MASTER'S THESIS

ALLEN SELVERSTON

Mechanism of Contraction in Barnacle Muscle

1966

VITA

Name of author: Allen Selverston

Place of birth: Chicago, Illinois

Date of birth: January 17, 1936

Undergraduate and graduate schools attended:

University of Illinois, 1953-1955
University of Southern California, 1959-1961
University of California, Berkeley 1956-1958; 1961-1962
B. A. in Physiology, June, 1962
University of Oregon, 1963-

Region of special interest: Excitation-contraction coupling in muscle; neurophysiological mechanisms underlying behavior.

Professional experience: Research physiologist, Biology Section, U. S. Naval Radiological Defense Laboratory, San Francisco, California.

Graduate teaching assistant in comparative neurophysiology, 1964.

APPROVED: Graham Hoyle

(Graham Hoyle, Advisor for the Thesis)

ACKNOWLEDGMENTS

It is a pleasure to acknowledge the interest and assistance given me by Dr. Graham Hoyle. His advice, encouragement and criticism were invaluable. I would also like to express my appreciation to Dr. J. H. McAlear and his staff at the Electron Microscope Laboratory, University of California, Berkeley, whose technical assistance made possible the addition of electron microscopy to this investigation. The research was conducted under a United States Public Health Service Traineeship, STI-GM-336-03.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
REVIEW OF THEORIES OF MUSCULAR CONTRACTION	5
MATERIALS AND METHODS	12
RESULTS	17
Phase Contrast Microscopy	
Electron Microscopy	
DISCUSSION	31
SUMMARY	40
BIBLIOGRAPHY	43
APPENDIX 1: PLATES	47
APPENDIX 2: MYOFIBRIL MEASUREMENTS	118

LIST OF FIGURES

Figure		Page
1.	Glycerol extracted fibril at rest length as seen in the phase-contrast microscope.....	49
2.	Fibril with narrow I-band.....	51
3.	Fibril in first stage of ATP-induced contraction.....	51
4.	Fibril having H-zones.....	53
5.	Fibril at 140 per cent of rest length..	55
6.	Stretched fibrils having bulging appearance.....	55
7.	Fibril with broad contraction bands....	57
8.	Fibril with narrow contraction bands...	57
9.	Fibril with contraction bands in doublet arrangement.....	59
10.	Fibril showing extraction of myosin and action.....	61
11.	Movie sequence of ATP-induced contraction: Number one.....	63
12.	Changes in A- and I-band widths from contraction sequence, Number one.....	65
13.	ATP-induced contraction sequence: Number two.....	67
14.	Fibril showing initial appearance of α-bands.....	69
15.	Fibrils showing triplet formation.....	69
16.	Fibril which had undergone an unequal ATP-induced contraction.....	71

Figure		Page
17.	Fibril unequally contracted showing triplet formation.....	71
18.	Unequally contracted fibril showing all banding changes.....	73
19.	Myosin and actin extraction of fibril shown in Fig. 18.....	75
20.	Extraction sequence of fibril with short I-bands.....	77
21.	Extraction sequence in a highly stretched fibril.....	79
22.	Extraction of fibril contracted down to α -band stage.....	81
23.	Electronmicrograph of fibrils fixed at rest length.....	83
24.	Electronmicrograph of fibril from a supercontracted fiber.....	87
25.	Electronmicrograph of supercontracted fibril showing passing through.....	87
26.	Electronmicrograph of fibrils from a supercontracted fiber; fibril length 3.5μ.....	89
27.	Electronmicrograph of fibrils supercontracted by natural nervous stimulation.....	91
28.	High magnification electronmicrograph showing passing through.....	93
29.	High magnification electronmicrograph showing alternating thick and thin filaments passing through Z-disc.....	95
30.	Contraction sequence of fibrils contracted by natural nervous stimulation..	97

Figure		Page
31.	Electronmicrograph of fibrils contracted by high potassium.....	99
32.	Electronmicrograph of fibrils contracted by high potassium	101
33.	Electronmicrograph of fibrils contracted by high potassium.....	103
34.	Electronmicrograph of fibrils in a potassium contracture.....	105
35.	High magnification electronmicrograph showing buckling up of thick filaments at Z-line.....	107
36.	Electronmicrograph showing extreme skewering of thick filaments.....	109
37.	Electronmicrograph of early potassium contracture.....	111
38.	Electronmicrograph showing effects of high ATP concentration.....	113
39.	High magnification electronmicrograph showing effects of high ATP	115
40.	Phase-contrast photomicrograph showing effects of high ATP concentration in a basic solution.....	117

INTRODUCTION

The basic morphological arrangement of filaments in all striated muscle appears to be about the same. The manner in which these filaments interact to produce isotonic or isometric contractions however is not yet completely understood. Workers interested in this mechanism are now divided into two main groups. The first group can be considered those who have accepted in principle the sliding filament concept of H. E. Huxley (Huxley, H. E., 1954). The second group has interpreted their data to be indicative of molecular changes in the filaments such as folding or coiling. The evidence favoring the sliding filament theory is overwhelming and therefore is the one most generally accepted. It suggests two kinds of relatively stiff filaments, one thick (110 Å) and one thin (50 Å), connected by cross bridges at regular intervals. In rabbit muscle the thin filaments are attached to the Z-line and extend into the thick filaments up to the level of the H-zone. The thick filaments are located in the central A-band. During contraction the thin filaments are thought to be pulled into the thick ones, causing a shortening of the sarcomeres. The length of the thick filaments remains constant until they reach the Z-line at which point further shortening causes

crumpling of their ends. Opposed to this concept are those who feel only part of the shortening may be due to filaments sliding past one another, much of it being due to a shortening of the thick filaments by some change in their molecular configuration or movement of their substance (Sjostrand, F. S., 1964; Hodge, A. J., 1955; DeVillafranca, G. W., 1963). They base their argument on a shortening of the A-band observed in the light microscope and on a measurable decrease in length of thick filaments when seen in the electron microscope.

There are two experimental approaches which can be utilized to elucidate the problem. The first consists of phase contrast microscopy and its modification, interference microscopy. The former is employed for the examination of individual fibrils while the latter was developed for the study of whole fibers (Huxley, A. F. and Niedergerke, R., 1958). In both, resolution is limited by the wave length of light and therefore can be used only to measure the relation between the bands. Their advantage, however, lies in the fact that the muscle can be manipulated while it is being observed. Thus the location of the protein components can be deduced by extraction with appropriate solvents or labeling with fluorescent antibodies. Glycerol extraction leaves the contractile proteins intact and in a condition which enables them to contract upon the addition of adenosine

triphosphate (ATP). The contraction mechanism in this model and that in the living muscle should be similar. (Szent-Gyorgyi, A., 1951). The ATP-induced contractions can be studied for changes in the banding pattern. The changes can be recorded either by still or cinephotography. Fresh material can also be made to contract or stretch and their banding changes can be similarly recorded.

In order to visualize the filaments directly, the second major technique, electron microscopy, must be employed. Because of the labile structures involved, as well as difficult and severe fixing, staining and sectioning procedures, the interpretation of muscle electron micrographs is often confusing. This problem may be in part responsible for the often contradictory interpretations put forward by various workers. Electron microscopy is of course useless for recording any chemical or physical changes imposed on the fibrils while they are occurring.

A synthesis of these two methods is required to determine the actual changes in molecular morphology which accompany contraction. The relation between the changes in the fibril banding pattern during shortening and electron micrographs showing what is actually happening to the filaments in the fibril at various stages of contraction should provide the evidence necessary for an explanation of the contractile mechanism.

Recently, the giant muscle fibers of the barnacle Balanus nubilus have been shown to have many interesting properties which make them valuable preparations for the study of the basic contractile mechanism (Hoyle, G. and Smyth, T., 1963). The fibers are of large size (up to 2mm. in diameter) and contain sarcomeres whose length averages four times that found in rabbit fibers. Also, whereas rabbit striated muscle contracts reversibly to about 40 per cent of its rest length, barnacle muscle can shorten to one sixth its resting length (Hoyle, G. and Smyth, T., 1963). Because of its large size, some of the structural changes which occur with contraction might be within the resolving power of the light microscope and since the A- and I-bands are comparable to vertebrate muscle, its mechanism of contraction over such a wide range would be of basic interest.

REVIEW OF CONTRACTION THEORIES

The sliding filament model of muscle contraction was put forward in 1954 on the basis of light microscopic investigations from two separate laboratories (Huxley, H. E. and Hanson, J., 1954; Huxley, A. F. and Niedergerke, 1954). It had already been found that the material in the A-band was myosin (Hanson, J. and Huxley, H. E., 1953). A. F. Huxley, using an interference microscope, was able to show in fresh whole fibers that the A-band length remained constant while the I-band widths became narrower during isotonic shortening. H. E. Huxley and Jean Hanson, using glycerinated rabbit muscle, were able to induce single fibrils to contract with dilute solutions of ATP and in addition remove the actin and myosin selectively at various stages of contraction. They showed clearly that myosin was located in the A-band and actin in the I-band. During ATP contractions the I/A ratio decreased and dense contraction bands formed at the Z-lines. Both Huxleys came to the conclusion that the sarcomere had a backbone structure of actin filaments extending from the Z-line to one side of the H-zone and possibly continuing through as S-filaments. Myosin filaments extended from one edge of the A-band to the other, and their lengths did not change until they came

in contact with the Z-lines. At this stage the myosin filaments would bunch up at the Z-line and form contraction bands. During contraction the actin filaments would be pulled into the myosin filaments thus giving the appearance in the light microscope of a shortening I-band and H-zone. The sliding filament hypothesis was given added support by electron microscopic evidence from H. E. Huxley's laboratory (Huxley, H. E., 1957). In cross section he was able to show that the filaments were arranged in a hexagonal array. In the region where the thick and thin filaments overlapped, each thick filament was surrounded by six thin filaments. In the H-zone only the thick filaments arranged hexagonally, could be seen. In order to see the correct relationship between thick and thin filaments, sections had to be cut thin enough so that only a single layer of thick filaments were in the plane of section. In one lattice plane a 250 Å section would include one thick filament alternating with one thin filament, (actually two thin filaments, one above the other). If the block was then rotated by 45 degrees and the section thickness reduced, the section would show one thick filament alternating with two thin filaments.

When rabbit muscle fibers had a small area of their sarcolemma depolarized, a localized contraction could be observed initiating at the point of the microelectrode, (Huxley, A. F. and Taylor, R. E., 1958) provided the

microelectrode was over the Z-band and not the A-band. The banding changes corresponded to ATP-induced contraction changes in glycinerated muscle. Furthermore, experiments which compared tension development and shortening with sarcomere length indicate contraction and tension development ceases to occur when the sarcomeres are stretched to a point where there is no overlap between thick and thin filaments (Huxley, A. F. and Peachey, L. D., 1961).

Extraction experiments and experiments in which fluorescein-labeled antibodies were used to locate specific contractile proteins (Tunik, B. and Holtzer, H., 1961) have demonstrated with a fair amount of certainty the location of the various proteins in the sarcomere. This was accomplished by making antibodies against myosin, heavy and light meromyosin and actin. The antibodies were then tagged with a fluorescent dye and washed over fibrils in various states of contraction. In rest length fibrils myosin was found to be located in the A-band and actin in the I-band. In fibrils which had shortened the I-band width decreased while the A-band width remained constant.

Natural and synthetic protein filaments have now been prepared and examined in the electron microscope by the negative staining technique (Huxley, H. E., 1963). The thick filaments correspond structurally with the thick filaments seen in the A-band of sectioned muscles. The

thin filaments, even after fragmentation by homogenization in a "relaxing medium", often remained attached to residual Z-line structures and also resembled closely the actin filaments seen in sectioned tissue. Surprisingly, when purified myosin was precipitated out, spindle shaped aggregates were formed which had projections at both ends but not in the central regions. The thin filaments or filaments of purified actin when combined with myosin also showed the same complex structure. This does away with one of the persistent criticisms of the sliding filament model, the question of the polarity of the filaments. Huxley's results show the filaments are structurally polarized so that in muscle those on one side of the Z-line point in one direction while those on the other side are oppositely oriented.

Hodge was among the first to employ thin sectioning techniques as well as phase and polarization microscopy to the study of muscle structure (Hodge, A. J., 1956). After comparing evidence from vertebrate and insect skeletal muscle as well as dipteran flight muscle, Hodge came to the conclusion that there was only a single continuous array of filaments from one end of the sarcomere to the other. These were thought to be linked by an axially periodic system of transverse filamentous bridges. The myosin was located primarily in the A-bands and during contraction migrated to the Z-lines where it formed contraction bands. Hodge felt

the transverse bridges compensated for the birefringence of the actin and was thus responsible for the isotropy of the I-bands, while the myosin was responsible for the birefringence of the A-bands. The ultrastructure of cross sections of dipteran flight muscle revealed a hexagonal array of filaments at all levels of the sarcomere (Hodge, A. J., 1955). They appeared to be joined by cross-links which he thought to be regularly repeating transverse bridges. The electron micrographs are not at all convincing and since he figured the overall diameter of the myofilaments to be 120 Å they probably represented the thick filaments.

Investigation of dipteran flight muscle by Hanson revealed shortening of only 8 per cent, however, her interpretation of the mechanism was in accord with the sliding filament model (Hanson, J., 1956).

Sjostrand studied the morphology of the individual myofilaments at various stages of shortening and came to the conclusion that they increase in diameter as the sarcomere contracts (Sjostrand, F. S. and Anderson-Cedergren, E., 1957). He found that when the muscle was stretched above resting length there was a corresponding increase in width of the I-band while the width of the A-band remained constant. Shortening below rest length, however, caused a decrease in the width of the A-band as well as the I-band width. The thick filaments were found to contain subfilaments 20 Å

thick and 100 Å long. The shortening of the A-band was attributed to folding of the subfilaments which represented cables of supercoiled α-helices. Additional support for the concept of shortening being due to molecular changes has come from DeVillafranca's investigations of Limulus muscle (DeVillafranca, G. W. and Philpott, D. E., 1961; DeVillafranca, G. W. and Marschhaus, C. E., 1963). He described the appearance of glycerol-extracted sarcomeres as having the typical banding pattern except for an absence of H-zones and M-lines. Electron micrographs did not disclose the presence of thin filaments in the A-bands except when the muscle was under tension during fixation. Even when under tension however, the sections were probably cut too thick to secure clear visualization of thin filaments. On the basis of ATP-induced contractions of glycerol-extracted muscle, movies of which show an apparent shortening of the A-band, DeVillafranca felt it was the A-band itself which was responsible for the shortening (DeVillafranca, G. W., 1961).

Recently there has been reported a similar shortening of the A-band in the barnacle Balanus aquilia (Baskin, R. J. and Wiese, G. M., 1964). In addition to new types of bands which formed (also described by DeVillafranca), fibrils under various states of shortening appeared to show a migration of A-band material to the Z-lines.

The literature examined leaves many questions about the contractile mechanism still unanswered while on several of the most basic points it is confusing and in disagreement. If we are to fit the contraction of Balanus muscle into the picture there are several points one must remain cognizant of. First is the question of how typical of all striated muscles is this particular preparation. Should the credentials of "typical" striated muscle be only its light microscopic appearance? There is, in addition, the nature of changes imposed on the muscle by glycerination and fixation, as well as the optical artifacts of phase-contrast microscopy.

Keeping these questions in mind, the giant fibers of Balanus were examined with both light and electron microscopy with the purpose of elucidating their mechanism of contraction. The large size of the fibers, the pattern of regular striations and the ability to supercontract were thought to make it a valuable addition to comparative investigations of muscle physiology.

MATERIALS AND METHODS

The barnacle Balanus nubilus was used throughout the investigation. Specimens were obtained by dredging and skin-diving from the waters of Puget Sound by the staff of the Friday Harbor Laboratory. They were transferred to Eugene in plastic bags filled with sea water and surrounded by ice. Upon arrival the barnacles were placed in large sea water aquaria which were kept in a cold room. The water was continually aerated and filtered.

Glycerination

Barnacles were dissected following the method of Hoyle (Hoyle, G. and Smyth, T., 1963). Generally the depressor muscles were used, their rest length being noted before removal from the shell. Whole bundles were then dissected free, leaving their origins and insertions intact, and placed in cold barnacle Ringers (in mM/L: Na^+ 476; K^+, 8; Ca^{++},; Mg^{++}, 12; Cl^-, 538; HCO_3^-, 10). The muscles were then tied onto applicator sticks with soft cotton thread. Some muscles were kept at rest length while others were stretched up to 180 per cent of rest length. Cold (0^oC) glycerol (50 per cent V/V buffered with Tris, .067M at pH 7.2) was added slowly to the barnacle Ringer solution to prevent contraction. After a few minutes the muscles

were transferred to fresh 50 per cent glycerol and placed in a freezer at -10°C. Contracted muscles for glycerination were obtained in two ways. One was to place the muscle in a bath of high K^+ barnacle Ringer (200mM). Some were allowed to go into a contracture isotonically while others were made to pull a slight load. The muscles were then tied to sticks and prepared as described. It was found that when the operculum connective was cut the depressor muscles all reflexly contracted pulling the plates deep into the shell. The muscles could be fixed in this natural supercontracted condition merely by filling the shell with cold 50 per cent glycerol and placing the whole animal in the freezer. After 24 hours the shell was removed and the contracted muscles were dissected free and placed in fresh glycerol. All muscles were allowed to remain in the freezer at least one week before use. They remained in good condition and could be used for several months.

Preparation of fibrils for Phase Microscopy

For contraction and extraction experiments one fiber was removed from the muscle and placed in a solution of .02M ATP in .05M KCl and .0067M Tris buffer at pH 7.0. If the fiber contracted the muscle was considered in good condition. Several fibers were then removed and placed in about 100cc. of buffered .05m KCl, pH 7.4 in a Waring blender. The fibrils were blended for approximately 30 seconds at

high speed and aliquots transferred to centrifuge tubes. The fibrils were centrifuged down and washed twice with .05M KCl at pH 7.0. Fibrils were finally suspended in 2cc. KCl and put in an ice bath. For observation one drop of the suspension was put on a slide and covered with a number 0 coverslip. Solutions of various kinds could be washed over the fibrils by applying them at one end of the cover slip and drawing them through with a small square of filter paper at the other end.

Contraction and Extraction

Fibrils could be made to contract by the addition of .02M ATP. Pure sodium ATP was obtained from the Sigma Chemical Company and dissolved in .05M KCl. pH was adjusted with NaOH. .001M MgCl was added.

Although several of the standard mycsin extracting solutions were tried, a solution with the following composition was found to work best: .6M KCl, .1M PO_4 buffer, .01M Na pyrophosphate and 10^{-3}M $MgCl_2$ at pH 6.5. This is Hanson and Huxley's modification to the Hasselbach-Schneider solution (Huxley, H. E. and Hanson, J., 1957; Hasselbach and Schneider, 1951; Hasselbach, W., 1953). Actin was removed by the addition of a solution containing 0.6M KI and 0.006M sodium thiosulfate (pH 5.5).

The fibrils were observed with a Leitz Ortholux microscope fitted with a Heine phase contrast condenser.

The objective was a 63x dry type which was found to give better contrast than the oil immersion objective. The microscope was fitted with a Nikkon 35mm. camera or a Bolex 16mm. movie camera. Still pictures were taken on high contrast copy film using a green filter. Movies were taken on Kodak Tri-x film with a high pressure mercury burner as light source and a green filter.

Electron Microscopy

Glycerol extracted fibers to be examined under the electron microscope were first washed in .05M KCl and cut into small sections. Fixation was in 2 per cent Osmium buffered with .1M PO_4 at pH 7.2 for one hour. Dehydration was carried out in acetone. Staining was accomplished at the 70 per cent stage by adding 1 per cent uranyl nitrate to the acetone and leaving overnight. Dehydration was completed the following day. The material was then placed in a 50 per cent epon-acetone mixture for two hours. After two hours the vials were uncapped and the acetone allowed to evaporate for five hours. The sections were then flat embedded in fresh epon and polymerized overnight at 60°C. Thin sections were cut on a Porter-Blum microtome which had gray interference colors, and were approximately 600-800 Å thick. Sections were mounted on copper grids and examined

either in a Bendix TR S 50 or Siemens Elmiskop I electron microscope. All electron microscopy was carried out at the University of California, Berkeley, with the technical assistance of Dr. J. H. McAlear and his staff.

RESULTS

General Morphology

Fibers were first selected from bundles which were glycinerated at various stages of contraction, from super-contracted to 140 per cent of rest length. Rest length fibers revealed a wide range of sarcomere lengths when examined by phase-contrast microscopy. Sarcomeres had the same appearance as vertebrate striated muscle with regard to A- and I-bands and Z-lines. Several differences were noted, however, such as an absence of H-zones and M-lines. The most obvious difference was the large size of the sarcomere. The average size of rest-length sarcomere was 10μ compared with the 2.7μ of rabbit psoas muscle. A typical fibril is illustrated in Fig. 1. The ratio of I-band to A-band in this fibril is .72. Other fibrils from similar bundles had ratios of from .25 to .85 and sarcomere lengths from 8μ to 14μ (Figs. 2 and 3). Only very rarely were fibrils with H-zones found (Fig. 4). Stretched fibers had sarcomeres with extended I-zones (Fig. 5). Often the stretched sarcomeres bulged in the middle as if the diameter of the A-band was greater than the diameter of the Z-line. In highly stretched fibers (over 150 per cent) there

appeared to be a gap between the A-band and the I-band (Fig. 10) and where fibrils were broken during the blending process, the break was usually at the A-I junction.

Contracted fibers had sarcomeres which mostly showed contraction bands, although about 15 per cent of the fibrils had sarcomeres with A- and I-bands. This would indicate that not all fibrils contracted equally during shortening. Contraction bands were not all of the same type. Some were broad and about 2.5μ apart (Fig. 7) while others were narrower and about 1μ apart (Fig. 8). Often contraction bands were observed to consist of doublets (Fig. 9) about 2μ apart. In all the contracted fibrils the width was increased up to four times that of the rest-length fibril. Despite the increased girth there was no loss of optical density in the contraction bands.

Early in the investigation an attempt was made to correlate the I/A ratio with sarcomere length in different sarcomeres. Unfortunately there did not seem to be a positive correlation, because there was a wide range of sarcomere lengths for each I/A ratio. Moreover, the basis for the postulate that the I/A ratio would decrease during contraction was the evidence obtained by Huxley and Hanson on single fibrils. Since the formation of contraction bands may occur by a different mechanism in this muscle it was necessary to observe their formation in single fibrils also.

Extraction of Contractile Proteins

Glycinerination of muscle removes all of the water soluble components of the sarcomere. Left are the contractile proteins actin and myosin bound firmly together because of the absence of ATP which acts normally as a plasticizing agent. Initially standard myosin and actin removing solvents were used to locate their position in the sarcomere. Guba-Straub solution (.3M KCl, 0.15M PO_4 buffer, $5X10^{-4}$ ATP at pH 6.5) was found to be almost totally ineffective for removing myosin. Equally ineffective was the Hasselbach-Schneider solution (0.47M KCl, .01M $NaHPO_4$, 10^{-3}M $MgCl_2$ and 0.1M PO_4 buffer at pH 6.5). Complete selective extraction of myosin could be achieved with Huxley and Hanson's modification of the Hasselbach-Schneider solution. This consisted only of raising the ionic strength of the KCl to .6M. Removal of actin could be achieved by the use of a KI-S_2O_3 solution. Results of a typical extraction experiment are shown in Fig. 10. This fibril was taken from a bundle which had been glycinerated at about 140 per cent of rest length. Washing with the myosin extracting solution removed the A-band entirely. Further washing with KI-S_2O_3 removed the actin from around the Z-bands. By this procedure myosin was found to be located in the A-bands and actin in the I-bands. The presence of actin and myosin in these positions correspond to their equivalents in vertebrate striated muscle. The

length of the actin however is proportionately somewhat less than in vertebrates. The length of the I-band could be measured after removal of the A-band and was found to be 1.5 μ. At rest length this would allow the actin filaments to just penetrate the myosin filaments. This would account for the absence of H-zones which are found in vertebrate sarcomeres as a result of the actin filaments penetrating deep into the A-band, except for an area in the center.

ATP Induced Contraction

With knowledge of the normal dimensions of the sarcomeres as well as the locations of the principal contractile proteins, the changes in banding patterns were studied in fibrils made to contract with ATP. The whole contraction sequence was filmed and single frames were studied individually. Analysis of a sequence is shown in Fig. 13. Results of the measurements are tabulated in the appendix. This fibril had already started to contract when filming was begun. Shortening proceeded down to 58 per cent of the initial length. The first changes one observes upon shortening are the formation of dense lines at the edges of the A-band (α-bands). While these are forming the I-bands are decreasing in width. There follows a gradual decrease in density of the A-band except for the center portion which increases its density. The overall length of the sarcomere shortens as well as the apparent width of the

A-band. (See Graph, Fig. 12). The α-bands increase in density and move to form triplets with the Z-disc. The center of the A-band has now shortened to a more discrete dense line and the whole fibril begins to show what are usually called contraction bands. These bands consolidate themselves by further drawing together of the triplets into single bands and further shortening of the fibril. The contraction bands are formed ultimately by thickened Z-discs (C_Z) alternating with narrowed A-bands (C_M).

During the process of isotonic contraction induced by ATP, many fibrils were found which did not contract completely or which had contracted from one end only. This could be explained by the non-uniform absorption of ATP by the glycinerated fibrils (Bowen, W. J. and Martin, H. L., 1963). In addition, the ATPase activity of myosin in glycinerated fibrils was reduced and this reduction no doubt occurred at different rates. As a result when ATP was added to a suspension, some fibrils would not contract at all, while others contracted vigorously. The fortuitous occurrence of partially contracted fibrils permitted a biochemical and histological study of the different stages of contraction as determined by the filmed sequences. The first change in banding which could be seen in an ATP-induced contraction was a decrease in the density of the outer thirds of the A-band, (Fig. 3). As the I-bands began

to narrow, dark lines (α-bands) appeared at the ends of the A-band. There was also a dark line which could sometimes be seen running vertically down the very center of the A-band, (M-line). This stage is well illustrated by Figs. 14 and 22. The triplets formed by α-bands and A-lines which were described in the movie sequences are shown in Figs. 15, 16 and 17.

When fibrils contracted from one end only, the sequence of banding changes supported those determined by analysis of filmed contractions. Figure 18 is an example of an unevenly contracted fibril. At one end of the fibril, sarcomeres show the typical banding arrangements seen at rest length. At the other end, contraction bands are observed to have formed from the merging of triplets at the Z-line and the formation of M-lines at the center of the A-band. The contraction bands can be designated as C_Z and C_M bands. Measurements of the fibril shown in Fig. 18 indicated the initial broad contraction bands formed when the sarcomeres had shortened only about 20 per cent. Also the distance between the outside borders of the C_Z-bands was approximately the length of the A-band before contraction. The A-band appears to shorten by about 85 per cent, but to say this is attributable to a shortening of the thick filaments is unwarranted. That the appearance of shortening is probably due to relative

increases in numbers of thick filaments at the Z-band and M-line will be shown in the section on ultrastructure.

The extraction of myosin and actin from an unevenly contracted fibril is illustrated in Fig. 19. Most of the myosin can be removed by the Huxley-Hanson solution, but a faint line can be observed where the middle of the A-band was. This line, and also the remaining actin, was removed by a KI-S_2O_3 solution, leaving only Z- and C_Z-lines. This extraction shows clearly the dual nature of the contraction bands, i.e., alternate C_Z-C_M bands.

In accordance with the sliding filament model, fibrils which were excessively stretched could not be induced to contract with ATP. Figure 21 illustrates such a fibril after it had been irrigated with ATP. Extraction of this fibril, however, showed resistant areas which must have undergone some change under the influence of ATP. These areas were located in a position where α- and C_M-bands would be expected to occur. Figure 20 shows a fibril in an early stage of contraction which also had resistant areas in the same places. Figure 22 illustrates a fibril which contracted down to the level of α- and M-band formation. Extraction left the α-bands intact, while all the free myosin and actin were completely removed. It cannot be said whether these resistant areas represent areas having new chemical linkages making them less soluble or

are areas in which there is only an increase in extractable material.

Ultra-structure

In order to correlate the banding patterns observed in the light microscope with the actual position of the myofilaments, glycinerated fibrils were examined under the electron microscope. Figure 23 shows a fiber fixed at rest length. The A-bands are composed of thick filaments about 6.2μ in length. No thin filaments are seen interdigitating with them but this may be due to the thickness of the sections. Thin filaments in the I-bands extend into the thick filaments a short distance. The width of the I-band in this particular example is about 1.5μ making the total sarcomere length 9.2μ. The short I-bands and long A-bands correspond fairly well with the location of the myosin and actin as determined by the extraction experiments (See Fig. 10). The Z-lines are composed of homogenous, irregular lines, more osmiophilic than the myofilaments. Glycineration apparently causes the sarcoplasmic reticulum to be broken up because there is no clear indication of where it might be connected to the sarcomere.

The length of the thin filaments allows them to penetrate only a short distance into the A-band. They may continue through the A-band as S-filaments, not visible in the thick sections which were made. The absence of actin

filaments throughout most of the length of the A-band would account for the fact that no H-zones are observed under phase contrast microscopy. The H-zones which are so prominent in vertebrate sarcomeres are due to the greater penetration of thin filaments into the thick ones. This increases the relative number of myofilaments in the outer regions of the A-band, making the central region less optically dense. The short actin filaments in Balanus would also explain why contraction bands form at about 80 per cent of rest length, for at this point the myosin would have been pulled up to the Z-lines.

Contracted Fibers

Fresh muscles were shortened prior to glycerination by the following procedures:

1. Cutting the scutal-tergal connectives causing a reflex contraction of the depressors.
2. Immersion in 200mM potassium barnacle Ringer causing contracture.

In addition, glycinerated fibers were placed in a .02M solution of ATP to which 10^{-3}M Mg^{++} had been added. These fibers shortened isotonically to about 50 per cent of their initial length.

Muscles contracted by natural nervous stimulation are shown in Figs. 24-29. It is evident at once that shortening

has proceeded by groups of thick filaments being pulled through the Z-disc on both sides of the sarcomere. The Z-disc is composed of dense bodies which have moved apart to allow passage of the filaments. Figure 30 shows sarcomeres taken from the same fiber indicating the probable sequence of the contraction. It begins as a skewering of the thick filaments towards each end of the sarcomere, (Fig. 30A). The sarcomere length here is about 5μ so the fibril has probably shortened somewhat. Thick filaments then penetrate the Z-disc (Fig. 30B) and the muscle shortens until large groups of thick filaments from adjoining sarcomeres have come to override one another (Fib. 30C); the sarcomere length here is 2.5μ. It is difficult to determine whether or not the thick filaments shorten because they come in and out of the plane of section and in many places appear to run together. However, they are no thicker in the heavily contracted stages than they are in the more extended ones. In a few places the thick filaments have not been able to penetrate the Z-discs and are bowed back. Higher magnifications show clearly the passage of the thick filaments through the Z-particles (Fig. 28). In some cases thick and thin filaments interdigitate as they pass through, although these thin filaments may actually be incomplete sections of thick ones (Fig. 29).

Figure 31 to 35 are low power electron micrographs of K^+ contractures. Shortening exceeds that produced by natural excitation but the Z-particles remain close together thus preventing the thick filaments from passing through. There is an enormous amount of buckling up in all directions as the thick filaments hit the Z-line. The length of the sarcomeres in most of the sections is less than 2 μ. This is a little shorter than the muscle was able to contract by natural stimulation. The high magnification shown in Fig. 35 indicates clearly how the Z-line has remained effectively closed to the passage of the thick filaments. The particles when forming a solid Z-line also appear to be of a smaller diameter than when the Z-line has opened up.

Electron micrographs of fibers which had been made to contract with ATP were not of sufficient quality to allow adequate interpretation. However, several electron micrographs (Figs. 36 and 37) suggest a possible mechanism. The shortening of the A-band would be the result of skewering so that the relative width of the A-band becomes shorter. The length of the thick filaments would remain the same, but the ATP would cause a virtual "tug-of-war" between actin and myosin on both sides of the A-band. The result would be some thick filaments going to one side and some to another. If the thick filaments were unable to penetrate

the Z-lines when they reached them, buckling up would occur as in K^+ contractures (Fig. 37). The relative increase of thick filaments at these points of crumpling up would result in the appearance of dense lines under the phase-contrast microscope at about the position where the lines do occur. As more and more thick filaments were pulled up to the Z-discs the density of the lines would increase to the point where under the light microscope they would appear to merge. In the case of ATP-induced contractions of glycinerated muscle therefore, contraction bands could be due to a crumbling up of filaments. There was, however, some electron microscopic evidence obtained which indicated there was good passing through of thick filaments. At this time the true configuration of thick filaments in glycine-rated fibrils supercontracted with ATP is not clear, and must await further investigation.

Effect of High ATP Concentration

When a high ATP concentration (.06M) was employed to induce contractions a very unusual type of shortening occurred. The fibril shortened about 30 per cent but lost density at the Z-lines, when observed in the light microscope. The contracted fibril assumed a dumb-bell shape with large bulges at the Z-lines. The A-bands and I-bands merged and lost optical density. A fiber was prepared for electron microscopy using a 0.06M ATP concentration to induce

contraction. Examination of the ultrastructure revealed a similar enlargement around the Z-disc area although the Z-particles themselves were not visible (Figs. 38 and 39). There was no sign of thin filaments, but thick filaments appeared almost normal. There was some flaring of the ends of the thick filaments and cross bridges were visible.

Electrostatic Effects on Filaments

It was noted in early attempts to remove myosin that when .04M ATP at a pH of 7.5 was irrigated over the sarcomeres, they swelled rather suddenly (Fig. 40A,B). The swelling up was accompanied by a decrease in optical density throughout the A-band. Often, however, a dark band remained in the center of the A-band and occasionally at its ends. The decrease in optical density could have been due to either the extraction of myosin or to the repulsion of myosin filaments. ATP has the same plasticizing effects on the actomyosin linkages as does pyrophosphate, so probably some myosin is removed. The sudden swelling still remains a mystery and is certainly worth further study. If there was a repulsion of thick filaments, changes in pH should cause similar effects in contractions induced by lower concentrations of ATP. The optimum pH for myosin ATPase activity is 6.5 and 9.2 for vertebrate myosin. It was found that fibrils would contract in a medium whose pH was as low as 6 and as high as 9.5. There were obvious differences in

the rate of contraction however, with faster rates at higher pHs. Contractions at lower pHs were slower and the fibrils remained optically dense. At pHs above 7.4 the fibrils contracted but lost optical density and at higher pHs seemed actually to lose myosin. It would appear that the amount of negative charge collected by the myosin molecule influences its relation both with actin and with adjoining myosin molecules. Further studies should be undertaken to elucidate further the effects of pH on extraction and contraction of glycinerated fibrils.

DISCUSSION

In the Balanus depressor muscles several unique features are seen which make reconsideration of existing theories of contraction necessary. There are two models of simplified contraction systems which have led to these theories. The first model consists of gel threads or fibers of purified actomyosin which has been extracted from whole muscle by alkaline 0.6M KCl and reconstituted (Szent-Gyorgyi, 1951). The second model is made by extraction of muscle in 50 per cent glycerol, removing salts, small organic molecules and water soluble proteins and leaving bundles of fibers which contain only contractile proteins. Both models contract and develop tension when induced with 2mM ATP in the presence of Mg^{++}. Glycerol extracted muscle models are more similar to live muscles than are actomyosin models and except for the absence of soluble sarcoplasmic materials, have the same appearance as fresh muscle in the electron microscope. Fresh muscle placed in a solution having a high concentration of potassium will also contract and develop tension. Kuffler (1946) was one of the first to show that a rise in external potassium concentration causes a rapid depolarization and prompt contraction. Single fibers in potassium contracture

could also develop tension greater than their tetanus tension (Hodgkin, A. L. and Horowicz, P., 1960). Shortening and tension development have also been measured in single fibers of Balanus by indirect stimulation (Hoyle, G. and Smyth, T., 1963c).

There is the a priori assumption by many physiologists that the mechanism of shortening and tension development in terms of molecular events is the same no matter what muscle system is employed. In addition there is a tendency to place muscles into three groups on the basis of their histological appearance. Muscles are considered striated, smooth or of cardiac type on the basis of their banding and to some extent their physiologic properties. Recent evidence, especially that obtained by a comparative approach, is proving that there is a much broader diversity of histological types than once assumed.

The mechanism of contraction in Balanus muscle requires consideration of both the method used to cause shortening and the histological changes accompanying each different method. The results of the present investigation indicate that the normal physiological contraction initiated by natural nervous stimulation differs markedly from shortening initiated by high potassium. Although the gross manifestations are the same, i.e., shortening, molecular events in terms of filament interaction are different.

Both self-contraction and potassium contracture can occur by thick and thin filament interaction following the Huxley model. What differs between the two is that during a normal contraction the Z-particles open to allow passage of thick filaments through them, and in a potassium contracture remain closed, effectively blocking the passage of thick filaments. The Z-particles apparently could serve a key function in the contraction process.

The ultrastructure of the Z-disc in vertebrate muscle has only recently been investigated (Knappeis, G. G. and Carlsen, F., 1962; Franzini-Armstrong, C. and Porter, K. R., 1964), and appears to be made up of rod-like projections of thin filaments. In longitudinal sections one thin filament lies between two thin filaments on the opposite side of the Z-line. In cross sections through the Z-line the thin filaments and the Z-filaments form a tetragonal pattern. The structural arrangement was interpreted to indicate that each thin filament on one side of the Z-line faced the center of the space between four thin filaments on the other side of the Z and that the interconnection was formed by four Z-filaments. There was no indication of the dense osmophilic particles shown in the Z-line of the present report. The ultrastructure of the Z-disc in invertebrate striated muscles has not been thoroughly investigated, however several reports are relevant to the problem.

A study of muscle fibers from the translucent part of the adductor of the oyster Crassostrea (Hanson, J. and Lowy, J., 1961) show dense particles. The muscle does not show Z-lines but the dense bodies appear in the planes where Z-lines would be expected. Similar dense bodies occur in many smooth muscles, for example in the mammalian uterus (Mark, 1956; Schoenberg, 1958) and in the anterior byssus retractor of Mytilus (Hanson, J. and Lowy, J., 1959). Kawaguti shows them in many invertebrate muscles such as earthworms and crabs in both striated and smooth types (Kawaguti, S., 1957, 1959, 1961).

The Z-particles are larger when separated than when forming a solid Z-line suggesting a change in their morphology during contraction. Smaller particles may coalesce to form larger ones or their orientation may change with respect to the longitudinal axis. It is also possible to suggest the particles being interconnected by a reticular system which would allow rapid communication across the Z-band during transmission of excitation. Supporting this, Garamvolgyi has been able to take electron micrographs of Z-discs by dissolving the rest of the sarcomere in .1 per cent lactic acid (Garamvolgyi, N., 1962). The discs show a fine network-like structure. If the fibrils were first suspended in distilled water and then extracted in Weber-Edsall's solution (.6M KCl, pH 7.6, plus pyrophosphate

and Mg^{++}) they yield Z-discs with a more expressed reticular structure.

There is a large body of evidence accumulating that the coupling device between depolarization of the sarcolemma and the development of linkages between thick and thin filaments is the sarcoplasmic reticulum (Porter, K. R. and Palade, G. E., 1956; Weber, A., 1963; Davis, R. E., 1963). A sarcoplasmic reticulum is found in Balanus muscle when the tissue is fixed unglycinerated; furthermore, it is found to be connected to the sarcomere mainly at the Z-lines. On the basis of this evidence it is possible to postulate a mechanism for supercontraction in Balanus. The initial event would be a depolarization of the muscle membrane as is well documented (See Hoyle, G., 1957). The excitation is transported to the individual fibrils via a reticular network through a still unknown mechanism but which ultimately leads to the release of calcium (Niedergerke, T., 1955; Podolsky, R. S., 1962). During normal excitation, and this may just be a difference in degree from a potassium contracture, (Bianchi, C. P. and Shanes, A. M., 1959) the Z-particles are somehow triggered to spread apart causing large gaps in the Z-disc. Instead of actin being pulled into the myosin, myosin is pulled by the actin filaments towards the Z-line and ultimately through it. Extreme shortening is possible due to the

ability of the thick filaments to slide over one another while thin filaments remain between them. The location of myosin in the Z-discs of contracted fibrils has been beautifully demonstrated by Tunik (Tunik, B. and Holtzer, H., 1961). Fluorescein-labeled rabbit antibodies were prepared against chicken myosin, heavy meromyosin and light meromyosin. In fibrils showing contraction bands, myosin and light meromyosin was found to be located in the bands formed at the Z-line while heavy meromyosin was found in those formed at the M-line and in two regions on both sides of the Z-line. Actin was found at the Z-line and M-line indicating part of the M-line may be formed by actin filaments bunching up in the middle of the sarcomere.

Contracted fibrils teased from the indirect flight muscles of Drosophila show a strong positively birefringent band at the level of the Z-band (Aronson, J., 1963). As the fibril shortened the width of this Z-band increased suggesting the positively birefringent Z-band resulted from interdigitation of A-filaments in adjacent sarcomeres. With shortening to about 35 per cent of their initial length (by exposure to ATP and EDTA) the cytological pattern suggested the A-filaments of alternate as well as adjacent A regions interdigitated.

During a potassium contracture the slow depolarization may not provide a trigger adequate to open the Z-line.

As a result, the thick filaments crash into the tightly packed Z-particles and buckle up near them. Whether thick filaments penetrate the Z-line or just hit them, there is an effective increase in thick filaments around the Z-lines which would lead to the appearance of contraction bands. Because an ATP-induced contraction would probably have no way of opening the Z-lines successfully, contraction bands are probably formed initially by doublets as in potassium contractures.

There is a recent report of electrically stimulated frog muscle showing dense material at the A-I junction which the author interprets to be early contraction bands (Sjostrand, F. S., 1964). The actomyosin models which contract under the influence of ATP and Mg^{++} can serve as a basis for contraction theories other than the sliding filament model. Sjostrand, for example, suggests that the contraction bands develop through a change in the arrangement of A- and I-band filaments at the A-I-band boundary. The A-band filaments would presumably dissociate into thinner branches forming a dense network by interaction with I-band filaments. The structural arrangement of the thick and thin filaments would serve to block active sites on the myosin. The splitting up of the myosin filaments would unmask the active sites and make possible the formation of an actomyosin complex. Electrical stimulation

was not undertaken during the present investigation because of inherent fixation difficulties, but this as well as continued efforts to determine the ultrastructure of ATP contracted fibers should be pursued.

The electrostatic effects of pH and high ATP concentrations deserve further examination also. The dispersal of the Z-band with high concentrations of ATP lends support to the postulate of the Z-band being triggered by an excitatory impulse. It may in fact be the accumulation of charge on the Z-particles which is responsible for a coulombic repulsion from one another.

Balanus muscle fits into the interpretation given by Hanson and Lowy to invertebrate muscle contraction mechanisms (quoted from Hoyle, G., 1964): (1) there are two kinds of filaments; (2) there are bridges between the filaments; (3) the filaments are not continuous along the length of the fiber; (4) the filament length is constant at different muscle lengths; (5) the relative position of the two kinds of filaments change as the muscle shortens. The mechanism derived from these points is equivalent to that proposed in vertebrates (Huxley, A. F. and Niedergerke, R., 1954; Huxley, H. E. and Hanson, J., 1954). One reservation of the Huxley theory, namely the fate of the thick filaments when they hit the Z-line, is explained. The extreme shortening of which the Balanus depressor is capable of is due to the overriding

of the thick filaments. There is no evidence to suggest that shortening, no matter how it has been induced, is caused by contraction of myosin molecules or an actomyosin complex. Balanus muscle, although striated has many properties of smooth muscle, so that further studies on this preparation, and on invertebrate muscles in general, should recognize the possibility of Balanus being an intermediate form.

SUMMARY

1. Depressor muscles from the barnacle Balanus nubilus were found to contain giant fibers which were beautifully striated and capable of contraction down to one-sixth their resting length.

2. Rest length sarcomeres averaged 10μ. A- I- and Z-bands were exactly like those observed in vertebrate skeletal muscle. M-bands and H-zones were seen only rarely.

3. Glycerinated fibrils were studied utilizing phase-contrast and electron microscopy.

4. Extraction of contractile proteins revealed myosin to be located in the A-bands and actin in the I-bands. I-bands were relatively shorter than those found in vertebrate sarcomeres.

5. Fibrils were induced to contract with .02M ATP in the presence of Mg^{++}. Contraction sequences were filmed and analyzed frame by frame for changes in the banding pattern. Shortening was found to consist of:

    a. decrease in width of I-bands.

b. Loss of optical density of A-band followed by the appearance of dense bands at both ends (α -bands), a dark area remaining in the middle of the A-band (M-band).

c. α -bands became broader and formed triplets with the lightening Z-line. M-band became thinner.

d. Contraction bands are formed by coalescence of α -bands at the Z-line alternating with M-bands.

This sequence is supported by still photographs of fibrils which had contracted unevenly and show the changes described.

6. Electron microscopy revealed the sarcomeres to be composed of alternating thick and thin filaments. Thin filaments appeared to be attached to the Z-particles and to extend into the thick filaments. Cross-bridges were observed on the filaments at regular intervals.

7. Electron micrographs of self-contracted fibers indicate the process of supercontraction occurs by an opening of the Z-lines allowing passage of groups of thick filaments to slide through them and over one another.

8. High potassium contractures can be postulated as being caused by the same interaction of thick and thin filaments. The Z-lines, however, do not open up and thick filaments buckle up as they crash against them.

9. No good ultrastructural evidence was obtained for the mechanism of ATP-induced contractions but analysis of banding changes suggests they are similar to potassium contractures.

10. The overall relation of Balanus muscle and its contraction mechanism to other types of muscle is discussed.

BIBLIOGRAPHY

nson, J., 1963. Overlap of the Birefringent Component of Adjacent A Regions During the Induced Shortening of Fibrils Teased from Drosophila Muscle. J. Cell Biol., 19, 107.

kin, R. J. and Wiese, G. M., 1964. Contraction-Band Formation in Barnacle Myofibrils. Science, 143, 134.

nchi, C. P. and Shanes, A. M., 1959. Calcium Influx in Skeletal Muscle at Rest, During Activity and During K Contracture. J. Gen. Physiol., 42, 802.

en, W. J. and Martin, H. L., 1963. A Study of Diffusion of ATP Through Glycerol-Treated Muscle. Arch. Biochem. Biophys., 102, 286.

, R. E., 1963. A Molecular Theory of Muscle Contraction. Nature, 199, 1068.

illafranca, G. W. and Philpott, D. E., 1960. The Ultrastructure of Striated Muscle from Limulus Polyphemus. J. Ultrastr. Res., 5, 151.

illafranca, G. W., 1961. The A- and I-Band Lengths in Stretched or Contracted Horseshoe Crab Skeletal Muscle. J. Ultrastr. Res., 5, 109.

illafranca, G. W. and Marschhaus, C. E., 1963. Contraction of the A Band. J. Ultrastruct. Res., 9, 156.

anzini-Armstrong, C. and Porter, K. R., 1964. The Z Disc of Skeletal Muscle Fibrils. Z. Zellforsch, 61, 661.

ramvolgyi, N., 1962. The Z and M Formations of Striated Muscle. Acta Physiol. Acad. Scient. Hung., 23, 223.

nson, J., 1956. Studies on the Cross-Striation of the Indirect Flight Myofibrils of the Blowfly Calliphora. J. Biophys. Biochem. Cytol., 2, 691.

on, J. and Lowy, J., 1959. Evidence for a Sliding Filament Contractile Mechanism in Tonic Smooth Muscles of Lamellibranch Molluscs. Nature, 184, 286.

Hanson, J. and Lowy, J., 1961. The Structure of the Muscle Fibers in the Translucent Part of the Adductor of the Oyster Crassostrea Angulata.

Hasselbach, W. and Schneider, G., 1951. Die L-Myosin und Aktingehalt des Kaninchenmuskels, Biochem. Z., 321, 462.

Hasselbach, W., 1953. Elektron Mikroskopische Untersuchungen an Muskelfibrillen bei Totales und Partielter Extraktion des L-Myosins. Z. Naturforsch., 80, 449.

Hodge, A. J., 1955. Studies on the Structure of Muscle. J. Biophy. Biochem. Cytol., 4, 361.

Hodge, A. J., 1956. The Fine Structure of Striated Muscle. J. Biophys. Biochem. Cytol., Supplement, 2, 131.

Hodgkin, A. L. and Horowicz, P., 1960. Potassium Contractures in Single Skeletal Muscle. J. Ultrastr. Res., 5, 151.

Hoyle, G., 1957. Comparative Physiology of Nervous Control of Muscular Contraction. Cambridge University Press.

Hoyle, G. and Smyth, T., 1963a. Giant Muscle Fibers in a Barnacle, Balanus Nubilus Darwin. Science, 139, 49.

Hoyle, G. and McAlear, J. H., 1963b. Mechanism of Supercontraction in a Striated Muscle Fiber. Science, 141, 712.

Hoyle, G. and Smyth, T., 1963c. Neuromuscular Physiology of Giant Muscle Fibers of a Barnacle, Balanus Nubilus Darwin. Comp. Biochem. Physiol., 10, 291.

Hoyle, G., 1964. Muscle and Neuromuscular Physiology in Physiology of Mollusca. Vol. I., Academic Press, New York.

Huxley, A. F. and Niedergerke, R., 1954. Structural Changes in Muscle During Contraction. Nature, 173, 971.

Huxley, A. F. and Niedergerke, R., 1958a. Measurement of the Striations of Isolated Muscle Fibers with the Interference Microscope. J. Physiol., 144, 403.

Huxley, A. F. and Taylor, R. E., 1958b. Local Activation of Striated Muscle Fibers. J. Physiol., 3, 426.

Huxley, A. F. and Peachey, L. D., 1961. The Maximum Length for Contraction in Vertebrate Striated Muscle. J. Physiol., 156, 150.

Huxley, H. E. and Hanson, J., 1954. Changes in the Cross-Striations of Muscle During Contraction and Stretch and Their Structural Interpretation. Nature, 173, 973.

Huxley, H. E., 1957. The Double Array of Filaments in Cross-Striated Muscle. J. Biophys. and Biochem. Cytol., 5, 631.

Huxley, H. E., 1963. Electron Microscope Studies on the Structure of Natural and Synthetic Protein Filaments from Striated Muscle. J. Mol. Biol., 7, 281.

Kawaguti, S. and Ikemoto, N., 1957. Electron Microscopy on the Smooth Muscle from the Body Wall of the Earthworms, Pheretima Communissima and Eisenia Foetida, Biol. J. Okayama Univ., 3, 223.

Kawaguti, S. and Ikemoto, N., 1959. Electron Microscopic Patterns of Earthworm Muscle in Relaxation and Contraction Induced by Glycerol and Adenosine-Triphosphate. Biol. J. Okayama Univ., 5, 57.

Kawaguti, S., Ikemoto, N., and Takeoka, T., 1961. Electron Microscopic Study on the Gut Muscle of the Horseshoe Crab. Biol. J. Okayama Univ., 7, 53.

Knappeis, G. G. and Carlsen, F., 1962. The ultrastructure of the Z Disc in Skeletal Muscle. J. Cell Biol., 13, 323.

Kuffler, S. W., 1946. The Relation of Electrical Potential Changes to Contracture in Skeletal Muscle. J. Neurophysiol., 9, 367.

Mark, J. S. T., 1956. An Electron Microscope Study of Uterine Smooth Muscle. Anat. Rec., 125, 473.

Niedergerke, R., 1955. Local Muscle Shortening by Intracellularly Applied Calcium. J. Physiol., 128, 12.

Podolsky, R. J., 1962. The Structural Changes in Isolated Myofibrils During Calcium Activated Contraction. J. Gen. Physiol., 45, 631A.

Porter, K. R. and Palade, G. E., 1956. Sarcoplasmic Reticulum in Striated Muscle Fibers. J. Biophys. Biochem. Cytol., 2 (Supp.), 163.

Schoenberg, C. F., 1958. An Electron Microscope Study of Smooth Muscle in Pregnant Uterus of the Rabbit. J. Biophys. Biochem. Cytol., 4, 609.

Sjostrand, F. S. and Anderson-Cedergren, E., 1957. The Ultrastructure of the Skeletal Muscle Myofilaments at Various States of Shortening. J. Ultrastruct. Res., 1, 74.

Sjostrand, F. S., 1962. The Connections Between A- and I-Band Filaments in Striated Frog Muscle. J. Ultrastr. Res., 7, 225.

Sjostrand, F. W., 1964. Ultrastructural Changes in Skeletal Muscle, Myofibrils in Connection with Contraction. Nature, 201, 45.

Szent-Gyorgyi, A., 1951. Chemistry of Muscular Contraction. 2nd Edit., Academic Press, N. Y.

Tunik, B. and Holtzer, H., 1961. The Distribution of Muscle Antigens in Contracted Myofibrils. Determined by Fluorescein-labeled Antibodies. J. Biophys. Biochem. Cytol., 11, 67.

Weber, A., 1963. On the Mechanism of the Relaxing Effect of Fragmented Sarcoplasmic Reticulum. J. Gen. Physiol., 46, 679.

APPENDIX I

PLATES

Figure 1. A fibril viewed under phase-contrast microscopy shows A- and I-bands as well as Z-lines, features common to all striated muscle. H-zones were seen only in a few preparations. The sarcomeres measure just over 10μ in length and 3μ in diameter. (There are actually two fibrils here as can be seen by the Z-lines going out of register in several places.) The I:A ratio is .72 for each sarcomere.

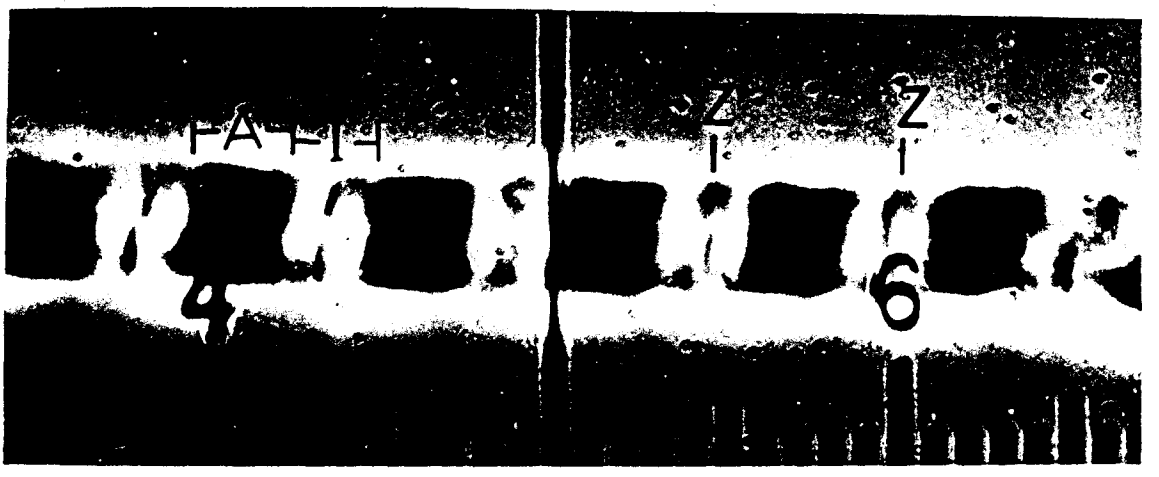

Figure 1