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THE RÔLE OF MYOFIBRILS, SARCOPLASMA AND SARCOLEMMA IN MUSCLE-CONTRACTION

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I

In the field of physiological research on the skeletal muscle, numerous problems still remain to be studied. In the field of research based on the living muscle, however, the studies have practically been systematized. At home and abroad, a large number of papers have been published announcing the results of researches on muscle structure, elasticity, processes involved in contraction, and on various phenomena observed in the active muscle.[5]

Since the development of the study of isolated muscle fibers, an outgrowth of the study of the whole muscle, there has been particularly great activity in further detailed studies. These researches base their observations on an attempt to preserve the actual uninjured living conditions of the muscle in order to clarify the mechanism of muscle contraction.

Recently, in contrast to this method, a second procedure has been evolved. In this, protein fiber extracted from the muscle is utilized. By means of this method, SZENT GYÖRGYI[15] and others have further developed the relationship between myosin B and ATP. This was recognized as the fundamental change in muscle contraction and had evoked great interest as combining the chemical process with the physical. Thus, in this country, researches in this field have become widespread in recent years.[4][12]

However, according to my experiments,[5] the phenomena observed in protein fibers cannot fully explain some of the phenomena observed in living muscle fibers, and at times one even encounters contradictory phenomena. Therefore, I first separated the living fiber (though this was considered the ultimate unit in the physiological study of the muscle) and isolated the myofibrils. Next, abopting necessary processes, I tried experiments of changing them until they resembled the extracted protein fibers.[6] In other words, I planned to produce characteristically different fibers in graded steps between the living muscle fiber and protein fiber. This type of research may also be found in those on glycerol-treated muscle or frozen-strip preparations. How-

ever, there seemed to be no study on myofibrils based on the viewpoint such as the one I contemplated.

First, I tried to isolate the myofibrils in Ringer's solution. However, I found that the myofibrils contracted greatly as soon as the sarcolemma was stripped. If we stretch them after a certain lapse of time, we can extend them to the original length, but their properties are considerably different from those of living myofibrils. I experimented with various solutions and found that the extent of contraction was smaller when the muscle fibers were put in a KCl solution of more than 0.3M. Such isolated myofibrils were used in the early experiments.[7] However, there still occurred a contraction of more than 10%, and these myofibrils could not be said to be quite similar to the living muscle fibers.

In the course of these experiments, I wondered if the contraction had not occurred when the myofibrils were being isolated because, they were immersed in oil in order to avoid both the diffusion of sarcoplasma and the effects of injurious electric current. With this thought in mind, I continued the experiments and found that if the liquid element around the muscle fiber was reduced to the lowest possible minimum, not only was there no contraction of the myofibrils when the sarcolemma was stripped, but also the isolated myofibrils retained the properties quite similar to those of the living muscle fibers.[8] The following is a comparison between the myofibrils isolated according to this procedure: with myofibrils isolated in a solution; with myofibrils bathed in WEBER EDSALL'S solution (KCl 0.6 M, $NaHCO_3$ 0.04 M, Na_2CO_3 0.01 M); with myosin B, etc.

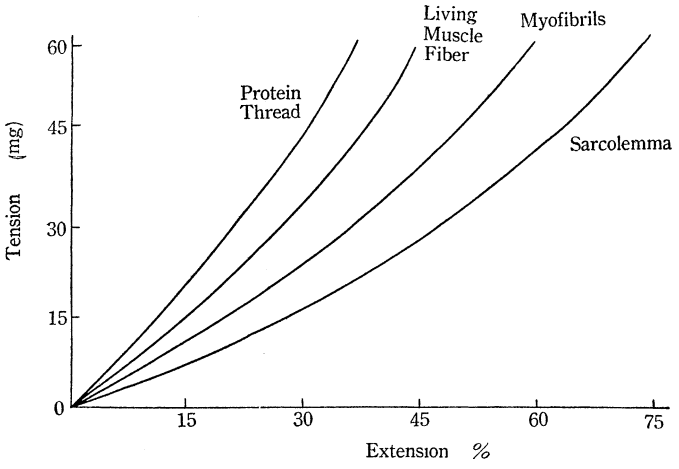
II

A comparison of the viscoelasticity of the myofibrils isolated in oil with that of the living fibers and other fibers, is shown in Table I and Fig. 1. In this Table, we can change the elasticity of the protein fibers in various ways

Table 1.

Load mg	0	15	30	45	60
Diameter of fiber (f)	Extension %				
Living muscle fiber (60±20)	0	15±4	28±5	40±5	48±6
Myofibrils (separated in KCl solution) (27±7)	0	19±3	32±4	47±3	56±4
Protein thread (16±3)	0	6±3	21±5	30±7	39±6
Sarcolemma	0	25 (63~140)	50 (70~227)	62 (12~400)	65 (31~510)

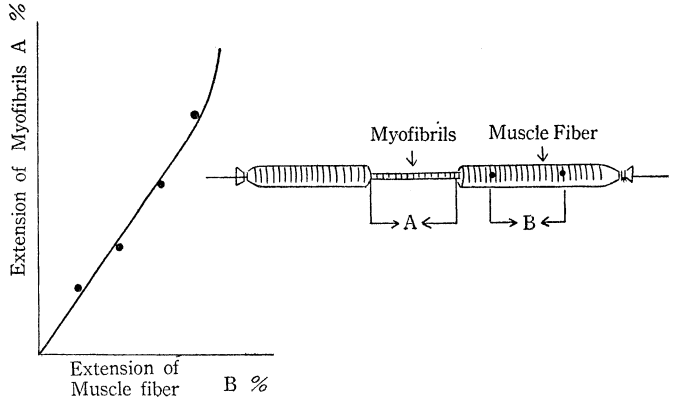
Fig. 1. Length-tension curve of living muscle fiber, myofibrils, protein thread and sarcolemma



according to the method of obtaining them, but, if we obtain the fiber by immersing protein in water, and then, after once drying it up, swell it in water again, it is generally of greater elasticity than the muscle fiber. As shown in Table I and Fig. 1, the elasticity of the myofibrils isolated in solutions is greater than that of living fibers, but, when we consider their size, we find that they are smaller in the reverse order. In contrast, the sarcolemma is greatly tensile. Here, I should like to add that, according to RAMSEY and STREET[14], the muscle's elasticity is due to the sarcolemma, a point which is stressed to some degree, but, according to my experiments, the sarcolemma does not represent the elasticity of the muscle fiber.

However, in the method shown in Fig. 2, namely, after making one part

Fig. 2.

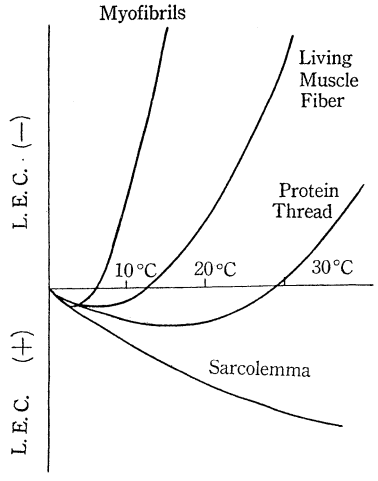


exclusively the myofibril, and having the other part marked with distinguishing marks, and then stretching the whole, if we compare the elasticity of the myofibrils isolated in oil with that of the living muscle fibers, we find that they stretch in the same ratio as their size, as seen in the Figure.[10]

Next, examining the linear expansion coefficient, as shown in Fig. 3, we find that the coefficient of the myofibrils is minus and its absolute value is

Fig. 3.

Length-Temperature relation of myofibrils, living muscle fiber, protein thread and sarcolemma



large. In contrast, the protein fiber has a wide temperature range of plus, and the sarcolemma is always plus.[13] That is to say, from the view-point of both elasticity and thermo-elasticity, the properties of the myofibrils isolated in oil are quite different from those of the other fibers.

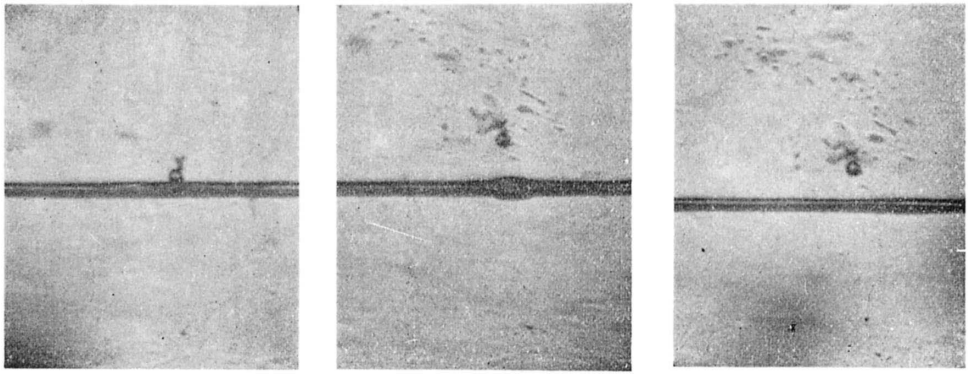
Next, we consider the contractive power of the isolated myofibril. As shown in Table 2 and Fig. 4, the myofibrils isolated in oil show a delicate temporary contraction. When these myofibrils are stimulated by a flow of electric current, or by bathing in minimum volume solutions, they contract according to the time relation shown in Fig. 4. If the electric current continues, a long-

Table 2. The temporary contraction of myofibrils by H_2O.

Temp. °C	Phase of contraction sec.	Plateau sec.	Phase of relaxation sec.
5～ 7	3～15	2～ 3	3～14
10～12	2～ 5	0～ 1	2～ 4
20～25	1～ 3	0～0.5	1～ 3

Fig. 4 a. Mechanical record of contraction of myofibrils

Fig. 4 b. Photographic records of contraction of myofibrils



Rest Contraction Relaxation

period contraction will occur. When bathed in solutions, their contraction or relaxation has no relation to the variety of solutions. If the solutions are soluble in water, the myofibrils are not affected by the volume of concentration; furthermore, even a piece of crystal can cause temporary contraction.

Naturally, there are some exceptions. K_2HPO_4 of more than 0.6 M, or KCl of more than 0.3 M does not cause contraction so readily. Furthermore, with substances considered to possess great adhesive power, as $MgCl_2$ and heavy metals, or pharmaceuticals like quinine, nicotine, acid, etc., it is apt to change into irreversible contraction; that is, when repeated again and again, it becomes a continuous irreversible contraction. With H_2O or ATP rolutions, or NaCl solution, temporary contraction can be caused by considerable repetitions. When the volume of the solutions becomes great, it changes into a continuous contraction, but when, the volume of the solution was small, I was able to obtain both contraction and relaxation after 100-300 repetitions. Moreover, as far as this experiment is concerned, ATP is not different from H_2O or NaCl, nor is any action shown, which is clearly different from the others.

In contrast, the myofibrils isolated in solutions do not show such contraction.

Next, the myofibrils isolated in oil contract easily through heating. Their thermo-contracture is in fact stronger than that of living fibers, as shown in

Table 3. Thermo-contracture of myofibril (M.F.), living muscle fiber (L.M.) and protein thread (P)

Temp. °C	M.F. %	L.M. %	P %
15	100	100	100
25	81	83	—
35	60	73	—
45	38	57	—
55	—	34	103
65	—	—	88
75	—	—	86

Fig. 5

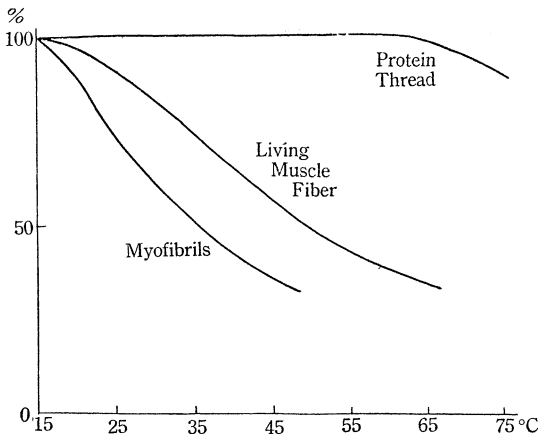


Table 3, Fig. 5.[3] In contrast, the fibers isolated in solutions have a far smaller contration potential. And protein fibers hardly cause thermo-contracture.[2] There are many details still to be studied concerning the quantitative relation of the tension of the myofibrils isolated in oil and others at the time of contraction, but it is generally thought that it resembles the contraction tension of the living muscle fibers; and the changes in birefringen (Table 4) or in the hystologic structure at the time of contraction coincide with those of the living muscle fibers.

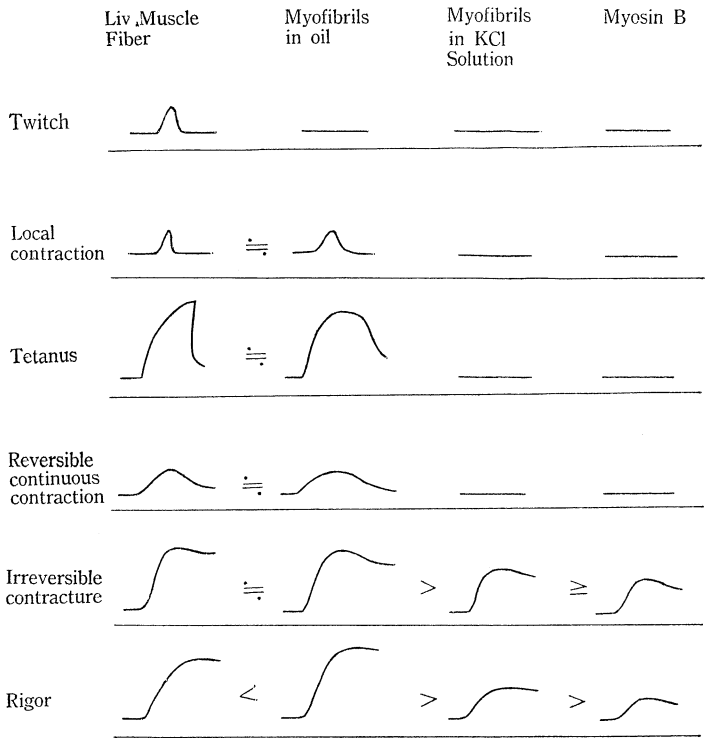
Table 4.

Stimulus	Diameter of fiber (μ)	Rest	Contraction
Electrical Current	20~30 μ	36±5	15±7
$MgCl_2$ (0.1 Mol)	„	31±8	9.5±8
Na_4—ATP	„	34±7	13±9

III

Taking into consideration the foregoing properties together with additional observations, in summary we can state that, while in the living muscle fibers there occurs a conductive contraction for the contraction period of 1/6—1/10 sec., in twitch, there is no contraction in the myofibrils isolated in oil (Fig. 6).

Fig. 6.



Yet it still remains questionable whether it does not occur at all in myofibrils isolated in oil; i.e., when the muscle is stimulated by various compounds, contracture appears, but, when the contraction occurs in one part of the muscle fiber, the contraction wave which originates in that part appears.[16] This contraction velocity is less than 1 msec. per second.

We can classify the movements observed in muscle fibers into several forms, and this contraction wave appears readily enough, even if the muscle fibers are narcotized with various narcotics, or bathed in saccharose or other solutions, in other words, even when the muscle does not twitch.

One great difference between the myofibrils isolated in oil and the living fibers is that the former are stripped of sarcolemma. One could presume that the irritability is caused by the action of the sarcolemma. Therefore, the fact that the irritability has vanished through the action of narcotics or other agents would also mean the disappearance of conductive contraction. Thus, the observation of the conductive contraction wave in the muscle fibers immersed in narcotics or other agents seems to indicate that the power to cause conductive contraction still remains in the myofibrils. However, because it seems that rapid conductive contraction does not appear at all when we immerse

the isolated muscle in oil, we can say that one of the rôles of the sarcolemma is to cause a rapid conductive contraction. Nevertheless, even if the sarcolemma is present, as conductive contraction hardly appears when the surrounding solution is oil, it can be considered that the surrounding solution is an important factor in conductive contraction.

Next, we are concerned with non-conductive and temporary contraction. This is a property observed in living muscle fibers and fibers isolated in oil. When we stimulate a part of the muscle by a micro electrode, a non-conductive local contraction occurs. Further, when the muscle is stimulated electrically by the separation method, non-conductive, temporary contraction occurs in the separated part. This contraction process may be considered the same as the twitch. This contraction greatly resembles the temporary contraction observed in myofibrils isolated in oil, but the contraction period of the myofibrils is a little longer than that of the twitch. As technical problems are involved here, there are a few questions to be settled yet. However, I believe this temporary contraction is the fundamental form of the normal contraction mechanism of the muscle or of tetanus. That is, I believe that the normal contraction process of the muscle comes from the fact that such contraction as observed in the myofibrils is combined with the action of the plasma membrane of the muscle to be transformed into conductive contraction. Just as repeated electrical stimuli on the muscle will produce tetanus, a similar process can be produced on the myofibrils. Myofibrils isolated in solutions do not retain this property.

The reversible continuous contraction is also clearly observed in living fibers and in myofibrils isolated in oil, but, when myofibrils isolated in oil are caused to contract by $MgCl_2$, they retain their tensile property after washing. Myosin B fibers can be said to have lost this reversible contraction power.

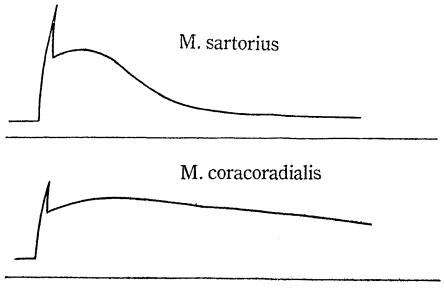
The data shown in Table 5 can be considered one of the characteristics of reversible continuous contraction. Reversible continuous contraction can be

Table 5.

	Duration of contraction	Consumption of creatine phosphate
Sartorius	30 sec.	2.8 m Mol/l
	2 min.	3.8
	5 „	3.9
	15 „	5.8
Coracoradialis	30 sec.	11.1
	2 min.	14.3
	5 „	15.3
	15 „	17.6

caused by electric stimulus, by idio-muscular contraction due to rapid extension; etc. This contraction is a leisurely process, as shown in Fig. 7, and

Fig. 7. Myogram of idiomuscular contraction



depending on the type of muscle, the manner of occurrence differs somewhat. In the so-called tonic muscle, a longer process is involved than the non-tonic muscle.[7]

In these contractions, there is practically no lactic acid,[11] but the decomposition of CP is observed.[1] Further, comparatively speaking, it is not accompanied by an increase in ortho-P. This process is different from that of twitch or tetanus, but, on the contrary, this reversible continuous contraction is observed clearly in narcotized muscles.[5] In cases where we must explain muscle contracture through the action of ATP and actomyosin, it would be helpful to bear this fact in mind.

Next, irreversible contraction can be produced in the living muscle fiber, or in the myofibrils isolated in solutions or in protein fibers. But, as mentioned before, when myofibrils isolated in oil are acted on by ATP or by $MgCl_2$, this contraction is clearly observed; especially when $MgCl_2$ acts upon myofibrils isolated in solutions, a contraction of more than 50% may be observed. When ATP or $MgCl_2$ is washed off, the contraction can be relaxed, but, in contrast, the muscle isolated in oil or the living muscle fiber cannot conversely produce contraction. Further, the tension at the time of contraction is very slight in the case of protein fiber.

Rigor can be produced readily through heat, water, etc., in living muscle fibers and in myofibrils isolated in oil, but not so readily in other fibers. Researches based on the relation between ATP and myosin B employing glycerol-treated muscle or frozen-strip preparation as intermediary have reached a high point and have been discussed as the fundamental mechanism of muscle contraction. However, such researches aim in one sense at an irreversible, long-period contraction process. (The expression "irreversible" may not be appropriate, for a change of solutions may change it to "reversible.") Moreover, a large field remains unexplored, as mentioned before, in order to clarify the process which occurs in the myofibrils in their living state.

From the data given above, it can be presumed that, in the living muscle fiber, when the nature of the liquid surrounding the myofibrils, that is, the component of sarcoplasma, is altered in part, that part of the myofibril contracts; that, when the changed component returns to its original state, it relaxes; and that, when the change is in one direction, contracture or rigor is produced. That is to say, no matter how the solution surrounding the myofibril changes, as long as it changes, the myofibril contracts; and, if the change is of a type that permits to return to the original state, the myofibril relaxes. Actually, contraction in the living muscle fiber can be produced in various ways.

Thus arises the problem: what changes in the physiological state of the solution produce twitch or tetanus? Details concerning this problem must await the results of further researches. However, since it has already been ascertained that an action current arises and there occurs a change in the distribution of the ions, contraction can be caused by these. This idea is supported by various facts. For example, after immersing the muscle fiber in oil with a small amount of RINGER's solution around it, if we inflict a wound on one part of the fiber, it contracts according to the amount of short circuit resistance of RINGER's solution. In other words, we can clearly observe the contraction of the myofibrils caused by an injury current.

According to the above-mentioned results, it is thought that the rôle of the sarcolemma is: (1) to produce rapid contractive conduction in its relation with the surrounding solution; (2) to maintain intact the extremely delicate properties of the sarcoplasma by separating, in one sense, the inner and outer solutions. Though its hitherto emphasized rôle as an elastical substance can be valued to some extent, its importance has been over-emphasized.

As to the rôle of the sarcoplasma, it is thought to be that of controlling the liquid state of the myofibrils, in order that the myofibrils may produce reversible contraction.

Although the foregoing discussion is conducted on the premise that the properties of the myofibrils and the sarcoplasma can be separated, this is only for convenience in explanatory discussion; for actually the properties of the two cannot be separated from each other. The properties of the myofibrils and the components of the sarcoplasma are connected in various ways, and, only through the existence of the myofibrils, can the components of the sarcoplasma retain their equilibrium. Therefore, we can say that a change in the sarcoplasma's components produces a change in the properties of the myofibrils. In actuality, it is thought that the change in the composition of the sarcoplasma causes the chain molecular structure of the myofibrils to change its state or stereochemical configuration (convalent bond, electro-statical bond, hydrogen bond, etc.).

Thus, my researches produced graded steps leading to the protein fiber. Of course, this is only a rough outline, and there remain many points to be further investigated. However, I firmly believe that this attitude of researching on the change in properties at various stages will be a contribution to the study of the contraction mechanism of the muscle from a physiological viewpoint.

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