimmune bases.

In some cases, (idiopathic, polymyositis and dermatomyositis) the autoimmune disease is restricted to muscle (and skin). The cases cover a wide age range. The onset and source may be acute, subacute or chronic. The chronic gradually progressive pattern is the most common. Cranial nerves are usually not involved although difficulty in swallowing may be present due to posterior pharyngeal striated muscle involvement. Proximal muscles are involved predominantly in dermatomyositis. There is more diffuse involvement in polymyositis but with a proximal predominance (limb girdle and neck flexors). In dermatomyositis there is edema and bluish (heliotrope) discoloration of the eyelids, with a scaly red rash on the face (in a butterfly pattern), shoulders, upper chest, back and extensor surfaces of the limbs, Sedimentation rate and muscle enzymes are significantly increased. Muscle biopsy indicates segmental muscle necrosis, regeneration and inflammatory infiltrates, *Figs 6-16.* EMG indicates not only myopathic features but also the presence of fibrillations.

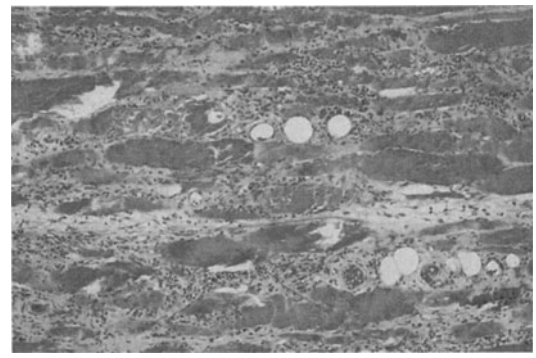

Figure 6-16. Muscle histopathology II: Polymyositis wide spread necrosis of muscle fibers is present with extensive infiltration by mononuclear inflammatory cells H&Ex25. (Compare to 6-15). Courtesy of Dr. Tom Smith, Neuropathology, U. Mass. Medical Center.

There is an increased incidence of underlying neoplasm in adults with dermatomyositis and polymyositis (particularly in older adults). Common sites are lung and ovary. Overall incidence in all cases of dermatomyositis and polymyositis -is 9% (Sigurgeirsson et al 1992).

The term overlap polymyositis refers to patients who develop polymyositis within the context of already existing autoimmune connective tissue diseases: systemic lupus erythematosus (SLE), rheumatoid arthritis, periarteritis nodosa, systemic sclerosis and Sjögren's syndrome. It has been estimated that 5-15% of all patients with these diseases will manifest polymyositis (Isenberg, 1984).

Most patients (60-70%) with polymyositis and dermatomyositis have a significant response to corticosteroid therapy. Non responders may require more definitive immunosuppressive therapy. Approximately one third of non-responders have another variety of myositis inclusion body myositis. Such cases have a male predominate relatively normal CPK and greater distal involvement (see discussion Dalakas 1991). Case 6-3 demonstrates dermatomyositis complicating ovarian malignancy.

Case History 6-3.

This 69-yr. old white female had partial resection of ovarian carcinoma in Dec. 1973 and received radiotherapy and chemotherapy in January and February 1974. Two weeks prior to admission she had developed progressive weakness of all four extremities with proximal more than distal involvement. Because of difficulty swallowing, she had been on a liquid diet for two weeks prior to admission. General Physical Examination: 1) There was superficial redness; swelling involving the skin of both upper extremities 2) Liver was enlarged.

Neurological examination

1) *Mental Status:* Intact

2) *Cranial Nerves*: Intact except absent gag reflex

3) *Motor System*: Weakness of all four extremities proximal greater than distal.

4) *Reflexes:* Deep tendon reflexes were depressed in the upper extremities and absent in the lower extremities

5) *Sensory System:* Intact.

Laboratory Data

1) *Erythrocyte sedimentation rate* increased to 50 mm per hour.

2) *Muscle enzymes* (SGOT LDH, CPK) all significantly increased with creatine phosphokinase elevated to 201/12 units.

3) *Antinuclear antibody titer* was not significantly elevated (test for SLE)

4) *EMG:* Abnormal - consistent with polymyositis.

Hospital Course:

The patient was treated with high dosage corticosteroids. - (Prednisone). Despite a significant improvement in muscle enzymes (CPK 201>9.9, SGOT.295>102 LDH 320 > 102), the weakness continued to progress. During the last week of life, she had three episodes of aspiration and increasing respiratory distress expiring 3-29-74.

Comment. This patient received a diagnosis of dermatomyositis complicating ovarian malignancy. In such patients deep tendon reflexes may be totally absent. Changes may be present on sensory or cranial nerve examination, suggesting a peripheral neuropathy. Peripheral nerve changes reflect - a) paraneoplastic effect of malignancy or b) the effects of chemotherapy.

The continued progression of the weakness in this patient is not unusual. The paraneoplastic polymyositis syndrome does not have the favorable response to corticosteroids found in the idiopathic syndrome.

DISEASE OF THE NEUROMUSCULAR JUNCTION

Diseases affecting the neuromuscular junction may be classified as to location: postsynaptic or presynaptic (see Newsome-Davis 1992, Engel 1984,Drachman 1986).

Postsynaptic Disorders-Myasthenia Gravis

The major primary disease due to neuromuscular junction pathology in this country and western Europe is myasthenia gravis. The prevalence is 5-7.5 per 100,000. This is a disease characterized by fluctuating weakness of voluntary muscle worse on exercise, improved by rest and by administration of anticholinesterase drugs. This is primarily a disease of adults with onset between ages 10 and 70. However neonatal and congenital cases are recognized. The peak age of onset is between ages 20 and 30. Under age 40, females are affected more often than men in a ratio of 70:30. After age 50, there is a male predominance of 60:40. Ten % of patients have an associated tumor of the thymus gland (thymoma). These patients tend to be older with a male predominance.

The symptoms usually first appear in the muscles supplied by the cranial nerves. The extraocular muscles for control of eye and eyelid movement are first affected in 60% of cases and are affected at some stage in 90% of patients. In some cases, with a relatively benign course, the symptoms may remain confined to the eye muscles, ocular myasthenia. The patient has a ptosis (closure or droop) of one or both eye lids, often most apparent on sustained upward gaze. Double vision (diplopia) results from a weakness of one or more extraocular muscles. The early occurrence of diplopia may be a reflection of the precise synchronization required between the two eyes in movement under normal conditions. A minimal weakness of one medial or lateral rectus muscle would disrupt such a precise synchronization. In the extensive series of Grob et al

with 1487 patients followed 1940-1985) 14% of patients continued to have localized ocular myasthenia.

Generalized myasthenia refers to progression to involve other cranial nerves and the extremity and trunk muscles. The patient will have difficulty swallowing (dysphagia), speaking (dysarthrias) and chewing (mandibular nerve supplied muscles). In addition a bilateral weakness of facial muscles may provide a suggestive myasthenic faces. The patient may support head or jaw with the hand to compensate for weakness. As the disease progresses, more generalized involvement of proximal limb muscles develop.

In some patients there is significant involvement of the muscles of respiration. In the series of Grob maximum level of weakness was reached during first year in 55% first 3 years in 70% and first 5 years in 85%. After 3-5 years, the disease stabilizes.

The prognosis depends on the age of the patient, the duration of the localized form of the disease and on the associated diseases: (thymoma, thyroid disorders other autoimmune disorders).

Myasthenia was once associated with a significant mortality due to respiratory insufficiency and infection but this mortality has now been markedly reduced. In the series of Gob, et al. for patients with maximum weakness in the 1940-1957 era - mortality was 31%, rate of remission 10%. In 1958-1966 era, the mortality was 15% rate of remission 10%. In the 1966-1985 era, mortality was 7% remission rate was 11%.

The diagnosis is readily apparent from a clinical standpoint: Fluctuating weakness a) no sensory finding b) Intact reflexes and no long tract findings. However there are several confirmatory diagnostic tests. Jolly in 1895 first described the electrical test that carries his name. Repetitive stimulation of a motor nerve result in a rapid decrease in the amplitude of muscle contraction "decremental response". Jolly demonstrated that the apparently fatigued muscle would still respond to direct galvanic stimulation.

In modern clinical neurophysiology the motor nerve is stimulated at a rate of 3-5 per second and the reduction in amplitude of the muscle action potential is measured. (Abnormal is greater than 10% reduction). An agent which blocks acetylcholine esterase, is then administered (edrophonium or neostigmine). This increases the duration of available acetylcholine at the neuromuscular junction (edrophonium or neostigmine) and the decremental response is reversed.

The edrophonium (Tensilon) test can be carried out in the outpatient office. This agent administered intravenously will often transiently reverse or reduce the signs of weakness. Clinical effect occurs in 30-60 seconds and lasts 4-5 min. Since related cholinergic agents (anticholinesterases) play a major role in therapy the test can also be performed to determine whether the patient is receiving too little on too much of the therapeutic agent.

A very specific test is the measurement of the blood level of the acetylcholine receptor antibody positive in 85-90% (see below).

Recently major advances have been made in our understanding of the underlying pathogenesis and pathophysiology of this disorder. This constitutes a major accomplishment for neurobiology that has been translated into improved results as regards therapy of the disease.

The role of the thymus appears critical from several standpoints. 1) The thymus has a central role in immunology. 2) The thymic hyperplasia involves the presence in the medulla of many germinal centers and surrounding T cell areas. Such germinal centers are rare in non-myasthenic thymus. 3) The thymus contains muscle like cells, and ACH receptors can be demonstrated when these cells are placed in tissue culture. Drachman 1978 has suggested that this receptor bearing muscle cells may be particularly vulnerable to immune attack. Some alteration of these cells by lymphocytes (perhaps triggered by viral infection of thymus etc.) could initiate any acute immune response directed against ACH receptors.

The essentials of therapy in myasthenia gravis follow from the neurobiology of the disease.

1) Initial therapy consists of anticholinesterase drugs. The main drug used is pyridostigmine (Mestinon) which has a somewhat longer duration of action than neostigmine. For some patients this will suffice particularly if only local ocular myasthenia is present and if this responds well.

2) Generalized myasthenia will almost always

require more definitive therapy directed at the immune systems.

a) The most definitive procedure is total thymectomy. As discussed by Rowland, (1987) there is now a consensus that all adults with generalized myasthenia should have this procedure relatively early in the course of the disease. In the "maximum thymectomy" series of 72 non-thymoma patients previously with moderate to severe generalized myasthenia reported by Younger et al. (1987), 46% of patients were in complete remission (on no medication). 33% were asymptomatic on 60-240 mg of pyridostigmine daily and 10% were asymptomatic on steroids. Approximately 90% then were in complete remission or asymptomatic. An additional 6% were improved. At least 1-4 years were required to see the maximum response to therapy. There was in significant decline in the titre of acetylcholine receptor antibody. Although this was not a matched control series, the results are in striking contrast to the contemporary results of non surgical treatment of moderate to severe generalized myasthenia (see Grob. 1987). Other major centers have confirmed these results. Schumm et al. (1985) has also recommended the procedure in patients with pure ocular myasthenia, if no spontaneous remission and no satisfactory response to cholinesterase inhibitors occurs in a 6-month period. In their series, thymectomy prevented the subsequent development of generalized myasthenia; none of 18 patients progressed over two years from ocular to generalized myasthenia.

Thymectomy was originally performed primarily to remove thymomas in patients who coincidentally also had myasthenia gravis; some improvement in the myasthenia also occurred (Blalock et al. 1939). Considerable experience in subsequent years has demonstrated a poorer response of the myasthenia in such cases of thymoma to thymectomy.

b) Other types of immunosuppression may also be employed in the pre or postoperative period or in those patients unable to tolerate thymectomy. These include corticosteroids, plasmapheresis (plasma exchange) and cytotoxic drugs (azathioprine).

Myasthenia Gravis

Adams and Victor (1989) have summarized the major historical landmarks in the study of myasthenia. Welch in 1877 and Erb in 1875 recognized the lack of any pathology in brain stem to explain the cranial nerve motor findings. The electrical studies of Jolly in 1895 suggested what has come to be recognized subsequently as the location of pathology: the neuromuscular junction. The term's pseudoparalysis as well as myasthenia gravis was originated by Jolly since no pathology was present at autopsy. He also recommended the use of an anticholinesterase physostigmine. Walker in 1934 noted the similarity of some of the signs to those produced by the poison curare and began the use of physostigmine. Buzzard in 1905 in a detailed clinical pathological analysis of 5 cases described the lesions in thymus; (Thymic hyperplasia which now has been noted in over 80% of patients) and the minor lymphocytic collections in muscle. Buzzard proposed that an autotoxic agent could produce all of these findings. He also noted the association of the disease with thyrotoxic cases. Simpson in 1960 proposed an autoimmune basis for the disease because of the increased incidence of other putative autoimmune disease: thyroiditis, lupus erythematosus and rheumatoid arthritis.

Electron microscopic studies by Zack et al. 1962 and by Engel and Sarta 1971, Engel et al 1976 had demonstrated significant changes in the postsynaptic region with shallow postsynaptic folds, a widened synaptic cleft. In contrast the presynaptic area was normal as regards the number and size of presynaptic vesicles. Fambrough, Drachman and Satyamurt; (1973) then demonstrated a marked (3 fold) reduction in acetylcholine receptors per neuromuscular junction in motor point nerve biopsies of myasthenic patients. Compared to control subjects, there were a decreased number of binding sites for a radioactive labeled snake poison, alpha bungarotoxin which can be purified from the venom of the cobra and krait. This toxin binds in an irreversible manner to the acetylcholine receptor. In 1973, Patrick and Lindstrom demonstrated that repeated immunization of rabbits with acetylcholine receptor protein derived from the electric organs of eels reproduced the disease. These ani-

mals develop all of the clinical and electrical features of myasthenia gravis. Antibodies to the ACH receptor protein could be identified attached to the ACH receptor. Moreover, normal animals receiving these antibodies also developed myasthenia.

Subsequently Lindstrom et al (1976) and several other groups reported a radioimmunoassay for acetylcholine receptor antibody. This is now a standard laboratory test for myasthenia gravis with a 90% detection rate in the serum of myasthenic patients. (The serum level does not correspond to the severity of the disease). Engel (1984) were able to demonstrate immune complexes at the postsynaptic junction in biopsies from myasthenic patients. When the immunoglobulin derived from serum of myasthenic patients is administered to mice, the characteristic myasthenic syndrome develops in the recipient animal (Toyka, et al. 1974). The syndrome of neonatal myasthenia also suggests a serum or plasma transmissible factor. 15% of children born to myasthenic mothers will manifest clinical signs of weakness lasting several weeks due to passive transfer of anti ACH receptor antibodies across the placenta. Circulating ACH receptor antibodies and electrophysiological findings can be demonstrated in these infants and even in additional neonates who do not manifest clinical weakness.

Subsequent studies reviewed by Drachman (1986) suggest several mechanisms for antibody action: 1) there is a significant increase in the rate of degradation of ACh receptors, 2) the antibody actually blocks the binding sites of the acetyl choline receptor and, 3) there is a complement mediated damage to and subsequent change in the geometry of the junction with a reduction in efficiency of transmission.

Cells cultured from patients with myasthenia gravis but not from control subjects can synthesize acetylcholine receptor antibody in vitro. The highest levels of production are obtained from thymic cells of patients with medullary thymic hyperplasia. Peripheral blood lymphocyte lymph nodes and bone marrow also produce the ACH receptor antibody.

Case History 6-4. NECH.

This 44-year-old, white, married, machine operator for several years had noted a general sensation of fatigue in his arms and legs at the end of a day. Approximately 6 to 7 weeks prior to admission, on Dec 7, 1975 the patient began to have more significant difficulty with a marked increase in the degree of weakness of the arms and legs. At the same time, the patient noted significant slurring of words, difficulty in swallowing, and drooping of the lids. All of these symptoms were transient; they were not present in the morning; they were clearly precipitated by exercise. For example, although the patient would initially have strong chewing movements, as soon as he began to chew for a short period he would develop fatigue of jaw muscles. The degree of ptosis was sufficient to result in difficulty in driving.

The patient had been receiving neostigmine 15 mg every 6 hours. He reported that 30 mg every 6 hours produced significant diarrhea and hypersalivation. In addition this dose exacerbated swallowing and chewing problems.

Past history: The patient had a long-standing problem of marked obesity.

Neurological examination: Four hours after neostigmine 15 mg.

1. *Mental status*: intact

2. *Cranial nerves*:

a. A significant bilateral ptosis of the eyelids was present. The degree of ptosis was markedly increased by exercise when complete closure of the left eyelid occurred.

b. On repetitive upward gaze, a bilateral weakness of superior rectus developed.

c. There was a bilateral facial weakness, worse on exercise, more marked on the right than on the left.

d. Jaw movements (opening, closing, and lateral movements) were weak. The degree of weakness was increased by exercise.

e. Lateral tongue movements, particularly on sustained pressure, became weak.

3. *Motor system*:

a. There was a significant weakness in shoulder abductors, elbows flexor and extensors, and handgrip. The degree of weakness was markedly increased by repetitive exercise.

4. *Reflexes:*

a. Deep tendon reflexes were symmetrical.

b. Plantar responses were flexor.

5. Sensory system: All modalities were intact.

Laboratory data:

1. Chest x-rays and tomogram studies of thyroid function were normal. Subsequent tests for lupus erythematosus were negative.

2. A tensilon edrophonium test was performed. This demonstrated an almost immediate eye-opening effect with the disappearance of the bilateral ptosis. The ptosis, however, had reappeared 3 to 4 minutes after injection of 10 mg of the agent.

Subsequent course: The patient was treated with pyridostigmine and stabilized on a dosage of 90 mg 5 times per day (approximately every four hours). On the dosage his ocular bulbar and generalized weakness stabilized until June 1973 when increasing generalized weakness developed. The patient had gradually increased his dosage to 180 mg every three hours. Respiratory distress increased and the patient was readmitted in extreme distress in the superior position. Tidal volume was zero. He had marked bilateral ptosis and diffuse skeletal muscle weakness. Analysis suggested the patient was in "cholinergic crisis." He was intubated, ventilated and received no medication for 60 hours. Repeat tensilon test now demonstrated marked improvement in respiratory effort and in skeletal weakness. Pyridostigmine was reinstituted and based on edrophonium tests, the patient was stabilized on 90 mg every 3 hours. A tracheostomy was performed and thoracic surgery consulted regarding thymectomy. The surgeons considered that the marked obesity constituted too great an operative risk for surgical thymectomy. The patient instead received radiation of the anterior mediastinum, 3000 rads over one month. At the time of discharge tidal volume was now 3000 cc. The patient did well with no additional problems over the next three years. An exacerbation occurred in 1976.

He was subsequently treated with long term prednisone therapy eventually being maintained an alternate day therapy. On March 26 1983, he had a respiratory arrest followed by cardiac arrest. Despite resuscitation he died after a one-month period of anoxic encephalopathy.

Other post synaptic syndromes: pharmacologic and toxic: (see also discussion above)

1) Acetylcholinesterase inhibitors: The usual action of acetylcholine esterase at the neuromuscular junction is to rapidly terminate the action of ACH. This allows high rates of transmission across the synapse. If acetylcholine esterase is inhibited, acetylcholine remains on the receptor and in the synaptic space.

There are two classes of acetylcholine esterase inhibitors:

a) reversible: (carbamates), and

b) relatively "irreversible" (organophosphates)

a) The reversible agents include those anti ACH esterase drugs used in the treatment of myasthenia gravis. An increased availability of acetylcholine is of value in counteracting the weakness due to decreased numbers of receptors in this syndrome. Excess amounts of such drugs produce excessive amounts of acetylcholine at the receptors as noted in the above case history resulting in increased weakness referred to as "cholinergic crisis" The dose of the drug must be reduced. In the management of such patients the edrophonium ("Tensilon") test is of value.

b) The relatively irreversible agents (organophosphates) constitute a much more serious problem - not only does a significant acute cholinergic effect occur but there is damage to the postsynaptic membrane and delayed effects on peripheral nerve. Senanagake and Karolliedde 1987 distinguished three syndromes. 1) The acute cholinergic syndrome is accompanied by autonomic effects fasciculations, and at times central effects (coma) 2) The intermediate syndrome occurring in 10% of patients after recovery from the acute syndrome 24-96 hours after the poisoning - identical to an acute myasthenic syndrome with respiratory cranial nerve and proximal limb muscle involvement. If the patient survives the respiratory problems, recovery may occur in 5-18 days. 3) A delayed distal motor neuropathy occurs in some patients at 2-5 weeks after exposure.

The organophosphates are the neurotoxic agents of chemical warfare. They are also extensively employed as pesticides. As discussed by Davis (1987) this is a major problem in the developing countries where the sale of such chemicals is often unregulated, the chemicals are often repackaged in unlabeled containers. The original containers are often reused. The storage

and use are often unsupervised and the population is often unable to read any warning label.

The magnitude of the problem is staggering. In Sri Lanka alone between 1978 and 1980, there were 80,000 patients admitted with pesticide poisoning and 6083 of these patients died. Seventy five % of all cases were due to organophosphorous. In Sri Lanka, the majority of complex cases were the result of ingestion with suicidal intent.

2) Agents that bind to the acetylcholine receptor and thus block access of acetylcholine to the receptor.

a) *d-tubocurarine* - competes for the receptor in a reversible manner. Initially a South American Indian arrow poison - now used in anesthesia.

b) *Repolarizing agents:* biquartenary ammonium salts. These resemble acetylcholine. They are agonists that initially produce depolarization and then block the site. They are utilized to produce muscle relaxation during general anesthesia and during mechanical respiration.

c) *Snake toxins*: derived from the venom of the cobra and krait. These snakes are common in India, Sri Lanka, Southeast Asia and Taiwan. The neurotoxic effect produced is often an acute myasthenic syndrome with respiratory paralysis. As discussed by Watt et al. (1986) and by Drachman (1986) for some of the toxins, - alpha cobra toxin, the binding is reversible and can be treated with anticholinesterase. For other toxins in the group, alpha bungarotoxin from the Taiwan Krait, the binding is less reversible and the effects of anticholinesterase less consistent. Some snake venoms also contain toxic agents that act at the presynaptic region (B. bungarotoxin of the krait).

1) *Eaton-Lambert (or Lambert-Eaton) Syndrome:* The patient usually an adult has predominantly proximal limb weakness which may be progressive but which fluctuates. In contrast to myasthenia, a) bulbar ocular and respiratory muscle involvement is not common. b) Exercise improves the weakness. c) Repetitive stimulation of the motor nerve produces an incremental response in the muscle action potential.

Most patients with this disorder have an underlying malignancy, most often a small cell carcinoma of the lung. The neuromuscular syndrome however may precede the appearance of the tumor by months or years. In some patients (1/3) a malignancy may not be identified, but there may be other evidence of autoimmune disease.

The underlying pathophysiology relates to a decrease in the release of acetylcholine quanta from the nerve terminal (Not only at the neuromuscular junction but also at cholinergic nerve endings in the autonomic nervous system). As discussed by Newsom-Davis (1992), this defect in release relates to immunoglobulin g antibody that binds to presynaptic Ca channels and prevents their voltage gated opening. As the action potential enters the presynaptic region, fewer Ca channels open, less Ca++ enters to catalyze the binding of vesicles to the surface membrane. Hence less ACH is released. The disorder may be transferred passively (via plasma from patients) to mice. The patients are improved by plasma exchange or by drugs that increase acetylcholine release. Treatment of the underlying malignancy may produce some improvement.

2) *Botulism*: The toxin of the bacteria Clostridium Botulinum produces an acute syndrome of paralysis involving extraocular muscles, cranial nerves muscles of respiration and then progressively descending to involve limbs. Cholinergic autonomic junction is also involved. Thus the pupil which is spared in myasthenia often is involved (dilated). Anaerobic bacteria that may contaminate improperly canned food or preserved meat produce the neurotoxin. Clusters of cases bring this problem to alteration the attention of public health officials. Many cases are fatal. In infants and some adults, the bacteria itself may be present in the gastrointestinal tract or in a wound producing the toxin. (See Bartlett 1986). The toxin binds rapidly to cholinergics nerve endings and blocks the quantal release of acetylcholine. Once the toxin enters the nerve ending, the available therapeutic antitoxin if administered has little action. The flaccid paralysis then is often long lasting (see Chia et al. 1986).

3) *Antibiotics:* The aminoglycoside antibiotics: neomycin, streptomycin, and kanamycin act to interfere with the quantal release of acetylcholine. In most patients this presents no signif-

icant clinical problem. When blood levels are high due to high dosage or renal failure and when the patient has myasthenia or a subclinical myasthenia. There may be the presentation of an acute paralysis. Most often there is an associated factor of anesthesia and the patient fails to regain normal ventilation after anesthesia has been discontinued. The effects may also be seen with another type of antibiotics.

4) *Spider venom:* Black widow. This toxin produces rapid release of quanta of acetylcholine storage vesicles. The clinical syndrome is characterized by severe muscle contraction followed by paralysis.