

## REVIEW ARTICLE

# Fifty years of muscle and the sliding filament hypothesis

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This review describes the early beginnings of X-ray diffraction work on muscle structure and the contraction mechanism in the MRC Unit in the Cavendish Laboratory, Cambridge, and later work in the MRC Molecular Biology Laboratory in Hills Road, Cambridge, where the author worked for many years, and elsewhere. The work has depended heavily on instrumentation development, for which the MRC laboratory had made excellent provision. The search for ever higher X-ray intensity for time-resolved studies led to the development of synchrotron radiation as an exceptionally powerful X-ray source. This led to the first

direct evidence for cross-bridge tilting during force generation in muscle. Further improvements in technology have made it possible to study the fine structure of some of the X-ray reflections from contracting muscle during mechanical transients, and these are currently providing remarkable insights into the detailed mechanism of force development by myosin cross-bridges.

**Keywords:** muscle; structure; contraction; X-ray diffraction; synchrotron radiation; MRC Laboratory of Molecular Biology.

## Early days at the MRC (1948–1952)

I came to the MRC Laboratory as a research student in the summer of 1948, when it was called the MRC Unit for Work on the Molecular Structure of Biological Systems, and consisted of Max Perutz and John Kendrew, who became my supervisor. Francis Crick joined the unit a short time later, and Jim Watson was there during my last year as a graduate student.

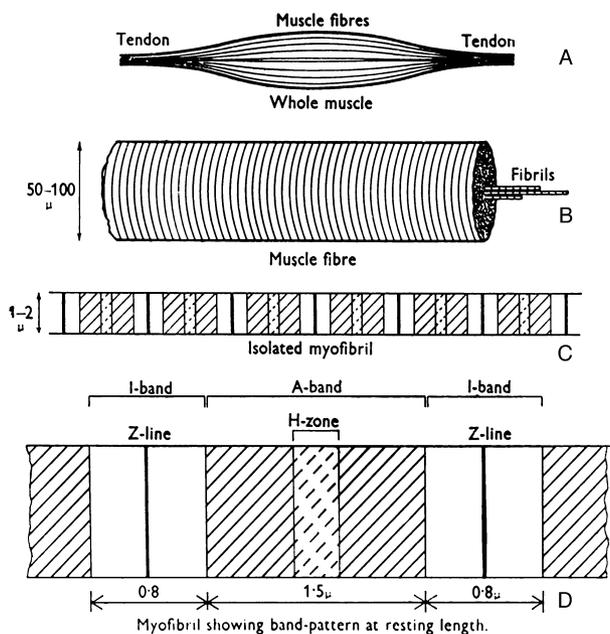
I had just finished Part II Physics in 1948, in my third year in Cambridge, a degree interrupted by four years of working on radar development in the RAF, during the war. Though extremely ignorant of biology, I had picked up the idea that there might be interesting applications of physics to biological and medical problems. Joining the MRC Unit sounded like a good way of following that line, with the advantage that I could stay on and perform research in Cambridge. This had been my ambition for many years, though in a different field.

I had just finished learning all about the remarkable ways in which the physical properties of matter – mechanical, thermal, electrical – could be accounted for by the properties and interaction of atoms, which depended on atomic structure. So it seemed obvious that now one needed to find out about the structure of biological systems, at the atomic and molecular levels, to understand how they worked. X-ray diffraction seemed to offer a way of doing just that, which this group was exploring, but of course I had no way of knowing just how extraordinarily fortunate I was to join them. Nor did we ever dream of quite how important those years would turn out to be.

I did recognize that I was quite fortunate, as Max, John and Francis were all such marvelous people to be with, and I admired and liked them very much. They created a light-hearted, stimulating intellectual environment, with high standards and ambitious objectives. It was so exhilarating to be back again in Cambridge, now as a research student, very soon after the end of World War II. The clouds of the 1930s had gone, we had won the war against Fascism – and many of us had helped to do so – and now there were all sorts of marvelous ideas and research flourishing around us – Hoyle, Bondi and Gold with their theory of continuous creation, Fred Sanger sequencing insulin, Martin Ryle doing great things in radio astronomy, the first EDSAC computer whirring away in the maths lab, Nikolaus Pevsner lecturing on Renaissance Art and Architecture in Italy – and great hopes for the Labour Government and a better world.

However, the work in the laboratory on hemoglobin and myoglobin was going slowly, and crystallography was not a subject that I found I enjoyed – my favorites had been experimental nuclear and particle physics. So I started working on muscle structure, which seemed to offer more opportunity for adventure. Essentially nothing was known about muscle structure at the submicroscopic level at that time, except that striated muscles had complicated repeating pattern of bands and lines (Fig. 1), and that there were filaments of a complex between two proteins, actin and myosin, whose individual structures were, of course, unknown. What the general structure of the complex was, no one knew either, and yet such knowledge was clearly essential in order to understand the mechanism of contraction. This mechanism was still completely mysterious – a situation that, as a newcomer to biology, I had at first found very surprising.

To begin to learn something about muscle filament structure, I knew that I would have first to look for X-ray reflections in the 100 Å range. This would require cameras with very narrow slits, which meant problems of X-ray



**Fig. 1.** Diagram showing different levels of structure in vertebrate striated muscle as recognized circa 1950, and approximate dimensions of band patterns within each repeating unit or sarcomere.

intensity, especially with hydrated biological specimens, as I wanted to look at muscles in the living state. Bernal had been the first to recognize that maintaining hydration was essential to obtaining informative X-ray patterns from protein crystals, and this had opened up the whole subject of protein crystallography. So it seemed possible that muscles, too, might give good patterns when in their native state, though the patterns might be very weak.

This was what began the long road of forever searching for higher intensity X-ray sources, and the MRC laboratory provided an ideal base for doing that, which was my good fortune. Kendrew and Perutz were very open-minded about research projects, and encouraged me in this venture. The first step was the acquisition of a prototype very fine focus ( $50\ \mu\text{m}$ ) X-ray tube giving high brilliance (Fig. 2) obtained via Kendrew and Bernal from Ehrenberg and Spear at Birkbeck College.

Using this tube and a miniaturized low-angle X-ray camera ( $5\ \mu\text{m}$  beam defining slit, 3 cm specimen-to-film distance), I was able to get my first diffraction patterns from live relaxed muscle, with quite practicable exposure times (a few hours for equatorial patterns and a couple of days for axial ones). There were indeed sharp reflections from a highly ordered structure, a tremendously exciting and promising finding [1].

On the equator, there were reflections whose relative spacings and intensities suggested that they came from a hexagonal array of filaments about  $450\ \text{\AA}$  apart and about  $100\text{--}150\ \text{\AA}$  in diameter (Fig. 3A). So there was a paracrystalline lattice of filaments, in a live muscle! A diagram from a muscle in rigor showed about the same lattice spacings but very different relative intensities (Fig. 3B) which I realized could be accounted for by the presence of a second set of filaments, located at the trigonal positions of the original hexagonal lattice. One can see this by constructing very



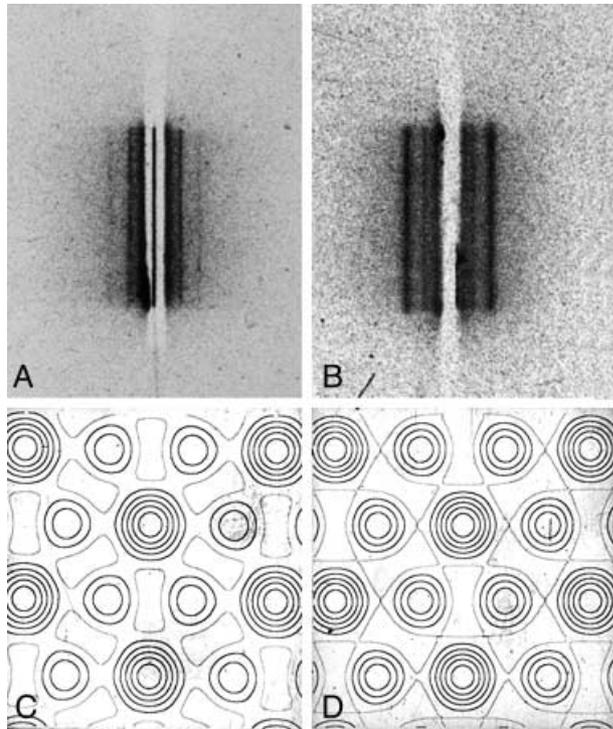
**Fig. 2.** Prototype model of Ehrenberg-Spear fine-focus X-ray tube used in early muscle work (diameter of tube is approximately  $3.5\ \text{cm}$ ). Anode connection is inside safety shield, at 40 kV.

primitive end-on Fourier projections, with plausible phases,  $\pm$  in this case (Fig. 3C). So I guessed that the original main set of filaments must be myosin and the second set, actin. That is, that the two contractile proteins were present in separate filaments, which therefore had to have cross-connections between them to interact, to become rigidly bonded in rigor, and to somehow produce shortening in contraction [2,3].

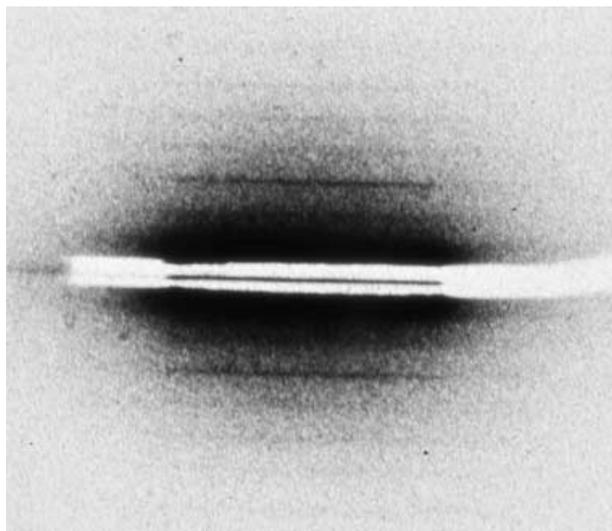
Axial X-ray patterns showed a pattern of reflections based on an approximately  $420\ \text{\AA}$  axial repeat (Fig. 4) with a very strong third order, which remained in rigor, while the other reflections became very faint. Intriguingly, the axial period did not change when the relaxed muscle was passively stretched! However, at that time I thought that the two sets of filaments were both continuous through the whole muscle sarcomere, and that the filaments giving the axial periodicity must develop gaps during stretch. This mystery was solved a year or two later, in 1953.

### Work at MIT (1952–1954)

The year 2003 is in fact another fiftieth anniversary, as well as being that of the DNA structure, and of Max Perutz's discovery of how to phase the X-ray reflections from protein crystals. It was in 1953 that Jean Hanson and I – Jean from the King's College London Biophysics Research Unit – this time an intentional collaboration! – began working together

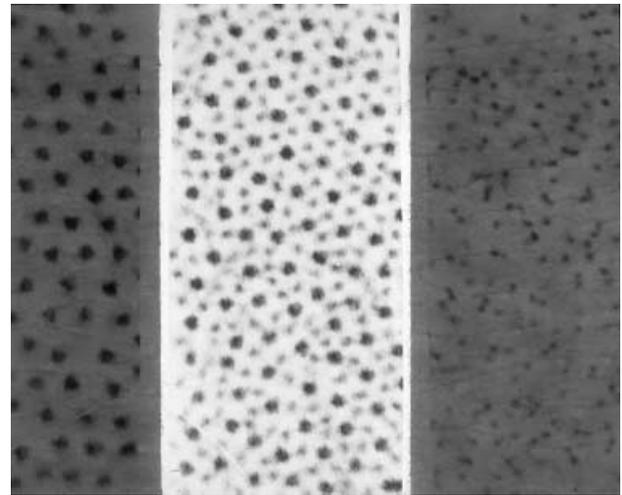


**Fig. 3. Equatorial X-ray diagrams (slit camera) from frog sartorius muscle.** (A) Live, resting muscle; (B) muscle in rigor; (C, D) corresponding Fourier projections showing electron density distribution in hexagonal lattice with  $a = 440 \text{ \AA}$ , with increased density at trigonal positions of lattice in rigor.



**Fig. 4. Axial X-ray diagram (slit camera) from live, resting frog sartorius muscle, showing long axial repeat, measured to be  $\approx 415 \text{ \AA}$  (actually  $430 \text{ \AA}$ ) with strong third order.**

at the Massachusetts Institute of Technology (MIT), following up projects we had started earlier at our respective MRC Units. In September 1953, we published the overlapping, interdigitating, double array of filaments model for the structure of striated muscle [4].

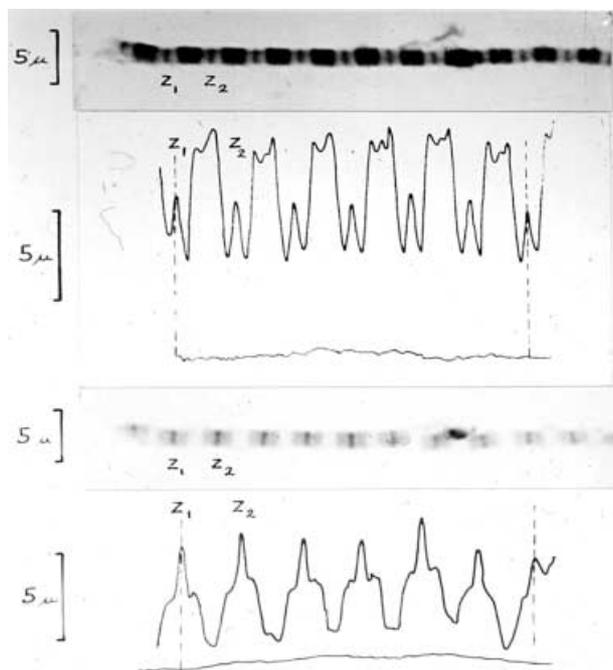


**Fig. 5. Electron micrograph of cross-section of frog sartorius muscle, showing end-on view of double array of filaments in overlap zone (centre picture), and of H-zone and I-band (flanking pictures, shaded).** (Note: Not from the 1953 paper [5], where reproduction was poor.)

I had moved to MIT (September of 1952) to learn electron microscopy in F. O. Schmitt's group, and to look for my double array of filaments using that technique. and in fact, I had soon found I could see them quite readily (Fig. 5) when I looked at thin cross-sections of vertebrate striated muscle [5], cut using a special microtome which Hodge, Spiro and I [6] had designed and built together for the different projects we were pursuing.

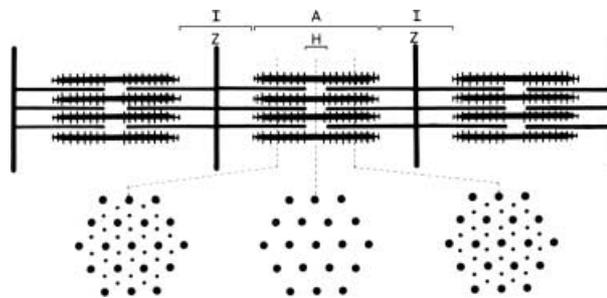
Jean, at the King's lab, had been using the newly developed phase contrast light microscope to look at isolated myofibrils, which gave superb images in that instrument, and she also had come to MIT to learn electron microscopy, arriving in January 1953. When she came, we decided to join forces and work together on muscle, using light and electron microscopy. We soon found that the application of myosin-extracting solutions to isolated myofibrils removed the extra density which gave the A-bands of muscle their characteristic appearance, leaving behind a ghost fibril, of segments bisected by the original Z-lines (Fig. 6). At the same time, the thicker filaments seen in the electron microscope were removed. So we realized that myosin, making up the thick filaments, was present only in the A-bands, and was responsible for the higher density there. The myosin filaments formed a partially overlapping array with the secondary array of actin filaments, which were attached to the Z-lines (Fig. 7). Force was developed in some way within the region of overlap. So it was clear that the constant axial periodicity I had seen by X-ray diffraction during stretch could be accounted for by some type of sliding filament mechanism, and that the contraction might occur by a similar sliding process, mediated by the crossbridges which I could see in the EM cross-sections [5].

Confirmation that this was indeed what happened came by the following year, when Jean and I had measured the changes in the band-pattern during ATP-induced contraction of isolated myofibrils, as seen in the phase contrast light microscope [7]. Both the actin and myosin filaments remained essentially constant in length, and the sarcomere



**Fig. 6.** Phase-contrast interference light microscope images of rabbit psoas myofibril before and after myosin extraction, plus density scans, showing removal of A-band density, leaving residual I-segments (actin-containing filaments). (For clarity, this is a later picture, not from 1953 paper [4].

length changes were accounted for by changes in overlap of the two arrays. The sliding force had to be developed in some way by the interaction of the myosin crossbridges with actin (Fig. 8). A. F. Huxley and Niedergerke reached a similar conclusion using observations on intact single fibres observed by interference microscopy [8], and the two papers were published together in *Nature* in May 1954. I had met A. F. Huxley briefly in Woods Hole, Massachusetts the previous summer, and had told him of our structural model and current work; and he had told me of the similar line on



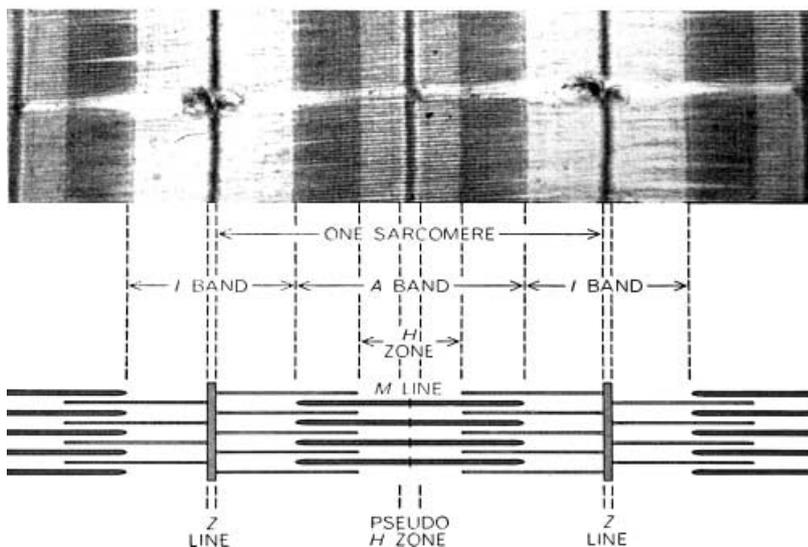
**Fig. 8.** Diagram of overlapping filament arrays and crossbridges believed to generate the relative sliding force between the filaments. Also shown are cross-sectional views at different regions of the muscle sarcomere.

band-pattern changes that he and Niedergerke were pursuing. So we agreed to co-ordinate publication, assuming we reached similar conclusions. Fortunately, we did, and these papers gave the basic description of the sliding filament model, which has remained essentially unchanged since then.

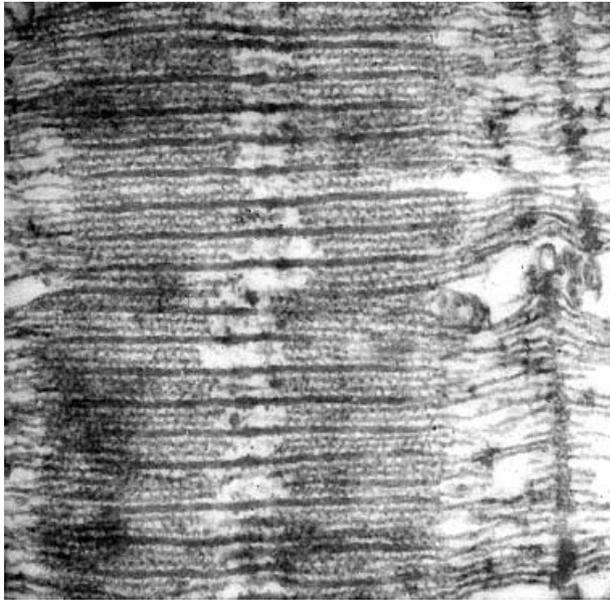
### London (1955–1962)

Two or three years later, I was able to get thin enough longitudinal sections to show the two types of filament, their overlap, and the crossbridges (Figs 9 and 10) very clearly with EM [9], but even this was insufficient to convince many people, who remained skeptical about the whole sliding filament theory. This was partly because the idea that the muscle filaments themselves must become shorter had become so ingrained, and because conclusions based on the relatively new techniques of EM and X-ray diffraction were still viewed with suspicion.

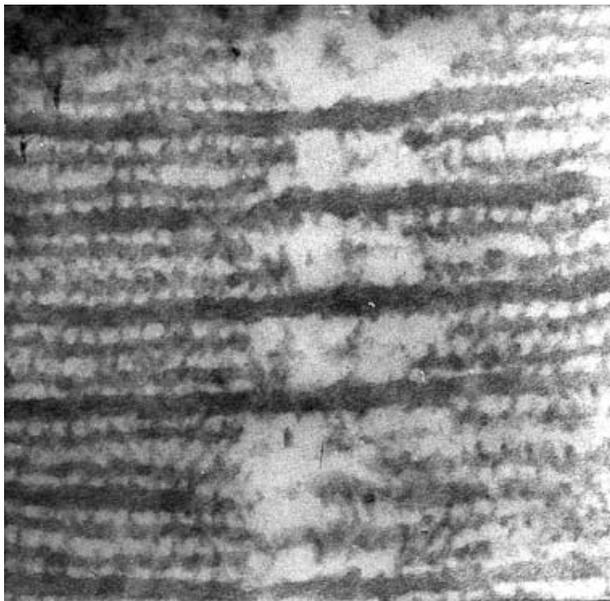
Subsequent EM work which I performed in Bernard Katz's Biophysics Department, at University College London, and later back in Cambridge at the new MRC Laboratory for Molecular Biology (LMB) on Hills Road (from 1962) used the negative staining technique, which I



**Fig. 7.** Longitudinal section of frog sartorius muscle, and diagram showing corresponding overlapping arrays of thicker (myosin) and thinner (actin) filaments.



**Fig. 9.** Very thin longitudinal sections (rabbit psoas muscle) showing single layer of filaments lattice, and hence individual thick and thin filaments and crossbridges between them (1957 micrograph).



**Fig. 10.** Higher magnification view of very thin longitudinal section on either side of H-zone. Axial compression during sectioning distorts relative dimensions, but crossbridges axial spacing is  $\approx 40$  nm and the thick filament diameter is  $\approx 12$  nm (1957 micrograph).

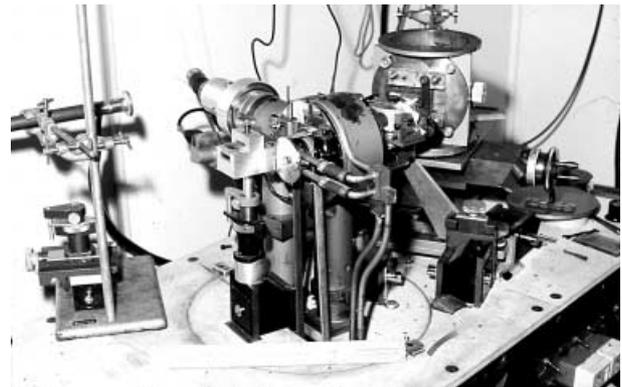
first described in work on Tobacco Mosaic Virus in 1956 [10]. I studied the structure of 'natural' filaments of actin and myosin, prepared directly from muscle by a simple technique, and of 'synthetic' filaments, prepared from purified proteins. The experiments showed that the actin and myosin molecules were arranged in their filaments with the appropriated structural polarity for the elements of force

developed by their individual molecular interactions to all add up in the appropriate directions within each sarcomere [11]. They also showed that myosin molecules could self-assemble into filaments with the requisite reversal of polarity at their midpoints.

### **The new MRC Laboratory in Hills Road, Cambridge (1962–1987)**

The next big hurdle was to get better X-ray data, and to begin the attempt to get data from contracting muscle in order to learn more about how the crossbridges produced the sliding force. This required more intense X-ray sources, and more efficient X-ray cameras, and the MRC LMB provided an ideal environment to develop and apply these techniques. By this time, rotating anode X-ray tubes, designed by Tony Broad, were already in standard use at the lab, where their increased intensity had been essential for the then relatively huge amounts of data collection necessary for solving the myoglobin and hemoglobin structures. Ken Holmes and I joined forces to put together a system suitable for the low-angle patterns from frog and insect flight muscle. Ken and Bill Longley had grafted a Beaudoin fine focus cathode (which Rosalind Franklin had introduced to Birkbeck, where Ken and Bill had been graduate students) onto the LMB-designed rotating anode (Fig. 11). Ken and I developed a focusing mirror large/aperture, focusing monochromator camera arrangement, which was enormously more efficient than the normal pinhole or slit collimator, and is now universally used in almost all synchrotron X-ray work. Later, Ken and I developed and had built at the MRC, the 'Big Wheel' type of large rotating anode X-ray generator (Fig. 12), which Gerd Rosenbaum helped into commercial production at Elliot Automation Ltd, UK.

So, we were finally able to get two-dimensional X-ray patterns from contracting muscle in 1964/5, and could see directly that the actin and myosin axial periodicities hardly changed in muscles which were contracting with substantial shortening [12], confirming that the filaments all remained constant in length. However, the myosin layer lines, coming from the helical arrangement in resting muscle of the myosin



**Fig. 11.** Holmes-Longley-Broad rotating anode X-ray tube, circa 1964, with bending mirror component only of a mirror-monochromator camera on left hand side, and monochromator-only camera on right hand side with cylindrical film holder to preserve focusing in high angle work.

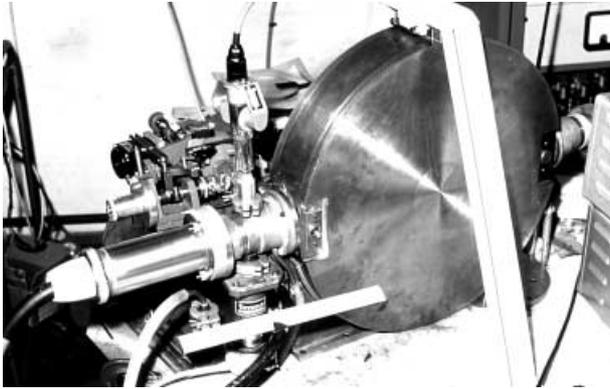


Fig. 12. Prototype 'Big Wheel' rotating anode tube in MRC (circa 1968).

crossbridges around the thick filaments, almost completely disappeared (Fig. 13), but a moderately strong meridional reflection remained at about 145 Å, about a 1.5% increase in spacing from the resting value. So the crossbridges had to have undergone substantial azimuthal (and perhaps radial) movement while interacting with actin (or at least during the transition from rest to contraction), while still maintaining enough of an axial periodicity to give the relatively strong meridional reflection [13]. Many other details of the layer-line patterns were now visible (Figs 14 and 15), and of the equatorial reflections too [14].

This all led to the 'Swinging Crossbridge Model' (it was, after all, the 1960s) in which the structural change responsible for developing force and movement was a change of tilt (or an 'equivalent change of shape') of myosin heads attached to actin, during the ATP hydrolysis cycle [15]. The heads were connected to the myosin filament backbone by a link (S2) which provided axial rigidity but allowed radial and azimuthal flexibility (Fig. 16).

These X-ray patterns were studied very extensively [16–18], and time-resolved data were obtained on the equatorial

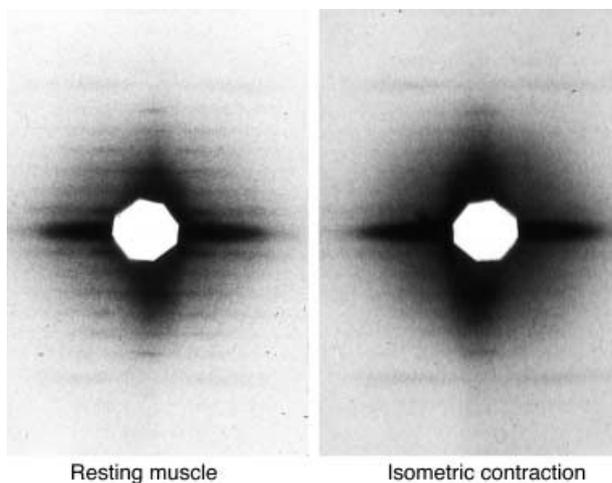


Fig. 13. Resting vs. contracting axial X-ray pattern from frog sartorius muscle, 15 min total exposure, mirror-monochromator camera, showing loss of myosin layer lines, and slightly strengthened actin 59 reflection.

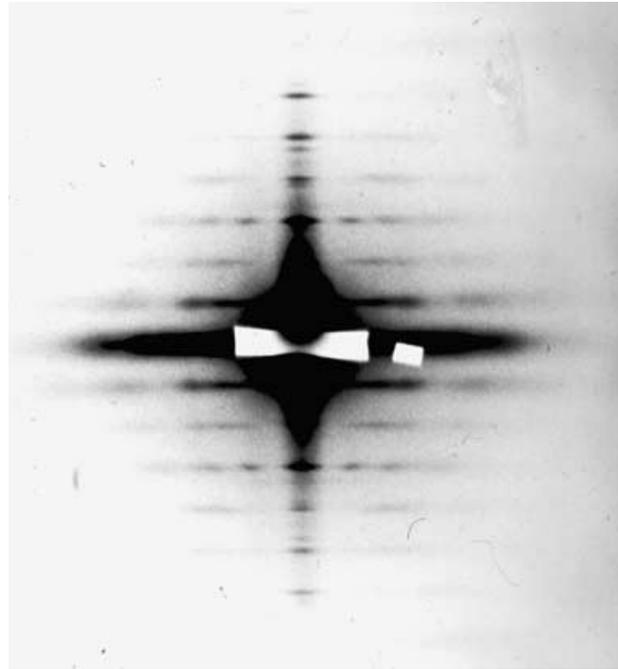


Fig. 14. High resolution X-ray diagram of myosin layer-lines in resting muscle, 430 repeat, strong meridional third order. Mirror-monochromator camera, Holmes–Longley–Broad fine focus rotating anode tube, 90 cms film distance, 20 hours exposure.

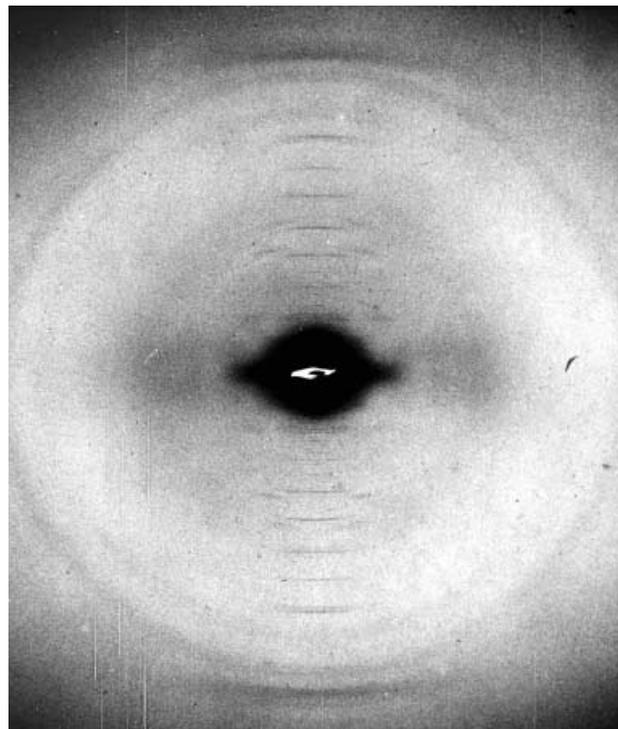
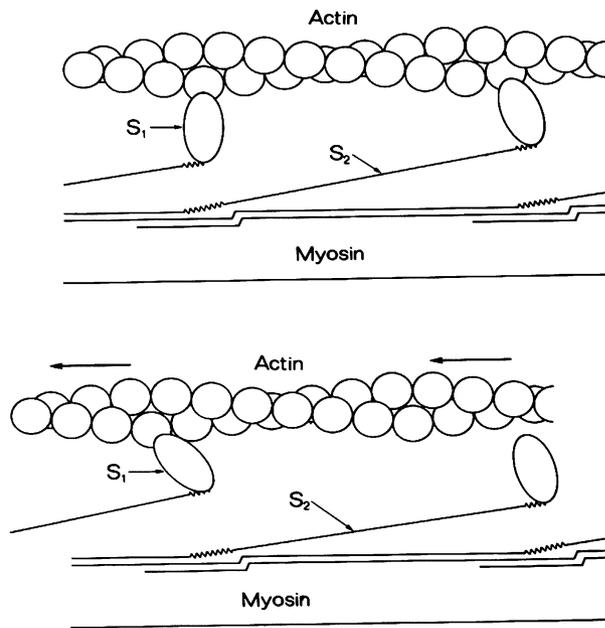


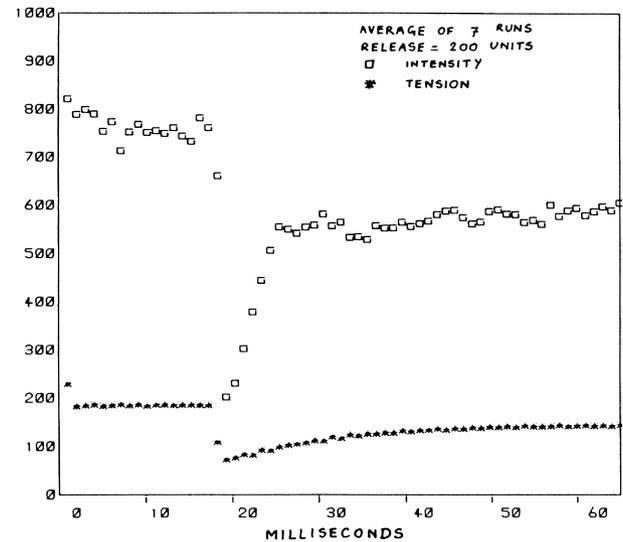
Fig. 15. Wider angle X-ray diagram showing higher angle actin reflections from resting muscle. Broader, stronger reflections at top and bottom of picture are the 5.1 Å helical reflections.



**Fig. 16. The swinging, tilting crossbridge-sliding filament mechanism (1969).** Force was developed when myosin S1 heads attached to actin either tilted (or underwent 'a change of shape'), and the resultant axial movement was transmitted to the myosin filament via the S2 portion of the myosin molecule.

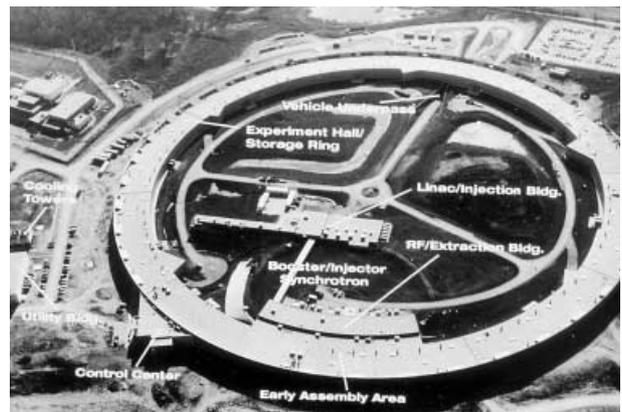
reflections during the onset and decay of contraction in single twitches of frog muscle. Nevertheless, we still needed direct experimental evidence that crossbridge movement was actually what happened during the force-producing actomyosin interaction. The problem was (and still is) that billions of individual crossbridge events happen asynchronously in a contracting muscle, so that all one normally sees is an X-ray pattern averaged over the whole crossbridge cycle, even in the shortest exposures. However, A. F. Huxley and Simmons showed that one can partially and temporarily synchronize these events, for a millisecond or so, by applying a small, very rapid, length change to a single muscle fiber [19].

So we now needed an even further large increase in X-ray intensity in order to be able to record a pattern within such a very small time interval – the first patterns in 1950 had taken hours or even days of total exposure time; and even with the mirror-monochromator-rotating anode tube set up, 10 or 15 min total exposure was needed for patterns with a minimum amount of detail. Fortunately, Ken Holmes, who was already thinking about unconventional X-ray sources while at the MRC lab, was able to show in 1971, with Gerd Rosenbaum and John Witz [20], that electron synchrotrons, specifically the one called DESY in Hamburg, could be used as a powerful X-ray source for diffraction experiments. However, many frustrating years of development took place before this potential began to be fully realized. Our work was performed both in Hamburg, at the EMBL outstation that was built there especially for this purpose, and at the NINA synchrotron at Daresbury, with John Haselgrove and Wasi Faruqi, using a camera which Uli Arndt helped to design [21].



**Fig. 17. Abrupt intensity decrease of myosin meridional reflection at 14.5 nm (M3) (□) approximately synchronous with tension decrease (\*) in A. F. Huxley-Simmons type quick release.** Time channels 1 msec (circa 1981, DORIS storage ring, EMBL Hamburg).

In 1981, greatly helped by the advent of electron (or positron) storage rings that provide a much larger, and relatively continuous, X-ray output instead of the short and temperamental duty-cycle of synchrotrons, and with electronic instrumentation largely developed in the MRC lab [22–24], we were finally able to achieve the required millisecond time resolution [25,26]. We were able to show that there was a large decrease in the intensity of the 145 Å meridional reflection during very rapid (< 1 ms) quick-releases in which relative sliding of actin and myosin filaments in each half sarcomere would be 10 nm or less, as in the A. F. Huxley-Simmons experiments [19] (Fig. 17). This was exactly what was expected to be the signature of a tilting cross-bridge mechanism, where the axial profiles of all the actin-attached cross-bridges become more spread out by the temporarily synchronous tilting towards the end



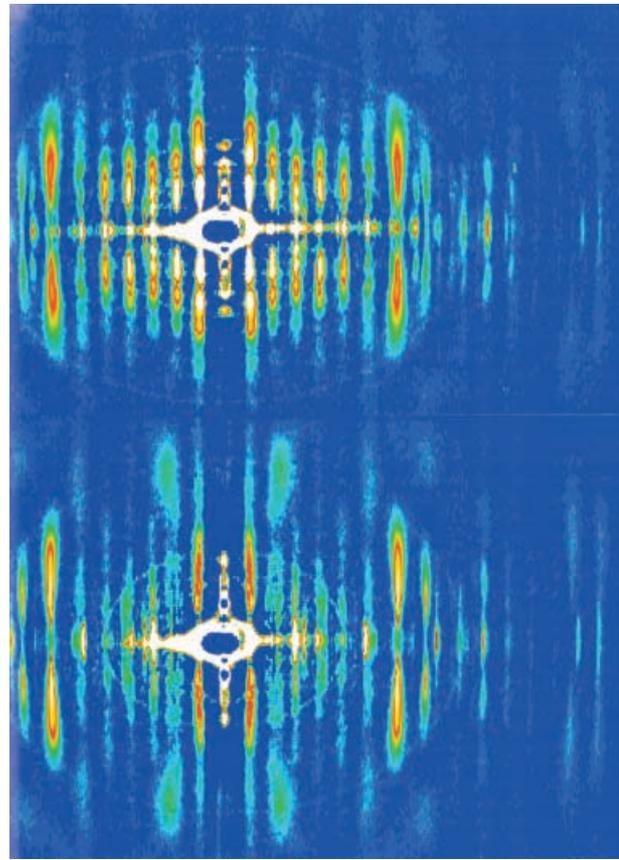
**Fig. 18. Advanced Photon Source (APS), Argonne Laboratory, USA.** Scale of the machine can be judged from automobiles and tractor-trailers on left-hand side of photographs.

of their working strokes. But while this evidence was strongly consistent with such a mechanism, it still did not provide conclusive proof, as some type of disordering process could conceivably have caused the intensity decrease. However, two new advances, in other areas of muscle work, then provided strong, independent lines of support for the sliding-filament, tilting-crossbridge mechanism.

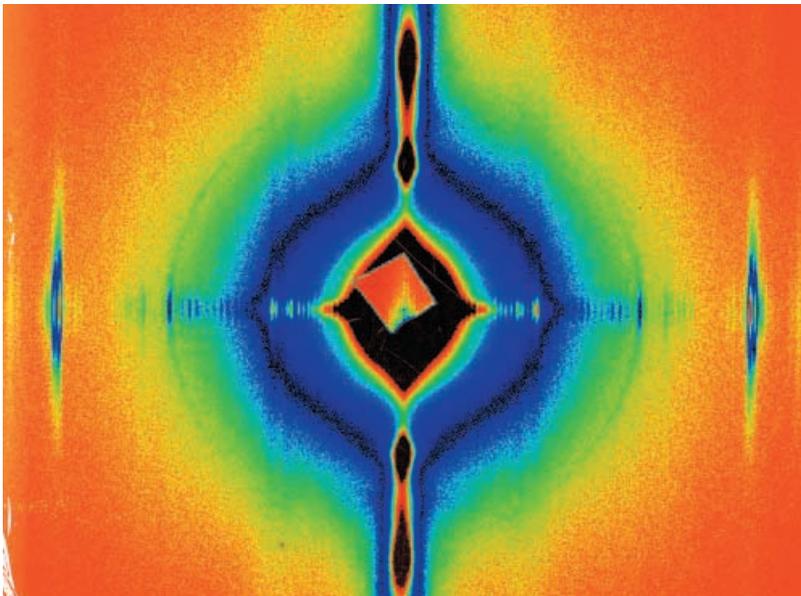
### Important new types of evidence (1983–2000)

The first was the introduction of *in vitro* molecular motility experiments, by Spudich and colleagues [27–30], and by Yanagida and colleagues [31–35]. In many of these experiments, fluorescently labeled single filaments of actin could be seen in the light-microscope, sliding unidirectionally in the presence of ATP, over substrates coated with myosin molecules, at velocities consistent with the maximum shortening velocity of the muscles from which the myosin was derived. This fully vindicated the original sliding filament hypothesis. The sliding was observed even when only myosin subfragment-1, i.e. the isolated head-piece of the molecule, was used, showing that the source of this movement is in the crossbridge itself, as visualized in the 1969 model [15], and not, for example, in the S2 region, or the myosin filament backbone. Later, even more remarkable experiments by Finer, Simmons and Spudich [36] showed that discrete steps of movement and force development could be measured (using optical traps) during the interaction of an actin filament with a single myosin molecular, and gave values in the expected range.

The second major advance came with the solution of the high-resolution X-ray crystallographic structure of the myosin S1 head by Rayment and his colleagues [37,38]. The most remarkable feature of this structure was the presence of a 8.5 nm long single  $\alpha$ -helical region extending out at the C-terminal end of the molecule, with the myosin light chains twisted around it, and presumably giving it strength and stability. This immediately suggested that this ‘neck’ region might function as a lever-arm, to amplify



**Fig. 19.** X-ray diagrams from frog sartorius muscle (fiber axis horizontal) recorded with CCD detector at the BioCAT beam line at the APS, in Argonne. Upper frame, resting; lower frame, isometric contraction. Background scattering has been subtracted electronically, and intensity displayed on false color scale. Note first meridional actin reflection (2.75 nm) and fifteenth myosin meridional reflection (2.86 nm) (resting value) on right-hand side of diagrams; also, strong actin second layer line reflections in contracting patterns, from tropomyosin/troponin movement. Recorded from 2 msec time frames, total exposure 100 msec.



**Fig. 20.** Very high-resolution axial diagram, isometric contraction. Myosin M3 reflection (14.5 nm) is the strong reflection at either side of the picture, and is split into subpeaks by the interference fringes (spacing approximately 900 nm). Camera, 5.7 m, BioCAT beamline.

atomic-scale movements around the enzyme site in the more globular part of the head structure into the 5–10 nm movements expected from the crossbridges. Later experiments have provided strong experimental support for this idea, particularly those of Cohen, Szent-Gyorgyi and their colleagues [39], in which scallop myosin heads, in different nucleotide states, were shown to have their lever arms oriented at the widely different angles expected in the tilting model.

Despite these successes, it still remained to be demonstrated explicitly that such movement actually takes place in a contracting muscle, and can be responsible for the observed physiological behaviour, particularly during transient length changes.

**Recent work (2000 onwards)**

I think that reasonably decisive evidence has now been obtained, some of it quite recently, with the present generation of purpose-designed, electron-storage ring X-ray sources such as the ESRF in Grenoble and the APS at the Argonne National Laboratory, near Chicago (Fig. 18). These give excellent two-dimensional X-ray patterns from muscle (Fig. 19) when used with CCD detectors. (I have been at the Rosenstiel Center, Brandeis University, Waltham, Massachusetts since retiring from the

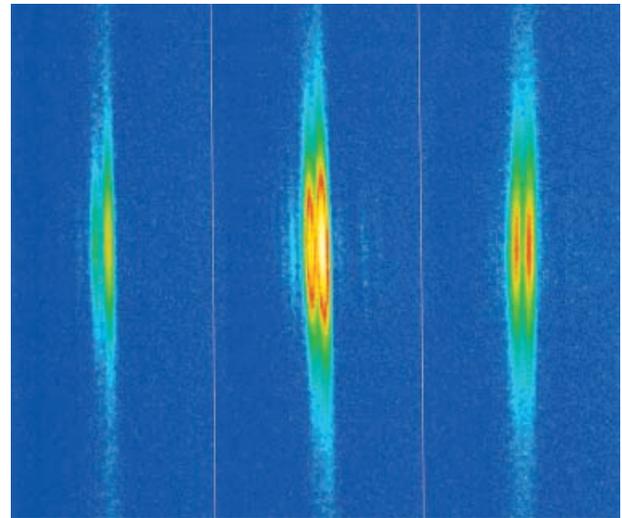
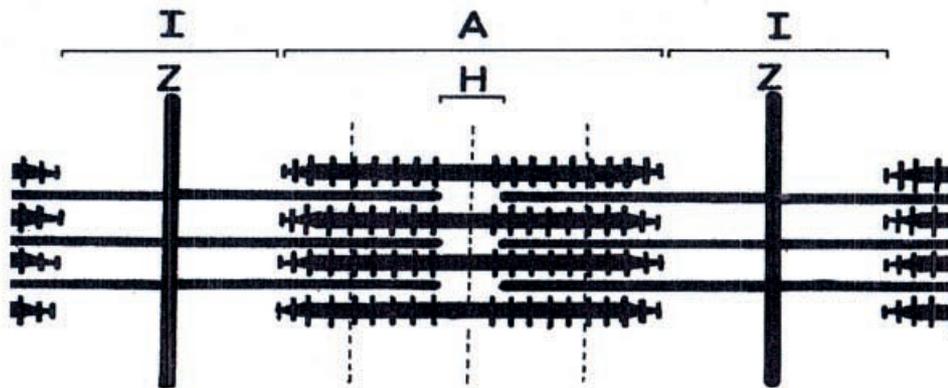


Fig. 21. Enlarged view of M3 reflection; isometric contraction, in centre photograph, quick release on left, quick stretch on right. False color gives imprecise impression of relative intensities.

MRC in 1987, and have used the BioCAT beamline at the APS ring quite extensively.) They also have very small electron-beam cross-sections, and so give X-ray beams that



Myosin filaments all have same precisely determined structure, with 2-fold rotation axis at H-line in centre of A-band.

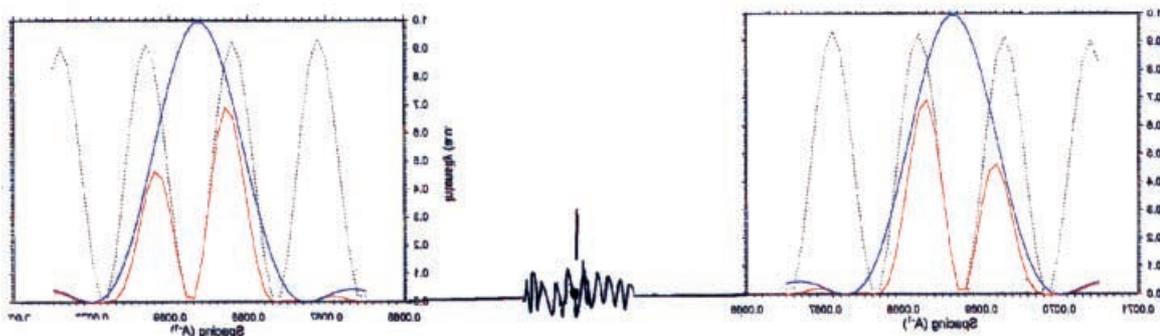


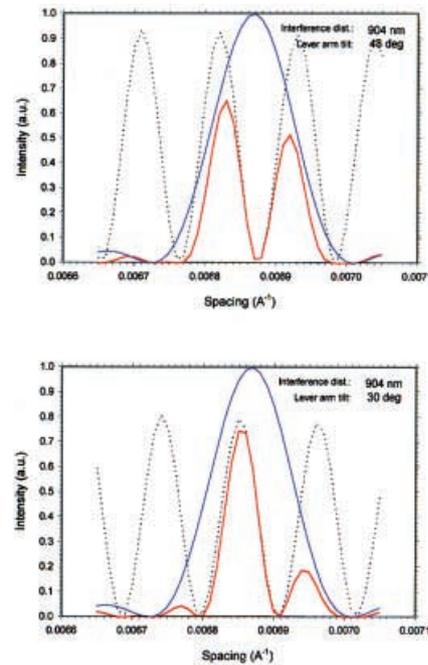
Fig. 22. Diagrammatic illustration of how bipartite structure of thick filaments brings about X-ray interference between diffraction from crossbridges, in either half of filaments. Lower diagram shows envelope (blue) of M3 reflection which would be given by either half of filament on its own, with sampled peaks (red) generated by interference fringes (black, dotted) which sample the envelope when the two halves diffract together.

can be focused to extremely small spots or narrow lines, which can be less than 100  $\mu\text{m}$  wide with a camera length of 6 m. At a wavelength of about 1  $\text{\AA}$ , this gives an order-to-order resolution of about 60 000  $\text{\AA}$ , and at the same time very high total intensity – more than  $10^{13}$  photons per second in the X-ray beam. This is more than 10 million times stronger than our sources 50 years ago, a factor of improvement hardly imaginable in the early experiments.

But why should the very high spatial resolution be such an advantage? The reason is that the myosin meridional reflections, especially the one at 14.5 nm (the basic axial period of the crossbridges) contains internal fine structure, which can give direct information about axial movements of the myosin heads on a nanometer scale, but which can only be seen at very high resolution (Figs 20 and 21). We noticed this fine structure in resting muscle many years ago [13,16] using a rotating anode X-ray generator and a 2.5 m long camera to give the necessary resolution, but as exposure times were then 20–30 h, we were unable to study it in contracting muscle, and did not think about it long enough to realize its potential usefulness. Bordas and Lowy were the first to see the splitting of the 14.5 nm reflection into two distinct peaks in contracting muscle, using the Daresbury synchrotron [40], but misinterpreted the pattern as arising from two distinct sets of crossbridges with slightly different spacings.

In fact, the fine structure arises from interference between the diffractions from the two halves of each of the thick filaments, which have a very precise construction, so that the axial periodicities of the crossbridges in the two halves have the same phase relationship to each other, in all thick filaments (Fig. 22). The centers of scattering mass of the two crossbridge arrays are a constant distance apart (approximately 900 nm) for a given average crossbridge configuration, so that the profile of the 14.5 nm reflection is sampled by interference fringes with this periodicity (because of the spatial displacement produced by sampling a sloping curve, the apparent spacing of the sampled pattern is in excess of 1000 nm, which can be misleading).

However, it was Lombardi and his colleagues [40] who first realized that the relative intensities of the sampled peaks would provide an extraordinarily sensitive indicator of any concerted axial movement of the myosin heads, and hence of changes in crossbridge configuration during a quick release. In a contracting muscle, the M3 reflection arises in large part from the population of tension-generating crossbridges attached to actin (this can be seen from the large decrease in intensity, down to 20% of the isometric value, produced by a quick release, as seen in the original experiments [25,26]). If the tilting crossbridge mechanism is correct, then in a rapid quick release, there will be a synchronous movement of the myosin heads towards the center of the A-bands as they all tilt over, and as the sets of actin filaments slide towards each other. This will alter the phase relationship between the two interfering patterns, and cause a shift in the fringe position. Essentially, the interference distance decreases by twice the axial movement of the center of mass of the attached myosin heads (somewhat less than the actin filament movement since the end of the myosin lever arm remains fixed in axial position on the thick filaments). This will be less than 1% of the total interference

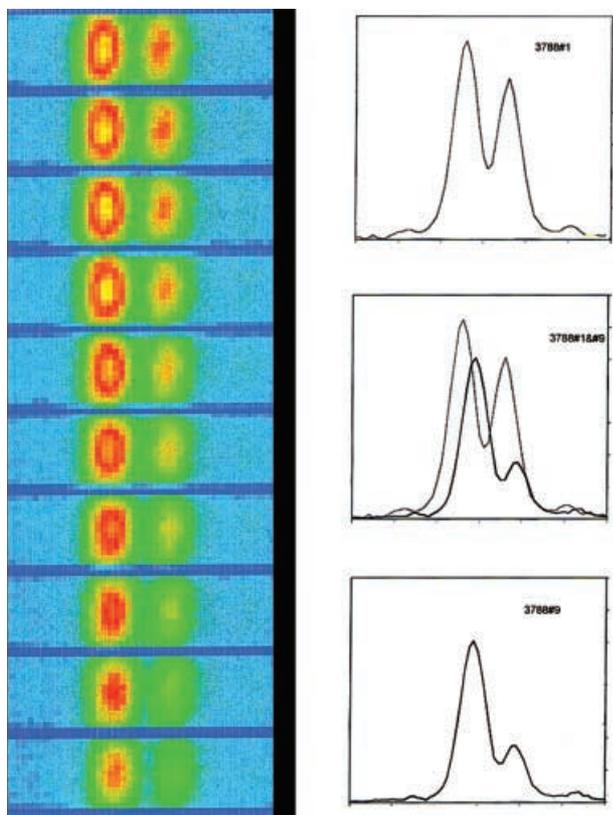


**Fig. 23. Profiles of M3 reflection, computed from high-resolution X-ray structure.** Blue trace, envelope of reflection given by a single 736 nm long array of myosin heads with a 14.56 nm repeat. Red trace, sampled peak produced by two such arrays, with appropriate symmetry, centers separated by 904 nm. Upper picture, lever arm  $48^\circ$  away from Rayleigh rigor position (catalytic subunit and lever arm approximately aligned). Lower picture, lever arm  $30^\circ$  away from rigor position, corresponding to an axial shift of 2.97 nm. Large change in intensity ratio is predicted.

distance, when the filaments move past each other by, say, 5 nm, but as the 14.5 nm reflection intercepts the fringe system at the 62nd order (approximately), small changes in the fringe spacing produce very substantial shifts in the fringe positions at the reflection. These in turn produce large changes in the relative intensities of the sampled peaks (Fig. 23). In fact, movements of a few angstroms can readily be detected and measured, providing an extremely powerful tool for studying and quantitating crossbridge behavior during the working stroke.

Lombardi and his colleagues have explored these effects in very elegant experiments on single muscle fibers [40–43], and we have pursued similar experiments on whole muscle [25,26,45–48]. It is perhaps surprising that the extents of crossbridge tilting are so similar, in the large number of different filaments (and fibres) illuminated by the X-ray beam in the whole muscle experiments, so that the fringe pattern can still be observed after a quick release. But indeed it is the case and the profiles of the fringes appear just as sharp as those seen with single fibres.

One can see the ratio of the intensities of the two peaks change by increasing amounts as one applies larger and larger quick releases to the muscle. The outer peak (i.e. at the slightly wider angle) becomes progressively weaker (Fig. 24), from an initial value of about 0.8 of the intensity of the inner one, to a saturating value of 0.25–0.35 at larger releases. This shows that the fringe pattern



**Fig. 24. Changes in M3 X-ray reflection during quick releases.** (Left) M3 reflections, electronically foreshortened (in vertical direction on display) recorded after each of a series of quick releases of increasing magnitude. (Right) Profiles of 1st and 9th frames, illustrating change in relative intensities of the two peaks in the sampled reflection. The spacing shift occurs as a result of: (a) a shift of the fringes due to crossbridge movement and (b) a shift in both the fringes and the underlying reflection due to compliance, these two effects being additive.

is moving outwards across the profile of the 14.5 nm reflection, and that therefore the centre of scattering mass of the crossbridges in each half sarcomere is moving inwards towards the M-line, thus, decreasing the interference distance between the two halves. At the same time, the total intensity in the reflection changes in the way already seen in the earlier experiments. This provides, finally, direct evidence for the type of crossbridge behaviour required by the sliding filament, tilting crossbridge model, i.e. axial movement of myosin heads attached to actin, with the predicted effects on total intensity of lever arm tilting. The saturation of the intensity ratio change shows that a fixed component is also present, probably due to diffraction from the unattached crossbridges and structures in the myosin filament backbone. It is also very noticeable that the overall intensity of the reflection at first increases slightly, at the smaller releases, and then decreases, at larger extents of shortening (up to about 10–12 nm of relative filament sliding). The initial increase is explained readily (as pointed out by M. Irving and his colleagues [49]) if, in the isometric muscle, the lever-arm is

**Table 1. People who have collaborated with the author in work on muscle, both in the MRC Laboratory of Molecular Biology, and elsewhere.**

Scientific	Technical	In other Laboratories
Uli Arndt	Tony Broad	Dick Birks
Wyn Brown	Mike Bitton	Joan Bordas
David DeRosier	Chris Bond	Jean Hanson
Wasi Faruqi	Barry Channing	Alan Hodge
John Finch	Mike Fordham	Tom Irving
John Haselgrove	Michael Fuller	Michel Koch
Sarah Hitchcock	Dave Hart	Bernard Katz
Ken Holmes	Keith Hopkins	Sally Page
John Kendrew	Chris Raeburn	Massimo Reconditi
Jake Kendrick-Jones	Tony Woollard	Bob Simmons
Marcus Kress	Alex Wynn	Dave Spiro
Peter Moore		Hernando Sosa
Vivian Nachmias		Alex Stewart
Raul Padron		
Tom Pollard		
Murray Stewart		
Andrew Szent-Gyorgyi		
Taki Wakabayashi		
Alan Weeds		

tilted out beyond the angle at which maximum axial alignment of the catalytic domain of the head and the lever arm occurs. As the lever arm tilts in an inward direction (i.e. towards the center of the sarcomere) during shortening, the alignment passes through a maximum, and then progressively decreases more and more with further shortening. The axial profile of the myosin head becomes wider and wider, and the M3 reflections shows the characteristic large intensity decrease.

I do not have space to go into the detailed features of these studies here, but I really do believe that, altogether, there is now incontrovertible evidence for the correctness of the tilting lever-arm model, although of course many important details still remain to be worked out.

In retrospect, it is remarkable what a lot of information was hidden in those original faint reflections, waiting to be recorded and understood. How fortunate it was that unexpectedly large improvements in technology, essential to extract that information, were indeed feasible; and how fortunate I was to have had the privilege of working in a laboratory so excellently planned that it was able to contribute to those developments, and to enable me to perform the experiments with the help of so many great colleagues and visitors who have been here (Table 1).

## References

- Huxley, H.E. (1951) Low-angle X-ray diffraction studies on muscle. *Discuss. Faraday Soc.* **11**, 148.
- Huxley, H.E. (1952) Investigations in biological structures by X-ray methods. The structure of muscle. PhD Thesis, University of Cambridge, Cambridge, UK.
- Huxley, H.E. (1953) X-ray diffraction and the problem of muscle. *Proc. Roy. Soc. B.* **141**, 59.

4. Hanson, J. & Huxley, H.E. (1953) The structural basis of the cross-striation in muscle. *Nature* **172**, 530–532.
5. Huxley, H.E. (1953) Electron-microscope studies of the organization of the filaments in striated muscle. *Biochem. Biophys. Acta* **12**, 387–394.
6. Hodge, A.J., Huxley, H.E. & Spiro, D. (1954) A simple new microtome for ultrathin sectioning. *J. Histochem. Cytochem.* **2**, 55–58.
7. Huxley, H.E. & Hanson, J. (1954) Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature* **173**, 973–976.
8. Huxley, A.F. & Niedergerke, R. (1954) Structural changes in muscle during contraction. Interference microscopy of living muscle fibres. *Nature* **173**, 971–973.
9. Huxley, H.E. (1957) The double array of filaments in cross-striated muscle. *J. Biophys. Biochem. Cytol.* **3**, 631–648.
10. Huxley, H.E. (1956) Some observations on the structure of tobacco mosaic virus. In *1st European Regional Conference Elect. Micro. Stockholm*, pp. 260. Wickse, Stockholm, Sweden.
11. Huxley, H.E. (1963) Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. *J. Mol. Biol.* **7**, 281–308.
12. Huxley, H.E., Brown, W. & Holmes, K.C. (1965) Constancy of axial spacings in frog sartorius muscle during contraction. *Nature* **206**, 1358.
13. 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.
14. 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.* **38**, 507–520.
15. Huxley, H.E. (1969) The mechanism of muscle contraction. *Science* **164**, 1356–1366.
16. Haselgrove, J.C. (1970) X-ray Diffraction Studies on Muscle. PhD Thesis, University of Cambridge, Cambridge, UK.
17. Haselgrove, J.C. & Huxley, H.E. (1973) X-ray evidence for radial cross-bridge movement and for the sliding filament model in actively contracting skeletal muscle. *J. Mol. Biol.* **77**, 549–568.
18. Haselgrove, J.C. (1975) X-ray evidence for conformational changes in the myosin filaments of vertebrate striated muscle. *J. Mol. Biol.* **92**, 113–143.
19. Huxley, A.F. & Simmons, R.M. (1971) Proposed mechanism of force generation in striated muscle. *Nature* **233**, 533–538.
20. Rosenbaum, G., Holmes, K.C. & Witz, J. (1971) Synchrotron radiation as a source for X-ray diffraction. *Nature* **230**, 434–437.
21. Haselgrove, J.C., Faruqi, A.R., Huxley, H.E. & Arndt, U.W. (1977) The design and use of a camera for low angle X-ray diffraction experiments with synchrotron radiation. *J. Phys.* **10**, 1035–1044.
22. Faruqi, A.R. & Huxley, H.E. (1978) Time resolved studies on contracting muscle using low-angle X-ray diffraction. *J. Appl. Cryst.* **11**, 449–454.
23. Faruqi, A.R. & Huxley, H.E. (1978) Time-resolved studies on contracting muscle using low angle X-ray diffraction I. Design of data collection system. *Nucl. Instr. Methods* **156**, 19–25.
24. Faruqi, A.R. & Huxley, H.E. (1978) New techniques in the study of time-resolved X-ray diffraction from muscle. In *Proceedings of the 4th Tamaguchi International Symposium on Biophysics*, pp. 367–401. Tsukuba University Press, Japan.
25. 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 during rapid mechanical transients, recorded using synchrotron radiation. *Proc. Natl. Acad. Sci. USA* **78**, 2297–2301.
26. 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.
27. Sheetz, M.P. & Spudich, J.A. (1983) Movement of myosin-coated fluorescent beads on actin cables *in vitro*. *Nature* **303**, 31–35.
28. Spudich, J.A., Kron, S.J. & Sheetz, M.P. (1985) Movement of myosin-coated beads on oriented filaments reconstituted from purified actin. *Nature* **315**, 584–586.
29. Kron, S.J. & Spudich, J.A. (1986) Fluorescent actin filaments move on myosin fixed to a glass surface. *Proc. Natl. Acad. Sci. USA* **83**, 6272–6276.
30. Toyashima, Y.Y., Kron, S.J., McNally, E.M., Niebling, K.R., Toyashima, C. & Spudich, J.A. (1987) Myosin subfragment-1 is sufficient to move actin filaments *in vitro*. *Nature* **328**, 536–539.
31. Yanagida, T., Nakase, M., Nishiyama, K. & Oosawa, F. (1984) Direct observation of motion of single F-actin filaments in the presence of myosin. *Nature* **307**, 58–60.
32. Yanagida, T., Arata, T. & Oosawa, F. (1985) Sliding distance of actin filament induced by a myosin crossbridge during one ATP hydrolysis cycle. *Nature* **316**, 366–369.
33. Harada, Y., Noguchi, A., Kishino, A. & Yanagida, T. (1987) Sliding movement of single actin filaments on one-headed myosin filaments. *Nature* **326**, 605–608.
34. Kishino, A. & Yanagida, T. (1988) Force measurements by micromanipulation of a single actin filament by glass needles. *Nature* **334**, 74–76.
35. Kitamura, K., Tokunaga, M., Iwane, A.H. & Yanagida, T. (1999) A single myosin head moves along an actin filament with regular steps of 5.3 nanometres. *Nature* **397**, 129–134.
36. Finer, J.T., Simmons, R.M. & Spudich, J.A. (1994) Single myosin molecule mechanics: piconewton forces and nanometre steps. *Nature* **368**, 113–119.
37. Rayment, I., Rypniewski, W., Schmidt-Base, K., Smith, R., Tomchick, D., Benning, M., Winkelmann, D., Wesenberg, G. & Holden, H. (1993) Three-dimensional structure of myosin subfragment-1: a molecular motor. *Science* **162**, 50–58.
38. Rayment, I., Holden, M.H., Whittaker, M., Yohn, C.B., Lorenz, M., Holmes, K.C. & Milligan, R.A. (1993) Structure of the actin-myosin complex and its implications for muscle contraction. *Science* **162**, 58–65.
39. Houdusse, A., Szent-Gyorgyi, A.G. & Cohen, C. (2000) Three conformational states of scallop myosin S1. *Proc. Natl. Acad. Sci. USA* **97**, 11238–11243.
40. Bordas, J., Lowy, J., Svensson, A., Harries, J.A., Diakun, G.P., Gandy, J., Miles, C., Mant, G.R. & Towns-Andrews, E. (1995) X-ray evidence that in contracting live frog muscles there exist two distinct populations of myosin heads. *Biophys. J.* **68**, 99s–105s.
41. Linari, M., Piazzesi, G., Dobbie, I., Koubassova, N., Reconditi, M., Narayanan, T., Diat, O., Irving, M. & Lombardi, V. (2000) Interference fine structure and sarcomere length dependence of the axial X-ray pattern from active single muscle fibers. *Proc. Natl. Acad. Sci. USA* **97**, 7226–7231.
42. Lombardi, V., Piazzesi, G., Linari, M., Vannicelli-Casoni, M.E., Lucii, L., Boesecke, P., Narayanan, T. & Irving, M. (2000) X-ray interference studies of the working stroke in single muscle fibres. *Biophys. J.* **78**, 134A.
43. Piazzesi, G., Reconditi, M., Linari, M., Lucii, L., Sun, Y.-B., Koubassova, N., Boesecke, P., Narayanan, T., Irving, M. & Lombardi, V. (2001) X-ray interference study of myosin head motions during rapid length changes of single muscle fibres. *Biophys. J.* **80**, 509a.
44. Piazzesi, G., Reconditi, M., Linari, M., Lucii, L., Sun, Y., Nagayanan, T., Boesecke, P., Lombardi, V. & Irving, M. (2002) Mechanism of force generation by myosin heads in skeletal muscle. *Nature* **415**, 659–662.

45. Huxley, H.E., Reconditi, M., Stewart, A. & Irving, T. (2000) Interference changes on the 14.5 nm reflection during rapid length changes. *Biophys. J.* **78**, 134A.
46. Huxley, H.E., Reconditi, M., Stewart, A., Irving, T. & Fischetti, R. (2001) Use of X-ray interferometry to study crossbridge behavior during rapid mechanical transients. *Biophys. J.* **80**, 266A.
47. Huxley, H.E., Reconditi, M., Stewart, A. & Irving, T. (2002) Crossbridge and backbone contributions to interference effects on meridional X-ray reflections. *Biophys. J.* **82**, 5A.
48. Huxley, H.E., Reconditi, M., Stewart, A. & Irving, T. (2003) What the higher order myosin meridional reflections tell us. *Biophys. J.* **84**, 139A.
49. Irving, M., Allen, T.S.C., Sabido-David, C., Craik, J.S., Brandmeier, B., Kendrick-Jones, J., Corrie, J.E.T., Trentham, D.R. & Goldman, Y.E. (1995) Tilting of the light chain region of myosin during step length changes and active force generation in skeletal muscle. *Nature* **375**, 688–691.