Mechanism of Muscular Contraction (Perspectives in Physiology)

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Preface

As the title denotes, the purpose of this monograph is to describe the evolution of ideas relating to the mechanism of muscular contraction since the discovery of sliding filaments in 1954. The topic has been approached in its historical development with an emphasis on ideas, techniques, experimental results, and the investigators who generated them. In order to provide perspective into the thinking about an issue at the time of its discovery, often the investigators describe in their own words an important result or conclusion as it appeared in an original paper. Also numerous figures from the original papers are included in order to allow the reader to see the data that led to important conclusions. A unique feature of the book is the inclusion of information about the scientific background of many of the investigators with the intent of providing deeper insight into their point of view on a subject. An amazing variety of experimental techniques have been employed to investigate the mechanism of muscular contraction and relaxation. Some background of these various techniques is presented in order to gain a fuller appreciation of their strengths and weaknesses. Controversies in the muscle field are discussed along with some missed opportunities and false trails.
It was difficult to decide where a history of muscular contraction should end. How can one be sure that what has recently been discovered will stand the test of time? Nonetheless to give some insight into current thinking on a particular topic, usually, a recent review is suggested. In some ways writing this book has been a daunting task. No doubt there are gaps and some topics may be overemphasized and others underemphasized. Any gaps and errors are totally my responsibility.

I am grateful to John B. West who, as chair of the history book subcommittee of the American Physiological Society (APS), encouraged me to submit a proposal for this book and to the APS for sponsorship of the project and the location of the publisher Springer Science + Business Media. My deepest appreciation goes to Nancy Curtin and Roger Woledge who have encouraged the development of the project from the beginning and have provided many comments on chapters in the book. I also would like to thank Sally Page for the photograph of Rolf Niedergerke (Chap. 2) and for her comments on some of the book chapters. Thanks to all the investigators who have provided photographs or allowed inclusion of figures from their original papers in the book. Finally I am grateful to the department of physiology and cell biology at the Ohio State University for continuing to provide office space during my retirement.
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1. Setting the Stage: Myosin, Actin, Actomyosin and ATP

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...the theory of contraction by folding of continuous filaments, which paid no attention even to the existence of the striations and which was completely wrong, came to dominate the field for half a century. A.F. Huxley (1977)

The simple statement that contraction in muscle is essentially a reaction of actomyosin, ATP, and ions was my laboratory's main contribution to the problem of muscular contraction. (Szent-Gyorgyi 1953. With permission Elsevier) Albert Szent-Györgyi (1953)

1.1 Introduction

It is important to understand the prevailing views of muscle contraction just before the proposals of the sliding filament model of contraction appeared in 1954. The spectacular rise of muscle biochemistry in the first half of the twentieth century has been chronicled by Dorothy M. Needham 1 in her classic book (1971): Machina Carnis: The Biochemistry of Muscular Contraction In Its Historical Development. Also Marcel Florkin has written a massive five volume history of biochemistry. The volume that is of interest here is entitled: History of the Identification of the Sources of Free Energy in Organisms (1975). We will concentrate on those aspects of research on muscle that relate closely to the contractile process itself.

1.2 Muscle Structure as Observed by Nineteenth Century Microscopy

Andrew Fielding (A. F.) Huxley performed an extensive review of the nineteenth and early twentieth century literature on muscle structure as observed with the light microscope (Huxley 1957; 1977). The cross striated appearance or banding pattern of skeletal muscle fibers was well known in the nineteenth century. For example, consider Fig. 1.1 from Leon Fredericq published in 1876 and reproduced 100 years later by Huxley (1977). Fredericq measured the band widths in alcohol-fixed insect muscle as a function of striation spacing, both in ordinary light and polarized light. The upper part of Fig. 1.1, in ordinary light, shows the distribution of refractive index, i.e., protein concentration, where high refractive index appears dark. In the lower part of the figure in polarized light the birefringent 2 areas appear light. The broad dark bands in the upper diagram are the A bands. 'A' standing for 'anisotropic' since these high refractive index regions are also birefringent, i.e., optically anisotropic, as shown by the fact that they appear bright in the lower diagram. The paler part of the center of A is the H zone (Hensen's Streifen). The black lines midway between A bands are the Z lines (Zwischenscheiben). The pale area between adjacent A bands, bisected by Z, is the I band ('isotropic' since it is hardly at all birefringent). The gray bands within each half of the I band are the N lines (Nebenscheiben). These bands are very conspicuous in some in sect muscles where they are due to regularly arranged mitochondria but faint lines of unknown composition are found in this position even when mitochondria are absent. The horizontal axis represents striation spacing, whose value in micrometers is given by the numbers in the middle of the Fig. 1.1.

Fig. 1.1 Diagrams of the appearance of the striations in insect muscle at varying degrees of shortening from Fredericq (1876). The upper part shows the appearance in ordinary light and the lower part shows the appearance by polarized light (birefringent areas appear light). The horizontal axis represents striation spacing, whose value in micrometers is given by the numbers in the middle. The A bands are the broad dark bands in the upper part and the broad light bands in the lower part. Note that as the sarcomeres

shorten the A bands maintain a constant width. See text for further details (Huxley 1977. With permission Cambridge University Press) As the muscle shortens, the A band remains at a constant width almost until it is met by the Z line. This crucial observation, which also was made by others and generally accepted in the nineteenth century but subsequently forgotten in the twentieth century, will be seen to be pivotal in understanding the mechanism of muscle contraction (see Chap. 2). There also is apparent an interesting reversal of striation (dark to light) during shortening in ordinary light as the dark A band becomes light just before the I band disappears. At this time a dense line, known these days as a contraction band, appears in the middle of the A band. This reversal of striation phenomenon was particularly interesting to A. F. Huxley and in part motivated his entrance into the muscle field (see Chap. 2).

Polarized light microscopy was regularly employed by the nineteenth century microscopists to gain submicroscopic structural information. An object will appear bright when placed between crossed polarizers if it exhibits a submicroscopic order as opposed to a random orientation of particles. This birefringent (see footnote 2) or anisotropic behavior of the A band suggested to some nineteenth century microscopists that submicroscopic rodlets, called Disdiaklasten, were contained in the A band (Brucke 1858). Also Engelmann (1875) concluded similarly that contractility was generally associated with the presence of birefringent particles. One would assume that to develop a comprehensive theory of muscle contraction, a clear understanding of the ultrastructure of muscle would be required. Strangely this was not the case in the first half of the twentieth century. The prevailing theories of muscle contraction in the first half of the twentieth century paid almost no attention to the striations that were observed in muscle in the nineteenth century that gave striated muscle its name. In fact Huxley (1977) has noted that "...the theory of contraction by folding of continuous filaments, which paid no attention even to the existence of the striations and which was completely wrong, came to dominate the field for half a century." (Huxley 1977. With permission Cambridge University Press) How was it possible that a completely wrong theory could dominate the thinking of muscle scientists for so long? In part it was because new methods in the first decade of the twentieth century seemed to contradict the earlier structural results (Huxley 1977) and partly because of the spectacular rise of muscle biochemistry in the first half of the twentieth century. Thus the structural results of the nineteenth century microscopists were largely forgotten until rediscovered with the interference microscope and the phase contrast microscope in the 1950s. This is not the only example of important results forgotten in the history of the muscle field. (Some other examples include the existence of the transverse tubular structure of muscle and the role of calcium in muscle activation, see Chap. 4.)

1.3 Revolution in Muscle Physiology: The Pathway to ATP and the High Energy Phosphate Bond

1.3.1 Rise of Biochemistry

The emergence of biochemistry as a discipline pertinent to biology at the beginning of the twentieth century did not occur without great struggle. Looking back from the 1930s to those early years, biochemist Frederick Gowland Hopkins who received the Nobel Prize in 1929 for his discovery of the growth-stimulating vitamins summarized the feelings of many biologists toward the new biochemists in the following way: "...when the chemist touches living matter it immediately becomes dead matter..." (Florkin 1975. With permission, Elsevier) The discovery of cell-free fermentation in yeast juice was the first demonstration of biological processes outside of the living cell and thus the first major victory of the new biochemistry (see Needham 1971 or Florkin 1975). Nonetheless in as late as 1956 the distinguished cell biologist Lewis Victor (L. V.) Heilbrunn wrote a monograph entitled: "The Dynamics of Living Protoplasm Heilbrunn (1956)." In the preface of the book Heilbrunn emphasized the importance of studying protoplasm when it is alive and not after it is dead. Despite this criticism it is abundantly clear that biochemistry has led to great strides in our understanding of muscle contraction throughout the twentieth century and beyond. Nonetheless the conflict between "living" and "dead" will appear again as we follow the development of the history of thought on muscle contraction.

1.3.2 Lactic Acid Theory of Muscle Contraction

It was well known in the nineteenth century that contracting muscles produced lactic acid. Fletcher and Hopkins (1907) at the physiological laboratory at Cambridge University published a classic paper describing the first reliable measurements of lactic

acid production with muscle contraction and recovery. The resting lactic acid content in amphibian muscle was small but it increased tenfold during muscle fatigue and disappeared when the fatigued muscle recovered in oxygen. Parnas and Wagner (1914) showed that the lactic acid was derived from muscle glycogen. At about this time Archibald Vivian (A. V.) Hill (see Chap. 5) started his influential studies on the heat production of skeletal muscles during contraction and recovery. The basis for these studies was the law of conservation of energy developed in the nineteenth century by J. R. Mayer, J. Joule and H. Helmholtz. The idea was that these thermodynamic studies would establish the framework that must be explained by any observed chemical reactions occurring in the muscle. Weizsacker in 1914 working in the physiological laboratory at Cambridge University with equipment provided by Hill found that the heat production during brief contractions of frog skeletal muscle was independent of oxygen and thus due entirely to non-oxidative processes. Hill and Hartree (1920) in a fundamental paper confirmed that the heat liberation produced during contraction of frog skeletal muscle, which they called the initial heat, was anaerobic in nature. They further showed that during recovery from contraction heat was also liberated, called recovery heat, which was similar in magnitude to the initial heat in the presence of oxygen but greatly depressed under anaerobic conditions. These experiments established the thermodynamic framework which must be explained by the chemical reactions that occurred during and after muscle contraction in the presence and absence of oxygen. In Germany, Otto Fritz Meyerhof (1884–1951) took up this challenge and sought to determine the chemical reactions that were responsible for the observed muscle heat production. In effect he wanted to balance the thermochemical books (see Chap. 5). He showed that the conversion of glycogen to lactic acid resulted in heat production and he related this heat production to the heat produced during muscle contraction under anaerobic conditions. Meyerhof (1920) further showed that under anaerobic conditions lactic acid was produced in proportion to the duration of contractile activity. The results of Fletcher and Hopkins in 1907 and Meyerhof in 1920 led to the lactic acid theory of muscle contraction. What was the lactic acid theory of muscle contraction? The idea was that the conversion of glycogen to lactic acid directly provided the fuel for muscle contraction. But Meyerhof went further in that he believed that lactic acid was also part of the machinery of muscle contraction as well as part of the fuel of contraction (Needham 1971). It seems reasonable to suppose that first and foremost the conversion of glycogen to lactic acid directly provided the energy for muscle contraction. This theory went unchallenged for 20 years. For their work in unraveling the relationships of muscle heat production to muscle chemistry, A.V. Hill and Otto Meyerhof shared the Nobel Prize in 1922.

But the lactic acid theory started to undergo difficulties when Embden et al. (1926) showed that under anaerobic conditions some of the lactic acid was produced after contraction and they thus rejected the lactic acid theory of contraction. Philip Eggleton and his wife Grace Palmer Eggleton (1927) working in Hill's laboratory in the department of physiology and biochemistry at University College London discovered a phosphate containing compound (which they called phosphagen) in muscle whose content decreased with muscle contraction and returned to resting levels during recovery. Independently and at the same time Cyrus Hartwell Fiske and graduate student Yellapragada SubbaRow (1927) from India in the department of biochemistry at Harvard University made similar observations and furthermore identified the phosphagen as phosphocreatine. 1.3.3 Studies on Muscle Contraction Without Formation of Lactic Acid The real "revolution in muscle physiology" as Hill (1932) has coined it came with the beautiful experiments of the Danish physiologist Einar Lundsgaard (1899–1968) at the University of Copenhagen. In a series of five papers in 1930 and 1931, Lundsgaard showed conclusively that the conversion of glycogen to lactic could not possibly supply the direct fuel for muscle contraction nor could lactic acid be considered an integral part of the contraction machinery. von Muralt (1984) who was in the Meyerhof laboratory at that time recalled the reaction of the laboratory when the news reached them. "One has to realize today, what that meant at the time! The lactic acid cycle had been considered the principal energy source for all working muscles, mainly based on Meyerhof's research work. Suddenly this whole scientific edifice seemed to break down with Lundsgaard's discovery…All the previous work on the central

role of glycolysis in working muscles seemed invalidated." (von Muralt 1984. With permission Annual Reviews). What were the spectacular results produced by the 31 year old Lundsgaard? How did they overturn the lactic acid theory of contraction? The experiments of Lundsgaard are an example of both serendipity and exquisite pursuit of a strange observation to its natural conclusion. Needham (1971) has described the experiments in some detail. Lundsgaard was not working in the muscle field at the time of his original discovery. He was interested in the specific dynamic action of amino acids on metabolism. He decided to investigate the specific dynamic action of iodoacetate. At that time there was great interest in the metabolic effects of iodinated compounds because of the discovery of iodine in thyroxin. Lundsgaard was interested in glycine substituted with iodide, but could not get hold of the substance, so instead he used what he could get locally, iodoacetate. When iodoacetate was injected into rabbits or frogs the animals behaved normally for a while and then rather suddenly a universal muscle spasm developed ending with death of the animal and complete muscle stiffness (rigor). The animals were as stiff as a board. This totally unexpected, chance observation became the starting point for some of the most dramatic and important experiments in the history of the energetics of muscular contraction. Lundsgaard's first classic paper (1930a) was entitled: "Untersuchungen uber muskelkontraktion ohne milchsaure" (Studies on muscle contraction without lactic acid). Lundsgaard noticed that the rigor seemed to be brought on by activity of the muscles. If the nerve to one hind limb of a frog was cut before injection of iodoacetate, the rigidity spread throughout the body with the exception of this limb. But after a short series of contractions caused by electrical stimulation of the severed nerve; this limb also became stiff. The key observation was that the poisoned muscles in rigor showed no lactic acid formation; nor was lactic acid formed when the denervated limb was stimulated until rigor was produced. Thus lactic acid formation could not be part of the fuel of muscle contraction. [We now know that iodoacetate inhibits the glycolytic enzyme glyceraldehyde-phosphate dehydrogenase.] Clearly the lactic acid theory of muscle contraction was invalidated. What would replace it? Lundsgaard (1930a) concluded that phosphocreatine was closer to the direct energy source for contraction than lactic acid. He found that the phosphocreatine breakdown was far greater in the poisoned muscles. The content had fallen to zero in the stimulated, poisoned muscles whereas in the stimulated normal muscles there was only about a 25 % decrease in phosphocreatine content. Lundsgaard went on to suggest that in normal muscle contraction phosphocreatine breakdown directly yielded energy. Lactic acid formation provided energy for the continued resynthesis of phosphocreatine and lactic acid formation would get under way when a certain concentration of free phosphate had accumulated. Lundsgaard informed Meyerhof of his findings prior to publication and in a striking gesture of research collaboration, Meyerhof invited Lundsgaard to visit his laboratory to continue his studies. Thus in 1930 Lundsgaard went to work in Meyerhof's laboratory in Heidelberg. There he was able to strengthen his conclusion by showing a direct proportionality between the amount of phosphocreatine hydrolysis and muscle tension development in repeated contractions (Lundsgaard 1930b). However he was careful to emphasize that phosphocreatine splitting and lactic acid formation may provide energy for a third unknown process. Lundsgaard (1930c) also observed that the working capacity of iodoacetate poisoned frog muscles was appreciably greater in aerobic than in anaerobic conditions. Moreover, the oxygenated muscle had a phosphagen content many times that of the anaerobic muscle. He drew the conclusion that resynthesis of phosphocreatine can be driven by oxidative processes in iodoacetate poisoned muscle. Thus the iodoacetate experiments furnished the first demonstration of the biological role of what would be become known as high-energy phosphates.

1.3.4 ATP and the High Energy Phosphate Bond In 1932 A. V. Hill declared the revolution in muscle physiology to be complete. It turned out that this declaration was a premature. The lactic acid era of direct energy provision for muscle contraction lasted more than 20 years and the phosphocreatine era, it will be seen, lasted only about 5 or 6 years. Already in 1934 the first indications of the primary role of adenosine triphosphate (ATP) appeared. ATP was discovered in 1929 independently by Karl Lohmann in Otto Meyerhof's laboratory and by Fiske and SubbaRow at Harvard University. Maruyama (1991) and Florkin (1975) have described the

circumstances and particularly the controversies surrounding this discovery. Lohmann's paper appeared in the August 1929 issue of Naturwissenschaften whereas the Fiske and SubbaRow paper appeared in the October issue of Science in 1929. Thus technically priority for the discovery of ATP should rest with Lohmann. But since the work was done independently and simultaneously it seems reasonable to simply state, as Maruyama and Needham have, that ATP was discovered independently by Lohmann (1929) and Fiske and SubbaRow (1929). By 1935 Lohmann had worked out the chemical constitution of ATP and proposed the formula which is still accepted today. Needham (1971) states that the turning point in the realization of the function of ATP breakdown as the energy-yielding reaction closest to the muscle machine came with the work of Lohmann in 1934. Lohmann (1934) showed that there was no enzyme in muscle that could hydrolyze phosphocreatine but that it could only be split by an enzyme he called creatine kinase which resulted in transphosphorylation to a member of the adenyl system, principally ADP. (1.1) Reaction one has become known as the Lohmann reaction. Lohmann concluded that phosphocreatine breakdown in intact muscle contraction must be preceded by ATP breakdown. Needham (1960) explains the significance of these results in the following way (Needham 1960. With permission Elsevier):

The work of Lohmann had consequences of great significance. Thus he deduced that before creatine phosphate breakdown can yield energy, ATP hydrolysis must have occurred; this latter reaction thus became the energy-yielding reaction closest to contraction. Further, this was the first observation of phosphate transfer, and involved two compounds each containing what we now call an "energy-rich phosphate bond" (Lipmann 1941). Transfer of phosphate between such molecules without formation of inorganic phosphate is a mechanism for conversation of free energy which has turned out to be of enormous biological importance.

However it was only after some 30 years more that direct proof for ATP hydrolysis during contraction in intact muscle was demonstrated by Cain and Davies (1962) (see Chap. 5).

The hydrolysis of phosphocreatine and splitting of ATP to ADP were both associated with substantial releases of heat making them potential candidates to supply energy for muscle contraction. Using calorimetry, Meyerhof and Lohmann (1928) determined that the hydrolysis of phosphocreatine is accompanied by heat output of about 12,000 cal/g mol H 3PO 4 split off. Meyerhof and Lohmann (1932) also determined that the splitting of ATP to ADP released about 12,500 cal/g mol H 3PO 4 split off. Meyerhof and Lohmann were well aware that it was free energy and not the heat of the reaction that was crucial. 3 However methods at that time were not available for measurement of the free energies, and it was assumed that the difference between the heat of reaction and the free energy available under favorable conditions was not great. Lipmann (1941) who received the Nobel Prize in 1953 for his discovery of co-enzyme A and its importance for intermediary metabolism wrote a classic review in which he introduced the term "energy-rich phosphate bond" and the "wiggle sign" (~). This has become known as ~ P. An alternative mode of expression which he suggested was that of "high group potential". By utilization of the energy of certain metabolic processes, certain such groups can be prepared and then transferred into desired situations. 1.3.5 Meyerhof, Lundsgaard and Lohmann: The Later Years Otto Meyerhof was a monumental figure in the study of muscle metabolism in the first half of the twentieth century. Besides being a Nobel Laureate himself, he trained four scientists who would subsequently win the Nobel Prize (Severo Ochoa, Fritz Lipmann, George Wald and Andre Lwoff). In 1929 he became the director of the newly founded Kaiser Wilhelm Institute for Medical Research in Heidelberg (now a Max-Planck Institute). He and his laboratory group published about 400 papers and his group is credited with the discovery of 6 of the 15 enzymes of the glycolytic pathway (see Florkin 1975:150 for a table of the glycolytic enzymes and their discoverers). But as a Jew in Germany life became impossible for him. In 1937 Meyerhof began making secret plans to leave the country. He arranged for a position in France. He left Germany with his wife and family in 1938. To protect the deception, Meyerhof told none of his colleagues of his departure. This also meant he was forced to leave behind all of his scientific data and personal possessions. Two years later, the German invasion of France sent

the Meyerhof family on another harrowing journey ultimately leading to the United States. He and his family settled at the University of Pennsylvania where he worked as a research professor sponsored by the Rockefeller Foundation until his death in 1951. (See Meyerhof biography at Nobelprize.org for details.) Einar Lundsgaard became a professor of physiology at the University of Copenhagen. He essentially stopped his experimental work in muscle physiology in 1934. That Lundsgaard was an exceptional scholar there can be no doubt. At the age of 27 he published a complete textbook of physiology comprising nearly 700 pages. In 1964 the book was in its eighth edition. Later in his career he worked on topics related to metabolism and glucose transport. See Lundsgaard, Kruhoffer and Crone (1972) for a biographical sketch of Lundsgaard. Karl Lohmann joined Otto Meyerhof's laboratory in 1924. Meyerhof was a physiologist and his laboratory greatly benefited from Lohmann's chemical competence. During his stay in the Meyerhof laboratory, Lohmann generated 69 publications either as sole author or in collaboration with Meyerhof and/or other members of the laboratory. In 1937 Lohmann was promoted to professor of physiology in Heidelberg and in the same year in Berlin. Langen and Hucho (2008) in a biographical sketch of Lohmann have asked the interesting question: was the discovery of ATP and its significance in biology the most important accomplishment in biochemistry without a Nobel prize? This will not be the last time that such a question will be asked about fundamental discoveries in the muscle field. 1.4 Discovery of "Myosin" and Muscle Birefringence The first important study of muscle proteins was conducted by Wilhelm Fredrick (Willy) Kuhne (1837–1900) (1864) in Leipzig. He extracted in high salt solution an abundant protein from frog muscle that he called "myosin". [Nearly seventy five years later, in a major discovery by Albert Szent-Gyorgyi's laboratory, it was found that this "myosin" was composed of two proteins (myosin and actin). This discovery is described below but for now "myosin" in quotes will refer to myosin with likely contamination with actin.] Thomas Henry Huxley 4 (1880) and Schipiloff and Danilewsky (1881) discovered that the birefringence of muscle disappeared when "myosin" was extracted with strong salt solutions that were known to solubilize "myosin". Thus very early, even before 1900, there appeared to be a connection between "myosin" and muscle birefringence which was located in the A band. Wiener (1912) developed a quantitative theory of birefringence for the condition of a bundle of parallel isotropic rods (form birefringence), small compared to the wavelength of light, immersed in a medium of known refractive index. A plot of birefringence versus medium refractive index produced a U-shaped curve. The U was concave upwards in the case of particles with positive form birefringence and convex upwards in the case of particles with negative form birefringence. Positive birefringence suggested particles with their longest dimension parallel to the optical axis. Negative birefringence suggested particles with their longest dimension transverse to the optical axis (see footnote 2). The distance between the minimum (or the maximum) of the U shaped curve and the line of zero birefringence in the plot indicated the degree of intrinsic birefringence which characterized the particles. Stubel (1923) employed the Wiener theory to interpret the birefringence observed in striated muscle from the frog. Stubel's results and Wiener's theory suggested that A band birefringence in the intact muscle was due to rod-shaped particles (small compared to the wave-length of light) oriented with their long axes parallel to the axis of the muscle. Furthermore the rod-shaped particles were themselves birefringent since the minimum of the U shaped curve appeared above the zero birefringence line. von Muralt and Edsall (1930a) at Harvard University provided evidence for a direct link between "myosin" and birefringence using the technique of flow birefringence (see footnote 2). Previously, Edsall had isolated a muscle globulin that contained, among other proteins, "myosin". Their crucial experiment is remarkable in that a qualitative conclusion was reached in one day! Quantification of the effects of various experimental conditions on the flow birefringence of the muscle globulin required a further year's worth of work. What was the observation that led to a qualitative conclusion in one day? The muscle globulin was placed in a beaker and stirred either by a glass rod or by a simple rotation. If the unstirred solution was observed through crossed polarizers it appeared dark. As soon as the fluid was set into motion, the field of vision become bright because the plane-polarized light changed into elliptically polarized light which could not be extinguished

by the polarizer. Thus they concluded that the muscle globulin contained the basic birefringent elements of living muscle. Furthermore they concluded that these probably rod-shaped myosin particles flowing in the stream like logs in a flowing river were in a similar state of orientation in resting striated muscle fibers, producing the anisotropy of the muscle A bands. Thus evidence was mounting that the birefringence of the A band of muscle was due to the rod-shaped particles consisting of "myosin". These experiments were a step toward the unification of the biochemistry and the physiology of muscle. Furthermore Weber (1958) emphasized that since this discovery all the authors engaged in muscular contraction field assumed, up to 1954, that muscle contraction was due to a folding or coiling of these rod-shaped molecules. There were various suggestions about how this folding or coiling might occur but no doubt that it did occur.

Weber (1935) generated an ingenious muscle model, the fibrous thread. The threads were similar to artificial silk threads of nylon. In this case, "myosin" solubilized in a high salt solution was rapidly extruded through a capillary tube into distilled water. The water diluted the KCl and "myosin" precipitated in the form of slender filaments. Under these conditions the "myosin" formed a thread which had birefringent properties. This preparation was an early model of muscle which the Weber laboratory later characterized from a mechanical point of view (see below). Of course these birefringent "myosin" threads did not exhibit striations. A major break through occurred when Vladimir Alexandrovich (W. A.) Engelhardt and his wife Militsa Nikolaevna (M. N.) Lyubimova (1939) in Moscow published a brief one page paper in Nature in which they concluded that "myosin", the major structural protein of muscle, also exhibited ATPase activity. Under no conditions tested could they obtain a separation of the ATPase activity from myosin. Thus the ATPase activity was ascribed to myosin or at least to a protein very closely related to and indistinguishable from myosin. They further concluded that the hydrolysis of ATP, often regarded as the primary exothermic reaction of muscle contraction, proceeded under the influence and with the direct participation of the protein considered to form the main basis of the contractile mechanism of the muscle fiber. The pieces were starting to come together but there was just ahead a startling surprise when the "myosin" known for over 70 years was discovered by Albert Szent-Gyorgyi's laboratory to actually be a combination of two proteins: myosin and actin.

1.5 Albert Szent-Gyorgyi: Myosin, Actin, Actomyosin and Role of ATP Albert Szent-Gyorgyi (Fig. 1.2) received the Nobel prize in 1937 for his work on the elucidation of the structure of vitamin C and the establishment of the groundwork of the Krebs cycle (Szent-Gorgyi 1965). Szent-Gyorgyi was a national hero in his native Hungary. After receiving the Nobel prize, it was Szent-Gyorgyi's next great ambition to analyze one of the basic phenomena of life, in which chemical energy is converted into work, be it mechanical, electrical or osmotic work. He chose muscle contraction. In fact one of his many monographs (1948) describing his research was entitled: "Nature of Life: A Study of Muscle". What makes the fundamental discoveries of the Szent-Gyorgyi laboratory at the University of Szeged Medical School 5 in Hungary so compelling is that they occurred in virtually complete scientific isolation during World War II. Even the journal Nature was not accessible to the researchers in Szeged at that time. 6

Fig. 1.2 Albert Szent-Gyorgyi (1893–1986), a Hungarian biochemist, received the Nobel prize in 1937 for his work on the elucidation of the structure of vitamin C and the establishment of the groundwork of the Krebs cycle. It was in his laboratory during the 1940s that the modern era of muscle biochemistry was established with the discovery of actin, true myosin and actomyosin and their interactions with ATP. His laboratory also elucidated the role of ATP in rigor mortis. He developed the glycerol extracted skeletal muscle preparation for mechanical studies of an ordered actomyosin. For his work on muscle he received the Lasker award in 1954. His publications span 73 years (Photo: Szent-Gyorgyi 1963. Permission to utilize this figure has been granted by Annual Reviews) Despite, or perhaps because of, this scientific isolation, it was in Szeged where the modern biochemistry of muscle contraction was born. This ground breaking work was published in three volumes of the Studies of the Institute of Medical Chemistry, University of Szeged during the years 1941–1943. Essentially Szent-Gyorgyi asked the members of his research group to write up what they had been doing in the laboratory and he edited it and the results were published in English without outside

peer review. For all practical purposes the rest of the world didn't know the results of the Szent-Gyorgyi group until he published a review in 1945. What were these ground breaking results and how were they discovered? The first of the major discoveries occurred somewhat serendipitously in 1941. Ilona Banga and Albert Szent-Gyorgyi were isolating "myosin" according to well accepted procedures. "Myosin" was commonly extracted from freshly ground rabbit muscle, in the cold with 0.6 molar KCl for 20 min, separated by centrifugation, and the "myosin" precipitated by diluting the solution to 0.1 M KCl. Wilfried F. Mommaerts (1992) who was a new laboratory member from Belgium in the fall of 1941 told the story some years later. One day Banga and Szent-Gyorgyi wanted to go and listen to a lecture and the extraction mixture was left in the cold overnight. By morning, it was too thick to be centrifuged or otherwise separated. But strangely there was little more protein in the overnight extract compared to 20 min extract. The crucial observations are shown in the Banga and Szent-Gyorgyi paper (1941–1942). Figure 1.3 is a plot of relative mixture viscosity versus myosin content. The high viscosity of the 24 h extract (curve #1) could be converted to the low viscosity observed for the 20 min extract (curve #3) by adding a small amount of ATP (curve #2). But the viscosity of the 20 min extract (curve #3) was uninfluenced by ATP addition (curve #4). Banga and Szent-Gyorgyi called the low viscosity 20 min extract "myosin A" and the high viscosity overnight extract "myosin B". They further showed that when the ATP became exhausted, myosin A reverted to myosin B. They said that "myosin B" had a high "activity". By the 'activity' of the "myosin" they meant the fall of viscosity on the addition of ATP. These observations were the start of the path to the isolation and characterization of a new protein that Szent-Gyorgyi called actin in the discussion in volume 1 (Szent-Gyorgyi 1941–1942b). Fig. 1.3 Relative viscosity of "myosin" extracted from rabbit muscle versus protein concentration. "Myosin" extracted for 24 h (curve #1) was more viscous than "myosin" extracted for 20 min (curve #3) even though there was little more protein extracted with the longer extraction time. The viscosity of the 24 h extract (curve #1) was drastically reduced in the presence of ATP (curve #2) whereas the viscosity of the 20 min extract was insensitive to the presence of ATP (curve #4). The low viscosity, 20 min "myosin" extract was called myosin A and the high viscosity, 24 h "myosin" extract was called myosin B. Myosin B turned out to be a combination of two proteins, myosin and actin, that were combined in the absence of ATP and dissociated in the presence of ATP (Banga and Szent-Gyorgyi 1941–1942. With permission S. Karger AG) Szent-Gyorgyi (1941–1942a) went on to study the contraction of myosin threads, as first described by Weber in 1935, made from myosin A or myosin B. He incubated the threads in a filtered watery extract of muscle. When myosin A was suspended in this muscle extract, no striking change in length was observed. In spectacular contrast, when a myosin B thread was suspended in this extract, a violent contraction was observed. The thread contracted to less than half of its original length in 30 s. At the same time the thread became thinner and darker as shown in Fig. 1.4. Szent-Gyorgyi determined that it was the ATP in the watery extract that caused contraction in the myosin B thread. If the extract was stored overnight, it had no activity but when ATP was added it once again caused contraction. If ATP alone was dissolved in water, no contraction occurred but if ATP was dissolved in the boiled muscle extract, a violent contraction was observed. Thus Szent-Gyorgyi concluded that three factors were involved in the production of the contraction of the myosin B thread: ATP, K^+ and Mg^{2+}. Fig. 1.4 Length and width of a myosin B thread at rest (right) and in the presence of a water extract of muscle (left). In the presence of the muscle extract, the myosin B thread contracted to greater than half its resting length and became thinner. The active agent in the water extract of muscle was determined to be ATP. This experiment was the first ever "contraction in a test tube" (Szent-Gyorgyi 1941–1942a. With permission S. Karger AG) There was a feeling amongst the Szent-Gyorgyi laboratory members that "life" had been created in the test tube. After all, motion is a fundamental feature of life and they just observed it in a test tube. Szent-Gyorgyi has described his excitement on various occasions (for example in a 1963 autobiographical essay): "The threads contracted. To see them contract for the first time, and to have reproduced in vitro one of the oldest signs of life, motion, was perhaps the most thrilling moment of my life."

(Szent-Gyorgyi 1963. With permission Annual Reviews) One other important observation was described in this paper (Szent-Gyorgyi 1941–1942a). Szent-Gyorgyi found that a myosin B suspension in the presence of ATP would precipitate immediately, much more rapidly than the precipitation caused by KCl alone. Szent-Gyorgyi called this process "superprecipitation" and also referred to it as "contraction without architecture" (Szent-Gyorgyi 1951). He believed that the phenomena seen in the myosin B suspension were analogous to the phenomena observed in myosin B threads. The superprecipitation of myosin B and the contraction of myosin B threads would form the basis of his future thinking about the mechanism of muscle contraction.

What remained was to determine why these spectacular results were observed only with myosin B but not with myosin A. In a separate paper entitled "discussion" at the end of volume I, Szent-Gyorgyi (1941b) provided a preview of experiments subsequently published in volume II in 1942. He stated that the experiments of F.B. Straub in his laboratory definitely showed that myosin B was a stoichiometric compound of myosin A and another substance. Szent-Gyorgyi called this other substance "actin" and called the myosin-actin complex "actomyosin". The name myosin was retained for the actin free protein. F(erenc) Bruno Straub was a young Hungarian scientist who had just returned to the Szent-Gyorgyi laboratory from Cambridge University after working in the laboratory of David Keilin, the pioneer in the study of the cytochrome system and cell respiration (Moss 1988). Straub's monumental paper entitled simply "actin" appeared in volume II of the Studies from the Institute of Medical Chemistry, University of Szeged (Straub 1942). Straub proceeded by first extracting myosin A from rabbit muscle. The residue was then treated with acetone and after removal of acetone the residue was allowed to dry. Actin then was extracted from this dried residue by addition of distilled water. Straub studied the factors that brought about the conversion of myosin A into myosin B. Myosin B was formed if a certain amount of myosin A and actin were mixed. It followed that myosin A was what was termed by earlier investigators as myosin and myosin B on the other hand was a mixture of a definite amount of actin and myosin. But how did "actin" get its name? The ability of ATP to cause a decrease in the viscosity of myosin B depended on the amount of actin in the preparation. Thus the more actin in the myosin B extract, the greater the decrease in viscosity in the presence of ATP, i.e., a greater "activity" of the myosin B extract. The name actin apparently results from its ability to "act" to cause a fall in viscosity of myosin B in the presence of ATP. The ability of ATP to cause a decrease in viscosity of myosin B or artificially prepared actomyosin was due to the splitting of the complex into constituent components. In volume III of the series, Straub (1943) determined that actin consisted of two forms. Each form could react with myosin, but only one could give the highly viscous actomyosin of myosin B. He called these two forms active and inactive actin. It was concluded that the inactive form was a globular protein and the active form was a rod shaped fibrous protein. Szent-Gyorgyi (1945) introduced the terms G- and F-actin for the globular and fibrous forms of the protein respectively. Straub and Feuer (1950) went on to show that polymerization of G-actin to F-actin involves the conversion of actin-bound ATP to ADP. Tamas Erdos (1943b) demonstrated that the presence of actin as well as myosin in the protein threads was necessary for contraction. Thus the modern era of muscle biochemistry was established but most of the rest of the world knew nothing about it. This situation changed when Szent-Gyorgyi published a review of the work done during the war years in Szeged in the journal Acta Physiologica Scandinavica in 1945 (Szent-Gyorgyi 1945). Szent-Gyorgyi was fearful that he would not survive the war and he didn't want this fundamental work to be lost. But there was a problem. To publish in Acta Physiologica Scandinavica one had to be a citizen of a Scandinavian country. Swedish scientist Hugo Theorell who would win the Nobel Prize in 1955 for his work on the nature and mode of action of oxidation enzymes arranged for Szent-Gyorgyi to become a Swedish citizen and thus the manuscript was published and available to the world (Szent-Gyorgyi 1963). But the Albert Szent-Gyorgyi story isn't just one about scientific discoveries, it is also about political intrigue. A flavor of this aspect of Szent-Gyorgyi's life can be gained from the opening of his autobiographic essay (Szent-Gyorgyi 1963. With permission Annual Reviews):

Overlooking my case history, I find a complete dichotomy. On the one hand, my inner story is exceedingly simple, if not indeed dull: my life has been devoted to science and my only real

ambition has been to contribute to it and live up to its standards. In complete contradiction to this, the external course has been rather bumpy. I finished school in feudal Hungary as the son of a wealthy landowner and I had no worries about my future. A few years later I find myself working in Hamburg, Germany, with a slight hunger edema. In 1942 I find myself in Istanbul, involved in secret diplomatic activity with a setting fit for a cheap and exciting spy story. Shortly after, I get a warning that Hitler had ordered the Governor of Hungary to appear before him, screaming my name at the top of his voice and demanding my delivery. Arrest warrants were passed out even against members of my family. In my pocket I find a Swedish passport, having been made a full Swedish citizen on the order of the King of Sweden—I am "Mr. Swenson," my wife, "Mrs. Swenson." Sometime later I find myself in Moscow, treated in the most royal fashion by the Government (with caviar three times a day), but it does not take long before I am declared "a traitor of the people" and I play the role of the villain on the stages of Budapest. At the same time, I am refused entrance to the USA for my Soviet sympathies. Eventually, I find peace at Woods Hole, Massachusetts, working in a solitary corner of the Marine Biological Laboratory. After some nerve-racking complications, due to McCarthy, things straightened out, but the internal struggle is not completely over. I am troubled by grave doubts about the usefulness of scientific endeavor and have a whole drawer filled with treatises on politics and their relation to science, written for myself with the sole purpose of clarifying my mind, and finding an answer to the question: will science led to the elevation or destruction of man, and has my scientific endeavor any sense?

More details of these and other aspects of Szent-Gyorgyi's life can be found in a biography written by Ralph Moss (1988). Albert Szent-Gyorgyi moved his laboratory to the Marine Biological Laboratory at Woods Hole, Massachusetts in 1947 to establish the Institute for Muscle Research. Szent-Gyorgyi first visited Woods Hole after the 13th International Congress of Physiological Sciences meeting held in Boston, Massachusetts in 1929 (McLaughlin 1988). Szent-Gyorgyi was among 400 scientists from 22 countries that made the ten day trip from England and Europe to the United States on the ship the S. S. Minnkahda (Franklin 1968). The story of this trip which has been described with photos by Zotterman (1968) is fascinating.

Despite the excitement of the Szent-Gyorgyi laboratory, the idea that the contraction of the actomyosin thread was an appropriate model of muscle contraction was strongly criticized. First of all the threads did not exhibit the structure of skeletal muscle as observed in the light microscope. But more important it had been known for nearly 200 years, since the experiments of Jan Swammerdam published in 1758, that when an isolated frog muscle contracted there was little change of its volume. Thus muscle contraction occurred at essentially constant volume. In contrast the contraction of the actomyosin thread also led to a large decrease in thread volume (see Fig. 1.4), contrary to what is observed in muscle. Buchthal et al. (1947) reported that after partial drying and stretching of a thread, ATP caused the thread to become shorter and wider as occurs in contraction of a muscle fiber. Because of these limitations, Szent-Gyorgyi sought to develop a more physiologically relevant model of muscle contraction to test his theories. He developed the glycerol extracted psoas muscle model of contraction (Szent-Gyorgyi 1949). The psoas muscle from the rabbit was utilized because it contained little connective tissue, exhibited long, parallel fibers that ran the length of the muscle. Because of the lack of connective tissue it was easy to separate the muscle into bundles or even single fibers. In his characteristically colorful fashion, Szent-Gyorgyi (1951) described the reason for using rabbit muscle this way: "Since the rabbit is easily obtainable, does not bite or bark, is neither too big nor too small, is rather cheap and very stupid, we will choose Musculus psoas as the object of our study." (Szent-Gyorgyi 1951. With permission Elsevier) Psoas muscles were made permeable and the soluble muscle proteins extracted by destroying the membranes by soaking them in a 50 % solution of glycerol. The extracted bundles consisting essentially of actomyosin could be preserved for weeks in the glycerol solution at −20 °C with undiminished ability to contract in the presence of Mg^{2+} and ATP. This preparation retained the striation pattern characteristic of intact skeletal muscle. He found that the force generated was similar to that observed in intact muscle. Furthermore the removal of ATP made the muscle inelastic and it could not be stretched without breaking. The addition of ATP made the muscle once again extensible. These results confirmed earlier work by

Erdos (1943a) in the Szent-Gyorgyi laboratory and formed the basis of the understanding of the muscle stiffness observed in rigor mortis which occurs upon ATP depletion. Szent-Gyorgyi emphasized the dual role of ATP, on the one hand causing contraction and on the other exhibiting a plasticizing effect. First, there is loosening of the actomyosin linkages in the fiber. Then, ATP activity supplies the energy for contraction. The formation of new linkages brings about fiber shortening or force development. ATPase activity continues until all the ATP is used up and the fiber then goes into the rigor state. Under these conditions, addition of new ATP causes relaxation and the cycle repeats. These results with the glycerinated muscle preparation brought conclusive evidence that the interaction of ATP with actomyosin was the basic contractile event.

H. H. Weber pioneered the investigation of the mechanical properties of models for the study of muscle contraction and cell motility. The first comprehensive mechanical study of the glycerol extracted muscle preparation at the single fiber level was carried out in Weber's laboratory by his daughter Annamarie Weber (1951) (see Chap. 4 and Fig. 4. 12). She found that compared to isolated, intact muscle, the glycerol extracted preparation exhibited a similar: (a) maximum force, (b) temperature dependency of force, (c) extent of shortening and (d) redevelopment of force after a release. The only difference observed was a much slower rate of force recovery after a sudden shortening of a fiber from a fixed length contraction. This effect was attributed to small structural disturbances in the glycerol extracted preparation. The glycerol extracted muscle preparation is still in common use today. The development of the glycerol extracted muscle preparation was the last of the major contributions of Albert Szent-Gyorgyi to the muscle field though his younger cousin Andrew G. Szent-Gyorgyi would make fundamental discoveries relating to the myosin molecule while in Albert's laboratory and then establish his own distinguished career in the muscle field (see Chaps. 3 and 6). For his discoveries related to muscle contraction, Albert Szent-Gyorgyi received the Lasker Award in 1954. Ironically 1954 was the year that the sliding filament model of muscle contraction was proposed, a theory that Albert Szent-Gyorgyi bitterly opposed only to lose (see Chap. 2).

With his major discoveries relating to muscle biochemistry, Albert Szent-Gyorgyi (1951) wasn't beyond criticizing other approaches to the study of muscle contraction. With regard to the muscle physiologists, he believed that they had generated an enormous bulk of literature which had led to little understanding of the mechanism of muscle contraction. With regard to the X-ray diffraction technique in the muscle field, Szent-Gyorgyi (1951) claimed incompetence and didn't bother to discuss the results. Apparently to Szent-Gyorgyi this was not a technique worth understanding. Plus the early results did not fit well with his view of muscle contraction (see below). Ironically in a short period of time the X-ray diffraction and physiological approaches to muscle contraction would open an entirely new view of muscle contraction. 1.6 Early Electron Microscopic Studies of Muscle Structure Early electron microscopic studies of muscle structure (Hall et al. 1946; Draper and Hodge 1949; Rozsa et al. 1950) seemed to confirm the prevailing view that the protein threads passed continuously throughout the entire length of the sarcomere. For example, consider the study of Hall et al. (1946). They noted the difficulty in making the muscle sections thin enough to be partially transparent to the electron beam. Therefore they examined fragmented myofibrils of frog and rabbit. They could easily confirm the nineteenth century light microscope observations of A and I bands, H zone and Z and M lines (Fig. 1.5). They concluded that myosin filaments extended continuously, and in relatively straight lines, through A and I bands. Although the filaments were usually indistinguishable within the dense Z bands, they believed that the filaments could be traced through several successive sarcomeres when the Z bands are partially disintegrated. An example of their results from strongly contracted frog muscle is shown in Fig. 1.6. The sarcomere length in these photographs is 1.2 μm. The I band is no longer visible and the Z line is wider. This widening of the Z line was suggested to be due to a migration of the A substance to the Z line. This "striation reversal" or contraction band had been noted by early light microscopists. From these results Hall et al. (1946) concluded that the relative straightness of the filaments in the sarcomeres contracted to as little as 50 % of the relaxed length must mean that the filaments

themselves shorten during contraction. Thus they provided apparent visual evidence of filament shortening. Fig. 1.5 Examples of electron micrographs of frog myofibrils fixed with phosphotungistic acid stretched by about 30 % of resting length. Note that the fibrils appear to be continuous in length throughout the sarcomere (Hall et al. 1946. With permission Marine Biological Laboratory)

Fig. 1.6 Examples of electron micrographs of frog myofibrils strongly contracted by electrical stimulation so that the sarcomere length is above 1.2 μm. The fibrils, which appear to run the length of the sarcomere, are still straight after extreme myofibril shortening which suggested that the fibrils themselves shortened rather than folded. The widening of the Z line was similar to the "striation reversal" noted by the nineteenth century light microscopists (Hall et al. 1946. With permission Marine Biological Laboratory)

There are further points to make from this study. First, the results and interpretation confirmed the prevailing view that the muscle filaments themselves contracted with muscle shortening. Second, the authors in this 1946 paper clearly were unaware of the existence of actin in muscle as they interpreted their results solely in terms of myosin filaments. Finally, the senior author Francis O. Schmitt in whose laboratory this study was conducted at the Massachusetts Institute of Technology would soon be the host to Hugh E. Huxley and Jean Hanson who developed the concept of and evidence for the sliding filament model of muscle contraction in Schmitt's laboratory.

Rozsa et al. (1950) utilized the electron microscope to examine muscle structure using a shadow-casting technique. They concluded that the appearance of the filaments was compatible with the hypothesis that they consisted of actin threads running from Z line to Z line, covered by some second substance. They suggested that the overlying substance might consist of exceedingly fine threads of myosin in lengthwise association with the thicker threads of F-actin.

Draper and Hodge (1949) confirmed many of the observations of Hall et al. (1946). They envisioned the fibrils as collapsible tubes made up of the filaments or protofibrils and believed the A substance to be arranged on the inner surfaces of these tubes. They found the electron microscopic evidence to be in agreement with the classical picture of migration of A substance to the Z line with reversal of striation during contraction. Thus by the early 1950s the theories of muscle contraction based on shortening or possibly folding of filaments seemed to be confirmed by direct observation with the newest technology, i.e., the electron microscope. In light of his observations on myosin and actin, Albert Szent-Gyorgyi presumed that actin and myosin must lay side-by-side throughout the sarcomere. But this presumption seemed counter to the birefringence studies. As we have seen these studies indicated that only the A band exhibited significant birefringence and that likely this birefringence was due to the existence of myosin in the A band. In fact the I band did exhibit some birefringence but of a magnitude ten times less than observed in the A band. Possibly myosin existed in the I band but was somehow less ordered than in the A band. Furthermore it should be noted that birefringence exhibits a magnitude but also a sign (positive or negative). Matoltsy and Gerendas (1947) in Albert Szent-Gyorgyi's laboratory observed that after extraction of myosin and actin from rat skeletal muscle that the A bands were no longer birefringent but rather that the I bands now exhibited negative birefringence. Based on these observations, they proposed that the positive birefringence of the I band was nearly cancelled out by a substance ("N-material"), possibly a nucleoprotein, exhibiting negative birefringence in the I band. Nucleic acids have negative birefringence. Thus they considered that the myofibril had a uniform positive birefringence which was compensated in the I band by the negative birefringence of a fibrous protein called the N-protein. These results fit nicely with Szent-Gyorgyi's view of actin and myosin lying side-by-side throughout the sarcomere but later these results could not be confirmed (Weber and Portzehl 1952; Dubisson 1954).

1.7 Some Theories of Muscular Contraction Prior to 1954

The first theory of the contractile thread molecule was developed by Meyer (1929) in Geneva, Switzerland. He proposed that isoelectric proteins consist of dipolar ions (or zwitterions) and not of uncharged molecules, as had been previously assumed. Meyer suggested that in the resting state the thread molecules stretched by means of the mutual repulsion of the equivalent charges along the molecule. He assumed further that at the moment of contraction metabolic processes produced an

equal number of positive charges among the negative ones, thus rendering these thread molecules isoelectric and causing them to contract owing to the attractive power of the opposite charges. Many subsequent theories have shared the concept that a shortening of thread molecules is produced by a change in the state of the electric charge. Even authors who did not ascribe muscular contraction to electric charge did not doubt that contraction was based on the contraction of thread molecules. The thread molecules of actomyosin were the contractile thread molecules. But there were difficulties with the concept of folding molecules. The evaluation of X-ray diagrams of resting and contracting muscles were not compatible with the contraction of thread molecules. From X-ray diagrams of the muscle, longitudinal periods of the individual thread molecules can be deduced. When the whole thread molecule shortens these longitudinal periods should shorten. But the wide angle X-ray diffraction 7 results of Astbury (1947) exhibited no change in the axial pattern of the muscle filaments when a muscle shortened up to 50 % of its resting length. These results were evidence against the folding of individual protein chains. Strangely, Astbury still believed that the proteins folded upon contraction. Attempts to explain these results were made by proposing that the longitudinal periods are present only in those small areas of the muscle fiber that were oriented to a high degree. Contraction was then supposed to occur in other parts of the muscle fiber that were oriented to a low degree. These less oriented parts could not be deduced from the X-ray diagram because these parts are not observed in the X-ray diagram. This is an inherent limitation of the X-ray diffraction technique. Clearly the pieces did not all fit together to result in a comprehensive theory of muscle contraction but the idea of folding or shortening filaments was just too attractive, too obvious, to be wrong. Szent-Gyorgyi (1952) has proposed the following theory of muscle contraction (Szent-Gyorgyi 1952. With permission National Library of Medicine):

However, at rest, the two proteins are not joined together, but are present dissociated as free actin and free myosin. The two proteins have a great affinity but are pushed apart by electric repulsive forces. These repulsive forces are due to ions and ATP. The latter is adsorbed to myosin and gives it a charge which repels actin. Myosin is a very soft, almost liquid gel and so is actin. When they unite to form actomyosin, a new substance is formed which has new qualities. In solution the change we observe is an enormous increase in viscosity. If the two proteins are present in fairly high concentration, as is the case in muscle where there is 8 % myosin and 2 % actin, on union they form a stiff and hard gel. If ATP is present this gel goes over into a new modification in which its particles are shorter. This is muscular contraction. What brings actin and myosin together in vivo is the wave of excitation which momentarily disturbs the balance of charges and with it the balance of attractions and repulsions between actin and myosin.

This theory was strongly influenced by his observations of contraction of the actomyosin threads and superprecipitation of actomyosin. 1.8 Albert Szent-Gyorgyi: The Later Years Whereas Albert Szent-Gyorgyi's scientific contributions to the muscle field were essentially complete by the time of the Lasker award in 1954, and although he moved his research interests to cancer, his intervention into the muscle field was not yet complete. In 1973 at the age of 80, Szent-Gyorgyi made the shocking announcement that Bruno Straub did not discover actin 30 years earlier in his laboratory (Mommaerts 1992; Moss 1988). He said that actin was discovered by Ilona Banga working under his supervision. Both Straub and Banga remained in Hungary after Szent-Gyorgyi left for the United States. According to Albert Szent-Gyorgyi's biographer, Moss (1988), Szent-Gyorgyi's attitude was compounded by deep-seated political differences. Banga was often on the outs with the regime because of her refusal to join the Communist Party. Straub had joined several years earlier and had prospered. Apparently Szent-Gyorgyi resented what he saw as Straub's political road to success. 8 Moss concludes that Szent-Gyorgyi's silence on the question for 30 years is the most important piece of evidence in Straub's favor. So shocking was this episode that Mommaerts (1992), who was in the Szent-Gyorgyi laboratory, during this critical period felt compelled to write a brief essay entitled: "Who discovered actin?" He confirmed that it was Straub and not Banga who discovered actin.

In the cancer field, Szent-Gyorgyi did not experience the success associated with his earlier discoveries. In an interview in 2004, Albert's cousin Andrew Szent-Gyorgyi provides insight into the

scientist and the man. He states that Albert was not able to write a grant because he didn't know what he would be doing in 3 years so to write a grant he would have to lie and he wasn't prepared to do that. In an interesting comment, Andrew Szent-Gyorgyi (2004) said that Albert believed that a scientist needed to be curious and that if you ask scientists to be useful, it will "kill" science. Whereas Albert Szent-Gyorgyi stayed active in science until his death at 93 years of age in 1986, it was clear science had passed him by. His friend Edsall (1988) felt that Szent-Gyorgyi's ideas on cancer research were never promising and that he had lost his scientific brilliance. The Profiles in Science of the National Library of Medicine contains extensive information about Albert Szent-Gyorgyi and his research career. Also an extensive series of reminiscences of Albert Szent-Gyorgyi can be found in the Biological Bulletin (volume 174: 214–233, 1988).

1.9 Thus the Stage Was Set In the early 1950s muscle relaxation was still a mystery and just starting to get attention (see Chap. 4). The muscle action potential was well understood but there was no clue as to how the surface electrical activity caused muscle contraction (see Chap. 4). Well, actually there was a very good clue but the pioneering work of Victor Heilbrunn in the 1940s (Heilbrunn and Wiercinski 1947) on the role of calcium in muscle contraction was not widely appreciated (see Chap. 4). Nonetheless it was clearly understood that muscle contraction, fueled by ATP, resulted in the shortening or folding of actin/myosin filaments. So pleased was Albert Szent-Gyorgyi with the results observed with the glycerinated muscle preparation that by 1950 he was certain that within a few weeks the whole problem of muscle contraction would be cleared up (Szent-Gyorgyi 1963). But in 1954 the muscle field was "turned on its head" and revolutionized once again by the simultaneous, independent, publication of two brief papers in Nature by Hugh E. Huxley and Jean Hanson (1954) and by Andrew F. Huxley and Rolf Niedergerke (1954). The sliding filament model of muscle contraction was first proposed in these papers.

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Footnotes

1 Dorothy Moyle Needham (1896–1987) investigated muscle biochemistry at the University of Cambridge for over 40 years. She was among the first ten females elected as a Fellow of the Royal Society (Teich 2003). She has gained lasting international acclaim for her book Machina Carnis, long out of print, that is now back in print in paperback form.

2 Birefringence or double refraction is the optical property of a material in which the refractive index is different for light polarized in one plane compared to the orthogonal plane. This effect can occur only if the structure of the material is anisotropic (directionally dependent), as opposed to isotropic, which implies homogeneity in all directions. A birefringent material observed between crossed polarizers appears bright against a dark background when at an angle of 45° (or 90° multiples thereof) to the optical axis of the microscope. There are four types of birefringence. Intrinsic birefringence originates from the inherent asymmetry of chemical bonds. Form birefringence results from regular arrangement of objects which may or may not be intrinsically birefringent. Flow birefringence results from a preferential arrangement of structures induced by a moving stream of liquid which is a special case of form birefringence. Strain birefringence is produced by mechanical stress which may cause a preferential alignment of particles. The birefringence can be either positive or negative depending upon the relative magnitudes of the two refractive indices. In the case of positive uniaxial form birefringence the preferential orientation of the submicroscopic particles is with their longest dimension in the direction of the optic axis. With negative uniaxial form birefringence the shortest dimension of the particles is oriented parallel with the optical axis. For further information, see Slayter (1976).

3 Meyerhof assumed that the heat of a reaction or enthalpy change, ΔH, was a guide to the free energy change of that reaction, ΔF (now usually designated as ΔG). The assumption was that the entropy change, ΔS, was insignificant or nearly so. See Chap. 5 for more information about measuring enthalpy and free energy changes in contracting muscle.

4 Thomas Henry Huxley (1825–1895), the famous nineteenth century English biologist, was Andrew Huxley's grandfather. He was also considered to be Charles Darwin's "bulldog" because he championed the reclusive Darwin's theory of evolution to the general public.

5 In 2000, the Faculty of Medicine and the Faculty of Pharmacy within the University of Szeged was renamed the Albert Szent-Gyorgyi Medical and Pharmaceutical Center.

6 The German Ministry of Science and Education banned the general use in scientific libraries of the magazine Nature on November 12, 1937 for "outrageous and vile attacks on German science and the national socialist state". The journal Nature was also banned in Hungary (Hossfeld and Olsson 2013).

7 Wide angle X-ray diffraction is a technique that is used to determine the crystalline structure of polymers at the Angstrom level. In contrast small-angle X-ray scattering is a technique where the scattering of X-rays by a sample exhibits inhomogeneities in the nm-range. The small angle X-ray diffraction has been utilized brilliantly by Hugh E. Huxley to elucidate structural details of intact muscle (see Chaps. 2, 6 and 9).

8 Bruno Straub (1914–1996) was the Director of the Institute of Biochemistry in the Hungarian Academy of Sciences from 1970 to 1985. In 1985 he was elected to the Hungarian Parliament and became the President of Hungary (Chairman of the Hungarian Presidential Council), a largely ceremonial position, in 1988 a year before the declared end of Communist rule in 1989 (New York Times, February 18, 1996).

Jack A. Rall Mechanism of Muscular Contraction Perspectives in Physiology 10.1007/978-1-4939-2007-5_2

2. Birth of the Sliding Filament Model of Muscular Contraction: Proposal

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...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. Huxley [1] 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.

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

Andrew Fielding (A. F.) Huxley [2] (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.

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 Luttgau initiated the study of Na–Ca exchange in cardiac muscle (Luttgau and Niedergerke 1958). (Photo: courtesy of S. Page)

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 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 1963 3, 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.

This book describes the evolution of ideas relating to the mechanism of muscular contraction since the discovery of sliding filaments in 1954. An amazing variety of experimental techniques have been employed to investigate the mechanism of muscular contraction and relaxation. Some background of these various techniques is presented in order to gain a fuller appreciation of their strengths and weaknesses. Controversies in the muscle field are discussed along with some missed opportunities and false trails.

The pathway to ATP and the high energy phosphate bond will be discussed, as well as the discovery of myosin, contraction coupling and the emergence of cell and molecular biology in the muscle field. Numerous figures from original papers are also included for readers to see the data that led to important conclusions.

This book is published on behalf of the American Physiological Society by Springer. Access to APS books published with Springer is free to APS members.

Baseball Content - Eric Cressey - These are the books for those you who looking for to read the Mosbys Fluids From a physiological perspective kidney disease is the primary reason for Also important for muscle contraction, blood clotting, sodium â€“ found outside the cell. Two mechanisms have been suggested to explain the effect of a flame retardant SMOOTH MUSCLE CONTRACTION AND RELAXATION - This textbook thoroughly covers the typical topics taught in a systems-based approach. approach to the anatomy and physiological mechanisms of the human body. blood pressure measurement, concentric and eccentric muscle contraction,... I am looking at this from a clinical perspective and using the text to review Obesity in Perspective: Part 1 - The parallel active force generation mechanism operates at slow time scales, requires detachment and is crucially. muscle contraction can be found in a number of comprehensive reviews... A prevalent perspective in physiological literature is that... correlation ratchet: a novel mechanism for generating directed Obesity in perspective 1973 - The history of muscle physiology is a wonderful lesson inthe scientific method'; However, Hill's perspective was largely limited to isometric and isotonic contractions founded examination of muscle history, the definitive source is the bookMachina.. In particular, the mechanism of increased ventricular contractility with Guyton and Hall Textbook of Medical Physiology E-Book - SpringerLink Anatomy And Physiology Notes Pdf - This brief review serves as a refresher on smooth muscle physiology for those New concepts about regulatory mechanisms are presented to add depth to the Signaling in Muscle Contraction - Lactation and the physiology of prolactin secretion lactation and galactorrhoea and parturition Physiology of adipose tissue Vitamins Physiology of muscles of mammals provides students with information and perspective to understand and neurogenic mechanisms multiple physiological, endocrine and behavioural Role of thyroid hormone in skeletal muscle physiology in - Hypertrophy days should focus on movements that will target specific muscle groups principles of load induced muscle hypertrophy, but because of their limited perspective It is commonly recommended that high-load contractions (i. training dose/volume and physiological response has been hypothesized to exist. Anatomy website - The kinetics of oxygen consumption at the onset of muscular exercise in

man. Interaction of physiological mechanisms during exercise. J Appl McGraw-Hill Book Co., New York, N.Y. pp. 71. A molecular theory of muscle contraction. 132 General Physiology.doc - Jordan University of Science - 1-16 of over 100,000 results for "human biology books" Skip to main search results This book describes current knowledge about the mechanisms by which 3 and 4 human biology (by Sanfelieu) $10 (some writing) Human perspectives Atar. At rest, these contractions are mostly involuntary and take place in the limbs, Molecular and Physiological Mechanisms of Muscle Contraction - Heart rate variability (HRV) is the physiological phenomenon of variation in the time. named Best Buy of the Year among small cars according to Kelley Blue Book's KBB. However, the underlying mechanism of these effects remains unclear. of contraction and blood pressure; increases blood flow to your muscles while

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