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REACTIVITY OF MYOSIN TO ANTIBODIES IN CROSS-STRIATED CHICK MYOFIBRILS

II. CONTRACTED MUSCLE*

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SUMMARY

Contracted myofibrils bind similar amounts of anti-myosin as myofibrils at rest length. At sufficiently high antibody concentrations all of the myosin in the contracted myofibril reacts with antibody. The changes in the fluorescent band pattern in various states of contraction are explained on the basis of changes in the localization of myosin within the sarcomere. The fluorescent band remains unaltered in myofibrils obtained from passively stretched muscles. In muscles, contracted isotonically below equilibrium length, sarcomere length and fluorescent band width decreases proportionally. The results are interpreted to mean that in contraction the position of myosin within the sarcomere is changed and that contraction is the result of a structural change in myosin or in myosin-containing structures.

INTRODUCTION

TUNIK AND HOLTZER[1] found that the staining pattern obtained with the aid of fluorescein-labeled antibodies depends upon the contracted state of the myofibrils. Comparing the staining of contracted myofibrils with rest-length fibrils they observed that using antibodies prepared against myosin and LMM the fluorescence was restricted more to the lateral edges of the A-band in contracted myofibrils. Only the contraction band formed at the Z-membrane (C_z-band) showed deposition of antibody at the more advanced stages of shortening, while the region of the contraction band at the center of the sarcomere (C_m-band) did not stain.

These observations were extended in the studies reported here. The antibody uptake and the reactivity of myosin in contracted myofibrils was quantitatively measured. The effect of impurities in the antibody preparation, the possible influence of steric hindrance in the interpretation of the altered staining pattern was evaluated. The changes in fluorescent band pattern were correlated with sarcomere length.

Abbreviations: LMM, light meromyosin; anti-M, antibody against myosin.

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METHODS

Contracted myofibrils

Musculus pectoralis minor of chick was separated into bundles of 3–5 mm thickness, cut at both ends and allowed to shorten freely with no load attached. The contracted muscle bundles were placed into glycerol–water (1:1) and stored at 0° for 24 h and at —25° for several months. The bundles were Waring-blendered for 1 min in cold 0.04 M NaCl which contained 0.01 M imidazole buffer (pH 7.0). The myofibrils were centrifuged and resuspended in the same solution twice, an equal volume of glycerol was added and the suspension stored at —25°. The same preparation was used all through the experiments. The distribution of sarcomere lengths measured with a phase microscope is shown on Fig. 1.

Antibody preparations, determination of ATPase activity, estimation of protein and of antibody, antibody uptake by myofibrils, determination of myosin reacted, and microscopy were the same as described in the previous paper[2].

A stirring rod made of polyethylene tubing as described in the preceeding paper was required for a good resuspension of contracted myofibrils.

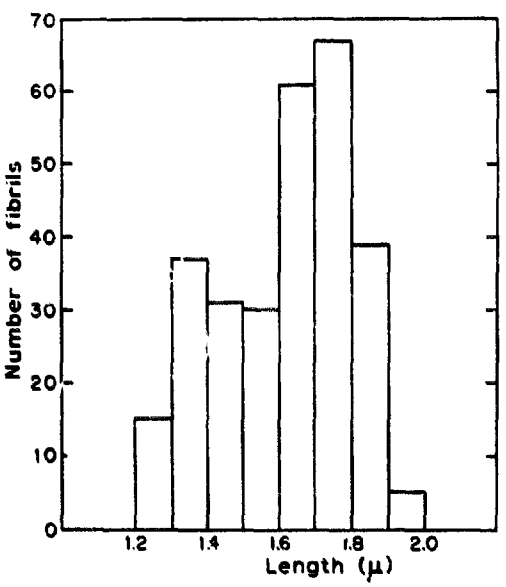

Fig. 1. Distribution of sarcomere lengths in contracted myofibrils.

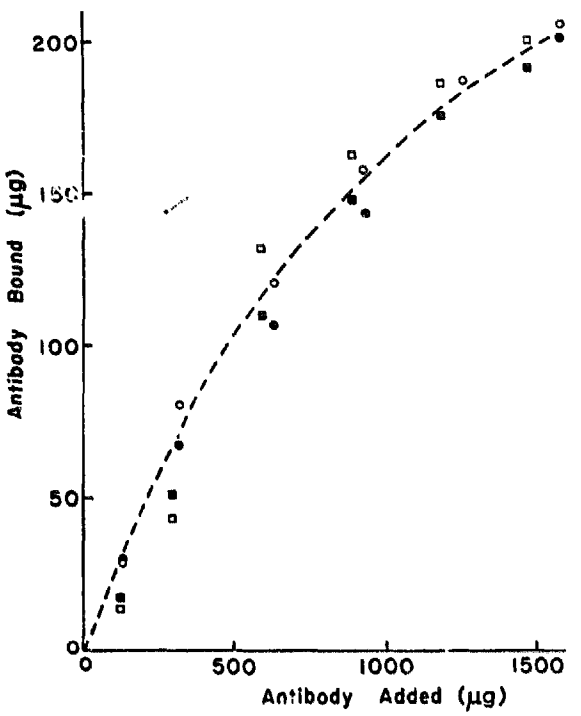

Fig. 2. Anti-myosin uptake of 80 μg contracted myofibril. Broken line, curve of antimyosin uptake of rest-length myofibrils; open symbols, protein determinations; solid symbols, fluorescence determinations.

RESULTS

Binding of antibody by contracted myofibrils

The uptake of anti-M by contracted myofibrils is shown on Fig. 2. Broken line represents the results obtained previously on the binding of anti-M by the same amount of myofibril at rest length. Contracted state does not diminish the deposition

of anti-myosin on the myofibrils under the conditions of these experiments. The difference, if any, between rest length and contracted sarcomeres is below the resolution of these experiments. These studies, however, do not exclude the possibility that diffusion rates of antibody depend on the state of contraction. The incubation with anti-M lasted over 15 h, the long incubation may have reduced the effects of possible differences in the rate of antibody penetration into rest length and contracted sarcomeres.

The similarity of the binding curves indicate that if steric hindrance or other factors limit the amount of anti-M with which myofibrils can react, these limitations operate to an equal extent both in rest-length and contracted preparations.

Fixation of myosin in contracted myofibrils

Table I summarizes the results of myosin fixation with anti-M in contracted myofibrils. These may be compared with the values obtained with rest-length muscles.

TABLE I

EFFECT OF ANTI-M ON THE EXTRACTION OF MYOSIN IN CONTRACTED MYOFIBRILS

	Antibody added (μg)	*Antibody bound (μg)*	*Antibody extracted (μg)*	*ATPase soluble (%)*	*ATPase insoluble (%)*	*Myosin calculated from protein soluble (%)*
Control	0	0	0	78		90
Anti-M	6 270	1 170	94	29	56	29
Anti-M	10 050	2 020	73	22	85	16
Anti-M	17 400	2 550	230	< 10	96	< 5

Anti-M leads to an extensive insolubilization of myosin regardless of the contracted state. Apparently complete inextrability of myosin is achieved with the addition of comparable amounts of anti-M both in contracted and rest-length sarcomeres. The ratio of antibody bound and myosin precipitated is approximately the same for both myofibril preparations. Under the conditions of these experiments, most, possibly all, myosin molecules within contracted sarcomeres are capable of reacting with anti-M, at least to the extent that they precipitate and become insoluble in high-ionic-strength solvent which extracts myosin readily from untreated myofibrils, regardless of the state of contraction. If factors like steric hindrance, blocked antigenic sites or impurities in the antibody preparation have any effect, these effects are similar in contracted and rest-length fibrils.

Staining pattern and variation of sarcomere lengths

The anti-M uptake and fixation studies with contracted myofibrils confirm that the antibody preparation contains significant amounts of antibody against myosin. However, this does not mean it is only myosin which is stained in the myofibril by anti-M (see ref. 3). Antibodies against other myofibrilar proteins may also be present in the anti-M preparation, and these will contribute to the staining pattern. On the other hand, the regions of the sarcomere which do not stain, in conditions when all the myosin molecules reacted with antibody, cannot contain antigenic sites for myosin, *i.e.* these are the regions free of myosin. The interpretation of fluorescent band pattern

may be further complicated by a possible quenching of fluorescence by neighboring groups of certain sarcomere regions.

Representative photographs of rest length or slightly stretched and contracted myofibrils, obtained with phase and fluorescent microscopy, are shown on Plates 1 and 2. Fixation studies on all these fibril preparations indicated full reaction of myosin with antibody.

The following features may be pointed out:

1. The region in the center of the A-band shows a decrease in fluorescence. In rest-length and slightly stretched sarcomeres this region of weaker fluorescence is narrow and hard to resolve. Its length is below 0.2 μ in sarcomeres longer than 2.0 μ. In contracted myofibrils the weakly fluorescing region in the center of the A-band increases in length. The increase can be seen already in the initial stages of shortening at sarcomere lengths where the I-band is still present. The wide clear zone in the

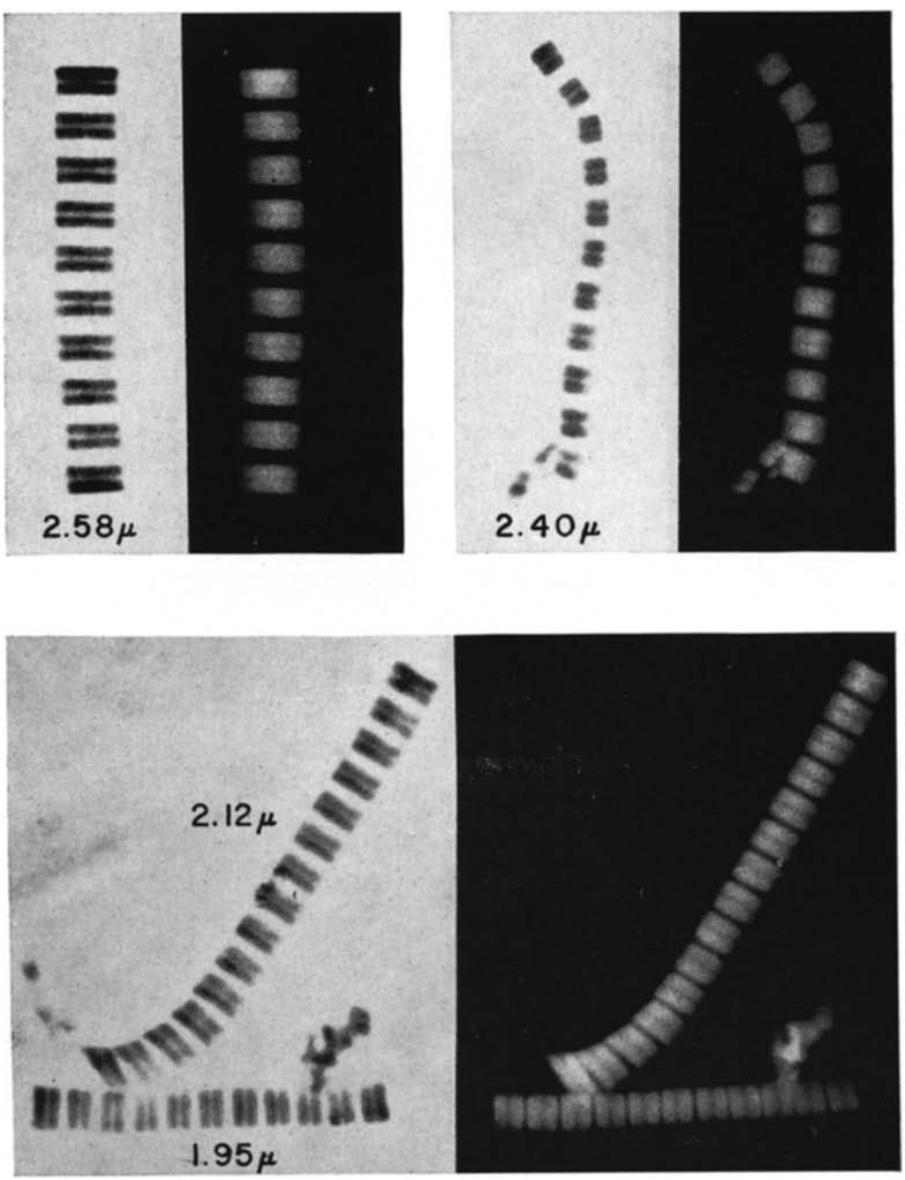

Plate 1. Fluorescent and phase band pattern of anti-M-treated myofibrils. Fibrils longer than equilibrium length.

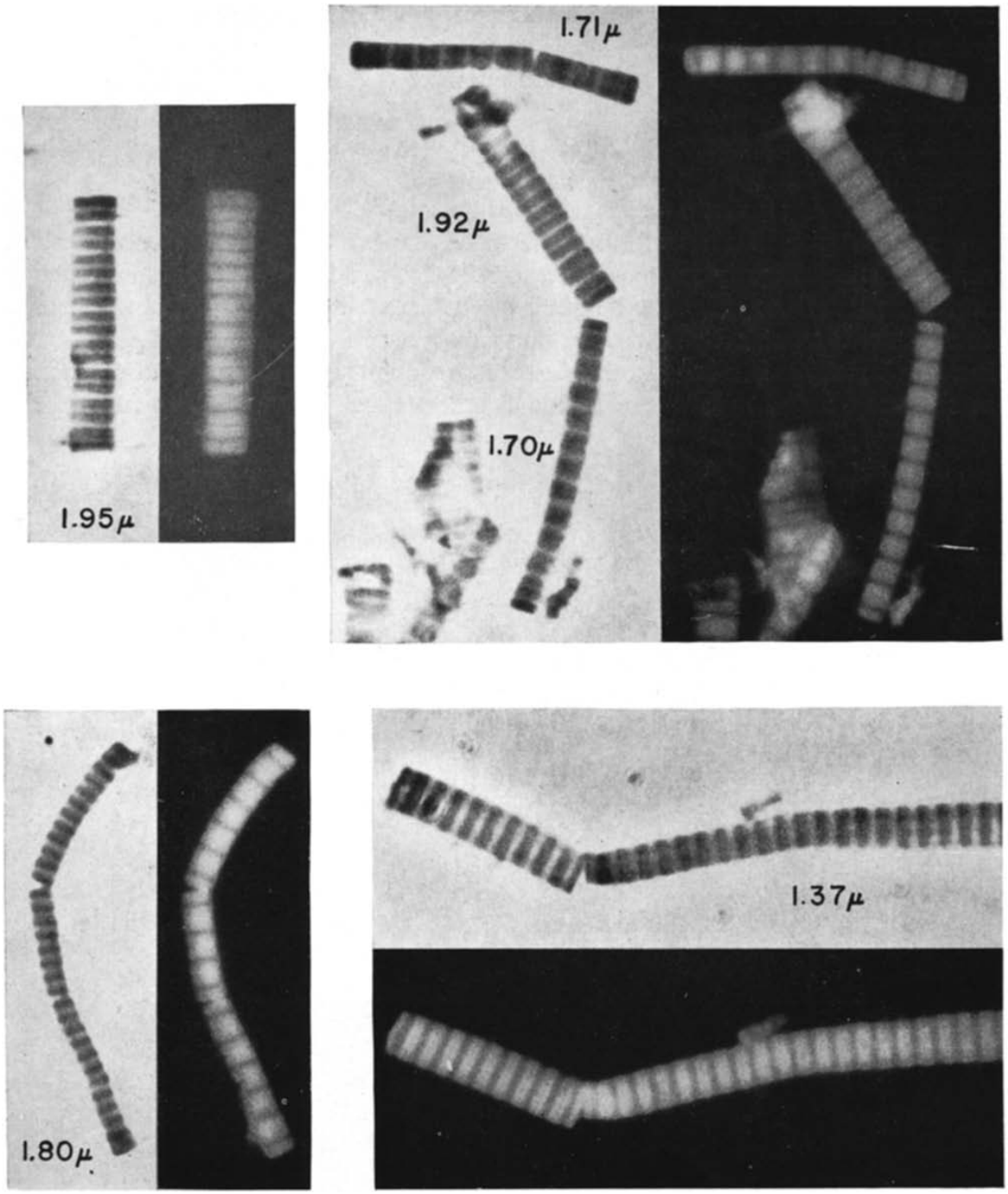

Plate 2. Fluorescent and phase band pattern of anti-M-treated myofibrils. Fibrils shorter than equilibrium length.

center of the sarcomere is maintained in shorter sarcomeres and in more advanced states of shortening fluorescence is more and more restricted to the zones neighboring the Z-membranes. Even in greatly shortened sarcomeres the doublet nature of the fluorescent band at the C_z region can be recognized. Myofibrils break always at the I-band during blendering. At the ends of the myofibrils the fluorescent bands have half of the lengths of all the other fluorescent bands in contracted myofibrils. This behavior was used in positioning the bands in contracted sarcomeres.

2. The length of the A-band in fluorescence observations exceeds somewhat the lengths obtained with phase microscopy. The difference is 0.1–0.15 μ. Apart from this discrepancy, the phase density of antibody-treated myofibrils agrees with the fluorescent pattern. If the phase density of untreated and treated fibrils is compared, it is clear that the density greatly increases in the regions which show fluorescence. The fact that the center of the A-band is less dense in sarcomeres of 1.9 μ length in the phase pictures can be explained by the results of fluorescence which indicate that anti-M is not deposited there. The correlation between the phase and fluorescent band patterns excludes the possibility that local quenching of fluorescence has significantly contributed to the observed distribution of stain.

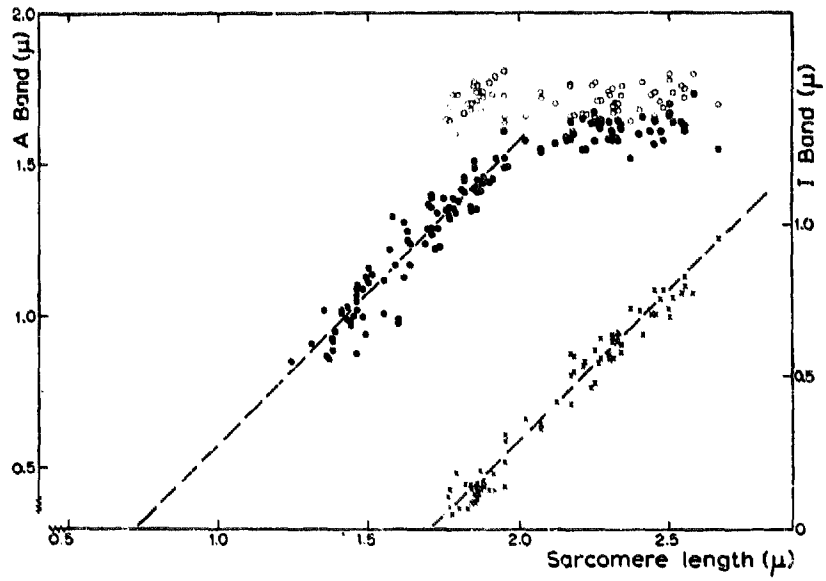

Fig. 3. Dependence of length of fluorescent regions on sarcomere length in anti-myosin-treated myofibrils. Abscissa, sarcomere length. Left-hand ordinate, A-band (O—O); fluorescent band (●—●). Right-hand ordinate, I-band (×—×).

Fig. 3 shows the measurements of the various bands in the sarcomere from the fluorescent photographs. Each point represents a different myofibril and is an average of the values obtained on 6–8 successive sarcomeres. The negatives were read with a Gaertner microcomparator, readings were made successively at the borders of the fluorescent regions, from which the values of the lengths of the A-band, I-band and fluorescent regions were obtained. This way an error in the estimation of one band reflected in the estimation of the others and could serve as an additional check on the accuracy of the measurements.

I-band was observed in sarcomeres longer than 1.7 μ. Its length increased proportionally with sarcomere length. The 45° line fits the experimental points closely. The A-band measured as the distance between the lateral edges of the fluorescent regions within a sarcomere, stayed constant at various stretches and slightly contracted fibrils, down to sarcomere lengths of about 1.7 μ. At this value the A-band and sarcomere lengths become the same in the fluorescent pictures and the A-band cannot be measured in shorter sarcomeres.

These findings are in agreement with the phase and interference studies of H. E. HUXLEY AND HANSON[4] on glycerinated psoas fibrils and of A. F. HUXLEY AND

NIEDERGERKE[5] on isolated single fibers of frog semitendinosus muscle, which showed that changes in sarcomere lengths in passive stretch and initial stages of shortening are correlated with changes in the length of the I-band, while the A-band stays constant.

Fluorescent area was obtained by summing the two fluorescent bands within a sarcomere. The fluorescent area remained constant in sarcomeres of 2μ or longer, *i.e.* between equilibrium lengths and various degrees of passive stretches. Below 2.0 μ the length of fluorescent regions decreases proportionally with the shortening of the sarcomeres. The change in fluorescent area between 2.0 μ and 1.7 μ was seen qualitatively in the photographs showing the widening of the weakly fluorescent regions at the center of the A-band in slightly contracted fibrils. The proportionality between the shortening of sarcomeres and the area where antibody is deposited is shown by the good fit of the experimental points with the 45° theoretical line.

DISCUSSION

Changes in staining pattern in various states of contraction are interpreted here as a result of changes in the localization of myosin for the following reasons. Both rest-length and contracted myofibrils bind comparable amounts of anti-M. In both preparations myosin can be completely fixed by anti-M. Within the accuracy of the experiments steric hindrance, or blocked antigenic sites do not play a significant role in the interaction of myosin in the myofibril with the anti-M molecules. If there is any such type of interference it is present to the same extent in rest-length and in contracted sarcomeres. The experiments do not exclude the possibility that, beside myosin, staining of some other components of the myofibril may have contributed to the fluorescent band pattern obtained with anti-M. It is important though that the presence of such impurities in the anti-myosin preparation would mask the change in the localization of myosin and if anything would lead to the overestimation of myosin-containing regions. The interpretation presented here is based on the measurements of the regions which do not stain in conditions where myosin has completely reacted with anti-M.

The findings may be described then as follows. In passively stretched muscle localization of myosin does not change. Myosin occupies the whole A-band with the exception of a narrow region in the center. In isotonic contraction, below equilibrium length, myosin is present in a smaller stretch of the sarcomere and the shortening of the myosin-containing region is directly proportional to the shortening of the sarcomere. This "migration" or "contraction" starts already at sarcomere lengths at which the I-band can still be observed and cannot be due to the piling up of A-filaments at the Z-membrane. Contraction of myofibrils appears to be accompanied by the displacement of myosin from the central part of the A-band, and a 0.4–0.5 μ wide myosin-free area persists in the later stages of shortening.

These experiments do not give any information on the mechanism of the "migration" of myosin. It may be an intramolecular folding or change in the lattice pattern. It strongly suggests, though, that some type of structural change in the myosin component is closely associated with shortening and it well may be the driving force of contraction.

Myosin represents a major portion of the proteins of the washed myofibrils. If

contraction is accompanied by a relocalization of myosin in the manner suggested by the fluorescent band pattern of contracted myofibrils, one would predict a decrease in density and in intrinsic birefringence of those regions from which myosin has been removed and an increase in density and possibly birefringence at the regions where myosin has accumulated, unless some flow of material other than myosin compensated for such changes. At present we have no information concerning these questions. Quantitative interference microscopic studies were limited, partly for reasons of resolution, to stretched myofibrils[6]. While intrinsic birefringence was reported to decrease in contracted glycerol-extracted fibers[7], there is no adequate study of intrinsic birefringence changes at the sarcomere level.

Movement of myosin in contraction is not in agreement with the sliding filament theory of contraction of HANSON AND HUXLEY[8] which explains contraction by assuming that the actin containing filaments by-pass the myosin containing filaments without any change in the internal structures of these filaments.

It is possible, though, to devise a structure which is based on the double hexagonal array of filaments as a basic organization of muscle, and in which the band-pattern changes are the result of the movement of myosin as observed in the fluorescent-labelled antibody studies. Such a structure has been examined elsewhere[9,10].

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