CONTRACTILITY AND ULTRASTRUCTURE IN GLYCEROL-EXTRACTED MUSCLE FIBERS

I. The Relationship of Contractility to Sarcomere Length

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ABSTRACT

This study was undertaken to determine whether glycerol-extracted rabbit psoas muscle fibers can develop tension and shorten after being stretched to such a length that the primary and secondary filaments no longer overlap. A method was devised to measure the initial sarcomere length and the ATP-induced isotonic shortening in prestretched isolated fibers subjected to a small preload (0.02 to 0.15 P0). At all degrees of stretch, the fiber was able to shorten (60 to 75 per cent): to a sarcomere length of 0.7 μ when the initial length was 3.7 μ or less, and to an increasing length of 0.9 to 1.8 μ with increasing initial sarcomere length (3.8 to 4.4 μ). At sarcomere lengths of 3.8 to 4.5 μ, overlap of filaments was lost, as verified by electron microscopy. The variation in sarcomere length within individual fibers has been assessed by both light and electron microscopic measurements. In fibers up to 10 mm in length the stretch was evenly distributed along the fiber, and with sarcomere spacings greater than 4 μ there was only a slight chance of finding sarcomeres with filament overlap. These observations are in apparent contradiction to the assumption that an overlap of A and I filaments is necessary for tension generation and shortening.

INTRODUCTION

Recent electron microscopic evidence leaves little doubt that the sliding filament model (1, 2) provides a satisfactory morphological description of the shortening process in vertebrate striated muscle. However, the molecular mechanism whereby tension is generated by a double set of parallel, discontinuous filaments remains to be elucidated. It has been suggested (1–7) that force is developed by an interaction between reactive sites on overlapping primary and secondary filaments. If this is the case, the capacity of a muscle to generate tension and to shorten should fail when the muscle is stretched to such a length that the two sets of filaments no longer overlap. Based on the filament length measurements of Page and Huxley (8), the maximum sarcomere length for contraction would be about 3.6 μ in frog skeletal muscle and about 3.8 μ in rabbit psoas muscle.

This prediction was confirmed in the experiments of Huxley and Peachey (9, 10) and Podolsky (11), whereas Carlsen et al. (12) observed a significant isometric tension development at sarcomere lengths of 3.7 to 4.3 μ. Two fundamental complications are inherent in all the experiments cited which were performed with living frog fibers. First, the distensibility of the living fiber is not uniform along its entire length; rather, there is a greater resistance to stretch at the tendon ends, and this...
mechanical inhomogeneity is reflected in corresponding differences in sarcomere length. Thus, even when the average sarcomere length is greater than 4.0 μ, there are, in the region of the myotendinous junction, sarcomeres less than 3.6 μ long which may account for all or part of the observed tension development (9, 10, 12). Secondly, as a consequence of the elastic recoil of the stretched living fiber, it is impossible to study the behavior of the fiber with loads less than the applied stretching force. That is, the stretching of the fiber involves change in length and change in resting tension, both of which could influence the behavior of the contractile mechanism.

An approach that reduces these difficulties involves the use of glycerol-extracted muscle fibers in which contraction is induced by the application of adenosine triphosphate (ATP). Since it is possible to use short segments of single fibers, the homogeneity of sarcomere length may be more easily controlled. If the fibers are stretched in the living state and extracted with glycerol while held at fixed length, they go into rigor and become stabilized at a new sarcomere length. It then becomes possible to vary the sarcomere length and the load independently.

Edman (13) has presented preliminary data showing that the isotonic shortening response of glycerol-extracted psoas muscle fiber bundles is a complex function of both the initial muscle length and the load. Of special interest in relation to the sliding-filament model is his finding that with light loads the amount of shortening seems to be independent of the degree of stretch. However, evaluation of these experiments is difficult since the sarcomere lengths of his preparations are unknown.

We have studied the relationship of isotonic shortening to initial sarcomere length in lightly loaded single glycercinated fibers. Particular attention has been paid to the determination of the magnitude and internal variation of sarcomere length and to the correlation of fibrillar ultrastructure with sarcomere length.

**METHODS**

**PREPARATION OF FIBERS:** Glycerinated rabbit psoas muscle fiber bundles were prepared according to the Szent-Györgyi (14) method. Fresh fiber bundles either at rest length or after different degrees of stretch were tied to sticks, and were extracted at ~2°C with a 50 per cent glycerol-water mixture containing 1 mM phosphate, pH 7.0. After 24 hours the fiber bundles were split into finer bundles and stored in fresh glycerol at −18°C. Bundles which showed signs of tearing during stretch were discarded. All preparations were extracted for at least three weeks before use.

**MEASUREMENT OF SARCOMERE LENGTH AND SHORTENING:** To minimize diffusion effects as well as to define more precisely the degree of stretch, all experiments were made with single fibers, most of which had diameters in the range of 35 to 65 μ. A fiber segment 6 to 10 mm long was laid on a glass slide; one end was attached with a bit of paraffin wax to a light (3 to 4 mg) platinum load, and the other end was connected in the same way to a small piece of mica attached to the end of a string. The fiber was carefully transferred to a buffer solution inside a plane parallel cuvette (volume, 4 ml; optical path, 1 cm) mounted on the stage of a horizontal measuring microscope. The buffer contained 80 mM KCl, 5 mM MgCl2, and 20 mM Tris, pH 7.0. The microscope was fitted with a Zeiss 15 X ocular micrometer with moveable cobweb and a Leitz U.M. 5 mineralogical objective which under our experimental conditions had a N.A. 0.4, magnification of 18, and a working distance of 6 mm. With this arrangement it was possible to measure with an accuracy of a few per cent the sarcomere length at any point on the fiber as well as the diameter and fiber length before and after the addition of ATP. In each experiment the sarcomere length was determined at 1 mm intervals (or in some cases, 0.5 mm intervals) along the entire length of the fiber. Each value was based on the measurement of the length of a row of 5 sarcomeres. The diameter was measured at 3 or more different points on the fiber. Following the determination of initial fiber length, 5 mM ATP (Sigma Chemical Company, St. Louis, Missouri) was injected into the cuvette and the mixture stirred with a fine stream of air bubbles. The fiber length was determined after 2 and 5 minutes, and the percentage of shortening was calculated from the five-minute value. In general, more than 90 per cent of the total shortening occurred in the first two minutes. All experiments were carried out at room temperature (20 to 22°C).

**ELECTRON MICROSCOPY:** Resting single fibers and thin bundles were washed in buffer solution and fixed in 2 to 3 per cent buffered osmium tetroxide for one hour. The fibers were dehydrated in a graded series of alcohol and alcohol-acetone mixtures and embedded in Vestopal W. Although it is recommended that acetone dehydration be used in conjunction with Vestopal embedding (15), we have obtained satisfactory results with alcohol dehydration. Following treatment with absolute alcohol the preparation was treated with successive alcohol-acetone mixtures (75:25 and 50:50, respectively). The 50:50
alcohol–acetone mixture was completely miscible with Vestopal and was used for the embedding procedure. In all cases the fibers were held at fixed length during fixation and during all the steps up to polymerization. Sectioning was carried out with glass knives on a LKB Ultrotome and the sections were stained with uranyl acetate. Where measurements of sarcomere length were desired, sections were made

![Figure 1](https://jcb.rupress.org/content/127/2/F1.large.jpg)

**Figure 1** Electron micrographs of glycerinated psoas muscle fibers at sarcomere lengths of 2.7 μ (a), 3.1 μ (b), 4.1 μ (c) and 4.5 μ (d). Note the reduction in the overlap zone as the fiber is stretched from a sarcomere length of 2.7 μ to 3.1 μ. At a sarcomere length of 4.1 μ the I filaments are completely withdrawn from the A band. Fibers were stretched in the living state and held at a fixed length during glycerol extraction. They were fixed in buffered OsO₄, embedded in Vestopal W, and the sections stained with uranyl acetate. The cutting direction is indicated by the inset diagram. × 30,000.
with the knife-edge parallel to the fiber axis. Electron microscopy was carried out with a JEOL type 5-Y microscope. Magnification was controlled by frequent calibrations against a replica of an optical grating with a spacing of 1.76 μ.

RESULTS

ULTRASTRUCTURE AND SARCOMERE LENGTH: The sarcomere length was determined by light microscopy and correlated with the filament array as it appeared in electron micrographs. In agreement with previous observations on glycerinated muscle (16), there was a progressive reduction in the overlap zone with increase in sarcomere length (Fig. 1). At the highest sarcomere lengths illustrated (4.1 and 4.5 μ), the primary and secondary filaments were detached and separated by a gap. The total I filament length in our preparations was about 2.2 μ, in agreement with the more extensive measurements of Page and Huxley (8), and the A filaments were about 1.5 μ in length. Thus we may reasonably assume that filament overlap will be lost at sarcomere lengths greater than ~3.8 μ.

ISOTONIC SHORTENING AND SARCOMERE LENGTH: The results of 139 measurements of isotonic shortening as a function of initial sarcomere length are shown in Table I and Fig. 2. Fibers from 31 different fiber bundles from 8 rabbits were used. Fourteen of the bundles had average sarcomere lengths greater than 3.7 μ. The loads applied per unit cross-sectional area, as calculated from diameter and total load, were 100 to 300 gm/cm². Since single glycerinated psoas muscle fibers can develop an isometric tension of 2 to 4 kg/cm² at rest length (17), the loads employed over the whole range of sarcomere lengths in these experiments were only 2 to 15 per cent of the maximum tension.

Table I shows that there was shortening at all degrees of stretch, the average shortening being 60 to 75 per cent (column 5). The last column gives the average length of the shortened sarcomeres calculated from the percentage shortening. Microscopic measurement could not be carried out because of the small sarcomere height and the irregular curling up of the filaments. Inhomogeneous shortening would be associated with sarcomeres shortened even more than the calculated figures indicate. In Fig. 2 the length of the shortened sarcomeres is plotted as a function of the initial sarcomere length. Sarcomeres with overlap, i.e. with an initial length of 2.6 to 3.7 μ shortened to about 0.7 μ. When the initial length was greater, the sarcomeres shortened less; at the maximum length examined (4.4 μ), the length of the shortened sarcomeres averaged 1.8 μ. On the basis of the sliding filament theory, one would expect shortening to fail when the sarcomeres were stretched to lengths greater than 3.8 μ.

INTERNAL VARIATION IN SARCOMERE LENGTH: The interpretation of our observations would present no problems if it could be shown that the stretched fibers contained localized regions in which the sarcomere length was less than that at which overlap disappears. In fact the internal variation in the sarcomere length was rather small. Table II shows data from 15 fibers, five chosen at random from the first group of fibers of Table I, five from the fifth group and five from the tenth group. The average maximum deviation from the mean was about 6 per cent, irrespective of the amount of stretch. This was only slightly greater than the 3 per cent deviation which Edman (13) found in slightly stretched glycerinated fiber bundles as measured by light diffraction.

The histograms in Fig. 3 show the distribution of sarcomere length measurements on the two groups of fibers with the highest degree of stretch, the lengths being in the ranges of 4.1 to 4.29 and 4.3 to 4.5 μ (excluding that group which had only a slight shortening). For the first group, 211 measurements were made on 26 fibers. Three of the measurements, made on three different fibers, fell outside of the normal frequency distribution curve. For the remaining 23 fibers, the lowest value recorded was 3.8 μ. For the 15 most highly stretched fibers, 133 measurements were made, and none were below 4.0 μ. It is of interest that this group of fibers had an average shortening of 60 per cent as compared to the 65 to 75 per cent shortening of the rest length fibers (Table I).

Since each optical measurement of sarcomere length represents an average of many fibrils, it would be of interest to know the distribution of measurements for individual fibrils in localized regions of the fiber. This question has been investigated by electron microscopy and an example of one of our experiments is described below.

A single stretched fiber about 10 mm long was held at fixed length in buffer solution under the microscope, and the sarcomere spacing was measured by ocular micrometer at 0.2 mm.
TABLE I
ATP-Induced Shortening from Initial Sarcomere Length of 2.6 to 4.5 μ. (Single Fibers from Glycerol-Extracted Rabbit Psoas Muscle)

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of experiments</th>
<th>Range of s.l.*</th>
<th>Average s.l.*</th>
<th>Shortening</th>
<th>MS. of shortening</th>
<th>Calculated shortened s.l.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>2.50-2.69</td>
<td>2.62</td>
<td>75</td>
<td>4</td>
<td>0.66</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>2.70-2.89</td>
<td>2.78</td>
<td>67</td>
<td>6</td>
<td>0.92</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>2.90-3.09</td>
<td>3.00</td>
<td>76</td>
<td>5</td>
<td>0.72</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>3.30-3.49</td>
<td>3.40</td>
<td>80</td>
<td>3</td>
<td>0.68</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>3.50-3.69</td>
<td>3.59</td>
<td>82</td>
<td>3</td>
<td>0.65</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>3.70-3.89</td>
<td>3.79</td>
<td>75</td>
<td>2</td>
<td>0.85</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>3.90-4.09</td>
<td>4.00</td>
<td>70</td>
<td>4</td>
<td>1.20</td>
</tr>
<tr>
<td>8</td>
<td>26</td>
<td>4.10-4.29</td>
<td>4.18</td>
<td>59</td>
<td>4</td>
<td>1.71</td>
</tr>
<tr>
<td>9</td>
<td>15</td>
<td>4.10-4.29</td>
<td>4.17</td>
<td>24</td>
<td>12</td>
<td>3.17§</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>4.30-4.50</td>
<td>4.37</td>
<td>60</td>
<td>7</td>
<td>1.75</td>
</tr>
</tbody>
</table>

*s.l. = sarcomere length.
† ME = mean error.
§ Fibers from six different bundles gave an average shortening of 59 per cent (group 8). Fibers from a single bundle gave an average shortening of 24 per cent. This point is omitted in Fig. 2.

Figure 2. Calculated average sarcomere length of shortened fibers as a function of initial sarcomere length in glycerinated rabbit psoas muscle fibers.

Intervals along the entire length of the fiber. The fiber was then fixed in the same chamber and prepared for electron microscopy, the length being maintained throughout the entire procedure. Before polymerization the fiber was cut into eight pieces, each of which was embedded in a gelatin capsule and sectioned. To control shrinkage during polymerization, a fiber from the same bundle as the experimental fiber was carried through the same procedure, cut into several segments, and the lengths of the segments determined before and after polymerization. The shrinkage was found to be less than 1 per cent. Electron micrographs were made of ten fields from each of the eight segments, and sarcomere length measurements were made on as many
individual fibrils as possible. Measurements were made only on fibrils where it could be verified by knife marks and filament continuity that the knife-edge had been parallel to the fiber axis. Table III shows the agreement between the two independent sets of measurements.

Of the 356 fibrils measured from electron micrographs, 2, or less than 1 per cent, had sarcomere lengths of 3.7 to 3.8 μ, just the length when the two sets of filaments become detached. In the case of the remaining fibrils there was no doubt about the existence of a gap between the two sets of filaments. The lowest value for sarcomere length recorded by light microscopic measurement was 4.18 μ. In all, the data indicate that our experimental results do not have a simple explanation in terms of heterogeneity of sarcomere length.

**DISCUSSION**

Several investigations of the relationship between sarcomere length and contractility in frog muscle fibers have now been reported. In experiments on isotonic contraction in single living semitendinosus fibers stimulated electrically, Huxley and Peachey (9, 10) observed that shortening failed to occur in those parts of the fiber which were stretched to sarcomere lengths greater than 3.5 μ. Podolsky (11) has reported that the "naked fibril" preparation of Natori (18) fails to shorten in response to local application of calcium ions if the sarcomere spacing exceeds 3.6 μ. On the other hand, Carlsen et al. (12) found that single fibers of the semitendinosus muscle still developed 30 per cent or more of the maximum isometric tension although...
TABLE III

Example of Measurements of Sarcomere Length in a Single Fiber by Light and Electron Microscopy

<table>
<thead>
<tr>
<th></th>
<th>Number of measurements</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light microscopic</td>
<td>49</td>
<td>4.34</td>
<td>0.10</td>
<td>0.014</td>
</tr>
<tr>
<td>Electron microscopic</td>
<td>356</td>
<td>4.38</td>
<td>0.26</td>
<td>0.014</td>
</tr>
</tbody>
</table>

The fibers had been stretched to such a degree that there was no longer any overlap along 99 per cent of the fiber.

The interpretation of these experimental observations meets with a number of difficulties. In the isotonic contraction experiments of Huxley and Peachey (10) the tension on the fibers is unknown, and their data still leave room for the possibility that the unshortened parts of the fiber underwent changes in elasticity. The presence of filament overlap at the tendon ends of living fibers, even at very high degrees of stretch, is especially troublesome in the case of isometric tension measurements. Under isometric conditions in stimulated stretched fibers there is, in fact, shortening of the sarcomeres at the tendon ends and stretch of the middle part of the fiber (10, 12), a finding consistent with the view that only sarcomeres with overlap can shorten. However, Carlsen et al. (12) have measured the amount of stretch of single semitendinosus fibers, and on the basis of the passive length-tension diagram have calculated that the contraction of the end sarcomeres can account for only about one sixth of the total isometric tension development. They argue that the middle part of the fiber must at least undergo an increase in stiffness upon stimulation. Of interest in this connection are the recent birefringence measurements of Eberstein and Rosenfalck (19) which indicate that in the electrically stimulated single fiber some ultrastructural change occurs even in the absence of

Figure 3 Histograms of sarcomere length measurements for the two groups of fibers with the highest average sarcomere lengths. Left, mean sarcomere length range, 4.10 to 4.29 µ, mean of means, 4.19 µ; right, mean sarcomere length range 4.30 to 4.50 µ, mean of means, 4.37 µ.
filament overlap. Gordon, Huxley, and Julian (20) have reported the preliminary results of a reinvestigation of this problem, using an improved technique in which only the tension generated in the middle part of the fiber is determined. They state that at sarcomere lengths greater than 3.6 μ the isometric tension developed in the middle part of the fiber is only about 2 per cent of the maximum.

Difficulties also arise from the fact that the resting tension in the living fiber is length-dependent and may itself influence the development of active tension. Some support for this possibility comes from Edman's experiments with glycerinated muscle (13). This point has been discussed by Podolsky (11), and his conclusion that tension development requires the presence of an overlap zone is strengthened by his finding that the elastic modulus of the highly stretched fiber is greatly reduced by the stripping away of the sarcolemma. The load carried by the contractile elements under these conditions is difficult to evaluate because of the presence of inert parallel structures (sarcoplasmic reticulum, etc.), but most probably the contractile elements carry a considerable initial tension.

The experiments described in this report demonstrate that the glycerinated fiber subjected to a small preload can develop tension and can shorten even at sarcomere lengths greater than the combined lengths of the A and I filaments. The use of a small load necessitates certain precautions since, in theory, the smaller the load, the fewer the number of overlapping sites required to lift the load. The problem of internal variation in sarcomere length becomes, then, the most critical point to consider in the evaluation of our results.

Our findings indicate that the stretch is quite evenly distributed along the length of the fiber and that in fibers with sarcomere spacings greater than 4.0 to 4.1 μ there is little chance of finding sarcomeres with a length below 3.8 μ. This conclusion is based on what we consider to be a valid statistical sampling of our experimental material. We cannot state that in any given fiber there are absolutely no fibrils with sarcomere lengths below 3.8 μ. However, we do feel justified in concluding that such fibrils, if not absent, are very few, and one can question whether so few fibrils can account for the necessary tension development and the 50 to 80 per cent shortening which is commonly observed. Assuming inhomogeneity in sarcomere length to be the reason for shortening and tension development, it is instructive to attempt to calculate, on the basis of the sliding filament model, how many fibrils with overlap would be required for tension development and shortening in a single preloaded fiber under specified experimental conditions.

Let us consider a fiber that is 50 μ in diameter and, for the sake of simplicity, only one sarcomere long. The load on the fiber is 3.5 mg, which was the usual load employed in our experiments. This is equivalent to a tension of about 170 gm/cm². We will make the following assumptions: (a) The combined lengths of the A and I filaments is 3.8 μ. (b) Under our experimental conditions the ATP penetrates throughout the fiber and maximally activates all of the myofibrils. This assumption seems justified, in view of a recent re-evaluation of the diffusion constant of ATP in glycerinated muscle (21) as well as morphological evidence (22). (c) The myofibrils are about 1 μ in diameter and occupy 80 per cent of the total cross-sectional area of the fiber. Thus, a fiber 50 μ in diameter contains about 2000 fibrils. (d) The fiber can exert a maximum isometric tension, P₀, of 2000 gm/cm². (e) The force developed, P, at any sarcomere length, S, is directly proportional to the length of the overlap zone and is equal to P₀ at that sarcomere length S₀, where the ends of the I filaments reach to the innermost cross-bridges of the A filaments (4, 20). Since the middle 0.2 μ of the A filament is free of cross-bridges (23), the sarcomere length for maximum tension should be about 2.4 to 2.5 μ. Unfortunately, there are no experimental data available on the relationship between maximum active tension and sarcomere length in glycerinated psoas fibers. (f) Virtually the entire preload is carried by the contractile element. There is, in fact, no experimental information on how a load is distributed between the myofibrils and extrafibrillar structures in glycerinated muscle. If most of the initial load were carried by extrafibrillar structures (e.g., sarcolemma), it might be possible for a small number of fibrils with overlap, bearing little or no load, to shorten and increase their zone of overlap before they had to develop tension. Furthermore, the shortening fibrils might exert a pull on adjacent Z lines, thus causing a development of overlap in neighboring fibrils. In this way a small number of fibrils could initiate shortening through a side-ward spread of overlap. Assuming shortening to
occur through a sliding of filaments and at
constant load, one would expect a constant length
of the shortened fiber regardless of initial length.
Our findings (Fig. 2) suggest that shortening from
an initial sarcomere length of up to 3.7 to 3.8 μ
occurred through sliding, whereas at higher
sarcomere lengths another mechanism intervened.
Evidence which supports this assumption is
presented in the succeeding paper (22).

On the basis of assumption (e), the force de-
veloped by a single fibril, P', is related to the
maximum force, P0, as follows:

\[
P' = \frac{3.8 - S}{3.8 - S_0}
\]

(1)

P' and P, and P0 and P0, are related by the ex-
pressions:

\[
P'N = P
\]

(2)

\[
P_0N_0 = P_0
\]

(3)

where N is the number of fibrils which are exerting
tension at any given sarcomere length and N0 is the
total number of fibrils in the fiber. Rearranging
Equations (2) and (3), substituting into Equation
(1), and solving for N, we have

\[
N = \frac{P_0(3.8 - S_0)}{P_0(3.8 - S)}
\]

(4)

N0 is 2000, S0 is 2.5 μ, and, under our chosen
experimental conditions, P/P0 is 0.09. Thus, if the
minimum sarcomere length is 3.7 μ, then the
number of fibrils of that sarcomere length which
must be present in a single sarcomere to lift a load of
3.5 mg would be 2940. Since this is more than the
total number of fibrils in the fiber, we would
conclude that a fiber with a uniform sarcomere
length of 3.7 μ would be unable to shorten. If
there were no fibrils of sarcomere length less than
3.6 or 3.5 μ, then the number of such fibrils
required to initiate shortening would be ~1200 or
~800, respectively. The above argument can be
generalized to a fiber of any length which is
shortening uniformly, since uniform shortening
requires that each sarcomere exert a force equal
to the applied load. In other words, if it is sup-
posed that in our fibrils which have been stretched to
average sarcomere lengths of 4.1 to 4.5 μ there
are fibrils with sarcomeres as short as 3.4 to 3.5 μ
which exert all the force, then at least 40 to 50 per
cent of the fibrils at each sarcomere level must
have sarcomere spacings of less than 3.8 μ. This
calculation admittedly involves some approxima-
tions and unproven assumptions, but, even if it is
in error by a factor of 3 or 4, the number of fibrils
would still be of an order of magnitude to easily
influence our sarcomere length measurements.
For example, if we suppose that the fiber in ques-
tion can exert a maximum tension of 4000 gm/cm²,
as has been reported (17), then the results stated
above would have to be reduced by a factor of 2.
In any case, it seems reasonable to assume that a
shortening of more than 50 per cent against a
force of 100 to 300 gm/cm² would require the
concerted action of a substantial number of
fibrils scattered throughout the fiber.

These calculations indicate that the ability to
shorten cannot be accounted for simply by vari-
ation in sarcomere lengths. One can argue that
sarcomere length is not a good measure of the
degree of overlap, since in highly stretched fibers
variations in filament alignment within the fibril
might cause overlap of A and I filaments. Fig. 1 c
and d indicate the existence of such a misalign-
ment. The analysis of this phenomenon requires
more detailed electron microscopic examination
of the individual fibrils and is reported in the
following paper (22).

We conclude, then, as follows: (a) Lightly
loaded glycerol-extracted fibers shorten at all
degrees of stretch corresponding to sarcomere
lengths from ~2.6 to 4.5 μ. This is contrary to the
theoretical predictions and in disagreement with
the results of isotonic shortening experiments per-
formed with living frog muscle fibers (9, 10, 11).
(b) While at sarcomere lengths ~2.6 to 3.7 μ the
fiber shortens to ~0.7 μ, the sarcomere lengths
of the shortened fiber increase with increasing
initial sarcomere lengths above 3.8 μ. (c) The
marked shortening of the highly stretched fibers
cannot be explained in terms of the variation of
sarcomere lengths within the individual fiber.

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