CHAPTER 5 THE STRUCTURE AND PROPERTIES OF MUSCLE

5.1 Introduction

The second half of Book 3 is about movement and the structure and properties of the many different tissues that contribute to locomotion. Chapters 5 and 6 are about muscle, the only component of the musculo-skeletal system capable of transforming chemical energy (i.e. ATP) into mechanical work. In this way, its role is analogous to that of the engine in a car. However, muscle differs from most chemically driven engines in several important ways. First, the muscles are located on or close to the moving parts, and are therefore stretched and twisted by all movements of the limb, whether or not the muscle itself has generated the force that caused the movement. Second, the transformation of chemical energy into mechanical work takes place within the temperature range of 0–40°C, very much cooler than the working temperatures of most engines.

This chapter describes the structure and properties of muscle as a tissue: obviously features of muscle related to its contractility have been the most thoroughly studied and so occupy most of the text, but we also describe other ways in which this massive, biochemically active tissue contributes to the metabolism of the body as a whole. Chapter 6 concentrates on what muscles can do, the mechanical and energetic consequences of how muscles are deployed and the neural mechanisms of muscle control. Muscle never operates in isolation: it is always associated with connective, skeletal and nutritive tissues that maintain and support it, and interact mechanically with it to maximize the power and efficiency of movement. The tissues associated with muscle are described in Chapter 7. Chapter 8 explains how a thorough understanding of how the mechanical and physiological properties of muscles are matched to those of the tendons and skeleton and how they work together to produce an integrated and efficient locomotory system. The concepts so developed help to explain the effect of body size on the energetic cost of transport, described in Chapter 3.

Muscle can be studied at many different levels: properties of isolated molecules, as well as livestock production for meat and the athletic performance of people and horses contribute to our understanding of this remarkable tissue. Indeed, one of the most exciting aspects of muscle biology is the opportunity to explain the properties and athletic performance of the whole animal in molecular terms. So, in these chapters, you should be prepared for abrupt transitions between molecular biology and explanations for animals’ habits and habitats.

Physicists, chemists and engineers, as well as biologists, have made major contributions to our present knowledge of the vertebrate musculo-skeletal system. Physicists and engineers are interested in the mechanical properties of individual tissues; chemists are interested in how such properties can be explained in terms of the chemical composition of the tissues. These scientists have concentrated on studying that small minority of tissues that consist of ‘pure’ muscle, ‘pure’ tendon or regularly shaped pieces of bone. In fact, as you may have noticed yourself, in most vertebrates (e.g. in poultry, beef cattle and...
the domestic pig) almost all the muscles are intimately associated with tendon and other connective tissues. Clearly, the properties of the individual tissues are best understood in terms of their roles in the musculo-skeletal system as a whole. Biologists and physicians are concerned with how the different tissues work together to generate movement, and how the activity of the entire system is controlled and coordinated by the nervous system.

5.1.1 Methods in the study of muscle tissue

Muscle is an exceptionally robust tissue which retains its elaborate histological structure and many of its metabolic capabilities for some time after it has been isolated from the animal. Many of the enzymes and structural proteins can withstand rigorous chemical separation procedures and the contractile components of muscle retain the ability to convert metabolic energy into movement, even after much of the rest of the cell structure has been removed by solvents, and after preservation for many months at −20°C. Such treatment would irrevocably damage most biochemical pathways in liver, intestinal or brain cells (although some individual enzymes may remain active after freezing and thawing). The robustness of muscle and skeletal tissues has made possible the demonstration of a huge range of properties, many of them involving grossly non-physiological conditions. Consequently, although a great deal of information about muscle and skeletal tissues is now available, it is not always clear which properties are relevant to their role in the living animal. It is therefore essential to combine studies on isolated molecules, organelles and cells with investigations into how living animals actually use their muscles and skeletal tissues during normal movement.

The situation is further complicated by long-established traditions of using only certain muscles for certain kinds of investigation. Frogs have been a favourite since the London-based physiologist Professor Archibald V. Hill began his classic studies in the 1920s: their long muscular hindlegs are adapted to both leaping and swimming so some muscles can contract very fast, while others are capable of producing powerful, sustained forces. Frogs are also a convenient size: their leg muscles are small enough for good diffusion of oxygen to be maintained for hours, and powerful enough to produce forces that can be easily measured (via the stout tendons attached to the muscle) using not too delicate apparatus. Finally, frogs are poikilothermic and the muscles of those native to the USA or northern Europe contract very well at room temperature and moderately well at 0°C, a temperature that is relatively easy to hold constant. But frog muscle proteins are unstable in solution, so rabbit muscle, particularly the psoas in the back, which consists mainly of long parallel fibres, is preferred for biochemical and many biophysical studies. While it is true that the biochemical differences between the muscles of frogs and rabbits are quite minor, we cannot be sure that it is valid to match the details of mechanical studies on one species with those from chemical studies on the other.

Insects are very small, but they share with frogs the advantages for physiologists of being poikilothermic. Their flight muscles have a highly ordered structure and produce exceptionally powerful contractions that involve very little shortening. The mechanical properties and fine structure of the muscles of the giant water bug Lethocerus (several different species of adult body mass about 3 g occur in the humid tropics of South America, Africa and southeast Asia) and large dung
beetles, such as those that feed on elephant dung, have been much studied. Although these large insects rarely fly in the wild when the temperature is below 30–35°C, their flight muscles contract well at room temperature.

Another advantage of insects has recently been exploited for muscle research: they can grow to maturity and breed quite well with flight muscles that are grossly defective due to gene mutations that cause the absence or alteration of particular muscle proteins. So rare mutants of insects such as the fruit-fly Drosophila can be bred in the laboratory and many different aspects of their muscles examined (Section 5.2.2). Vertebrates with comparable defects in their skeletal muscles would probably be unable to walk or swim, or even to breathe normally, and hence would die long before they reached breeding age.

The specialized muscles of even more exotic animals lend themselves well to certain kinds of investigations: some giant barnacles native to the Pacific Ocean have exceptionally large muscle fibres, and those of molluscs such as clams and oysters have a unique mechanism that enables the animals to hold their shells tightly closed for hours using almost no energy. Clearly modern muscle biology is a rich mixture of many different investigative techniques applied to many different kinds of muscle from a huge range of species.

5.2 The structure of muscle

Figure 5.1 (overleaf) illustrates the basic organization of the contractile components of vertebrate striated muscles: a whole muscle is composed of from scores to thousands of muscle fibres, each 10–200 µm in diameter (in vertebrates, usually 50–100 µm) and from 1 mm to several centimetres long. In any one muscle, most fibres are about the same length but, as explained in Section 6.4, skeletal muscle may have several different arrangements of muscle fibres and tendons. In general, large animals have somewhat longer muscle fibres than related smaller species: for example, it is obvious that the muscle fibres of mice could never be longer than about a centimetre. Other structures shown are discussed later in this section.

Bundles of muscle fibres (Figure 5.1, ring 2) are bounded by tough collagenous tissue which merges with tendon, bone and other structures that attach muscle to the skeleton, and extends around and between the muscle fibres. This collagenous tissue, together with a layer of fine, amorphous collagen called the basal lamina, adheres closely to the plasmalemma of each muscle fibre (see Section 2.4 and Figures 2.13 and 5.6). These structures together form the sarcolemma, but in many texts this term is used more loosely to mean just the plasmalemma of the fibre. The plasmalemma of some muscle fibres, especially large vertebrate fibres, is electrically excitable and forms propagating action potentials. In vertebrates, a single motor neuron* forms one or more neuromuscular junctions on the plasmalemma, but invertebrate muscle fibres are usually innervated by several different motor neurons. Arterioles, venules and smaller nerves, together with blood vessels and, in many mammals, adipose tissue, are found between and around the muscle fibres.

* Often spelt motoneuron or motoneurone.
Figure 5.1 The basic organization of the contractile components of vertebrate striated muscles, drawn to show the relationships between the components, not to scale. The sarcoplasmic reticulum (SR) and other regulatory structures are omitted for simplicity. In ring 4, the thin filaments are omitted. The repeating unit along the myofibrils, the sarcomere, consists of several bands and zones identified by letters.
In vertebrates and larger invertebrates, each fibre contains thousands of myofibrils, which are usually polygonal in cross-section, and average about 0.5–2.0 µm in diameter. The structure is shown diagrammatically in Figure 5.1, ring 4. Several myofibrils are visible in longitudinal section in Figure 5.2. They are often arranged in groups of from half a dozen to a score or more, as shown in Figure 5.3 (overleaf).

Between and around the groups of myofibrils there are from dozens to hundreds of relatively small nuclei (Section 2.4), mitochondria, several kinds of structural proteins and a system of internal membranes called the sarcoplasmic reticulum (SR) that has similarities with endoplasmic reticulum. The most important property of SR is its ability to take up calcium ions actively; in the relaxed state, the concentration of calcium ions around and within the myofibrils is only about 40 nmol l\(^{-1}\) (1 nmol l\(^{-1}\) = 10\(^{-9}\) mol l\(^{-1}\)), lower than that of the cytoplasm of other cells such as liver and gut, because most of the calcium is sequestered inside the vesicles of the SR. Like many kinds of cell membranes, the plasmalemma actively extrudes calcium ions from the muscle fibres. Recent studies combining electron microscopy with labelling with fluorescent antibodies indicate that in a wide variety of cells as well as striated muscle, most calcium pumping takes place in minute depressions in the plasmalemma called caveolae.

Figure 5.2  Electron micrograph of human striated muscle. The sample was taken by biopsy from the gastrocnemius muscle of the lower leg. A longitudinal section of several adjacent myofibrils, showing whole sarcomeres (S) with A-bands (A), I-bands (I), M-lines (M) and Z-lines (Z), plus mitochondria (Mt) and glycogen granules (G).
In vertebrates, the SR abuts closely to, but is not continuous with, another set of membranes called T-tubules (‘T’ stands for ‘transverse’) that form as invaginations and branches of the plasmalemma and extend deep into the muscle fibres. (T-tubules are shown in Figure 5.5.) As their name implies, T-tubules are predominantly, but not exclusively, transverse to the plane of the contractile apparatus. They are about 40 nm in diameter and, of course, they enclose part of the extracellular space. This property can be demonstrated by soaking the muscle in a medium containing large, lipid-insoluble molecules: these markers appear inside deep T-tubules, but not inside the muscle fibre itself.

The contractile proteins are assembled into regular, repeating units about 2.0–2.5 µm long called sarcomeres, arranged end to end along the length of each myofibril (Figure 5.1, rings 4 and 5, and Figure 5.2). For reasons that are still not entirely clear, sarcomeres of adjacent myofibrils are usually arranged in register, creating the pattern of dark and light bands across the myofibrils. These ‘striations’ were first seen in the mid-19th century by microscopists equipped with high-power light microscopes and it is to them that we owe the term ‘striated’ muscle.

Electron microscopy combined with chemical procedures that selectively leach out or disassemble certain proteins has revealed much about the internal organization of sarcomeres. Early staining techniques for electron micrographs demonstrated two different arrays of interdigitating longitudinal filaments, called the thin and the thick filaments, and at right angles to them, the Z-lines* and M-lines. The region of the sarcomere occupied by the thick filaments is called the A-band. The two regions near the ends of a sarcomere where the thin filaments extend beyond the region of overlap with the thick filaments are called the I-bands, as shown in Figure 5.1, ring 4.

To understand the three-dimensional organization of sarcomeres, compare Figure 5.1 (rings 5 and 6) with Figures 5.2 and 5.3, which were prepared from tiny pieces of the gastrocnemius muscle (the ‘calf’ muscle of the lower leg) of a normal adult human, excised with a biopsy needle. Figure 5.2 shows four complete sarcomeres, each in a different but adjacent myofibril, in longitudinal section, as shown diagrammatically in Figure 5.1, ring 5. The thin filaments are regularly arranged around the thick filaments, so in transverse section such as Figure 5.3, they appear as a geometrical pattern of large and small dots with or without tiny protrusions, as shown diagrammatically in the sixth group of rings in Figure 5.1. It is, of course, impossible to cut absolutely straight through a muscle fibre (its internal structure is invisible until the completed section is viewed in the electron microscope), and the myofibrils are not exactly aligned, so Figure 5.3 is a slightly oblique section that passes through the overlap region of the A-bands of the myofibrils at the top, the I-bands in the centre and the Z-lines in the lower right.

The lengths of the thick and thin filaments, and the exact pattern in which they are arranged, differ between species and (in invertebrates) between types of muscle in the same species. In vertebrate striated muscle, thick filaments are about 42 nm apart, and the spacing between thick and adjacent thin filaments is 22–30 nm.

* Also called Z-discs in some texts.
Insect flight is one of the most energetically demanding activities in the whole animal kingdom, and probably represents the highest mechanical power output of all poikilotherms. The wingbeat frequency of large tsetse flies is about 200 Hz which is audible as a low buzz; that of small flies such as mosquitoes and midges is up to 1000 Hz, and sounds like a whine. As well as generating fast, powerful contractions, the flight muscles of such insects are exceptionally highly ordered, and hence produce beautiful electron micrographs such as Figure 5.4b (overleaf).
Figure 5.4  Electron micrographs sectioned through the A-band regions of sarcomeres of (a) the human gastrocnemius muscle, and (b) the flight muscles of a tsetse fly.
Calculate the average spacing of the thick filaments (by measuring a row of ten or more densely stained thick filaments) in Figures 5.4a and 5.4b. What can you conclude?

At the magnification used here, the thick filaments average 3.4 mm (the equivalent of about 68 nm) apart in Figure 5.4a but only 3.2 mm (roughly 56 nm) apart in Figure 5.4b. However, the muscles in vertebrates contain proportionately more thick filaments than do the muscles in insects: the ratio of thin to thick filaments is 2:1 in all vertebrate striated muscles, and 3:1 in insect flight muscle. The ratio is as high as 5:1 or 6:1 in some insect leg and body muscles, and the thin filaments are arranged in various different patterns around the thick filaments.

Crossbridges are regularly arranged protrusions of the thick filaments in the regions where they overlap with the thin filaments, as shown diagrammatically in Figure 5.1, rings 5 and 6. They are often difficult to demonstrate in electron micrographs of vertebrate muscle and are only faintly visible in Figure 5.2. Crossbridges are more clearly seen, and apparently more regularly arranged, in insect flight muscle than in any other kind of muscle, so much of the information about their role in muscle contraction comes from the study of this tissue.

The other important structures visible in Figure 5.2 are the Z-lines and M-lines. Both have an intricate internal structure that is visible with very high resolution electron microscopy, and they contain several unique proteins. For reasons explained in Section 5.2.1, both thick and thin filaments are polarized, i.e. their molecular components are asymmetrical and are assembled so that they are ‘facing’ one way or the other. The thick filaments in the two halves of the sarcomere, i.e. either side of the M-line, are of opposite polarity. Up to five bands perpendicular to the filaments are visible in the M-lines of certain muscles, but the functional implications of the structural differences (compare Figures 5.2 and 5.6 with Figure 5.10) found between taxa (e.g. insects, worms and vertebrates) and types of muscle are unknown. Thin filaments of opposite polarity from adjacent sarcomeres are joined at the Z-lines, which, in three dimensions, appear to form discs that cap each sarcomere of each myofibril, like the two ends of a tin can (Figures 5.2 and 5.3).

In some muscles, particularly those adapted to high power output and rapid onset and termination of contraction, the T-tubules and sarcoplasmic reticulum are arranged in a regular pattern of parallel tubes and vesicles between the myofibrils, often concentrated around the Z-lines and M-lines.

How would such T-tubules and sarcoplasmic reticulum appear in thin sections seen in the electron microscope?
In cross-section or longitudinal section, these membranes appear as rings or parallel bands, called triads, situated between the myofibrils (as shown in Figure 5.5). However, as with many aspects of muscle structure, the abundance of these membranes and of mitochondria, and their arrangements in relation to the sarcomeres are very variable: there are differences between taxa, between different muscles of the same individual, and ontogenetic changes within a single muscle. The functional implications of the many different patterns have not been investigated in detail.

It has probably occurred to you by now that this orderly arrangement of sarcomeres and membranes could be thrown out of alignment each time the muscle shortens. It is now becoming clear that Z-lines and M-lines have pivotal roles in the muscle cytoskeleton that connects the contractile proteins to the membranes and maintains their functional relationships. You can see the cytoskeletal attachments of the plasmalemma to the Z-lines in Figure 5.6a. When a muscle fibre shortens, its whole sarcolemma is deformed into deep folds (Figure 5.6b) and remains closely applied to the sarcomeres only at the Z-lines. The protein that forms the mechanical connection between these components of the sarcomeres and the outer membrane is called desmin.

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**Figure 5.5** Longitudinal section of the same human muscle shown in Figures 5.2, 5.3 and 5.4 to show two triads of sarcoplasmic reticulum (SR) and T-tubules (T). Mt, mitochondrion.
Figure 5.6  Electron micrographs of sections of sarcomeres and sarcolemma of a human gastrocnemius muscle when (a) moderately contracted, with I-bands substantial and clearly visible, and (b) strongly contracted, with I-bands almost occluded. Note that the sarcomeres are 10% shorter in (b) than they are in (a).
5.2.1 Muscle proteins

Because they are chemically robust and many occur in, for a biological system, a relatively pure and concentrated form, muscle proteins are easier than most to isolate and so have been intensively studied. Dozens of proteins have been isolated, characterized and named, which gives the impression that muscle contains a wide variety of proteins. In fact, compared to other kinds of cells such as liver or white blood cells, muscle contains relatively few kinds of protein. It is just that we know more about more of them. The following account refers mainly to mammalian striated muscle and describes only those proteins for which the localization and/or function are fairly well established.

The thin filaments are about 1.1 µm in length and 5–10 nm in diameter and consist mainly of polymerized chains of the globular protein, actin, with much smaller quantities of regulatory proteins called troponin and tropomyosin. Although approximately round in shape, actin monomers are asymmetrical and they always assemble ‘nose to tail’, which confers polarity on the entire thin filament. Actin seems to be chemically almost identical wherever it is found, although its abundance differs substantially in different kinds of muscle. In frog muscle, each thin filament contains about 380 actin molecules and about 22% of the total protein in whole muscle fibres is actin.

Troponin is a complex of three globular proteins which together are about twice the size of an actin monomer. Tropomyosin is a rod-shaped, helical protein and in the thin filament, one troponin complex is bound to a particular point on each tropomyosin molecule. Actin forms a double helical chain, like two strings of beads twisted together, and the tropomyosin molecules, each with a troponin complex, lie one on either side of the chain. Tropomyosin molecules are arranged end-to-end, and there is one molecule of tropomyosin for every seven actin monomers.

In vertebrate striated muscle, thick filaments are both wider and longer than thin filaments, about 1.6 µm in length, and are composed mostly of staggered bundles of myosin molecules, about 300 per filament. At 43–50% of the total protein, myosin is by far the most abundant kind of protein in muscle. It is a large protein of about $M_r$ 520,000, that dissociates into two heavy chains of $M_r$ about 220,000 each, and four light chains of $M_r$ 20,000. Myosin occurs in several different isoforms that are produced by different genes. Although they differ only slightly in chemical structure and hardly at all in size, myosin isoforms are major factors in determining how fast a muscle can contract (Section 5.3.3).

The heavy chains form a long fibrous tail and two heads that act as enzymes and protrude from the thick filaments towards the adjacent thin filaments, forming the crossbridges with the light chains bound to them. The bundles of myosin molecules are assembled in a way that gives the thick filaments a clear polarity, with those of each half-sarcomere having opposite polarity. Some of the tails of the myosin molecules, the heads of which are forming crossbridges in the two regions of the sarcomere where the thick and thin filaments overlap, meet at the M-line (see Figures 5.2, 5.5, 5.6 and 5.7). Some of the unique proteins that form the M-line are probably involved in binding these myosin tails together. Others are enzymes, including creatine kinase, the enzyme that generates ATP from phosphocreatine.

The myosin heads, probably in conjunction with specialized sites on the thin filaments, are ATPases that convert chemical energy into mechanical force. Only two other known proteins have this remarkable property (the others being kinesin and dynein in neurons and in cilia and flagella) which is the essence of muscle contraction.
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Attachment, detachment and/or changes in the angles of the crossbridges result, under appropriate mechanical conditions, in the thin and thick filaments sliding past each other. The many detailed studies of the mechanism of this process and its control are beyond the scope of this course. J. T. Finer and others working at Stanford University, California and London, have built some very ingenious apparatus that enables them to measure the forces generated by a single isolated myosin molecule interacting with actin. They find that for each ATP molecule hydrolysed, a myosin head (which forms the crossbridge, see Figure 5.1, ring 5) can move about 11 nm along an actin filament, and can produce about 3–4 pN (1 piconewton = $10^{-12}$ newtons) of force. The whole sarcomere shortens, almost entirely from a symmetrical reduction in the lengths of the I-bands. When all (or a large proportion) of the sarcomeres of a myofibril contract more or less simultaneously, these small events sum to produce substantial forces in the myofibril as a whole.

Recent improvement in techniques for cutting and staining material for the electron microscope has demonstrated super-thin filaments, less than 5 nm in diameter, in vertebrate muscles. These filaments consist of titin, which, with an $M_r$ of $3 \times 10^6$ and length of up to 1 µm, is the largest single-chain protein so far described in any organism. As illustrated in Figure 5.7, titin molecules extend from the M-line to the Z-line along the thick filaments and into the I-band. Z-lines are the points of attachment of titin and actin filaments between adjacent sarcomeres. The amino acid sequence of titin, and hence the form of its folding is different in the A-band and the I-band. Vertebrate striated muscle is about 10% titin, and current research suggests that each thick filament contains six of these huge proteins and that they may contribute to the assembly and alignment of the myosin molecules, and hence to the pattern of crossbridges.

Another recently discovered fibrous protein in muscle is nebulin, which spans the entire length of the thin filaments. At an $M_r$ of around $8 \times 10^5$, nebulin is also a very large molecule, though only about a quarter of the size of titin, and it accounts of 5% of the total muscle protein. Nebulin may determine the length of the thin filaments, and hence of the sarcomere as a whole. But its exact arrangement in the thin filaments, and its role in maintaining the arrangement and relative abundance of actin, troponin and tropomyosin, are not yet known, so it is not shown in Figure 5.7.

The Z-lines that ‘cap’ each sarcomere consist mainly of actin from the ends of the thin filaments and α-actinin and, in insect flight muscle, another very large protein called keitin. Kettin and α-actinin seem to bind the thin filaments.
together at the ends of each sarcomere (Figures 5.2, 5.3, 5.5 and 5.6). The Z-lines of adjacent myofibrils are connected by a filamentous network of desmin. These proteins form the internal cytoskeleton that binds the myofibrils together and maintains their highly ordered structure; they are probably essential to effective communication between the fibre membranes and the contractile proteins, which, as explained in Section 5.3.2, is crucial in controlling contraction.

Several more proteins have been isolated from muscle and identified, including some very minor constituents such as dystrophin (Section 5.4.3) which accounts for only 0.002% of the total muscle protein. Sophisticated electron microscopy combined with immunocytochemistry has revealed the normal location and probable physiological function for many of such minor constituents of muscle. As a result of such intensive study, striated muscle is now one of the best known of all vertebrate tissues from the chemical and micro-anatomical point of view: our ‘map’ of the structure and arrangement of liver cells or neurons is not nearly as complete.

5.2.2 Sarcomere assembly

The unique properties of muscle arise from the precise ordering of the many different proteins described above rather than from the chemical composition of any one of them. The two most abundant muscle proteins are by no means confined to muscle: actin is found in almost all eukaryotic cells (where, among other functions, it is a component of the mitotic spindle), and fibrous proteins with many features in common with myosin occur in many kinds of animal cells. But only in muscle are these proteins arranged in ways that enable them to contract rapidly and to generate high power. Crystallographers, biochemists, electron microscopists and more recently geneticists have studied what substances and conditions are essential to the formation of this all-important order.

Several lines of evidence indicate that sarcomeres can assemble themselves. Heart muscle of vertebrates differs from skeletal muscle in that it generates its own electrical signals that initiate contraction (so hearts continue to beat for several seconds after excision, extendable to many minutes of activity under appropriate conditions). Heart muscle myofibrils can be dissociated into their component filaments by incubating them with the proteolytic enzyme trypsin. If the enzyme is then removed, the filaments reassemble spontaneously in a few hours to form normal-looking sarcomeres, some of which start to contract. This process takes place even if drugs such as cycloheximide that inhibit protein synthesis are added to the incubation medium. However, the presence of Z-lines with at least some actin filaments attached to them seems to be essential to reassembly.

In Drosophila, the major proteins of the flight muscles are generated by different genes from those that produce the proteins for the muscles of the legs, mouthparts, etc. Thus flies carrying mutant genes for flight muscle proteins are viable except that they cannot fly: they feed, walk, mate and breed normally and do not appear to suffer any inconvenience. The study of such lineages of Drosophila has revealed more about what materials are essential to the formation of certain components of sarcomeres. Figure 5.8a shows the flight muscles of a mutant fly that cannot synthesize flight muscle actin, Figure 5.8b shows those of another lineage of flies in which the gene for flight muscle myosin is defective and Figure 5.8c is of flies bred to have both mutations.

What can you conclude from the electron micrograph appearance of such deficient muscles?
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Figure 5.8  Flight muscles of mutant fruit-flies Drosophila. (a) A longitudinal section of muscle fibre that cannot synthesize flight muscle actin. Note the arrays of thick filaments and rudimentary M-lines. (b) Longitudinal section from a fruit-fly that does not synthesize flight muscle myosin. Note the Z-lines with thin filaments emerging from both sides. (c) The ‘flight muscles’ of a fruit-fly that has both mutations. The mitochondria and some membranes form normally, but there are no arrays of contractile proteins.
Thick filaments and M-lines can form in the absence of actin (Figure 5.8a) and thin filaments are viable without myosin, but both are necessary for the formation of regular, discrete sarcomeres. Furthermore, normal-looking Z-lines are clearly visible in Figure 5.8b, demonstrating that they can form in the absence of myosin. Flies that are heterozygous for both mutations have only one of the normal two genes for each protein, so although all necessary proteins are present, their relative abundance is abnormal. Electron micrographs of the muscles of such flies show filaments of widely different lengths and very few assemblages that could be called sarcomeres. Too much of certain proteins is as disruptive to the formation of normal sarcomeres as too little. In the mutant flies, the proteins are synthesized in the wrong proportions, and so very few filaments of exactly the right length for sarcomere formation assemble themselves.

**Summary of Section 5.2**

Muscles consist of bundles of fibres composed of myofibrils, several different kinds of membranes, nuclei and mitochondria. The contractile proteins are organized into sarcomeres, which consist of A- and I-bands, and M- and Z-lines, and are arranged end to end along the myofibril. Actin and smaller quantities of nebulin, troponin and tropomyosin form the thin filaments and myosin and titin form the thick filaments. The heads of the myosin molecules form crossbridges; part of the heads acts as an ATPase.

### 5.3 Muscle mechanics

The most spectacular, and most widely discussed, property of muscle is its ability to generate force actively using ATP. Muscle does not use chemical energy to become longer, but, like all elastic materials, it can store and later release some of the mechanical energy applied to it. The response of relaxed and semi-relaxed muscle to externally applied forces plays a major role in determining the form of all movements, because all actions require muscles to be moved as well as to produce forces that tend to cause movement. If you have ever had a minor injury in a muscle, you may have been surprised by the range of ordinary movements that seem to involve that muscle, even if you are trying hard not to ‘use’ it actively. As well as generating forces themselves, muscles are stretched and twisted by external forces and by the actions of other muscles as part of all normal activities. So before we address the active, ATP-using properties of muscle, it is appropriate to describe briefly the passive properties.

#### 5.3.1 Passive properties of muscle

The passive mechanical properties of muscle are familiar to all cooks: although it is soft and pliable, muscle resists large deformations, and when twisted or pummelled, it tends to spring back to its original shape. These properties can be quantified accurately by stretching the muscle by a known fraction of its original length while measuring the tension (i.e. the force required to overcome resistance to extension).

Figure 5.9 shows the tension measured when various freshly excised muscles were stretched without being stimulated to perform active contraction; the muscles were not permanently damaged by the procedure because they were
stretched only to lengths from which they would recoil elastically to their original length when released. You can see that the basic shape of all the curves is similar; there is an initial phase in which the muscle is extended by quite small forces, but for most of the range, tension is proportional to stretch. However, the bee flight muscle is very stiff and hardly stretches at all even when quite large forces are applied, while the muscles in the ‘foot’ of snails are readily extensible, and can be extended to 160% of their normal length in the body without injury. These passive properties are referred to collectively as the internal elastic components of the muscle.

Some of these passive elastic properties can be demonstrated in muscle in which the connective, vascular and nervous tissues within and around myofibrils have been dissolved away.

- What can you conclude from such observations about the physical basis of passive elastic properties of muscle?

Much of the internal elasticity of muscle must arise from properties of the sarcomeres themselves. Before the discovery of titin and nebulin (Section 5.2.1), the molecular basis of the passive strength and internal elasticity was not clear: the I-band seemed to be the weakest point, as it was hard to see how actin, being a globular protein, could have much mechanical strength. Current research indicates that titin is the main agent of mechanical continuity in relaxed muscle. The regions of the super-thin filaments in the I-bands are more extensible than those in the A-bands: they may crumple during active contraction, or stretch when the whole muscle is stretched, then recoil when the sarcomere relaxes or is released. The linear arrangement of these very long molecules suggests that the elasticity arises from folding and unfolding of portions of the protein rather than from molecules sliding past each other.

- In view of this explanation, how would you expect the structure of sarcomeres in bee muscle to differ from those of snails?

Bee muscle has relatively very short I-bands. A comparison of the bee flight muscle in Figure 5.10 (overleaf) with the mammalian muscle in Figure 5.2 illustrates this difference very clearly: at a length of 2.4 μm, the sarcomeres of the flight muscles of insects such as bees are about the same length as those of vertebrates, but the insect A-bands are 2.2 μm long, compared to about 1.6 μm in almost all vertebrate muscles.
Figure 5.10 Longitudinal section of a single sarcomere in the flight muscle of a bumble bee *Bombus*. Notice that the structure of the M-line of this invertebrate muscle is different from that of human muscle in Figure 5.2. Mt, mitochondrion.

More extensible muscles have longer sarcomeres but the long I-bands make such sarcomeres mechanically weak, which seems to set the upper limit to their maximum possible length. Sarcomeres 10–15 µm long have been reported from crabs and other crustaceans, but those of vertebrate muscles are all between 2 and 3 µm long. Collagenous connective tissue also contributes to elasticity, and snail and mussel muscle have much more connective tissue between fibres than bee muscle.

The proteins that form the cytoskeleton and maintain the structure of the thick filaments seem to be different in invertebrate and vertebrate muscles. True titin is known only from vertebrate muscles, but many arthropods and nematodes have a protein that is chemically similar although smaller ($M_r$ about $8 \times 10^5$) and that may have a similar role. The thick filaments of insect flight muscle and many molluscan muscles contain relatively large quantities of a protein unique to invertebrates called paramyosin.

The elastic properties of titin, combined with its role in maintaining the array of myosin molecules, may keep the A-band exactly in the centre of the sarcomere, thereby preventing it from becoming unstable by generating unequal forces in its two halves. If titin molecules are broken by exposing whole sarcomeres to radiation, the A-band deviates from the exactly central position that it invariably occupies in all natural muscles.
Muscle contraction

Initiation

In most striated muscles, the sequence of events leading to contraction begins when an action potential in its motor neuron reaches the neuromuscular junctions and depolarizes the plasmalemma. In many vertebrate muscles (and a few insect muscles), the plasmalemma and its extensions, the T-tubules, can produce regenerative action potentials in the same way as neurons can. Action potentials thus travel rapidly over and through the entire muscle. Action potentials do not propagate over the plasmalemmas of most invertebrate muscles, but their motor neurons branch to form many more neuromuscular junctions, which together depolarize the membrane. In either case, the electrical changes can be picked up by fine wires inserted into or near the muscles and amplified to produce a display that indicates which muscles are active.

If conduction velocity of action potentials over the plasmalemma is $5 \text{ m s}^{-1}$, how long does an action potential take to travel over a $5 \text{ cm}$ long muscle fibre in which the neuromuscular junction is at its centre?

The time taken for the action potential to travel the $25 \text{ mm}$ from the centre to the ends of the fibre is $5 \text{ ms}$. Depolarization in the plasmalemma and T-tubules causes (by a mechanism not understood in detail) the SR to release its calcium ions, abruptly raising their concentration around the contractile proteins by over a hundredfold to about $10 \mu\text{mol L}^{-1}$. If ATP is present and various other chemical conditions are met, the influx of calcium initiates the mechanisms that lead to hydrolysis of ATP and the generation of active contraction.

Myosin by itself hydrolyses ATP only very slowly: both thin and thick filaments are necessary to produce the rate of ATPase activity observed in intact muscle. The major steps in this process can be investigated by studying various combinations and arrangements of the muscle protein in vitro, to which free calcium ions are added to induce ‘contraction’. Such observations show that the calcium binds to the troponin complex in the thin filaments, which alters the arrangement of the tropomyosin lying against the actin in a way that exposes the active sites on the actin. With their inhibitory covering of tropomyosin temporarily removed, these sites interact with the ATPase on the crossbridges of the adjacent thick filaments to accelerate ATP breakdown and cause contraction.

The depolarization in the plasmalemma wanes after a few milliseconds unless it is maintained by a series of action potentials arriving in quick succession. With repolarization of the plasmalemma, the SR accumulates the calcium it released, quickly reducing the concentration of Ca$^{2+}$ ions inside the fibre and terminating active force generation. The SR, like other ion-pumping membranes, uses ATP to take up the calcium ions against a concentration gradient.

Modern research techniques enable biologists to identify which components of the filaments are necessary for hydrolysing ATP and generating movement. When mixed with pure actin, pure myosin ‘contracts’ by wriggling along the actin filament. At a maximum velocity of about $9 \mu\text{m s}^{-1}$, the movement is not fast compared to that of intact muscle, but it can readily be measured under the light microscope, and is widely used as an assay of motility. Thus assessed, the motility of myosin isolated without the light chains around its enzymic heads is less than one-tenth that of myosin from the same source (chicken breast muscle) with its light chains in place, but the rates of ATP breakdown are similar in both cases.
What can you conclude from these observations about the structure of the crossbridges?

The light chains are not essential for ATPase activity but they are necessary for the generation of movement: in other words, the two processes take place in different parts of the crossbridge region of the myosin molecule. Other kinds of experiments indicate that, under certain mechanical conditions, the crossbridges can detach and re-attach without using more ATP, which also points to a separation between enzymic activity and movement generation.

The stimulus regime

The mechanical contraction itself lasts much longer than the chemical events that initiated it. The muscle plasmalemma, like the membrane of neurons, is capable of supporting another action potential only a few milliseconds after the first has passed. Unlike action potentials, mechanical contractions can summate, so the timing of a sequence of electrical stimuli to muscle is a major determinant of the maximum force generated and the duration of a contraction.

How natural trains of action potentials generated by the nervous system determine muscle activity in vivo is described in Section 6.5; for analysing the relationship between stimulus regime and mechanical response, it is usually more convenient to apply artificial pulses to an isolated muscle. The data in Figure 5.11 were obtained from an intact freshly excised muscle that was fixed firmly at one end and attached at the other end to apparatus that measured the tension produced with minimum compliance (i.e. the apparatus was so stiff that the muscle could hardly shorten at all).

To most people the word ‘contraction’ means shrinkage or shortening, usually as a result of internally generated forces. Muscle physiologists use the term to refer to the active state of the muscle which, if the mechanical constraints allow it, result in the muscle becoming shorter; in many cases, the contracting muscle exerts tension on the tendon or bone to which it is attached, but, as we shall see in Chapters 6 and 8, it may not undergo any net shortening at all. It is also important to remember that muscles contract at constant volume: they get thicker as they get shorter, and do not in any way shrink or shrivel, nor do they deflate on relaxation.

Dissecting out the motor neurons with the muscle is difficult, so for studying isolated muscles in the laboratory, it is convenient to simulate natural action potentials by applying brief depolarizing pulses directly to the plasmalemma. The mechanical response of a typical limb muscle to a single such artificial stimulus is called a twitch and is shown in Figure 5.11a. The stimulus, and the depolarization that it elicited in the plasmalemma and T-tubules lasts less than 5 ms, but the mechanical events continue for at least 40 ms. Maximum tension is reached after about 30 ms, but the relaxation phase takes longer than the contraction phase, so the curve is asymmetrical.

For the conditions mentioned above, what fraction of the total rise time of the twitch does the time for conduction of action potentials over the plasmalemma represent? How could this delay be reduced?

* The flight muscles of insects with very high wingbeat frequency such as tsetse flies (Section 5.2) are specialized. Their contraction time is much shorter, often as little as 3 ms.
The conduction time is about 17% \((5/30 \times 100)\) of the rise time of the twitch. It would be shorter if the neuromuscular junctions were closer together, muscle fibres were shorter or action potentials travelled faster across the plasmalemma. These features are found in muscles adapted to very fast, powerful movement for which close synchrony between all the sarcomeres of all the myofibrils is essential. In many postural muscles, electrical stimuli lasting only a few ms may produce mechanical responses that last hundreds or ms or even seconds.

Contractions elicited by several successive stimuli can summate, giving rise to **tetanus**, in which the maximum force generated is four times larger than that of a single twitch. In the tetanic contraction shown in Figure 5.11b, the muscle is stimulated with brief pulses at 10Hz. At this frequency, about two-thirds of the first twitch is already completed before the next stimulus starts active contraction again, so the time course of force production is not linear and the contraction takes almost a second to reach its maximum. Force generation continues for as long as the stimuli are applied and then declines with a similar time course to the twitch in Figure 5.11a, although it takes longer because the maximum contraction is greater. If the muscle is stimulated at 20Hz (Figure 5.11c), the force from the first twitch has only just begun to wane before the second stimulus arrives, so the twitches fuse to produce a steeply rising, almost smooth contraction of much greater maximum force than the single twitch and the lower-frequency tetanus.

Twitches fuse to form tetani because, as explained at the beginning of Section 5.3, all muscles are to some degree extensible when relaxed; much of the active contraction performed during the initial phase of a twitch is dissipated in stretching the internal elastic components of the muscle itself, and is therefore not available to do work on external loads. However, if, as happens during tetanus, a second stimulus arrives before the active contraction of the first one has decayed, a greater proportion of the contraction it initiates is available to perform work on an external load, because the internal elastic components have been stretched by the first contraction.

Would you expect (a) the maximum twitch tension, (b) the total rise time of the twitch, (c) the ratio of maximum tetanus tension to maximum twitch tension, to be greater or smaller in short muscle fibres with numerous neuromuscular junctions and high conduction velocity of action potentials than in longer, more slowly conducting fibres with fewer neuromuscular junctions?

A greater density of neuromuscular junctions and high conduction velocity promote near-synchronous activation of all the sarcomeres so the internal elastic components would quickly be fully stretched by the active contraction, producing higher maximum twitch tension and a shorter time to peak tension. However, because the mechanical conditions of the twitch in such muscle fibres are more similar to those of a tetanic contraction, the ratio of maximum tetanus tension to maximum twitch tension would be lower in fibres adapted to very fast, powerful movement than in those that contract more slowly.

Looking closely at Figures 5.11b and 5.11c, you can see that the total force produced in response to the second stimulus does indeed appear to be greater than that elicited by the first stimulus. In the high-frequency tetanus (Figure 5.11c), the force produced by the muscle gradually wanes after about 0.6s, although the stimulation frequency is unchanged. This effect, called **muscle**
fatigue, almost certainly involves several different mechanisms: transmission failure at the neuromuscular junction, depletion of the energy supply, and changes in the mechanisms that control contraction are among the processes implicated in this phenomenon, which is familiar to everyone who performs strenuous or repetitive actions.

Like the rates of most chemical reactions, the rates of increase in force production and the relaxation of muscles proceed much more slowly at lower temperatures. Certain muscles of homeothermic animals can produce steeply rising contractions like those shown in Figure 5.11, but the contractions of the muscles of poikilothermic slugs or sea anemones may take several seconds to reach peak force.

How would you expect the minimum frequency at which trains of twitches fuse to form tetani to change with temperature?

Twitch contractions would fuse to form tetani at a lower frequency in cooler muscles because each twitch lasts longer at lower temperatures.

The maximum speed of contraction, and the form and duration of the response to standard stimuli differ enormously between different muscles. Differences in sarcomere dimensions affect contraction velocity in the way expected from the crossbridge theory of muscle contraction. Muscles with longer A-bands have longer thick filaments with more crossbridges and so can contract faster. Those with longer I-bands (but not longer A-bands) can function at a greater range of lengths but the maximum shortening velocity is lower because a smaller proportion of the whole length of the muscle is overlap region (see Figure 5.1, rings 5 and 6).

5.3.3 Fibre types

In Section 5.3.2, we described how the work performed and time course of the contraction of a whole muscle depend upon external factors such as mechanical constraints, frequency of stimulation, state of fatigue of the muscle and temperature. However, there are also intrinsic differences in the structure and metabolism of individual muscle fibres within a whole muscle. The most important of these intrinsic differences are the maximum rate at which the contractile apparatus is capable of generating tension, the metabolic fuel used, and the way in which the motor nerves and plasmalemma initiate contraction. Most physiologists recognize four main types of fibres in mammalian skeletal muscles; similar categories of fibres can be distinguished on slightly different criteria in birds, reptiles, amphibians and fishes.

Tonic fibres (sometimes called ‘slow’ fibres) contract very slowly, and it is usually impossible to identify a twitch response to a single stimulus (see Figure 5.11a). They require very little energy to develop and maintain tension and their deep red colour shows that they are rich in myoglobin, the oxygen-binding pigment that takes up oxygen from the haemoglobin in the blood. Their economical metabolism and plentiful reserves of oxygen mean that they can sustain prolonged activity because they fatigue very slowly. Tonic fibres are rare in the muscles of most mammals.
Muscle fibres that can produce a twitch contraction in response to a single stimulus are called phasic fibres, although the time course of the twitch may be quite different from that shown in Figure 5.11a. There are three main types of phasic fibres that differ in the rates at which they fatigue and in the sources of their metabolic energy. The energy supply of slow phasic fibres comes from both oxidative phosphorylation and glycolysis. They usually contain many mitochondria and are rich in myoglobin. They can therefore contract repeatedly or continuously for long periods without significant fatigue.

The two main types of fast phasic fibres are distinguished mainly by the metabolic pathways by which ATP is synthesized. Fast oxidative fibres also derive most of their energy from oxidative phosphorylation, and therefore contain numerous mitochondria; as long as the blood supply is maintained, they are resistant to fatigue during prolonged exercise. Fast oxidative fibres are usually rich in myoglobin and so are red. The other group, the fast glycolytic fibres, generate ATP from glycolysis and hence have very few mitochondria; they contain very little myoglobin and so are pale pink or white. The maximum rate of ATP breakdown in fast glycolytic fibres is several times greater than that in slow phasic fibres. Glycolytic fibres contract at about the same speed as fast oxidative fibres but their small store of high-energy phosphate compounds is quickly exhausted and they fatigue very rapidly, sometimes within a second or two, when stimulated repeatedly.

Synchronous, powerful activation of all the fibres of a muscle can generate forces large enough to break the bones to which they are attached. Simultaneous stimulation may be achieved artificially (e.g. by an electric shock), but it occurs very rarely, probably never, as part of natural movements, and indeed there seem to be neural control mechanisms that prevent it from happening (Section 6.5.1).

During many activities, including steady walking, most of the fast glycolytic fibres are moved passively by the forces produced by the other fibres; the movements of high velocity and high power produced by the glycolytic fibres are important only for brief bursts of strenuous activity, such as escape swimming, take-off in flight, and running very fast.

The different types of fibres can be distinguished using histochemical techniques: small pieces of muscle are frozen within a few minutes of the animal’s death and the thin sections cut from it are treated in ways that reveal differences in fibre biochemistry. Sometimes stains that combine selectively with particular enzymes are used. For example, the section in Figure 5.12a has been stained to show the distribution within the muscle of succinate dehydrogenase, a key enzyme of the tricarboxylic acid (TCA) cycle which takes place in the mitochondria. Fibres in which oxidative phosphorylation is an important source of ATP appear darker, but glycolytic fibres, which have few mitochondria, take up very little stain. Histochemists also take advantage of the fact that myosin ATPase (Section 5.2) of fast fibres is unaltered even by quite strong alkalis, whereas that of slow fibres retains its affinity for the stain only in acid solution.

To what category does the fibre indicated by the small arrows in Figure 5.12 belong?

It is a slow phasic fibre; it appears much paler than its neighbours in Figure 5.12b, but it takes up large quantities of the stains used in Figures 5.12a and 5.12b, but it takes up large quantities of the stains used in Figures 5.12a and
5.12c, so it and the other oxidative fibres here appear darkly stained. These and other histochemical methods can be combined to identify the major types of fibre in vertebrate muscles.

It may seem strange that the fastest contractile machinery of mammalian muscles should have the fewest mitochondria and a very low reserve of oxygen. Fast glycolytic fibres consume energy very rapidly and there is not sufficient space between the myofibrils for as many mitochondria as would be necessary to sustain the supply of aerobically generated ATP. These fibres are able to produce fast, powerful contractions because they consist mostly of myofibril; only a small fraction of their volume is occupied by mitochondria and other supply apparatus.

Would you expect fast glycolytic fibres to be able to use fatty acids as an energy source as readily as they use glucose?

No. Most vertebrate tissues can metabolize fatty acids only by aerobic pathways, so these fast fibres use glucose almost exclusively. Free glucose in the muscle and surrounding blood is exhausted after only a few seconds of exercise; more glucose is quickly produced by breakdown of glycogen in the muscle fibres, but these energy stores are enough for only a few hundred contractions, or about five minutes of strenuous exercise in humans.

The other types of fibre utilize both fats and carbohydrates. The provision of lipids to fuel muscular activity during exercise may be the main reason why intermuscular adipose tissue, which is always only a small fraction of the total adipose tissue in the body, occurs in close association with skeletal muscle. It has several physiological properties consistent with a role as a local fuel supply for adjacent muscle fibres. Intermuscular adipose tissue is relatively abundant in most meat animals and humans compared with small mammals such as rats, but it is also abundant in certain muscles of some wild mammals, such as fin whales, where it is intimately associated with the bundles of muscle fibres (Figure 5.13).
Chapter 5   The Structure and Properties of Muscle

High-resolution electron microscopy and immunohistochemistry (the use of antibodies linked to pigments or fluorescent molecules to identify and locate particular proteins) have demonstrated differences between fibre types in the structure and protein content of the M-lines and Z-lines and in the isoforms of myosin light and heavy chains (Section 5.2.1). Isoforms are slightly different forms of proteins usually distinguished by the capacity of antibodies raised against a protein extracted from one source to bind to another. Tonic fibres with ‘slow’ myosin isoforms occur in narrower fibres that contain proportionately more nuclei (and less cytoplasm) than those in which ‘fast’ myosin predominates.

Fast fibres tend to have fewer visible bands in the M-line and narrower Z-lines than slow fibres, and ‘slow’ and ‘fast’ myosin appear to be distinct proteins that form slightly different thick filaments, but all these features have not been consistently found in all the fibres identified on histochemical criteria to belong to one or other category. The picture that emerges from these complicated and fast-moving studies is of continuous variation in structure and composition of muscle fibres rather than discrete categories: even in large muscles such as the flight muscles of the chicken breast (pectoralis), very few fibres have been found to be identical in every detail. Different isoforms of the subunits of myosin are now known to be important determinants of contraction velocity, but since we do not know which of the other features compared determine energy utilization, the functional implications of this wide range of structures and compositions are unclear.

Although it is technically possible to study single fibres dissected from a whole muscle, physiologists usually find it quicker and more convenient to make use of those few specialized muscles in which most of the fibres are of only one type. In most mammals, certain postural muscles around the spinal column and the soleus muscle in the calf region of the lower leg, which steadies the ankle joint during standing, consist of about 85% slow phasic fibres. The sartorius muscle of the thigh and the muscles that flex and extend the fingers and toes (known as flexor digitorum longus (FDL) and extensor digitorum longus (EDL), see Figure 2.14) usually consist mainly of fast phasic fibres. It is important to emphasize, however, that the terms ‘fast’ and ‘slow’ refer to the relative rates of contraction; as with most other aspects of metabolism (Section 3.3), the absolute rate at which chemical energy is converted into mechanical work depends upon body size. Thus the fast fibres of a horse probably have a lower maximum intrinsic speed of shortening than the slow fibres of a mouse.

The criteria upon which fibres are distinguished and the neural control of invertebrate muscles differ in several important ways from that of vertebrates, but similar physiological principles apply: muscle is a very active tissue that needs a copious supply of ATP to sustain contraction. Notice the large mitochondrion lying close beside the contractile proteins in bumble bee flight muscle (Figure 5.10) and the numerous mitochondria almost filling the spaces between the myofibrils in Figure 5.4b, as they do in the human muscle shown in Figure 5.3.
5.3.4 The determinants of the fibre-type composition of muscles

Most mammalian muscles resemble the biceps femoris muscle of the guinea-pig (Figure 5.12) in consisting of a mixture of several types of fibre; different combinations of fibres are stimulated to contract according to the speed and form of the movement. The principal leg muscles of people leading an ordinary life consist of about equal numbers of slow phasic and fast phasic fibres. However, the fibre-type composition of the homologous muscles of trained athletes can be very different. Up to 76% of the fibres in the leg muscles of sprinters, who specialize in running very fast for 0.5–3.0 min, are of the fast glycolytic type, as are 88% of the muscle fibres of thoroughbred horses trained for short-distance flat racing; the limb muscles of greyhounds are said to be 97% fast phasic fibres, most of them deriving energy from anaerobic glycolysis.

Champion marathon runners can cover 42.2 km (more than 26 miles) in a little over 2 h, although 3–4 h might be more typical for an amateur athlete. As few as 21% of the fibres in the large leg muscles of such athletes are of the fast glycolytic type, the rest being mostly fast oxidative fibres that use mainly fatty acids as an energy source during prolonged, strenuous exercise. However, all muscle fibres have to use a certain amount of glucose as well as fatty acids, which, after the first few seconds, is obtained from the breakdown of glycogen stored in the muscles themselves or in the liver.

What simple measurement would indicate when the muscles of an animal or person who is exercising vigorously starts to use substantial quantities of lipid?

The respiratory exchange ratio (RER) is 1 when carbohydrate is the only fuel, but decreases when fat is being oxidized. In vigorous exercise, the striated muscles become the largest consumers of energy and oxygen, so the RER measured for the whole body reflects the metabolism of the locomotory muscles fairly accurately. The thigh muscles of a resting, well-fed person contain about 0.1 mmol g\(^{-1}\) glycogen but this carbohydrate reserve is halved after 20 min of fast running, and exhausted after 80–90 min, long before the much larger stores of lipid in adipose tissue are significantly depleted. Switching to lipids as the main fuel source for muscles early in the bout of exercise and using mostly oxidative fibres helps to spare the precious glycogen, and thereby enables athletes to keep going longer, but glycogen depletion is always the main cause of severe exhaustion after prolonged, strenuous exercise.

The leg muscles of saddle horses bred and trained to carry heavy riders over long distances consist of about 67% fast fibres. Many of the differences between individuals in fibre-type composition seem to be genetically determined; in this sense, champion athletes and racehorses are born, not made. But there is also some evidence that physical training can cause certain fast glycolytic fibres to acquire the biochemical machinery that enables them to derive energy from the aerobic metabolism of fatty acids; so even people who are not endowed with the appropriate physique can improve their athletic performance by practice.

In newborn mammals, all muscle fibres are relatively small and more or less similar in structure and biochemical properties, depending mainly upon oxidative metabolism. All muscle fibres grow rapidly during infancy and childhood (Section 2.4) and, by weaning, most of the larger fibres have become fast glycolytic.
whereas the smaller ones remain oxidative. Several lines of evidence indicate that the neural input to the muscle influences this maturation, and is certainly essential to the transformation of fibres from one type to another in fully formed muscle.

Investigators have interchanged the innervation of the soleus muscle (85% slow phasic fibres) and the adjacent EDL muscle (mainly fast phasic fibres; Section 5.3.3) in the hindlimb, allowed the motor neurons to establish sound contacts with their new fibres, then studied their mechanics of contraction and their composition. Relatively large mammals such as cats or rabbits have to be used for such experiments because rat motor neurons are too small to be handled, but the subjects are able, indeed encouraged, to exercise freely. A year after the operation, the total duration of a single twitch (see Figure 5.11a) of soleus muscles that had received the EDL innervation was only 25±1 ms, compared with 87±1 ms in similarly treated animals in which the innervation was unaltered. There were also differences in the properties of the light and heavy chains of myosin and in the activities of oxidative and glycolytic enzymes. Similar experiments have shown that fast fibres could be made slower, and slow fibres faster by cutting their motor neurons and allowing them to be re-innervated by different nerves.

Advances in microelectronics have enabled muscle physiologists to determine experimentally whether these effects arose from the pattern or frequency of action potentials or from some chemical factor. Small stimulator capsules were placed in the abdomen and connected by fine wires to certain muscles in one hindlimb with the homologous muscle on the other side serving as a control. The muscles could be thus stimulated continuously for up to 20 weeks, enabling investigators to simulate or oppose the effects of removal of the muscles’ own motor neurons and their reinnervation by different ones. The duration of a single twitch, the isoform of myosin and various aspects of energy metabolism were found to have changed in muscles thus stimulated for as little as 12 weeks. None of these features changed in cross-innervated muscles that were stimulated for a similar period in the mode characteristic of their original innervation.

How do these experiments confirm the suggestion that the pattern of electrical activity in the plasmalemma rather than trophic effects determines the chemical composition and mechanical properties of muscle fibres?

Changes in electrical activity without cross-innervation alter the type of fibres in the muscle, but cross-innervation without changes in electrical activity do not. The changes occurred much faster under artificial stimulation than ever happens naturally (in less than a quarter of the time) because the stimuli were applied continuously; normally the muscle would not be used all the time, and hence would receive the action potentials in the characteristic pattern only intermittently. Over a period of weeks or months, the pattern of electrical activity in the plasmalemma somehow activates the genes for certain kinds of myosin, troponin and M-line proteins which form fast or slow fibres, and activates changes in metabolism and fuel utilization.

The fibre-type composition of muscle can also alter under selective breeding and indeed evolutionary changes in the relative masses of muscles, and in the proportions of different fibres, are some of the most important changes that equip related species for different habits and habitats. The breast muscles of ducks and pigeons are dark red and consist mainly of oxidative fibres, which are active during steady, prolonged flight. Interspersed between them are some fast glycolytic fibres which may be used to provide additional power for short periods during take-off.
From your own experience, what would you expect the fibre composition of the flight (i.e. breast) muscles in domestic hens and turkeys to be?

The flight muscles of these birds are pale because almost all the fibres are of the fast glycolytic type and blood perfusion is very low. Wild turkeys and poultry live in dense forests and they fly mainly in brief but strenuous bursts, often from feeding sites on the ground to the safety of nearby trees; they do not migrate and rarely fly above the forest canopy. Farm turkeys and hens kept in small cages do not fly at all. Artificial selective breeding has produced birds with relatively massive breast muscles that are nearly white, indicating that their blood supply is even more sparse than that of their wild ancestors.

Could selective breeding ever produce breast muscle that was pure white?

No. Even if the muscles do not need oxygen and other blood-borne nutrients for contraction, such supplies are certainly necessary for growth and maintenance. Like most modern domestic livestock (Section 1.3.2), poultry are selected to grow unnaturally fast so their muscles must receive a certain amount of blood. However, the difference in colour between a chicken breast muscle, and those of wild duck, goose or pigeon gives you an idea of how much more blood perfusion is required to sustain vigorous exercise than to support even rapid growth.

Flight muscles are used only for flight, but the leg muscles are essential for both standing and running. These activities involve different kinds of fibre, so some compromise has to be reached in animals that do a great deal of standing and running. Ratites (emus, ostriches, rheas and cassowaries) do not fly but they can run very fast over long distances to escape from predators. If undisturbed, these exceptionally large birds spend most of the day standing or walking slowly while eating, and lie down only to sleep or incubate their eggs. Australian biologists have recently studied the gross anatomy and fibre-type composition of the leg muscles of their native ratite, the emu (*Dromaius novaehollandiae*). The largest muscle of the lower leg, the gastrocnemius, was found to consist almost entirely of fast fibres, 55–72% fast glycolytic and 28–25% fast oxidative. Only the much smaller digital flexor muscles resembled the situation in the homologous muscle of most other birds in containing a mixture of approximately equal numbers of slow, fast glycolytic and fast oxidative fibres.

What can you conclude from these observations about the roles of these muscles in standing, walking and running?

The gastrocnemius must be used almost exclusively for fast running: it is not equipped to contribute to standing or slow walking. These actions must be powered and maintained by the digital flexor muscles, and the gastrocnemius is probably relaxed and electrically silent in undisturbed birds.

This arrangement, in which muscles of different metabolic capacities have similar anatomical connections and are used for contrasting functions, is also common in fishes. As in fishes, most of the work most of the time is performed by quite small muscles, and the largest, most conspicuous muscles are inactive except during brief periods of vigorous exercise, such as chasing prey or escaping from predators.
Summary of Section 5.3

Muscle contraction is started by depolarization of the plasmalemma, normally following the arrival of an action potential at the fibre’s neuromuscular junction. The depolarization spreads through the T-tubules and causes the release of calcium ions from the sarcoplasmic reticulum, and thereby causes ATP breakdown and contraction. Studies of isolated muscle protein suggest that the myosin light chain is essential for generating movement but not for ATPase activity. In vitro studies of isolated muscles show that a single stimulus produces a twitch, and a train of stimuli, a tetanus. Production of force wanes during prolonged tetanic contractions because the muscle fatigues. The four main types of muscle fibres differ in their maximum rate of contraction and their energy metabolism and in the kind of myosin they contain. The large differences between muscles in different species, and between different muscles in the same individual, in the intrinsic rate of shortening and hence in the form and duration of the twitch and tetanus contractions can be explained in part by the types of fibres of which they are composed. Muscles with similar anatomical connections but composed of different types of fibres may have very different functions. Fibres change in ‘type’ during ontogeny; changes in fibre type can also be induced by cross-innervation or by artificially stimulating the muscle fibre for days or weeks.

5.4 Muscle in whole-body metabolism

Most textbooks leave the story here, but since the main theme of this course is the physiology of the animal as a whole, it is appropriate to mention some other ways in which muscles contribute to whole-body metabolism. Movement might be the most important function of muscle, and is certainly the most widely studied, but it is not its only role. Like bone, liver, adipose tissue and indeed most other tissues, muscle is capable of many different metabolic pathways and contributes to several physiological functions. Muscle is the most massive tissue in the body (except in very obese or atrophied individuals) and thus is by far the largest repository of proteins, and a major participant in protein turnover (Section 2.1.2). The previous sections have concentrated on the structure and properties of the 80% of the volume of muscle fibres occupied by myofibrils and the 10% or so that is membranes and mitochondria. The remaining 10% of muscle volume performs several biochemical processes not directly related to contractility. Although these processes represent only a small fraction of the muscles’ own energy expenditure, they make an essential contribution to the metabolism of other body tissues.
5.4.1 Properties of muscle other than contractility

Muscle is the major site of disposal of glucose that enters the blood following a meal rich in carbohydrates and thus is central to glucose homeostasis of the body as a whole. The muscle plasmalemma contains many insulin receptors and its capacity to take up glucose is stimulated rapidly and effectively by insulin secreted into the blood in response to a rise in blood glucose. Once inside the muscle, some of the glucose may be oxidized at once, but most of it is converted into glycogen and stored as such granules in and around the fibres, as shown in Figure 5.14.

The turnover of glycogen in muscle is fairly rapid. Abrupt increases in glycogen can cause the skeletal muscles to swell noticeably, enough to make tight clothes feel tighter over the hips and thighs, within about half an hour of eating a large carbohydrate-rich meal after many hours of fasting.

Why does uptake of glycogen cause muscles to swell?

Glycogen granules always have about three times their own mass of water associated with them, so the muscle takes up more water from the extracellular space as well as the glucose.
Recent experiments have also demonstrated that skeletal muscle is the major source of the amino acid glutamine. Although constituting only 3% of most proteins (including muscle proteins), glutamine accounts for 35% of the free amino acids in the muscle cytosol, reaching concentrations as high as 20 mmol L\(^{-1}\) in some human muscles, compared to about 0.5 mmol L\(^{-1}\) for other amino acids.

Can you suggest a role for these free amino acids?

They may arise from the breakdown of muscle proteins and could be the raw material for the synthesis of more such proteins (Section 2.1.2). However, this role cannot account for the very high concentration of glutamine in the cytosol compared to its abundance in muscle proteins. An alternative interpretation is suggested by the observation that lymphocytes and some other cells of the immune system have a high capacity to utilize glutamine, both as a fuel and as a raw material for synthesis. Sepsis (i.e., the presence of foreign bacteria) stimulates the immune system and accelerates the rate of lymphocyte proliferation, thereby greatly increasing the system’s need for fuel and substrates, including glutamine. Experiments using rats show that under such conditions, the skeletal muscles of the hindleg (and probably those elsewhere in the body as well) release more glutamine into the blood, suggesting that a major function of muscle’s ability to synthesize and accumulate glutamine is to supply the metabolite to the immune system when required. What role, if any, the contractile components of muscle play in these metabolic pathways, and whether strenuous mechanical activity affects their efficiency, are currently under investigation.

How would an animal’s ability to combat infection be affected by major damage to muscle (e.g., from a gunshot wound, or being crushed by a motor vehicle)?

Such injuries could impair the muscle’s ability to supply the immune system with glutamine. Although for a long time the study of wound healing concentrated on local processes in particular tissues (see Chapter 2), it is now becoming clear that all major injuries (surgical or accidental) have many effects on the body’s metabolism as a whole, among them glutamine metabolism.

5.4.2 Protein turnover in muscle

As described in Section 2.1.2, the constituents of tissues are continuously replaced, and such turnover is an integral part of the mechanisms of their growth and their capacity to adapt to new activities and to repair injury. Being near the surface of the body and around the limbs, and sustaining high strain forces, muscle is very susceptible to mechanical damage — bruises, sprains and worse. It is also continually adapting to changes in habits and activities: physical training both increases muscle mass and improves the efficiency of muscle metabolism, but we quickly lose ‘fitness’ after a few weeks of relative inactivity. Both processes are due in part to changes in the relative rates of degradation and synthesis of contractile proteins and internal membranes, and hence in the addition or loss of myofibrils from muscle fibres.

In striated muscle, calpain (Section 2.1.2) is the major agent of breakdown of myofibrillar proteins. Perhaps surprisingly, the enzyme does not cleave myosin and actin, but vigorously attacks desmin, nebulin, and parts of titin, Z-lines and the troponin complex.
Which aspects of myofilbril structure would break down first?

Nebulin and troponin are parts of the thin filament and desmin connects the Z-lines in adjacent myofilbrils (Section 5.2.1), so the I-bands and the Z-lines would be the first features of the muscle to be disrupted.

How could the activity of such an enzyme be increased by running and other strenuous physical activity?

Calpain is a calcium-activated enzyme (Section 2.1.2). One form, m-calpain, is active at calcium concentrations of around 1 mmol l\(^{-1}\), and the other, µ-calpain, is active at micromolar calcium concentrations (3–50 µmol l\(^{-1}\) Ca\(^{2+}\)). The calcium concentration in the cytosol of resting muscle is low (Section 5.3.2), lower than that of almost all other types of cells, and too low for either form of calpain to become active. The agent of the coupling between excitation of the plasmalemma by its motor neuron and contraction of the sarcomeres is the release of calcium ions from the SR into areas around the contractile proteins (Section 5.3.2). More contraction means that relatively high concentrations of calcium prevail for a larger proportion of the time, increasing the chance of calpain being activated.

As explained in Section 2.4.1, more mechanical activity also stimulates growth and regeneration of fibres, so normally the net effect of more frequent or more prolonged periods of higher intracellular calcium is a slightly higher rate of turnover of the muscle fabric, which may improve the muscle’s strength and its ability to take up and use oxygen and fuels efficiently. These effects contribute to physical training that improves muscle performance and endurance.

5.4.3 Muscular dystrophy

Human diseases of the contractile mechanism itself are rare, possibly because fetuses with severely defective contractility would not survive gestation. But several disorders of the muscle membranes and the neural control of movement are both devastating and moderately common. One of the best known is Duchenne muscular dystrophy, named after the French neurologist and physician, Guillaume Duchenne, who, in the 1840s and 1850s, made the first thorough study of the disease and the first serious (though unsuccessful) attempts to treat it. The disease affects boys from early childhood and is due to a defective gene carried on the X chromosome. It involves progressive weakening and atrophy of skeletal and heart muscle, usually leading to death from cardiac or respiratory insufficiency before the age of 25. During the last ten years, many different kinds of investigations, from molecular biology and ultrastructure to comparative medicine and studies of muscle mechanics and growth, have combined to elucidate several major features of the disease, and to indicate some possible therapies. The following brief account illustrates how these diverse approaches come together, and is not intended to be comprehensive or conclusive.

In the early stages of the disease (around the age of 2–4 years), affected boys are not disabled and indeed some of their muscles, notably the gastrocnemius, are often larger than normal, but biochemical examination of blood samples reveal exceptionally high levels of enzymes derived from muscle fibres, such as creatine kinase (CK).
How could you explain the presence of such enzymes in blood serum?

They must have diffused out of muscle fibres in which the sarcolemma was torn or had become leaky.

By the age of 5–7, the muscles are noticeably wasted and strength is diminished. Electron micrographs (Figure 5.15) of small samples of muscle from such boys showed clearly that the immediate cause of these symptoms is disordered, incomplete sarcomeres and damaged myofibrils. Figure 5.15a shows two stages of breakdown of the muscle fibres. The lower fibre is necrotic: the muscle proteins have been reduced to a homogenous mass, and almost no ordered

Figure 5.15 (a) Adjacent muscle fibres showing two stages of breakdown in Duchenne muscular dystrophy.
(b) Fragments of myofibrils broken up by calpain. The cell in the centre is a polymorphonuclear leucocyte with a large, irregularly shaped nucleus, and that on the lower right is a part of a macrophage.
structure is visible. The other (upper) is only slightly abnormal, with wavy Z-lines and abnormally large T-tubules. The large macrophage in the centre is full of cell debris. On the upper left of Figure 5.15b, the myofibrils are reduced to a mass of A-bands (consisting mostly of thick filaments), the Z-lines having been broken up by calpain. Cells of the immune system, including a macrophage (Figure 5.15a) and a polymorphonuclear leucocyte (Figure 5.15b), have moved into the dying muscle fibre. By the time these defects are well advanced, the abnormally high levels of CK in the serum decrease and continue to decline as the children become older. Cells of connective tissue and adipose tissue may accumulate outside the dying muscle fibres.

After an intensive search for laboratory animals in which to investigate the disease, scientists eventually found several mutant strains of mice, and certain cats and dogs that develop many, but not all, of the symptoms of muscular dystrophy observed in humans. Much of the detailed information about the initial stages and progress of the disease comes from the comparative study of these animal models.

In 1987, scientists investigating the disease identified a gene apparently associated with muscular dystrophy (in boys as well as mice), and named its product dystrophin, but it was not until several years later that the protein produced by this gene was successfully isolated and characterized. Dystrophin is a large protein, $M_r 4,270,000$, with structural resemblances to $\alpha$-actinin and other cytoskeletal proteins, but it is present in only very small quantities, constituting only 0.002% of the total muscle protein (2% of membrane-associated muscle proteins). It occurs on the inside of the plasmalemma of all normal striated muscles, and in many other tissues including non-striated muscle, certain neurons and the retina and cornea of the eye, but is greatly reduced or absent from dystrophic muscles.

The protein can be located in electron micrographs of muscle using specific antibodies to which particles of gold are attached, so each dystrophin molecule is marked by an electron-dense blob (Figure 5.16). As you can see from Figure 5.16, in normal muscle dystrophin is scattered thinly but fairly evenly across the plasmalemma. This labelling procedure never reveals any dystrophin in biopsies from boys with Duchenne muscular dystrophy, but the protein may be present in people suffering from other kinds of muscular dystrophy.

By studying the form of contractions of muscles isolated from certain strains of mice that develop muscular dystrophy, biologists showed that single twitches of dystrophic muscles developed less maximum force and lasted longer than those of the homologous muscles of normal mice.

What can you conclude from these facts about (a) the athletic ability of a dystrophic mouse or child, and (b) the underlying mechanisms?

The actions would be weaker and slower than those of a normal child or mouse. But the differences might be smaller than expected, because additional fibres may be recruited (Section 6.5) which together would partially compensate for the weakness of each fibre. The reduced peak force is to be expected if dystrophic muscle contains fewer functional sarcomeres. But this defect alone could not cause the twitch to last longer: the duration of contraction depends upon the SR releasing and taking up calcium ions. Measurements of Ca$^{2+}$ ion concentration showed that although the maximum levels were similar in dystrophic and normal muscle fibres, the concentration did not return as quickly or as completely to the very low calcium level typical of normal relaxed muscle.
How could slightly more calcium around the sarcomeres for slightly longer at each contraction cause them to atrophy?

More calcium could activate calpain, and accelerate the breakdown of sarcomeres. The Z-lines are the first to be degraded (Section 5.4.2) by the enzyme and can be badly disrupted without visible damage to the rest of the sarcomere.

How would the muscle respond to an increased rate of muscle breakdown?

The mechanisms of regeneration and repair (Section 2.4) would be activated and would form new myotubrils, or even whole new muscle fibres, that replaced the disintegrating ones. Almost complete repair is, in principle, possible because in muscular dystrophy, the collagenous components of the sarcolemma remain intact, providing a cytoskeletal framework upon which new contractile material can reassemble. If regeneration mechanisms were operating efficiently, all defective myotubrils would heal and muscle function should be unimpaired. In young boys who are not yet obviously disabled by the disease, there is much evidence of regeneration; electron micrographs of biopsy samples reveal satellite cells (Section 2.4, Figure 2.13)-dividing and new myotubrils forming in damaged fibres. But these regeneration processes wane in older boys and become barely detectable in the advanced stages of the disease.

Why does connective tissue develop in place of the degenerated muscle of older boys?

Figure 5.16   An example of immunogold labelling of dystrophin in a normal human gastrocnemius muscle. The primary antibody was raised against one end of the dystrophin protein and conjugated to tiny particles of gold. The gold appears in this electron micrograph as dense spots, concentrated close to the plasmalemma, as indicated by arrows.
As pointed out in Section 2.4.2, in any damaged tissue that is slow to repair itself, fibroblasts proliferate and form scar tissue before regeneration is complete, often preventing further growth of the ‘proper’ tissue. This process happens extensively in human muscular dystrophy.

During the late 1980s, the absence of dystrophin was noted in certain dogs, cats and strains of mice, of which the best known is called the mdx-mouse. The defects appeared spontaneously and genetic studies of the lineages in which they were observed showed that most were X-linked, although the structure and position on the chromosome of the genes involved were different in each species. Both biochemical and electron microscope studies of muscle (see Figure 5.16) showed conclusively that dystrophin was absent from the membranes of their skeletal muscles. The interesting point is that although the primary defects are almost identical, and the genetics impressively similar, the courses of the diseases are remarkably different from that of human muscular dystrophy.

After a brief period at 2–3 weeks old in which blood creatine kinase is abnormally high and fibre necrosis can be detected in the muscles, mdx-mice recover, the creatine kinase in the blood declines and many become larger and stronger than normal mice. The mdx-mice breed successfully and many live as long as genetically normal mice. Dystrophin deficiency in cats was only noticed because the affected animals appeared stiff, not because they were weak or atrophied. Far from being wasted, many such cats have hypertrophied muscles. However, in dogs that lack dystrophin, symptoms similar to human muscular dystrophy are evident early in life; all affected puppies are small and wasted, and some die from muscular weakness during their first year. As in boys, the sarcomeres are disordered and connective tissue replaces the atrophied muscles. However, the dogs that survive their first year improve and live many more years, some developing massive muscles similar to those of ‘dystrophic’ cats.

- How do these observations on cats, dogs and mdx-mice help to explain the course of human muscular dystrophy?

They show that the debilitating symptoms of human muscular dystrophy that lead to early death are not an inevitable consequence of the absence of dystrophin: cats and mdx-mice thrive, although their dystrophin genes are mutated and so produce a defective protein. Far from becoming wasted, their muscles grow massive and strong.

- What treatment for human muscular dystrophy does this conclusion suggest?

Symptoms could be cured if genetically normal myoblasts (Section 2.4) could become established and grow to replace the dystrophin-deficient muscle fibres. Physicians are trying such therapy, but there are always immunological problems associated with introducing foreign tissues into the body and the introduced myoblasts do not migrate far into the muscle.

The observations on mdx-mice show that the decrease with age of the serum CK (also observed in boys) is not due simply to fewer muscle fibres being left to leak enzymes into the blood: there is actually more muscle in the mice, so their sarcolemmas must have become less leaky. Although, in the early stages of the disease, high serum creatine kinase, necrotic muscle fibres, etc., occur in very
young mdx-mice, their muscles respond by growing so much that they become hypertrophied, and there is no space within them for connective tissue to grow. The sarcolemma seems to strengthen with advancing age, so it becomes less permeable or less susceptible to mechanical damage.

What normal ageing process could explain this change?

The collagen content of muscles increases steadily from early in the juvenile period onwards (Section 4.5.2). Most collagen is extracellular, accumulating in and around the sarcolemma.

The problem of muscular dystrophy now becomes one of growth, tissue regeneration and ageing rather than of muscle physiology per se, and that is where we must leave this story. Although the emphasis has now shifted, it is clear that mechanical studies, our understanding of the organization of the cytoskeleton, membranes and contractile apparatus, and of interactions between muscle and adjacent tissues, and a knowledge of genes for muscle proteins were all essential to reaching this conclusion. Without such detailed knowledge, the mechanism of this disease would not have been elucidated.

The story of muscular dystrophy illustrates the intricacy of the biochemical mechanisms that regulate turnover and contractility of muscle: the absence of one rare protein can lead, over many years, to self-destruction of the whole muscle mass. Perhaps we should be more impressed by how well the system normally retains its intricate structure and continues to function efficiently for so long in the face of over-exertion, bruising, oxygen deficiency and temporary nutrient shortages.

Summary of Section 5.4

In addition to its major roles in maintaining posture and producing movement, muscle is a major short-term repository of glucose and takes up and releases glutamine which is an essential fuel for the immune system. A primary defect of Duchenne muscular dystrophy is the lack of dystrophin, a protein associated with the plasmalemma. Its absence probably weakens the sarcolemma, making it more susceptible to mechanical damage which prevents the muscle fibres from maintaining their normal low internal calcium concentration. Raised concentrations of calcium stimulate fibre breakdown, which in small species such as mice may be balanced by fibre growth, leading to normal or hypertrophied muscles, but in humans produces gradual atrophy of muscles. Affected humans become progressively disabled as regeneration and repair fail to keep pace with destruction of muscle fibres.
Objectives for Chapter 5

When you have completed this chapter, you should be able to:

5.1 Define and use, or recognize definitions and applications of each of the bold terms.
5.2 Describe the gross anatomy, fine structure and chemical composition of a typical vertebrate muscle.
5.3 Outline some differences in the composition and structure of muscles from different kinds of animals and explain their functional implications.
5.4 Describe some passive mechanical properties of living muscle and explain why a knowledge of these properties is essential to our understanding of the role of the muscles in the intact animal.
5.5 Outline the main events leading to contraction of muscles in situ and describe the mechanical response of muscle to one or several artificial stimuli in vitro.
5.6 Describe the main types of muscle fibre in mammals and explain why their properties are believed to represent intrinsic differences in the energy transduction system of the muscle.
5.7 Describe the contribution of muscle to the carbohydrate, lipid and protein metabolism of the whole body.
5.8 Outline our current understanding of the root cause and progression of Duchenne muscular dystrophy and explain briefly what kinds of laboratory studies contributed to the theories.

Questions for Chapter 5

(Answers to questions are at the end of the book.)

Question 5.1 (Objective 5.2)
Arrange the following components of muscle in order of increasing size: crossbridge, muscle fibre, myofibril, myoglobin, myosin, nebulin, sarcolemma, sarcomere, T-tubule, titin, Z-line.

Question 5.2 (Objectives 5.2 and 5.3)
Identify the features of muscle listed below as constant (i.e. universal to all striated muscle, although they may differ in abundance) or variable (i.e. present in only certain muscles or substantially different in chemical composition or arrangement between species or between different muscles of the same species):
- Actin, ATPase, I-bands, myosin, M-lines, sarcolemma, sarcomeres, sarcoplasmic reticulum, sliding filaments, titin, troponin, T-tubules.

Question 5.3 (Objective 5.3)
Describe the fine structure and composition of (a) an insect muscle adapted to high-frequency, powerful contractions over short distances; (b) a vertebrate muscle adapted to generate force at a wide range of lengths.
Question 5.4 (Objective 5.4)
Why is it essential for muscles to be extensible? What would happen if all muscles were inextensible?

Question 5.5 (Objective 5.5)
Which of the following reasons (a)–(e) explaining why the total force during a twitch is less than that during tetanus are correct?

(a) Contraction can take place only while the sarcolemma is depolarized by the motor neurons; because the electrical events on the sarcolemma last only a short time in a twitch, the contraction is terminated prematurely.

(b) For a muscle in vitro, only a small minority of the muscle fibres are stimulated to contract during a twitch.

(c) During a twitch, some of the force produced by the sarcomeres is dissipated in stretching the internal elastic components of the muscle.

(d) During a tetanus, all the different types of muscle fibre contract at the same speed, thus producing greater total force.

(e) When the stimulus frequency is low enough for the mechanical force to rise in ‘steps’, the external force generated by the mechanical response to the second stimulus is usually greater than that to the first, but smaller than that to the last stimulus of a prolonged tetanus.

Question 5.6 (Objective 5.6)
Describe the major differences in the structure, composition and normal function of oxidative and glycolytic muscle fibres in mammals and birds.

Question 5.7 (Objective 5.7)
(a) List three biochemical mechanisms essential to the role of muscle in removing glucose from the circulation.

(b) List three observations that suggest that glutamine is more important as a fuel for other tissues than as a constituent of muscle proteins.

Question 5.8 (Objective 5.8)
How has the study of laboratory and domestic animals with gene defects similar to those of boys suffering from Duchenne muscle dystrophy helped scientists to understand the biochemical mechanisms behind the disease?