Chapter 5

Muscle

Our bodies contain three kinds of muscle—skeletal, smooth and cardiac—classified according to their structure and function. Muscle cells are excitable and contain the proteins necessary for contraction. Muscles convert chemical energy into mechanical energy—movement. **Skeletal muscles** are characterized by the presence of thin, light and dark bands (striations) that are seen to lie across fibres when viewed through a microscope. These muscles form some 40% of the fat-free body weight. They are under voluntary control and are the only tissue through which we can directly influence our environment. In contrast to skeletal muscles, **smooth muscles** (which are also called involuntary or visceral muscles) lack transverse striations and are not under conscious control; they are found in viscera and blood vessels. **Cardiac muscle**, like skeletal muscle, is striated and like smooth muscle is not under conscious control; it generates the pressures required to drive blood around the vascular system and is described in Chapter 15.

5.1 Skeletal muscle

Skeletal muscle cells, known as fibres, are large and multinucleate, and are characterized by transverse striations and the ability to contract rapidly. The two functions of contraction of skeletal muscle are to maintain or move one component of the skeleton relative to its neighbour, and to produce heat. The force necessary to do this is generated by a regular array of actin and myosin filaments, the contractile proteins, and is fuelled by adenosine triphosphate (ATP) hydrolysis. The contraction of each muscle fibre is preceded by the generation, near the motor end-plate, of an action potential which travels along the muscle membrane and down the transverse (T) tubules. The action potential synchronizes and initiates each contraction by promoting the release of Ca^{2+} from the sarcoplasmic reticulum (SR). The elevation of intracellular Ca^{2+} stimulates the binding of myosin cross-bridges to actin; the subsequent flexing of the cross-bridges generates force. The force generated by a muscle is dependent on both the frequency of the action potentials in the muscle fibres and the number of muscle fibres activated by motor neurones. Each motor neurone innervates a number of muscle fibres that together form the basic functional contractile unit called the motor unit. The tension developed by muscles is used to move limbs or to resist their movement, to close sphincters that control the emptying of hollow organs, to move the tongue and regulate the vocal cords, and to perform other specialized functions. The heat produced by muscles is used to maintain body temperature, either by non-shivering mechanisms regulated by hormones or by shivering which is under direct neural control. The needs of the body for a range of contractions, from slow and sustained to fast and brief, are

satisfied by the presence of muscles having different fibre types.

Basic biomechanics and contractions

Skeletal muscles are attached to bones via tendons. At the joints between bones, muscle contraction causes movement of the skeleton. If this movement takes the bones away from each other then the muscle is an extensor, e.g. triceps, and if the bones are brought closer together by the muscle contracting, it is a flexor, e.g. biceps. Many muscles exist as flexor–extensor antagonistic pairs. Muscles vary enormously in their capacity to generate force (tension) and in the rate at which this force can be developed. The maximal force that muscles develop is proportional to their cross-sectional area (up to 40 N cm^{-2}); thus, the 'strength' of a muscle is dependent on the number of muscle fibres and on their diameters, as well as the orientation of the fibre bundles. Under the influence of testosterone the cross-sectional area of muscles increases, leading to greater muscular strength in the average man compared to the average woman. As the contractions of muscles depend on the shortening of a large number of subcellular units (sarcomeres) arranged in series, the speed with which a muscle changes length depends on the number of units in the series, on the rate of their change in length, and on the magnitude of any external applied force opposing the shortening of the muscle. Muscles contain varying amounts of fibrous and connective tissue that also contribute to their mechanical properties (see also tetanic contraction).

Muscles are said to be contracting when the contractile machinery is active and energy is being consumed. The term contraction applies whether the muscle is shortening, remaining at constant length or lengthening. In the latter case, the contractile process may be activated but the muscle as a whole may be forced to lengthen by the imposition of external force. Such **eccentric** contractions are a feature of normal muscle function, and are essential to our ability to move ourselves about while opposing the force of gravity. All types of contraction are employed in everyday use, but it is convenient to study muscle contraction when either the length of the muscle or its load is constant. When the length remains constant (**isometric** contraction), we measure the force (tension) generated by the contractile machinery. When the load remains constant (**isotonic** contraction), we measure the rate of shortening of the muscle. These forms of contraction are used in everyday activities, e.g. isometric contractions are involved in the maintenance of posture and isotonic contractions in the lifting of limbs.

Cellular structure of skeletal muscle

Skeletal muscles cells are known as muscle fibres and are some of the longest cells in the body. They are formed from the fusing of several cells during embryogenesis, and hence are multinucleate, and range in length from a few mm to up to 5 cm, with a diameter of 50–70 μm. To help maintain synchronous activity individual fibres retain only a single neural contact near their midpoint.

Muscle fibre force is generated by intracellular contractile proteins arranged into **myofilaments** (Fig. 5.1). The myofilaments are in bundles, called **myofibrils**, which run the whole length of the fibre. Each myofibril is surrounded by the **sarcoplasmic reticulum (SR)** (Fig. 5.2), and between the lateral cisternae of the SR are fine **T tubules** opening out on the surface membrane (the **sarcolemma**). The complex of a T tubule and the two adjacent SR cisternae is known as a **triad** (Fig. 5.2), and in human muscles these are located at the junction of the A and I bands, see below and Fig 5.1.

Sarcomeres and contractile proteins

Myofilaments are arranged into **sarcomeres**, which are considered to be the basic contractile units of muscle fibres. Each sarcomere is approximately 2 μm in length and its limits are defined by a Z disc (line) at each end. From the Z line, **thin actin** myofilaments (approximately 5 nm wide and 1000 nm long) project towards the middle of each sarcomere (Fig. 5.3 and 5.4), and in the central region of each sarcomere the filaments inter-

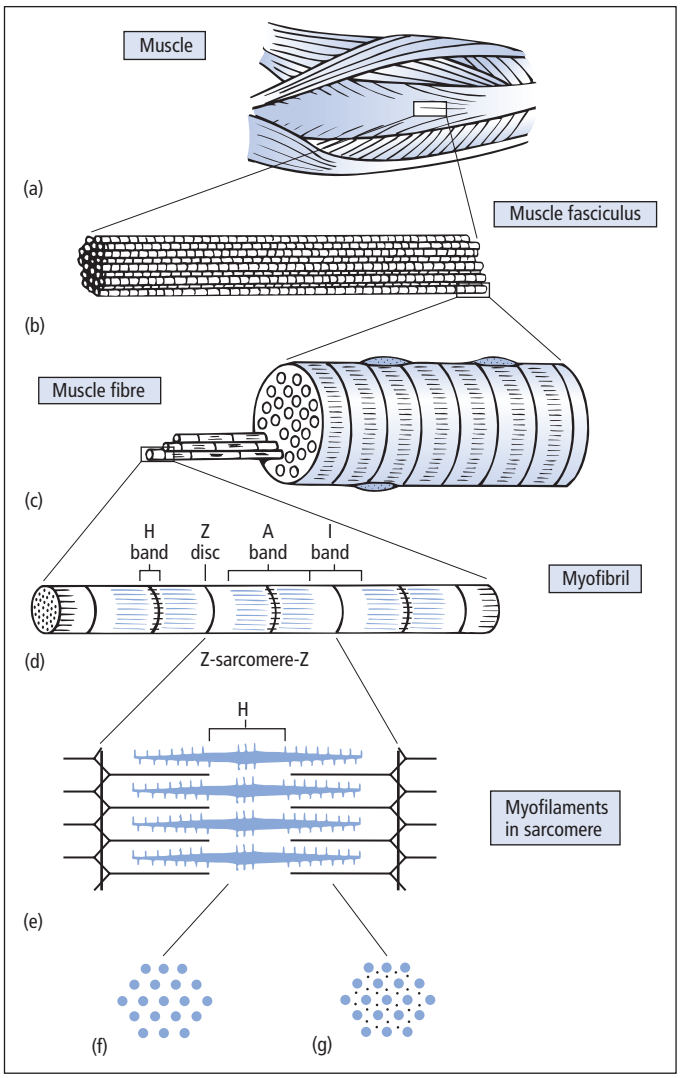

Fig. 5.1 Organization of muscle structure from whole muscle to myofilament (a–e) and transverse sections (f, g) showing the pattern of myofilaments. (Adapted from Bloom, W. & Fawcett, D.W. (1975) *A Textbook of Histology*, 10th edn, p. 306. Saunders, Philadelphia.)

digitate with **thick myosin** filaments (approximately 12 nm wide and 1600 nm long); each thick filament is surrounded by a hexagonal array of thin filaments (Fig. 5.1f,g).

Each thin filament is composed of two chains of globular actin molecules in a helical arrangement with two other proteins, tropomyosin and troponin lying in the grooves between the actin chains (Fig. 5.3a). Each thick filament is composed of myosin molecules (Fig. 5.3b) aligned with their tails parallel and pointing towards the middle of the filament (Fig. 5.3c). Their heads are helically arranged along the filament and form **cross-bridges** with the actin filaments.

The striated appearance of skeletal (and cardiac) muscle fibres is a result of the serial and parallel repetition of the myofilaments and the differing abilities of the actin- and myosin-containing regions to transmit light. As polarized light is not transmitted through the myosin-containing region (i.e. it is anisotropic), this region is called the A band (Fig. 5.1c). Light is transmitted through the

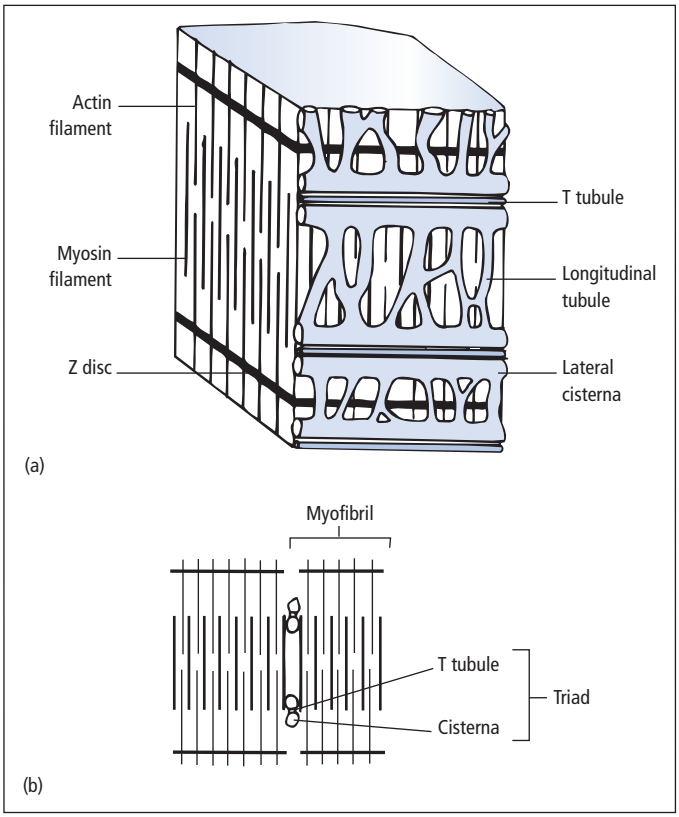

Fig. 5.2 (a) Diagram illustrating the sarcoplasmic reticulum and T tubules in mammalian skeletal muscle. (b) A transverse section through sarcoplasmic reticulum and T tubules illustrating the relationship between a T tubule and two adjacent lateral cisternae (a triad).

actin-containing region (i.e. it is isotropic) and so it is referred to as the I band. In the middle of the A band where the myosin and actin filaments do not overlap, there is a lighter H band which marks the region devoid of cross-bridges, and in the middle of this is a finer dark M line. The Z disc lies in the middle of each I band.

Cytoskeletal proteins, extracellular matrix and muscular dystrophies

The repeated cell shortenings associated with muscle contraction requires that both the regular array of the myofilaments be maintained, and that the cell membrane and associated structures withstand the deformations that occur. These important requirements are met by a large group of proteins—some of which have only been identified in the last decade—contributing to the alignment and stabilizing of myofibrils, maintaining anchorage to the surface membrane and extracellular matrix (basement membrane), and transmitting force laterally across the sarcolemma. The term **costamere** is used to encompass the subsarcolemmal structures that perform this function, arranged circumferentially in register with the Z discs.

The regular structure of skeletal muscles is maintained at rest and during changes in length by a network of stable filaments formed from such proteins as **titin** and **nebulin**. To date, titin is the largest protein in our bodies and a single molecule of it can stretch from the Z disc to the M line. In addition to stabilizing the sarcomeric structure by linking myosin filaments to the Z lines, titin is also responsible for the passive visco-elasticity of the myofibre. Nebulin, another huge protein, is a more rigid molecule linking Z discs to actin filaments. Several other proteins have been identified includ-

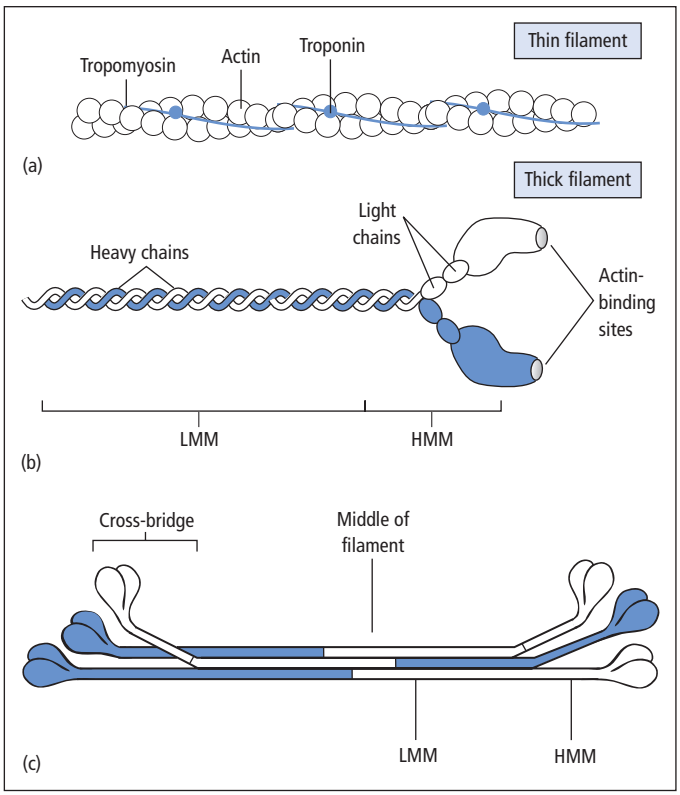

Fig. 5.3 (a) Actin filament composed of two chains of actin monomers arranged in a helix. In the grooves between these chains lie strands of tropomyosin and at regular intervals of about 40 nm are troponin molecules. (b) Myosin molecule composed of two filamentous heavy chains with globular heads bound to two pairs of light chains. The molecule can be cleaved by enzymes to produce a light meromyosin (LMM) fragment and a heavy meromyosin (HMM) fragment; the point of enzymatic cleavage is thought to be a region with some degree of flexibility. (c) The arrangement of myosin molecules in a filament. The LMM segment projects towards and forms the middle of the filament and forms the core, while the HMM segment extends to form cross-bridges.

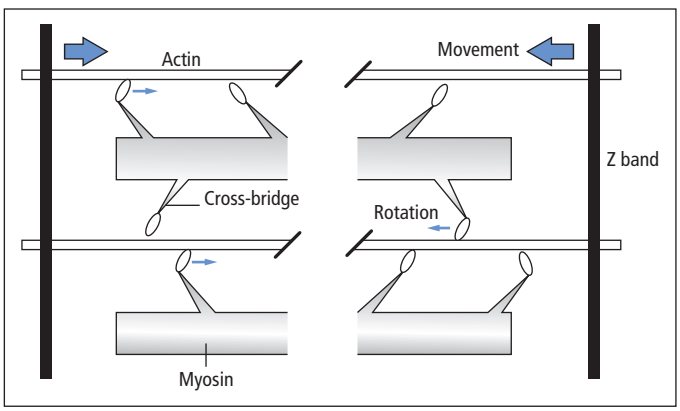

Fig. 5.4 Relative movement of actin and myosin filaments. This is accomplished by the rotation of the cross-bridge head, which contains the myosin ATPase. A second flexible point appears to exist where the cross-bridge joins the backbone of the filament.

ing **desmin**, a strong inelastic molecule that connects adjoining Z bands in a myofibril with adjacent myofibrils, and **skelemin** and **talin**, also with suspected roles in stabilization and linkage to the cell membrane.

A number of inherited diseases of muscle have been shown to be due to mutations in the molecules associated with costameres, including notably, some forms of dystrophy. The X-linked muscle wasting Duchene dystrophy for example, is caused by mutations in the gene encoding dystrophin. This protein forms a complex, which acts

to connect the cytoskeleton to the basement membrane. The sarcolemma of dystrophic fibres is easily damaged during contraction, leading to excessive Ca^{2+} entry and a cycle of fibre degeneration and regeneration, until the regenerative potential is exhausted.

Resting membrane potential and action potentials

The resting membrane potential of skeletal muscle fibres is –75 to –85 mV. The basis of this membrane potential is similar to that found in other excitable cells; that is, a high intracellular concentration of K^+ and a selective permeability that favours potassium (see Chapter 1). The membrane potential of healthy skeletal muscles is stable and thus contractions have to be initiated by stimuli triggering action potentials. These triggers are the transmission of action potentials from the motor nerves to the muscle. The local depolarization (end-plate potential) generated by the interaction of neurally-released acetylcholine with the acetylcholine (nicotinic) receptors (see Chapter 1), is more than sufficient to initiate an action potential at the sarcolemma surrounding the end-plate. The ionic basis of the action potential in muscle is similar to that in nerves, i.e. the depolarizing phase is caused by a rapid increase in conductance to Na^+. However, in mammalian muscle the major contributor to repolarization is Cl^- influx, rather than K^+ efflux. Having many Cl^- channels and few K^+ channels in the muscle fibre membrane is an advantage during repetitive activity (e.g. during exercise); it minimizes K^+ accumulation in the T tubules, which could cause prolonged depolarization of the muscle fibre, and minimizes rises in K^+ concentration in the plasma, which could disrupt the rhythmic activity of the heart. The total duration of a muscle action potential may be several milliseconds longer than that of an axonal action potential. Once initiated in the middle of each muscle fibre, the action potentials are conducted at about 4–5 m s^{-1} towards both ends of the fibre by local current flow, as in unmyelinated axons (Chapter 4). Thus in a 3 cm long muscle fibre an action potential with a velocity of 5 m s^{-1} will activate a 15 mm length of fibre (from endplate-to-end) in 3 ms, so that activation is essentially instantaneous with respect to the timing of the changes in $[Ca^{2+}]$ elicited by this activation, as discussed below.

Contractile process: the sliding filament theory

If a muscle changes its length, the sarcomeres also change in length. However, the length of the thin and thick filaments remains the same, with the change in muscle length resulting from the filaments sliding over each other. This is the basis of the **sliding filament theory** developed by Hansen and Huxley in the 1950s; the muscle shortens but the myofilaments remain the same length. The forces generated during contractile activity arise in the regions where actin filaments overlap the cross-bridges, i.e. myosin heads. During contractions the myosin cross-bridges attach to adjacent actin filaments and flex towards the centre of the sarcomere (Fig 5.4 and 5.5), thereby generating a tension. The muscle shortens when the active tension generated between actin and myosin exceeds any passive tension applied to the muscle externally. As activation occurs at both ends of the myosin filament, the opposing actin filaments are drawn in towards the centre, the Z bands are pulled closer and the muscle fibre shortens.

Muscle contraction has at least three requirements:

1 that the actin and myosin must interact;
2 that the myosin cross-bridges must flex; and
3 that the system must be able to convert chemical energy into mechanical energy.

Of these, number two still engages muscle physiologists in vigorous debate; for example how far can a myosin head flex? Early X-ray diffraction studies of muscles at rest and in *rigor mortis* (see below) showed that the cross-bridges could have two stable positions—the resting position and the flexed position. More recent pulsed irradiation–diffraction (synchrotron) studies of muscles have demonstrated movements of the cross-bridges during contractions; but as with X-ray diffraction, critics would point to the large degree of interpretation needed when applying these techniques to living

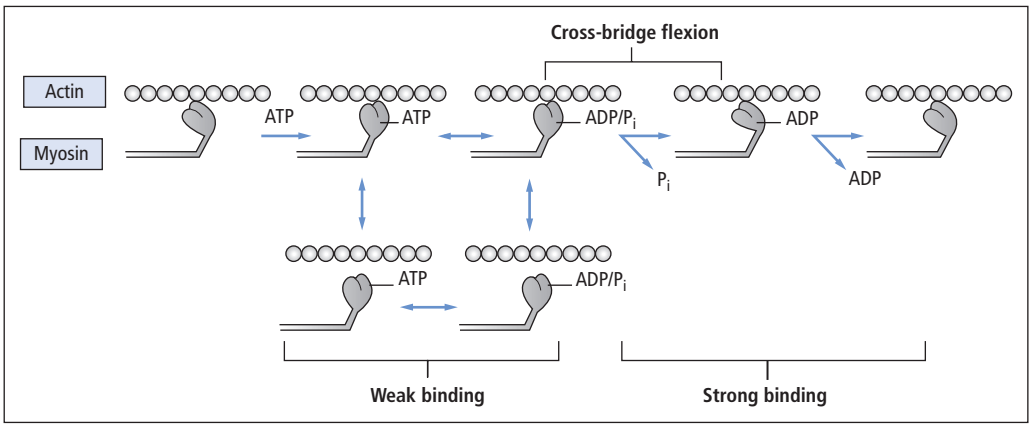

Fig. 5.5 The cross-bridge cycle. See text for explanation.

biological systems. Unravelling the mechanism involved in the transformation of chemical energy to mechanical energy has also proved elusive.

There is overwhelming evidence that ATP is the fuel used to operate the contractile machinery, and the myosin cross-bridges contain an ATPase. The rate of contraction of the sarcomeres appears to depend on the speed with which this enzyme can hydrolyse ATP. The interaction between actin and myosin is a multi-step process in which they may bind either strongly or weakly (Fig. 5.5). When neither ATP nor the products of its hydrolysis, adenosine diphosphate (ADP) and inorganic phosphate (P_i), are bound to the myosin head, the two proteins remain strongly bound. Consequently, muscles in which ATP is severely depleted (e.g. following death) are stiff and inextensible, a state referred to as **rigor**. When ATP or ADP/P_i are bound, the myosin rapidly attaches to, and subsequently detaches from, actin, in a weak binding relationship. This ATP-induced dissociation of actin and myosin allows the 'recocking' of the myosin head from its strongly bound flexed position in readiness for the next working stroke. When P_i is released from the myosin head, myosin undergoes a conformational change that results in both its strong binding to actin and the flexing of the myosin head (the working stroke), which generates the force to drive contraction. The subsequent release of ADP from myosin ensures strong binding of actin and myosin until the next cycle of ATP binding and hydrolysis.

As a single cycle of cross-bridge attachment and detachment produces only a movement equivalent to 1% of the length of a sarcomere, it requires repetitive cycling to achieve shortening of the sarcomere and hence, muscle. Thus in each cycle the cross-bridge attaches to the actin, flexes, and then dissociates before returning to its initial configuration and a new binding site on the actin filament. These repetitive cycles throughout the sarcomere must also be asynchronous from surrounding myosin filaments, to ensure that the force exerted on an actin filament is maintained during a contraction. This activity results in the actin filament being pulled between the myosin filaments. When the muscle is unable to shorten, the elastic properties of the muscle fibres allow the cross-bridge mechanism to operate and force to be generated. Consider for example, pushing on a wall; force is generated in the arms of your muscle but they do not shorten (and the wall does not move). Actively resisting extension (**eccentric contraction**) is an important part of muscle function, both dynamic and static.

Length–tension relationship

A testable hypothesis arising from the sliding filament theory is that force should be directly

proportional to the amount of interaction between the thick and thin filaments, as the more they overlap the more cross-bridges will be formed. As shown in Fig. 5.6 this hypothesis has proved to be correct, most impressively, in experiments performed on single muscle fibres.

In a single living muscle fibre the force generated can be shown to be related to the degree of overlap of the actin and myosin filaments (Fig. 5.6). It can also be seen that at long lengths, when there is no overlap of the actin and myosin filaments, the fibre is incapable of generating a force. In the intermediate range, when overlap of filaments is optimal, the force generated is maximal; at shorter lengths, the actin filaments overlap and interfere with each other and the force decreases. Eventually, at very short lengths (60–70% of the normal resting length), the Z discs will be pulled against the myosin filaments and the external force will again fall to zero. At this point the contractile machinery may still be active but the energy is used to distort the myosin filaments.

These findings relating to the length–tension relationship at the cellular and molecular level also apply to an entire muscle in our body. Thus, maximal muscle tension is generated when the muscle is approximately at normal resting length in the body. The relationship between the length of a muscle and the contractile (active) force that it develops can be examined by measuring the forces generated by a muscle at different lengths. Two forces can be measured: the **passive** force and the **total** force. When a relaxed (unstimulated) muscle held between a movable clamp and a force transducer (Fig. 5.7) is progressively stretched, an increasing force (tension), derived from an increasing resistance to stretch, can be measured (Fig. 5.7b). As the contractile machinery is not active, this force is passive and is due to the resistance exerted by elastic elements in the muscle—both extracellular components and also the elongation of myofilaments.

The force generated by stretch is not directly proportional to increase in length, as the elastic modulus increases with lengthening of the muscle. Some muscles, for example the back muscles of humans and the hind-leg muscles of kangaroos, which contain large amounts of elastic extracellular matrix material, can strongly resist extension by using purely passive mechanisms.

In Fig. 5.7b we can see that the **active** force developed when the muscle is stimulated at various lengths shows a similar relationship with length to that of the single muscle fibre. Thus the maximal active force is seen to occur near the natural resting length, and to decrease with changes in length from this position, just as it did at the level of a single sarcomere. The curve of **total** tension (Fig. 5.7b) is the sum of both the **passive** force (as described above) and the **active** force. (The amplitude of the

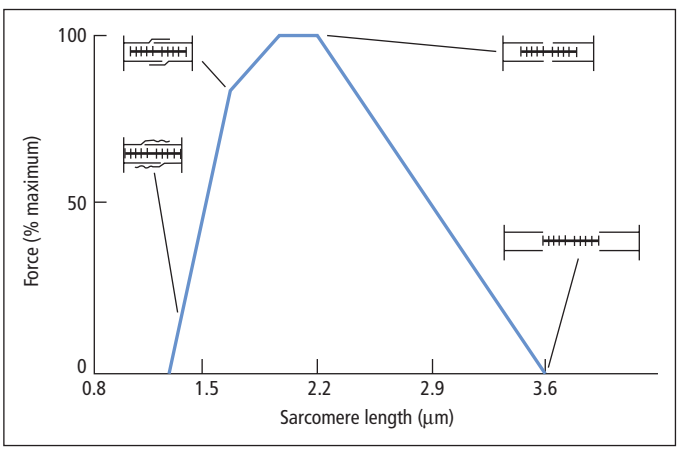

Fig. 5.6 The relationship between the contractile force and sarcomere length in a single muscle fibre. The insets illustrate the degree of overlap of the myofilaments at the sarcomere lengths indicated. (After Gordon, A.M., Huxley, A.F. & Julian, F.J. (1966) *J Physiol*, **184**, 170–92.)

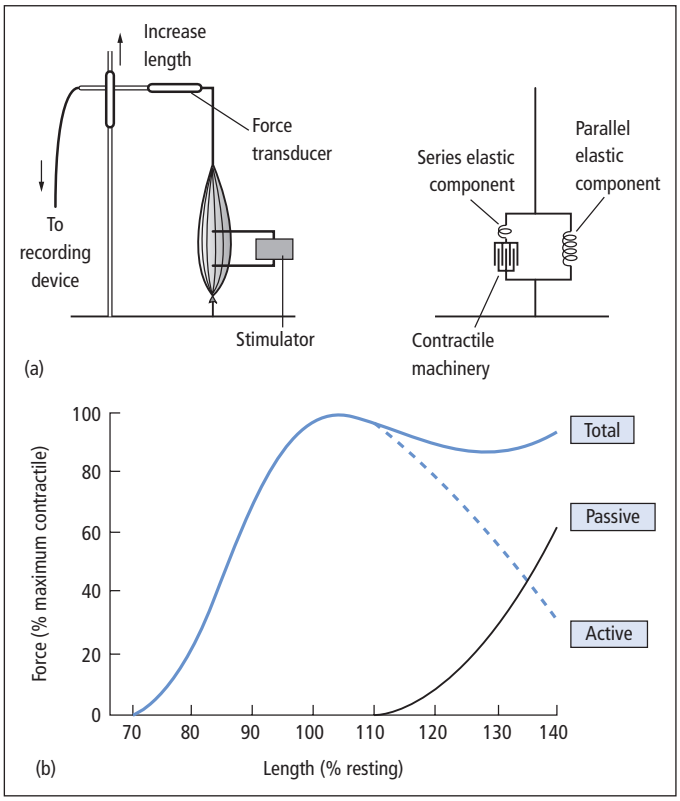

Fig. 5.7 (a) The experimental set-up used to study isometric contraction at different lengths (left) and a model of the contractile and elastic elements in muscle (right). (b) The relationship between force and muscle length. Note that the total force generated at each length is the sum of the active force generated by the contractile elements and the passive force due to extension of the elastic elements.

active force at any length is obtained by arithmetically subtracting the passive force from the experimentally measured total force.)

Excitation–contraction coupling

The term excitation–contraction coupling is used to discuss how events following the action potential at the sarcolemmal membrane lead to contraction. This coupling is accomplished via cytoplasmic Ca^{2+} and two regulatory proteins associated with the thin filament, tropomyosin and troponin. At rest this regulation is such that myosin ATPase activity is very low and thus the sliding of filaments cannot occur.

Myosin ATPase activity, and the contraction of muscle, depends on the interaction between the actin and myosin filaments, which is regulated by cytoplasmic Ca^{2+} (Mg^{2+} is also necessary for myosin ATPase activity but is in adequate supply). At rest the free cytoplasmic Ca^{2+} concentration is so low (10^{-8} mol L^{-1}) that little interaction occurs. However, during activity the concentration rises sharply (~10^{-5} mol L^{-1}); thus the free cytoplasmic Ca^{2+} regulates the development of tension within a muscle fibre and its control is vital.

Tropomyosin and troponin regulate contraction at the molecular level

In resting muscle, the free cytoplasmic Ca^{2+} concentration is low because the SR contains a membrane-bound pump (a Ca^{2+}–ATPase) that actively binds Ca^{2+} and then transports it to the lateral cisternae. However, this Ca^{2+} can be released by depolarization of T tubule membranes that come into close apposition with the lateral cisternae (Fig. 5.8). The T tubule membrane contains receptors that are sensitive to voltage (dihydropyridine receptors; DHP) and are mechanically linked to the Ca^{2+}-release sites on the SR. An action potential

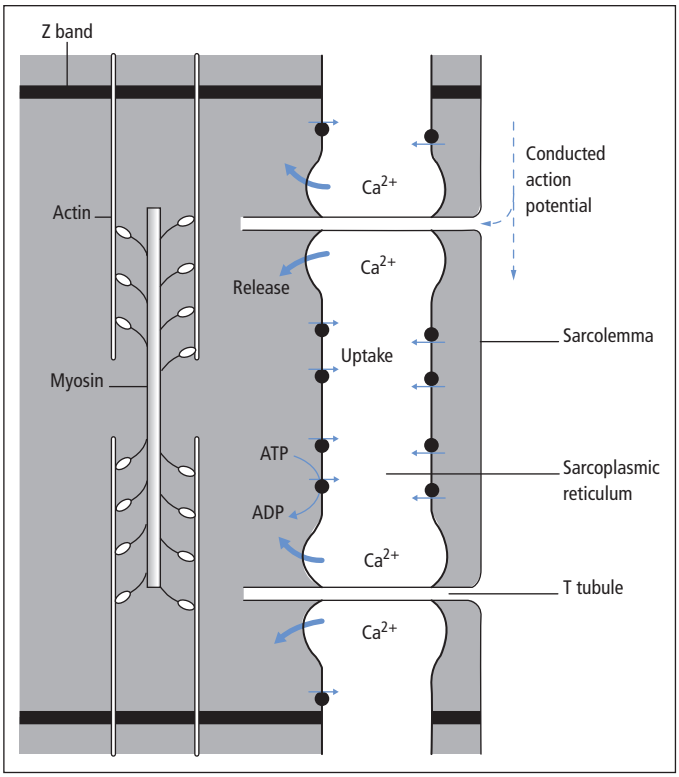

Fig. 5.8 The release of Ca^{2+} from the sarcoplasmic reticulum and its reaccumulation by an active transport process.

propagating along the muscle and down the T tubules is sensed by the DHP receptors; this causes a conformational change at the SR, which opens the Ca^{2+} channels resulting in an elevation in the free intracellular Ca^{2+} concentration. The abundance of the SR and fast speed of action potential propagation ensures that the contractile activity in adjacent myofibrils is synchronized. The released Ca^{2+} binds to troponin.

At rest the interaction between actin and myosin (and the myosin ATPase activity) is inhibited by the **troponin–tropomyosin** complex (Fig. 5.3a). The troponin (Tn) component is a complex molecule spaced regularly along the actin filament, which has specific tropomyosin-binding (TnT), calcium-binding (TnC) and inhibitory (TnI) subunits. The inhibitory effect of the complex on actin–myosin binding is removed when Ca^{2+} binds to the TnC subunit. The change is associated with movement of the tropomyosin strands that lie in the grooves between the strands of actin molecules. With this movement of the tropomyosin subtle changes in the conformation of actin occur, which unmask binding sites for the myosin cross-bridges and hence greatly enhances its interaction with myosin.

For **relaxation** to occur the central SR takes up the Ca^{2+} released from the lateral SR, using the Ca^{2+}–ATPase pump. As the cytoplasmic [Ca^{2+}] falls, Ca^{2+} is removed from TnC and this results in tropomyosin returning to its actin-blocking position. The myosin ATPase is then no longer activated, the filaments do not slide and therefore no further cross-bridges are formed. (Recall the role of ATP in this cycle—it is required for cross-bridge dissociation.)

In summary, for muscle to contract its motor neurone must produce an action potential and release ACh at the motor end-plate, which will depolarize the sarcolemmal membrane. This is sensed by DHP receptors and produces a conformational change in the lateral SR Ca^{2+}-release channel, and

thus Ca^{2+} rises and binds to TnC. This causes tropomyosin to move and reveal the myosin-binding sites on actin, and hence cross-bridges form. There will be many cycles of cross-bridges attaching and detaching, resulting in the filaments sliding and muscle contractions as ATP is hydrolysed by myosin ATPase.

The biochemistry of contraction

As mentioned above, ATP is the immediate source of energy for muscle contraction and is also required for Ca^{2+}-pumping back into the SR. However, very little ATP (~5 mM) is stored in a muscle—sufficient for only a few contractions. Thus ATP must constantly be supplied and renewed within the muscle cells. The short-term reserve for replacement is creatine phosphate (CP; also known as phosphocreatine), which forms a dynamic balance with free ATP; the enzyme creatine phosphokinase (CPK) ensures that this equilibrium is reached rapidly. Striated muscles have about 30 mM CP to buffer ATP, thus ATP is hydrolysed to ADP in the reaction:

$$ATP \rightarrow ADP + P_i$$

but the level of ATP is rapidly restored by the reaction:

$$ADP + CP \overset{CPK}{\rightleftharpoons} ATP + C$$

As the last reaction is reversible, the CP is restored by the production of new ATP. How metabolism supplies the new ATP depends upon the individual's stored reserves and the availability of oxygen, and in addition, on the biochemical preference of different muscles. ATP may be derived from the metabolism of glucose and free fatty acids from blood, or from reserves of glycogen and lipid droplets in muscle fibres. The storage of glycogen is a characteristic of skeletal muscles.

Under anaerobic conditions, which may occur if the muscle is working hard, the breakdown of muscle glycogen proceeds via the glycolytic pathway to lactic acid. The end-product is lactic acid rather than pyruvic acid, and the oxidized nicotinamide adenine dinucleotide (NAD) generated by the conversion of pyruvic acid to lactic acid is used in an earlier step. As discussed below, some muscle will obtain a major portion of the ATP necessary for contraction from anaerobic metabolism. During aerobic conditions both fatty acids and pyruvate can enter the citric acid cycle via acetylcoenzyme A, and thus a far greater amount of ADP is converted to ATP. For example, anaerobic metabolism of 1 mol of glucose generates 2 mol of ATP, but aerobic metabolism in which pyruvate is further catabolized by the citric acid cycle generates 38 mol of ATP per mole of glucose.

The whole process, that is contraction and relaxation, operates with an efficiency of conversion of metabolic energy into external work of the order of 10–20%; the remainder is dissipated as heat.

Heat production

Our everyday experiences reveal that muscular work is accompanied by the liberation of heat and that the amount of heat generated by muscles is proportional to the effort. The rate at which muscles produce heat may increase during maximal contractile activity to 20–50-fold the resting level. To a large extent the amount of heat ($m W g^{-1}$ tissue) produced by a muscle depends on the physiological characteristics of the muscle; fast-contracting muscles produce about six times more heat that slow-contracting muscles. As these two types of muscles have similar abilities to develop force ($N cm^{-2}$ cross-sectional area), it is clear that fast-contracting muscles are less efficient than slow-contracting muscles. Shivering when exposed to cold results in an increase in the production of heat by muscles; with intense shivering it may rise to some eight times the resting level.

Precise measurements of the heat released from an isolated muscle contracting at a fixed length reveal that heat production is maximal at the *in vivo* length and diminishes with either increases or decreases in length.

Twitches and tetanus: the frequency–force relationship

Shortly after a muscle is stimulated by a single stimulus there is an increase in muscle tension,

which then decays. The time course of an action potential and a contraction is shown in Fig. 5.9a. The time taken for the development of peak tension varies from 10 to 100 ms; its rate of decline also varies and both depend on the type of muscle being studied. A single contraction of this type is called a **twitch**. If the muscle is stimulated a second time, before it has had time to relax completely, the second response may add to the first and a greater peak tension is developed. This is referred to as **mechanical summation**. If the muscle is stimulated continuously, it fails to relax completely and during the period of stimulation the tension fluctuates (Fig. 5.9b). With increasing frequency of stimulation the maximum tension is increased, the oscillations become smaller and, eventually, at **fusion frequency** a smooth **tetanic contraction** is produced. The tension produced in tetanus may be two to three times as great as that produced in a twitch. Note that while the twitch provides a useful experimental measure of the properties of the muscle fibres, it is not a behaviourally useful action since the time course of the twitch is usually too short for a behavioural response to occur. Skeletal muscles, therefore, are activated in normal behaviour by volleys of action potentials which produce fused contractions.

The substantial difference between the maximal tensions reached in a twitch and a tetanus has been attributed to the physical properties of the muscle and to changes in the cytoplasmic Ca^{2+} concentration. First, muscles are not rigid and the forces generated by the contractile machinery are transferred to limbs by elastic structures (the tendons and my-

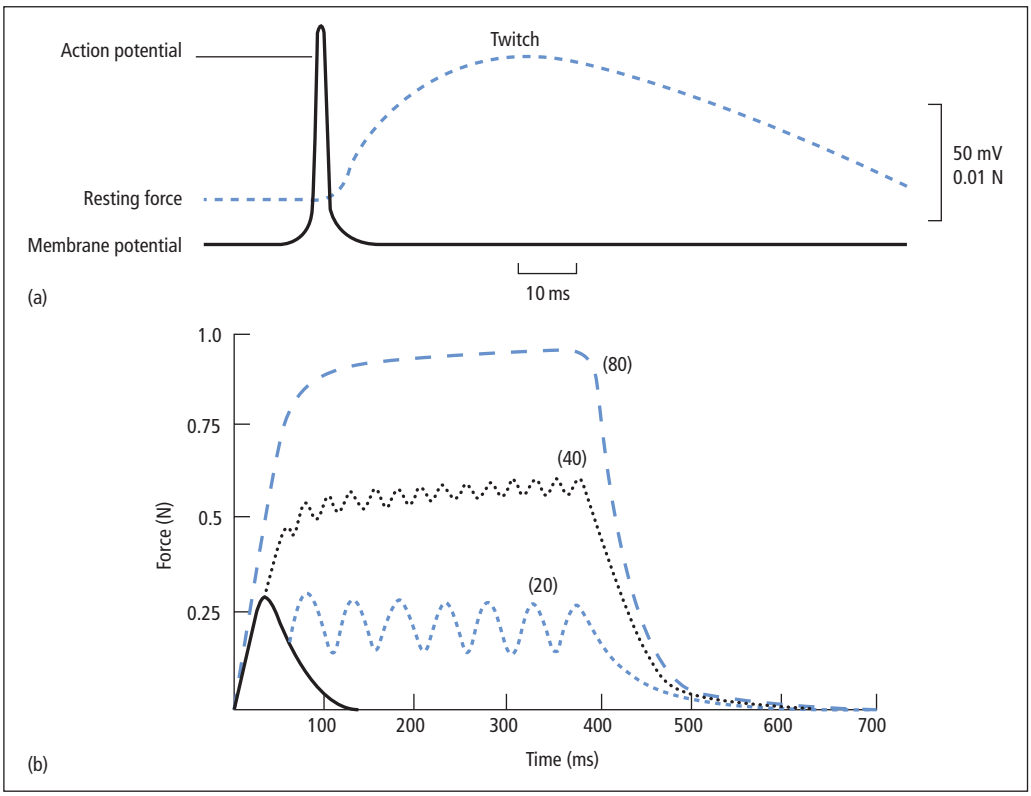

Fig. 5.9 (a) The time course of an action potential recorded intracellularly from a single fibre and the accompanying isometric twitch recorded from many fibres. (b) Isometric contractions from a rat extensor digitorum longus muscle showing the response to a single stimulus and to bursts of increasing frequencies (Hz), indicated in parentheses.

ofilaments). These are embedded in a viscoelastic medium (the cytoplasm, sarcolemma, sarcolemmal connective tissue and the connective tissue around fibre bundles), and many of these elements are arranged parallel to the contractile machinery. Thus much of the energy consumed in a twitch is used in overcoming the damping action of these elements. With continued activation, as in a tetanus, the elastic elements are already stretched and the maximum muscle tension is attained. Second, there is evidence that a higher level of cytoplasmic [Ca^{2+}], and hence muscle activation, is reached during a tetanus.

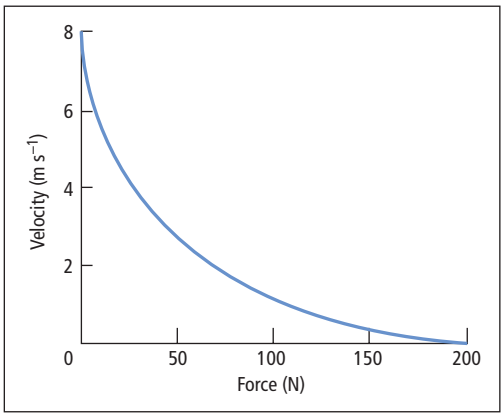

Fig. 5.10 The effect of force (load) on the velocity of shortening of human muscle. (Adapted from Wilkie, D.R. (1950) *J Physiol*, **110**, 249–80.)

Force–velocity relationship

The length–tension curve has described the ability of muscles to develop tension when the muscle is held at fixed lengths (isometric contractions). But, as mentioned earlier, the movement of limbs may be associated with the shortening of muscles under a constant load (isotonic contractions). It is an everyday experience that the lighter the load, the more rapidly it can be lifted. In fact, both the rate and the degree of muscle shortening depend on the load. The relationship between the rate of shortening, and the load carried, by a muscle is illustrated by the force (load)–velocity curve.

This relationship is determined by measuring the rate of shortening of a muscle as it lifts a variety of loads. The muscle is not initially subject to each load as this would alter the starting length of the muscle. However, before it can shorten the muscle must obviously first lift each load. Such an event is called an **after-loaded contraction**. When stimulated tetanically, an after-loaded muscle starts to contract. Initially, and until the tension exceeds the load, the contraction is isometric. After this, the muscle shortens isotonically and continues to shorten until it reaches the length at which (according to the length–tension curve) the maximal force it can develop is equal to the load. It is clear that with zero load the time required initially to shorten (the latency) will be minimal and the velocity of the contraction maximal (Fig. 5.10); as the load is increased the latency is increased and the velocity decreases. Finally, when the load is too heavy, the velocity of shortening is zero and the muscle is contracting isometrically. It can be seen from the force–velocity curve that the **power** (force × velocity) that a muscle develops is not constant. The power output of a muscle is in fact optimal when both the load and the velocity are moderate—hence the advantage of multiply-geared bicycles.

The reasons for the shape of the force–velocity curve are not known. One suggestion is that the myosin cross-bridges move continually as a result of thermal agitation and that there is only a limited space within which a cross-bridge and an actin site can interact. If this is correct and the actin filament is moving, the probability of successful union will decrease as the velocity of movement increases. Thus, at high velocities few cross-bridges are formed and the force is low because it is dependent upon the number of cross-bridges. Accordingly, the velocity of shortening will increase until the force generated by the muscle equals the load. If the force is either greater or less than the load, the velocity will either increase or decrease, respectively, which will in turn decrease or increase the number of bridges formed and the force generated. This idea is supported by the observation that the velocity of shortening in isotonic contractions is relatively constant.

Muscle fibre types

The diversity of muscular activity requires that muscles have different properties. Thus, some muscles are called upon to maintain a high level of tension for long periods without fatigue while others are required to produce intermittent rapid movements. These two extremes of activity are illustrated by the postural soleus muscle that reaches a peak tension in 80–200 ms (Fig. 5.11), and the extraocular eye muscles that develop their peak tension in 7–8 ms. The soleus muscle contains predominantly slow-contracting muscle fibres, and the extraocular muscles mainly fast-contracting muscle fibres. Muscles that have to perform both endurance and rapid actions have a more even mixture of these fibre types. When the properties of the slow (**type I**) and fast (**type II**) muscle fibres are compared, pronounced differences are evident. The slow fibres have a low myosin ATPase activity and a high capacity to produce ATP by oxidative phosphorylation, which is aided by a well-developed blood capillary network and high levels of intracellular **myoglobin**. The latter is an O_2-binding protein (like haemoglobin; see Chapter 13), which both facilitates the diffusion of O_2 into these muscle cells and stores a small quantity of O_2 in the cells. The simultaneously high concentration of myoglobin and high capillary density in these muscles have led to the use of the term 'red muscle'.

There are two distinct groups of fast-contracting fibres. Both have a greater diameter and a higher myosin ATPase activity than the slow fibres, but their resistances to fatigue differ (Fig. 5.11b). The resistance to fatigue is correlated with a high ox-

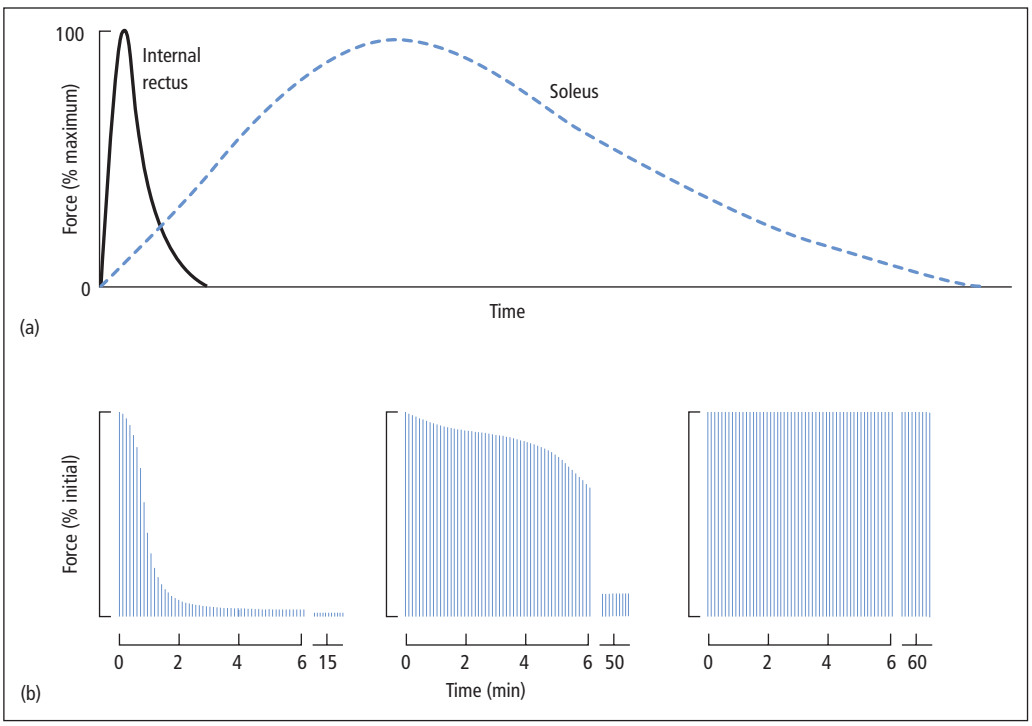

Fig. 5.11 (a) Isometric twitch contractions of cat internal rectus and soleus muscles scaled to the same peak height. (Adapted from Cooper, S. & Eccles, J.C. (1930) *J Physiol*, **69**, 377.) (b) Fatigue of fast (left), intermediate (middle) and slow (right) muscle fibres that were stimulated through their nerve supply at 40 Hz for 330 ms once each second. (From Burke, R.E., Levine, D.W., Tsairis, P. & Zajac, F.E. (1973) *J Physiol*, **234**, 723.)

idative capacity and those fibres with a high resistance are often referred to as **intermediate** fibres, required for example to perform marathon running. The largest and fastest contracting type II fibres (the so-called **fast** fibres) have a poorly developed oxidative metabolism and depend largely on glycolysis for the production of ATP; consider for example running the 100 m sprint. A summary of these and other properties of the different fibre types is given in Table 5.1. Thus differences at the molecular, biochemical and histological level underpin the broad physiological performance differences of our muscles.

Regulation of contraction at the gross level

The total force generated by a muscle depends on the number of active fibres and the level of activity in each fibre. Each motor axon entering a muscle makes contact with a number of muscle fibres; each of these fibres is innervated by a single terminal branch of that axon. Thus, groups of muscle fibres are activated synchronously.

Motor units

A **motor unit** comprises a motor neurone and the group of muscle fibres innervated by the branches of its axon (Fig. 5.12). Motor units vary greatly in size, ranging from one or two muscle fibres in the smallest units in muscles controlling the fine movements of fingers or eyes, to more than 2000 in the largest units in limb muscles. All the muscle fibres in a motor unit tend to be very similar in their properties; so the terms type I and type II are used for both motor units and muscle fibres. In general, the type I units of slow muscles are rather similar in size and are not particularly large; in contrast, type II units of fast muscles range from very small to very large. The larger a motor unit is, the larger the axon and the nerve cell body of the motor neurone supplying it. This probably reflects the need for production by the cell of all the materials needed to keep every one of its nerve terminals functioning.

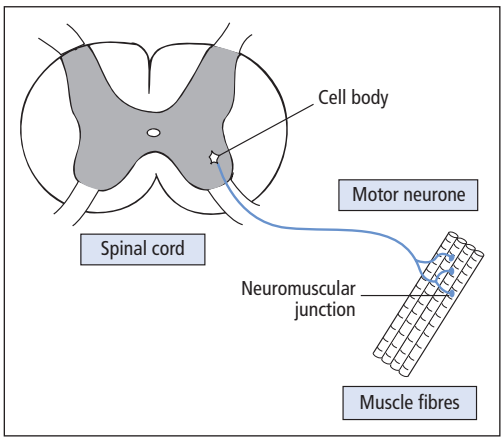

Fig. 5.12 A motor unit, consisting of a motor neurone and the muscle fibres that it innervates.

Table 5.1 Characteristics of type I and type II muscle fibres.

	Type I	Type II	
	High oxidative	High oxidative	Low oxidative
Rate of contraction	Slow	Fast	Fast
Myosin ATPase activity	Low	High	High
Main pathway for ATP production	Oxidative phosphorylation	Oxidative phosphorylation	Glycolysis
Number of mitochondria	Many	Many	Few
Myoglobin content (muscle colour)	High (red)	High (red)	Low (white)
Capillary density	High	High	Low
Glycogen reserves	Low	Intermediate	High
Rate of fatigue	Slow	Intermediate	Rapid
Fibre diameter	Small	Intermediate	Large

Gradation of tension

Increments in tension can result from an increase in the force generated by individual motor units or by bringing into action (**recruitment**) additional units. Extracellular recordings of the electrical activity of muscle fibres (electromyography) have shown that both these events occur, but not at the same rate. Thus, the initial development of muscle tension is thought to be due largely to recruitment of units. As explained below there is, in addition, an increase in firing frequency but the contribution of this to increments in tension is thought to be important mainly in the generation of larger forces.

The recruitment of motor units is not random but occurs in an orderly fashion from small to large. Low tensions are produced and precisely controlled by the selective activation of a number of small units. In fact, under most circumstances, a small proportion—paradoxically, the smallest ones—do most of the work. The largest units are activated only when a maximal effort is required and even then their activity is often brief.

Recruitment of motor neurones

The ordered recruitment from the **pool** of neurones supplying a muscle arises because the smallest cells are the most easily excited. The smaller surface area of the small motor neurones results in these cells having a higher input resistance. When similar excitatory synaptic currents are generated in the small and larger motor neurones, the small ones reach threshold first. As the intensity of excitatory synaptic activity in a motor neuronal pool increases, larger and larger motor units are recruited, and at the same time the frequency of discharges increases. However, there are also neural mechanisms that limit the discharge frequency of individual motor neurones to a frequency appropriate to the type of muscle fibres they innervate.

It should be noted that the contractions of skeletal muscles are not regulated solely by the motor units. These activities also make use of sensory information, including that from the muscles and limbs involved. The role of the muscle receptors (the muscle spindles and Golgi tendon organs) in motor control is discussed in Chapter 8.

Development and maintenance of skeletal muscles

The speed with which muscles can contract and their ability to do work are not constant throughout life, but change as a person grows and ages; their performance is also influenced by exercise. The development, growth and maintenance of muscles are all dependent on the presence of an intact motor nerve supply.

Development of muscles

Skeletal muscle fibres are derived from cells of embryonic mesodermal origin. These myogenic precursors have their origin in the **somites**, the tissue blocks that are adjacent to the developing brain and spinal cord (Fig. 3.7). Myogenic precursors (myoblasts) migrate from the somites to the appropriate position in the body where, under the influence of unknown environmental signals, they may exit the mitotic cycle and fuse with one another to produce multinucleate embryonic muscle fibre myotubes. This process occurs in two stages: an early generation of primary myotubes defines the anatomy and fibre organization of the adult muscle, and acts as a scaffold to guide the formation of secondary myotubes. The number of fibres in skeletal muscles appears to be genetically determined, but the expression of their full genetic capacity is dependent on the normal development of the nerve supply to the muscles. If during early development the motor nerves fail to maintain contact, the muscles will be smaller than normal due to a decrease in the number of their fibres.

As well as influencing the number of fibres in a muscle, some property of the neural input also appears to influence fibre type. This has been demonstrated in a number of ways, but most obviously in that the muscle fibres within a motor unit are homogeneous with respect to such properties as contraction time, resistance to fatigue, enzymes of anaerobic and aerobic metabolism and myosin ATPase. These properties are determined early in

development but they are not irreversible and changes can be seen in both developing and adult muscles, for example after denervation (see below). The ability of nerves to regulate the properties of muscles is referred to as a trophic influence but it is not known precisely how this influence is exerted. There is good evidence that nerve-induced muscle activity at the appropriate frequency (tonic low frequency for slow muscles and phasic high frequency for fast muscles) is important. Maintained low-frequency activation leads to a sustained rise in intracellular Ca^{2+}, which stimulates calcineurin, a Ca^{2+}-regulated phosphatase, leading to the activation of genes coding for slow-fibre specific contractile proteins. There is also evidence suggesting that specific messengers, **myogenic regulatory factors**, are released by motor nerves to influence the muscle fibres that they innervate. Experimentally, if the normal input to a muscle is cut and replaced with a nerve of a different type, the properties of the skeletal muscle will gradual transform to those of the muscle type previously innervated by the nerve. An exciting therapeutic use of this knowledge has been to transform the properties of skeletal muscle, e.g. latissimus dorsi *in vivo*, to become more like cardiac muscle in terms of non-fatigability, and to use it for cardiac assist in patients with failing hearts, by forming an additional ventricle.

Effects of training

Type I fibres make up about 30–40% of the cells in human muscles and they are about the same size in men and women (the mean diameter being ~60 μm). Type II fibres are larger in men (average diameter 69 μm) than in women (50 μm). The higher levels of testosterone are thought to underlie the larger size, and hence strength, of skeletal muscle in men, although female body builders show that musculature can be greatly increased in women by training. Two distinct responses to regularly performed strenuous exercise can be seen in muscle: hypertrophy of the fibres with an increase in strength (e.g. weight-lifters) and an increased capacity for aerobic metabolism (e.g. long-distance runners, cross-country skiers, swimmers).

Endurance exercise training gives rise to an increased capacity for oxidation of pyruvate and long-chain fatty acids. This is due to an increase in the density of mitochondria and hence in the amount of enzymes; for example, those of the tricarboxylic acid cycle and those involved in the activation, transport and oxidation of long-chain fatty acids. There is an increase in capillary density and myoglobin, which speeds the rate of diffusion of O_2 from cell membrane to mitochondria. Trained individuals have increased intramuscular stores of triglyceride and lowered concentrations of serum triglycerides, and their muscles can utilize lipids directly from blood.

The consequences of these changes are that during submaximal exercise, trained individuals derive more energy from fat and less from carbohydrate than do untrained individuals. Furthermore, in the trained individual, liver and muscle glycogen stores are better maintained during exercise and a greater proportion of oxygen is extracted from the blood supply to muscles.

Fatigue has two major causes: an inability to maintain an adequate motor drive from the central nervous system and a failure of excitation–contraction coupling. Fatigue resulting from voluntary exercise is normally evident before significant depletion of muscle energy reserves has occurred and, no matter how severe the exercise, muscle energy supplies are never depleted to the point of inducing rigor. (Recall that ATP is required for cross-bridges to detach). The process of fatigue is always reversible and training results in both its onset being delayed and its intensity reduced.

Effects of ageing

The ageing process results in a decrease in the size, speed and strength of skeletal muscles, and also a reduction in their fibre number. The age-related loss in skeletal muscle mass is referred to as **sarcopenia**. The death of type II motor neurones is an important factor, as it results in the denervation of type II muscle fibres, some of which will then die, while others will attract new input from nearby type I nerve terminals. This process results in an overall slowing of muscle contractile responses, a

decrease in motor unit number, and an increase in motor unit size. In addition, motor unit fibres may become clumped within the muscle belly, in a way analogous to that of reinnervated muscles shown in Fig. 5.13. The potency of synaptic transmission also decreases with age due to structural changes at the neuromuscular junction.

Effects of damage to nerve or muscle

After the nerve to a muscle is sectioned, there are changes in both the muscle and the axons. Changes in the muscle fibres are particularly pronounced. Within a few days of denervation there is a small decrease in the resting membrane potential of the muscle fibres and an increase in their sensitivity to applied acetylcholine, due to the insertion of newly synthesized acetylcholine receptors throughout the sarcolemma (i.e. including the extrajunctional regions). A few days later the fibres develop spontaneous activity (**fibrillation**) due to instability of their membrane potential. Other changes, such as a pronounced decrease in the ability to develop tension, a change in enzymic composition and a decrease in fibre diameter (**atrophy**), may take longer to develop. In humans, the fibres may shrink down to some 10 μm unless they are reinnervated. If muscle fibres remain denervated for prolonged periods (months to years), they will gradually be replaced by connective tissue and fat.

When the nerve to a muscle is sectioned, some of the motor neurones die but others regenerate their axons. However, in higher vertebrates there is little or no specificity in the re-establishment of nerve–muscle connections. Regrowth of axons is aided and directed by the presence of the old nerve sheaths (hence the accurate suturing together of the cut ends of a nerve is important). Normally fibres belonging to a particular motor unit are well scattered across the muscle, but after regeneration they clump together (Fig. 5.13) as if the ingrowing nerve fibre made connections with all the muscle fibres in its immediate vicinity. Moreover they develop the characteristics of the motor neurone providing their input. However, reinnervation may not always be successful; when a whole limb is denervated there is very little evidence of orderliness in nerve regeneration to muscles, and normal coordination of movement is never fully restored.

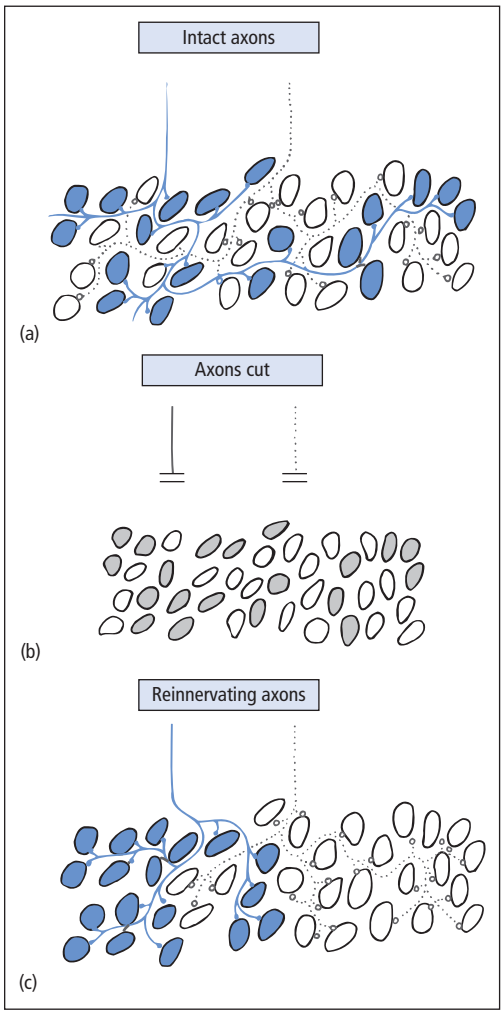

Fig. 5.13 Reinnervation of muscle fibres. (a) Prior to denervation the fibres innervated by each motor neurone are intermixed. (b) The muscle fibres atrophy following section of the nerve. (c) After reinnervation the muscle fibres of each motor unit tend to be grouped together.

It is generally taken that damaged muscle fibres cannot divide and therefore lack the capacity for regeneration. They do however have endogenous stem cells—known as satellite cells because of their peripheral location—which are activated by damage and effectively repair the muscle. The small

mononucleate satellite cells normally lie beneath the basal lamina of mature muscle fibres. These cells appear to be a special generation of myoblasts which migrate into the muscle region from the somite during development, but which do not then contribute immediately to the formation or growth of a muscle fibre. The appropriate stimulus triggers them to undergo mitosis, increase in number and ultimately fuse to repair the damaged fibres or to make new multinucleate muscle cells. It is actually possible to remove a muscle, mince it, pour the mince back into the appropriate place in the animal, sew up the skin, and produce a new but smaller functional muscle. Regeneration of the muscle in such cases is critically dependent on the presence of the nerve.

5.2 Smooth muscle

Smooth muscle has a wide range of functions including the regulation of gastrointestinal motility, the diameter of blood vessels and bronchioles, and uterine contractions. The characteristics and control of smooth muscle vary with location, enabling it to perform, in a tailored way, its tissue-specific functions. Smooth muscles control the movement of material through most hollow organs; for example, they propel material in the gastrointestinal tract, they restrict flow in arteriolar blood vessels and bronchi, and they expel material from the uterus, bladder and vas deferens. Smooth muscles also control piloerection and influence the dilator and constrictor muscles of the iris, thus affecting the amount of light reaching the retina. Smooth muscle cells usually exist in bundles or sheets. They are thin elongated cells, which may be connected to their neighbours electrically by low-resistance gap junctions that help coordinate contractile activity. Contraction is initiated by an increase in the concentration of intracellular Ca^{2+}, which acts through calmodulin, and phosphorylation of myosin light chain, i.e. regulation is thick-filament based. The contractions of smooth muscle are slower than those of skeletal muscle but more efficient. Smooth muscles vary in their level of activity from those that show more or less continuous activity, e.g. vascular tone, to those that are quiescent for prolonged periods, e.g. urinary bladder. Activity in smooth muscles depends on a number of factors, including the character of the smooth muscle cells, their environment, neural input and hormones. All neural influences are exerted by the autonomic nervous system; some tissues are innervated by only one division, while others are innervated by both the parasympathetic and sympathetic divisions. Factors such as stretch, pH and oxygenation help to couple smooth muscle activity to the varying demands of the body.

Smooth muscle structure

A connective tissue sheath, the **epimysium**, surrounds the smooth muscle of each organ. Thin septa extend inwards from the epimysium to form the **perimysium**, which contains fibroblasts, capillaries, nerves and collagenous elastic fibres. The perimysium divides smooth muscle into discrete **bundles** (or sheets) of fibres. These bundles range from 20 to 200 μm in width, and anastomose with one another; these anastomoses can be seen at roughly 1 mm intervals along a fibre bundle (Fig. 5.14). An exception in which the smooth muscle is not organized into bundles is found in arteriolar walls, which may be only a couple of cell diameters in thickness.

The individual smooth muscle cells within a bundle are fusiform, or irregular elongated cells 2–10 μm in diameter, and vary in length from about 50 μm in arterioles to 400 μm in most other organs, and up to 600 μm in the pregnant uterus. They interweave and overlap with each other (Fig. 5.14) to form a network interlaced with collagen; smooth muscle cells may synthesize much of the collagen found in the extracellular space, i.e. they are both contractile and secretory cells. Damage to vascular smooth muscle cells can cause excessive matrix production and proliferation, causing a pathological narrowing of vessels.

Individual smooth muscle cells come into close contact with 10 or so neighbouring cells; at these points they may be connected by specialized intercellular junctions of relatively low electrical resistance called **gap junctions**. At these junctions the sarcolemma of the cells is separated by 3–5 nm and

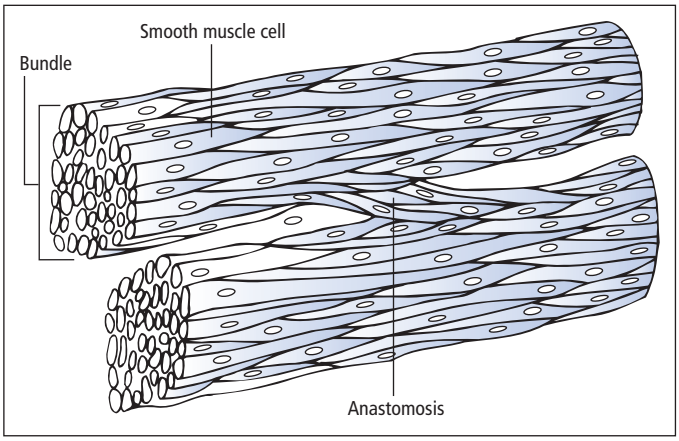

Fig. 5.14 Smooth muscle cells are arranged in bundles that are interconnected by an anastomosis.

the gap is bridged by structures that allow small ions to pass from cell-to-cell. The relatively low electrical resistance of these junctions allows current, which may have either an excitatory or an inhibitory effect, to pass from cell-to-cell. Alteration in gap junction permeability, e.g. by pH, provides a mechanism for changing the activity of smooth muscle. Where bundles exist, the direct coupling of cells within each bundle can result in the bundles being a functional (contractile) unit.

Pronounced differences between the structure of smooth muscles and striated muscles are seen at the ultrastructural level. Smooth muscle cells possess few mitochondria and a single nucleus—usually centrally located. The SR was originally considered to be poorly developed but recent confocal imaging techniques have shown it to be abundant in most smooth muscles, coming very close to the sarcolemma and encircling the nucleus (Fig. 5.15). The SR of most smooth muscles has both release channels gated by both Ca^{2+} and inositol triphosphate (IP_3). There is no specialization at the neuromuscular junction, and the myofilaments of actin and myosin are irregularly arranged. The actin filaments appear to be inserted into specialized structures in the sarcolemma—the dense bodies; costameres in skeletal muscle may be analogous to the better-known dense bodies. Long actin filaments radiate out in a longitudinal direction from dense bodies and there is a much higher ratio of actin to myosin compared with skeletal muscle. Smooth muscle cells lack troponin, and as detailed below, activity is regulated by another Ca^{2+}-binding protein, **calmodulin**, which is associated with the thick filaments. Various proteins have been identified in association with smooth muscle actin filaments, e.g. calponin and caldesmon, and appear to play a role in regulating contraction.

Contractile activity of smooth muscle: phasic and tonic

The variety of activity required by the different tissues containing smooth muscle leads to considerable variations in contractile activity and mechanisms of excitation. In some organs, only localized contractions occur (e.g. intestinal sphincters), while in others the whole organ may be involved (e.g. bladder). The contractions can be phasic with regular undulations of contraction and relaxation, or tonic with a steady level of force being produced over a long period of time (Fig. 5.16). The contractions of smooth muscle are slower than those of skeletal muscle. When excited by a single stimulus, there is often a long latency, a slow rise to peak tension (>1 s) and then a slow decline to the resting state. In many tissues, this single contraction may take several seconds and in some tissues last for minutes, e.g. uterine contractions in labour. Some smooth muscles, e.g. blood vessels, produce a more-or-less steady level of contraction

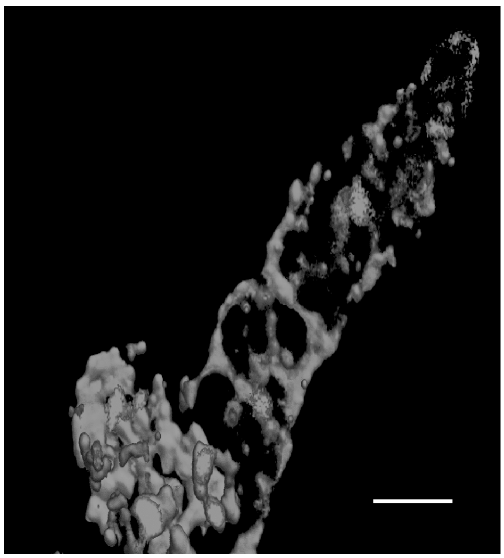

Fig. 5.15 Image of the sarcoplasmic reticulum in a smooth muscle cell from the ureter, obtained using confocal microscopy. Scale bar, 5μm.

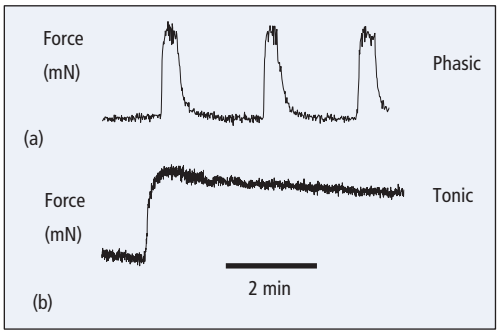

Fig. 5.16 The contractions of smooth muscle may be (a) phasic or (b) tonic.

for many hours, referred to as tone or tonic activity. With trains of action potential or hormonal stimulation the forces generated by smooth muscles can reach levels similar to those found in skeletal muscles (30–40 N cm^{-1}). However, unlike many skeletal muscles, smooth muscles can maintain their tension at a high level for long periods and over a wide range of muscle lengths. It seems probable that the low activity of the smooth muscle myosin ATPase may account for both the slow development of force and the relatively low O_2 consumption during contractions (<1/100 that of skeletal muscle). The efficiency of smooth muscle contraction is low, i.e. ~25% work/ATP compared to skeletal muscle, but its economy, the product of force and time per ATP, is greater. As with striated muscle the rate-limiting step in the cross-bridge cycle is P_i release. In smooth muscle the cross-bridge life-time is longer than in skeletal, which will also contribute to its greater economy. The ability to contract over a wide range of lengths (up to four times the resting length) may be a result of the irregular arrangement of the myofilaments, and is clearly advantageous to their physiological role in surrounding organs, such as the bladder, as they fill with their contents.

Regulation of contraction—role of myosin phosphorylation

As with skeletal muscle, the force generated by smooth muscle is controlled by the level of intracellular free Ca^{2+}. In smooth muscle during stimulation, this Ca^{2+} may come from the interstitial fluid as a result of a change in membrane permeability, or it may be released internally from the SR as occurs in skeletal muscle. The incoming Ca^{2+} contributes substantially to the rising phase of the smooth muscle action potential (see below), and visceral smooth muscles will stop contracting in the absence of external Ca^{2+}. As a result of these changes, the cytoplasmic concentration of Ca^{2+}

may rise from a resting level of 10^{-8} mol L^{-1} to 10^{-6} mol L^{-1} or higher.

With this rise in concentration, more Ca^{2+} combines with the regulatory protein calmodulin to activate a highly specific protein kinase that phosphorylates the light (small) chains in the head of each myosin molecule. This is a prerequisite for the activation of the smooth muscle actin–myosin complex. This kinase is myosin light chain kinase, MLCK, and it phosphorylates a sereine residue, and thereby greatly increases the actin-activated myosin ATPase activity. The activity of MLCK is a target for modulation by some hormonal second messengers. Inactivation of the contractile mechanism is accomplished by the lowering of the intracellular concentration of Ca^{2+} and the activity of a phosphatase (myosin light chain phosphatase; MLCP) that dephosphorylates myosin light chain (Fig 5.17). The activity of MLCP is a key target for many hormonal second messengers, and in particular by phosphorylation; MLCP activity is greatly reduced if it is phosphorylated. This inhibition of MLCP can therefore lead to an increase in force without a change in $[Ca^{2+}]$, a process termed Ca^{2+}-sensitization in which the normal sigmoidal relation between $[Ca^{2+}]$ and force is right- or leftward-shifted. Thus, force production in smooth muscle may be viewed as the balance between the activities of MLCK and MLCP (Fig. 5.17).

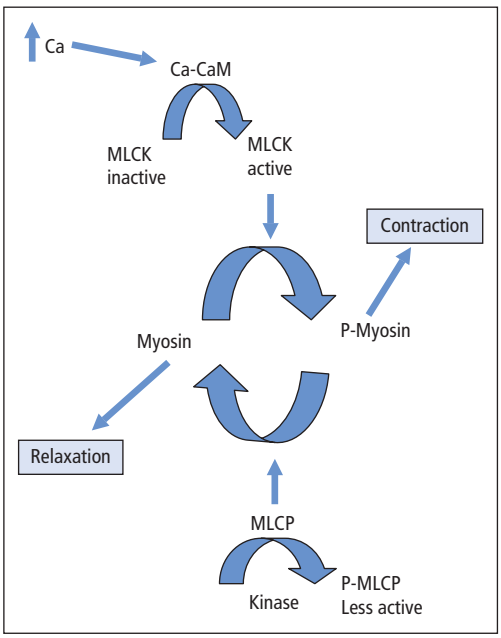

Fig. 5.17 Regulation of smooth muscle contraction occurs by altering the activity of myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP). CaM, calmodulin.

Thin filament based regulation

While myosin phosphorylation is the dominant regulatory mechanism, recent evidence has supported an additional, thin filament based regulatory system operating in smooth muscles. This mechanism appears to be based around removal of the inhibitory influences of caldesmon and calponin on myosin ATPase, by phosphorylating them. The kinases required for this phosphorylation are stimulated when agonists bind to their receptors on the smooth muscle membrane.

The Ca^{2+}-independent pathways, i.e. sensitization, and thin filament-based regulation, will augment the contractile process initiated by excitation and Ca^{2+} entry through voltage-gated Ca^{2+} channels. However it is now recognized that some agonists act to modulate smooth muscle force without changing membrane potential; a process referred to as pharmaco-mechanical coupling to distinguish it from the usual electro-mechanical coupling. These agonists bind to receptor-operated channels, ROC, as opposed to voltage-operated channels, VOC, and produce IP_3 and other second messengers. The IP_3 will stimulate the release of Ca^{2+} from the SR, and other second messengers will activate the kinases that modify the regulatory mechanisms described above.

During tissue relaxation the Ca^{2+} that entered the cell for contraction is transported to the extracellular fluid or re-sequestered into the SR. Expulsion of Ca^{2+} from the cell is energy-dependent and due to the activity either of a Na^+–Ca^{2+} exchange mechanism or a Ca^{2+}-dependent ATPase.

Finally, in muscles that contract for long periods, there may be a decrease in the level of phosphorylation of myosin light chain while tension is maintained. It appears that dephosphorylation of the

cross-bridge while it is attached to actin may slow its dissociation. This 'latching' of cross-bridges may provide an energetically efficient means of maintaining tension.

In summary, the regulation of contraction in smooth muscles is more varied than in striated muscles and need not correlate with [Ca^{2+}], membrane depolarization or myosin light chain phosphorylation.

Electrical activity is varied in smooth muscles

The resting membrane potential of many smooth muscles is in the range of –55 to –70 mV, and its basis is similar to that found in other excitable cells (see Chapter 1). There is, therefore, the same tendency for K^+ efflux leading to hyperpolarization, as the E_m for K^+ is –90 mV, and a strong inward driving force for Ca^{2+}, both electrically and chemically. One notable feature of smooth muscle cells is the relatively high [Cl^-] and the modulation of excitability via Cl^- efflux, producing depolarization (as electrically it is equivalent to positive charge entering).

A description of electrical activity in smooth muscle cells is more complex than that of striated muscles because of their diversity. Not all smooth muscles exhibit action potentials, but in those that do they may be spike-like, but somewhat slower than in skeletal muscle, or plateau-type action potentials, as seen in cardiac cells (e.g. in the ureter; Fig. 5.18). A depolarization of some 20 mV is required to reach threshold and initiate an action potential, which reaches a peak of about 10 mV. If the stimulus is maintained, repetitive firing may occur,

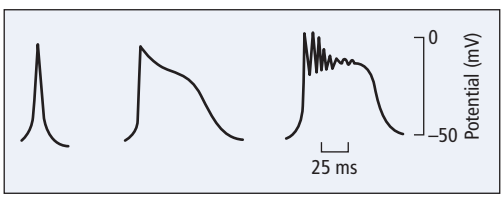

Fig. 5.18 Action potentials of smooth muscle may be spike-like (left), plateau-like (middle) or a mixture of these (right).

the frequency depending on the degree of depolarization.

In contrast to the action potential in nerves and skeletal muscles, in smooth muscles Ca^{2+}, not Na^+ ions are responsible for the inward current. The threshold depolarization is that required to open the voltage-sensitive Ca^{2+} channels. Thus the magnitude of the overshoot of the action potential is not directly proportional to E_{Na}, and removing Ca^{2+} ions from the bathing fluid abolishes action potentials, while increasing Ca^{2+} produces larger action potentials. Drugs that block these Ca^{2+} channels are used in the treatment of smooth muscle over-activity, for example hypertension.

Slow waves are a feature of some smooth muscles, e.g. gastrointestinal (GI). These are rolling changes in membrane potential of some 20 mV occurring over a timescale of many seconds and even minutes, rather than milliseconds. At the peak of the depolarization produced by the slow wave, trains of action potentials may be triggered. Thus contractile activity will map to the slow wave frequency. The ionic mechanism responsible for the generation of the slow waves is not fully understood at present.

As in cardiac muscle, some smooth muscles, e.g. ureter and GI tract, have pacemaker areas and/or specialized cells responsible for triggering electrical activity that can be transmitted rapidly to many cells via gap junction coupling. As discussed below, smooth muscles containing these pacemakers are spontaneously active. Other spontaneously active smooth muscles, e.g. uterine, have no anatomically defined pacemaker region but are suspected of having these cells distributed throughout them. However, this remains to be established. The mechanism underlying pacemaking activity is best understood for GI smooth muscle.

Types of smooth muscle

As mentioned above, the activity of smooth muscles may be phasic (rhythmical) and dependent on spontaneous mechanisms; other tissues are quiescent until stimulated by an incoming signal. The former have often been referred to as unitary (cells acting together) and the latter as multiunit (cells

acting independently) smooth muscles, respectively, but these divisions are now of little use as they represent extremes, and smooth muscles may be considered to be a continuum, between these extremes. Smooth muscles may be conveniently divided into three groups according to their membrane properties; namely, spontaneously active, electrically inexcitable and intermediate.

Spontaneously active smooth muscle

Many visceral organs containing smooth muscle contract rhythmically (e.g. stomach, small intestine, ureter, uterus). As this coordinated activity is maintained without nerves and hormones, it must be initiated and coordinated by the smooth muscle cells, i.e. it is **myogenic** (as in the heart). Such activity usually depends on the spontaneous generation of action potentials, and the presence of a conducting system (the gap junctions). Two types of mechanism are responsible for the spontaneous generation of action potentials, **pacemaker** potentials and **slow waves**.

In some smooth muscles (e.g. ureter) there is a focal pacemaker region (in the case of the ureter this is in the renal pelvis) where groups of cells will depolarize to threshold; the subsequent action potentials are then conducted through the tissue. As mentioned earlier, in other smooth muscles, e.g. the uterus, the pacemaker regions are not constant in location, and it is thought that all regions within these tissues have the capacity to assume the role of pacemaker.

The rhythmic activity of the stomach and intestine results from the regular generation of depolarizing potentials (pacemaker potentials) in the highly specialized interstitial cells of Cajal (see Chapter 19). Thus the term myogenic can be misleading as it is not necessarily muscle cells that initiate the electrical activity, although it does occur in cells contained within the muscle. This initial depolarization spreads throughout the smooth muscle (Fig. 5.19). Not all activity in the GI tract can be attributed to slow waves triggering action potentials; for example in the fundus and body of the stomach the slow waves are larger but the spikes are smaller and occur only at the beginning of the slow wave. In this area the contraction may be independent of action potentials and can be triggered by the slow wave exceeding the membrane potential for the initiation of contraction.

The spontaneous contractile activity of smooth muscles can be altered by nervous and hormonal activity, which may be either excitatory or inhibitory upon the underlying mechanisms, producing rhythmicity. For example, in the intestine acetylcholine, the transmitter released from parasympathetic nerves, causes the smooth muscle to depolarize. As a consequence, the number and frequency of action potentials on each slow wave are increased and the contractions are more forceful. In contrast, the inhibitory action of noradrenaline, the transmitter released from sympathetic neurones to the detrusor (bladder) muscle, and the inhibitory actions of non-adrenergic non-cholinergic autonomic neurones to the gut (see also Chapter 19) are due to hyperpolarization and movement of the membrane potential away from threshold. This may result in complete cessation of contractile activity while the spontaneous fluctuations in membrane potential continue at a subthreshold level. Hormones modify the activity of spontaneously active smooth muscles by a variety of mechanisms. Oxytocin for example can act on uterine smooth muscle cells to increase Ca^{2+} influx and the release of Ca^{2+} from the SR, as well as by decreasing Ca^{2+} efflux via the Ca^{2+}–ATPase of the plasma membrane. By affecting both the $[Ca^{2+}]$ within the cells, and the relationship between $[Ca^{2+}]$ and myofilaments force production (sensitivity), the normal phasic activity of uterine cells can be transformed into the strong and long-lasting contractions associated with labour.

Electrically inexcitable smooth muscle

This term applies to an extreme, but not unimportant, group of smooth muscles that do not generate action potentials (e.g. bronchial, tracheal and some arterial smooth muscles). In these tissues, the membrane potential remains stable until the tissue is stimulated. Stimulation may be the result of neurotransmitter release or the activity of paracrine or endocrine agents (e.g. histamine, bradykinin).

Stimulation is accompanied by depolarization and subsequent contractions. In tissues with a sparse innervation, excitation can spread because of the presence of gap junctions. The physiological advantage of these muscles may reside in their generally slow and sustained response to nerve stimulation.

Intermediate smooth muscle

This category includes smooth muscles in the iris, piloerector, blood vessels, vas deferens and seminal vesicles. Like electrically inexcitable smooth muscle they have a stable resting membrane potential and when stimulated they exhibit spike-like action potentials. The cells are linked by gap junctions, but conduction is decremental and so the contractions fail to spread throughout the tissue. The force of contraction is proportional to the frequency of the action potentials and is usually under neural control.

Activation of smooth muscle

Contraction of all smooth muscles is dependent on changes in the intracellular Ca^{2+} level. This can occur as a result of inherent myogenic mechanisms, which regularly depolarize the muscle fibres, or as a result of neural or hormonal action. Contractions may also be induced by other means. For instance, some smooth muscles are relatively plastic when slowly stretched, but rapid stretching results in a depolarization and contraction. Such behaviour may be important in **myogenic autoregulation** of blood vessels (p. 387). In other tissues, local agents modify the force of contraction (e.g. the actions of O_2 and CO_2 on blood vessels of the lungs, and histamine on bronchial smooth muscle).

In tissues such as arterioles where the dominant influence is exerted by the nerves, irrespective of their type, excitation is usually the result of a depolarization (an **excitatory junction potential**; Fig. 5.20a). In the case of inhibition, hyperpolarization of the smooth muscle membrane occurs (an **inhibitory junction potential**; Fig. 5.20b). In nearly all cases, the increase in conductance arises as a result of the neurotransmitter interacting with specific surface receptors on the muscle fibres; one notable exception appears to be nitric oxide which is released as a neurotransmitter but acts directly on cytoplasmic guanylate cyclase in the smooth muscle to cause relaxation (p. 35). If the neuromuscular junction has a relatively small junctional

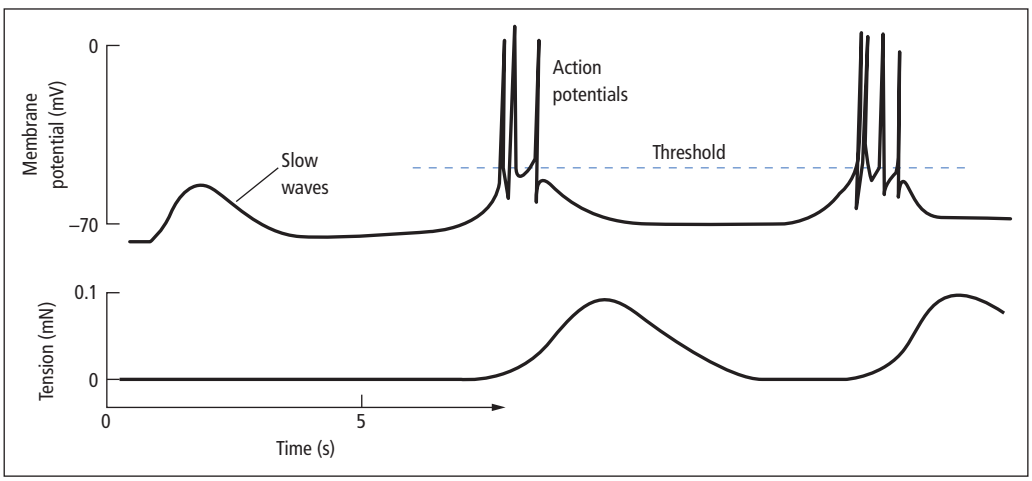

Fig. 5.19 Rhythmic depolarizations (slow waves), action potentials and contractions recorded from a strip of smooth muscle from the small intestine. The slow waves initiate action potentials on reaching threshold, and cause the muscle to contract. (In the stomach, the slow waves are usually larger in amplitude and may initiate contraction.)

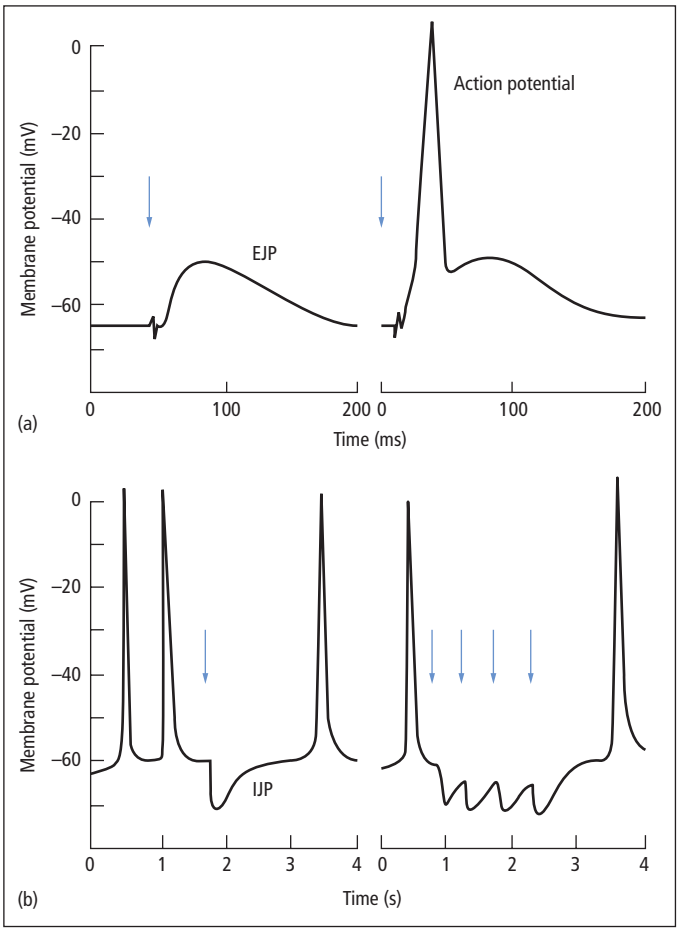

Fig. 5.20 Junction potentials in smooth muscle. (a) Excitatory junction potentials (EJP) recorded intracellularly from the vas deferens following stimulation (arrows) of its sympathetic nerve supply (left, subthreshold EJP; right, suprathreshold EJP leading to an action potential). (b) Inhibitory junction potentials (IJP) recorded intracellularly from longitudinal intestinal muscle following stimulation (arrows) of the intramural nerves (left, single stimulus; right, repetitive stimuli).

cleft (20 nm) then the junctional potential is distinct, with a fast rate of rise, lasting about 0.5 s; wider (400–500 nm) neuromuscular junctions appear to respond more slowly to nerve stimulation. In fact in some tissues (e.g. the tunica media of blood vessels), many of the muscle fibres may not be directly innervated; they may, however, be under some neural influence, as current will spread from neighbouring innervated regions (through gap junctions).

The contractile activity of many smooth muscles is influenced in a tissue specific manner by hormones and paracrines, which are discussed more in the relevant chapters, reflecting the highly specific nature of the receptors on the plasma membrane.

Some hormone receptors are expressed on many different smooth muscles and hence these hormones, e.g. adrenaline, will have widespread activities; for example, adrenaline can change the contractile activity of many tissues (e.g. blood vessels, bronchioles, the intestines). Similarly, the highly potent prostaglandins and thromboxanes have pronounced effects on a number of smooth muscles. For instance, prostaglandin F_2 is a potent stimulator of uterine contractility (and is used to induce labour at term), and of intestinal and bronchial smooth muscles.

Smooth muscle pathophysiology

It is clear from the widespread distribution of smooth muscle in the body that its control and

correct functioning will be vital to our health. Many of the body's homeostatic mechanisms work via the autonomic nerve system affecting smooth muscle function. Consider for example what happens when blood pressure is elevated; its detection by baroreceptors alters autonomic activity and reduces the constriction of arterioles (as well as changing cardiac activity), to restore blood pressure. If a patient has elevated blood pressure then drugs to reduce the contraction of arterioles will be given; e.g. Ca^{2+}-channel blockers or drugs to reduce the effects of noradrenaline. Not all dysfunctions of smooth muscle can be so well controlled; for example, premature labour occurs when the mechanisms triggering the coordinated contractions of childbirth occur too early in pregnancy, jeopardizing the fetus. The aetiology is usually unknown, and it is difficult to predict. It is almost impossible to stop the uterine contractions once labour has started, but Ca^{2+}-channel blockers, β mimetic drugs, or oxytocin antagonists may be effective for long enough (2 days) to administer steroids, which bring forward surfactant production in the baby's lungs and greatly increase its chances of survival. Many other common conditions such as asthma and bladder instability, also involve smooth muscle problems. The key to correcting the pathophysiological conditions affecting smooth muscles lies in obtaining a more complete understanding for each of the physiological processes and their modification.