THE FORCE GENERATING COMPONENTS

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THE CROSSBRIDGE MECHANISM OF MUSCULAR CONTRACTION AND ITS IMPLICATIONS

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SUMMARY

The basic features of the sliding-filament crossbridge mechanism are reviewed briefly, and some recent objections involving supposed changes in A-filament lengths are discussed. X-ray diffraction studies on live muscles show no sign of a decrease in axial spacing during contraction, and it is unlikely that a stepwise shortening or depolymerization of A-filaments would provide a plausible contraction mechanism. Thus electron microscope observations which occasionally are reported to show such length changes probably arise from experimental artefact, of which there are many sources.

The factors which govern tension and speed in muscle contraction are described. Since all vertebrate striated muscles which have been studied have A-bands of at least approximately the same length, they are likely to have rather similar maximum isometric tensions. The design probably matches this tension to the strength of the filaments themselves. The large variations in shortening speeds between different muscles and different animals arise because of corresponding variations in the rates of particular steps in the crossbridge cycle and in the rate of ATP splitting by the actin-myosin complex involved. Questions concerning the nature and the speed of the activation mechanism are also discussed.

INTRODUCTION

This conference is concerned with the design and performance of muscular systems, specifically with the way they are organized and operated to develop force and optimize speed; with the components involved in the provision of energy for the development of power; and with the factors that limit performance and adaptability. My role as the first contributor is to discuss the structural basis of muscular contraction, from the point of view of the light that structural knowledge may cast on the way in which these various design requirements are accommodated in muscles, and on the limitations that the present design, and the laws of physics and chemistry, may impose on the various aspects of their performance.

THE BASIC MODEL

I shall start by summarizing very briefly the basic structural features of striated muscle, as we know them at present. All muscles, as well as many other motile

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systems, contain the two principal contractile proteins actin and myosin, whose combined interaction with ATP converts chemical energy into mechanical work. The molecules of actin and myosin are organized into separate but overlapping filaments, and in striated muscles (with which this meeting is exclusively concerned) these filaments form a very highly ordered structure. This structure is contained in myofibrils, usually about a micron or so in diameter, which contain many hundred filaments side by side. In turn, many hundreds or thousands of parallel myofibrils will be contained within the cross-section of a single muscle fibre. Along the length of each myofibril there is a repeating pattern of striations which is generated by an alternation of partially overlapping arrays of actin and myosin filaments, and the unit of repeat is known as the sarcomere. Its resting length is in the range 2·3–2·6 μm in many vertebrate striated muscles. The myosin filaments, which in all vertebrate striated muscles seem to have exactly the same length (1·55 μm), are arranged in register in the centre of each sarcomere and form the A-bands. The actin filaments, which are usually about 1 μm in length, are attached to plate-like structures called Z-lines at either end of the sarcomere. Thus the actin filaments lie side by side on their own in the I-bands (each half-I-band being about 0·45 μm in length at resting sarcomere lengths in vertebrate muscles), then overlap with the myosin filaments in the A-bands, and terminate before the centre of the sarcomere, leaving a gap of about 0·4 μm, the H-zone. The general arrangement is illustrated in Fig. 1.

The muscle structure can change its length by a process in which the actin and myosin filaments remain essentially constant in length but where the extent of overlap of the filament arrays changes, the actin filaments being withdrawn further from the arrays of myosin filaments (A-bands) during stretch, and moving further into the array during shortening. During active shortening, the same overall sliding movement occurs, still without change in filament length.

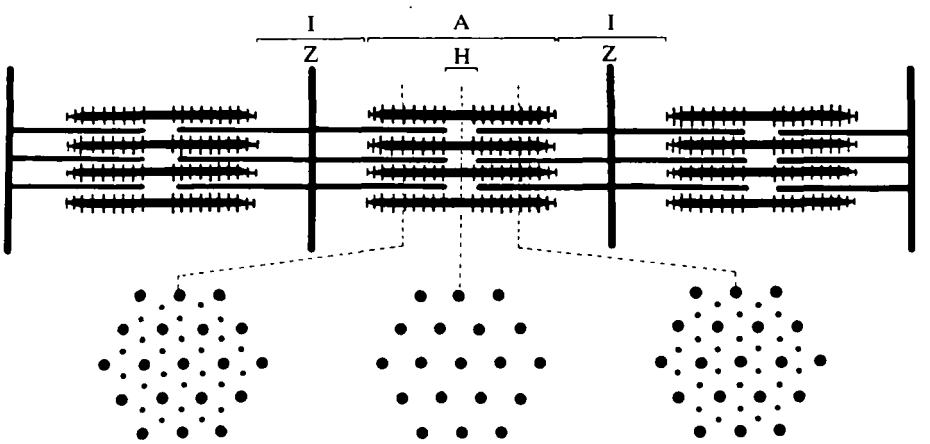

Fig. 1. Diagrammatic representation of the structure of striated muscle, showing overlapping arrays of actin- and myosin-containing filaments, the latter with projecting crossbridges on them. For convenience of representation, the structure is drawn with considerable longitudinal foreshortening; with filament diameters and side-spacings as shown, the filament lengths should be about five times the lengths shown.

The force to produce the sliding is generated by projections on the myosin filaments, the crossbridges, representing the enzymatically active portions of the myosin molecules, which undergo repetitive interactions with the actin filaments and pull them along in the appropriate direction, i.e. towards the centre of the A-band.

This model for contraction was proposed thirty years ago this year (H. E. Huxley & Hanson, 1954; A. F. Huxley & Niedergerke, 1954) and it would be appropriate to ask whether any subsequent experiments have significantly altered the original hypothesis. I believe I can say, and I think most workers in the field would agree, that this has not been the case. In all the well-documented work on vertebrate striated muscles at rest length or longer, by a variety of techniques (H. E. Huxley & Hanson, 1954; A. F. Huxley & Niedergerke, 1954, 1958; Hanson & H. E. Huxley, 1955; A. F. Huxley, 1957; Gilëv, 1962; Page & H. E. Huxley, 1963; H. E. Huxley, 1963, 1964, 1965; Page, 1965, 1968), the measurements showed that the length of the A and I filaments remained constant, and even a most persistent critic now recognizes this fact (Pollack, 1983). The function of crossbridges as tension generators is, I think, virtually universally accepted.

CURRENT EVIDENCE CONCERNING CONSTANT FILAMENT LENGTHS

Additional evidence against any A-filament shortening is provided by the fact that the 14·3 nm axial periodicity of the thick filaments, which can be measured by X-ray diffraction during the contraction of an intact muscle, shows no sign of any decrease in spacing during contraction whether the muscle is allowed to shorten or not, and the reflection may even increase in intensity when the muscle goes from the relaxed to the contracting state (H. E. Huxley & Brown, 1967; H. E. Huxley *et al.* 1982).

At sarcomere lengths below rest-length, where the I-bands become very short and where the absence of resting tension impairs the order and orientation of the structure, there have been a number of reports of relatively small apparent changes seen in the electron microscope in A-filament lengths (10–15 %) in vertebrate muscles (e.g. Herman & Dreizen, 1971; Samosudova, Lyudkovskaya & Frank, 1972), but to the best of my knowledge there is no good evidence that these represent a genuine length change taking place in the intact muscle, and indeed there are other reports indicating no length change (Hagopian, 1970). It is not easy to measure the position of the end of the A-filaments (which are tapered) with an accuracy of 50–70 nm if there is any disorder present, and a greater tendency for A-filaments to shorten slightly during fixation and embedding at sarcomere lengths where no resting tension is present can by no means be excluded. Also, deviations of the long axis of the myofibrils from the plane of sectioning are all too likely in heavily shortened muscle and are quite difficult to detect; they will always lead to underestimates of filament lengths.

There have been occasional reports of much larger amounts of A-band shortening (e.g. Samosudova & Frank, 1971; Bossen, Sommer and Waugh, 1978) but these appear to occur when the experimenters are not fully aware of a number of experimental pitfalls, viz: the effect of section compression in the cutting direction (if the fibres are not appropriately orientated during sectioning); the effect of

foreshortening due to major misalignment of myofibrils in the viewing direction; electron-optical magnification artefacts which can inadvertently be introduced by non-planarity of the specimen supporting grid or by simple setting errors unless careful checks are carried out; and shrinkage effects caused by inappropriate fixation. A further element of confusion is sometimes introduced by reports of A-band shortenings, which turn out to refer to instances where the sarcomere length has decreased substantially below 1·5 μm and contraction bands have formed at the Z-lines.

The only situation in which a genuine change in A-band length during contraction has been shown to occur sometimes in vertebrate striated muscle (though not under physiological conditions) is when glycerol-extracted muscle fibres are made to contract from a somewhat stretched length in ATP (H. E. Huxley, 1964; Sjöstrand & Jagendorf-Elfvin, 1967). In some sarcomeres, a denser band appears at the ends of the A-bands (although long I-bands can still be present), as though some filament crumpling was taking place there. As suggested in the first work cited, this might arise if some of the myosin crossbridges were not able to carry out their cyclic interactions with actin properly after exposure to glycerol and prolonged loss of ATP, so that actin and myosin filaments became stuck together in some places. A similar explanation may account for the A-band shortening produced when ATP is added to myofibrils embedded in a fibrin clot (Hasselbach, Sommer & Von Graf, 1975).

The important point to recognize is that in the case of intact muscle, X-ray diffraction measurements of constant axial periodicity *exclude* the possibility that any uniform shortening along the length of the thick filaments takes place during contraction, whether the muscle is above or below rest length. Obviously, such measurements could not readily detect a local stepwise telescoping of thick filaments by 14·3 nm intervals, but there is no plausible way that such a localized phenomenon could be linked to energy release at all the interacting actin-myosin sites in the overlap region of a sarcomere, as would have to be the case to account for the observed energy release. Of course, it is conceivable that the thick filaments depolymerize from their ends under certain conditions, but such an effect would not provide a contraction mechanism, and it is difficult to see how – or why – such a process could be linked to variations in sarcomere length when the sarcomeres were substantially longer than the A-bands. It is the constancy of the axial period of the thick filaments during a normal physiological contraction which leads one to suspect that electron-microscopic observations of A-band shortening arise from experimental artefact. And in the papers reporting such observations, the possible sources of such artefacts are often only too apparent.

OBSERVATIONS ON *LIMULUS* MUSCLE

The only persistent and puzzling reports of significant apparent changes in A-filament length have arisen from studies on the muscles of the horseshoe crab *Limulus* (Dewey, Levine & Colflesh, 1973; Dewey *et al.* 1977*b*). However, the interpretation of the original observations has now been thrown into considerable doubt by recent

studies (Levine & Kensler, 1984) on the substructure of *Limulus* thick filaments. Unlike vertebrate thick filaments, the *Limulus* filaments show considerable variation in length (3·5–5·5 μm), and it had been suggested that this was the result of a structural change in the filaments during contraction and was associated with a significant increase in diameter, supposedly produced by shortening (Dewey, Colflesh, Walcott & Levine, 1977*a*) at constant total filament mass. However, Levine & Kensler (1984) have found that the detailed substructure of the filaments seen in the electron microscope – groups of four crossbridges at 14·3 nm axial intervals (Stewart, Kensler & Levine, 1981) – is identical in both long and short filaments. Similarly, the 14·3 nm axial period of these muscles, as measured by X-ray diffraction, is unchanged during a normal physiological contraction (Y. Maeda & H. E. Huxley, unpublished observation). This makes mechanisms of contraction in these muscles based on filament shortening much less plausible, and depolymerization of A-filaments, even if it occurs, would not seem like a good way of producing force. It further supports the likelihood that a variety of filament lengths is always present in these muscles irrespective of their state of activity, and that the apparent predominance of shorter A-bands and shorter filaments in muscles which had been caused to contract and shorten was a sampling artefact.

Altogether then, I consider that the conventional sliding-filament model remains the most reasonable basis for interpreting various aspects of muscular contraction.

ADVANTAGES OF A CYCLIC MECHANISM

Given the observed constancy of filament length, it is apparent that the function of the overlapping filament structure is to provide platforms on which the arrays of interacting protein molecules are mounted with the appropriate structural polarity. This arrangement makes it possible for a large number of actin and myosin molecules to interact in parallel and to do so repetitively during the overall contraction and shortening of the muscle. This is such an advantageous system that it is difficult to understand why people should propose mechanisms which do not have these properties!

The biochemical properties of actin and myosin seem equally appropriate to the efficient operation of such a system. Myosin hydrolyses ATP only very slowly unless it is allowed to combine with actin, when the splitting rate is greatly accelerated. Thus only those crossbridges which attach to an actin filament and can perform useful work will split their bound ATP. Moreover actin and myosin in solution undergo detachment and reattachment in the course of splitting each molecule of ATP. When ATP is bound to actomyosin, dissociation takes place very rapidly, and then a much slower change has to take place before tight binding of the myosin can occur again and the split products can be released (Lymn & Taylor, 1971; Stein, Chock & Eisenberg, 1982). An approximation to this cycle is shown in Fig. 2.

The operation of this cycle in a muscle has been demonstrated with particular clarity in recent experiments by Goldman and his colleagues (Goldman, Hibberd & Trentham, 1984), in which ATP is rapidly released inside a skinned muscle fibre by

the action of a laser flash on an inert precursor ('caged-ATP'). With the fibre initially in a state of rigor (i.e. most crossbridges attached), under tension, and in the presence of calcium, the release of ATP was followed first of all by a decrease in tension and stiffness, and then by an increase in tension and stiffness to the values appropriate for an isometric contraction. Clearly, the binding of ATP produced first crossbridge detachment and subsequently reattachment and tension generation. Other details of the experiments indicated that reattachment took place to a different site on the actin from that where the crossbridge had originally been bound.

Thus I think that there can be no doubt about the reality of the crossbridge attachment-detachment cycle and of the concept that some structural change in the crossbridge is responsible for force development. The 'rapid release' experiments of A. F. Huxley & Simmons (1971) and Ford, Huxley & Simmons (1977) indicate that an individual crossbridge can remain attached to and exert a force on an actin filament while that filament moves longitudinally by about 14·0 nm. It can then be shown from energetic considerations that at least one-half of all the myosin heads must be attached to actin during very slow shortening. If force is exerted over a shorter distance, the proportion of heads involved will be correspondingly smaller.

DETAILED BEHAVIOUR OF THE CROSSBRIDGES

The really interesting uncertainties about the mechanism are concerned with the detailed molecular changes within the crossbridges whereby they are able to exert a longitudinal force on the actin filaments. One possibility is that there is a change in shape or in the effective angle of attachment of the myosin head (S_1) to actin (H. E. Huxley, 1969) which produces a longitudinal change of position of about 14·0 nm in the distal part of S_1 (i.e. near the S_1–S_2 junction) relative to the attachment site on actin, during the so-called working stroke of the crossbridge and that this is coupled to

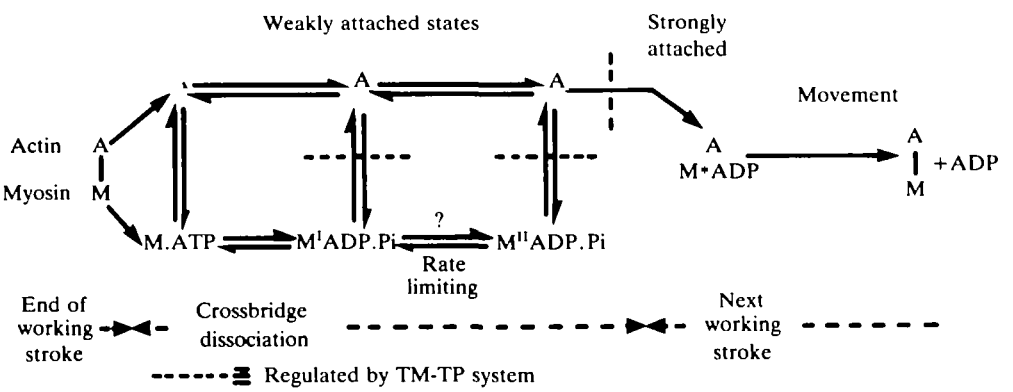

Fig. 2. Schematic diagram showing crossbridge attachment-detachment cycle. For explanation see text.

one of the steps in the splitting of ATP. Since the ATPase site in myosin is located in the S_1 region, this model is very plausible in theory. In practice, the problem has been to obtain experimental evidence for a structural change, in myosin heads attached to actin, which normally takes place rapidly and asynchronously in a contracting muscle. There are now rapid X-ray diffraction measurements (H. E. Huxley *et al.* 1981, 1983) which show changes in the longitudinal position of parts of the myosin head during the working stroke of the crossbridge. However, if these were accomplished by a simple rotation of the S_1 heads about a site on actin, one might in general expect to see substantial changes in the intensities of the equatorial X-ray reflections. However, only rather small changes are observed. It is possible to devise rotation models in which, for some special structural reason, such equatorial changes are extremely small, so we cannot exclude this type of model, but nor can we point to positive X-ray evidence in its favour. A more plausible model perhaps is one in which a shape change takes place within the S_1 structure which results in a more predominantly longitudinal movement of the region distal to actin.

Another possibility is that the primary change in crossbridge structure takes place in the S_2 region, e.g. a helix-coil transition (Harrington, 1979) and that this is somehow linked to the attachment-detachment cycle of the crossbridge. This type of model has not been specified very precisely, and many objections can be raised against it. Some of them involve rather long and intricate technical arguments that would be inappropriate here, but the most straightfoward one is that the site at which ATP is attached and split is located on the S_1 head of the myosin molecule. It is therefore probable that the energy transduction mechanism is located in the same place, rather than several tens of nanometers away near the S_2–LMM junction. However, even if S_2 does not play an active role in force development it may well be that it functions as a partially elastic element in the force generating mechanism, and its elasticity may show time-dependent effects. Probably the safest position at present is to keep a fairly open mind about a range of crossbridge mechanisms until some really decisive piece of experimental evidence appears!

Notwithstanding the uncertainties about the details of the changes in crossbridge structure which produce contraction, one can still use the general framework of that model to think about the problems of the design and performance of muscles, and a good starting point is to consider what features of the structure determine the tension that a muscle can develop and the speed with which it will shorten.

FACTORS GOVERNING TENSION AND SPEED

We will assume that the interaction between a myosin and an actin molecule follows very much the same structural and chemical pathway in different muscles since these proteins and the structures they form are rather highly conserved, especially amongst the vertebrates. What does vary over quite a wide range (Barany, 1967; Close, 1965) are the reaction *rates*, i.e. the actomyosin ATPase rates. Thus it seems likely that an individual crossbridge would always be capable of generating more or less the same tension over more or less the same working stroke and hence performing about the

same amount of work, but that different muscles would differ in the speed at which the crossbridge cycling could take place.

The identification of the rate-limiting step in the actomyosin ATPase reaction has provided a fascinating problem for enzyme kineticists and muscle biophysicists. When the reaction takes place in solution, with a relative absence of constraint on the movement or change in structure of the molecules, the rate-limiting step may well be different from that in an intact muscle; and even in a muscle, the limitation may vary with the velocity of shortening. In a muscle held isometrically, developing tension but not performing external work, it is obviously desirable to reduce crossbridge cycling and ATP splitting to a minimum, and very low ATPase levels have indeed been measured in such isometric muscles (Homsher, Irving & Wallner, 1981). Thus an essential step in the attached phase of the cycle cannot be completed quickly unless the crossbridge is able to proceed to the end of its working stroke. This may be the products release step or the binding of the next molecule of ATP. In a muscle shortening against a moderate load, where there is good evidence that a high proportion of bridges are attached, but with many of them not developing tension (Podolsky, St. Onge, Lu & Lymn, 1976; H. E. Huxley, 1979), the most obvious possibility for the rate-limiting step is the one at which an attached but inactive bridge changes state in some way – perhaps by phosphate release – and develops tension. By adjusting this rate constant, one could vary the velocity of shortening that could be maintained against a given fraction of the maximum isometric load. Alternatively, there might be a rate-limiting step subsequent to tension generation, even by a moving crossbridge, which had to occur before a new attached tension generating state could be produced (Goldman *et al.* 1984). At very small or zero loads and maximum velocity of shortening, yet another step may become rate-limiting, perhaps the detachment step (A. F. Huxley, 1957). In the case of solution measurements, the steps corresponding to the working stroke of the crossbridge and its detachment are likely to take place very quickly since the system is free of externally applied mechanical constraints and the effective rate-limiting step at V_{max} will be one that occurs before the working stroke. According to Eisenberg and his colleagues (Stein *et al.* 1982), this step represents a change of state in the myosin molecule which can occur whether it is attached to actin or not.

There is obviously room for a great deal of interesting comparative work on the details of the actomyosin link-ATPase cycles for muscles with different shortening velocity characteristics.

The total isometric tension produced in a muscle will be proportional to the number of myosin crossbridges which are attached to actin filaments in one half A-band of the muscle, integrated over the whole cross-section of the muscle. Within each half-sarcomere, all the individual crossbridge tensions would be expected to add up in parallel, whereas the tension in the two halves of an A-band will add up in series, as will those in successive sarcomeres along the whole length of a muscle. Thus the parameters which determine the maximum isometric tension of a muscle are the number of myosin molecules in each half A-filament, and the

number of A-filaments per unit area, i.e. a quantity that depends on their lateral separation.

PRACTICAL LIMITS ON TENSION

The lateral spacing of the A-filaments does not vary much amongst the vertebrate striated muscles where it has been measured (H. E. Huxley, 1953, 1968) and is about 40·0 nm. It is larger in insect muscles (Miller & Tregear, 1972) but here the number of myosin molecules per unit length, and the length of the A-filaments, is larger too. This spacing is presumably determined by the minimum size that a crossbridge needs to have in order to incorporate the structural features necessary for its functioning. The optimum packing of such crossbridges is achieved by the double hexagonal array of actin and myosin filaments. The density of longitudinal packing is probably limited by the length of the working stroke of the crossbridges (believed to be about 14·0 nm) and by the fact that there must be room for them to act asynchronously. The observed axial repeat of groups of crossbridges is always 14·3 nm in all muscles studied and there appear to be three myosin molecules at each 14·3 nm repeat in vertebrate striated muscle (Squire, 1975). Thus the only way in which the tension generated by a muscle could in theory be substantially increased without running into inherent limitations in the structure of the force-producing mechanism itself would be by increasing the length of the A-filaments (and A-bands).

In practice, the lengths of the A-bands in all vertebrate striated muscles are all virtually the same, namely 1·55 μm approximately. A recent book (Hoyle, 1983) has reported substantial variation between different vertebrate species, but inspection of the original papers quoted (Page, 1965; Fernand & Hess, 1969; Gradwell & Walcott, 1971; Bossen *et al.* 1978) shows that this is not the case. The instances of lengths significantly different from 1·5 μm all arise either because of a simple misquotation of the measurements in the original paper or an error of reading of the magnification marker on the micrographs by the quoting author, or a deliberate action in the experimental protocol of the quoted author to section fibrils in random orientations. Longer and more variable length A-bands are genuinely observed in some arthropods (e.g. crayfish 3·95 μm, Zachar & Zacharova, 1966; crab, from 2·5 μm up to 5–6 μm, Franzini-Armstrong, 1970), but these have less regularly arranged actin filaments and thicker myosin filaments than vertebrate striated muscles, so that comparisons become much more complicated. If one restricts the discussion to vertebrate striated muscles, then the constancy of A-band length suggests that there is a limitation on the tension per unit area that the muscle structure can safely transmit, and that the limit is reached with A-bands of the observed lengths, developing tensions around 3 kg cm^{-2}. This interpretation would be supported by the fact that muscles make up a very substantial proportion of an animal's mass, and that almost the whole cross-section of all muscles is occupied by the arrays of actin and myosin filaments. Thus it appears that evolution has maximized the tension generated by muscles, that the limiting factor lies in the strength of the filaments themselves or

the attachments at the Z-lines, and that muscles are therefore as large and as tightly-packed with filaments as possible.

PRACTICAL LIMITS ON SPEED

In a classic paper many years ago, A. V. Hill (1950) drew attention to the fact that despite very large differences in size, most animals show very much smaller differences in the maximum speed at which they can move. He showed by a dimensional argument that this would be the expected consequence for animals of similar construction with muscles capable of exerting similar tensions per unit area, as the available measurements indicated. It is the velocity of shortening of the muscle which varies with the size of the animal, being inversely proportional to its linear dimensions. Thus the smallest animals (e.g. mice) have muscles with the highest shortening velocities. This means that the velocity of sliding of actin filaments past myosin filaments is highest in these animals, and that the characteristic ATPase activities of these actomyosin systems are correspondingly higher. This has indeed been found to be the case (Barany, 1967).

Thus it appears that between different vertebrate species muscles are matched in speed to the general requirements of their situation by keeping constant all the main structural parameters, such as A-band length or sarcomere length, and by changing the characteristic rate at which the crossbridges go through their cycle of combination with actin and splitting of ATP. Even within a given vertebrate species, more than one type of myosin has been identified as well as several different types of fibre (Close, 1972). However, there do not seem to be many cases where a detailed physiological and biochemical comparison has been made of several different muscles in the same species. It is also not clear at present whether one pays a price in the efficiency of energy conversion in an actomyosin system capable of high shortening velocities, and this should be an interesting topic for comparative studies.

WHY STRIATIONS?

Given that one has a contractile system based on thick filaments of a certain optimum length (i.e. the maximum consistent with the strength of the filament system), one must next ask what factors would influence the optimum length of the thin filaments, and why should the system be assembled with all the thick filaments in register, and with all the thin filaments in register.

The minimum length of thin filament required will be given by the length of the crossbridge region in one half A-filament (since for maximum efficiency all crossbridges should be utilized) plus the distance of shortening required in each half sarcomere. One might have supposed that this latter quantity would vary somewhat from one muscle to another in a given animal, but as far as I know, all the I-filaments in every muscle in a given vertebrate species either have the same length as each other, or have a range of lengths with the same maximum. There are small differences between

different species (Page & H. E. Huxley, 1963; Page, 1968). Thus it seems that the solution has been to use muscles with a standard structure (allowing them to shorten to about 70 % of their length at full crossbridge overlap before I-bands disappear) and presumably to arrange their anatomical disposition so that the range of sarcomere lengths over which they are required to operate falls into their most efficient region. It would be interesting to know the working range of sarcomere lengths *in situ* for a variety of muscles.

Given that there is an optimum length for the thick and for the thin filaments, and an optimum degree of overlap, then the simplest packing arrangement and the one which would maximize the number of interacting sites is the one normally observed, i.e. in register arrays of myosin and of actin filaments, giving the same degree of overlap throughout. Such a system is also mechanically and hydrodynamically more satisfactory than one lacking axial registration of filaments, in which unwanted interference between filaments will occur. A further advantage of the sarcomere-type organization, with filaments held rather rigidly in register by transverse structures at M-lines and Z-lines, is that the relative statistical variation in the number of interacting crossbridges in adjoining half-sarcomeres is greatly reduced (since the contracting unit is not just one pair of actin and myosin filaments, but 500–1000 such pairs, reducing the effective variation by $\sqrt{500}$–$\sqrt{1000}$). Thus any tendency for longitudinal 'dithering' of actin or myosin filaments, which would lead to additional ATP usage, is substantially reduced.

REGULATION OF CONTRACTION

The other part of the mechanism which we need to consider is the control system, i.e. the on-off switching provided by the calcium release and sequestering system, acting on the regulatory proteins troponin and tropomyosin in vertebrate striated muscle. The design of the membrane system to allow the whole cross-section of a muscle fibre to be switched on in a very few milliseconds is well known and need not be described again here, but we may note that the organization of the contractile material into myofibrils only a micron or so in diameter in fast muscles and enveloped in reticulum reduces diffusion times to a minimum. The mechanism of operation of the regulatory system on the thin filaments continues to be a matter of some debate, which is probably too complicated to go into here. However, recent time-resolved X-ray diffraction measurements (Huxley, Kress & Faruqi, 1984) have shown very clearly that tropomyosin movement of the type proposed in the steric blocking mechanism (H. E. Huxley, 1972) does indeed take place and that it occurs earlier after the initial stimulus than any of the other structural changes in the muscle filaments during contraction. Also, there is good evidence that the number of attached but non-tension generating crossbridges increases during contraction, indicating that part of the regulation occurs at the attachment step itself, in addition to the regulation demonstrated at later steps in the cycle (Chalovich & Eisenberg, 1982). For fine muscular control, however, it is necessary to be able to switch muscles off very quickly too, and an additional system which may be responsible (Gillis, Thomason, Lefevre &

Kretsinger, 1982) for doing this has been discovered in recent years in certain types of muscles. These contain in their sarcoplasm large amounts of the calcium binding protein parvalbumin (Perchere, Derancourt & Haiech, 1977). This cannot bind calcium fast enough to interfere with the activation of the muscle by calcium released from the reticulum and rapidly bound to troponin, but it binds calcium more strongly than troponin and so ultimately captures the calcium from troponin and does so at a faster rate than the reticulum is capable of during relaxation. Thus relaxation is speeded up. However, the reticulum binds calcium even more tightly than does parvalbumin, so that after a delay the system returns to its initial state, with nearly all the calcium in the reticulum.

The extent to which muscle activity is regulated *in vivo* at a finer level than full on/off switching is a more complicated matter and perhaps there will be more discussion of it in the course of this Symposium. There are obvious possibilities for such regulation by altering the strength and the speed of calcium binding by troponin (and perhaps parvalbumin?) and it is also possible for different steps in the crossbridge cycle to be affected in a more direct way by myosin phosphorylation.

CONCLUSION

Overall, we can see plenty of variation and adaptation of muscle as far as fine molecular and biochemical details are concerned, but remarkable conservation in the basic ultrastructural organization, especially among the vertebrates.

REFERENCES

BARANY, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. *J. gen. Physiol.* **50**, 197–218.

BOSSEN, E. H., SOMMER, J. R. & WAUGH, R. A. (1978). Comparative stereology of the mouse and finch left ventricle. *Tissue & Cell* **10**, 773–784.

CHALOVICH, J. M. & EISENBERG, E. (1982). Inhibition of actomyosin ATPase activity by troponin-tropomyosin without blocking the binding of myosin to actin. *J. biol. Chem.* **257**, 2432–2437.

CLOSE, R. (1965). The relation between intrinsic speed of shortening and duration of the active state of muscle. *J. Physiol., Lond.* **180**, 542–559.

CLOSE, R. I. (1972). Dynamic properties of mammalian skeletal muscles. *Physiol. Rev.* **52**, 129–197.

DEWEY, M. M., COLFLESH, D. E., WALCOTT, B. & LEVINE, R. J. C. (1977*a*). Changes in length, diameter and volume of the thick filaments of *Limulus* striated muscle during sarcomere shortening. *Biophys. J.* **17**, 160a.

DEWEY, M. M., LEVINE, R. J. C. & COLFLESH, D. E. (1973). Structure of *Limulus* striated muscle. *J. Cell Biol.* **58**, 574–598.

DEWEY, M. M., WALCOTT, B., COLFLESH, D. E., TERRY, H. & LEVINE, R. J. C. (1977*b*). Changes in thick filament length in *Limulus* striated muscle. *J. Cell Biol.* **75**, 366–380.

FERNAND, V. S. V. & HESS, A. (1969). The occurrence, structure and innervation of slow and twitch muscle fibres in the tensor tympani and stapedius of the cat. *J. Physiol., Lond.* **200**, 547–554.

FORD, L. E., HUXLEY, A. F. & SIMMONS, R. M. (1977). Tension responses to sudden length change in stimulated frog muscle fibres near slack length. *J. Physiol., Lond.* **269**, 441–515.

FRANZINI-ARMSTRONG, C. (1970). Natural variability in the length of thin and thick filaments in single fibres from a crab, *Portunus depurator*. *J. Cell Sci.* **6**, 559–592.

GILEV, V. P. (1962). A study of myofibril sarcomere structure during contraction. *J. Cell Biol.* **12**, 135–147.

GILLIS, J. M., THOMASON, D., LEFEVRE, J. & KRETSINGER, R. H. (1982). Parvalbumins and muscle relaxation: a computer simulation study. *J. Mus. Res. Cell Motil.* **3**, 377–398.

GOLDMAN, Y. E., HIBBERD, M. G. & TRENTHAM, D. R. (1984). Initiation of active contraction by photogeneration of adenosine 5′-triphosphate in rabbit psoas muscle fibres. *J. Physiol., Lond.* **354**, 605–624.

GRADWELL, N. & WALCOTT, B. (1971). Dual functional and structural properties of the interhyoideus muscle of the bullfrog tadpole. *J. exp. Zool.* **176**, 193–218.

HAGOPIAN, M. (1970). Contraction bands at short sarcomere length in chick muscle. *J. Cell Biol.* **47**, 790–796.

HANSON, J. & HUXLEY, H. E. (1955). The structural basis of contraction in striated muscle. *Symp. Soc. exp. Biol.* **9**, 228–264.

HARRINGTON, W. F. (1979). On the origin of the contractile force in skeletal muscle. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5066–5070.

HASSELBACH, W., SOMMER, J. R. & VON GRAF, H. (1975). A-band shortening in contracted skeletal muscle fibrils. (Abstr.) *Fedn Proc. Fedn Am. Socs exp. Biol.* **34**, 1433.

HERMAN, L. & DREIZEN, P. (1971). Electron microscope studies of skeletal and cardiac muscle of a benthic fish. I. Myofibrillar structure in resting and contracted muscle. *Am. Zool.* **11**, 543–547.

HILL, A. V. (1950). The dimensions of animals and their muscular dynamics. *Sci. Prog., Oxford* **150**, 209–230.

HOMSHER, E., IRVING, M. & WALLNER, A. (1981). High energy phosphate metabolism and energy liberation associated with rapid shortening in frog skeletal muscle. *J. Physiol., Lond.* **321**, 423–436.

HOYLE, G. (1983). *Muscles and their Neural Control*. New York: John Wiley & Sons.

HUXLEY, A. F. (1957). Muscle structure and theories of contraction. *Prog. Biophys. biophys. Chem.* **7**, 255–318.

HUXLEY, A. F. & NIEDERGERKE, R. (1954). Structural changes in muscle during contraction. Interference microscopy of living muscle fibres. *Nature, Lond.* **173**, 971–973.

HUXLEY, A. F. & NIEDERGERKE, R. (1958). Measurements on the striations of isolated muscle fibres with the interference microscope. *J. Physiol., Lond.* **144**, 403–425.

HUXLEY, A. F. & SIMMONS, R. M. (1971). Proposed mechanism of force generation in striated muscle. *Nature, Lond.* **233**, 533–538.

HUXLEY, H. E. (1953). X-ray analysis and the problem of muscle. *Proc. R. Soc. B* **141**, 59.

HUXLEY, H. E. (1963). Electron microscope studies on the structure of natural and synthetic filaments from muscle. *J. mol. Biol.* **7**, 281–308.

HUXLEY, H. E. (1964). Structural arrangements and the contraction mechanism in striated muscle. *Proc. R. Soc.* **160**, 442–448.

HUXLEY, H. E. (1965). Structural evidence concerning the contraction mechanism in striated muscle. In *Muscle*, (eds W. M. Paul, E. E. Daniel, C. M. Kay & G. Monckton), pp. 3–28. New York: Pergamon.

HUXLEY, H. E. (1968). Structural difference between resting and rigor muscle; evidence from intensity changes in the low angle equatorial X-ray diagram. *J. mol. Biol.* **37**, 507–520.

HUXLEY, H. E. (1969). The mechanism of muscular contraction. *Science, N.Y.* **164**, 1356–1366.

HUXLEY, H. E. (1972). Structural changes in the actin- and myosin-containing filaments during contractions. *Cold Spring Harb. Symp. quant. Biol.* **37**, 361–376.

HUXLEY, H. E. (1979). Time-resolved X-ray diffraction studies on muscle. In *Cross-Bridge Mechanism in Muscle Contraction*, (eds H. Sugi & G. H. Pollack), pp. 391–401. Tokyo: University of Tokyo Press.

HUXLEY, H. E. & BROWN, W. (1967). The low angle X-ray diagram of vertebrate striated muscle and its behaviour during contraction and rigor. *J. mol. Biol.* **30**, 383–434.

HUXLEY, H. E., FARUQI, A. R., KRESS, M., BORDAS, J. & KOCH, M. H. J. (1982). Time-resolved X-ray diffraction studies of the myosin layer-line reflections during muscle contraction. *J. mol. Biol.* **158**, 637–684.

HUXLEY, H. E. & HANSON, J. (1954). Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature, Lond.* **173**, 973–976.

HUXLEY, H. E., KRESS, M. & FARUQI, A. R. (1984). Time-resolved X-ray diffraction measurements of the activation mechanism in striated muscle. *Biophys. J.* **45**, 10a.

HUXLEY, H. E., SIMMONS, R. M., FARUQI, A. R., KRESS, M., BORDAS, J. & KOCH, M. H. J. (1981). Millisecond time-resolved changes in X-ray reflections from contracting muscle, recorded using synchrotron radiation. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2297–2301.

HUXLEY, H. E., SIMMONS, R. M., FARUQI, A. R., KRESS, M., BORDAS, J. & KOCH, M. H. J. (1983). Changes in the X-ray reflections from contracting muscle during rapid mechanical transients and their structural implications. *J. mol. Biol.* **169**, 469–506.

LEVINE, R. J. C. & KENSLER, R. W. (1984). Crossbridge arrangements in shortened *Limulus* thick filaments. *Biophys. J.* **45**, 348a.

LYMN, R. W. & TAYLOR, E. W. (1971). Mechanism of adenosine triphosphate hydrolysis by actomyosin. *Biochemistry N.Y.* **10**, 4617–4624.

MILLER, A. & TREGEAR, R. T. (1972). Structure of insect fibrillar flight muscle in the presence and absence of ATP. *J. mol. Biol.* **70**, 85–104.

PAGE, S. G. (1965). A comparison of the fine structures of frog slow and twitch muscle fibres. *J. Cell Biol.* **26**, 477–497.

PAGE, S. G. (1968). Fine structure of tortoise skeletal muscle. *J. Physiol., Lond.* **197**, 709–715.

PAGE, S. G. (1969). Structure and some contractile properties of fast and slow muscles of the chicken. *J. Physiol., Lond.* **205**, 131–145.

PAGE, S. G. (1974). Measurements of structural parameters in cardiac muscle. In Ciba Foundation Symposium **24** *The Physiological Basis of Starling's Law of the Heart*, pp. 13–30.

PAGE, S. G. & HUXLEY, H. E. (1963). Filament lengths in striated muscle. *J. Cell Biol.* **19**, 369–391.

PERCHERE, J. F., DERANCOURT, J. & HAIECH, J. (1977). The participation of parvalbumins in the activation-relaxation cycle of vertebrate fast skeletal muscle. *FEBS Lett.* **75**, 111–114.

PODOLSKY, R. J., ST. ONGE, R., LU, L. & LYMN, R. W. (1976). X-ray diffraction of actively shortening muscle. *Proc. natn. Acad. Sci. U.S.A.* **73**, 813–817.

POLLACK, G. H. (1983). The crossbridge theory. *Physiol. Rev.* **63**, 1049–1113 (see p. 1063, para. 4).

SAMOSUDOVA, N. V. & FRANK, G. M. (1971). Changes in the ultrastructure of the contractile apparatus of cross-striated muscle with the tonic type of contraction. *Biofizika* **16**, 244–253.

SAMOSUDOVA, N. V., LYUDKOVSKAYA, R. G. & FRANK, G. M. (1972). Changes in the ultrastructure of the slow and intermediate muscle fibres of the frog on tonic contraction. *Biofizika* **17**, 1107–1116.

SJÖSTRAND, F. S. & JAGENDORF-ELFVIN, M. (1967). Ultrastructure studies of the contraction-relaxation cycle of glycerinated rabbit psoas muscle. *J. Ultrastruct. Res.* **17**, 348–378.

SQUIRE, J. (1975). Muscle filament structure and theories of contraction. *A. Rev. Biophys. Bioeng.* **4**, 137–163.

STEIN, L. A., CHOCK, P. B. & EISENBERG, E. (1982). Evidence for a '6-state' kinetic model of the actomyosin ATPase. *Biophys. J.* **37**, 264.

STEWART, M., KENSLER, R. W. & LEVINE, R. J. C. (1981). Structure of *Limulus* telson muscle thick filaments. *J. mol. Biol.* **153**, 781–790.

ZACHAR, J. & ZACHAROVA, D. (1966). Potassium contractures in single muscle fibres of the crayfish. *J. Physiol., Lond.* **186**, 596–618.