BIOGRAPHICAL MEMOIRS

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Elected FRS 1960

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Hugh Esmor Huxley devoted his life to understanding how muscles contract. He was born in Birkenhead and entered Christ’s College, Cambridge, in 1941 to study Physics. Joining the RAF in 1943 as an Acting Pilot Officer, he later moved to the Malvern Telecommunications Research Establishment where his pioneering work on developing H2S Mk IVA airborne radar over two years to 1947 led to his being elected a Member of the Order of the British Empire in 1948 while still an undergraduate. He started X-ray research on living muscle with Sir John Kendrew at the Medical Research Council Unit in the Cavendish Laboratory and showed that skeletal muscle is made of a hexagonal array of thick and thin filaments. In 1952 he moved to the Massachusetts Institute of Technology (MIT) to study muscle ultrastructure by electron microscopy, where he was joined by Jean Hanson, and in 1954 they published the sliding filament hypothesis (7)†. Back in London he produced ultra-thin sections of muscle barely 150Å thick, which showed cross-bridges between the filaments, and in 1960 was elected a Fellow of the Royal Society. His research at the MRC Laboratory of Molecular Biology from 1962 led to his proposal of the swinging cross-bridge model. His ambition of studying cross-bridge movement in living muscle by X-ray diffraction in the millisecond time range required ever stronger X-ray sources and more sensitive detectors. The development in the 1970s of beam lines from synchrotron radiation opened a new perspective that fascinated him for the rest of his working life. From his last work at Argonne National Laboratory with Massimo Reconditi, Hugh finally convinced himself that he had incontrovertible evidence for the tilting lever-arm model.

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Early life

Hugh was born in Birkenhead, Cheshire, in 1924, where his grandparents had moved from North Wales in the nineteenth century. His father had been in the Royal Engineers during the First World War, then worked as an accountant for the Post Office and later became Head of the Accounts Branch in Liverpool. Hugh’s mother was a schoolteacher, but had stopped working when Hugh’s elder sister, Helen, was born. Hugh described his parents as ‘people of remarkable intellect, great readers, lovers of music with great moral strength and power of judgement’ (35). The family moved to Prenton when Hugh was four and he attended the same elementary school as his father. At 11 he won a scholarship to Park High School, which had very good teachers because this was during the Depression when work was hard to find. His chemistry teacher, Mr Meeve, had an Oxford degree: one word of his advice he never forgot: ‘always look very closely at what is happening in an experiment because you may see something that no one else has ever seen before’ (35). Although Hugh’s interests in science began with chemistry, by the time he was about 12 he became more interested in physics and building structures with Meccano was very important for developing his early skills. He also built short-wave radio receivers and received signals from amateur stations as far away as the Pacific. He became Head Boy and was good at cross-country running, doubtless helped by the 100-mile cycle-rides taken on Sundays through the North Wales mountains. This gave him a great interest in hiking and, later, in skiing.

Atomic physics and quantum theory were exciting subjects and Cambridge was the obvious choice for university; this is where the atom had been split and where Rutherford was Head of the Cavendish. In addition, his sister Helen had won a State Scholarship to Newnham College to read English. Walking round the Backs in Cambridge seemed like absolute heaven after living in an economically depressed area like Birkenhead.

Hugh entered Christ’s College in 1941 with a State Bursary to study ‘Physics with radio’, a special programme to prepare physicists to work on radar during their war service. He chose Christ’s because his sister was being supervised by Dr A. L. Peck, an English don there. Hugh always regarded Helen as being extremely clever and himself as rather average. His acceptance at Christ’s convinced him that he might not be ‘so stupid after all’! He had also been offered a scholarship to study engineering at Liverpool University, but with Liverpool docks a favourite target for the Luftwaffe, this was not an ideal venue. Hugh remembered cycling through the tunnel under the Mersey and seeing the middle of Liverpool in flames with overhead tram wires drooping down all over the road. He described it as ‘an apocalyptic sight, very impressive and very, very memorable!’ (35). Figure 1 shows photographs of Hugh in the early 1940s.

During the war years, courses in Cambridge were shortened for those about to do military service. At the end of his first year, Hugh gained a First in the preliminary examination to Part I of the Natural Sciences Tripos, and was judged to have passed Part I completely. This allowed him to concentrate solely on his Physics in his second year and he could have taken Part II of the Tripos and completed his degree after two years, but instead chose the preliminary examination to Part II Physics, which would allow him to return after the war to

† Numbers in this form refer to the bibliography at the end of the text.
War service

Hugh entered war service in August 1943. Having always been very keen on aeroplanes, he enlisted as Acting Pilot Officer in the Emergency Commission Technical Branch, RAF Volunteer Reserve, to work on radar and was promoted successively to Pilot Officer in February 1944, Flying Officer in August 1944 and Acting Flight Lieutenant in February 1947.

Because of the importance Churchill attached to high altitude (typically over 15 000 feet) night bombing of Germany, the highest priority had been given to the development of airborne ground-scanning height-to-surface radar, H2S. In July 1943, H2S was successfully used by the Pathfinder squadrons in the destruction of Hamburg (project Gomorrah). Hamburg proved an ideal target because the aircraft came in over the sea and the contrast of the coastline and rivers on the radar gave exceptionally accurate positioning; however, later bombing, particularly of Berlin, proved much less successful: considerable improvements in navigation systems were required.

Hugh’s early training took place at RAF Cosford, a maintenance and technical training unit where he had essentially two jobs: part of his work was to repair broken radar sets, while the other involved rapid installation and testing of specialized equipment, notably advanced radar for De Havilland Mosquitoes of Pathfinder squadrons. When Mosquitoes entered service in 1941 they were the fastest operational aircraft in the world, but by 1943, with little in the way of defence, losses had become so high in the bombing of Berlin that their use had to be
Additional radar equipment was urgently needed to detect attacking German night fighters. Codenamed Fishpond, this became operational in October 1943 and Hugh described how, over a 72-hour period, he installed around 50 sets of this radar. By the spring of 1944 almost every H2S aircraft of both Pathfinder and main bomber force carried Fishpond and the results in stemming aircraft losses were dramatic.

At the very end of the war in Europe, in April 1945, Hugh moved to the Telecommunications Research Establishment (TRE) in Malvern where he was assigned to the Bombing Development Unit as the full-time technical officer with a sergeant and aircraftsman as support. He spent much of the rest of his service based in Malvern and with the Radar Research Flying Unit at Defford, but liaising with various bomber stations, particularly the Bomber Development Unit at Feltwell in East Anglia, where he flew with test crews on practice bombing missions in the Midlands and elsewhere. He was ultimately the chief liaison officer from TRE and had a specially equipped Lincoln bomber (successor to the Lancaster) under his control. Hugh described this as ‘a great job’ (35).

H2S Mk IVA, on which Hugh was working, was the first purpose-designed airborne radar with a stabilized view of the ground and an analogue computer that linked the radar automatically to the bomb release mechanism. Because poor wind correction was one of the biggest causes of bombing error, the display also incorporated a wind-correction capability to take account of drift due to wind. Hugh discovered large errors in the Plan Position Indicator—the cathode ray display—which arose from instability of the current in the magnetic deflection coils. His measurements revealed significant warming of the coils during use: this changed their resistance and hence the current. Although fans were installed to reduce the heating, Hugh found that by rewiring the DC amplifier feeding the coils for current negative-feedback instead of voltage negative-feedback, as used in the original design, this instability could be greatly reduced. In his notebook he wrote, ‘The incorporation of these or similar modification is considered absolutely imperative for the successful operation of H2S Mark IV A equipment’, and in a video interview with the authors, in 2004 (35), he described this as his first ‘Eureka moment’.

Subsequent flight tests in September 1945 showed considerable reduction in errors. Hugh was able to write in his notebook: ‘since the last Post Design Services report was written, the shift and scale drifts which were giving rise to considerable anxiety have been overcome.’ Experiments conducted at Central Bomber Establishment showed that residual drift was less than 1% in shift sensitivity and scale. In his last flight entry, ending bombing trials at the Bomber Development Unit at Feltwell on 8 November 1945, he wrote: ‘Results fairly reasonable: 300 yards error (!)’

Hugh might have hoped for a speedy return to Cambridge, but with so many individuals returning to the University, release from active service was inevitably phased and he was not in the first group for demobilization. Indeed, letters from Cambridge must have caused him great anxiety. A letter from Lawrence Bragg (FRS), the Cavendish Professor, dated 26 May 1945, stated:

We are not prepared to consider you for research until you have taken Part II of the Natural Sciences Tripos in Physics. . . . we think we should inform you that, on such evidence as we have at present, we are doubtful if we could eventually support your application for a research grant to enable you to work at the Cavendish. We cannot of course be definite now, and much would depend on your Tripos results and reports on your war work between now and when you leave it.
Hugh’s inevitable disappointment was greatly compounded when, in February 1946, he received a letter from Christ’s informing him of the College’s decision to refuse readmission of those, like Hugh, who had qualified for the BA degree under the rules prevailing during the war, even if they had decided to defer their degrees in order to qualify for a research grant by subsequently taking Part II. The College had taken this decision because of the large numbers involved and limitations of accommodation and facilities. Fortunately, Hugh’s expertise was greatly valued by the Air Ministry. In a letter dated 27 October 1945, the Director of Personnel wrote:

We have considered the fact that Flying Officer Huxley may be leaving the Service in August 1946, but we are, at the present juncture, unable to select a suitably qualified officer who will still be in the Service after August 1946. We propose therefore to allow Flying Officer Huxley to continue his training and carry out his duties for as long a period as he is available for service.

Hugh expressed his disappointment at the unfairness of College’s decision in a letter to the Senior Tutor in September 1946 and made arrangements for private accommodation in Cambridge before reapplying for admission in 1947, because the ban had been justified on the basis of limitation of accommodation in College. Wisely, the Senior Tutor relented and Hugh was able to return to residence and complete his undergraduate course: had this not been so, history would have been very different and muscle research denied one of its most outstanding researchers.

In early 1946 Hugh was selected as the technical officer to participate in a demonstration of different types of British radar in Australia and New Zealand. The mission comprised senior officers including experts in bombing and navigation. The Lancaster/Lincoln bomber, Thor, left Manby in late March and flew to Darwin via Malta, Cairo, Karachi and Singapore, then in early May to Auckland, returning to Manby on 12 June (figure 2). Promoted to Acting Flight Lieutenant in February 1947, Hugh continued test flying H2S in Lincoln bombers at Manby during the early stages of delivery of production units until his last flight on 10 July 1947. It had all been exciting and he had met a lot of interesting and very different kinds of people whom he would not otherwise have met, but he was delighted to get back to Cambridge to complete his course.

Many of the qualities that characterized his later research were evident in this early work. He loved doing experiments with his own hands, and working with electrical and mechanical devices became a lifetime passion. His work was characterized by his critical analysis, his technical skill and great tenacity. Moreover, his correspondence with the College revealed not only his tenacity, but his enterprise and great courtesy in his dealings with the authorities.

As an important postscript to his radar work, he was awarded an MBE (Member of the British Empire) in the military section of the New Year’s Honours list of 1948. The Master of Christ’s and University Vice-Chancellor, Charles Raven, sent a letter of congratulation, stating ‘you have brought honour to the College’.

RETURN TO CAMBRIDGE—X-RAY DIFFRACTION ON MUSCLE

Hugh returned to Cambridge for the Michaelmas term 1947. He describes how exhilarating it was to be back in Cambridge ‘with the clouds of the 1930s gone, war against Fascism won and a great deal of exciting work going on’ (35). He knew he had to get a first-class degree
to stay on in Cambridge to do research, but did not feel that he had done particularly well in his final examinations. When some weeks later on a cycling holiday in France he received a telegram from his sister Helen, ‘congratulations—first—idiot!’, Hugh was overjoyed and described this as one of the happiest evenings of his life, cycling down from Perpignan to a little harbour town where he was staying in a youth hostel and seeing this golden future in front of him. He was actually going to do research in Cambridge after all (37)!

Hugh had always been socially conscious and felt he wanted to do something important and of value to society. However, the dropping of the atomic bombs on Hiroshima and Nagasaki radically changed his views about doing research in nuclear physics. David Schoenberg (FRS), his final year Physics supervisor, introduced him to Max Perutz (FRS) and John Kendrew (FRS). Perutz and Kendrew were members of a small group financed by the Medical Research Council (MRC) to do X-ray crystal structure determination of proteins. Hugh became Kendrew’s first research student. (A few years later, the unit, the embryo of what was later to become the successful and famous Laboratory of Molecular Biology (LMB), grew with the arrival of Francis Crick (FRS) (1949), James Watson (ForMemRS 1951) and Vernon Ingram (FRS)). Kendrew first entrusted Hugh with calculating the Patterson function of diffraction data from sheep haemoglobin crystals but Hugh felt that this tedious job was unbefitting the human condition and persuaded Kendrew to let him do something else.

Kendrew was interested in applying the X-ray diffraction method to sub-cellular organelles and agreed that Hugh might look at muscle. Hugh had come across Dick Bear’s X-ray diffraction patterns from air-dried muscle and was amazed to discover no one knew how muscles contracted.

Figure 2. Photograph of Thor Lincoln bomber and crew (HEH is fourth from right) at RAF Manby on 4 June 1946. (Photograph courtesy of the RAF.)
Antoni van Leeuwenhoek’s (FRS) pioneering microscopy in the seventeenth century showed that vertebrate skeletal muscle fibres are built from repeating units now known as sarcomeres, each a few microns long. Light-microscopic observations in the nineteenth-century German literature showed that sarcomeres are delineated by Z-membranes (English usage Z-line) and contain striations of higher and lower refractive index—now termed A (anisotropic) and I (isotropic) bands from their appearance in a polarizing microscope. The I-bands flank the Z-lines and the A-bands lie in the middle of the sarcomeres. When muscles contract, the sarcomeres shorten and the X-ray band patterns alter, but these observations did not explain how muscle works.

The main constituent of muscle is the protein myosin, discovered in 1864 by Willie Kühne by extracting an ‘Eiweiß’ from minced meat with concentrated salt solutions. Nearly 80 years later, Brunó Straub and Albert Szent-Györgyi showed that Kühne’s myosin contained a second protein that they called actin. Actomyosin (actin+myosin) has ATPase activity and fibres drawn from actomyosin gels shortened when ATP was added (Szent-Györgyi 1942). Early electron microscopy of fixed myofibrils (Hall et al. 1946) revealed filamentous structures of variable diameter, which packed side by side and extended continuously over many sarcomeres. Alternating A-bands, I-bands and Z-lines were visible. Along with Albert Szent-Györgyi, these authors concluded that during a contraction the filaments themselves must shorten (see Needham 1971 (FRS)).

Bear’s X-ray diffraction pictures from dried muscle were not very informative, but diffraction from wet muscle was very weak. Nevertheless, the tradition of the Perutz group was to keep things wet. Thus, Hugh set about building an X-ray camera capable of resolving spacings of 300–400 Å to take diffraction photographs of wet ‘living’ frog sartorius muscle. As an X-ray source, he used a copy of the micro-focus X-ray generator that had been developed by W. Ehrenberg and A. Speer at Birkbeck College London. Using this, together with a miniaturized low-angle X-ray camera (5 μm beam-defining slit, 3 cm specimen-to-film distance), he obtained the first diffraction patterns from live relaxed muscle, with exposure times of a few hours for equatorial patterns and a couple of days for axial ones (figure 3). His system was 10–20 times faster than conventional systems. The X-ray films revealed sharp reflections from a highly-ordered structure in living muscle, an exciting finding (1).

**MUSCLE CONTAINS A HEXAGONAL LATTICE OF MYOSIN AND ACTIN FILAMENTS**

On the equator, there were reflections whose relative spacings and intensities suggested that they came from a hexagonal array of filaments about 450 Å apart and 100–150 Å in diameter. An X-ray diagram from a muscle in rigor showed approximately the same lattice spacings, but very different relative intensities. This could be accounted for by the presence of a second set of filaments located at the trigonal positions of the hexagonal lattice. Hugh guessed that the main set of filaments must be myosin and the second set actin: the two contractile proteins were in separate filaments. Moreover, there would need to be cross-connections between them in order to become rigidly bonded in rigor and to produce shortening in contraction (2, 3). These ideas turned out to be correct and laid the foundations of modern muscle research. They were strikingly original and completely at variance with the consensus view at the time.

Axial X-ray patterns showed a series of meridional reflections based on an approximately 420 Å axial repeat with a very strong third order. This remained strong in rigor, while the
other reflections became very faint. He also noted that the pattern of axial reflections remained unchanged when muscles were passively stretched by up to 40% of their length, demonstrating that stretching the muscle did not affect the underlying structure. The implications of this important observation were not immediately apparent to Hugh, who, at that time, accepting conventional wisdom, thought that both sets of filaments must be continuous through the whole muscle sarcomere. He ascribed the axial reflections to orders of the repeat of the actin filament and thought that this filament must develop breaks during stretching, whereas the myosin, which was thought to do the contracting and stretching, apparently gave no signal. It later became clear that the strong meridional reflections observed were in fact the first six orders of a 429 Å helical repeat of myosin filaments. In his thesis, Hugh also reported that the near-meridional 59.0 Å reflection, which comes from actin, is also invariant under stretch. Thus, he actually had evidence in his thesis that both sets of filament spacing are invariant under stretch. Had he appreciated this he might have been able to propose the sliding filament hypothesis a year or two earlier. Hugh described this as ‘a very vexing error!’ (41).

Dorothy Hodgkin (FRS) was Hugh’s external examiner for his PhD degree in June 1952. At the viva she suggested that if, on going into rigor, there were more overlap between interdigitating filaments, one could conveniently explain Hugh’s changes in the strength of
the equatorial reflections, an idea she had developed during the train journey between Oxford and Cambridge. Thus, Dorothy Hodgkin became the first person to propose a sliding filament mechanism for muscle contraction. However, her idea was based on a misunderstanding: she assumed that the muscle would contract on going into rigor. Hugh pointed out rather forcefully that Dorothy had not read his experimental procedures: he had been careful to maintain the muscle at constant length while going into rigor. A decade later Elliott et al. (1963) showed that changes in sarcomere length in a relaxed muscle do indeed produce intensity changes in the way Dorothy had envisaged. Subsequent X-ray experiments with improved cameras confirmed that the intensity changes Hugh had seen on going into rigor were caused by outward movement of myosin cross-bridges towards the trigonal points of the lattice as they attach to actin filaments.

**Massachusetts Institute of Technology—Sliding filaments**

Believing that he could only progress by using electron microscopy, in September 1952 Hugh moved to F. O. Schmitt’s laboratory at the MIT in Cambridge, Massachusetts, supported by a Commonwealth Fellowship. Schmitt’s laboratory was one of the earliest to use electron microscopy (EM) on biological specimens. Hugh first worked on developing a microtome capable of making very thin sections (6), then examined glycerinated* frog sartorius and rabbit psoas muscle myofibrils in thin sections after embedding in poly methyl methacrylate. Transverse sections clearly showed the hexagonal arrangement of the filaments: thick filaments in the A-bands and thin filaments in both A- and I-bands (4) (figures 4 and 5). In this paper, Hugh also points out that the extensibility of muscle fibres in the presence of ATP is most easily explained by a process in which the two sets of filaments slide past one another.

Jean Hanson (FRS) from King’s College London arrived at MIT in January 1953, also bent on learning EM. Jean was experienced in phase contrast microscopy and F. O. Schmitt’s lab had a phase contrast microscope, in those days still a rarity. Jean and Hugh began a very fruitful collaboration using both light and electron microscopy (41). One of their first experiments was to treat glycerinated rabbit muscle myofibrils with pyrophosphate, which had been used by Szent-Györgyi to relax muscle, and observe these preparations in the phase contrast microscope. They immediately saw that dilute pyrophosphate extracted the A-bands, showing that the A-bands were made of myosin. Hugh had earlier assumed, as had others at the time, that myosin was attached to the Z-lines with actin lying alongside. Further experiments with solutions that specifically extracted myosin (Hasselbach & Schneider 1951) showed the same result. Myosin was confined to the A-bands. The residual structure was composed largely of actin. Subsequent use of procedures for extraction of actin totally destroyed the organized structure of the myofibrils. Hugh wrote (38):

> [...] and so suddenly the veil dropped from our eyes. It was a real Eureka moment as we realized there wasn’t any ‘extra’ A-substance, that the A-substance and myosin were the same thing and

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* Albert Szent-Györgyi discovered that immersing muscle fibres in buffered glycerol solutions renders the cell membrane permeable while leaving the muscle proteins intact. Glycerinated rabbit psoas muscle myofibrils became a widely-used model system.
that the thick filaments must only be present in the A-band, partially overlapping the thin filaments which ran from the Z-lines to the H-zones.

When asked in the video interview in 2004 (35) what the most exciting moment of his scientific career had been, Hugh responded: ‘Oh, I think extracting myosin from the A-band. Yes, that clinched it!’

Hugh and Jean were now buzzing with excitement about how muscle could contract through the sliding of sets of interdigitating filaments. The resulting Nature paper (5) did not push sliding filaments, however, because Schmitt advised them not to spoil a good paper with a lot of speculations as to how the system might work. Although Hasselbach (1950) had also discovered that the A-bands contained myosin, the idea that myosin was confined to the A-band found a lot of resistance. Albert Szent-Györgyi, in Woods Hole, was not amused. He knew how muscles contract. Myosin is a negatively charged polymer: when you add calcium ions it undergoes a phase change and shortens. However, if myosin does not extend all the way between the Z-lines this cannot work! Albert Szent-Györgyi even published a paper purporting to demonstrate that myosin was not confined to the A-bands (Szent-Györgyi et al. 1953). Ironically, the first author on this paper was Albert’s younger cousin, Andrew Szent-Györgyi, who was to become a life-long friend and scientific colleague of Hugh’s.

Hugh often went down to the Marine Biological Laboratory in Woods Hole during summer weekends, and stayed with Andrew and Eva Szent-Györgyi. It was known that myosin was a dimer, but characterization was difficult because myosin is soluble only in 0.6 M KCl. Andrew Szent-Györgyi made an important advance in myosin biochemistry by preparing ‘heavy meromyosin’ (HMM) and ‘light meromyosin’ (LMM)—proteolytic fragments of myosin. The N-terminal HMM, which was soluble in low salt concentrations, contained two large globular domains and retained both ATPase and actin binding activities. The C-terminal fragment,
Figure 5. (a) Longitudinal section of frog sartorius muscle and diagram showing the overlapping A- and I-bands with adjacent sarcomeres separated by the Z-lines (37). The thin (actin) filaments are attached to the Z-lines and their polarity reverses at the Z-line. The thick filaments, made of myosin, constitute the A-bands. The areas of non-overlap between thick and thin filaments constitute the I-bands. In the centre of the H-zone is a distinct region without cross-bridges (the pseudo H-zone). During contraction, the length of the A-bands does not alter, but the I-bands shorten, suggesting that the filaments slide. (b) Cross sections through the A-bands showing hexagonal lattices of thick filaments with thin filaments at trigonal points in the overlap zone (see figure 4). Myosin molecules assemble spontaneously to form bipolar thick filaments with the ‘cross-bridges’ sticking out as shown in the diagram. (Image from (37) used with permission from John Wiley & Sons.)

LMM, which was insoluble at low ionic strength, was an extended two-stranded α-helical coiled coil that forms the part of the molecule responsible for the aggregation to form thick filaments. Thus, the myosin molecule was composed of a pair of cross-bridges attached to a two-stranded α-helical tail (Szent-Györgyi 1953). Hugh found a more receptive audience for the idea of sliding filaments in Andrew Huxley (later Sir Andrew Huxley PRS) to whom he reported his observations in Woods Hole in the summer of 1953. He told Andrew about both the microscope observations and also how they supported the X-ray work of his PhD thesis.
Andrew reported to Hugh his observations with Rolf Niedergerke on the constancy of A-band length in intact frog sartorius muscle fibres, observed using an interference microscope he had built; this led to an agreement to publish their evidence for a sliding filament mechanism simultaneously. Over the winter of 1953 Hugh and Jean succeeded in filming a contraction series using very low concentrations of ATP to slow the contractile process. The results confirmed that the A-bands remained constant in length during shortening and they further found that very clean myosin-extracted preparations showed clear I-segments with constant length thin filaments. From energetic considerations, they speculated that the actin filament needed to be pulled a distance of about 100 Å in each step of the contraction process with the splitting of a single ATP (35).

The two *Nature* papers were published together on 22 May 1954 ((7) and Huxley & Niedergerke 1954). Hugh and Jean were both surprised and disappointed that their paper was not published as the first of the pair, both because it was submitted to *Nature* before the Huxley and Niedergerke paper (a point made by Andrew Huxley when submitting his paper to *Nature* (Archives of Sir Andrew Huxley, Trinity College Library, Cambridge)) and because it contained a great deal more information. Perhaps Huxley and Niedergerke’s observations might be regarded as being closer to the native state of muscle because they worked on single muscle fibres rather than isolated myofibrils (41). In his Sherrington lecture XIV, published in 1980, Andrew Huxley acknowledged the extent to which Hugh’s and Jean’s contributions in their early work provided pivotal information in support of sliding filaments (Huxley 1980). Both groups had demonstrated constancy of A-band width during stretch and contraction and also that the darker bands seen at the centre of the A-band in high levels of contraction implied overlap of thin filaments, but Andrew specifically credits Hugh with the demonstration of a double array of filaments by low-angle X-ray diffraction in his thesis, the location of myosin in the A-band in (5) and evidence for constant distance between the Z-line and the edge of the H-zone, which itself provided evidence for constancy of length of thin filament (7).

Even with all this evidence, there was by no means universal acceptance of the sliding filament model: the concept of shortening filaments had become ingrained. Moreover, chemical fixation had given rise to artefacts and EM had acquired a bad reputation. This led to considerable scepticism. In particular, Sjöstrand & Andersson (1956) reported that only a single set of continuous filaments is present in striated muscle. In Hugh’s view, their published longitudinal sections were artefacts that could be explained because they contained more than one layer of filaments.

**University College — the swinging cross-bridge**

Hugh and Jean returned to England in 1954. Hugh returned to the MRC Unit in Cambridge and to a research fellowship at Christ’s College. However, he did not settle down in Cambridge, where, for personal reasons, he became deeply unhappy. Moreover, there was no EM. In 1956 he sought peace of mind by resigning his fellowship and moving to London, to the Biophysics Department at University College under the direction of Bernard Katz (FRS). Hugh had the support of Max Perutz and Sir Harold Himsworth (FRS), the Head of the MRC, who used his influence with the Wellcome Trust to get a Siemens Elmiskop 1 electron microscope installed in the Biophysics Department of University College, London. He moved to a pleasant
Hugh Esmor Huxley

Figure 6. (a) The hexagonal lattice of vertebrate skeletal muscle in transverse section showing the myosin filaments on the lattice points and the actin filaments (thin) in trigonal positions. (b) The trace of longitudinal section shown in figure 7, about 150 Å thick (dashed lines) passing through the lattice points. Note that there are two actin filaments between each myosin filament. (Adapted from (9).)

flat in Hampstead and immersed himself in work. After a couple of years of depression and headaches, his mood now lightened. Skiing in Davos helped a lot.

Hugh’s primary objective was to get much better EM images to demonstrate the double array of filaments in longitudinal sections. The basic problem was that the ‘thin’ sections of muscle that electron microscopists were using at that time were 600 Å or more in thickness and longitudinal sections showed only a confused image of superimposed filament layers. Hugh realized that ultra-thin (150 Å) sections were necessary to see thin filaments without them being obscured by overlying layers. He improved fixation and staining methods but, more importantly, he greatly improved the design of the microtome that he had developed at MIT. With much patience, he obtained ultra-thin sections barely 150 Å thick (9).

Longitudinal sections of about 150 Å thickness cut in the plane shown in figure 6 showed the presence of two thin filaments between adjacent thick filaments. Cross-bridges between thick and thin filaments could clearly be seen. Success in thin sectioning depended on embedding the fixed muscle in a suitable epoxy resin. During the war, a group at Duxford Aerodrome (near Cambridge), who had been developing laminated propellers, had collected a selection of epoxy resins of various hardness. Hugh availed himself of this epoxy resin library to find a plastic medium of the correct consistency for ultra-thin sectioning. The images he published in 1957 have never been surpassed (figure 7).

It was reasonable to suppose that the large N-terminal domains of HMM constituted the cross-bridges. Moreover, in the EM pictures, these projections could be seen at various angles to the filament axis. Emboldened by this observation, Jean and Hugh proposed that the cross-bridges bind to actin in a cyclical manner, linked to ATP hydrolysis, and go through a rowing-like motion to move actin past myosin (later termed the swinging cross-bridge hypothesis) (8). The step size was estimated as 5–10 nm. In 1958, Hugh summarized this work in an article in Scientific American (10). The review was amazingly perceptive. Hugh spent the rest of his life showing that it was correct.

Hugh’s improvements in EM techniques gave him the opportunity to examine systems other than muscle. In collaboration with Bernard Katz (FRS) he produced stained images of neuromuscular junction (11). With Geoffrey Zubay, he made significant developments to the method known as ‘negative staining’: if you immerse a biological macromolecule in a solution of a heavy metal salt on a microscope grid and let it dry to form a glass, the electron micrograph shows a cast of the macromolecule in the heavy metal glass. This method found wide application and became very important in unravelling virus structure. Initially, they used phosphotungstic acid, but later turned to uranyl acetate, which proved to be a much less
destructive negative stain. They also found a trick for improving visibility: holey grids. The microscope supports used for EM work are thin carbon films produced by sputtering carbon onto a thin film of collodion or formvar adhering to the copper grid. The collodion is then dissolved away to leave the carbon film as a specimen carrier. Unfortunately, the carbon film itself adds substantially to the random speckling (referred to as ‘noise’) in the image. If you breathe on the collodion films, some of the tiny droplets in your breath react with the film to produce holes. These in turn result in holey carbon films on the grid. The negative stain happily spreads across these nano-holes to produce thin films of unsupported stain. Rather naturally, negative stain on holey grids gives the best visibility. With this improvement, Hugh had hoped to see the RNA strand in tobacco mosaic virus. He failed to achieve this, but in an end-on view he could see the central channel in the virus. Later, he published papers with Zubay on the structure of microsomal particles from *E. coli* and the protein shell of turnip yellow mosaic virus (12, 13).

**Laboratory of Molecular Biology—muscle proteins**

About this time plans were being finalized for the MRC Unit for Molecular Biology to be moved from the Cavendish Laboratories to the new MRC Laboratory of Molecular Biology (LMB) on the Addenbrooke’s Hospital site in south Cambridge. Max Perutz was appointed Director, with John Kendrew as Deputy Director. They would jointly head the Structural Studies Division. Fred Sanger (FRS) would move from the Biochemistry Department to head...
the Division of Protein Chemistry and Francis Crick with Sydney Brenner (FRS) would head the Division of Molecular Genetics. It was planned that Hugh should join the laboratory as part of the Structural Studies Division. The LMB was officially opened by the Queen in May 1962 (figure 8).

Hugh moved back to Cambridge in 1962 to the newly opened LMB and a research Fellowship at King’s College. His corner set in Bodley’s Court overlooking the Cam became a venue for a bi-weekly gathering of friends and colleagues to listen to records of classical music. Hugh had a great love for music and, with his talent for building scientific equipment, had constructed an excellent record player. He also provided drinks and delicious cakes from Fitzbillies, a well-known nearby cake shop.

At the LMB he set up a Siemens Elmiskop 1 and undertook a detailed EM study on the structure of natural and synthetic filaments from striated muscle as revealed by negative staining (14). Native filaments were prepared by homogenization of glycerinated myofibrils from rabbit psoas muscle in a relaxing medium containing ATP and EDTA. These were characteristically 1.5–1.6 µm in length with tapered ends and, with the exception of a bare central zone of about 0.2 µm, they were covered with small projections (the cross-bridges). Filaments could also be prepared from purified myosin by lowering the ionic strength from 0.6 to 0.15 M. These synthetic filaments were also characterized by a bare central zone. Projections occurred at either end. These images provided evidence for bipolar self-assembly of myosin, giving structures very similar to native thick filaments.

Hugh examined myosin, heavy meromyosin (HMM) and light meromyosin (LMM) in the EM by shadow casting. These were revealed as rod-shaped molecules. Both myosin (mean length 1520 Å) and HMM (up to 800 Å in length, but more heterogeneous) had two globular heads at one end, while LMM gave rod-like molecules of 610 Å average length.
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solutions of LMM in 0.6 M KCl were reduced to an ionic strength of 0.15 M, paracrystals were formed with an axial periodicity of 430 Å and a light band of about 100 Å.

Hanson & Lowy (1963) had shown the structure of filamentous actin (F-actin) to be a helix of 13 monomers arranged in six turns with a repeat of about 360 Å. The appearance of actin filaments is of two chains of monomers winding round each other in a steeply pitched double helix (referred to as the ‘long-pitch helix’). Jean continued to work on muscle, concentrating on muscle from invertebrates, and, with Jack Lowy, did pioneering work on actin structure. Tropomyosin was localized in the long-pitch groove of the actin double helix. When Sir John Randall (FRS) retired from King’s College in 1970, Jean Hanson and Maurice Wilkins (FRS) became joint heads of the Biophysics Department. Very sadly, Jean Hanson died very suddenly in 1973 of meningitis.

Hugh found that addition of either myosin or HMM to F-actin resulted in a characteristic arrowhead pattern with an axial period of 366 Å arising from the myosin heads binding stereospecifically to the actin filaments. He called this ‘decorated actin’. When I-segments from intact glycerinated muscle were incubated with HMM, they also formed arrowheads, with the arrowheads pointing away from the Z-line. This demonstrated the bipolar organization of the I-bands, which arises as a property of the Z-line. Bipolarity of both thick and thin filaments is an essential requirement of the sliding filament mechanism.

The projections on the thick filaments that comprise the ‘head’ (N-terminal) portion of myosin form the cross-bridges between the two sets of filaments. At the end of this paper (14) Hugh speculates that,

if an actin filament can only interact with myosin filaments which are appropriately oriented, and if the result of this is a relative force directed along the actin filament, then such a filament would tend to be maintained in motion in a constant direction when placed in a suspension of myosin filaments in the presence of ATP. Furthermore, an oriented gel of actin in which a preponderance of filaments was polarized in one direction might tend to propel itself along under similar circumstances. Possibly some such mechanism is involved in the streaming of cytoplasm.

This was later demonstrated by Sheetz & Spudich (1983), an achievement that gave Hugh great pleasure and satisfaction.

The year 1963 also marked the publication with Sally Page (15) of a very detailed analysis of filament lengths under different conditions of fixation and embedding to distinguish changes arising during preparation from actual differences in living muscles. The thoroughness with which these experiments were conducted shows the extent to which Hugh was concerned to respond to those who argued that his EM observations were a consequence of experimental artefacts. Hugh and Sally concluded that filament lengths were identical in both resting and contracting muscle and at all sarcomere lengths greater than 2.1 µm. The thick and thin filaments in frog, toad and rabbit muscles all had lengths of 1.6 µm and 2.05 µm, respectively, and the fine periodicity visible along the thin filaments was found to be 406 Å.

In the late 1940s, A. V. Hill (FRS) had shown that the whole cross-sectional area of a frog sartorius muscle fibre becomes fully activated at 0°C in 40 ms, a time too short for any activating substance to reach the innermost regions of a fibre. In 1964 Hugh demonstrated that ferritin can enter the transverse tubular system (16) which forms the central elements of the triads identified by Porter & Palade (1957). No ferritin was observed in the longitudinal elements of the sarcoplasmic reticulum, but these results demonstrated that the tubules of the transverse system open directly into extracellular space. The existence of this continuity
greatly simplifies the inward conduction of the muscle stimulus, facilitating calcium to be released very rapidly to myofibrils throughout the fibre.

**MARRIAGE TO FRANCES**

The middle 1960s marked a great change in Hugh’s personal life. During his lonely bachelor life in London, he had met Dave Robertson, whom he described as ‘a very lively and charming man’. Robertson’s wife, Dody, was very keen on theatre and knew interesting theatrical people: this stimulated Hugh’s interest in theatre, something that stayed with him throughout his life. The Robertsons later moved to the Boston, Massachusetts, area and in 1964 Hugh met Frances Maxon Fripp at the Robertson’s house in Wayland, Massachusetts. They got on very well together and corresponded extensively. Frances had studied history of Art and Music at Wellesley and shared Hugh’s interest in theatre. Frances came to Cambridge briefly in the summer of 1965 and they married in Concord, Massachusetts, on 16 February 1966. Frances brought her three teenage children and an old English sheepdog into the marriage and, after a brief period living in an ancient thatched house in Meldreth (10 miles southwest of Cambridge), they moved into ‘Binstead’, a well-appointed house on Herschel Road, Cambridge, which they rented from St John’s College. Finding English schooling not to their tastes, the Fripp children slowly drifted back to Boston. Frances gave birth to Olwen in December 1970, named after Hugh’s mother, Olwen (née Roberts). The family later moved to Chaucer Road and Olwen went to school in Cambridge until the family moved to Concord when Hugh retired in 1987. This enabled Olwen to complete her schooling in Massachusetts and then go to Harvard.

Hugh’s five-year Fellowship at King’s College was not renewed in 1967, but he was immediately offered a Fellowship at Churchill College, which he described as more informal and friendly, particularly to women, visitors and American wives. Frances greatly enjoyed Churchill and Hugh was both happy and honoured to be connected with this most scientific of Colleges.

Both Frances and Hugh were enthusiastic about spending their summers in Woods Hole where, in 1972, they had built a summer home. For many years, Frances spent considerable time directing and acting with the Woods Hole Theatre Company; she also wrote three plays—Hugh’s favourite was the first, *Flowering Spurges or the Road to Compostela*, which won the Promising Playwrights award from the Colonial Players of Annapolis. Both Frances and Hugh greatly enjoyed sailing. They imported two English-made sailing boats; one, a 16-foot Wayfarer (named *Aquarius*) became Frances’s pride and joy. Hugh lavished much attention on maintaining the other, a 28-foot, twin-keeled yacht named *Sarabande*, which also allowed him to go out to the middle ground to catch blue fish as they ran past Martha’s Vineyard. After Gordon Research Conferences gatherings, Hugh would invite friends and ex postdocs to visit Woods Hole to enjoy not always uneventful sailing trips to Tarpaulin Cove or Martha’s Vineyard.

**THE SWINGING CROSS-BRIDGE MODEL**

In 1963 Mike Reedy came from Seattle with his family as a postdoctoral fellow in Hugh’s group. Reedy was interested in the structure of the Z-line. Hugh taught him all he knew
about sectioning muscle for EM. A year after he arrived, Reedy’s research programme was subjected to a radical change of direction. Ken Holmes (KCH) was collaborating with Richard Tregear, from John Pringle’s (FRS) lab in Oxford, on the structure of insect flight muscle. We discovered that the effect of ATP on the low-angle X-ray diffraction pattern from glycerinated insect flight muscle was dramatic: with ATP, the 145 Å meridional reflection was strong; in rigor (without ATP), the 145 Å meridional reflection vanished completely. To explain this result, Reedy cut ultra-thin longitudinal sections of the flight muscle embedded in epoxy resin with and without ATP for observation by EM: with ATP, the cross-bridges were at right-angles to the filament axis; without ATP, they pointed down at about 45° to the filament axis. Thus, it appeared that the myosin cross-bridges could take on two radically different conformations (Reedy et al. 1965). This result was easily integrated into the swinging cross-bridge model. Moreover, it demonstrated that the swinging of the cross-bridge might be monitored by following the strength of the 145 Å meridional reflection.

At the end of the 1960s, using a fine-focus rotating anode X-ray tube and improved X-ray optics, Hugh was able to analyse the structural changes that occur when muscles go from rest to rigor and to examine actively contracting muscles in as little as 10–20 minutes. The spacing of the 143.5 Å meridional reflection changed by only 1% in contracting muscle (to 145.0 Å), but its intensity was reduced by about one-third compared with resting muscle. This was interpreted as a longitudinal movement or tilting of the cross-bridges (18). In a following review in *Science* (19) Hugh was also concerned with explaining the ability of cross-bridges to interact with actin over a range of possible actin–myosin filament distances. The distance between actin and myosin filaments becomes significantly greater during a contraction. He made use of new information about the molecular structure of myosin resulting from the preparation of the globular subfragment-1 (S1) and α-helical subfragment-2 (S2) by proteolysis of HMM (Lowey et al. 1969). His model proposed two flexible ‘hinges’ (between S1 and S2 and between S2 and LMM, i.e. at the sites of proteolysis), so that the cross-bridges can interact with actin over a range of inter-filament spacings while maintaining the same orientation relative to actin (figure 10).

The presence of these flexible couplings means that the force-developing mechanism is likely to be located near the attachment site of the globular myosin heads to actin. Provided that the hinges and the connection S2 moiety are inextensible, any change of orientation of
The swinging, tilting cross-bridge–sliding filament mechanism (19). Force is developed when myosin S1 heads attached to actin either tilt (or undergo ‘a change of shape’), and the resultant axial movement is transmitted to the myosin filament via the S2 portion of the molecule. The flexibility of the linkages between S1 and S2 and between S2 and LMM allows the cross-bridges to reach out to attach to actin over a range of inter-filament spacings. (Figure from (37) used with permission from John Wiley & Sons.)

Figure 10. The swinging, tilting cross-bridge–sliding filament mechanism (19). Force is developed when myosin S1 heads attached to actin either tilt (or undergo ‘a change of shape’), and the resultant axial movement is transmitted to the myosin filament via the S2 portion of the molecule. The flexibility of the linkages between S1 and S2 and between S2 and LMM allows the cross-bridges to reach out to attach to actin over a range of inter-filament spacings. (Figure from (37) used with permission from John Wiley & Sons.)

the heads attached to actin will give rise to a sliding force between the filaments. This is the ‘swinging cross-bridge model’ and the rowing-like motion of the cross-bridge is referred to as the ‘working stroke’ or ‘power stroke’.

In 1970 Hugh collaborated with Vivienne Nachmias to explore the interaction of HMM with actin from Physarum. They found the same arrowhead decoration that had been observed with rabbit actin, providing evidence for conservation of the actin structure over a long evolutionary history. The authors suggested that muscle contraction may have evolved from a primitive form of cytoplasmic streaming (20). It appears that all known myosins rely on an interaction with actin similar to that occurring in muscle. They appear to use a swinging cross-bridge to ‘row’ the actin filament past the myosin filament and, by analogy with the arrowhead appearance of decorated actin filaments (figure 11), all known myosins, with the exception of Myosin VI,* move along actin in the direction of the ‘barbed’ end, as in muscle. (The ‘barbed’ end is the end closest to the Z-line, so that decorated filaments always have the arrowheads pointing away from the Z-line.)

Peter Moore, who had come to work with Hugh as a postdoctoral fellow, obtained further information about the structure of S1 decorated actin and thin filaments by three-dimensional reconstructions from negatively stained EM images (21), using the reconstruction technique

* Myosin VI uses a special add-on structure to reverse the direction of motion, but the basic mechanism is the same (Wells et al. 1999).
Figure 11. Electron micrograph of negatively stained preparation of actin filaments ‘decorated’ with myosin S1, showing well-developed ‘arrowhead’ formations (21). The simplified model of ‘decorated’ actin, based on 3-D reconstruction, shows S1 attached to the central core of uniquely arranged actin subunits in a characteristically tilted and slewed configuration and it is this that gives rise to the appearance of the arrowheads. (Images courtesy of Professor Peter Moore.)

of DeRosier & Klug (PRS) (1968). The reconstruction showed that the barbed appearance of decorated actin arises from the curvature and slewing of the subfragment-1 molecules (figure 11).

The reconstruction, which represented the ‘rigor’ state (i.e. no ATP), was therefore identified with the structure at the end of the working stroke or ‘power stroke’. Structural data on the ephemeral ‘beginning of power stroke’ have yet to be found. Comparison of structures computed for purified F-actin with isolated thin filaments showed the presence of additional mass in the latter lying between pairs of actin subunits. In line with other publications appearing at this time, the authors proposed that this additional mass was tropomyosin, following the long-pitch actin helix.

In 1970, Hugh delivered the Croonian Lecture, summarizing the results presented above (22). In William Croone’s bequest he stipulated that the lecture should be on the ‘nature and laws of muscular motion’. Hugh’s lecture was unusually apposite, since he did just that. At the end of his lecture, Hugh outlined the next steps that should be taken to discover the details of the contraction process. These included the crystallization of myosin subfragment-1 for X-ray crystallographic analysis, improved electron microscope techniques to give meaningful fine detail below the then 20 Å limit and much more intense X-ray sources so that the time resolution in X-ray experiments could match that available in mechanical and chemical measurements. This programme was indeed carried out, although not all by Hugh.
Much of it required revolutionary technology: indeed, muscle research drove the technology development, which extended over the ensuing 40 years.

It is a matter of deep regret to many working in the field that Hugh was never awarded the Nobel prize, although he was nominated for both the Chemistry and Medicine prizes on several occasions by many colleagues, including Sir Andrew Huxley, the principal author of the other paper published in 1954 (Archives of Sir Andrew Huxley, Trinity College Library, Cambridge).

It was becoming clear that, for a detailed understanding of the myosin cross-bridge as a molecular machine, it would be necessary to determine the atomic structure of the cross-bridge by X-ray crystallography. In 1967 Hugh appointed Alan Weeds to set up a muscle biochemistry group. Weeds had worked in Susan Lowey’s laboratory at the Children’s Cancer Research Foundation at Harvard Medical School in 1967–68 producing S1 ‘cross-bridges’ by controlled digestion of myosin with insoluble papain; he later used chymotrypsin to prepare homogeneous S1 (Weeds & Taylor 1975) in the hope that homogeneity would allow the S1 to crystallize. This turned out to be very difficult, probably because the myosin cross-bridge can take on multiple configurations. Crystallization was finally achieved in 1984 by Ivan Rayment and Don Winkelmann at the Rosenstiel Center in Brandeis University, Massachusetts (Rayment & Winkelmann 1984). By this time, Hugh had moved to Boston and was able to oversee these important developments as Director of the Rosenstiel Center.

**REGULATION BY TROPOMYOSIN/TROPONIN—THE STERIC BLOCKING MECHANISM**

Muscle contraction is controlled by the calcium level in the sarcoplasm: low calcium, relaxation; high calcium, contraction. In 1964, Sutsuro Ebashi (ForMemRS 1977) and Fimiko Ebashi discovered a protein in muscle that they called troponin (Tn) (Ebashi & Ebashi 1964). Troponin conferred calcium sensitivity on the ATPase activity of actomyosin and was shown to be a complex of three proteins: troponin-T (TnT), troponin-I (TnI) and troponin-C (TnC; the calcium-binding component).

In 1968 Jim Spudich came to the LMB to work with Hugh on the EM of decorated actin filaments (figure 12). He was curious about the function of tropomyosin and troponin in controlling S1 binding to the thin filament and collaborated with Weeds’ assistant, Susan Watt, to develop a method of making pure F-actin free of contamination with tropomyosin and troponin. The ensuing publication containing the actin preparation procedure became the most cited paper in the actin literature (Spudich & Watt 1971). They showed that the complex of troponin with tropomyosin (Tm.Tn) inhibits both acto-HMM and acto-S1 ATPase when free of calcium and that the complex binds to actin in a ratio of one Tm.Tn to seven actin subunits.

In 1972, Spudich, with Hugh and John Finch, compared the structures of actin paracrystals alone and paracrystals containing Tm.Tn (23). A major difference was the presence of ‘stripes’ repeating every 385 Å when Tm.Tn was present. This spacing was identical to that of the sharp meridional reflection seen in X-ray diffraction patterns of resting muscle (18), suggesting that the stripes arose from the binding of the Tn complex to every seventh actin along the long-pitch helix. Three-dimensional image reconstructions showed that each Tm molecule runs along the two-start long-pitch actin helix and was apparently associated more closely...
with one actin chain than the other. The authors compared S1 decoration of purified actin with that of actin-Tm.Tn (in the absence of calcium) and found that the pattern of decoration was significantly altered in the presence of 0.2 mM pyrophosphate (added as an analogue of ATP): binding of S1 to actin was inhibited by the presence of Tm.Tn. The reconstructions from images of actin paracrystals with Tm.Tn revealed that the position of the Tm in the calcium-free state overlapped with part of the S1 binding site as demonstrated by Peter Moore’s reconstructions of decorated actin (21). Tropomyosin in the absence of calcium apparently inhibits the actomyosin ATPase by blocking part of the actin-myosin binding site. This conclusion led to the so-called ‘steric blocking hypothesis’.

The year 1972 was marked by the Cold Spring Harbor Symposium on muscle contraction. It was an auspicious time for the meeting: the cross-bridge cycle arising from combining Hugh’s structural swinging cross-bridge model with Lymn and Taylor’s rapid reaction analysis of the actomyosin ATPase provided a model of how muscle works (Lymn & Taylor 1971). In the symposium, Hugh (24) and others put forward the ‘steric blocking hypothesis’: an on–off switching mechanism, in which azimuthal movements of tropomyosin strands in the long-pitched grooves in the actin filaments controls the access of myosin heads to their specific binding sites on actin subunits. Experimental support for this idea came from the observation of a diffraction peak rather far out on the second actin layer-line (shown in figure 13 as ‘$J_4$’) that, after stimulation, appeared rapidly in the X-ray diffraction pattern from a muscle. This had been observed on film in particularly long exposures of contracting muscle (and rigor muscle). The phenomenon was analysed by Hugh (24) and Haselgrove (1973). From its position, the peak can be identified as arising from a $J_4$ Bessel function: i.e.
Figure 13. The changes in the X-ray fibre diffraction pattern from a frog sartorius muscle on going from relaxation to isometric contraction. The muscle fibre axis is vertical. Data were recorded with a CCD detector at the BioCAT beam line at the Advanced Photon Source, Argonne National Laboratory, in 2003 (37). The data were collected in 100 ms. Note the loss of the ‘myosin layer lines’ and the appearance of the ‘$J_4$’ term on the second actin layer-line. The strong sixth and seventh order actin layer-lines (marked as ‘actin’) do not alter very much, nor do the equatorial reflections. The white bar on the 145 Å meridional reflection shows the position of the wire one-dimensional counter used for the traces shown in figure 14. (Image from (37), used with permission from John Wiley and Sons.)

its strength reflects the degree of four-foldedness of the thin filament. As shown by Spudich et al. (23), in relaxed muscle (no calcium) where there is no $J_4$, the tropomyosin lies snugly next to actin, held there by troponin, thereby blocking myosin binding. On activation (calcium binding to TnC), the troponin releases the actin and the tropomyosin swings round by 20–30° to make a four-fold symmetrical structure (two actin strands + two tropomyosin strands at about right-angles that give a strong $J_4$ signal), allowing the myosin cross-bridges to bind. In rigor muscle, the binding is so strong that the myosin cross-bridges push the tropomyosins out of the way (thereby also strengthening the four-foldedness of the thin filament). Although greeted with some scepticism, the steric blocking hypothesis has stood the test of time.

Hugh spent the summer of 1972 as a visiting professor at Brandeis University. Here, he worked with Andrew Szent-Györgyi and with Szent-Györgyi’s postdoctoral fellow, Sarah Hitchcock, who was studying the details of the interactions of the component proteins of troponin with actin and tropomyosin. They found that calcium, through binding to Tn-C, affected the binding of Tn-I to actin, thereby controlling the movement of tropomyosin (25). Subsequently, Sarah Hitchcock went to the LMB Cambridge to work with Hugh.
Hugh wished to follow the changes that occur to the X-ray diffraction pattern when a resting muscle is stimulated and allowed to contract. EM techniques lead only to a static description of the components of the sarcomere; X-ray diffraction is the only method able to report structural changes in contracting muscle. Unfortunately, conventional X-ray sources were far too weak to allow meaningful experiments. Since an isolated muscle could be kept contracting for only a few seconds at a time, it was impractical to answer even the most basic question—how does the overall average pattern change during contraction? Moreover, since the changes in muscle structure that produce contraction take place on a timescale of milliseconds or less, experiments to determine their time courses, and to detect transient changes, were far out of range.

In 1964 Ken Holmes, with Bill Longley at LMB, had produced a more intense X-ray source by combining rotating anodes and fine-focus electron guns. In addition, Holmes passed on his experience with focusing monochromators. A bent quartz crystal can provide a clean monochromatic X-ray beam focused to a line. Hugh combined this with a bent glancing-angle mirror used to focus the beam in the second dimension to produce an intense point-focused X-ray beam. The aperture of the monochromator was large, allowing a 5 mm length of muscle to be illuminated by a beam that converged to a 100–200 µm point on the X-ray film. Using this optical system with the Holmes–Longley X-ray tubes, two-dimensional X-ray diffraction patterns from a frog sartorius muscle showing the myosin and actin layer-lines could be recorded on film in about 15 min (17).

Employing this set-up, Hugh and his collaborator, Wynn Brown, examined the diffraction from living frog sartorius in rest, contraction and rigor. Diffraction data from contracting muscles held at constant length were obtained by illuminating a series of muscles to collect data from a total of 1000 1 s tetani. The experiments allowed a description of the changes in the diffraction that occurred in going from resting muscle to force-generating muscle (18). They found that the actin and myosin periodicities did not alter on activation of the muscle, in accordance with the sliding filament model.

A resting frog or rabbit muscle gives a detailed myosin diffraction pattern from the helically arranged cross-bridges based on a 430 Å axial repeat (see figure 13). The distance between cross-bridges along the helix axis is 143.3 Å, which gives rise to a strong third order meridional X-ray reflection at this spacing. Huxley and Brown showed that on activation, most of the detailed myosin diffraction pattern was lost, but the strong meridional reflection was retained.

Even with these improvements, Hugh needed much higher X-ray intensity (see (36) for a historical account). The limited tensile strengths and heat conductivities of available metals precluded any further substantial improvements in rotating anode X-ray tube design. The future lay elsewhere. In 1971 Rosenbaum et al. reported that the radiation emitted tangentially from the ring of the 6 GeV electron synchrotron at Deutsches Elektronen-Synchrotron (DESY) Hamburg, if used with a Guinier focusing monochromator, yielded an X-ray beam at least ten times more intense than the best conventional X-ray source. Planned improvements, such as the electron storage ring Doppel-Ring Speiser (DORIS), would lead to a further projected increase in intensity of a factor 100–1000. Moreover, future developments appeared to be open ended.
Hugh was chairman of a committee set up to advise on technology for the nascent European Laboratory for Molecular Biology (EMBL) to be built in Heidelberg, Germany. He pushed for the establishing of an EMBL X-ray outstation at DESY to exploit synchrotron radiation as an X-ray source. Rosenbaum, John Barrington Leigh and Holmes constructed the first synchrotron X-ray beam line, using the DESY electron synchrotron as a source. Hugh and his co-workers set up a similar X-ray beam line on the electron synchrotron NINA (National Institute for Northern Accelerators) at the Daresbury Laboratory, near Manchester (27), but the anticipated intensity was never achieved and the physiological apparatus was later transferred to the X13 X-ray beam line at the EMBL Outstation at DESY on the DORIS storage ring, which gave much greater X-ray intensity than the synchrotrons. Detailed layer-line patterns from muscle could now be recorded on film in a minute.

In the meantime, Hugh, John Haselgrove and A. R. (Wasi) Faruqi were developing one-dimensional position-sensitive wire detectors for time-resolved experiments on contracting muscle (28). Using conventional X-ray sources, the time course of the large changes in intensity of the [10] and [11] equatorial reflections between resting and isometrically contracting muscles could be measured (26). Thus, Hugh’s team was prepared for the move to the storage ring source, but their first success at DORIS was with a two-dimensional X-ray image intensifier television detector that had been brought temporarily to Hamburg by Jim Milch, a visitor from the Reynolds’ laboratory in Princeton. This detector gave a display of the myosin layer-line pattern in about 1 s. Thus, it was possible to see that the layer-lines faded out as the muscle was stimulated, and re-appeared as it was allowed to relax (26). Another ten years were to elapse before two-dimensional X-ray detectors with fast readout became generally available (see figure 13); CCD detectors and optimally designed storage rings finally allowed muscle diffraction patterns to be obtained in 100 ms (37).

Hugh pushed on using one-dimensional wire detectors. The low-angle reflections from the myosin cross-bridges could be recorded about 1000 times more rapidly than with the best conventional X-ray sources. Data were collected from various relevant parts of the muscle X-ray diagram by positioning the detector appropriately, but this proved a tedious and frustrating experience and greatly extended the time of the experiments.

Intensity changes in the myosin layer-lines were studied initially. On stimulation and tension development, there was a large decrease in the intensity of the off-meridional myosin layer-lines (see figure 13) and the time course of this decrease was closely synchronized to the onset of tension. As soon as contraction was over, the cross-bridges quickly returned to their regular helical positions around the thick filament backbone (31). These data provided evidence that myosin cross-bridges undertook axial and azimuthal movements during tension development. Indeed, the intensity decreases preceded tension development by 10–20 ms, indicating that cross-bridge movement occurs before the tension changes.

When a muscle goes into rigor, most of the cross-bridges bind to actin filaments, as in decorated actin: this binding leads to a pronounced strengthening of the actin diffraction pattern. Hugh was disappointed to find that active muscle showed no strengthening of the actin layer-lines, which appeared to show that cross-bridges were not binding to actin. This observation was used as an argument to discredit the swinging cross-bridge theory, especially since stiffness measurements appeared to show that a large percentage of the cross-bridges took part in a contraction. Hugh proposed (correctly) that the result showed that only a small fraction of the cross-bridges, perhaps about 20%, were involved at any time in developing
force and the experimental setup was not sensitive enough to see the small signal arising from the actin-bound cross-bridges.

Andrew Huxley and Robert Simmons (FRS) had earlier shown that the rapid release of a contracting muscle fibre over distances up to 100 Å per half sarcomere (i.e. 1% of its length) led to a tension recovery within a few milliseconds (Huxley & Simmons 1971). This result was explained by the cross-bridges going through a working stroke of up to 100 Å per cross-bridge to re-establish tension. The quick release synchronized the movement of the cross-bridges. Hugh reasoned that if the working stroke was indeed accomplished by tilting the cross-bridge then it would be accompanied by a big change in the intensity of the 145 Å meridional myosin reflection.

Hugh now had enough X-ray intensity to see the effects of quick release on the strong X-ray reflections from a contracting frog muscle. The rapid shortening of a muscle, by 50–100 Å per half sarcomere, produced an almost immediate large decrease in intensity of the 145 Å meridional reflection, followed by a rapid partial recovery of intensity over the next 6 ms, and then a more gradual recovery back to the original value during the next 50 ms (figure 14) (30, 32). A similar drop in intensity (but without the rapid recovery) was produced by a small rapid stretch applied to an isometrically contracting muscle. These results showed for the first time that a structural change was indeed taking place in the myosin cross-bridges as the actin and myosin filaments moved past each other in active shortening. The most straightforward interpretation is that the reflection is generated by an elongated part of the cross-bridge structure, which is oriented predominantly perpendicular to the filament axis in an isometrically contracting muscle and gives rise to sharp concentrations of density with the 145 Å periodicity. Upon quick release, the cross-bridges tilt as they go to the end of their working stroke and the sharp concentrations of density at 145 Å intervals become smeared out, resulting in an intensity decrease which accompanies force regeneration.

In 1983 Hugh’s group moved to the new EMBL beamline X33 in the HASYLAB (Hamburger Synchrotronstrahlungslabor) at DESY Hamburg, which gave much better working conditions and higher intensity (in excess of $1 \times 10^{11}$ photons s$^{-1}$). They were
surprised how quickly the ‘$J_4$’ peak appeared—well before any tension was developed (33). Moreover, they showed that, in muscles stretched to sarcomere lengths at which no overlap was present between actin and myosin filaments, the increase in the intensity on the second actin layer-line still occurred even though no active tension developed. This showed that the change in the tropomyosin position was an intrinsic property of the thin filaments, brought about by calcium release in the activation process. The steric blocking mechanism was alive and well.

Much of this work (30–33) was done in collaboration with a student, Markus Kress, who Hugh described as an ‘extraordinarily clever experimentalist and a remarkable theoretician, who worked out a whole lot of things that no-one has ever worked out since’ (35). Hugh also described him as ‘a great adventurer who had been across the Sahara on a camel!’ Markus worked with Hugh for four years until his sudden death in September 1985. Although Markus was a diabetic, there was no evidence that his death was due to an insulin overdose or to foul play and it has remained a mystery ever since. Hugh was deeply affected by this tragedy, not the least because Markus was well into writing up what would have been an outstanding PhD thesis. He was Hugh’s closest scientific colleague for several years and perhaps closest ever in terms of their relationship and respect for one another. His death left Hugh very much on his own and contributed to his decision to move to the USA. In a letter to Andrew Huxley, Hugh described Markus as ‘gifted both as experimenter and theoretician, very mature and meticulous’ and that his death ‘was an appalling loss to the field’.

**Alpbach**

In 1970 Hugh and Aaron Klug organized a very successful meeting on EM at the Royal Society in London. Two days later a meeting on the same topic was organized by Walter Hoppe in Hirschegg, an Austrian ski resort. Hugh was impressed by the format of the Hirschegg meeting, which was followed two years later by a similar meeting organized by Max Perutz and Walter Hoppe in the outstandingly beautiful Tyrolean village of Alpbach. Hugh and Ken Holmes then resolved to hold muscle meetings in Alpbach on a three year cycle to interleave with the Gordon Conferences on muscle. The first of the Alpbach Muscle Meetings, held in 1974, took place in an insalubrious cellar room that was used by the local brass band for rehearsals. Somewhat to our surprise, everyone with a name in muscle research turned up. At the end of the week, Andrew Huxley was given the unenviable job of summing up. Finally, after a hiatus of almost 20 years, in the last decade of the twentieth century, X-ray structure analysis of crystalline myosin in three different conformations provided an explanation of muscle contraction at atomic resolution. In the meantime, the series of Alpbach meetings established themselves as a forum for heated discussions of how myosin cross-bridges might swing. Hugh could also go skiing. Figure 15 shows Hugh and Ken Holmes celebrating the thirty-third anniversary in 2007.

In 1975, together with Aaron Klug, Hugh became joint head of the Division of Structural Studies and three years later, following Max Perutz’s retirement as Director, he was appointed Deputy Director, with Sydney Brenner as Director. With retirement looming, Hugh wanted to continue his research long into the future, but was uncertain how long he might be able to stay at the LMB, particularly in view of Brenner’s views about continuing employment of retired workers. Throughout his career he had been offered many interesting appointments in
the USA, but had always turned them down, feeling that none of them could compete with the LMB. He also had a strong feeling of loyalty to the United Kingdom. However, he was offered a professorial appointment at the Rosenstiel Basic Medical Sciences Research Center at Brandeis, which he accepted. In 1987 the family moved to Concord, Massachusetts, within easy reach of Brandeis. The house they bought was in Nashawtuc Road, the very street in which they had married in 1966. It was in a heavily wooded area with land going down to the Concord River. Here it was possible to go for long walks in summer and cross-country ski in winter and Frances enjoyed ice skating on flooded land nearby.

Hugh returned to EM in an attempt to capture ephemeral states of the muscle fibres by rapid freezing (34). In 1988 he accepted the Directorship of the Rosenstiel Center, in part because this absolved him of teaching, and he carried out his duties with diligence, though administration and management were not his forte. At the age of 76, when the Directorship was not renewed, he took on a number of teaching duties, giving courses on biological motors with David DeRosier and later teaching introductory cell biology. His greatest contribution to the success of the Rosenstiel Center was his example as an experimentalist \textit{par excellence}. In 1997 he became Professor Emeritus and continued his research supported by NIH grants.

**ADVANCED PHOTON SOURCE AT ARGONNE**

In 1992 Hugh started going to Cornell to use CHESS, the synchrotron source. Tom Irving was running the beam line and Hugh demonstrated small changes in spacing of both the actin and myosin periodicities both in quick releases and when the tension was altered by stretching or slowly releasing the muscle. In 1997 the Advanced Photon Source, an electron storage ring at the Argonne National Laboratory, became available as the world’s brightest...
X-ray source. The BioCAT team led by Tom Irving set up excellent beam lines for low-angle X-ray scattering with fast X-ray detectors. These provided Hugh with the opportunity to investigate a phenomenon first explored with single muscle fibres by Vincenzo Lombardi and his collaborators: the 145 Å meridional reflection is actually split into two closely spaced reflections that arise from interference between the two halves of the sarcomere. Because it is an interference phenomenon, careful measurement of the splitting allows the average position of the cross-bridges attached to actin to be measured with high precision. Using intact frog muscles, Massimo Reconditi and Hugh were able to work out the distribution of cross-bridges during a contraction. Perhaps surprisingly, most of the cross-bridges are near the beginning of their working stroke. This work was reported in two papers (39, 40) that demonstrate Hugh’s characteristic rigour and analytical ability. They are a fitting conclusion to Hugh’s scientific career and provide an elegant vindication of the swinging cross-bridge.

RETIREMENT YEARS

The LMB celebrated Hugh’s seventieth birthday with a three-day symposium in September 1994 and he chose his undergraduate College, Christ’s, as the venue. He greatly enjoyed the meeting, with testimonies to his outstanding research being given by Max Perutz, Ken Holmes, John Kendrew and Professor Carolyn Cohen. Throughout his time in America, Hugh and Frances came back to Europe annually, frequently visiting Cambridge, where they gave parties for former colleagues and friends. Without these parties, the close communication of this group would have been lost. Hugh and Frances never lost their enthusiasm for drama and music, and spent several days in London attending theatre performances. His love of theatre, opera, books and good wine completely belied the C. P. Snow theory of the two cultures: Hugh straddled both with consummate ease. Their transatlantic trips also involved holidays in their favourite haunts on the Continent. Alan Weeds has lasting memories of a talk on Silk Road cities of Samarkand and Bukhara that Hugh gave at the LMB after a visit in 1965, before he married Frances. Hugh had promised that he would take her at the next opportunity, and in 2008 Alan invited them to join him on an Art History tour of this part of the ancient Silk Route (figure 16). Hugh demonstrated his strong powers of observation on the long journey over the ‘Red Sand’ (Kyzylkum) desert in Uzbekistan by pointing out how the distant horizon clearly demonstrated the curvature of the Earth. In Samarkand they visited the remains of an ancient astronomical observatory, built in 1428 by Ulugbek, grandson of Timur. The observatory had contained a quadrant of 40 m radius, parts of which were discovered in excavations in 1908. Ulugbek had used this to measure the length of the sidereal year and obtained a value just one minute different from our current value. This immediately excited Hugh’s interest and persistent questions: neither his curiosity nor his critical faculties had been dulled by the advancing years.

MEMORIES AND CONSEQUENCES

In June 2012 Hugh wrote to 60 former overseas visitors to the LMB between 1957 and 1986, mostly postdoctoral workers from the United States, inviting them to describe the scientific problems they addressed and how the environment at the LMB influenced their subsequent careers. The book contains a total of 41 contributions and makes fascinating reading. There
are many references to the British habit of taking morning and afternoon tea: the rather crowded canteen provided opportunities for vibrant and informative scientific exchanges. From the number of these visitors who went on to be leaders in their fields (six of whom won Nobel prizes for their subsequent work), it is clear that the LMB played a crucial role in the development of much of biomedical science in the last 50 years. Published in the summer of 2013, this volume expressed Hugh’s gratitude to the LMB he greatly loved and to which he had contributed so much. He received the first hardbound copies just before his sudden and untimely death on 25 July 2013 in Woods Hole.

Hugh’s death marked the end of an era. Active to the last, he combined a wonderful experimental ability with a very analytical mind. He was also a gentle humanist and a man of great integrity. When in 1960, just eight years after receiving his PhD and at the age of only 36, he was elected a Fellow of the Royal Society, he was the youngest Fellow of the Society (his supervisor, John Kendrew, was elected in the same year). He was later elected to the US National Academy of Sciences. Memorable among the many awards that he received, in 1971 Hugh was awarded the Louisa Gross Horwitz Prize (to his delight, Columbia University paid for two transatlantic flights on the supersonic Concorde) and the prize money was used largely to finance their summer home in Woods Hole. In 1975 he received the prestigious Gairdner Award and in 1997 he was awarded the highest honour of the Royal Society—the Copley Medal. The citation reads:

In recognition of his pioneering work on the structure of muscle and on the molecular mechanisms of muscle contraction, providing solutions to one of the great problems in physiology.

**Honours and awards**

1948  Member of the Order of the British Empire (Military)
Hugh Esmor Huxley

1953 Fellow of Christ’s College, Cambridge
1960 Fellow of the Royal Society
1962 Fellow of King’s College, Cambridge
1963 Feldberg Prize for Experimental Medical Research
1964 Doctor of Science, Cambridge University
    Honorary Foreign Member, Leopoldina Academy
1965 Honorary Foreign Member, American Academy of Arts and Sciences
    Hardy Prize for Biological Research
    Harvey Lecturer, New York
1967 Fellow of Churchill College, Cambridge
1969 Honorary Doctor of Science, Harvard University
    Dunham Lecturer, Harvard University Medical School
1970 Croonian Lecturer, Royal Society
1971 Honorary Foreign Member, Danish Academy of Sciences
    Louisa Gross Horwitz Prize
1974 Honorary Doctor of Science, Chicago University
    Feltrinelli International Prize for Medicine
1975 Gairdner Award
    Baly Medal, Royal College of Physicians
1976 Honorary Doctor of Science, University of Pennsylvania
    Honorary Member, American Society of Biological Chemists
1977 Royal Medal, Royal Society
1978 Foreign Associate, United States National Academy of Sciences
1981 Honorary Member, American Association of Anatomists
    Honorary Member, American Psychological Society
    Honorary Fellow, Christ’s College, Cambridge
1983 E. B. Wilson Award, American Society of Cell Biology
1987 Albert Einstein World Award of Science
1990 Franklin Medal
1991 Distinguished Scientist Award for the Biological Sciences, Electron Microscopy
    Society of America
1997 Copley Medal, Royal Society

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Biographical Memoirs


(35) 1996  A personal view of muscle and motility mechanisms. *Ann. Rev. Physiol.* 58, 1–19, with additional information from a video interview between Professor Huxley and Drs K. Holmes and A. Weeds in 2004 (courtesy of the MRC Laboratory of Molecular Biology).


