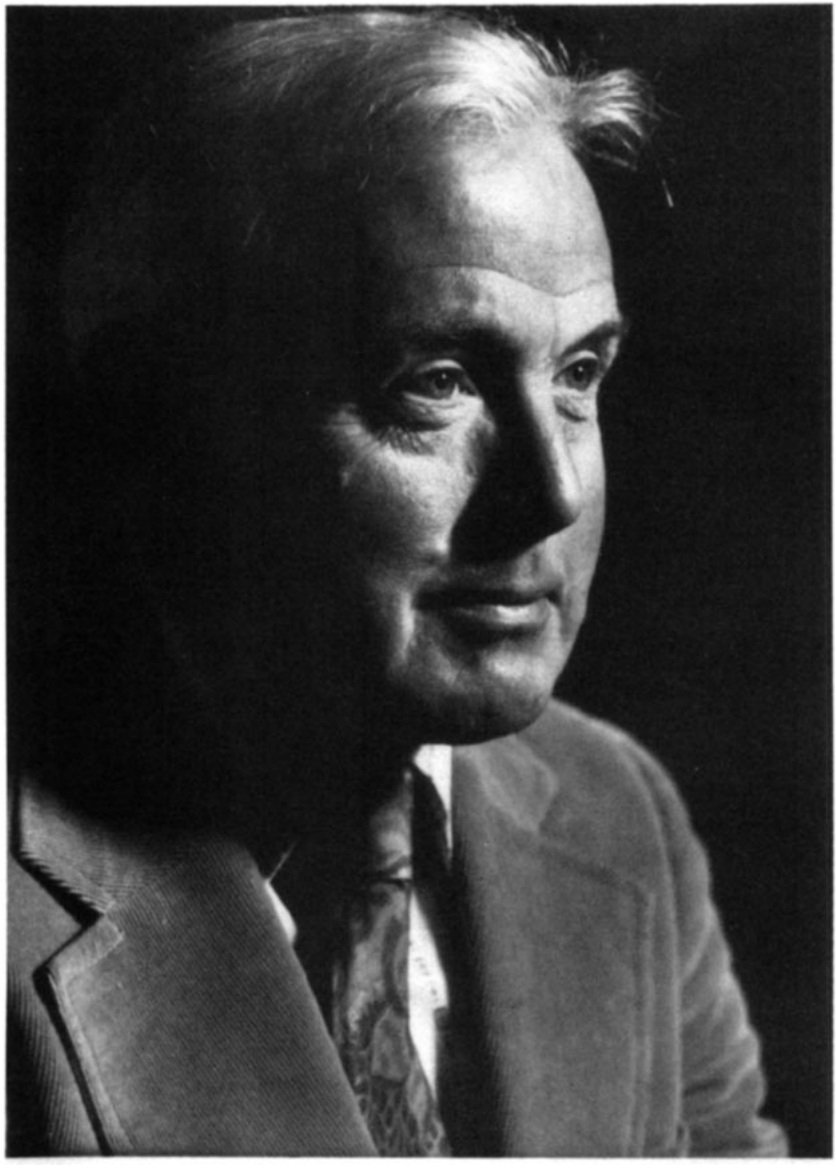

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Annu. Rev. Physiol. 1996. 58:1–19

A PERSONAL VIEW OF MUSCLE AND MOTILITY MECHANISMS

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KEY WORDS: molecular structure, contraction mechanism, X-ray, electron microscopy, autobiography

ABSTRACT

This is a personal account of some of the successive steps in our understanding of the structural mechanism of muscle contraction during the last 45 years. It describes how I, as an ex-physicist, came to be studying muscle by X-ray diffraction in 1949; how the concepts of the double array of actin and myosin filaments and, later, the overlapping filament model and the sliding filament mechanism were developed; and how further electron microscope findings of the structural polarity of muscle filaments led to the suggestion that analogous structures and mechanisms might be involved in cellular motility. The article describes briefly how synchrotron radiation has made it possible to obtain detailed structural information about contracting muscle with millisecond time resolution and discusses some of the recent major advances in the field and the prospects of reaching a full understanding of the contraction mechanism.

Introduction

In 1995 the muscle and cell motility field is in a very exciting state, as can be seen from several articles in this volume. There have been major advances in the last few years, including the publication of high resolution X-ray structures of actin and myosin subfragment 1 (16, 37, 43, 44), the observations stemming from the development of various types of in vitro motile systems (36, 38, 40, 46, 50), and the use of laser tweezers to measure the forces and displacements produced by single working molecules (4, 9), that have greatly sharpened our perceptions of the underlying molecular events. Although these experiments have amply confirmed the general picture of sliding filaments driven by crossbridges, there is still sufficient uncertainty about the detailed molecular mechanism to leave open the possibility that Nature may be using crossbridges in a

0066–4278/96/0315–0001$08.00

very subtle and unexpected way. This adds a further touch of drama to an already intriguing and significant chase. It is just over 40 years since the the original papers about the overlapping filament model and the sliding filament mechanisms were published in 1953 and 1954 (11, 17, 30), and I have been invited to write a more personal review of some of the developments in the field and some account of how I came to be involved in this work in the first place.

My first involvement in muscle work was in 1949 as a research student in a small group supported by the Medical Research Council, at the Cavendish Laboratory in Cambridge. This was the group that eventually grew into the MRC Laboratory of Molecular Biology, but at that stage it consisted of Max Perutz, John Kendrew (my PhD supervisor), Francis Crick, and myself. I was supposed to be working on the X-ray analysis of crystalline proteins, but I had grown restive at the lack of concrete results in that field (this was several years before Perutz showed that the heavy atom technique could work on proteins), and as a sideline, I was exploring the use of a microcamera that Kendrew had suggested to me, a device employing a narrow glass capillary to collimate down an X-ray beam to allow patterns to be recorded from very small selected areas of biological specimens. Reading through Perutz's reprint collection during long night vigils over water-cooled X-ray generators, I became intrigued by the problem of muscle structure and the contraction mechanism. I previously had no biological training, as I will explain presently, and was amazed to find out that the structural changes involved in contraction were still completely unknown. At first I planned to obtain X-ray patterns from individual A-bands, to identify the additional material present there. I hoped to do this using some arthropod or insect muscles that have particularly long A-bands, or even using the organism *Anoplodactylus lentus* Wilson, which my literature search revealed had A-bands up to 50 μm in length! However, getting the microcamera built was a lengthy process, and in the meantime I also became very interested by Schmitt et al's early work on muscle ultrastructure in the mid-1940s (10), and the X-ray diffraction patterns that Bear (2, 3) had obtained from air-dried specimens. He had used such material because of the very long exposure times necessary, so as to get more protein into the X-ray beam than a fully hydrated muscle would allow. But I had learned from Perutz that the whole secret of getting good high-resolution X-ray diagrams from protein crystals was to maintain them in their native fully hydrated state, in their mother-liquor. So I wondered whether a whole host of new details might not spring to light if one could obtain a low-angle X-ray diffraction pattern from a live, fully hydrated muscle. I knew from the earlier work on dried material that the size of the structural units present was likely to be in the hundreds of Angstroms range, and so I set about constructing a slit camera with the necessary high resolution.

The key to success in this endeavor lay in the use of a microfocus X-ray

generator, developed by Ehrenberg in Bernal's laboratory (7). With its 50 μm spot size, this generator gave a gain in brilliance (i.e. X-ray intensity per unit area of the source) by about 120 times over the sealed-off fixed anode X-ray tubes of that period. Kendrew, who knew Bernal well from wartime days, had first been interested in this generator in connection with microcamera work, for which it was well-suited, and through his good offices, I was able to obtain an early prototype of the device. I built a high-voltage supply from surplus parts, using the Van der Graf principle, and soon had an X-ray source that (viewed at a 10:1 angle) was ideal for a miniature low-angle-camera using 5–10 μm-wide slits, a film distance of a few centimeters, and a medium power microscope to view (hopefully) the resultant patterns.

And patterns there were! As soon as I had overcome the elementary technical problems of keeping an isolated frog sartorious muscle in good condition for the duration of the X-ray exposures (hours and sometimes days), I was able to see a number of equatorial reflections based on a hexagonal unit cell of 400–450 Å spacing. I took this lattice to represent a continuous array of contractile filaments spaced out across the myofibrils. A little later, using the Szent-Györgyi glycerinated muscle preparation, I found that muscles in rigor showed the same hexagonal reflections, but with greatly altered relative intensities. This showed that there must be some lateral redistribution of material within the same hexagonal lattice, leading to additional concentrations of material around the trigonal points in the lattice.

I then arrived at the correct interpretation of the overall lateral structure, but by somewhat faulty reasoning. I supposed that the actin and myosin must be present in separate filaments, with myosin probably occupying the hexagonal lattice points (because there was more of it) and actin filaments lying in between, more or less randomly in resting, relaxed muscle, but becoming fixed at the trigonal points of the lattice in rigor (i.e. no ATP) by symmetrically arranged crosslinks to the myosin filaments. The idea of an actomyosin complex forming in the absence of ATP was common at that time from the work of Szent-Györgyi and of HH Weber and Portzehl, but the concept of the two proteins being in separate filaments interacting via crosslinks was new. However, at that time I visualized the crosslinks as relatively thin structures and did not consider the possibility that their mass distribution might be the major factor affecting the intensity of the X-ray reflections, rather than the temperature factor of the actin filaments. And, of course, I also assumed that the array of filaments was continuous throughout the length of each sarcomere.

Some time later (in 1951) I found that the muscles from frogs caught in the wild from Fens around Cambridge (on very early morning bicycle expeditions) gave much better X-ray patterns than muscles from the cold-room frogs. Their patterns showed clear reflections in the meridional area, some corresponding to the series already identified by Astbury (1) and by Bear (3) as arising from

actin (the 59, 51 Å pair of reflections and the 27 Å repeat), and also others at high spacings, greater than 400 Å, with a strong third order repeat, which originally I supposed probably also came from actin. However, the most remarkable thing about these reflections was that all their spacings remained unchanged when the relaxed muscle was stretched. I reasoned that the contractile material itself must be extended by the stretch (rather than some separate elastic component) because the large passive compliance disappeared immediately when the muscle was activated. I supposed that in the dissociated, relaxed state, actin and myosin filaments could move independently in response to the extension. I envisaged that the actin filaments remained constant in structure, that the myosin filaments were extensible and were responsible for passive tension, and that there was overlap between filaments throughout the sarcomere. Upon activation the rigid actin filaments became crosslinked to myosin, making the whole structure immediately much less extensible, and shortening was produced by partial depolymerization of actin combined with rearrangement of myosin subunits during their interaction. I supposed that the myosin filaments, unlike actin, did not have a very regular internal structure and therefore did not show up in the X-ray diagram.

With hindsight, one can wonder why I did not then entertain the possibility that the very low-angle set of reflections at the higher spacings might come not from actin but from myosin. I can only plead that I was confused both by the numerology of the spacings, by somewhat inaccurate values of their relative values (recorded on two different cameras and relying on micrometer eyepiece measurements on grainy film), and by not knowing which if any reflections were off meridional. This was in the very early days of helical diffraction theory, and reflections at about 59 and 51 Å seemed consistent with a 410 Å fundamental period, approximately (since the seventh and eighth orders would occur at 58.57 and 51.25 Å), whereas the 27 Å reflection seemed to index on either 405 or 432 Å. My long-period repeat seemed to measure around 420 Å, and my failure to pursue these discrepancies cost me the opportunity to have predicted the whole structure and its behavior from the X-ray patterns alone!

I have described this matter in some detail because it added considerably to the sense of revelation some two years later when Jean Hanson and I, working together at Massachusetts Institute of Technology, discovered that the myosin filaments were present only in the A-bands and that it was the actin filaments that were attached to the Z-lines. However, more of that later.

Toward the end of my time as a research student in Cambridge, during 1951 and 1952, the first version of a rotating-anode X-ray generator built at the MRC by Tony Broad began to function. I was able to reduce the exposure on some of the axial patterns to a few hours, short enough to at least begin to contemplate some heroic experiments involving many many frogs in order to

obtain the crucial patterns from contracting muscle, but still too long to actually carry them out. I did, however, perform one heroic experiment, by repeatedly pouring an actin preparation (supplied by Andrew Szent-Györgyi through John Kendrew's good offices) down a thin capillary for 24 h in an effort to record the much sought after low-angle pattern from pure oriented actin—but to no avail!

My PhD viva, in June 1952, with Sir Lawrence Bragg and Dorothy Hodgkin as examiners, produced one exchange that later aroused some interest, and which I recounted at a Royal Society muscle meeting in 1964 (although it was not recorded). Dorothy had been impressed by the large increase in intensity of the equatorial (11) reflection that I had shown occurred when a muscle went into rigor. Being unfamiliar with the preparations in question (live and glycerinated rabbit psoas muscle), she had assumed that the muscles shortened considerably during the onset of rigor, and therefore wondered whether the increase could be due in some way to increased overlap between the filaments. I responded that I had taken great care not to allow the fiber bundles to shorten and to check that they had not done so. I had done this in order be sure that the intensity changes that I saw were produced by the lateral rearrangements during crosslinking of actin and myosin filaments on their own, without extraneous factors. Indeed, as later work showed (25), this was in fact precisely the case. Nevertheless, her intuition, although based on a misunderstanding and lacking a realistic model of the sarcomere structure, had considerable elements of truth in it. However, at the time I was slightly irritated by what I considered was her failure to read my "Methods" section carefully, and I took her suggestion much less seriously than (as I gathered some years later) she had done! Indeed, I quite dismissed it, and it was not until Gerald Elliott and his colleagues (8) showed in 1963 that changes in sarcomere length in a relaxed muscle do indeed also produce intensity changes, in the way she had envisaged, that I appreciated how close she had been.

But in 1952, these were early days still, and I was off to MIT as a post-doc on a Commonwealth Fund Fellowship to learn electron microscopy in Frank Schmitt's lab and to see the New World. This all proved so exciting that there was never any time to write up the X-ray work properly (except as brief conference abstracts) (19, 21), and that work was overtaken by the microscopy results that Jean Hanson and I obtained at MIT. However, perhaps now I should explain a little more why my approach to biological problems has always had a mainly physical orientation and how I came to be in the MRC Group in the first place, at such a crucial time in scientific history.

I was born in 1924, in Birkenhead, Cheshire, of Welsh parentage; families who had moved to Merseyside from North Wales in the previous generation but who still had strong ties to the Welsh countryside. They were schoolteachers and shopkeepers and government employees— my father was a Post Office

accountant who later became Head of the Accounts Branch in Liverpool, but in the 1920s and 30s we were fairly poor—it was the Depression, and Merseyside was a particularly depressed area. However, both my parents were people of remarkable intellect, great readers, lovers of music, and with great moral strength and power of judgment. With the help of extremely good local secondary schools (i.e. State schools), they instilled in my sister and me the idea that if we worked hard and tried hard enough we could win scholarships to University, perhaps even to Cambridge. My sister, who is about seven years my senior, was the first to succeed (she got a first in both parts of the English Tripos at Newnham, the first woman, I believe, to do so) and of course that spurred me on to greater efforts. My main interest was in atomic and nuclear physics, enthralling subjects for a 12-year-old schoolboy in the 1930s, and when I gradually realized that the main center for experimental research was in Rutherford's laboratory in Cambridge, my course was set.

My scientific and technical inclinations had started in the usual way with the Meccano constructions and chemistry sets, electric motors and shocking coils, and continued into building short-wave radio receivers and getting up very early in the morning when ionospheric conditions were best for receiving amateur stations from the Pacific, using a directional aerial system. My most ambitious experiment had been an attempt—unsuccessful—to make diamonds by dissolving carbon in molten metals in a home-made electric furnace. I was fortunate in having excellent schoolteachers in physics and chemistry and seven years of instruction by each of them in high school. Those were the days! One piece of advice was particularly memorable: "Always look very closely at what is happening in an experiment—you may see something that no one has ever noticed before!" Biology was not taught at all in school—it was a subject considered more suitable for girls—and perhaps that was just as well.

Atomic physics, relativity and quantum theory—or what little I knew of it—were to me then subjects of magical interest, offering glimpses of the ultimate nature of reality, and perhaps the opportunity to make some significant contribution oneself, to one of man's supreme intellectual endeavors. Moreover, my social conscience was persuaded that this would not be a purely abstract and selfish activity by my belief (around 1940) that nuclear power would be needed eventually to replace other sources of fuel, even if it took hundreds of years to discover how to do so.

But by that time, Merseyside was being bombed quite heavily, and I learned to recognize the terrifying hiss of the parachute on a descending landmine and have vivid memories of cycling through the smoking ruins of the center of Liverpool with sagging tangles of wires from the overhead tramcar cables making a quite apocalyptic spectacle. However, after two years in the sixth form, one of them as school captain (not in recognition of my athletic ability, although I was quite good at cross-country running, probably helped by 100

mile Sunday cycle rides through the North Wales mountains), I managed to get to Cambridge, in 1941.

Cambridge, even in wartime, was everything I had ever dreamed of, and when I was able to go on directly to Part II Physics in my second year, i.e. to do nothing else but advanced physics (plus electronics as a wartime training program), my heaven was complete. However, although my greatest ambition was to do research in nuclear physics, preferably in Cambridge, I felt very restive at playing no direct part in, nor even being very near to, the great wartime events that were taking place. So in 1943 I chose to join the Royal Air Force as a radar officer and to come back after the war to finish my degree, rather than spend the war inside some research laboratory. Basically, I suppose I wanted to have some adventure first. I was not successful in getting myself into Europe at the opening of the Second Front, but I did have an extremely interesting time doing flight trials on experimental radar systems, at Malvern and with Bomber Command, (often as it turned out, not far from Cambridge), as well as spending some time on an operational bomber station. I was scheduled to go as liaison with the first RAF Bomber Command Groups in the Far East, after the war ended in Europe.

Other events intervened. I heard the radio announcement of the dropping of the first atomic bomb on Japan with qualified surprise because the nature of such a chain reaction had been published in the popular science magazine *Discovery* several years previously, and during the war I had heard leaks of the enormous scientific and technical effort that was being put into the project in the United States. However, it was not until some time later that I realized the effect the bomb had on my idealistic attitude towards nuclear physics. I remember reading a magazine, *Life* probably, in the Officer's Mess at RAF Marham, with a full page picture spread giving a brief history of the physics involved. It included a gallery of photographs of all the distinguished faces who were my great heroes— Curie, Planck, Einstein, Bohr, Rutherford, Millikan, Compton, Heisenberg, Schrodinger, Pauli, DeBroglie, Chadwick, Dirac, who had done all those marvelous experiments and dreamed up those elegant theories—followed by a line of photographs of some of the survivors from Hiroshima. It was devastating.

At first I thought very seriously about switching to economics, but eventually it seemed to make more sense to at least finish a degree in a subject for which I had shown some aptitude. So, resisting some inducements to stay in the Air Force and help develop high altitude navigation systems, I went back to Cambridge in October 1947 when I was finally demobilized, back to Part II Physics again. I was sure, for a start anyway, that I wanted to do scientific research involving physics, but far away from its wartime uses, and I felt hopeful that interesting applications must exist in some form of medical research. The first task, however, was to be sure of getting a good enough degree

to be eligible and desirable as a research student, and it was hard work because my memorizing capacity seemed to have deteriorated sadly in the intervening years.

I was very fortunate in my supervisors, one of whom, Dr. David Shoenberg, had worked with Kapitza and happened to know Kendrew and Perutz through a college connection. They had been given some space in the new Austin Wing of the Cavendish Laboratory, where Sir Lawrence Bragg was Head of the Physics Department and very interested in this new application of X-ray diffraction to more complicated molecules, i.e. proteins. I knew nothing about proteins, and I had not been particularly enamored by what I had learned about crystallography and space groups, but it sounded as though it might lead somewhere interesting. However, I felt pessimistic about my performance in the Tripos exams and went off cycling in the south of France to await the worst. However, when I received my sister's telegram "Congratulations: First: Idiot." in Perpignan, I knew that my ambition of doing research in Cambridge was to be realized. But it took a year or two to find my own direction, as I have described earlier. After getting my PhD, I went to MIT.

Even in those early days of electron microscopy, it was apparent to me that the combination of that technique (which gave one real tangible images, but with all sorts of artifacts) and X-ray diffraction of intact specimens (which gave one true data but in an enigmatic form) would together provide a very powerful means of deciphering these hitherto hidden but very important biological structures and organelles. I knew I had the ideal material for this task and that was what brought me to Frank Schmitt's lab at MIT, where Dick Bear and Cecil Hall were also working at that time. After some months of work and the joint development of a new microtome with Alan Hodge and Dave Spiro, I was able to obtain convincing electron micrographs of the double array of filaments as seen in cross-sections, and I believed I could also see the cross-bridges between thick and thin filaments. However, at that time I still assumed that both types of filament were present throughout the sarcomere—otherwise, how could muscle shorten over such a wide range of lengths? The A-substance seemed to be just some mysterious extra material.

Jean Hanson's arrival in MIT (also to learn electron microscopy) focused my thinking much more sharply on the significance of the striations in skeletal muscles and on the various contradictory data in the literature about the changes in them during contraction. Up to that time, I had never actually seen what the striations looked like in the light microscope, but Jean had taken many beautiful photographs of rabbit and insect myofibrils, as seen in phase contrast—a relatively new technique then—and we wondered whether the A-substance diffused throughout the sarcomere in some way upon activation, and how it would change with muscle length. We drew up a long list of experiments to compare corresponding images as seen in phase-contrast light microscopy and

in thin-section electron microscopy while exposing the muscle to various relaxing, activating, and extracting solutions. Almost immediately, we obtained some very startling results. After several initial problems with unbuffered pyrophosphate, we found that the solutions standardly used to extract myosin from whole minced muscle would, when applied to myofibrils, selectively extract the A-band material. At first, still thinking in terms of my earlier model with myosin connected to the Z-lines and actin filaments floating in between, we wondered if perhaps it was actin that was first extracted from this very finely minced muscle. However, we soon convinced ourselves that that was not the case (we could see the same effect in coarsely minced muscle, in electron microscope sections) and so, within a day or two, with a tremendous sense of revelation, we realized what the overlapping filament array structure was really like, and how it enabled so many of the previous observations to fall into place.

I realized that in my electron microscope cross-sections of muscle, the areas that did not show double-hexagonal arrays of thick and thin filaments, but only disordered distributions of thin filaments, were not examples of bad fixation, but were sections through I-band regions, and in a similar way I was able to recognize areas showing thick filaments alone as being sections through H-zones.

We quickly wrote up the work for *Nature* (11), sticking very close to the experiments and the immediate deductions from them because Frank Schmitt warned us against spoiling the paper with a lot of speculation about how the system might work! However, our minds were very full of ideas in this direction, and as I looked repeatedly through Jean's earlier light microscope photographs, I noticed that although sarcomere lengths varied somewhat between different fibrils in these glycerinated psoas muscle preparations (which had gone into rigor at different lengths during the glycerination procedure), the A-bands all seemed to be the same length. It was clear that we needed to make detailed observations of myofibrils as they shortened during ATP-induced contractions. This material would enable us to take advantage of the very clear and unambiguous images that the fibrils gave in phase contrast, as compared with normal images of single whole fibers, which were usually beset by optical artifacts arising from their much greater thickness and the resultant overlay of out-of-focus images of the repeating band pattern.

During the early summer of 1953 (20), I finally got around to writing a short paper about my electron microscope results confirming the presence of the double-hexagonal array expected from the X-ray diffraction studies. In the discussion, I pointed out that my earlier observation of a constant axial period during passive stretch would be compatible with a process in which the two sets of filaments slid past each other and that possibly a similar process might occur during contraction. Looking back on it now, it might have been fairer

to have associated Jean somehow with this suggestion at that time because a vital part of its genesis was our discovery of the partially overlapping filament arrays. But at the time it seemed such an obvious possibility that I didn't think very much about this aspect, and neither did she, as far as I know, when she read the manuscript. Frank Schmitt sent the manuscript off to *Biophyica et Biochimica Acta* with his blessing, after some delay.

The main problem now was to get convincing evidence—good photographs—of what the A- and I-bands were doing during contraction. This was not easy because it all happened so quickly, even at cold-room temperature, and it took us some time to get together the necessary microcine-photography set-up, with high-intensity Xenon lamps. I used to go down to the Marine Biological Laboratory in Woods Hole quite frequently during summer weekends, staying with Andrew and Eve Szent-Györgyi. I also visited Andrew's older cousin, Professor Albert Szent-Györgyi, who was not at all enthusiastic about the finding that myosin filaments were confined to the A-band!

However, I did find a more receptive audience for our overlapping filament model and the X-ray and electron microscopy results in Andrew Huxley (no known relationship), who was visiting from England. I found that he was also working on band pattern changes in intact fibers, with Ralph Niedergerke, using a new type of interference light microscope that he (AFH) had built. He told me that they too had some preliminary indications of constant A-band length, and we agreed to communicate again when we had all got our experiments working properly and were nearing the publication stage.

During the latter part of that summer (1953), I also drove out to California via Yellowstone and back via New Mexico, Texas and New Orleans, camping out the whole way (part of the terms of my Fellowship was to see more of the USA!). I had the great fortune to be a participant in the remarkable Pasadena Conference, at which Perutz showed how protein structures could be solved by X-ray diffraction, and Crick and Watson described the DNA double helix and its implications, with Pauling and Delbruck and others to cheer them on.

Back at MIT in the fall, Jean and I were somewhat disappointed with the resolution of our ciné pictures, but found that we could supplement these with photographs taken with a still camera at various successive stages of contraction, by repetitive irrigations of the fibril preparation with small amounts of highly dilute ATP. We also found out how to make very clean myosin-extracted preparations after various degrees of shortening, in which we could see the I-segments very clearly and measure their lengths (admittedly in the absence of myosin, but the less clear-cut edges of the H-zone in intact fibrils gave consistent values) which, as we expected, remained constant, like the A-bands. However, we remained somewhat uncertain as to the situation at shorter lengths, when a dark line appeared in the center of the sarcomere, which we thought might represent the actin filaments coiling up in some way when

interacting with myosin at greater degrees of shortening. It was not until several years later that I was able to obtain electron micrographs showing the double-overlap behavior convincingly, and it took very much longer (47) before micrographs of muscles rapidly frozen during contraction could rule out more conclusively the possibility of significant amounts of actin-folding or depolymerization during force development and shortening. Eventually, we were satisfied with our measurements, and as is well known, arranged that our paper and the corresponding one from Andrew Huxley and Ralph Niedergarke be published together in *Nature* (17, 30).

At that time, and for a while subsequently, we entertained two distinct possibilities for the mechanism that produced the sliding force. From energetic considerations and from the structural data, we estimated that under maximum load, the actin filaments needed to be pulled along a distance of about 100 Å each time about one third of the myosin molecules split one ATP (not too far from current values!). One possibility was that this represented the extent of movement of a crossbridge during its working stroke. This was a simple solution, but we were concerned about how such a large movement could be produced by changes in chemical bonding at the angstrom level. The other possibility we considered was some type of vernier mechanism, involving small sequential changes in periodicity in the actin filaments, perhaps brought about when myosin crossbridges attached to them, in a zipper-like manner. In this way, a change of one or two angstroms per monomer could be magnified into 50–100 Å of movement by the successive interaction of 50 crossbridges; analogous mechanisms could be devised involving similar small changes in myosin. Of course, both mechanisms were quite speculative at the time, and we mentioned them only very briefly in the 1954 *Nature* paper. However, we did write them up much more explicitly for a Society for Experimental Biology Symposium in the summer of 1954, when we had both returned to England. The Conference Proceedings volume was published the next year (12), but did not, I think, enjoy a very wide readership. We gradually discarded the vernier mechanism, largely because of the difficulty of getting it to work in stretched muscles at small degrees of overlap, and by the time of writing the 1957 paper (23), showing crossbridges very clearly in very thin longitudinal sections of rabbit muscle, I was almost entirely convinced that they must have, somehow, a working stroke of 50–100 Å.

A Digression on Cell Motility

One of the first glimmerings of the idea that sliding filaments might be involved in movement in cells beside muscle cells arose very serendipitously early in the 1960s. After the success of seeing the double array of filaments and crossbridges in well-oriented thin sections of muscle in the electron micro-

scope, I had spent almost two fruitless years trying to see significant internal structure in the filaments (1956–1958). For some sort of relief I had been making brief forays into virus structure, encouraged by Rosalind Franklin and Aaron Klug, who were doing X-ray work at Birkbeck College, just round the corner from my lab in Bernard Katz's Biophysics Department at University College. The first success I had was with tobacco mosaic virus (TMV) (in 1956) where, using the negative staining technique that I discovered by accident at this time (22), I could see the central channel in this long rod-shaped virus. After some unsuccessful attempts to get informative images of the nucleic acid component in TMV and in a number of small spherical viruses, using uranyl acetate as a positive stain, I went back to negative staining again, by which time Brenner & Horne had arrived at a simple procedure for carrying it out more reproducibly. In turn, I found that unbuffered uranyl acetate functioned as a superb negative stain, and Geoff Zubay and I had a lot of fun with ribosomes (33) and with turnip yellow mosaic virus (TYMV) (34), the first small spherical virus to be shown by electron microscopy to have fivefold symmetry (the protein subunits form a pentakis dodecahedron).

This success encouraged me to think of applying the same technique to muscle structure. First, one had to take the muscle apart in some way, which I found ridiculously easy to do by homogenizing relaxed muscle in a Waring blender. It was very gratifying to see so many thick filaments all the same expected length (1.5–1.6 μm), but a much more surprising and far-reaching result lay in store (24). Some years earlier I had discovered that heavy meromyosin would bind strongly to the I-segments left behind after myosin extraction from myofibrils, giving a large increase in density as seen in the phase-contrast light microscope. I therefore wondered what the effect would be on the appearance of isolated actin filaments in the electron microscope, expecting that the myosin heads would probably look just as disordered and degraded as they did on the thick filaments. But, of course, quite the contrary was the case. The myosin heads were obviously well preserved and well ordered in a beautiful double-helical structure, matching that of the actin filament structure underneath, which Jean Hanson and Jack Lowy had first observed (13). After looking at my pictures of these "decorated" actin filaments for about two days, the significance of what I was seeing suddenly dawned on me! They were structurally polarized, which meant that all the attached myosin heads and all the underlying actin sites must be oriented in the same direction. Clearly, such a polarity is what one would expect in a sliding filament system, but somehow the requirement had never occurred to me before—nor to anyone else, as far as I know!

After that, it did not take very long to show that actin filaments were indeed attached to the Z-line with the appropriate polarities and that each half of a myosin filament had a corresponding structural polarity, reversing at the M-

line. So it all made a great deal of sense in terms of the crossbridge mechanism for muscle contraction. But it also showed that the direction of the force acting on an actin filament would be defined by the actin filament itself, which would only be able to interact with appropriately oriented myosin heads. So I argued (24) that an oriented gel of actin filaments whose polarization was predominantly in one direction might be able to propel itself along past myosin in the presence of ATP, and that this might have something to do with cytoplasmic streaming! At that time, actin was only thought to be present in muscle, but when it was discovered in many other cells, the idea became a lot more plausible, even if it was not exactly right. After Vivianne Nachmias and I (42) found that even slime mold actin could be decorated in just the same way with rabbit actin (a remarkable example of conservation), I became very intrigued with actin's possible movements and tried in many many ways—as did many others, no doubt—to construct an in vitro motility system in which bundles of actin filaments might be seen to move, in the light microscope, when interacting with myosin and ATP. We were all defeated for many years by actin's habit of forming bundles of mixed polarity (although Paul Matsudaira and I came close by using filaments grown from acrosomal bundles) and by the tricky problem of finding suitable surfaces for myosin to attach in a functioning state.

So I was absolutely delighted (though of course a little envious!) when Jim Spudich (a former colleague) and Mike Sheetz first did their famous experiment with Nitella (46), and even more so as the Spudich and Yanagida groups (40, 50) have continued to produce more and more elegant experiments on sliding actin filaments, culminating in those involving force and movements produced by single myosin molecules (9, 36). The corresponding experiments with kinesin and tubulin, a system first put on a clear basis by Vale and his colleagues (48), have been equally gratifying, and the whole subject area is discussed in several review articles in the present volume.

These in vitro sliding experiments were particularly helpful at that time because they provided renewed confirmation, by a novel, independent, and unexpectedly direct method, of the reality of actin filaments actively sliding along myosin heads whose tails were attached to a fixed support. This was important because by the mid-1980s, confidence in a straightforward sliding filament mechanism for muscle contraction had been significantly eroded by the failure of several types of spectroscopic experiments to show structural behavior of crossbridges of the kind expected (i.e. more than one attached configuration). An additional embarrassment was the virtual absence, from the X-ray pattern of isometrically contracting muscle, of low-order actin layer line reflections showing clear evidence of myosin head attachment in any defined configuration. There were also persistent claims by some people of A-band shortening. The in vitro studies re-established in everyone's mind, I believe, the conviction that the sliding force had to be generated by the myosin heads

and that our task, as originally, was still to find out how they did it! So let me return to the muscle story itself.

Why Has It Been So Difficult to Discover the Detailed Mechanism?

By the early 1970s, the time of the Cold Spring Harbor Symposium on muscle contraction (5), it was generally accepted that the sliding filament moving crossbridge model was correct. The much more detailed X-ray data (15, 27), including informative results from contracting muscle showing a major decrease in intensity of the myosin layer lines, confirmed and extended many aspects of such a model, and the Lymn/Taylor and Huxley/Simmons results gave important support from biochemistry and physiology (18, 41). In fact, several people asked me what I was going to work on next, now that the muscle problem was essentially solved, and were puzzled and disappointed when I said I would continue working on muscle because I did not think the evidence was really there yet.

What I had in mind was the fact that there was still very little direct evidence about what the crossbridges were actually doing during their force-producing interaction with actin. They were certainly moving from their more regular positions around the myosin filament backbone and moving towards actin (as shown by the X-ray data) (15, 27), but there was no direct evidence as to whether and when they actually attached to actin, although of course, it was entirely reasonable that they should do so. Similarly, there was no evidence as to what sort of structural changes in the myosin head, elsewhere in the molecule, or within the actin monomers, might be responsible for the generation of force and movement during the working stroke. It seemed to me that one could not just leave a problem when such crucial information was still lacking, particularly as new developments in X-ray diffraction (synchrotron radiation) (45) and electron microscopy (three-dimensional reconstruction) (6) offered excellent new ways to approach the question. If it were indeed a simple straightforward tilting mechanism, with most of the crossbridge population attached and generating force during isometric contraction and going steadily through repetitive cycles during shortening, then the evidence for this should be quite readily accessible when the new techniques were applied.

In practice, things were not so straightforward; it took another 10 years before synchrotron radiation began to fulfill its promise. In the meantime, we had to do the best we could with higher power rotating anode X-ray generators. One of the most obvious experiments was to apply a fairly quick release by about 2% of muscle length to an otherwise isometrically contracting whole muscle and look at the intensity changes in the equatorial reflections, which we could now do (with some difficulty) on a time-resolved basis using labo-

ratory X-ray sources. Because there was such a large change upon activation and tension development (increase in intensity of [11], decrease of intensity [10]) (equatorial reflections), I expected that when all the crossbridges detached and went through one or two cycles of movement following the quick release (the tension fell to zero and then recovered again), there would be interesting intensity changes in the equatorial pattern. But there were none. This convinced me that there must be some very strange features of crossbridge behavior and that the problems were still certainly worthy of attention.

The powerful X-ray beam lines at the synchrotron radiation source at the European Molecular Biology Laboratory Outstation at DESY, Hamburg, developed in the early 1980s, enabled us to do many of the experiments that seemed an impossible dream in the 1950s, and we obtained many informative results (28, 29). The large and abrupt change in the intensity of the 143 Å meridional reflection, which Bob Simmons, Wasi Faruqi, and I found takes place almost simultaneously with a rapid quick release (32), remains the best and indeed almost the only experimental evidence we have that axial changes in crossbridge structure are very closely associated with the working stroke. [Importantly, it has now been shown by Irving and his colleagues (35) on single fibers that this change occurs during the tension redevelopment phase.] But my favorite experiment remains the one involving the actin second layer line and the evidence it provides that tropomyosin movement is responsible for switching on the actin filaments, as Haselgrove and I and Vibert, Lowy, and others had suggested earlier (14, 26, 49). To see that reflection flashing up immediately after electrical stimulation of the muscle, at a time significantly before any tension had developed, was a thrilling experience for Marcus Kress, Wasi Faruqi, and myself (39). We had feared that the changes might be too faint to see.

However, even with our best efforts and a lot of beam time, we were unable to see any reproducible changes in equatorial intensities produced by short quick releases, and this remains a big puzzle. There are a number of other related effects, for instance, delays in the onset of the 143 Å reflection spacing change at the beginning of fast shortening (31), which seem to show crossbridge interaction with thin filaments over longer distances than the tension generating part of the working stroke is likely to be (in any straightforward model of a lever arm). It seems as though very rapid completion of the working stroke leaves the crossbridge in a state where it can detach and re-attach again very quickly to a different actin monomer, closer to the Z-line. Whether this is merely a quirk of nucleotide-binding rates, or two head interactions, or whether it is telling us something very important about the basic mechanism of force development remains to be seen.

The high resolution structure of the myosin head (44) with its more globular catalytic, actin-binding domain and its elongated regulatory domain, projecting

out sideways when the head is attached to actin, obviously provides a structural basis for the tilting crossbridge mechanisms that have long been postulated. The location of the normal binding sites for optical and spin labels in the catalytic domain, which might not have to change very much during a working stroke, could perhaps explain why changes in orientation of strongly attached heads have been so difficult to detect. However, there still seems to be a large amount of disorder in the attached heads during isometric contraction. I have made great efforts in recent years to obtain informative electron micrographs of thin sections of rapidly frozen, freeze-substituted contracting muscle, but although an ordered component is clearly present, it is quite sparse, as indeed one would expect from the low-angle X-ray diffraction patterns of contracting muscle. This may merely mean that only a very small fraction of crossbridges are in a tension-generating state at any one time (which is quite plausible), but it still remains true that a really decisive demonstration of an adequately large and specific change in the configuration of an attached crossbridge during the working stroke (or an equivalent change in structure of a myosin head produced by biochemical manipulation) has still to be produced. Thus the challenge to really understand the mechanism remains.

I consider myself very fortunate to have moved from physics to biology when I did. My motives for leaving physics were somewhat mixed. In part, as I have indicated, there was dismay and disillusion that the first practical consequences of all that beautiful work in atomic and nuclear physics had been the atom bomb, and my reluctant conclusion that I would never be able to enjoy working in that field without feelings of guilt. This was reinforced by the fact that my own contribution to society at that time had been to help improve target identification radar for Bomber Command. I was also influenced by another worry (which I think was exaggerated)—that the days when an individual experimenter could make a difference in the nuclear physics field were over, and that in the future just a few very good theoreticians, which I could never aspire to be, would tell great hoards of experimenters what to do and then interpret their results for them. In fact, there have been many important original contributions by individual experimenters since then (1947).

My reasons for going into biology were not clearly formulated, nor could they be at that time, especially given my ignorance of the subject. I had a vague notion that there must be many techniques in physics that could be applied to research in biology and medicine and that therefore a physicist, working with a "real" biologist, might be able to make a useful and interesting contribution. When I found that there was such a possibility, right there in the Cavendish Laboratory in Cambridge, it was an easy decision and an extraordinarily fortunate one for me. Not only was I "present at the Creation," so to speak, of the DNA double helix and the solution of protein structure by X-ray crystallography and much of the founding of molecular biology, but I benefited

enormously from the company of people with very clear minds and great (and justified) optimism about what could be accomplished. Moreover, since my own work prospered, I was able to enjoy a long and enthralling association with the MRC Laboratory of Molecular Biology in Cambridge, arguably the leader in the field, during one of the greatest periods of scientific development, certainly as great as the revolution in physics in the first part of this century, which had caught my imagination as a child. I sometimes regret that all the advanced physics I learned never had any application, but it encouraged in me the belief that everything in Nature could be explained rationally, eventually, and that after Part II Physics, understanding the basics of any other subject would be relatively easy!

A further piece of good fortune was that structure turned out to be so important for biological mechanisms and that my early faith that a combination of X-ray diffraction and electron microscopy would provide an extremely powerful tool for deciphering structure and function in numerous fields was fully confirmed. In the area of muscle and cell motility, the techniques for obtaining structural and mechanical information have been able to keep up very well with the questions arising from other techniques, over a long period of time. Just when it becomes possible to change individual amino acid residues in a myosin molecule, the high resolution three-dimensional structure of the myosin head is solved, so that interesting residues can be chosen. At the same time, it becomes feasible to measure forces and displacements on individual molecules, and perhaps even to follow the chemistry on single molecules too. There is still a great deal to learn from the X-ray diagrams of intact muscle, especially as brighter and brighter synchrotron sources become available; and a way still has to be found to obtain more detailed and reliable high resolution electron microscope images from rapidly frozen muscles during tension development and rapid length change. So there are plenty of interesting things still to do and good problems still to solve!

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