THE CONTRACTION OF GLYCEROL-TREATED FIBERS IN THULET'S SOLUTION

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Ever since Szent-Györgyi introduced the so-called glycerol-treated muscle fiber, its contraction under the action of ATP has been looked upon as exhibiting the basic features of muscular contraction.

Laki and Bowen (1955) demonstrated that glycerol-treated fibers contract, not only in ATP solution, but also in Thulet's solution (mercury potassium iodide; HgI_2 - KI). In this reagent, the reversibility of the shortening could be demonstrated (Bowen and Laki, 1956). When the shortened fibers were cooled down, an elongation could be observed. Such a behavior is in accord with the idea that shortening is due to a transition of long contractile elements to their short modifications (Mandelkern, et al., 1959).

The interest in this kind of contractile mechanism diminished when the double array of filaments in muscle fibers was demonstrated, and a force generating mechanism based on the sliding of these filaments was postulated. According to the sliding mechanism, the two sets of filaments seen in electron microscopic pictures represent myosin and actin and their temporary combination utilizing the energy from ATP splitting leads to contraction and the performance of work (Huxley, 1965).

In this paper, we would like to present experiments which

further illustrate the similarity of the contraction brought about by ATP and by the Thulet's solution. The experiments presented here do not contradict the theory of sliding filaments but are against the assumption that sliding is the force generating mechanism in the glycerol-treated fibers.

Microscopic observations.

The behavior of the fibers in HgI_2 - KI solutions made up by dissolving mercuric oxide in potassium iodide solution (Laki and Bowen, 1955) was examined under the phase contrast microscope. These experiments were carried out the following way: Fiber bundles about 4 cm. long and containing 12-20 single fibers were fixed at their ends on microscopic slides. A small drop of quick drying "Duratite" cement applied to the ends held the fibers in place. To achieve different degrees of shortening, the cement drops were placed closer together allowing a slack in the fibers. When the reagent was added to the fibers, these adjusted themselves to the preset lengths. In the case of stretched fibers, first one end was fixed on the slide. After the reagent was added to the fiber bundle, it was stretched out to the required length and the other end was tied down with the cement. To obtain the micro-photographs, cover slides were placed over the preparations and pictures were taken with a Polaroid Land camera applied to a microscope. Fig. 1 illustrates these experiments and the results are summarized in Table 1.

It is seen in Table 1 that the length of the A-band, I-band and the sarcomere of the unstretched fibers in Thulet's solution is in the expected range (Bowen and Field, in press). It is quite apparent that in the shortened fiber, it is principally the I-band which is shortened. Conversely, when the fiber is stretched, it is the I-band which elongates. In the case given in the table, the I-band lengthened 3 times, compared to the unstretched fiber. Undoubtedly, under these

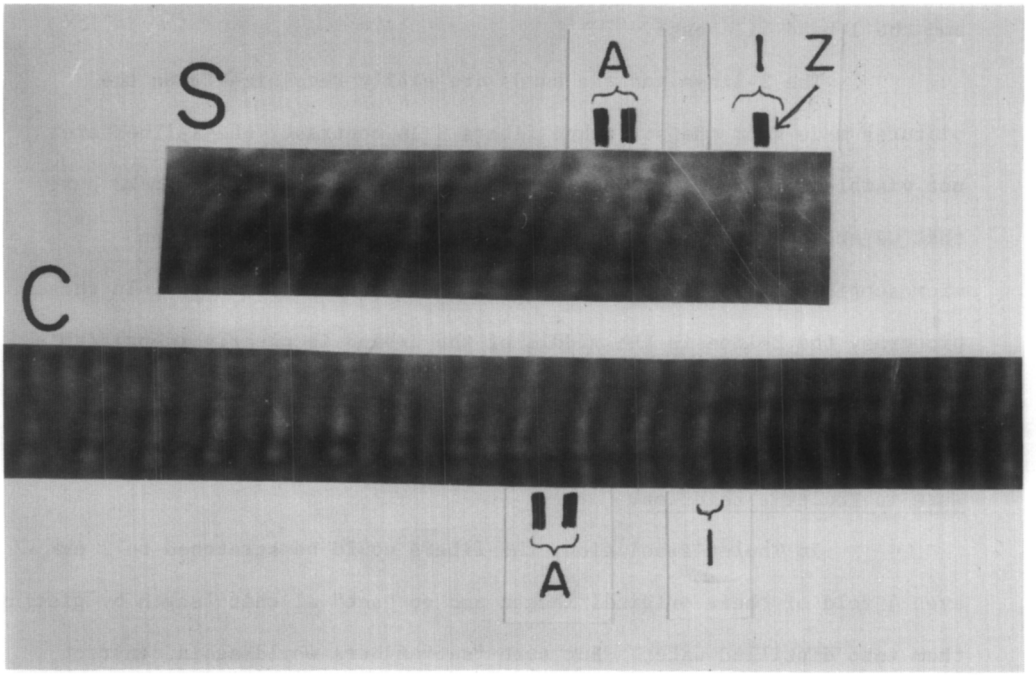

Fig. 1.

Table 1

	The length in microns of the various regions of the glycerol-treated fibers		
	Sarcomer	A-band	I-band
Stretched	4.6	1.54	3.1
Unstretched	2.6	1.50	1.10
Contracted	2.02	1.34	0.68

For explanation, see text.

stretched conditions, no overlapping can be expected between the A-band and the I-band filaments.

The Z-lines and the bands are easily recognizable on the pictures made from the stretched fibers. In contrast, the Z-lines are not visible on pictures of the shortened fibers. In order to make sure that we are not confusing the A-bands with the I-bands, electron microscopic pictures were also made from the shortened fibers. In these pictures, the Z-line in the middle of the I-band is clearly identifiable. The measurements on these pictures corroborate those made on the light microscope pictures.

Work in Thulet's solution.

In Thulet's solution, the fibers could be stretched to 2 or even 4 fold of their original length and be "set" at that length by placing them into distilled water. But such "set" fibers would again contract when put back into Thulet's solution.

When we studied the ability of the fibers in this reagent to lift weight, it became apparent that in contrast to the contraction in ATP, the fibers could not carry out work. We found, however, that if the fibers were stretched beyond their original length and set, they would shorten and perform work when placed back in the reagent. This behavior does not fit into a theory which postulates the generation of contractile force from periodic connections between the sliding filaments.

It is seen from Fig. 2 that the extent of shortening of the stretched fiber depends on the load. The length-tension diagram, however, does not have the usual shape. At the point where the filaments can be expected to reach each other, the fiber ceases to shorten further with loads. Further shortening occurs only when the fiber does not carry a load. Microscopic observation shows that under unloaded conditions, the extended fiber shortens to a stage where the sarcomere length, the width of the A- and the I-bands, is the same as we find when the unstretched fibers shorten.

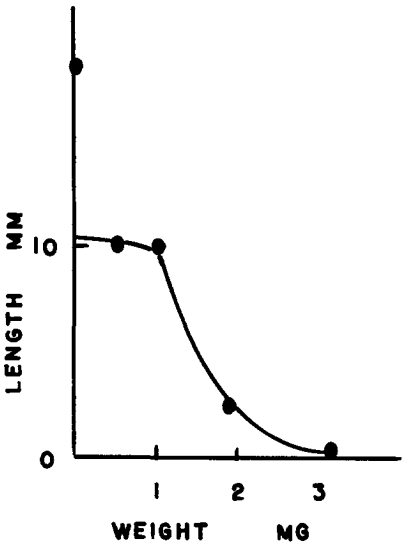

Fig. 2.

Quick stretch and quick release.

In ATP, as well as in Thulet's solution, the fibers exhibit the phenomenon of quick stretch and quick release. When the fiber is suddenly stretched, there is a corresponding increase in tension, but soon this extra tension drops back almost to the original level. Conversely, when the fiber is released, the tension drops to a low level but returns to the original level. In Thulet's solution, the recoveries are not as complete as in ATP solution, but the similarity in the two solutions is unmistakable (Fig. 3). In these experiments, the tension was measured with a transducer, as described by Bowen (Bowen and Martin, 1958).

Discussion.

In ATP, as well as in Thulet's solution, the extent of shortening can be controlled by adjusting the conditions (Bowen and Laki, 1956). If we let an unstretched fiber contract part of the way in ATP, no change takes place when this fiber is placed into Thulet's solution, which other-

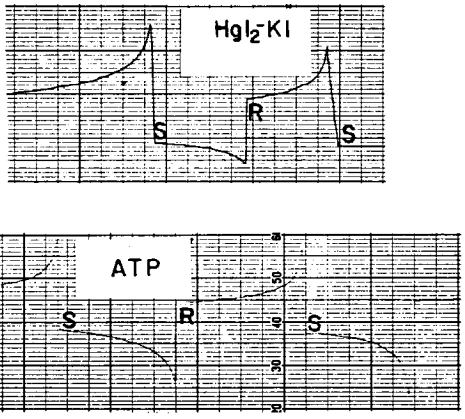

Fig. 3 Quick stretch and release behavior of the glycerol-treated fibers in HgI_2 - KI and in ATP solutions. S refers to stretch and R to release. The speed of the chart was 1 inch per minute.

wise would bring about the same extent of shortening. If in ATP solution, the shortening is due to a sliding mechanism and in Thulet's an order-disorder transition, then by shifting the fiber from ATP to Thulet's solution, irrespective of what happened in ATP, shortening should take place. If the fiber is allowed to reach total shortening in ATP and is placed in Thulet's solution where the shortening would be a partial one rather than total, the fiber elongates. The experiments presented here further indicate that the same machinery operates in the shortening brought about by ATP or by Thulet's solution. (The fuel supplying the energy in the two cases is undoubtedly different).

Recent experiments of Guba indicate that the basic material of the myofilaments is "fibrillin" (Guba, personal communication) which in the A-band may be coated with myosin. This myosin "coat" may not take an active part in muscular contraction. The rest of myosin, dispersed between the filaments, is free to carry out a "superprecipitation"-like process. It seems to us that we have to go back to the original

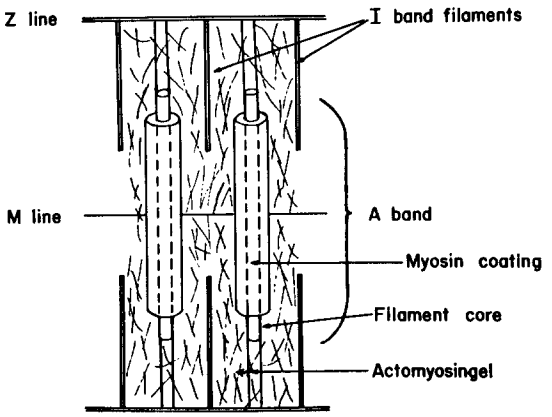

Fig. 4.

idea of Szent-Györgyi according to which the contractile machinery is the actomyosin gel (see Fig. 4).

In Thulet's solution, the contracting agent essentially is KI. How KI brings about contraction is not known, but it is a fair assumption that it brings about an order-disorder transition of the contractile proteins (Mandelkern, 1964).

It was surprising to find in working with the stretched fibers in Thulet's solution that these were unable to carry out work after shortening reached the state where the sliding filaments would be expected to begin to touch each other. As an explanation, it is proposed that the molecules of the sliding filaments while under load are strained and are in such a conformation that they readily form bonds when the sliding filaments come into contact with the stationary ones. This bonding prevents further movement but when the load is removed, the molecules take up their unstrained conformation and the shortening can resume.

In this respect, the situation is different in ATP solution in which the fibers carry out work in spite of the interdigitation. It looks then that in this case, no bonding takes place between the

filaments. Apparently ATP not only supplies the fuel for the work but also acts as lubricant and prevents connection between the filaments.

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