

Chapter 2

Birth of the Sliding Filament Model of Muscular Contraction: Proposal

...it is postulated that stretching of the muscle takes place, not by an extension of the filaments, but by a process in which the two sets of filaments slide past each other...one may note the possibility that an analogous process is involved in contraction.

Hugh E. Huxley (1953b)

Koscak Maruyama remembers Jean Hanson shouting: "I know I cannot explain the mechanism yet, but the sliding is a fact" (Maruyama 1995. With permission Oxford University Press)

K. Maruyama (1995)

The motion pictures taken by A. Huxley of living muscle can leave little doubt in the spectator's mind about the basic correctness of the theory. (Szent-Gyorgyi 1960. With permission Elsevier)

A. Szent-Gyorgyi (1960)

2.1 Introduction

The official date of the "birth" of the sliding filament theory of muscular contraction is May 22, 1954. On this day the journal *Nature* published two papers consecutively under the general title: "Structural Changes in Muscle During Contraction". The first paper by Andrew F. Huxley¹ and Dr. Rolf Niedergerke was entitled: "Interference microscopy of living muscle fibres". The second paper by Dr. Hugh Huxley and Dr. Jean Hanson was entitled: "Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation". But the story of sliding filaments begins before May 22, 1954. In order to understand and appreciate the experiments that were done and why they were done, it is necessary to review the scientific background of each of the investigators.

¹ Andrew Huxley did not work for a Ph.D. at Trinity College in Cambridge and thus he is the only one of the four authors on the classic 1954 papers who is not listed as "Dr.". During his time at Trinity promising young researchers would receive a research fellowship. Alan Hodgkin (1977) also did not work for a Ph.D.

2.2 The Investigators: Andrew Huxley and Rolf Niedergerke, Hugh Huxley and Jean Hanson

Andrew Fielding (A. F.) Huxley² (1917–2012) (Fig. 2.1) has described his research in physiology as “the mechanical engineering of living machines” (Huxley 2004a). A substantial part of his work has been the design and construction instruments needed for his research. Huxley conducted his first research with Alan L. Hodgkin (1914–1998) at the laboratory of the Marine Biological Association at Plymouth, England, in the summer of 1939. At that time the 22 year old Huxley had just finished his undergraduate education at Trinity College, University of Cambridge. Hodgkin invited him to join in an attempt to measure the transmembrane resting and action potential in the squid giant axon. The squid giant axon was discovered by the anatomist John Zachary (J. Z.) Young (1936). It is a single axon which is actually a syncytium of many cell bodies and it could reach a diameter of 500 μm or more. Huxley devised a method of inserting an electrode down the center of the vertically mounted axon. This worked at once, but the experiment often failed because the capillary scraped against the surface membrane. Huxley rectified this problem by introducing two mirrors which allowed one to steer the electrode down the middle of the axon by simultaneously viewing the position of the capillary through a horizontally mounted microscope from right to left and front to back. Hodgkin (1992) has commented that Huxley was a “wizard with scientific apparatus” and that he solved technical problems in an incredibly short period of time. This assessment is the first of many examples of Huxley’s wizardry in the design of equipment to solve experimental problems. During the summer of 1939, they recorded for the first time an intracellular action potential that exhibited an overshoot above zero potential. This observation was fundamental because it disproved the then prevailing view developed by Bernstein (1902) that the action potential consisted of a disappearance of the resting potential due to a general increase in permeability, allowing all kinds of ions to freely enter or leave the axon. This result was published in a brief letter to *Nature* (Hodgkin and Huxley 1939). Soon after these experiments were completed there was a stoppage of research because of the start of World War II during which Huxley worked on anti-aircraft gunnery for the next 5 years. Hodgkin and Huxley (1945) eventually published this work in-full.

Andrew Huxley returned to Cambridge and to research in late 1945/early 1946. He was joined in Alan Hodgkin’s laboratory by Robert Stampfli (1914–2002) from Alexander von Muralt’s institute in Berne. Together they published a series of papers that provided strong evidence for the saltatory conduction of the action potential in single myelinated nerve fibers from the nerves of frogs (Huxley and

²Andrew Huxley is a member of the famous Huxley family. His grandfather was Thomas Henry Huxley, the well known nineteenth century biologist who was Charles Darwin’s “bulldog” (see footnote #4, Chap. 1). Andrew Huxley’s half-brothers were the famous writer Aldous Huxley (1894–1963), author of the book *Brave New World*, and evolutionary biologist Julian Huxley (1887–1975), the first Director General of United Nations Educational, Scientific and Cultural Organization (UNESCO). For a biography on the Huxley family, see Clark (1968).

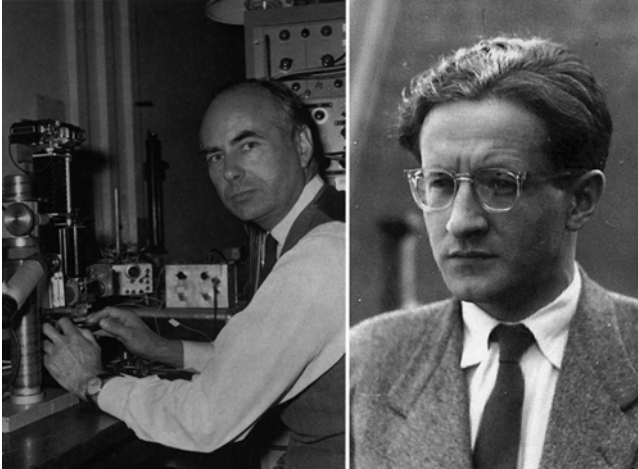


Fig. 2.1 The investigators: Andrew Fielding Huxley (*left*) and Rolf Niedergerke (*right*). Andrew Huxley (1917–2012) received the Nobel Prize along with Alan Hodgkin in 1963 for the elucidation of the ionic mechanism of the nerve action potential. He switched his research field to muscle in 1951 and thereafter made fundamental discoveries relating to muscle structure, activation and cross-bridge function over a period of 40 years. He became Sir Andrew Huxley in 1974. Huxley was the President of the Royal Society (1984–1995) and Master of Trinity College Cambridge (1984–1990). He gave up his laboratory after nearly 60 years of research in 1998. In 2005 the Andrew Huxley building at University College London opened. The building houses researchers from the departments of physiology and pharmacology. He was not related to Hugh Huxley. See an autobiographical sketch (Huxley 2004a). (Photo: Huxley 1974. With permission John Wiley & Sons Inc.) Rolf Niedergerke (1921–2011), born in Germany, came to the Andrew Huxley's laboratory from Berne, Switzerland, in the fall of 1952. After the completion of their collaboration, Niedergerke moved into the cardiac muscle field where he worked for over 40 years as a faculty member in the Department of Biophysics at University College London. His classic paper with Hans-Christoph Lüttgau initiated the study of Na–Ca exchange in cardiac muscle (Lüttgau and Niedergerke 1958). (Photo: courtesy of S. Page)

Stampfli 1949, 1951a, b). Stampfli remembered this collaboration (Stampfli 1992. With permission Cambridge University Press):

...many who had difficulties getting their problem straight used Huxley as a human computer. Working with him was thus a great privilege. Not only I, but Hodgkin and Katz, appreciated his unfailing logic and mathematical talent. On such occasions, Huxley not only proved to be a brilliant thinker, but also showed an amazing knowledge of biology, physics, and chemistry and an excellent memory as well.

Hodgkin and Huxley wanted to test their hypotheses related to the ionic mechanism of the action potential. But there was a major problem. The major problem was that the action potential was changing with voltage and time as it traveled down an axon. During 1948 Hodgkin visited Kenneth S. Cole at the University of Chicago and learned that he and George Marmont had developed promising approaches to solving these problems. Marmont (1949) eliminated the propagation of the action

potential in the giant squid axon by developing a “space clamp” wherein the membrane voltage changes occur over an isolated part of the membrane, thus avoiding the complications introduced by spread of current in a cable-like structure. Cole (1949) succeeded in applying electronic feedback to control the membrane current or voltage, “voltage clamp”, at a fixed value during an action potential [also see Cole (1968) for a historical perspective]. Hodgkin could see that these techniques would allow a test of their ideas about the ionic mechanism of the action potential. Once back in England in 1948, he and Huxley, along with Bernard Katz (1911–2003), made modifications to the voltage clamp technique. In 1949 they performed the experiments elucidating the ionic mechanism of the action potential and the roles of Na^+ and K^+ in squid giant axons. Amazingly the data that led to the 5 classic papers, 128 pages in all, in the *Journal of Physiology* (Hodgkin et al. 1952; Hodgkin and Huxley 1952a, b, c, d), and the eventual Nobel Prize for Hodgkin and Huxley in 1963³, was collected in approximately 1 month on 20 or so squid axons! Hodgkin (1977) believed that they were able to obtain the results so quickly because they had spent a long thinking and making calculations about the kind of system which might produce an action potential of the kind seen in squid nerve. This method of “thinking ten experiments and doing one” was typical of Andrew Huxley’s later approach to muscle research.

After collecting the data in 1 month, it took another 2 years to completely analyze the results. Hodgkin (1977) describes numerous reasons for the delay. One of the reasons was that the Cambridge computer was inoperative for 6 months and Andrew Huxley had to use a hand calculator to solve numerically the nonlinear differential equations used to fit the data for the time course of potential change if there were no feedback. It took up to 3 weeks to generate a simulated propagated action potential! Even though the simulations fit the data beautifully, they were disappointed with the results because no mechanism could be found. Hodgkin (1963) believed that no real progress at the molecular level could be made until much more was known about the chemistry and fine structure of the membrane. So they settled for the “more pedestrian aim” of finding a set of mathematical equations which might plausibly represent the movement of electrically charged gating particles (Hodgkin 1977). Even that was not easy. Their formulation is still considered useful today (see Hille 2001).

There are a number of themes that emerged from this research that influenced Andrew Huxley’s subsequent approach to solving scientific problems. These include: (1) working with the simplest living tissue possible, preferably single fibers, (2) making time resolved measurements, (3) developing new experimental tools needed to do the best possible experiments, (4) thoroughly analyzing data, (5) generating mathematical relationships to quantitatively explain the data, and (6)

³The Nobel Prize in Physiology or Medicine in 1963 was awarded jointly to John Carew Eccles, Alan Lloyd Hodgkin and Andrew Fielding Huxley “for their discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane”. Hodgkin and Huxley did not work with Eccles, an Australian scientist, who investigated the physiology of synapses (Eccles 1964). Hodgkin (1992) has described the “near-miss” of the Nobel Prize in 1962 and the ceremony in 1963.

thinking carefully about the possible experiments and results before actually doing the critical experiment.

Rolf Niedergerke (1921–2011) (Fig. 2.1) joined Andrew Huxley's laboratory in the autumn of 1952 (Niedergerke and Page 1992). He was born in Germany and at the time was working in Alexander von Muralt's Institute in Berne. Huxley had asked Robert Stampfli to recommend someone who would be capable of dissecting single skeletal muscle fibers. Niedergerke had worked previously on single myelinated nerve fibers. Besides having taught himself the dissection of single muscle fibers, Niedergerke introduced Andrew Huxley to the nineteenth century German literature on light microscopy of muscle (Huxley 2004a). This introduction encouraged Huxley to thoroughly evaluate the nineteenth and early twentieth century literature on muscle structure. This evaluation led to his conclusion that much that was known and accepted in the nineteenth century with regard to muscle striations was subsequently forgotten in the twentieth century (see Sect. 1.2 and Huxley 1957, 1977).

Hugh Esmor (H. E.) Huxley (1924–2013) (Fig. 2.2), who is not related to Andrew Huxley, received his undergraduate education in physics at the University of Cambridge (Huxley 1996, 2004b). After service in the Royal Air Force as a radar officer, he started graduate work at the University of Cambridge in the Medical Research Council (MRC) unit for research on the molecular structure of biological systems in 1948. This unit evolved from the famous Cavendish Laboratory headed by the eminent crystallographer W. Lawrence Bragg and eventually became in 1962 the world famous MRC Laboratory of Molecular Biology which by the early twenty-first century could claim 13 Nobel Prize winners and 14 Nobel Prizes (Fred Sanger won two Nobel Prizes). During Hugh Huxley's time as a graduate student, the laboratory members included John Kendrew (Huxley's Ph.D. advisor), Max Perutz, Francis Crick and James D. Watson. It must have been an incredibly stimulating atmosphere as all four of these scientists would go on to win Nobel Prizes in 1962⁴. Whereas no doubt a stimulating environment, it was not always pleasant to have lunch with Francis Crick who was notorious for challenging the ideas of colleagues. Watson (1968) remembered that Hugh Huxley found it difficult to enjoy Crick's continuous "inquisitive lunchtime attacks". Hugh Huxley was totally immersed in the Cambridge scientific environment from 1948 to 1987 with the exception of a few crucial years on leave at MIT (1952–1954) and time at University College London (1955–1962).

⁴The Nobel Prize in Chemistry in 1962 was awarded jointly to Max Ferdinand Perutz (1914–2002) and John Cowdery Kendrew (1917–1997) "for their studies of the structures of globular proteins". Perutz solved the so-called phase problem and this solution proved to be the breakthrough that opened up the whole field of protein crystallography. Perutz elucidated the 3D structure of hemoglobin and Kendrew the 3D structure of myoglobin. The Nobel Prize in Physiology or Medicine in 1962 was awarded jointly to Francis Harry Compton Crick (1916–2004), James Dewey Watson (b. 1928) and Maurice Hugh Frederick Wilkins (1916–2004) "for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material". They discovered the famous double helix of DNA. See Watson's (1968) entertaining account of the race to the double helix.

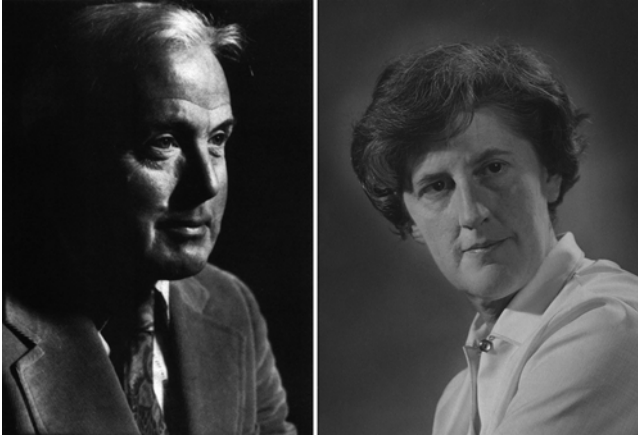


Fig. 2.2 The investigators: Hugh Esmor Huxley (*left*) and Jean Hanson (*right*). Hugh Huxley (1924–2013) investigated the structural mechanism of skeletal muscle contraction for 60 years. He pioneered many advances in utilization of X-ray diffraction and electron microscopy, including ultrathin sectioning and negative staining, for the study of muscle contraction. He was the first person to propose the sliding filament mechanism of muscle contraction (Huxley 1953b) and the titling cross-bridge theory of contraction (Huxley 1969). His demonstration of “arrowheads” in “decorated” thin filaments (actin filaments plus myosin in the absence of ATP) (Huxley 1963) helped open up the field of nonmuscle motility. After retirement from the MRC Laboratory of Molecular Biology in Cambridge in 1988, he continued his research at the Rosenstiel Basic Medical Sciences Research Center, Brandeis University. A fellow of the Royal Society at the age of 36, he received the Copley Medal, the society’s highest honor, in 1997. He was elected into the National Academy of Sciences in 2003. (Photo: Huxley 1996. With permission Annual Reviews.) Jean Hanson (1919–1973) pioneered the utilization of phase contrast microscopy to investigate structural changes in contracting myofibrils. She began her collaboration with Hugh Huxley when they were both at MIT in 1953. They collaborated from 1953 to 1960, alternating first authorship on each publication. Thereafter she concentrated on investigating the generality of the sliding filament model of contraction by examining insect and smooth muscles. Along with Jack Lowy, she elucidated the first structure of actin filaments (Hanson and Lowy 1963). Hanson became director of the Biophysics Research Unit at King’s College London in 1970 and was at her scientific peak when in 1973 she died suddenly of a meningococcal infection. See a biographical memoir by Randall (1975, photo) and papers in her honor in the *J Muscle Res Cell Mot.* 25: 2004. Photo: with permission of The Royal Society)

For his graduate student research, Hugh Huxley employed the X-ray diffraction technique to examine the structure of isolated living skeletal muscle. Earlier work by Astbury (1947) utilized wide angle X-ray diffraction to examine muscle structure at the level of a few Angstroms. In contrast Huxley employed low or small angle X-ray diffraction to probe muscle structural repeats in the 100–400 Å range. Since the diffraction angles were small⁵ and thus the reflections of interest were close to

⁵The X-ray diffraction pattern is recorded in reciprocal space which means that reflections farther from the origin (wide angle reflections) are due to repeating structures that are close together and reflections near the origin (low or small angle reflections) are due to repeating structures that are further apart.

the undiffracted X-ray beam, a narrow slit in the X-ray camera was required to be able to measure intensities and positions close to the undiffracted beam. This criterion created problems with low X-ray intensity, especially with hydrated biological specimens, like muscle. The net effect is that it required many hours, sometimes days, of illumination to get sufficient intensity of the diffracted X-rays. Thus results were limited to states in which the muscle would be stable for long periods of time, i.e., resting muscle and muscle put in rigor. Huxley was able to record a diffraction pattern from live relaxed muscle isolated from frogs in a few hours for equatorial patterns and a couple of days for axial patterns. Equatorial reflections arise from transverse structural repeats in the muscle and axial reflections (also called meridional reflections) are due to structures that repeat along the length of the muscle. Huxley was unsuccessful in obtaining results from contracting muscle and indeed it would be years before X-ray intensity was sufficient to probe changes in the intensity and positions of reflections associated with muscle contraction (see Chaps. 3 and 6).

On the equator in resting muscle from the frog there were reflections whose relative spacings and intensities suggested that they came from a hexagonal array of rods about 450 Å apart (Fig. 2.3, bottom) (Huxley 1951, 1953a). Huxley speculated that these rods were composed of myosin molecules. On stretching the resting muscle, the transverse distance between rods decreased according to the inverse square root of the muscle length as would be expected from the known constant volume properties of muscle. A X-ray diagram from a muscle in rigor (either from frog muscle or Szent-Gyorgyi's glycerinated rabbit psoas muscle) showed about the same lattice spacings as in the resting muscle but very different relative intensities of the first two lines of the pattern (Fig. 2.3, top) (Huxley 1953a). Huxley speculated that the pattern could be accounted for by the presence of a second set of filaments composed of actin, located at the trigonal positions of the original lattice. The idea was that the existence of myosin-actin linkages in the absence of ATP stabilized the secondary array of filaments in the interstices of the primary array, thus enabling them to be detected by the X-ray diffraction method. This was an important observation and the first time that changes in the X-ray pattern could be related to a change in the state of the muscle (rest to rigor). Based on the early electron microscopic observations of muscle (Hall et al. 1946), Huxley assumed that both sets of filaments ran the total length of the sarcomere.

In contrast the axial X-ray patterns showed a pattern of reflections based on an approximately 420 Å axial repeat which remained unchanged in rigor. Intriguingly, the axial period did not change when the relaxed muscle was stretched. This work which appeared in abstract form (Huxley 1951, 1953a) and in his Ph.D. dissertation was never published in full because the intervening work at the Massachusetts Institute of Technology with Jean Hanson was all-consuming (see below).

The X-ray diffraction technique has both major advantages and disadvantages. A major advantage is that the muscle structure is probed in a living state, at least at rest, whereas electron microscopy requires fixation, staining and embedding which could lead to artifactual changes in structure. In a sense the X-ray technique provides a control for these possible artifacts. There are two major disadvantages.

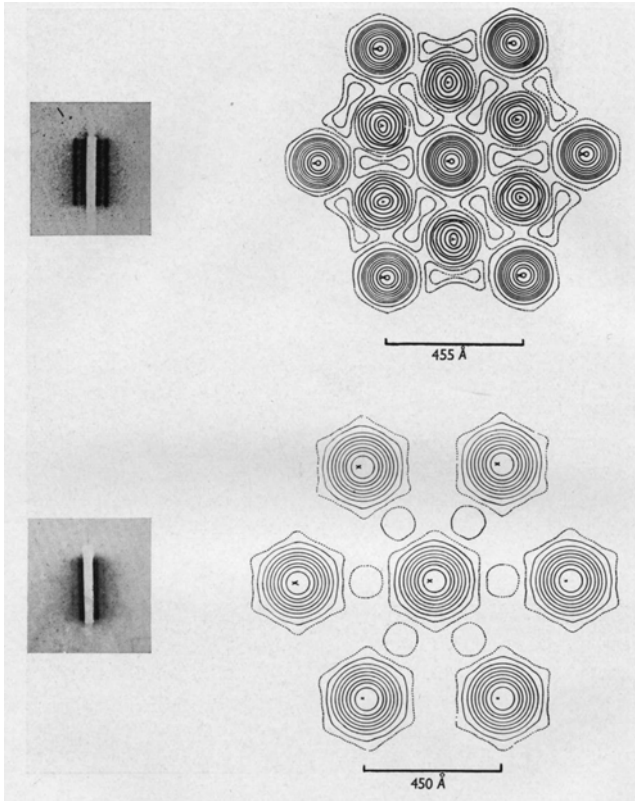


Fig. 2.3 Low angle X-ray diffraction diagrams of muscle at rest or in rigor. Reflections and electron-density distribution from: *Bottom*: resting living muscle isolated from frog and *Top*: glycerol-extracted psoas muscle from the rabbit in rigor. On *left*: transverse (equatorial) reflections. Muscle axis vertical. Two reflections (the one nearer to origin is designated 10 and the outer one 11) are visible as closely spaced vertical lines on either side of the center slit. These reflections are expected from a hexagonal lattice of filaments separated by about 450 Å. On *right*: possible electron-density distributions in the fiber, seen end-on, based on X-ray reflections. In the resting muscle the primary hexagonal array of filaments is visible and the region in between the filaments is of rather uniform density, suggesting that the material there is randomly arranged. In the rigor muscle, the lattice dimensions are not significantly different, i.e., the reflections are in the same position, but the 11 reflection is now much more intense. This result strongly suggested the presence of a second set of filaments (seen end-on) possibly occurring at specific sites in between the basic hexagonal array of primary filaments (Hanson and Huxley 1955. With permission Elsevier)

The first is the long exposure times required to observe a pattern which, at that time, precluded measurements on contracting muscle. The second disadvantage, at least to the outsider, is the “enigmatic” nature of the X-ray pattern. One measures spacings and intensities and then must deconvolute this information by building a molecular model that can reproduce the observed X-ray pattern. Clearly direct observation by electron microscopy would greatly enhance the conclusions reached

from the X-ray data. In order to learn the electron microscopic technique, Hugh Huxley took a temporary leave of absence from Cambridge and went to Francis O. Schmitt's laboratory at the Massachusetts Institute of Technology in September of 1952 for 2 years.

Throughout his on-going 60 year career, Hugh Huxley has pioneered improvements in the X-ray diffraction and electron microscopic techniques in search for the structural mechanism of striated muscle contraction.

Jean Hanson (1919–1973) (Fig. 2.2) was trained as a zoologist. She joined the Biophysics Research Unit at King's College, London, in 1948 (Randall 1975). Schick and Hass (1950) and Perry (1951) had recently shown that it was possible to isolate myofibrils, from mammalian skeletal muscle, which exhibited unimpaired function (ATPase activity) and normal structure (striation pattern). Hanson (1952) employed phase contrast microscopy⁶, which was a relatively new technique to biology at that time, to examine changes in the striation pattern of these "living" skeletal muscle myofibrils. These myofibrils isolated from various skeletal muscles were "living" in the sense that they were not fixed or stained as was the convention for microscopic observation at that time. Also the myofibrils were excellent microscopic objects since they were only 1–2 μm in diameter. She examined, in a phase-contrast microscope, the changes in band-pattern that took place when myofibrils contracted during treatment with ATP. The experiment was ingeniously simple: a drop of dilute ATP solution was placed at one edge of a coverslip and drawn through the preparation by means of filter paper placed at the opposite edge. The myofibril contracted slowly (about 10 s) to about 60 % of its original length. Before treatment, the myofibril had well-marked A and I bands (respectively black and white in positive phase-contrast illumination), with black Z lines (2.6 μm apart), and with a white line in the middle of each A band. During the earliest phase of contraction, the I bands rapidly disappeared; the myofibril became uniformly dark grey in color and no bands could be distinguished in it. Then a series of sharply defined black lines, the contraction bands, appeared. The zones between the contraction bands became progressively paler, and further contraction brought the contraction bands closer together. The fully contracted myofibril had a simple pattern of narrow contraction bands, 1.5 μm apart.

⁶Phase contrast microscopy. There is little absorption of light rays passing through living cells and thus they are essentially transparent. The cells do contain constituents that exhibit small differences in refractive index. These inclusions do not affect the amplitudes of the light rays but do cause the light waves to differ in phase according to the path that they have taken through the cell. The image formed by such rays consists of a pattern of phase differences of uniform brightness, and as such is essentially invisible. Frederick Zernike, of Groningen, produced a visible image in these circumstances by deliberately advancing or retarding the main beam, after it traversed the specimen, by one-quarter of a wavelength, without disturbing the diffracted rays. Consequently, when the whole beam was reunited, conditions for interference existed, and the transparent specimen produced an image where refractive index differences are now observed as differences in transparency. Thus changes in phase became changes in intensity. Zernike received the Noble Prize for this discovery in 1953. For more information on phase contrast microscopy, see Slayter (1976).

Even though Jean Hanson was an expert using phase contrast microscopy to examine muscle striations, she, like Hugh Huxley, wanted to extend her vision of muscle by learning electron microscopy. Thus Jean Hanson also went to Francis Schmitt's laboratory in February of 1953. There she met Hugh Huxley for the first time and a remarkable collaboration began. Hugh Huxley saw the banding pattern of muscle for the first time while looking through the microscope at Jean Hanson's myofibrils. It wouldn't be the last time.

Thus we see that the investigators came from very different scientific backgrounds and brought different but in the end complementary approaches to the problem of measuring and interpreting the changes in the striation pattern during skeletal muscle contraction and stretch.

2.3 Overlapping Arrays of Filaments and the First Proposal of Sliding Filaments

The first paper from the Hugh Huxley and Jean Hanson collaboration appeared in September of 1953 in *Nature* (Hanson and Huxley 1953). It was in this paper that the first evidence was provided for the overlapping arrays of filaments containing actin and myosin. Using phase contrast and polarized light microscopy and electron microscopy, they examined structural changes in myofibrils at various stages of extraction of myosin. In isolated myofibrils irrigated with a myosin extraction solution containing 0.6 M KCl the "A-substance" disappeared in 1–2 s. This result was confirmed with electron microscopy as the thick filaments were no longer visible and only thin filaments remained in the A band. From these results, they concluded that myosin is primarily concentrated in the A band in muscle and that it is responsible for the high density and the birefringence of the A bands and furthermore that actin is present as long filaments which extend continuously through the A and I bands. That myosin was concentrated in the A band and accounted for the myofibril birefringence was a confirmation of earlier, more indirect, studies (see Sect. 1.4). This observation also eliminated the possibility that myosin filaments extended the whole length of a sarcomere as was earlier proposed. Thus there are two sets of filaments and these myosin and actin filaments overlap. This was a crucial observation.

The official birth of the sliding filament model of muscle contraction is associated usually with the classic papers of Huxley and Niedergerke (1954) and Huxley and Hanson (1954). In fact the model was first proposed by H. E. Huxley in the August of 1953 (Huxley 1953b) based on electron micrographs generated in F. O. Schmitt's laboratory. Huxley employed a thin sectioning technique developed in the Schmitt laboratory by Alan Hodge, David Spiro and himself (Hodge et al. 1954). Huxley examined transverse sections of frog sartorius and rabbit glycerinated psoas muscle in the electron microscope. He described what he called "a most remarkable compound array of filaments". Two different types of filaments were present. The

larger filaments formed a very regular hexagonal array. They were spaced 200–300 Å apart⁷ and their diameter was about 110 Å. The smaller filaments, whose diameter was about 40 Å were also arranged in a regular manner. Each one was located symmetrically in between three of the primary filaments, which it shared with the six nearest neighbors. In the H zone a simple hexagonal array of filaments was observed and no secondary filaments were ever observed. Huxley also claimed to observe “bridges” extending between the filaments but the results are unconvincing on this point. Thus the electron microscopy results confirmed Huxley’s speculation based on the X-ray diffraction results that there are two arrays of filaments. Furthermore these results and those of Hanson and Huxley (1953) indicated that these filaments *partially overlapped* rather than running the whole length of the sarcomere as Huxley and others had previously assumed.

In the last paragraph of the discussion, Huxley (1953b) introduced the concept of sliding filaments into the literature for the first time (Huxley 1953b. With permission Elsevier):

This phenomenon finds a ready explanation in terms of the arrangement of actin and myosin filaments described above, if it is postulated that stretching of the muscle takes place, not by an extension of the filaments, but by a process in which the two sets of filaments slide past each other; extensibility will then be inhibited if the myosin and actin are linked together. In terms of the distribution of actin and myosin described above, this process clearly involves ‘I-band filaments’ being pulled out of the A-band during stretch...It is not considered appropriate to discuss here the various models which may be devised to describe the details of such a mechanism...However, one may note the possibility that an analogous process is involved in contraction.

With regard to the statement that it is *not considered appropriate* to discuss various models of contraction, Huxley (2008) has said: “Our otherwise very amiable and supportive department head at MIT, Professor Schmitt, remained quite skeptical and forbade us to say anything about possible contraction mechanism in our 1953 paper about the overlapping filament model-‘Do not spoil a good experimental paper with a lot of speculation!’” (Huxley HE 2008. With permission Elsevier) Thus Huxley only noted the “possibility that an analogous process is involved in contraction” in the last line of the paper. From a historical point of view, this restriction might have resulted in an unintended injustice to Hanson and Huxley. Nonetheless these fundamental observations and this hypothesis strongly influenced the thinking of Hugh Huxley and Jean Hanson as they devised future experiments with the phase contrast and electron microscope to test this hypothesis.

Over 50 years later, Hugh Huxley expressed some regret that Jean Hanson’s name wasn’t associated with the first proposal of the sliding filament model of contraction. Huxley (1996) stated: “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.”

⁷Hugh Huxley noted (1953b) that the differences in spacings of the elements in the hexagonal array as observed in the X-ray pattern (450 Å) of living muscle isolated from frogs and in the electron micrographs (200–300 Å) must be indicative of shrinkage of the tissue in preparation for electron microscopy.

(Huxley 1996. With permission Annual Reviews) It is clear that they were equal partners in the development of the hypothesis and collecting supportive data. In support of this conclusion, they had agreed to alternate first authorship on successive joint papers (Huxley 2008). Their research collaboration resulted in six major publications (four with original research and two major reviews) from 1953 to 1960 with first authorship alternated on successive publications.

So it is clear that before the publication of the classic 1954 papers, Hanson and Huxley had the idea of partially overlapping, sliding filaments already in their mind. Andrew Huxley and Rolf Niedergerke also came to the same conclusion in 1953.

2.4 Andrew Huxley and the Development of an Interference Microscope

After analyzing and publishing the work with the voltage clamp, Andrew Huxley (2004a) could not see how to carry the analysis of excitation and conduction to a deeper level. He and Hodgkin predicted the existence of “gating currents”, currents that would control ion permeability, but could not detect them⁸. So Huxley was looking for new experimental challenges. He became interested in muscle after giving lectures to first-year students in Trinity College in 1948. More accurately he became interested in the light microscopy of muscle. It was the so-called “reversal of striations” and formation of “contraction bands” observed with muscle shortening described by the nineteenth century microscopists that caught his attention. Huxley (2004a) thought that this observation might give a clue to the mechanism of contraction. Plus it was of interest to him because of his interest in microscopy.

The challenge as Huxley saw it was to determine the changes in the striation pattern of living, vertebrate skeletal muscle fibers during activation, force development and shortening. The experimental preparation of choice was the single skeletal muscle fiber of the frog for two primary reasons. First, much was known about the mechanical and energetic properties of frog skeletal muscle from the work of Hill (1965) and others. Second, and possibly more important, Frank W. Ramsey and his wife Sibyl F. Street (1940) showed that it was possible to dissect frog muscle fibers in the living state and do elegant mechanical experiments with them.

These proposed experiments presented numerous challenges but foremost in Huxley’s mind (Huxley 2004a) was the fact that with the ordinary light microscope it was virtually impossible to obtain a satisfactory image of the refractive index differences associated with the striations of frog muscle fibers since these fibers exhibit large diameters ranging from about 50 to more than 100 μm . Polarized light microscopy would give a satisfactory image but the nineteenth century work had shown that the phenomenon of reversal of striations does not show up with polarized light. Phase

⁸The gating current was not actually detected until the 1973 by C. M. Armstrong and F. Bezanilla in sodium channels of the squid giant axon and by M. F. Schneider and W. K. Chandler (1973) in frog skeletal muscle excitation-contraction coupling.

contrast microscopy shows refractive index differences well on thin specimens, like the myofibrils employed by Jean Hanson and Hugh Huxley, but not on thick specimens such as frog muscle fibers. Andrew Huxley concluded that what was needed was an interference microscope. He envisioned an interference microscope in which the light that passed through the muscle fiber was combined with coherent light that had bypassed the fiber; the path differences due to the refractive index differences in the fiber would then be converted to intensity differences by interference and could be observed unambiguously by eye or recorded photographically. It is clear that a main attraction to this aspect of muscle research was that it allowed Andrew Huxley the opportunity to pursue his deep interest in light microscopy, an interest that he had harbored since boyhood. So Andrew Huxley, the optics expert and the wizard at solving experimental problems, decided to build his own interference microscope.

The high powered version of this interference microscope (Huxley 1954) was operational when Rolf Niedergerke arrived in Cambridge in the autumn of 1952. During the months of March, 1953 to January, 1954 Huxley and Niedergerke did the experiments that constituted the results that appeared in the classic 1954 paper in *Nature* (Huxley and Niedergerke 1954) and in the later full publication (Huxley and Niedergerke 1958). In March of 1953 they made a cine film of muscle fiber shortening in response to a slowly increasing current (Huxley 1977). During the shortening, the A band remained at a constant length and contraction bands appeared. Huxley has described what they saw (Huxley AF 1977. With permission Cambridge University Press):

The contraction bands we were looking for did appear, but not where we expected: as the fibre shortened below its slack length the first 'contraction band' to appear was a narrow dense line at the middle of the A band, not opposite the middle of I. On more extreme shortening, however, a second set of dense lines did appear at the latter position. These bands would be nicely explained if, in addition to the rodlets needed to explain the constancy of the A-band width, there was a second set of filaments in each repeat of the striation pattern, crossing the I band and overlapping with the A-band rodlets. The first set of dense lines would then be due to collision between successive sets of these I filaments, and the second set of dense lines would be due to collision between successive sets of A-band rodlets.

This film suggested to Huxley and Niedergerke that muscle shortening occurred via a sliding filament system. Thus Hugh Huxley, Jean Hanson, Andrew Huxley and Rolf Niedergerke were all thinking in terms of muscle shortening occurring via a sliding filament system before the summer of 1953. But neither group was aware of the results of the other group until the summer of 1953.

During that summer, Hugh Huxley and Jean Hanson exchanged experimental results and interpretations with Andrew Huxley when they met for the first time at the Marine Biological Laboratory at Woods Hole. Andrew Huxley spent the summer of 1953 at the Marine Biological Laboratory at Woods Hole. There he learned of the experiments of Hasselbach (1953) who had dissolved away the myosin from fragmented muscle and examined the residue with the electron microscope, finding that the actin was in the form of filaments held together at their centers by the Z line. This result suggested to Andrew Huxley that the second set of filaments that he and Niedergerke postulated were composed of actin. Later during that visit, he met Hugh Huxley and Jean Hanson. He told them of their observations with the interference

microscope and the idea that length changes in muscle took place by relative sliding movements of two interdigitating sets of filament. They showed him the electron micrographs of transverse sections of muscle that established the existence of two sets of filaments that subsequently were published by Huxley (1953b) later that same year with the brief mention of the sliding-filament theory. They agreed to communicate again when papers were nearing the publication stage (Huxley 1977) and in 1954 agreed to publish the papers together in the journal *Nature*.

2.5 Birth of Sliding Filaments: *Nature*, Volume 173, Pages 971–976, May 22, 1954⁹

The investigators, their backgrounds, experimental tools and experimental evidence generated before 1954 have been described. Now it is time to consider the two classic papers that represent the “birth” of the sliding filament model of muscle contraction. But in what order should the papers be considered? Surely it makes little difference. Just as it makes little difference in what order the papers were published in *Nature* in 1954 since they were published back-to-back. But it turned out that the order of publication made a great deal of difference to Hugh Huxley and Jean Hanson. Many years later Hugh Huxley remembered (Huxley HE 2008. With permission Elsevier):

Since we had already published the essential first part of the story there, and since that paper was a direct extension of our earlier published work (including X-ray and e-m evidence), we rather naturally assumed that our paper would lead. In the event, the paper by A. F. Huxley and R. Niedergerke came first, whereas ours seemed (to us) to be tacked on as supporting evidence. Presumably ‘*Nature*’ decided that observations on intact muscle fibers would trump ones made on isolated myofibrils contracting in ATP, irrespective of previous publication history. This seemed an important matter at the time, and was somewhat of a disappointment for us. We were pleased to have our ideas confirmed by the intact muscle data.

In a sense this discussion relates to the one considered in Chap. 1 concerning the relevance of data collected from “dead” muscle pieces, i.e., myofibrils, to that collected from intact, living muscle. Nonetheless history has declared that because the data and conclusions in the two papers so strongly reinforced each other that the order of publication or discussion makes little difference. In fact we will go from Andrew Huxley/Rolf Niedergerke to Hugh Huxley/Jean Hanson not because one paper is more important than the other but because the observations on living muscle in the first paper were extended in the second paper on myofibrils in crucial ways that led to a deeper understanding of and support for the basic hypothesis.

⁹In these classic papers, Andrew Huxley and Rolf Niedergerke referred to the “widths” of the A and I bands whereas Hugh Huxley and Jean Hanson referred to the “lengths” of the A and I bands. In this context “width” and “length” meant the same thing. The quotations from these papers will be unchanged but elsewhere in the chapter, reference will be made to the “lengths” of the A and I bands. Also the H band and H zone were sometimes referred to interchangeably. We will refer to the H zone.

2.6 Interference Microscopy of Living Muscle Fibres by A. F. Huxley and Dr. R. Niedergerke. *Nature*. 173: 971–973, 1954

The rationale for the experimental approach in this first publication by Huxley and Niedergerke in the muscle field has been described. Huxley and Niedergerke chose to employ interference microscopy to monitor changes that might occur in striation spacing during passive stretch, activation, isometric contraction, and isotonic contraction of single muscle fibers, diameters 30–80 μm , isolated from frogs. To make the time resolved measurements of striation spacing, they employed cine photography with light flashes that were synchronized with the stimulation pulses that occurred at intervals of about 20 ms. These were technically demanding experiments since the experiments were conducted at room temperature where the isometric twitch reached a peak tension in about 40 ms (Huxley and Niedergerke 1958).

Passive stretch When the sarcomere length of the fiber was changed either slowly or rapidly from about 2.0–4.2 μm almost the whole of this change of length took place in the I-bands (Fig. 2.4). The length of the A band remained constant at 1.4–1.5 μm except for a fall to about 1.3 μm as the sarcomere length was reduced in the range of 2.5–2.0 μm . Huxley and Niedergerke felt that this fall may not be real since its amount was less than the resolving power of the optical system.

Isometric twitches No changes were observed in the lengths of the A and I bands during the time course of an isometric contraction.

Isotonic contractions During active muscle shortening in an isotonic twitch or short tetanus starting at an initial length of about 3.2 μm , the I band became narrower and the width of A band remained constant down to a sarcomere length of 2.5 μm and thereafter falling slightly down to a sarcomere length of 2.0 μm (Fig. 2.5). On further shortening below 2.0 μm , Huxley and Niedergerke observed a shortening of the A bands in all cases but there were additional phenomena which were not the same in every experiment. The full meaning of the statement that the phenomena were not the same in every experiment becomes clearer in the full publication of this work that appeared in 1958. This point will be taken up under “*loose ends*” below.

Summary and conclusions In summary these results showed that the changes in the ratio of widths of the A and I band depend simply on the length of the fiber and are unaffected by activation or by tension development. Huxley and Niedergerke went on to conclude (Huxley and Niedergerke 1954. With permission Nature Publishing Group):

The natural conclusion, that the material which gives the A-bands their high refractive index and also their birefringence is in the form of submicroscopic rods of definite length, was put forward by Krause, and receives strong support from the observations reported here. The identification of the material as myosin (Hasselbach 1953 and Hanson and Huxley 1953), and the existence of filaments (presumably actin) extending through the I-bands and into the adjacent A-bands, as shown in many electron microscope studies, makes very attractive the hypothesis that during contraction the actin filaments are drawn into the A-bands,

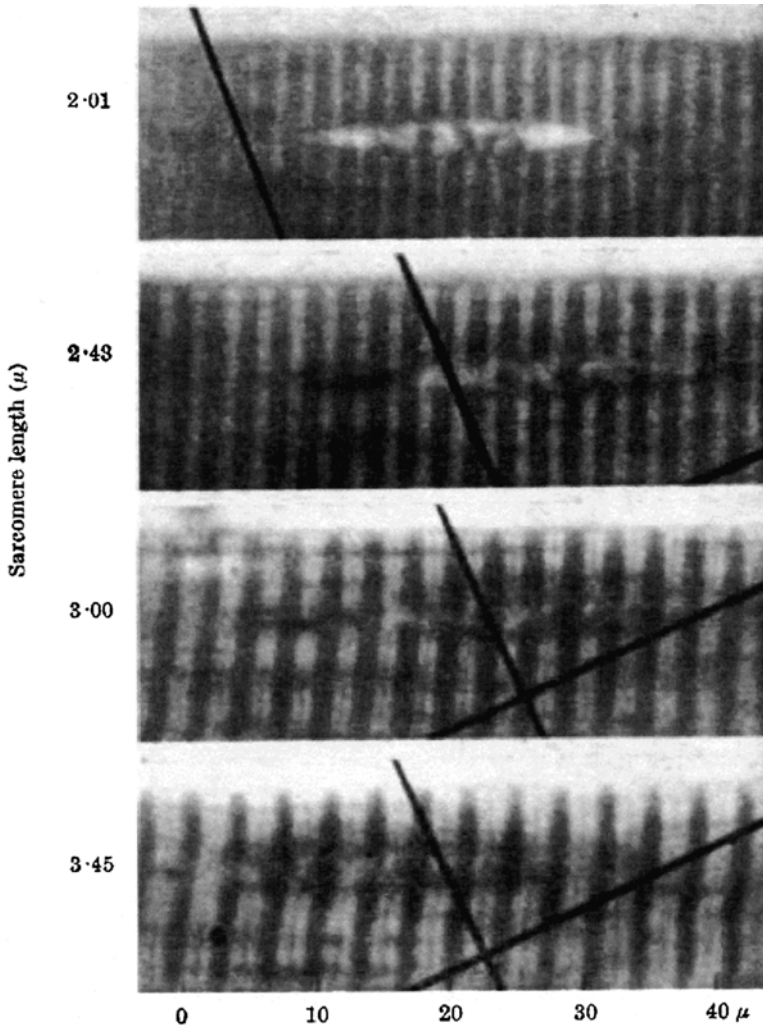


Fig. 2.4 Changes in the band pattern during passive stretch of a single living muscle fiber. Sarcomere lengths are indicated beside the photographs. Almost all the change of sarcomere length is in the I band (*light*) with little change in A band (*dark*) length (Huxley and Niedergerke 1954. With permission Nature Publishing Group)

between the rodlets of myosin. (This point of view was reached independently by ourselves and by H. E. Huxley and Jean Hanson in the summer of 1953. It has already been mentioned by one of those authors [Huxley, 1953] and is further discussed by them in the accompanying article.)

Thus Andrew Huxley and Rolf Niedergerke and Hugh Huxley and Jean Hanson independently and essentially simultaneously developed the concept of the sliding filament model of muscle contraction. But Andrew Huxley and Rolf Niedergerke

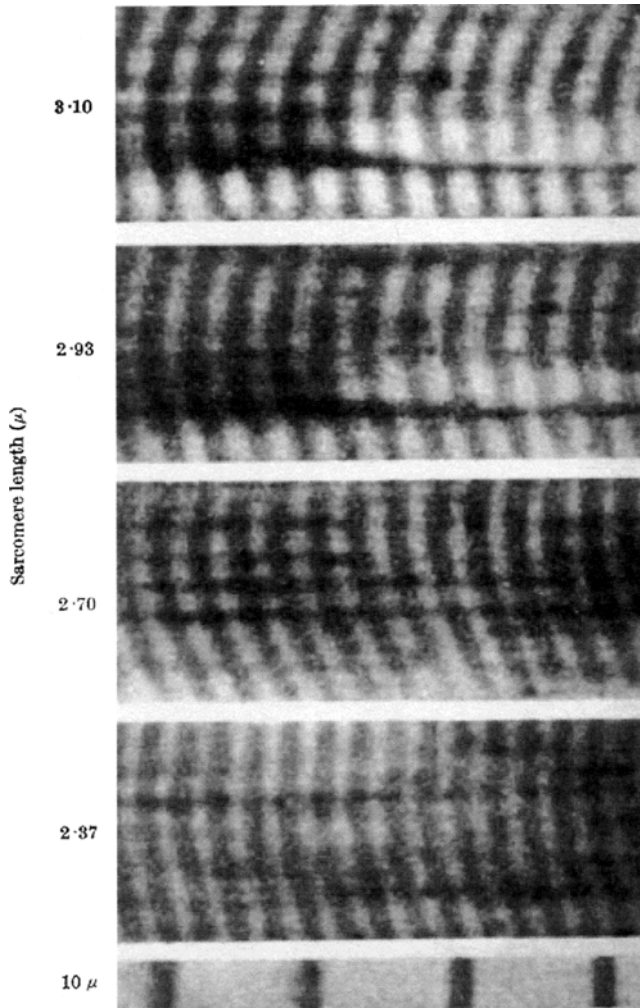


Fig. 2.5 Changes in the band pattern when a single living muscle fiber shortens during a brief isotonic tetanus. Sarcomere lengths shown beside the photographs. As observed with passive stretch (Fig. 2.4) the A bands (*dark*) remain almost constant in length as the muscle fiber shortens (Huxley and Niedergerke 1954. With permission Nature Publishing Group)

clearly acknowledged that Hugh Huxley was the first to propose in the literature a sliding filament model of muscle contraction (also see Huxley 1977).

Predictions They went on to make three important predictions concerning muscle behavior if the sliding filament model is correct (Huxley and Niedergerke 1954). The first prediction was the most crucial prediction. If a relative force between actin and myosin is generated at each of a series of points in the region of overlap in each sarcomere, then the tension per filament should be proportional

to the number of these points, and therefore to the width of the zone of overlap. If the myosin rods are $1.5\ \mu\text{m}$ long and the actin filaments $2.0\ \mu\text{m}$, the isometric tetanus tension should fall linearly as the fiber is stretched over the range of sarcomere lengths from 2.0 to $3.5\ \mu\text{m}$. This prediction was in “fair agreement” with observation. The “fair agreement with observation” refers to the experiments of Ramsey and Street (1940) who observed a linear fall of isometric force as a muscle fiber was stretched beyond rest length. But Huxley and Niedergerke no doubt realized that the “fair agreement” may not be a precise agreement. In fact analysis of the mechanical results of Ramsey and Street, who did not measure sarcomere lengths, suggested that a muscle fiber may still generate appreciable isometric tension at lengths beyond $3.5\ \mu\text{m}$. This discrepancy was real and it turned out to be associated with a difficult technical problem that was solved in two classic papers by Gordon et al. (1966a) (see Chap. 3).

The second prediction was that the speed of contraction should be correlated with the resting sarcomere length. Muscles with long sarcomeres should shorten more slowly than muscles with short sarcomeres. This would be expected if the relative sliding velocity between actin filaments and myosin rods in any one zone of overlap was the same for muscles of different sarcomere lengths since the number of sarcomeres shortening in series per unit length is inversely proportional to sarcomere length. This prediction has not been adequately tested, in part, because only later was it realized that myosin molecules came in various isoforms that split ATP at different rates and thus resulted in different velocities of muscle shortening (Barany 1967). The third prediction was that a muscle with longer sarcomeres would be capable of producing a greater isometric tension. This prediction is based on the idea that there would be more overlap of thick and thin filaments and thus more tension generating structures in parallel which would lead to greater tension development. This prediction has been verified in arthropod muscles which exhibit long sarcomeres and thus increased overlap of thick and thin filaments (Jahromi and Atwood 1969).

Loose ends The full publication of these results didn't appear until 1958 (Huxley and Niedergerke 1958). Whereas it isn't completely clear why there was such a long delay between the initial publication in *Nature* and the full publication in the *Journal of Physiology*, long delays to full publication would be a recurring characteristic of the Huxley laboratory. Numerous reasons may have contributed to the delay in publication. First, there were other important experiments on-going [see Huxley and Taylor (1958) in Chap. 4]. Second, considerable time was spent developing an important theoretical model of muscle contraction (Huxley 1957). Third, the analysis of the data was exceedingly thorough with micro-densitometer tracings of the photographic negatives to obtain an unbiased measure of band lengths. Finally there were some loose ends.

These experiments were originally planned to determine whether contraction bands (and reversal of striations) were formed and if so under what conditions. As it turned out their results were equivocal on this point. When fiber shortening was induced by a current that increased gradually over a period of a few seconds, two different types of changes were observed on different occasions. In March and April

of 1953, upon shortening from 2.0 to 1.8 μm , the A bands became gradually shorter until they appeared as thin lines and then on shortening to about 1.7 μm another thin dense line (corresponding to a contraction band) appeared midway between each two members of this series. These results suggested a sliding filament model of contraction, i.e., the first set of dense lines being formed by folding of the ends of the secondary filaments when they meet at the centers of the A bands and the second set (contraction bands) by folding of the primary filaments when they meet at the Z lines (Huxley and Niedergerke 1958).

But in later experiments (January 1954), the ratio between the lengths of the A and I bands did not change noticeably as the sarcomeres shortened from 2 μm down to 1.5 μm (Huxley and Niedergerke 1958). In other words the second series of experiments indicated a progressive shortening of the A bands without disappearance of the I bands, a result inconsistent with the sliding filament model. This discrepancy must have been very troubling to them but since they couldn't see how either experiment was wrong, they had no choice but to report both results in the full 1958 paper even though they were in conflict with each other. They concluded that the formation of contraction bands was observed only in extreme shortening, and then only occasionally.

It was only some years later that Andrew Huxley and postdoctoral fellow Albert M. Gordon (1962) discovered the reason for this discrepancy. With the slowly increasing current of stimulation, the myofibrils near the surface of the fiber remained straight while shortening whereas the myofibrils in the interior became wavy in appearance. In the straight fibrils the contraction-band pattern developed, while in the wavy fibrils narrow I bands remained and the A bands shortened progressively. It was concluded that the contraction-band pattern corresponded to active shortening whereas the pattern with shortened A band corresponded to passive shortening due to contraction of the surface myofibrils. Thus they concluded that these results supported the sliding-filament theory of muscular contraction. Nonetheless there is still some mystery related to the original results. Why did Huxley and Niedergerke get the correct answer in March and April of 1953? Many years later, Andrew Huxley (1977) commented that there was still no explanation why that preparation in early 1953 gave contraction bands, indicating active shortening, over its whole cross-section while later preparations appeared to contract only very locally unless they gave action potentials. If the January 1954 results were obtained in March and April of 1953, Andrew Huxley would have gone to Woods Hole in the summer of 1953 with a very different interpretation of the band pattern changes with extreme muscle shortening than that reached by Hugh Huxley and Jean Hanson and history might have turned out differently.

With regard to the sliding filament model of contraction, Huxley and Niedergerke (1958) emphasized that their results established a number of points relevant to the theory of contraction which could only be demonstrated on whole fibers. These points included: (a) the constancy of A band length, and other changes of the striation pattern required by the theory, were demonstrated in fibers in which the contractile system was in a normal condition, (b) the A band length was shown to be

independent not only of fiber length but also of tension and of activation in twitches and tetani and (c) the A band length was shown to be unaffected by rapid stretch.

Thus living muscle fibers exhibited changes in the striation pattern with changes in length that were consistent with a sliding filament model of contraction. But were these results sufficient to prove the correctness of the proposed model? The results were certainly consistent but not really sufficient to prove the theory. There was a crucial missing piece to the puzzle. Huxley and Niedergerke made no comment about changes in the H zone length with changes in fiber length in their 1954 paper but did state in the 1958 paper that the H zone was not sufficiently sharply outlined to justify measurement. If there is truly relative sliding of filaments with changes in muscle length, there must be predictable changes in the length of the H zone. Hugh Huxley and Jean Hanson were very aware of this fact and explored this point, and many others, carefully.

2.7 Changes in the Cross-Striations of Muscle During Contraction and Stretch and Their Structural Interpretations by Dr. Hugh Huxley and Dr. Jean Hanson. *Nature*. 173: 973–976, 1954

Hugh Huxley and Jean Hanson employed the isolated myofibril preparation that she had developed earlier (Hanson 1952) and that they were currently using (Hanson and Huxley 1953). With their 2 μm diameter, the rabbit myofibrils were ideal objects for phase contrast microscopy. The contraction of the myofibrils in the presence of low levels of ATP was much slower than would have occurred in intact muscle and thus allowed them to photograph the changes in band pattern as they occurred. Plus they often worked at 2 $^{\circ}\text{C}$ to further slow contraction. They also developed an ingeniously simple technique to stretch the myofibrils. A suspension of myofibrils, mounted as a very thin layer on a slide under a coverslip, was examined in the microscope until a myofibril was found with one end embedded in a fiber fragment adhering to the coverslip, and its other end in a fragment attached to the slide. Movement of the coverslip in the appropriate direction produced the desired stretch or would permit the myofibril to shorten if ATP was present. Whereas these experiments were not demanding in the technical sense that the Andrew Huxley and Rolf Niedergerke experiments were demanding, they were demanding in another sense. Huxley (2004c) remembered that it was grueling work with long hours peering through the microscope in the cold room, searching for the ideal myofibril and the ideal contraction series. They took turns, a few days at a time, because of the eye strain and both worked very long hours.

Myofibrils contracting freely at room temperature in ATP Using cine photography, they found that the I bands shortened from a resting length of about 0.8 μm until they disappeared completely (Fig. 2.6). During this shortening the A bands remained constant at about 1.5 μm . Importantly, they observed changes in density

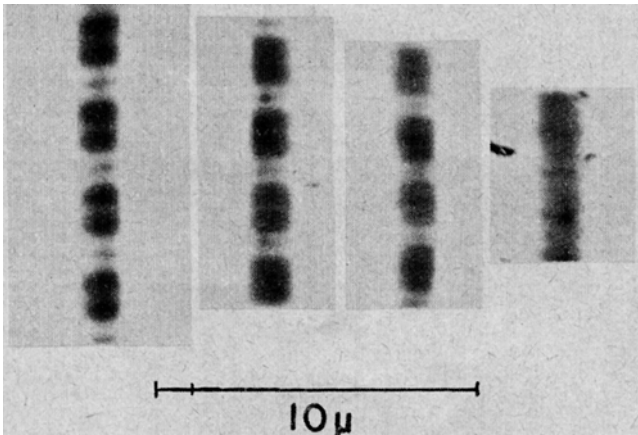


Fig. 2.6 Myofibril contraction photographed in the phase contrast microscope. The same four sarcomeres of one myofibril photographed during contraction (from *left to right*) induced by ATP from rest length down to 50 % of rest length where contraction bands formed. Note almost no change in A band length despite dramatic change in sarcomere length. Also note on the extreme left the light region in the A band (H zone) and its disappearance with sarcomere shortening. Magnification: $\times 4,000$ (Huxley and Hanson 1954. With permission Nature Publishing Group)

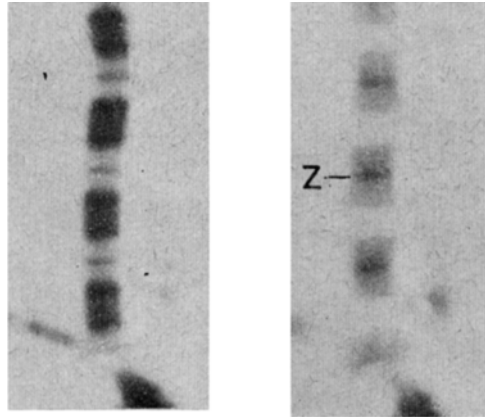
within the A band during shortening. The H zone, originally of low density, first became indistinguishable from the rest of the A band and was then replaced by a narrow zone which was more dense than the rest of the A band. At a slightly shorter sarcomere length, a very dense line became visible at either end of the A band. When the I bands disappeared at about 65 % rest length, contraction bands formed at the lines of contact of adjacent A bands. Thus their results provided the missing piece of the puzzle that Huxley and Niedergerke (1958) could not resolve in their experiments. In fact they acknowledged this in their 1958 paper when they pointed out that the crucial demonstration of variation of the H band width with sarcomere length was given by Huxley and Hanson (1954) on isolated myofibrils. Also the results of Hugh Huxley and Jean Hanson, somewhat ironically, provide a more consistent demonstration of the contraction bands that Andrew Huxley sought to study than did the Huxley and Niedergerke results.

Isometric contraction of myofibrils in ATP The A and I band lengths were unchanged during isometric contraction.

Passive stretch Only the I bands changed in length. The A bands remained at constant length but the central region become somewhat less dense, as though the H-zone were lengthening; the length of the less dense region increases as stretch proceeds. The process was reversible.

Myosin extraction Using the myosin extraction procedure developed by Hasselbach (1953), they found that the “ghost” myofibrils contained a faint backbone structure with a density which was about the same as that of the original I bands and thus they believed that the backbone was actin (Fig. 2.7). In stretched

Fig. 2.7 Stretched myofibril (115 % of rest length) before (left) and after (right) extraction of myosin. Photographs of extracted fibrils were printed by Huxley and Hanson so as to give adequate contrast and the myofibrils were in fact less dense than they appear here (Huxley and Hanson 1954. With permission Nature Publishing Group)



myofibrils, where there was originally a longer zone of low density in the center of the A band, the length of the gap was correspondingly greater than in an extracted myofibril at rest length. Myosin extraction of shortened myofibrils did not change the contraction bands. Surprisingly, the ghost myofibrils were still structurally intact. Although no material was visible in this gap in extracted myofibrils, they concluded that some structures must bind to the actin filaments and cross the gap because these myofibrils could be stretched elongating the gap and shortened spontaneously when released. This was a reversible process. They provisionally called these structures S-filaments because they could be stretched.

Electron microscopy They also described preliminary results with electron microscopy of stretched and contracted myofibrils before and after myosin extraction that were consistent with the light microscopic results but showed no images.

Discussion and conclusions They concluded that a “fairly simple model” could explain the results. In their words (Huxley and Hanson 1954. With permission Nature Publishing Group):

The backbone of the muscle fibril is made up of actin filaments which extend from the Z-line up to one side of the H-zone, where they are attached to an elastic component... which for convenience we will call the S-filaments. The S-filaments provide continuity between the set of actin filaments associated with one Z-line and that associated with the next... Myosin filaments extend from one end of the A-band, through the H-zone, to the other end of the A-band, and their length is unaltered by stretch or by contraction down to the point where the sarcomere length is equal to the length of the A-band; when contraction beyond this point takes place, the ends of the myosin filaments fold up and contraction bands form. Thus myosin and actin filaments lie side by side in the A-band and, in the absence of adenosine triphosphate, cross-linkages will form between them; the S-filaments are attached to the myosin filaments in the centre of the A-band by some more permanent cross-linkages.

In this model, plastic stretch takes place when the actin filaments are partly withdrawn from the A-band, leaving a long lighter central region and stretching the S-filaments. Only the I-bands and the H-zones increase in length, the length of A-band remaining constant...

Contraction takes place in this model when the actin filaments are drawn into the A-band (until the H-zone is filled up) and are then folded up in some way to produce more extensive shortening. Thus, when the model is allowed to shorten, only the I-bands decrease in length until adjacent A-bands are pulled into contact with the Z-lines...

They speculated further that the driving force for contraction might be the formation of actin-myosin linkages when ATP, having previously displaced actin from myosin, was enzymatically split by the myosin. Thus it is clear that Hugh Huxley and Jean Hanson had developed a very deep vision, with strong supporting evidence, of a sliding filament theory of muscle contraction.

By the autumn of 1954 Jean Hanson and Hugh Huxley had returned to their respective laboratories in London and Cambridge. They soon followed up on their 1954 publication with a publication (Hanson and Huxley 1955) emanating from a presentation given by Jean Hanson at a Symposium of the Society for Experimental Biology held at Leeds, England, in September of 1954. In this paper she and Hugh Huxley reviewed their previous results and the results of others and added to them with more electron microscopic images and more extraction experiments where now not only myosin but also actin was extracted from myofibrils. Also they took the opportunity to speculate more freely than was possible in the earlier papers. It is a very important review and it deserves wide recognition.

Hanson and Huxley did further extraction experiments and for the first time reconstitution experiments with the myofibrils. Actin extraction with potassium iodide solution (Szent-Gyorgyi 1951) after myosin extraction left a backbone of virtually zero optical density in the myofibrils (Fig. 2.8). The Z lines, however, were not removed. In an addendum to the paper, Hugh Huxley described an interesting experiment that indicated that when the myosin extracted ghost myofibrils were irrigated with myosin, these “reconstituted ghosts” were capable of contraction in the presence of ATP.

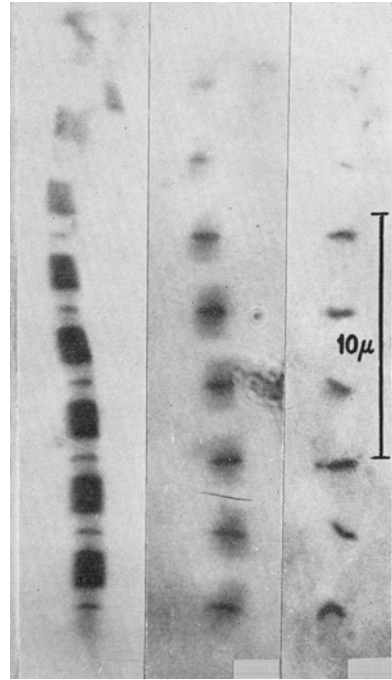
They put all of these results together in Fig. 2.9. The length of the A bands (i.e., the length of the myosin filaments) remains virtually constant until the I bands have disappeared (~65 % rest length). The ends of the A bands are then in contact with the Z lines, and further shortening is accompanied by the formation of contraction bands at these lines, presumably by crumpling of the ends of the myosin filaments.

The paper also contains interesting speculation about how “cross-linkages” between actin and myosin might operate (Hanson and Huxley 1955. With permission Elsevier):

...each myosin-actin linkage can pull the actin filament along a distance of, say, 132 Å, by the contraction of a branch of the myosin molecule; the branch would not, of course, give rise to any overall change in length of the myosin filaments (this type of model was mentioned both by ourselves and A.F. Huxley & R. Niedergerke in previous papers).

The 132 Å step size came from their estimate of the relative numbers of actin and myosin molecules in the filaments. They furthermore envisioned that at each step, a considerable proportion of all the actin-myosin linkages on the filament, if not all of them, were broken and reformed again. This speculation is remarkably similar to current day beliefs concerning how the filaments slide (see Chap. 9).

Fig. 2.8 Myofibril band patterns after extraction of myosin and then actin. One stretched myofibril photographed before treatment (*left*), after extraction of myosin by pyrophosphate treatment (*middle*), and then after treatment with potassium iodide solution to remove actin (*right*). After both myosin and actin have been removed only the Z lines and a ‘backbone’ of virtually zero optical density remain (Hanson and Huxley 1955. With permission Elsevier)



Loose ends What about the invisible S-filaments? Maruyama (1995) noted in a review describing the birth of the sliding filament model of muscle contraction that neither Hugh Huxley nor Jean Hanson mentioned S-filaments after 1955. In fact in a brief review in the journal *Endeavour* in 1956, Hugh Huxley (1956) described the sliding filament model of contraction and showed a diagram with filaments connecting the ends of the thin filaments in the middle of the sarcomere but he did not mention S-filaments in the review. After a while the S-filaments were dropped all together from the typical diagram showing the structure of the sarcomere (Huxley 1965). We know now that there is an elastic filamentous protein called titin (also known as connectin) that links the myosin filaments to the Z line. When myosin is dissolved away, most titin filaments retract toward either side of the Z line but a few opposing filaments from both Z lines appear to bind each other keeping continuity of the sarcomere (Maruyama 1995). (See Chap. 7 for more on the titin molecule and its role in sarcomeric structure and function.)

There is a historical curiosity associated with the observations in the classic 1954 work. Hanson and Huxley (1955) in their review point out that similar changes also were recorded by Harman (1954) using cine photography of myofibrils contracting and relaxing while in contact with active mitochondria. John W. Harman, at the University of Wisconsin, was investigating the relationship of mitochondrial oxidative capacity and structure to myofibril contractility and structure using phase contrast microscopy and electron microscopy (Harman and Osborne 1953). He exhibited a cine film in 1954 at the Federation meeting in Atlantic City, New Jersey,

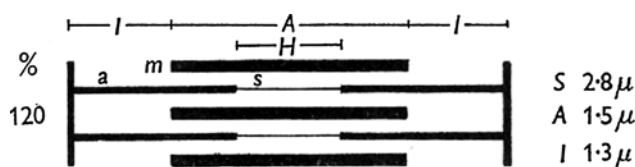


Fig. 3.

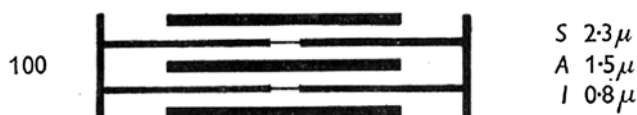


Fig. 4.



Fig. 5.



Fig. 6.



Fig. 7.

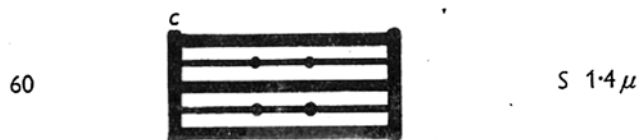


Fig. 2.9 Schematic representation of the sliding filament model of contraction. Proposed arrangement of filaments in one sarcomere of a myofibril at different sarcomere lengths. In order from top down: stretched to 120 % rest length while relaxed; at rest length; in isometric contraction; contracted to 90 % rest length; to 80 % rest length; to 60 % rest length. Sarcomere lengths (S) and A and I band lengths are given to the right of the diagrams, and lengths of sarcomeres expressed in % of rest length are given to the left. *s* "S filament", *a* actin filament, *m* myosin filament, *s.e.c.* series elastic component, *c* contraction band (Hanson and Huxley 1955. With permission Elsevier)

that showed that the A band stayed constant in length during contraction of myofibrils. Since his interest was in mitochondrial structure and function, he did not carry this observation further and thus his observation became a “footnote” to the birth of the sliding filament theory of contraction.

Should one of the classic 1954 papers be given greater weight in the development of the sliding filament model of contraction than the other? Certainly Hugh Huxley and Jean Hanson provided more crucial details, particularly with regard to changes in the H zone with stretch and contraction. But remember, their work was done on “dead” pieces of muscle whereas Andrew Huxley and Rolf Niedergerke worked on living muscle. One can only conclude that each paper provided crucial information that the other didn’t and as such both should be honored. With regard to publishing the two papers together, Huxley (2004b) has remarked: “Fortunately, we did, and these papers gave the basic description of the sliding filament model, which has remained essentially unchanged since then.”

2.8 Scientific Reception of the Sliding Filament Model of Contraction

Despite the fact that the basic description of the sliding filament model has remained essentially unchanged since the classic papers of 1954, the papers received a mixed reception at the time of their publication. There are various reasons for this less than enthusiastic reception. First, the results “flew in the face” of dogma (see Chap. 1). For example, Albert Szent-Gyorgyi was not convinced that the myosin filaments were confined to the A-band (Huxley 1996). Second, the weight of the results and interpretations were based on light microscopy, sophisticated light microscopy to be sure, but still based on an old fashion technique compared to the emerging exotic technology of electron microscopy. Also it is true that the light microscopy observations were at the very limit of what was possible. Third, and probably most important, even though Hugh Huxley’s electron micrographs supported the light microscopic results, his electron micrographs were not completely convincing. In fact his interpretation was not in agreement with the earlier results and interpretations generated in the same laboratory in which he was working at MIT (Hall et al. 1946). Thus his mentor there, F. O. Schmitt, was understandably skeptical (Huxley 2008). And there was more skepticism (Hodge 1956; Spiro 1956; Sjostrand and Andersen 1956). Alan Hodge and David Spiro also worked in the Schmitt laboratory at the same time as Huxley. The three investigators developed a thin sectioning technique together (Hodge et al. 1954). But neither one of them agreed with the Huxley and Hanson interpretations. Hodge (1956) felt that the data on balance favored the ‘classical’ model, in which a continuous skeletal framework of myofilaments traversed all bands of the sarcomere with the band pattern arising from interstitial materials. Hodge was critical of the sliding filament model. First, he argued that Huxley had not demonstrated directly that the thick filaments were not continuous with the thin filaments. Second, it was not shown that the secondary array of

dots seen in Huxley's (1953b) transverse electron micrographs actually represented filaments or that these presumed filaments were continuous with the I-bands. Third, the postulated S-filaments had not been observed. Spiro (1956) interpreted his electron micrographs to indicate that the thin filaments *became* thick filaments as they went in the A-band. In his view shortening of muscle beyond equilibrium length was accompanied by a progressive transformation of the thin filaments into thick filaments, i.e., on shortening some mechanism caused filaments to aggregate in the form of thick filaments. There must have been some lively discussions in the Schmitt laboratory during this period.

The 1954 Nature papers would become classic and the sliding filament model of contraction would eventually become the new dogma. Nonetheless in the view of many investigators in the mid 1950s it was far from proven that muscle contracted via the sliding of two sets of filaments. Much more work would have to be done to prove the point.

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