

**SKINNED FIBRES OF SKELETAL MUSCLE
AND THE MECHANISM
OF MUSCLE CONTRACTION**

—A Chronological Account of the Author's Investigations into Muscle Physiology—

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Reprinted from
Jikeikai Medical Journal
Vol. 33, Supplement

December, 1986

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PRELIMINARY REMARKS

About 50 years ago (1936), when I first became interested in muscle physiology, the main theme in this area was the excitation of nerve and muscle fibres, for instance, the “all-or-none” response and its applicability. At that time, many reports had been published, and with regard to the all-or-none response, a brisk dispute between Prof. G. Kato and Prof. H. Ishikawa over the theory of excitation conduction became the centre of interest in our physiological society, that is the former’s new opinion regarding decrementless conduction against the latter’s old opinion regarding decrement conduction of narcotized nerve (Kato 1924)¹⁵.

This dispute led to an idea proposed by Kato’s group, that the single nerve fibre would be useful for confirmation of the decrementless theory. T. Shimizu of Kato’s laboratory was the first to isolate a single nerve fibre (1930), prior to which a single muscle fibre had been isolated by T. Kamaya (1930) (Kato 1934)¹⁶.

When I began my research with skeletal muscle, the use of single muscle fibres was already widespread in Japan. In our laboratory, under the direction of Prof. S. Uramoto the histological changes occurring in the sarcomere during twitch and tetanus were investigated in single muscle fibres by means of high-speed microscopic cinematography (Natori 1951). The main aim of this research was to examine the all-or-none response under various conditions, but these experiments caused me to change my direction of study from the irritability of excitable tissue to research on the contraction mechanism of skeletal muscle.

At that time, the “Ganzheitsbezogenheit” of H. Driesch was the dominant philosophical concept behind physiological research in Japan. This concept was promoted by Prof. K. Hashida (1932)⁷ and Prof. S. Uramoto responded to this opinion. I was also influenced deeply and believed that there is a limit to the analytic investigation of life from the standpoint of physiology.

The above concept implies that life is a whole phenomenon and that through fundamental individuality, each living body is distinct from others and maintains its nature. This aspect of individual maintenance is the determinate character of the living body as a whole. Even though the outer and inner conditions of the living body are always changing, the body continuously maintains its united organization, that is, the phenomena revealed in the respective parts of the living body are related to its whole. These phenomena occur inevitably, but they are not independent from others and exist in the field of the whole. The processes occurring in such parts are intentional and there is a degree of unification.

“Ganzheitsbezogenheit” led to the idea that the cell is the ultimate unit of life which is possible to separate from a living organism. In other words, if the cell is disassembled, the characteristics of life must be lost. From this standpoint, it may be appropriate to consider the single muscle fibre as the limiting unit of living muscle, so that an understanding of the problems of the mechanism of contraction can be acquired on the basis of the accumulated knowledge of its properties derived from a number of different investigation procedures. When we pursue the problem along these lines, however, we face a limit.

Before 1938, I had been working on the action potential of nerve and muscle under various conditions, but acting on the above motive I subsequently began to investigate the visco-elastic properties of skeletal muscle fibres, and the contraction processes induced by various forms of stimulation such as electrical, mechanical and chemical (1938–1947). The results of these studies were collectively published in *Muscle Physiology* (Natori 1951).

The physiological study of muscle has been advanced by investigations of its structure and function in the living state.

In the course of my studies, however, serious trouble and deadlock in my experiments obliged me to break down the barrier imposed by “Ganzheitsbezogenheit”. One of the turning points was the research conducted on the thermo-elastic properties of muscle fibres. I tried to measure the expansion coefficient (α) of a single muscle fibre¹⁸), in order to further expand the knowledge existing on the visco-elastic structure of living muscle fibre. The results indicated, however, that the α value of a muscle fibres was influenced markedly by changes in temperature gradient and that α exhibited a negative value at temperatures over 8°C, returning to a positive value at temperatures below 5°C. Also, in the Japanese toad, marked seasonal variation in α was observed. These results suggested that the application of molecular theory might be difficult for an exact analysis of the visco-elastic properties of muscle fibre.

More importantly, difficulties in the molecular analysis of muscle fibres in the living state arose due to the fact that the muscle fibre is composed of several different parts, such as the sarcolemma, sarcoplasm, and myofibril, so that the α value of each part cannot be determined from the measurement of the fibre as a whole.

From these experiments it was considered necessary to connect experimentally the subcellular findings with the molecular ones, despite the conceptual restriction imposed by “Ganzheitsbezogenheit”, in order to elucidate analytically the relationship between the structure and function of muscle. If the muscle fibre could be decomposed (deconstituted) while maintaining its living state as far as possible, and its

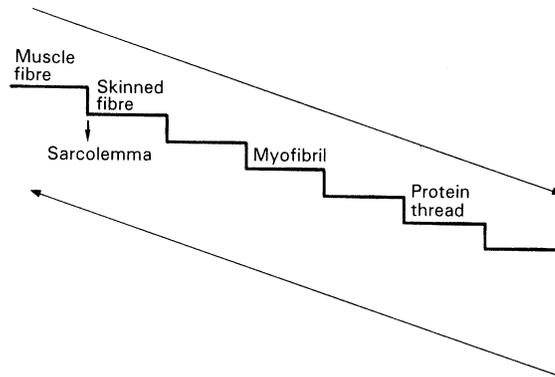


Fig. 1. Schematic illustration of “decomposition and reconstruction” of muscle fibre (Natori 1980).

properties clearly defined at each step of gradual decomposition by extracting its component substances toward the final step as shown in Fig. 1, one would be able to find a way to reconstruct the physiological properties of a living muscle fibre from those of the extracted substances. In other words, although the total could not be regarded simply as a sum of the parts, it was considered that the accumulated findings with respect to “decomposition and reconstruction” would enable us to understand the nature of the living muscle fibre. This was the motive of my research described herein.

*PROPERTIES OF SARCOLEMMA-FREE MYOFIBRIL BUNDLES
(SKINNED MUSCLE FIBRES)*

I. Method of isolation of skinned muscle fibres

With the aforesaid motive in mind, I tried to isolate myofibrils which still retained their *in situ* properties (Natori 1949*, Natori 1950, Natori 1950*, Natori 1951 and Natori 1952a).

At the beginning of this trial, on the basis of the chemical composition of the sarcoplasm reported previously, various solutions such as Ringer's solution, the Na and K concentrations of which were exchanged reciprocally, using 120 mM KCl solution and so on, were applied for removal of the sarcolemma. However, the mechanical removal of the sarcolemma in the above solutions induced irreversible contractions. For instance, if we tried to remove the sarcolemma in 120 mM KCl solution, the myofibrils contracted markedly as soon as the sarcolemma was injured. If we stretched them for a certain length of time, the fibres became extended until they equalled the slack length of the intact muscle fibre, but their properties were demonstrated in various tests to be considerably different from the properties of living myofibrils.

In the course of these trials, I wondered whether contraction would occur if the sarcolemma were to be removed in oil, thus avoiding both diffusion of the solution into and out of the muscle fibre and the generation of injury current.

The application of oil immersion was found to be successful and this marked the beginning of a series of experiments of skinned muscle fibres.

In the early years, I used the name "isolated myofibrils", "denuded myofibrils" or "sarcolemma-free bundle of myofibrils" for muscle fibres with the sarcolemma removed. Since the term "skinned fibre" later became popularized, I have recently been using the name "skinned muscle fibre". In Japan, the term "Natori's fibre" is also used to mean "skinned muscle fibre".

Technique of preparation of skinned muscle fibre: I usually used a single muscle fibre from m. sartorius or m. adductor magnus of the Japanese toad (*Bufo vulgaris japonicus*) or bullfrog (*Rana catesbeiana*) as the specimens.

The fascia of skeletal muscle *in situ* is removed beforehand and a thin bundle of muscle fibres is separated. From this bundle *in situ*, a single muscle fibre is directly isolated without transfer to a bath of Ringer's solution, placed on a glass plate, and then paraffin oil is immediately poured over the fibre.

One end of the muscle fibre is then held in the tip of a pair of forceps and the sarcolemma of the held portion is slit with the apex of a tapered knife. The sarcolemma is then stripped from this part toward the other end of the muscle fibre for a certain length. (Fig. 2). This denuded muscle fibre is used as the mechanically prepared skinned muscle fibre.

The procedure of stripping the sarcolemma is easier for bullfrog muscle than for that of the Japanese toad, since slight prolonged contractions are often evoked

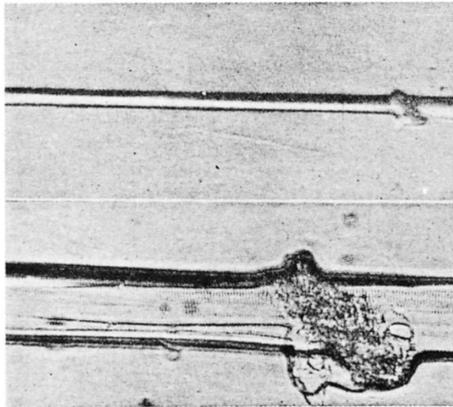


Fig. 2. Skinned fibre of *m. sartorius* of bullfrog isolated in a bath of paraffin oil.

successively during the process of sarcolemma peeling in the case of Japanese toad muscle, such contractions blocking the direction of peeling and causing tearing of some myofibrils. As is mentioned later, however, the threshold voltage of electrical stimulation necessary to evoke twitching is generally lower in skinned muscle fibres in which slight contractions have been evoked successively during peeling of the sarcolemma than in skinned muscle fibres isolated without successive local contractions.

When a muscle fibre is split longitudinally at a stroke from the snapped portion mentioned above toward the other end of the muscle fibre for a certain length, the sarcolemma with some myofibrils is peeled and two bundles of myofibrils are separated. These skinned muscle fibres, particularly those of Japanese toad, are generally recommendable preparations for experiments on the propagating contractions induced by electrical stimulation (Fig. 28).

2. Elastic properties of skinned muscle fibres

Having found a method of isolating skinned muscle fibres, I wondered whether it would be possible to isolate a bundle of myofibrils without tearing. At that time, attention had been given to the fact that the sarcolemma, responsible for the elastic properties of muscle fibres and myofibrils, is a rather plastic structure (Ramsey and Street 1940)²². The skinned muscle fibre, however, did not tear even when stretched to over twice its resting slack length, regardless of whether the sarcolemma was removed or not.

I therefore attempted to facilitate an accurate comparison of viscoelastic properties between the intact muscle fibre and the separated bundle of myofibrils (Natori 1954). Part of the sarcolemma of a single muscle fibre was peeled to expose a region of myofibrils and the other part was left intact. The respective rates of extension of both parts were estimated when this whole fibre was stretched. An example of these experiments is given in Fig. 3. This study revealed that the rates for both parts

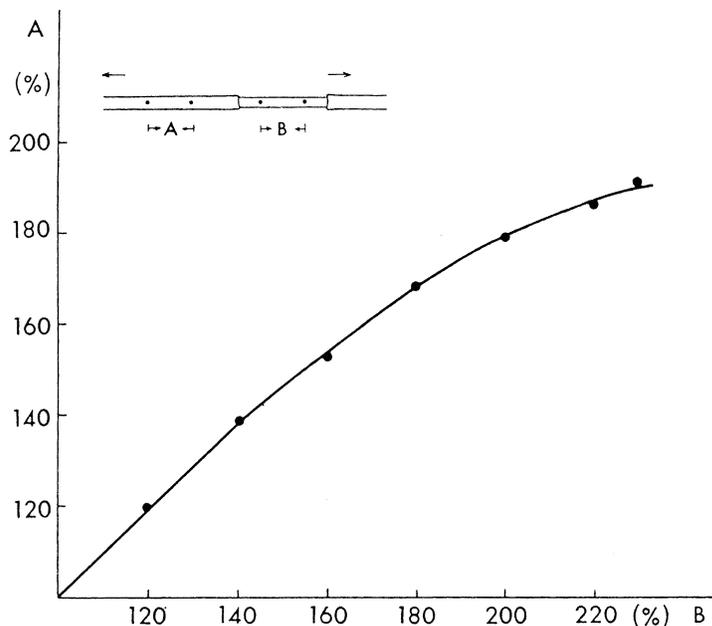


Fig. 3. Ratio of extension between intact and skinned parts upon stretching of a muscle fibre. Lengths of A and B without stretching were both 1 mm. *M. sartorius* of bullfrog (Natori 1954c).

closely resembled each other within a range of 0 to 40% extension with regard to a comparison of their cross-sections; the elastic modulus of the myofibril was thus found to be analogous to that of the living muscle fibre within the above range of extension.

Subsequently, a further theme was investigated. It was found that skinned fibres prepared in paraffin oil from the skeletal muscle of Japanese toad or bullfrog were not torn by stretching, even to a sarcomere length of 6–8 μm (Natori *et al.* 1974, Umazume *et al.*²⁵). According to reports by H.E. Huxley and Hanson (1960)¹⁴, it was considered that the overlapping parts of the thick and thin filaments of contractile protein would disappear and that the thin filaments would be dislocated from the A band, at sarcomere lengths of over 3.6 μm . If the structure of the sarcomere were mainly maintained in the overlapping portion between thick and thin filaments, the sarcomere might be expected to be torn off.

With regard to this matter, certain defense mechanisms should be worth consideration. One possibility is that among the many myofibrils of the skinned fibre, some sarcomeres exist with thin filaments emerging from the thick filaments while others do not, the two types of sarcomere being mixed among each other.

Furthermore, there is a fairly high possibility that certain parallel elastic components exist around myofibrils which might prevent sarcomere breakage. As will

be mentioned later, the internal membrane or internal elastic network might play the role of such a sustaining structure.

Structural changes in the sarcomere during stretching (Natori *et al.* 1974): Sarcomere length obtained from the diffraction pattern produced by a gas-laser light beam was used as the standard scale for determining the critical length before the skinned fibre snapped. A sample skinned muscle fibre, stretched to 2.2 times the length of L_0 (the length before stretching), is shown in Fig. 4. It was often observed that the skinned muscle fibre was not torn even when stretched to 3 times L_0 .

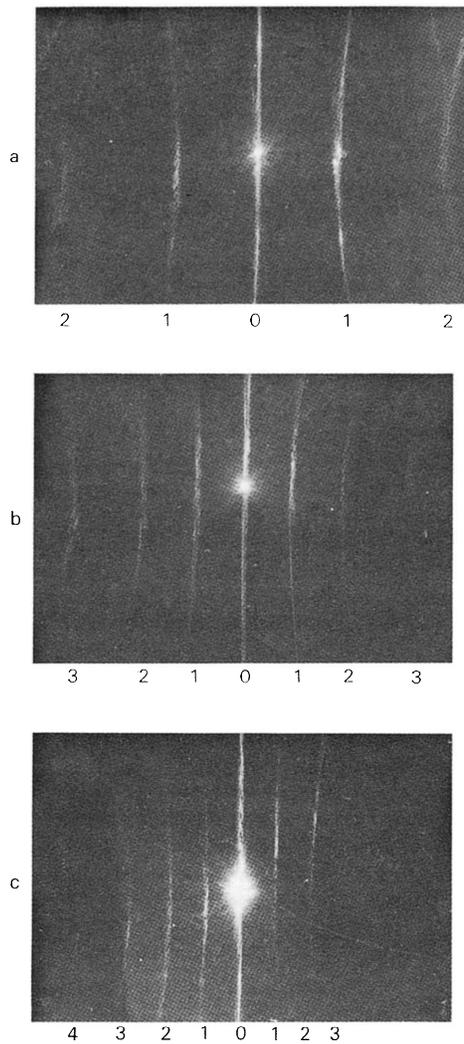


Fig. 4. Examples of laser diffraction patterns produced when skinned fibres of *m. sartorius* of bullfrog were stretched; sarcomere lengths calculated from a, b, and c, were 2.2, 3.6 and 5.3 μm (Natori *et al.* 1974).

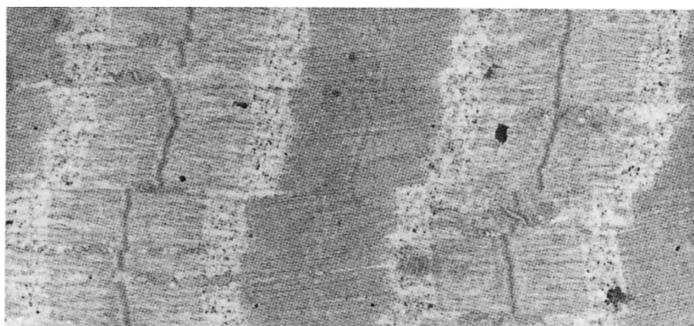


Fig. 5. Electron micrograph taken when *m. hyoglossus* of bullfrog was stretched. Spaces are produced between the A and I bands, although some filaments are observable to be left (Natori *et al.* 1974).

Electron micrographs of stretched muscle fibre are shown in Fig. 5. When the sarcomere length exceeded $3.6\mu\text{m}$ as the muscle fibre was stretched, slippage of thin filaments from the A-band was observed. According to the electron micrograph of the stretched muscle fibre, the arrangement of thin filaments which moved out of the A-band did not differ from that before moving (Fig. 5).

Fig. 6 shows the stretch curve of a skinned muscle fibre which reveals the temporal changes occurring in the stretching of the skinned region after application of a certain load. In order to keep the stretching speed low, application of viscous resistance was applied. When a skinned muscle fibre was slowly stretched, the stretching speed of the sarcomere initially decreased at the point where the length approached 150% of L_0 , and then increased. When the length approached 280–290% of L_0 under a considerable load, the sarcomere was torn in many cases.

As the results showed that no tearing of the sarcomere occurred even when the sarcomere length reached beyond $6\text{--}7\mu\text{m}$ after stretching of a skinned muscle fibre, and that regularity of the sarcomere arrangement was maintained by reference to the diffraction pattern, I speculated that a certain parallel elastic structure was preventing breakage of the sarcomere when the fibre was stretched.

This view was later coupled to Maruyama's discovery of connectin. On the other hand, I found that muscle fibres treated with collagenase could be easily isolated, the sarcolemma easily removed, and that the elasticity of such skinned muscle fibres isolated from collagenase-treated muscle fibres was changed markedly, the stiffness of the skinned fibres showing a decrease (Natori *et al.* 1974).

Since at that time it was considered that collagen might be absent in skinned muscle fibres, the possibility was discussed as to whether there was some contamination of protease with collagenase and if so, whether some elastic protein existed in skinned muscle fibre which was perhaps dissolved by the above contamination.

In 1975, Prof. K. Maruyama came to our laboratory and demonstrated a new

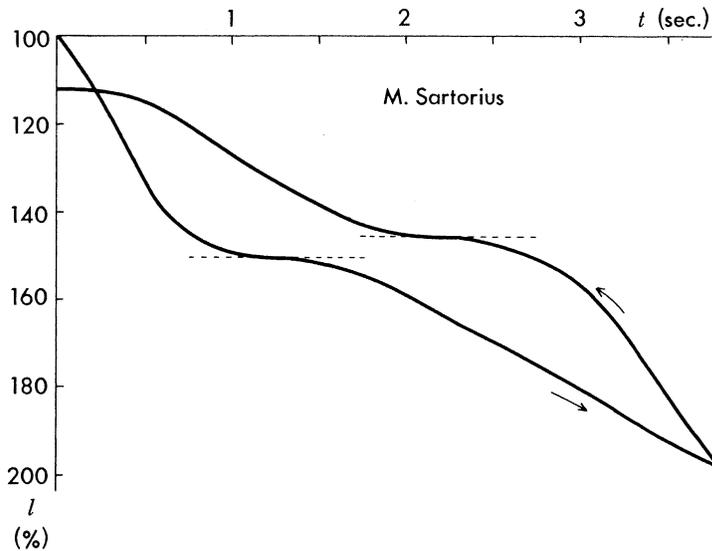


Fig. 6. Stretch curve of skinned fibre isolated from *m. sartorius* of bullfrog; temperature is 18°C (Natori *et al.* 1974).

elastic protein which differed from collagen. Maruyama said that this might be an intracellular elastic protein. From this viewpoint, we checked the properties of Maruyama's protein and a short report was contributed to *Nature* (Maruyama *et al.* 1976b).

This report, which I co-authored, stated; "The localization of the elastic protein in myofibrils is still obscure, but it seems that it is responsible for their continuity. After extraction of skinned fibre of frog muscle with an alkaline solution of pH 11.5, the elastic structure seemed to remain intact, although other structures visible under the microscope disappeared. It developed tension when stretched and behaved as an elastic body (Fig. 7). Treatment with 0.1 N NaOH resulted in a considerable decrease in tension generation, suggesting that the elastic properties had been damaged. The muscle elastic protein makes at least some contribution to the intracellular elasticity of muscle fibres".

The amino acid composition of elastic protein from pure myofibrils and fibre fragments of rabbit psoas muscle reported by Maruyama is shown in Table 1 (Maruyama *et al.* 1977). Maruyama later named this new elastic protein connectin.

From the result, it was subsequently considered possible that the connectin filament would not only serve to reinforce the T-tubule and SR system but would also contribute an elastic function by linking the thick and thin filaments. The possibility of connectin filaments binding to thick filaments was also suggested by the marked decrease in birefringence of the skinned fibre after the application of 1 M KCl solu-

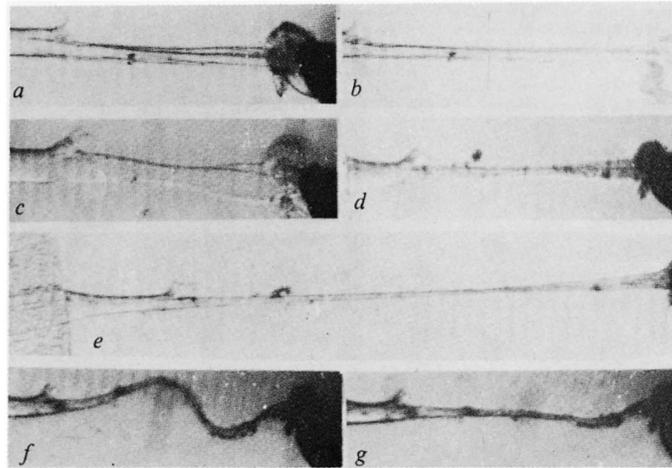


Fig. 7. Passive stretching of 'ghost' skinned fibres.
 a. Skinned fibres of *m. sartorius* from bullfrog in paraffin oil, 20°C.
 b. Stretched skinned fibres.
 c. Skinned fibres treated with alkaline solution (1 M KI, 0.1 M Na₂S₂O₃, pH 11.5).
 d. After 20h in alkaline solution, fibres were washed with Endo's solution (107mM K methansulphonate, 4mM MgSO₄, 4mM ATP, 2mM EGTA, 20mM Tris maleate, pH 6.8) (Maruyama *et al.* 1976b).

Table 1. Amino acid compositions of connectin preparations from rabbit psoas muscle.

	Pure myofibrils		Fibre fragments	
	Alkaline	Urea-SDS	Alkaline	Urea-SDS
Hyp	2	2	2	2
Asp	95	93	92	96
Thr	67	65	64	69
Ser	60	61	58	63
Glu	121	124	131	131
Pro	67	64	62	61
Gly	73	75	71	75
Ala	77	91 ^a	77	93 ^a
Cys/2	2	2	3	2
Val	67	68	67	66
Met	26	25	25	25
Ile	60	62	61	61
Leu	72	73	73	78
Tyr	33	34	33	34
Phe	31	31	31	32
Lys	74	58	77	50
His	21	21	22	16
Arg	52	52	51	46

^aSee the text. (Maruyama *et al.* 1977)

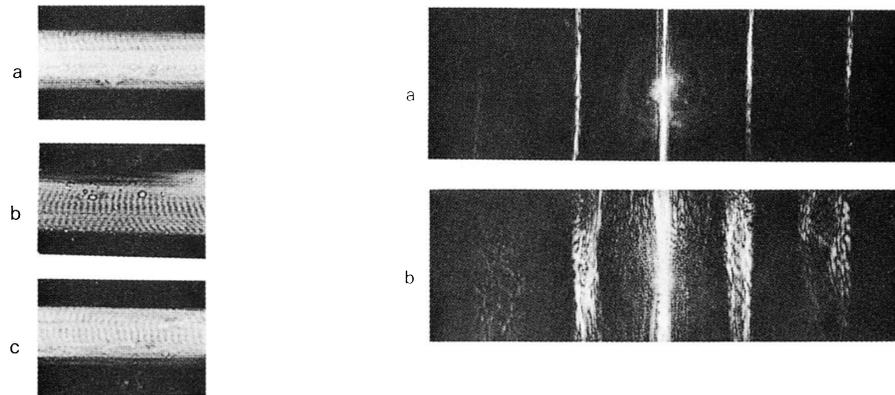


Fig. 8. Changes in skinned muscle fibres produced by application of 1 M KCl solution.
M. sartorius of bullfrog.
 Birefringence (left).
 a. Before application of 1 M KCl solution.
 b. After application of 1 M KCl solution.
 c. Recovery of the value of birefringence by addition of distilled water.
 Diffraction pattern (right).
 a. Control.
 b. Scattering of diffraction pattern by application of 1 M KCl solution
 (Natori Rb. *et al.* 1980).

tion and by the marked widening of the A-band after the application of slight stretch force (Natori Rb. and Natori R. 1983). As shown in Fig. 8, within 1 minute after application of 1 M KCl solution (pH 6.8) to the skinned muscle fibre at a resting slack length, no distinguishable change could be found in the figures of sarcomeres by phase-contrast microscopy, while sarcomeric birefringence was decreased remarkably. As for the diffraction pattern of 1 M KCl solution-treated fibre, each spectrum was scattered. After a lapse of more than 2 minutes, some changes were visible even in the figures observed by phase-contrast microscopy. The border between the A- and I-band in the sarcomere became somewhat zig-zag in appearance, and with the lapse of time, disorder of the striations occurred. When a small amount of distilled water was added drop by drop through a micropipette to a 1 M KCl-treated skinned fibre, the value of birefringence increased to the initial level of the control sarcomere, but no sample showed complete recovery of birefringence in the skinned muscle fibre.

When a skinned muscle fibre in paraffin oil was over-stretched, the figure observed by differential interference microscopy disclosed that the A-band was slightly increased in width, with a zig-zag borderline. In comparison with the extension of the A-band, the I-band was much more widened.

When a small amount of $1 \mu\text{g}/\text{ml}$ trypsin solution, which would partially dissolve connectin filaments, was applied to a skinned muscle fibre in paraffin oil, a slight extension (up to ca. $3 \mu\text{m}$ in sarcomere length) was produced, the birefringence in

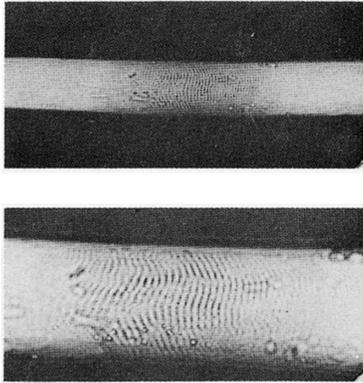


Fig. 9. Change in striation of a skinned muscle fibre by local application of 1M KCl solution containing $0.75 \mu\text{g/ml}$ trypsin. *M. sartorius* of bullfrog. Temp. 20°C (Natori Rb. *et al.* 1980).

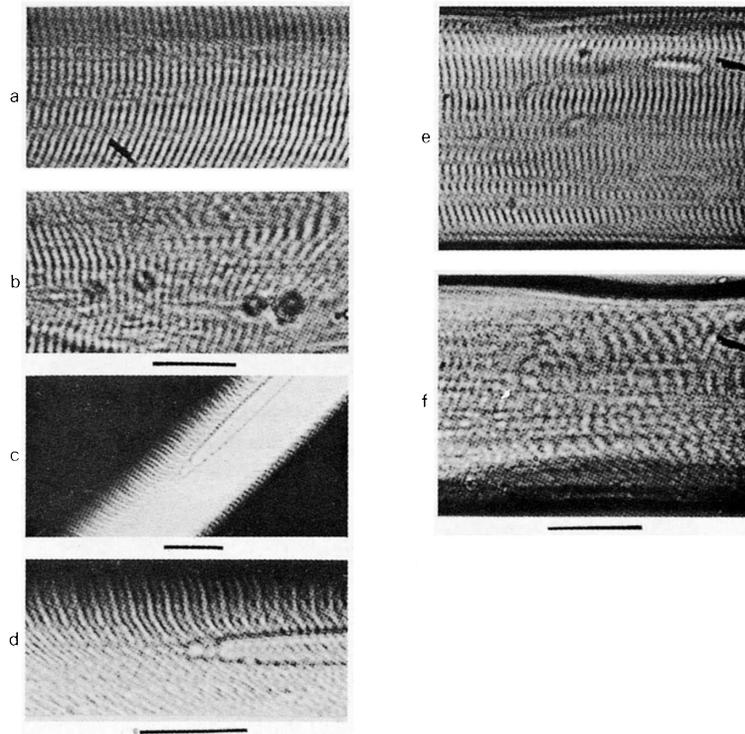


Fig. 10. DLM (Differential-interference micrograph) and PLM (Polarization micrograph) of skinned muscle fibres following application of trypsin. *M. adductor magnus* of bullfrog. Temp. 24°C .
 a. Control of DLM.
 b. After 10 minutes of local application of $1 \mu\text{g/ml}$ trypsin.
 c. PLM, after 2 minutes of application of $0.75 \mu\text{g/ml}$ trypsin.
 d. The same.
 e. DLM, control.
 f. After 5 minutes of local application of 1M KCl containing $1 \mu\text{g/ml}$ trypsin. Scale bar is equivalent to $30 \mu\text{m}$ (Natori Rb. *et al.* 1980).

the trypsin-treated part being reduced, and the sarcomere of this part being increased in length (Fig. 9). With the lapse of time, the widening of the A-band became visible, but at first, the part corresponding to the I-band exhibited marked extension as in the case of over-stretching. Even when scarcely any change was elicited by 0.7–1 $\mu\text{g/ml}$ trypsin in the profile of the sarcomere, the treated part displayed greater lengthening than the non-treated part upon application of stretch force. When 1 M KCl solution containing 0.7–1 $\mu\text{g/ml}$ trypsin, was applied to a part of the skinned muscle fibre in oil, more prominent widening of the A-band was induced in a short time than when trypsin alone was applied (Fig. 10).

Fig. 11 shows micrographs obtained by transmission electron microscopy (TEM) of a sarcomere stretched to a length of ca. 5 μm . These micrographs revealed the following: (1) Thick and thin filaments were drawn out of each other. (2) In this region of dislocation, filament-like structures of small diameter were observed together with glycogen particles which had migrated into the same area. (3) The lines formed by the free ends of the thin filaments, drawn apart from the thick filaments, were aligned nearly parallel with the Z-line, these thin filaments being indicative of some slackening. (4) By contrast, thick filaments were always maintained in a straight line, and

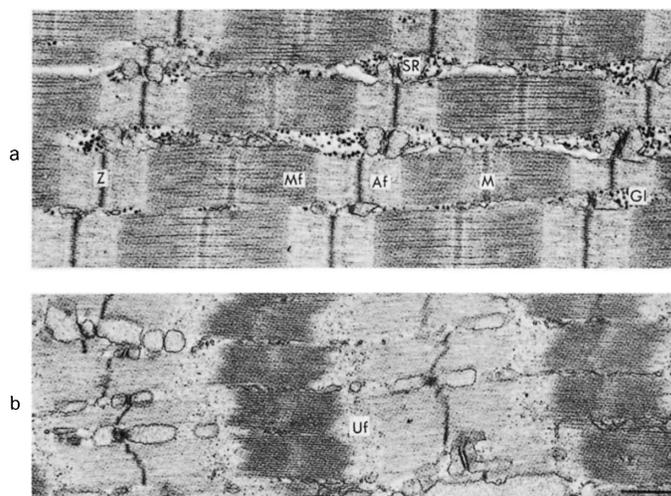


Fig. 11. TEM (Transmission electron micrograph) of longitudinally sectioned muscle fibres.

M. hyoglossus of bullfrog.

a. Non-stretched.

b. Over-stretched.

Scale bar is equivalent to 1 μm .

Mf: thick filaments, Af: thin filaments, Z: Z-line, M; M-line, SR: sarcoplasmic reticulum, G: glycogen particles.

Fine filaments (Uf) are observed in the area of separation of thin from thick filaments (Natori Rb. *et al.* 1980).

their ends showed a zig-zag alignment. From these TEM observations, it may be considered that both ends of the thick filaments would be connected with the Z-disc on both sides, thus being pulled by them.

As shown in Fig. 12, TEM observations of stretched muscle fibres also revealed the presence of very thin filaments, even finer than the thin filaments in the area of dislocation between the thick and thin filaments.

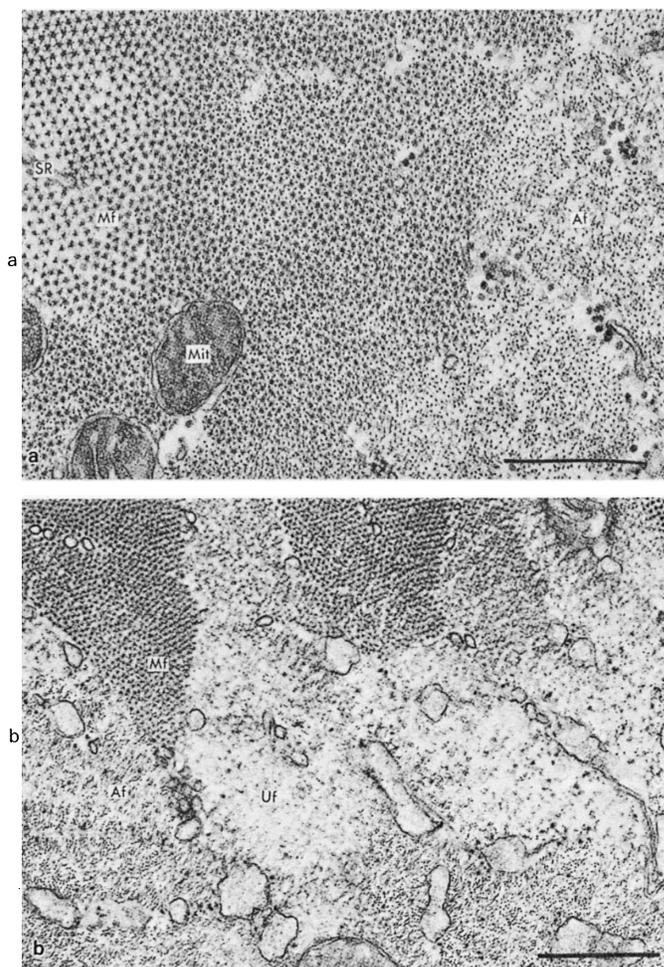


Fig. 12. TEM of cross-sectioned muscle fibres.

M. hyoglossus of bullfrog.

a. Non-stretched.

b. Over-stretched.

Scale bar is equivalent to $1\ \mu\text{m}$. Mit: mitochondrion.

In a non-stretched state the hexagonal arrangement produced by overlapping of thick and thin filaments shows separation of each from the other, leaving behind fine filaments (Natori Rb. *et al.* 1980).

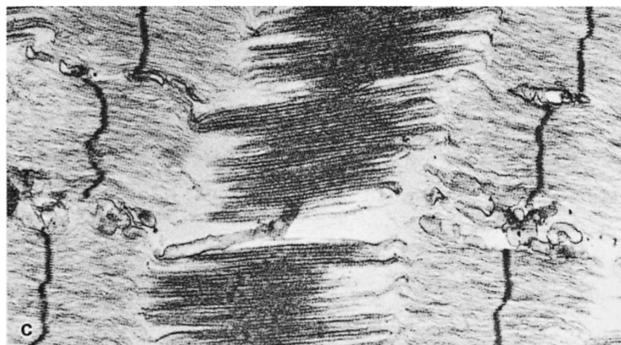


Fig. 13. TEM of over-stretched skinned muscle fibres in relaxing solution. *M. sartorius* of bullfrog. The arrangement of thick and thin filaments represents a zig-zag profile and thin filaments appear to be in a relaxed state despite the stretching of the fibre (Natori Rb. *et al.* 1980).

When a skinned muscle fibre placed in a relaxing solution was stretched, TEM revealed nearly the same arrangement of thick and thin filaments as that of a stretched muscle fibre, except that thin filaments showed a much more relaxed profile (Fig. 13).

A muscle fibre isolated from *m. sartorius* was immersed in relaxing solution containing $0.7 \mu\text{g/ml}$ trypsin and immediately a skinned fibre was prepared by removing the sarcolemma. A few minutes after stretching, the fibre was subjected to fixation for TEM. Also, a bundle of muscle fibres was immersed in the above-mentioned trypsin-containing relaxing solution for 20–30 minutes, and a TEM specimen prepared. In the case of the non-stretched muscle fibre, as shown in Fig. 14a, the extension of the thick filaments became inconspicuous following the application of trypsin, and the thin filaments were markedly relaxed, being folded in some sarcomeres.

In TEM micrographs of the skinned muscle fibre immersed in a relaxing solution containing $0.7\text{--}1 \mu\text{g/ml}$ trypsin, the profile of the M-line was observed much more clearly than in the case of the non-treated skinned fibre, and the bundles of thick filaments were separated into various small groups which gradually became scattered with the lapse of time. However, the thick and thin filaments were not dissolved within a few minutes after application of $0.7\text{--}1.0 \mu\text{g/ml}$ trypsin. In stretched skinned fibres some thick filaments tended to gather more on one side of the Z-disc than on the other, thus causing a loss of uniformity in their arrangement (Fig. 14).

From the TEM observations described above, it is probable that some elastic structure made up of ultra-thin filaments exists in the sarcomere of skeletal muscle.

In connection with these experiments, I wondered whether the skinned muscle fibres were over-stretched evenly without irregular changes in the length of the sarcomeres and whether removal of the stretching force would lead to sliding of the thin filaments between the thick filaments. With regard to this matter, one aspect

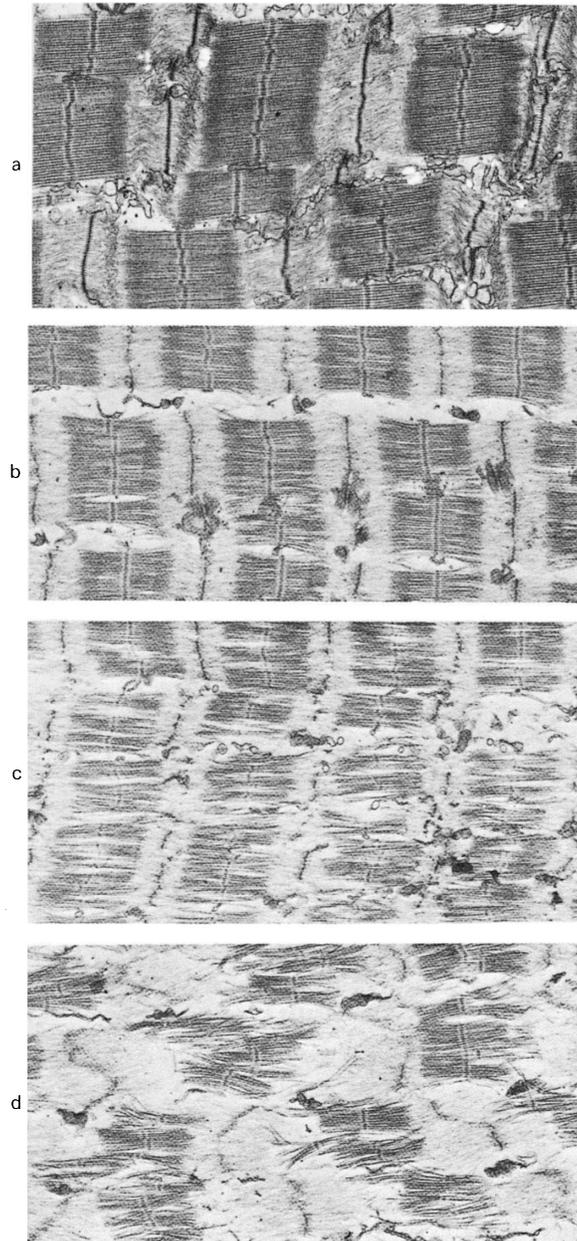


Fig. 14. TEM of muscle fibres following application of a relaxing solution containing $1\mu\text{g/ml}$ trypsin.

a. Thin filaments exhibit a relaxed and partially folded arrangement.

b~d. Time course of disorder observed in arrangement of thick and thin filaments.

Bundle of thick filaments are separated into small bundles and small bundles of thick filaments gradually assume a scattered arrangement.

Black arrow indicates the lapse of time of about 5 minutes (Natori Rb. *et al.* 1980).

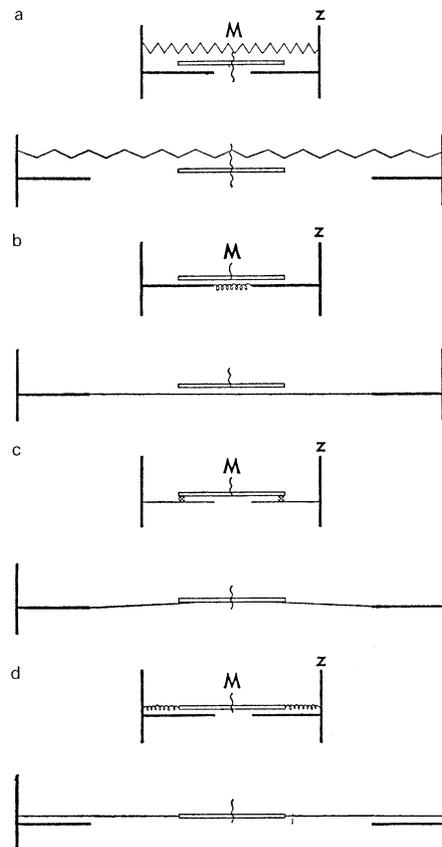


Fig. 15. Schematic illustration of some possible examples of the elastic structure of the sarcomere (Natori 1980).

of our scheme was proposed in the symposium, "Cross-bridge mechanism in muscle contraction" (Natori 1980). This scheme is represented in Fig. 15.

By TEM, the thick filaments of skeletal muscle fibre are almost without exception located in the middle part of the sarcomere. Even though they may be fixed at the M-line by some elastic structure manifested by TEM, this is not yet sufficient to explain why they should constantly reside there. If, as shown in Fig. 15d, both ends of the thick filaments are bound to the Z-discs by some elastic filaments, then the constant location of thick filaments in the middle part is highly probable. It is very plausible that connectin, extracted by Maruyama, may have a very thin filamentous structure and is sustained at both ends of the thick filament. Whichever of the cases represented by Fig. 15b, c and d, may be presumed, the involvement of connectin seems to be assumable.

Regarding this question, by means of immunoelectron microscopic study using polyclonal antibodies against native connectin, Maruyama *et al.* found that there are

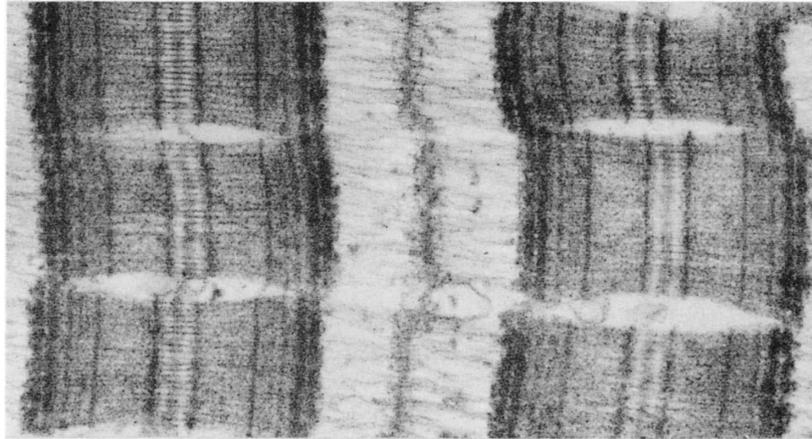


Fig. 16. Immuno-electron micrograph of a skinned fibre.
 M. semitendinosus of bullfrog.
 Skinned fibres were fixed with formalin followed by the treatment with antiserum raised against native chicken breast muscle connectin. A striped appearance due to the binding of anti-connectin antibodies can be seen (Yoshioka *et al.* 1986).

five distinct antibody-binding stripes in each half of the A-I junction region and that the connectin structures are directly linked to Z lines (discs) from the thick filaments (Fig. 16). Deposits of antibodies were also recognized in the I band and Z lines.

Addendum (Natori 1954, Natori 1954)*

Table 2 shows the difference of properties between the tonic and non-tonic muscles. Some differences were observed in skinned fibres between both kinds of skeletal muscle and also between the large bright (thick) and small dark (thin) fibres in the same muscle of Japanese toad.

When part of the sarcolemma of intact muscle fibre was torn in Ringer's solution, contracture was induced in the denuded myofibrils. In large bright fibres, after tearing of the sarcolemma, the skinned portion simply contracted and did not become fragmented. On the other hand, in the small dark fibres, contracture occurred together with escape of small granules from the interiors of myofibrils into the Ringer's solution, followed by myofibril fragmentation (Fig. 17).

No clear difference in the elastic skeleton between the large bright and small dark fibres has yet been demonstrated, however, the above results suggest that the elastic skeleton of small dark fibres may be weaker than that of large bright fibres and that the elastic properties of small dark fibres may account more significantly for the elastic properties of the sarcolemma than those of the large bright fibres.

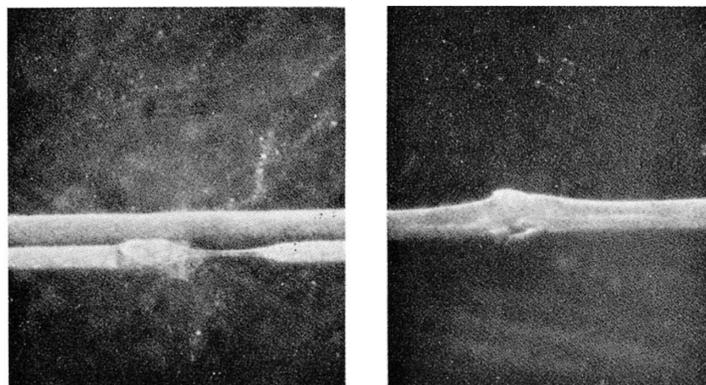


Fig. 17. When the sarcolemma was injured, the small granules escaped from small dark fibres are much more numerous than that from large bright fibres and the myofibrils are cut off. *M. sartorius* of Japanese toad (Natori 1956).

Table 2

	Tonic muscle fibre	Non-tonic muscle fibre	
Elasticity	$3 \times 10^4 - 5 \times 10^5$	$4 \times 10^4 - 5 \times 10^5$	(dyne/cm ²)
Birefringence	2.60×10^3	2.56×10^3	
Duration of twitch	0.14-0.17	0.15-0.16	(sec)
Critical fusion rate of tetanus	10-15	16-20	(/sec)
Half-value period of idio-muscular contraction	60-80	7-11	(sec)
Degree of shortening in dry test	20-24	20-24	(%)
Contractility of separated myofibrils	#	#	

(Natori 1956)

3. Contraction of skinned muscle fibres by electrical stimulation

At the time when I first isolated the sarcolemma-free bundle of myofibrils, I supposed that this fibre did not respond to electrical stimulation, since the sarcolemma was considered to be exclusively responsive to electrical stimulation due to its membrane property. However, it was found that the application of an electrical square pulse actually induced contraction of skinned muscle fibres. I subsequently tried various experiments on the contraction of skinned muscle fibres by applying electrical current.

a. Isotonic contraction of skinned muscle fibres by rectangular current (electrical square pulse) (Natori and Isojima 1962)

When a square pulse (1 sec duration, several volts intensity) was applied to a skinned muscle fibre in paraffin oil, transient contraction, like the twitch of an intact muscle fibre, was produced in the neighbourhood of the stimulating electrode contact at both the making and breaking of the current.

Table 3. Thresholds of the local twitch upon making and breaking of a rectangular current (square pulse) of 1 sec duration. Skinned fibres of *m. sartorius* from Japanese toad.

at make (V)	at break (V)
1.4	2.4
1.3	2.7
1.9	2.8
2.4	3.6
1.3	2.4

(Natori and Isojima 1962)

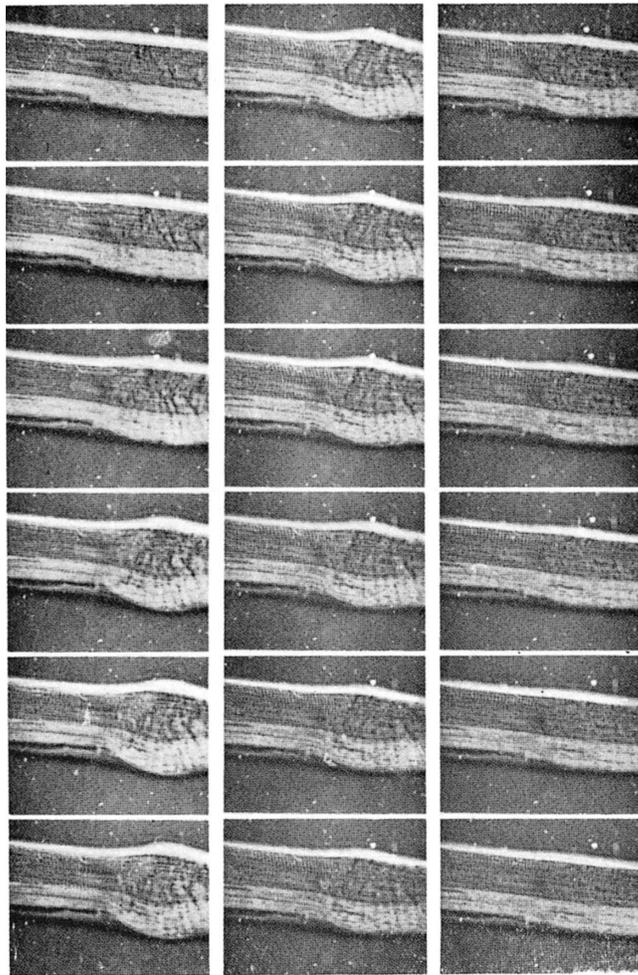


Fig. 18. Local tetanus of skinned muscle fibres produced by alternating current (200 Hz). *M. sartorius* of Japanese toad (Natori and Isojima 1962).

The thresholds of these contractions are shown in Table 3. The duration of contraction induced by a square pulse of 1–5 msec duration estimated from cinematographic recordings varied from 0.15 to 0.2 sec at a room temperature of ca. 18°C. When the intensity of the square pulse was increased over the threshold for local contraction, the zone of local response became enlarged and propagation of contraction was observed.

A series of stimulations with square pulses above the threshold intensity, and 1–5 msec in duration produced repetitive local twitch responses to each pulse. When the frequency of the pulse was increased to over 10–15/sec, a local prolonged contraction, like tetanus, was induced. As shown in Fig. 18, a local tetanus was induced by application of alternating current.

b. Isometric contraction of skinned muscle fibres by application of electrical square pulse

As shown in Fig. 19, both ends of the skinned part of a muscle fibre were cut and one end of the skinned muscle fibre was tied to a mechano-transducer (SHINKO 6001 F). A pair of platinum electrodes tied to micromanipulators was used as both the lead wire for electrical stimulation and the fastener of the skinned muscle fibre. The tip of each platinum electrode was cut sharply and both ends of the skinned muscle fibre were coiled 2–3 times around these tips, in this procedure the skinned muscle fibre being tightly fastened to the tip of each platinum electrode. A very thin copper wire connected to the electrical stimulator was placed at right angles to the platinum electrode tied to the mechano-transducer, so as to exclude the possibility of recording extra tension.

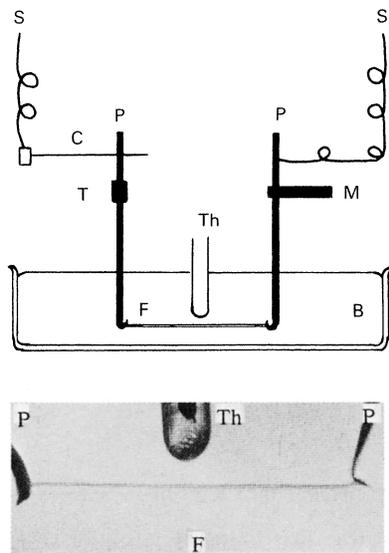


Fig. 19. Experimental arrangement used for recording of tension curve.

F: skinned muscle fibre, P: platinum electrode, T: mechanotransducer, M: micromanipulator, S: electrical stimulator, C: thin copper wire, Th: tip of thermopile, B: bath of paraffin oil (Natori 1985c).

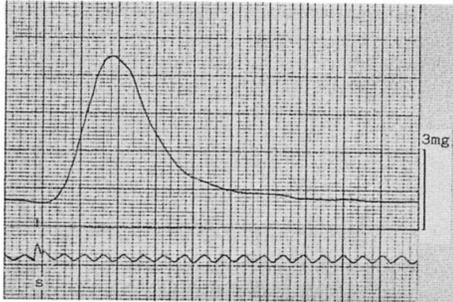


Fig. 20. Tension curve of twitch.

Skinned fibre of m. adductor magnus of bullfrog.

Stimulation (s): square pulse of 5 msec duration and 20 V intensity. Time mark: sine wave of 20 Hz.

Tension curve was obtained at a temperature of 15°C. (room temperature 23°C) (Natori 1985c).

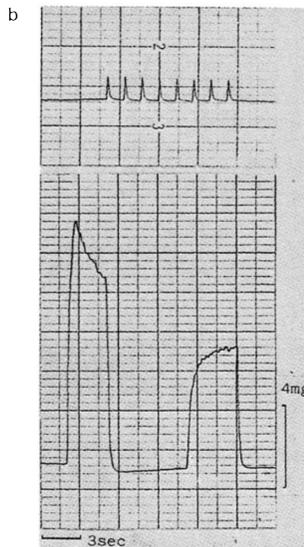
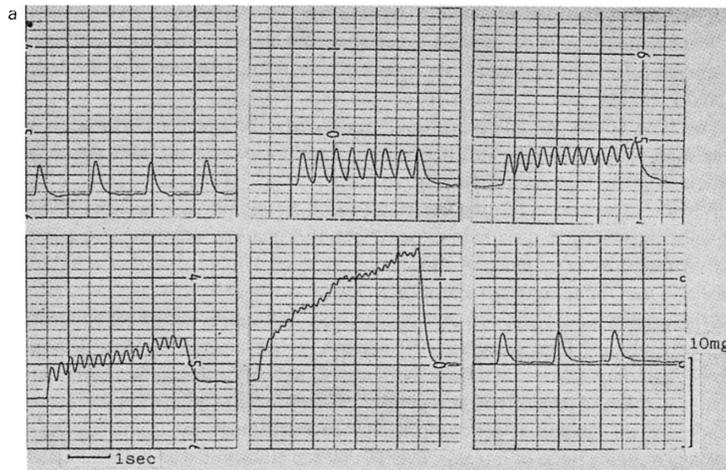


Fig. 21. Incomplete and complete tetanus in skinned muscle fibres.

a. Skinned fibres of m. sartorius from bullfrog.

Stimulation: square pulse of 8 msec duration and 20 V intensity.

This record was obtained in January (room temperature 16°C). Twitch led to complete tetanus by increasing the frequency of the square pulses and tension-residual after relaxation gradually increased.

b. Skinned fibres of m. sartorius from bullfrog.

Stimulation: square pulse of 5 msec duration and 20 V intensity.

In the tetanus curve on the right, direction of stimulating current was changed inversely from that of the curve on the left, and also in the left curve, the electrode on the mechano-transducer side was the anodal electrode. (room temperature 20°C) (Natori 1985c).

When an electrical square pulse of 5 msec duration was transmitted to a skinned muscle fibre, a twitch was induced over a certain intensity of current. The threshold voltage of stimulating current required to produce a local twitch in a skinned muscle fibre from Japanese toad was lower (2–6V) than in that from bullfrog (6–10V). A seasonal variation of threshold voltage was observed with both Japanese toad and bullfrog. Twitching of skinned muscle fibres from Japanese toad was observed all year round, the lowest threshold voltage (2–3 V) being demonstrated during February and March, but twitching of skinned muscle fibres from bullfrog was rather seldom observed in January (Natori Rb. 1983)¹⁹.

Tension curves of twitches are shown in Fig. 20. Tension decreased gradually during repetitive occurrences of twitching and the decrement of force was more marked than that of repetitive twitching of intact muscle fibre in Ringer's solution. This may suggest that a lowering of the polarization level of the internal membrane of a skinned muscle fibre advances more rapidly than that of the plasma membrane of an intact muscle fibre in Ringer's solution. When the direction of stimulating current flow was changed inversely or when an interval of stimulations was given while repetitive twitches were occurring, tension heights of twitches were somewhat elevated.

As shown in Fig. 21, incomplete and complete tetanus was induced in skinned muscle fibres by application of frequent stimuli, as in the case of tetanus in intact muscle fibre. In general, locally confined tetanus was induced and whole tetanus along the full length of the skinned area was rather seldom observed.

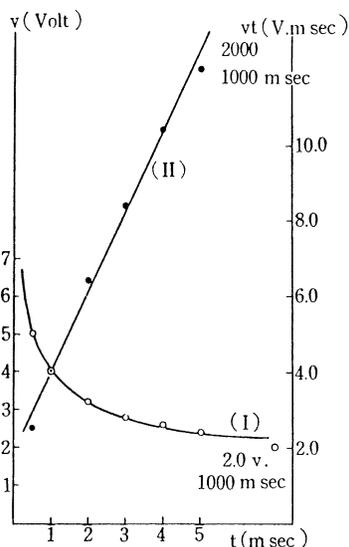


Fig. 22. The strength-duration curve of skinned muscle fibres. (I) is a v-t curve and (II) a vt-t plot. *M. sartorius* of Japanese toad (Natori and Isojima 1962).

c. Electrical stimulation and its relation to contraction response (Natori and Isojima 1962)

(1) The voltage-duration relations of square pulses capable of evoking local twitching: Threshold voltages were measured by applying a square pulse when the duration of the pulse was changed. Figure 22 shows the strength-duration relation of a skinned muscle fibre. The $vt-t$ plot indicates a linear relation.

(2) The accommodation of stimulation in skinned muscle fibres: The relation between the threshold voltage (V) and the halfperiod (τ) of an exponentially increasing current applied to a skinned muscle fibre is shown in Fig. 23. The half-period of the current was adjusted by changing only the capacity. As shown in Fig. 23, the (V)–(τ) relation was linear, indicating the accommodation of the skinned muscle fibres.

(3) The threshold intensity and frequency relation of alternating current for local twitching: The frequencies of the wave employed ranged from 50 to 7000 Hz. The induction of contracture was unavoidable, even to the slightest extent, at the point of contact of the stimulating electrode during stimulation with alternating current. For this reason, the threshold was tested by stimulation with a wave of 100 Hz, before and after each series of measurements, and only the cases which give the same value in both tests were taken as the experimental data. The relation between the threshold intensity and frequency is presented in Fig. 24.

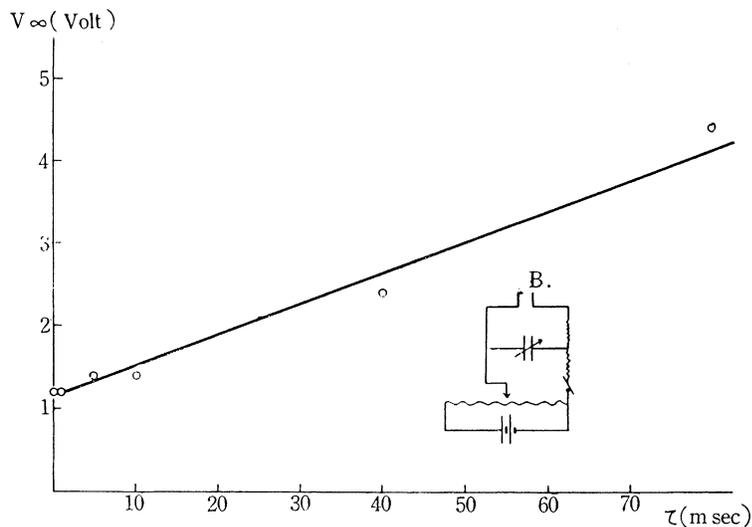


Fig. 23. The relation between the threshold and half-period of exponentially increasing currents on skinned muscle fibres.

The scheme at the lower right shows the electric circuit employed for the experiments.

M. sartorius of Japanese toad (Natori and Isojima 1962).

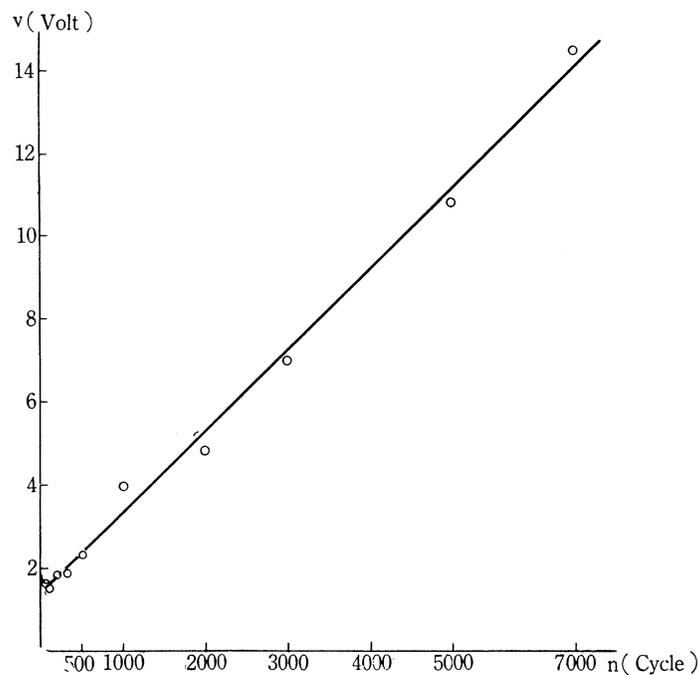


Fig. 24. The threshold intensity and frequency relation of alternating current with skinned muscle fibres.
M. sartorius of Japanese toad (Natori and Isojima 1962).

d. Propagated contraction produced by electrical stimulation

When the intensity of a square pulse was increased a little above the threshold voltage, the local contraction of skinned muscle fibres was spatially augmented and abruptly changed into a propagated contraction (Fig. 25). The propagated contractions were observed to originate from the anodic side of the stimulating electrodes. The starting portion of the contraction was reversed by reversing the direction of the stimulating current.

The velocity of propagation ranged from 700 to 1,000 $\mu\text{m}/\text{sec}$, but higher velocities of propagation as great as 2,000 to 3,000 $\mu\text{m}/\text{sec}$ were often observed, as shown in Table 4. These variations in the propagating velocity of the contraction wave depended on the temperature of the paraffin oil used for immersing the skinned muscle fibre, but I cannot deny that a difference existed between the specimens with regard to changes in excitability, since the excitability was remarkably related to the technique of peeling the sarcolemma.

At that time, no relation between the velocity of the contraction wave and the diameter of the skinned fibre had yet been confirmed.

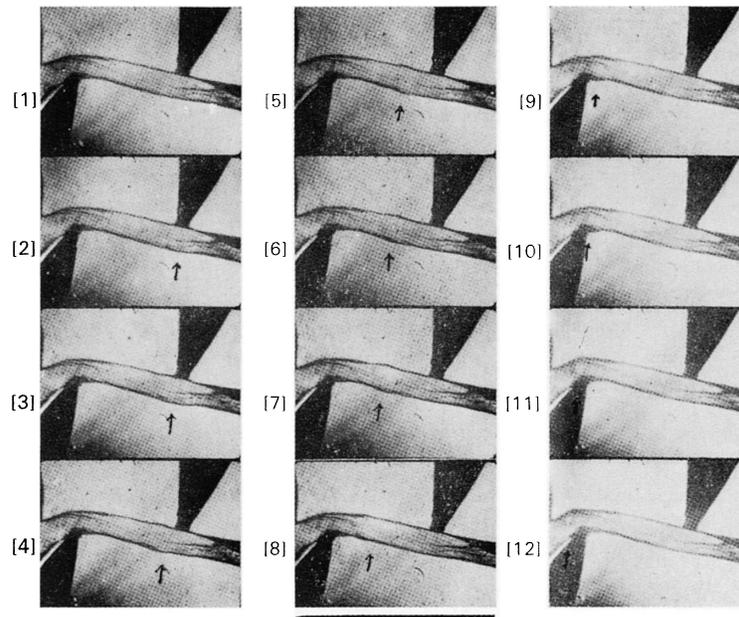


Fig. 25. Cine photographs of propagated contraction induced in skinned muscle fibres. *M. sartorius* of Japanese toad. Cine film was taken at 16 frames per second. Scale bar equivalent to $500\mu\text{m}$ (Natori 1965a).

Table 4. The velocity of propagation of contraction induced in skinned muscle fibres.

Diameter of bundle (μ)	Length of contraction wave (μ)	Velocity of propagation (mm/sec)
50	55	0.55
40	60	0.7
50	60	0.7
45	65	0.95
45	75	1.8
35	85	2.1
40	100	2.8

- 1) The propagated contractions were evoked by a single square pulse of 5-msec duration through platinum electrodes.
 - 2) The diameters of skinned fibres cited in the table are the widths of the myofibril bundle lying on a glass plate. Due to some possible change in the shape of cross-section of the skinned fibre, the value cited above does not always indicate the true diameter.
 - 3) The length of contraction wave was measured from cine photographs.
 - 4) The mean velocity of propagation was calculated from the distance of propagation per second measured from successive frames of cine film.
- (Natori 1965a)

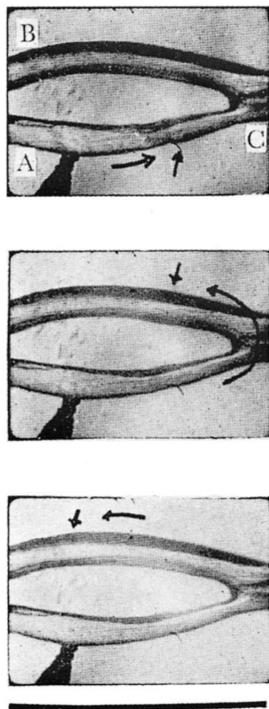


Fig. 26. The transverse propagation of contraction in skinned muscle fibres. *M. sartorius* of Japanese toad. Scale bar equivalent to $500\mu\text{m}$ (Natori 1965a).

A skinned muscle fibre was cut at its middle portion into two bundles in a longitudinal direction so that the two bundles formed a closed ring. The anodal stimulating electrode was placed on one branch (A in Fig. 26), while the cathodal electrode was placed on the other branch. The contraction wave evoked in A was propagated to B through the portion connecting the two branches (C in Fig. 26). When the direction of stimulating current was reversed, the direction of propagation was also reversed, *i.e.*, from B to A through C.

The minimum width of the local contraction in which the contraction changed to a propagated one was ca. $50\mu\text{m}$ (18°C) and the wavelength of the propagated contraction was in most cases more than $70\mu\text{m}$.

A schematic diagram of propagated contraction in a skinned fibre is shown in Fig. 27 (Natori 1972b). The contraction wave was propagated without decrement along the skinned portion of the muscle fibre, but was propagated decrementally after the contraction wave had entered the intact portion of the muscle fibre (Fig. 27b). The contraction was propagated along a ring of skinned muscle fibre, as mentioned above (Fig. 27c), but even in a skinned muscle fibre the contraction sometimes turned back after reaching the intact portion (Fig. 27d), and the propagation took place in both directions as shown in Fig. 27e. When the contraction waves started from both ends

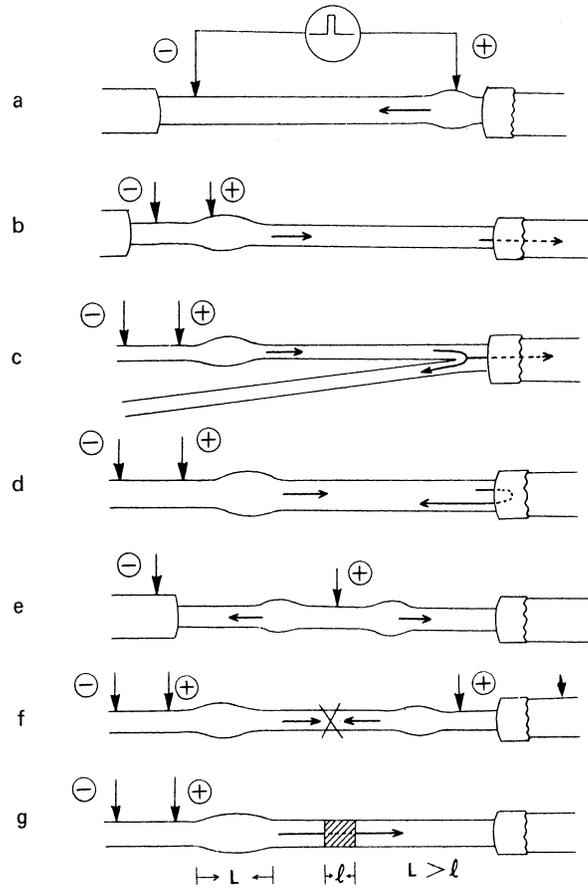


Fig. 27. Schematic diagrams of propagated contractions in skinned muscle fibres (Natori 1972b).

Table 5. Change in the threshold of propagated contraction and the total number of repetitive occurrences of propagated contraction when a skinned muscle fibre was stimulated at a frequency of once per sec.

Threshold (volt)	Total number of repetitive occurrences
2.2	53
2.4	45
3.0	23
3.8	12
4.5	10

(Natori 1972b)

of the skinned muscle fibre, they collided with each other and disappeared in the region of collision (Fig. 29f). Furthermore, when a very small quantity of tetrodotoxin (TTX, 1×10^{-4} g/ml) or MnCl_2 (100mM) was applied to the middle portion of a skinned muscle fibre, the propagated contraction stopped at this portion, but when this portion was narrow, contraction was propagated beyond it (Fig. 27g). Although the length of the treated portion was difficult to measure accurately and bearing in mind that diffusion must also be taken into consideration, it appeared that when the contraction wavelength was more than twice the length of the treated portion, propagation beyond the treated portion was possible.

The threshold voltage which induced propagated contraction was gradually elevated by repetitive stimulation of 1/sec as shown in Table 5, but propagated contractions could be induced repetitively 200 or more times.

e. The change in electrical potential during propagation of contraction in skinned muscle fibres (Natori 1963, Natori 1975)

The excitable properties of skinned muscle fibres described above suggest that some changes in potential similar to the case of an action potential would be expected to be detected in a skinned muscle fibre upon excitation. When a skinned muscle fibre is stimulated in paraffin oil by a single square pulse of short duration (1–5 msec), propagated contraction is induced.

The large bright muscle fibres used in the experiments were isolated from hind limb muscles (m. adductor magnus, m. sartorius) of Japanese toad, since from these, we can easily obtain specimens of low threshold voltage for induction of propagated contraction (Fig. 28).

A pair of Ag-AgCl stimulating electrodes was placed in contact with one of the ends of the skinned part of the muscle fibre. Changes in electrical potential were recorded by means of a glass capillary microelectrode inserted into the skinned region, while an Ag-AgCl electrode was used as the neutral recording electrode. Glass capillary microelectrodes were filled with 3 M KCl solution and suitable electrodes giving an electrical resistance of 20–60M Ω were selected. The tip diameter of the microelectrodes was 0.3–0.5 μm .

The arrangement of the stimulating electrodes is shown in Fig. 29a. The interpolar distance of the stimulating electrodes was about 0.2 mm, and the anodic electrode was placed nearer to the recording electrode. In some cases, a small portion of the fibre lying between the electrodes remained unskinned (Fig. 29b). A microelectrode was inserted into the skinned muscle fibre at a distance from the anodic stimulating electrode, and an Ag-AgCl electrode was brought lightly into contact with the skinned part at the other end of the fibre. In some experiments a portion between the two recording electrodes was not skinned (Fig. 29b).

In well-prepared skinned muscle fibres, the contractions evoked by square pulses of 1-msec duration were propagated as far as the other end of the skinned region.

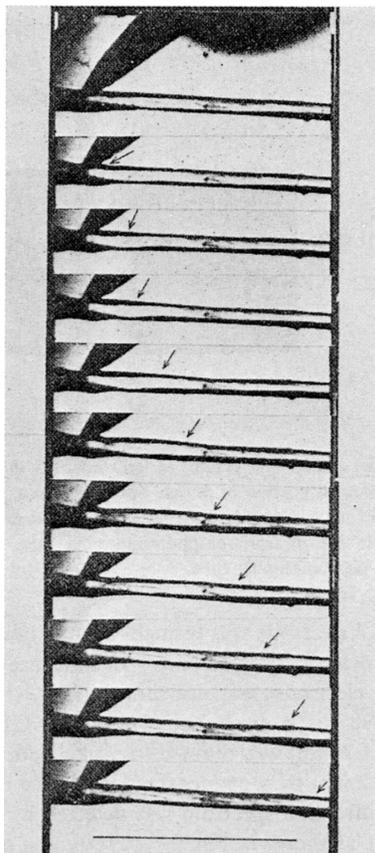


Fig. 28. Cine photographs of propagated contractions in skinned muscle fibres induced by electrical square pulse. *M. adductor magnus* of Japanese toad. Cine film was taken at 30 frames per second. Scale bar, 1 mm (Natori 1975).

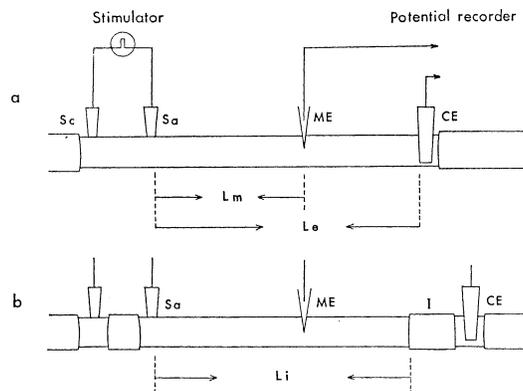


Fig. 29. Schematic illustration of the method used for recording of potential change in skinned muscle fibres.

Sa: anodic stimulating electrode (Ag-AgCl), ME: microelectrode, CE: Ag-AgCl electrode, I: unskinned portion, Le: distance between Sa and CE, Lm: distance between Sa and ME, Li: distance between Sa and I (Natori 1975).

The propagating velocities were 3–4 mm/sec at 17–18°C, and similar propagating contractions could be evoked more than 30 times by stimulation every 3 sec.

The amplitude of the recorded potentials varied from one preparation to another but never exceeded 10 mV. As shown in Fig. 30, the time course of the potential change was far slower than that of the spike potential of intact muscle fibre, and the period of the rising phase of the potential was about 30–40 msec or sometimes more. A propagated contraction was sometimes observed to be reflected at the boundary with the unskinned portion after it had travelled along the skinned part. In Fig. 31c, two potential deflections are seen, the second of which is the reflected one.

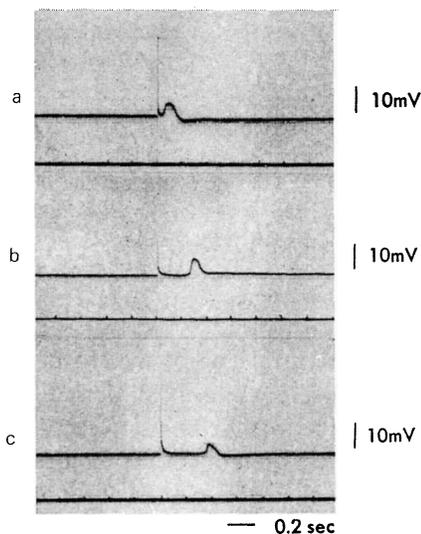


Fig. 30. Records of potential change in propagating contraction of skinned muscle fibres. *M. adductor magnus* of Japanese toad. 18°C.
 a. Distance between Sa and ME (Lm): 0.3 mm. The resistance of ME (Re): 50 MΩ.
 b. Lm: 0.8 mm, Re: 30 MΩ,
 c. Lm: 1.3 mm, Re: 40 MΩ.
 The first spike in each electrogram is an artifact due to stimulation (Natori 1975)

f. Temperature dependency of contraction induced in skinned muscle fibres (Natori 1972b, Natori 1985c)

The velocity of the propagation of isotonic contraction of skinned muscle fibres depends on the temperature of the paraffin oil used for immersing the specimens. $Q_{10} = 1.9\text{--}2.0$ for the same specimen, as shown in Table 6, but the velocity varied according to the specimens used, for example, it was 2 mm/sec with one specimen, while it was 3 mm/sec with another at the same temperature.

The tension height of the isometric twitch also depends on the temperature. Figure 33 shows the tension curves of twitching at various temperatures.

Paraffin oil at ca. 4–5°C was poured into a bath in which a skinned muscle fibre was immersed, and the temperature of the paraffin oil, which gradually increased up to room temperature (ca. 23–25°C), was monitored by means of a digital thermo-

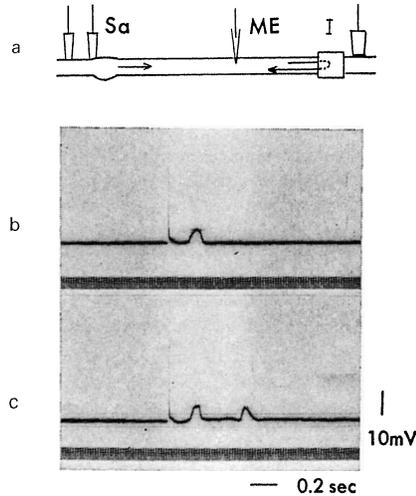


Fig. 31. Changes in potential of a reflected contraction wave.

Skinned fibers of *m. sartorius* from Japanese toad, 16°C.

a. Schematic diagram of reflected contraction wave in skinned muscle fibre.

b. A case in which reflection from point I did not take place.

c. A case in which reflection from point I did take place (Natori 1975).

Table 6. The temperature dependency of the velocity of propagation of contraction induced by a square pulse.
Skinned fibres of *m. sartorius* from Japanese toad.

Temperature (°C)	9-11 A	19-21 B	B/A
Velocity of propagation (mm/sec)	1.9	3.9	2.05
	1.7	3.0	1.76
	2.1	3.9	1.85
	2.3	4.5	1.95
	1.8	3.4	1.88
	1.96	3.74	1.91

(Natori 1972b)

meter, the thermopile tip of which was situated near the skinned muscle fibre (Fig. 19). The skinned muscle fibre was stimulated at various paraffin oil temperatures, as shown in Fig. 32.

g. On- and off-contraction observed with skinned muscle fibres (Natori 1985c)

As mentioned above, when a rectangular current (square pulse) of long duration (over 0.5 sec) was applied to a skinned muscle fibre, contractions were observed upon both making (on) and breaking (off) of the stimulating current.

As shown in Fig. 33, when the duration of the square pulse was increased stepwise from 10 to 100 msec, the tension of twitch gradually increased (graded response), regarding in duration from 100 to 500 msec, the tension curve of contraction shifted from a twitch to somewhat prolonged contraction and tension increased. With the

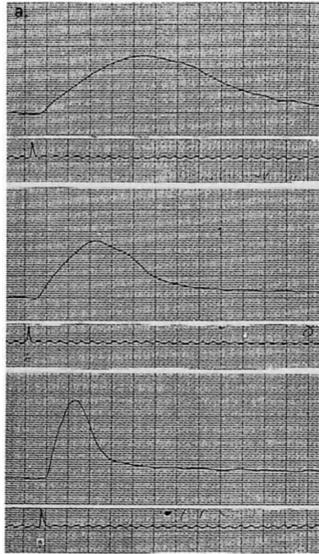


Fig. 32. Time course of tension development of twitch with temperature of paraffin oil used for immersing skinned muscle fibres.
a. Skinned fibre of m. adductor magnus of bullfrog.

Tension curves of twitch were recorded at paraffin oil temperatures of 6°, 8°, and 10°C, respectively.

s: mark of stimulation; sine wave of time mark is 20Hz.

b. Skinned fibre of m. sartorius from bullfrog.

Tension curves were recorded at temperatures of 12°, 14°, 18° and 21°C, respectively (Natori 1985c).

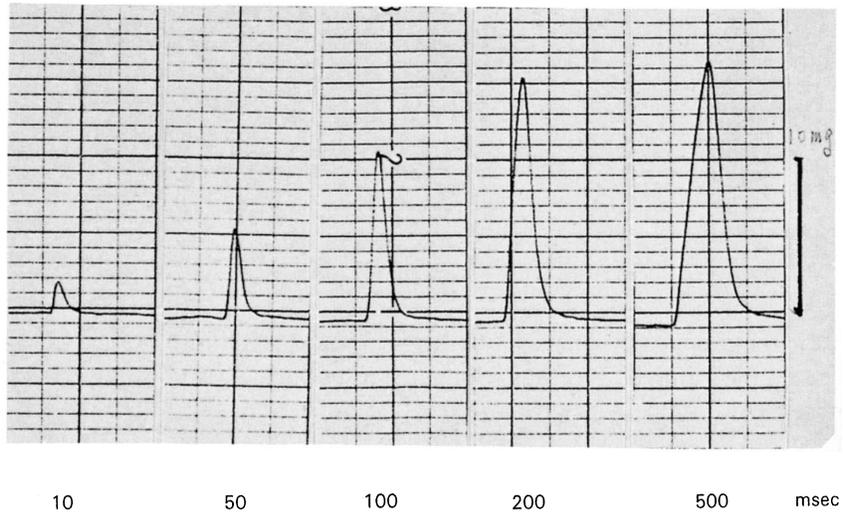
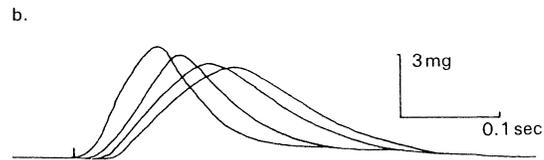


Fig. 33. Tension curve of contraction induced by square pulses of various durations. Skinned fibre of m. adductor magnus from bullfrog. Stimulation: square pulse of 10–500 msec duration and 30V intensity. Tension was gradually increased by prolongation of duration of square pulses.

application of a square pulse of over 200-msec duration, off-contraction often followed on-contraction and sometimes the tension of both the on- and off-contraction became piled up without separation of the tension curve.

The time course of tension development of the on-contraction, particularly the phase of relaxation, was slower than that of the twitch produced by a square pulse of short duration, and the tension development of the off-contraction was much slower than that of the on-contraction (Fig. 34a).

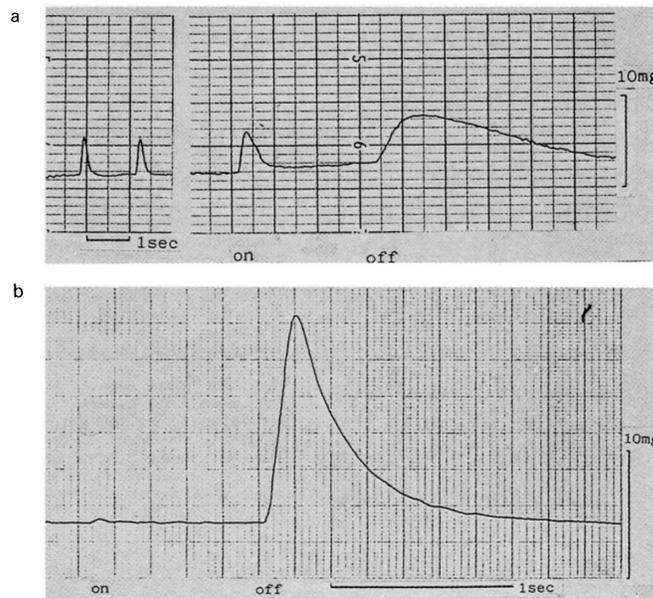


Fig. 34. On- and off-contraction induced in skinned muscle fibres.

a. Skinned fibre of *m. sartorius* from Japanese toad.

Stimulation: square pulse of 5 msec duration and 5 V intensity was used for twitch production, and a square pulse of ca. 1 sec duration and 10 V intensity was used for on- and off-contraction.

b. Skinned fibre of *m. adductor magnus* from bullfrog.

Stimulation: square pulse of ca. 1 sec duration and 30 V intensity.

This tension curve was obtained after repetitive occurrence of twitches, so that tension of twitches was decreased markedly. A very small on-contraction and a large off-contraction are observed. Temperature, 18°C (Natori 1985c).

When a square pulse of long duration (over 0.5 sec) and high intensity (over 20V) was transmitted to a skinned muscle fibre after marked decrease in the tension of the twitch by repetitive occurrences of contraction, marked off-contraction was induced, in spite of a very small on-contraction (Fig. 34b). When repetitive on- and off-contractions were induced by repetitive application of a square pulse of long duration, the tension of on-contraction was decreased markedly and only off-contrac-

tions were observed. When the direction of stimulating current was changed inversely after repetitive off-contractions, the tension of which decreased markedly, the tension of off-contraction recovered again somewhat.

h. Excitability and the effect of certain anions (Natori 1965b, Natori 1972b)

The propagated contraction of a skinned muscle fibre was induced when the threshold voltage, as measured by local twitching, was low (2–3 V) immediately after the preparation of the specimen, but in the case of a specimen having a high threshold voltage from the beginning, the propagated contraction was difficult to observe. Then, with a specimen in which propagated contraction was expected to be induced by electrical stimulation, the relation between the occurrences of propagated and local twitching was examined after various types of treatment.

It is well known that a muscle fibre immersed in Ca-free 120mM KCl solution loses its responsiveness to electrical stimulation. A skinned muscle fibre isolated from a fibre which has already been immersed in 120mM KCl solution also does not respond to electrical stimulation. However, when a skinned muscle fibre with a response to electrical stimulation previously abolished by immersion in Ca-free 120mM KCl solution is placed in a bath of paraffin oil containing dispersed microscopic crystals of NaCl or CaCl₂, the parts in contact with the crystals again begin responding to the repetitive square pulses. The excitability can thus be restored. On the other hand, if a very small amount of CaCl₂ is applied to a portion of the skinned region, local contracture is evoked at that portion, surrounded by a zone where the threshold with regard to responsiveness to electrical stimulation is lower than that before the application of Ca ions. As to the lowering of the threshold, hardly any difference was observed between the effect of NaCl and that of LiCl. CaCl₂ was more effective in lowering the threshold than NaCl, because the former can evoke a larger contracture than the latter (Table 7). In the case of CaCl₂ application, the restoration of excitability begins with a sudden large contraction and then the local twitch occurs in response to the square pulse.

Table 7. Comparison of the thresholds in the portions adjacent to a contracture evoked by applying a minute droplet of salt solution to a skinned fibre.

	EXP. NO.	NaCl	LiCl	KCl	CaCl ₂	MgCl ₂
Threshold at intact portion (Volt)	1	1.8	2.0	2.1	2.1	2.2
	2	2.0	1.8	1.8	1.8	2.0
	3	1.9	2.1	1.8	1.8	1.8
Threshold at portion adjacent to contracture (Volt)	1	1.2	1.4	2.0	1.2	1.8
	2	1.6	1.5	1.7	1.6	1.9
	3	1.4	1.6	1.8	1.3	1.8

The threshold was measured by a single square pulse of 5-msec duration at a temperature of 18°C. (Natori 1965b)

When a small amount of NaCl or CaCl₂ was applied to a skinned muscle fibre, small periodic contractions were often observed together with local twitching upon application of a square pulse. As will be mentioned later, these small periodic contractions propagate very slowly, the velocity of propagation being generally below several $\mu\text{m}/\text{sec}$.

After immersing a bundle of muscle fibres ca. 1 mm in diameter in normal Ringer's solution for 10–20 min at 18°C, a single muscle fibre was isolated and used for obtaining a skinned muscle fibre. The threshold of propagated contraction in this skinned fibre was nearly equal to that in the skinned muscle fibre isolated from a muscle fibre taken directly from a muscle *in situ*. However, the probability of a skinned muscle fibre being unable to respond to electrical stimulation with propagated contraction was higher in the specimen isolated after immersion in normal Ringer's solution. This probability is determined by certain technical difficulties such as removal of solution adhering to the surface of a single muscle fibre, and so on. Therefore, it is very difficult to express this probability quantitatively. However, when the duration of immersion in normal Ringer's solution was longer than 30 min, the threshold of twitching was slightly elevated and, in most cases, the propagated contraction was not observed in skinned fibres isolated from these muscle fibres.

When a skinned fibre was isolated after muscle fibres had been immersed in a solution in which the Na ion of Ringer's solution was replaced by choline ion, in most cases, such a skinned specimen exhibited local twitching for 2–3 min, but was sometimes unable to exhibit propagated contraction. Even when a skinned muscle fibre exhibited propagated contractions, these ceased to be induced after the specimen had been repetitively stimulated between five and to ten times. When muscle fibres were immersed in Ringer's solution containing TTX ($10^{-5}\text{g}/\text{ml}$) for 2–3 min, the same observation as in the case of immersion in choline-Ringer's solution was obtained.

When a single muscle fibre was immersed in 15 mM KCl Ringer's solution for 2–3 min, the skinned specimen was unable to exhibit more than ten propagated con-

Table 8. Maximal occurrences of local twitch of skinned fibre isolated from a single muscle fibre which was immersed in a salt solution for 2–3 min.

	Ringer	15 mM K-R	20 mM K-R
Maximal occurrences of repetitive local contraction	400–700	200–300	100–200
	30 mM K-R	120 mM KCl	choline-R
	50–100	100–200	100–200

A skinned muscle fibre was stimulated once per sec until the contraction became very weak. The figures recorded in this table have been rounded up, since the maximal occurrence of local twitch varied considerably with different specimens under nearly the same conditions. (Natori 1972b)

tractions in response to repetitive stimulations of 1/sec at 8–15°C. The maximal number of occurrences of local twitches evoked by repetitive stimulation was smaller in this kind of specimen than in the normal specimen, as shown in Table 8, but the time course of each local twitch was similar to that in normal fibres.

A skinned muscle fibre isolated from a muscle fibre which had been immersed in 120mM KCl or 96mM K₂SO₄ solution, did not respond to a square pulse of short duration (1–5msec), but did respond to a direct current of long duration (over 0.5 sec) with an off-contraction which was made to relax by making the current flow again just like the off-contraction of a depolarized muscle fibre (Isojima and Natori 1976a).

When very minutely powdered L-carnosine or a tiny amount of 1 M L-carnosine was applied to a portion of a skinned muscle fibre which had been repetitively stimulated by square pulses and in which the twitch tension was markedly decreased, tension increased again and, as shown in Fig. 35, marked potentiation of tension development was observed (unpublished).

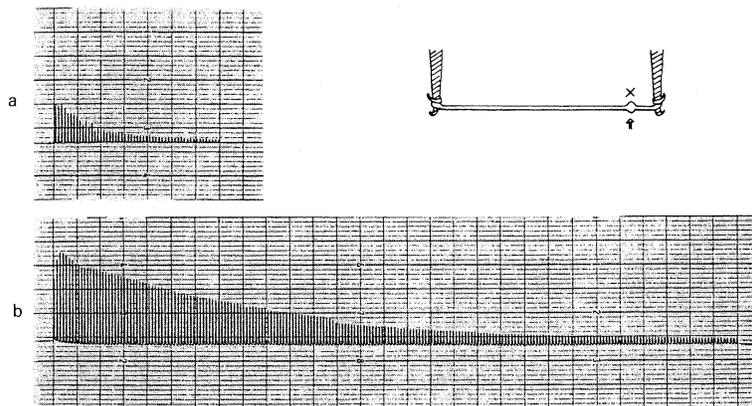


Fig. 35. The effect of L-carnosine upon the tension development of skinned muscle fibres.

Skinned fibre of *m. sartorius* from bullfrog.

Stimulation: square pulse of 10msec duration and 40V intensity.

a. Repetitive twitching as control.

b. Micropowdered carnosine was applied to a portion (x) of the skinned fibre. Small contracture was produced in the treated portion and tension heights increased markedly.

Views on the excitability of skinned muscle fibres

Experimental findings on the responses of skinned muscle fibres to electrical stimulation are briefly summarized as follows.

Skinned fibres in paraffin oil isolated from Japanese toad or bullfrog skeletal muscle responded to a square pulse with a twitch, and frequent stimuli evoked a tetanus.

nus. These tension curves of skinned muscle fibre were nearly the same as those of intact muscle fibre.

The temperature dependency of twitch tension of skinned muscle fibres was also nearly similar to that of intact muscle fibres.

The voltage-duration relation of square pulse stimuli which were capable of evoking local twitching could be expressed by the equation $i = a + b/t$ except in the range of extremely short duration. The frequency-threshold relation using alternating current was nearly linear in the range of 100–7,000Hz, and the relation between the threshold and the half-period of exponentially increasing currents revealed accommodation of the stimulus.

The fact that the electrical excitability of skinned muscle fibres is qualitatively similar to that of intact muscle fibres suggests that within the skinned muscle fibre there is some mechanism which plays a role similar to that of the plasma membrane in a muscle fibre.

On the other hand, when the intensity of a square pulse was increased to a little above the threshold voltage for a local twitch, local contraction was spatially augmented and was abruptly changed into a propagated contraction with a point of origin on the anodic side.

A change in electrical potential similar to an action potential was observed during propagation of contraction, although the time course was much slower than that of an action potential in intact muscle fibres.

The above research brought us to the following interpretation of the process of reception of electrical stimulation.

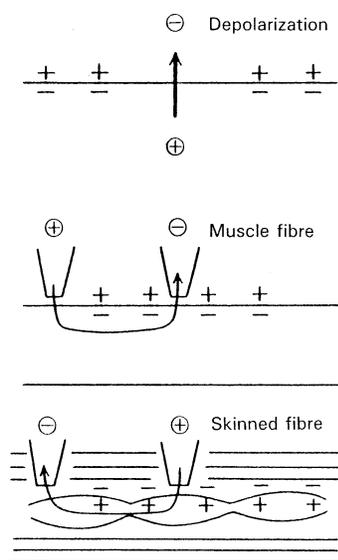


Fig. 36. A schematic illustration of excitation evoked by electrical stimulation in a muscle fibre and a skinned muscle fibre.

As shown in Fig. 36, if there is a tubular or saccate structure made up of excitable membrane and the composition of the solution within this structure is nearly similar to the outer solution of an intact muscle fibre, this would support an explanation that the portion receiving electrical stimulation is on the anodic side of the stimulation electrode, as opposed to the cathodic side in the case of excitation of an intact muscle fibre (Natori 1969).

When a skinned muscle fibre was partially split longitudinally into the shape of a ring of myofibril bundles, a contraction starting at a point on one of the half-circles was propagated to the other half-circle by transverse propagation through the portion connecting them.

If polarizable membranes surrounding each myofibril are connected with each other in the form of a network, there is a possibility that a change at the polarizable membrane would be propagated transversely along this network.

Such a hypothetical network of polarizable membranes would not always be needed if the transverse propagation were due to diffusion of certain chemical substances such as Ca ion, but if the polarizable membranes were situated at the T-tubules, triadic junction and sarcoplasmic reticulum (SR), the existence of a network structure would seem more probable.

When I first found that contraction of a skinned muscle fibre was induced by electrical stimulation, I supposed that there was a certain physico-chemical phase boundary in skinned muscle fibre which played a role in the reception of stimulation, since at that time, I had no knowledge of the electron microscopical structure of the internal membrane system of skeletal muscle, and I arbitrarily named this presumed phase boundary the sarcoplasmic phase boundary. This corresponded to the internal membrane system.

Early in the period of research using skinned muscle fibres, I noticed that those fibres which did not contract on application of salt solution (as will be mentioned later) never responded to an electrical square pulse, while those which lost their responsiveness to electrical stimulation still contracted when salt solution was applied. This observation led me to suggest that in order to be able to respond to electrical stimulation, the skinned muscle fibre must have appropriate physicochemical conditions existing in the sarcoplasmic phase. The easiest way to explain this appropriate condition is to postulate that a phase boundary in which certain species of ions are appropriately distributed is formed at the periphery of the myofibrils. As mentioned above, when a muscle fibre was immersed in 120mM KCl or 96mM K_2SO_4 solution, it became contracted and unresponsive to an electrical square pulse of short duration. The skinned fibre separated from the thus-treated muscle fibre did not respond to electrical stimulation. However, when this inactive skinned muscle fibre was placed into a bath of paraffin oil in which NaCl was dispersed, and repetitively stimulated by an electrical square pulse, it again began responding. Other salts such as $CaCl_2$ had a similar restoring effect upon skinned fibres isolated from muscle fibres treated

with potassium salt solution, and it was noticeable that in most cases of Ca^{++} application the restoration began with a large spontaneous contraction followed by a local twitch occurring in response to electrical stimulation. The restoring effect of NaCl was more durable than that of CaCl_2 and other salts. These results suggested that a certain level of intracellular sodium ions might be essential for the maintenance of the physiological state, particularly with regard to the excitability of skinned muscle fibres (Natori 1965b).

The off-contraction of a skinned muscle fibre, particularly the response of a skinned fibre isolated from depolarized muscle fibre by immersion in isotonic KCl or K_2SO_4 solution, also supports the existence of a polarizable membrane system within the muscle fibre (Isojima and Natori 1976).

As shown in Fig. 35, carnosine prevents the decrement of force due to repetitive occurrence of twitching. Although the mechanism responsible for prevention remains to be elucidated, this result suggests that carnosine in living muscle fibres may possibly play a role in the maintenance of excitability of the internal membrane system.

To summarize, it may be considered that there is a membrane system within the skinned muscle fibre which has properties similar to those of the plasma membrane and that depolarization of this membrane system is coupled with contraction and its repolarization with relaxation, although there is also a difference in properties between the plasma membrane of muscle fibre and its internal membrane.

As mentioned above, the tension developed upon on-contraction increased almost in proportion to the duration of the square pulse in the range of 10–100msec (graded response) and the change in potential during propagation was also much slower than that of the action potential of the muscle fibre. These results indicate that the time constant of the internal membrane in response to electrical stimulation is slower than that of the plasma membrane of the muscle fibre, the on-contraction of a skinned muscle fibre failing to demonstrate the all-or-none response which was observed in the twitch of intact muscle fibre.

It seems likely that this internal membrane may correspond morphologically to the T-tubule, triadic junction and sarcoplasmic reticulum, particularly to the T-tubule. If the T-tubule is the portion which accepts electrical stimulation it may be considered that such a very thin tubular structure may be related to the difference in response between the two membranes.

It still remains unknown whether the membrane structure of triadic junction is freely or selectively permeable to micromolecular ions, or has a synaptic property like an end-plate. If the transmission of an impulse at the triadic junction resembles that at an end-plate, transmission at the former would be expected to be inhibited in a skinned muscle fibre isolated after immersion in choline-Ringer's solution or TTX Ringer's solution, as mentioned above.

At any rate, I am tentatively of the opinion that the propagation of contraction in skinned muscle fibres induced by electrical stimulation involves a change of potential

in the internal membrane, that it is actually found to be a slow potential change as mentioned above, and that, although it is still not clear as to what portion of the internal membrane is involved in the evoked potential change, the T-tubule seems to be the most probable candidate.

When a muscle fibre was immersed in 20 mM KCl-Ringer's solution, the effect of 30 to 60 sec immersion was nearly the same as that of 15mM KCl-Ringer's solution, although the maximal number of occurrences of the local twitches evoked by repetitive stimulations per 1 second was smaller in the case of 20mM solution than in the case of 15mM solution, as shown in Table 8. This may suggest that 20mM K is the upper limit of concentration for the induction of twitching in a skinned muscle fibre. However, if the immersion time was as short as 5–10sec, the twitch produced was similar to the normal one even in the case of 25 mM KCl-Ringer's solution. It was observed that with a skinned muscle fibre isolated immediately after a K-contraction by immersion in 120mM KCl solution, a twitch could be observed in response to a square pulse of 5–10msec duration.

Although it may be considered that the diffusion of K ions through the T-tubule from the external solution takes less than several seconds and that a considerable change in the composition of the solution inside the T-tubule occurs, it is still conceivable that some part of the T-tubule could accept the electrical stimulation owing to negligible change in the chemical composition.

However, the results mentioned above seem insufficient to account for the change in excitement of the internal membrane, particularly with regard to the changes in the T-tubule, triadic junction and sarcoplasmic reticulum.

4. Local contraction and periodic contraction in skinned muscle fibres

a. Local contraction induced by application of certain solutions (Natori 1951, Natori 1954a,c)

When I first tried to make an intact preparation of a myofibril bundle by direct removal of the sarcolemma, one trouble which I encountered was that if the separation was carried out in Ringer's solution or artificial tissue fluids, irreversible contraction of the fibre was inevitably induced. I thought that such a fibre would no longer maintain the normal properties of a living muscle fibre. Therefore, when I found that a myofibril bundle could be isolated in oil without the occurrence of irreversible contraction, I decided to test whether or not this myofibril bundle would contract by application of Ringer's solution or artificial tissue fluids.

When a tiny drop of a solution was applied to a part of the separated myofibril bundle, local contraction occurred at that portion, and when the applied solution diffused out, it appeared that relaxation promptly followed. If the volume of solution was large, irreversible contraction was caused.

At that time, I considered the following process to responsible for this contrac-

tion. It may not be so unreasonable to consider that the applied solution must have disturbed the equilibrium of the physico-chemical state of the sarcoplasm accompanied by alteration of ion distribution. This may lead us to speculate that such alterations in the physicochemical state surrounding the myofibrils could be a direct source of the contraction. If this were true, the cycle of contraction and relaxation could be explained by assuming that the alteration in the equilibrated sarcoplasmic phases induces the contraction of the myofibrils and that the restoration of the initial equilibrated state produces the relaxation.

The reasons why a myofibril bundle without contracture could not be isolated in the solution are now clearly explained. Prof. S. Ebashi and his colleagues¹⁻³⁾ have established that it is partly because of localization of intracellular Ca^{++} in the sarcoplasmic reticulum (SR) and the role of Ca^{++} as a trigger of contraction.

When a tiny amount of a solution containing Ca ions was applied to the middle portion of a skinned muscle fibre, slow propagating contraction was sometimes observed (Fig. 37), but the velocity of propagation was much slower than that of the propagated contraction induced by electrical stimulation, as shown in Table 4 (Natori *et al.* 1976).

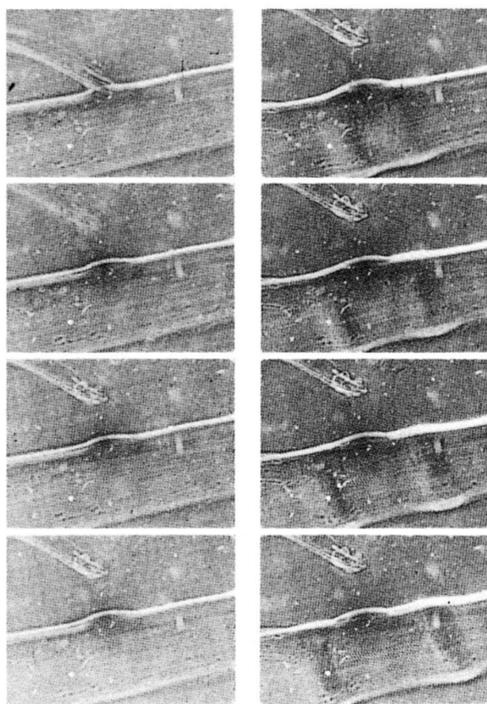


Fig. 37. Propagation in both directions of contraction waves induced by application of a very small amount of CaCl_2 solution. Skinned fibre of *m. sartorius* from Japanese toad. Cine film was taken at 16 frames per sec. Scale bar: 0.1 mm (Natori R. and Natori Rb. 1979).

b. Periodic contraction in skinned muscle fibres (Natori 1955, Natori 1968, Natori et al. 1976, 1979)

In skeletal muscle fibres, propagating bead-like contractions, surge-like contractions (das galvanische Wogen) and small ripple-like contractions are often induced by various causes.

(i) Periodic contraction induced by KCl solution of high concentration (Natori 1955)

On applying a tiny amount of 1–3 M KCl solution to a portion of skinned muscle fibre, contracture of this portion occurred immediately, and local periodic contractions were sometimes induced in its immediate neighbourhood. Periodic contractions, as shown in Table 9, exhibited various period times. These periodic contractions became successively prolonged, and in most cases contraction no longer occurred after being repeated about 10 times. The time taken for periodic contraction was about 0.3–0.6 sec.

Table 9. Conduction velocity and periodic time of repetitive contraction of skinned muscle fibre caused by KCl solution. *M. sartorius* of Japanese toad.

Temp.	0	5	10	20	°C
Conduction velocity	20	60	300	480	μ /sec
	120	160	280	800	
	70	130	500	680	
Periodic time	60	15	11	6	sec
	60	30	12	2	
	20	10	5	3	

(Natori 1955)

When the above periodic contraction was induced, propagation of contraction was sometimes observed. The propagation velocity was generally about $30\mu\text{m}/\text{sec}$, and decreased *en route*, the conduction distance in most cases being under 1 mm.

The area of contraction caused by KCl gradually spread to both sides of the skinned region, and the contracted portion moved in parallel with the occurrence of periodic contraction.

When an agar electrode containing 0.3–1 M KCl or NaCl was placed in contact with part of the skinned muscle fibre and an electrical current transmitted (0.1–0.5 V), periodic contraction occurred a few seconds later and continued for a few minutes. These contractions were generally observed on the anodal side of the applied electrodes, rather than on the cathodal side. The portion of induced periodic contraction gradually moved along the long axis of the skinned muscle fibre.

When a periodic contraction was induced in one of these regions, a small contraction wave frequently occurred. These contraction waves were observed more frequently in comparison with the above periodic contractions induced by application of KCl only (Table 10).

Table 10. Conduction velocity and periodic time of repetitive contractions of skinned muscle fibre caused by galvanic current.

Temp.	0	5	10	20	°C
Conduction velocity	30	210	400	740	μ /sec
	~ 160	~ 320	~ 530	~ 860	
Periodic time	10	8	3	1	sec
	~ 120	~ 15	~ 10	~ 4	
Distance of propagation	100	100	100	100	μ
	~ 600	~ 700	~ 650	~ 800	

KCl (0.3–1M) was applied, followed by electric current.
(Natori 1955)

(ii) Caffeine-induced ripple-like contraction of intra-muscle fibres (IRC) (Natori 1968, Natori 1972b, Isojima and Natori 1966*)

In 1940, it was found in our laboratory that repetitive propagated contractions of muscle fibres could be evoked by the application of Ringer's solution containing quinine (Natori 1951). Such contractions were also evoked by application of caffeine or various solutions such as acids and alkalis. IRC were observed around a contraction induced by locally applying a tiny amount of several-mM caffeine-Ringer's solution to a skinned muscle fibre in paraffin oil.

When an intact single muscle fibre of Japanese toad or bullfrog skeletal muscle was immersed in Ringer's solution containing 1–2mM caffeine, minute rhythmic contractions were induced (Fig. 38).

In these contractions, individual groups each consisting of several sarcomeres were observed to exhibit repetitive contractions at a frequency of several per second independently from each other. The contractions generally exhibited decrement propagation.

In one form of IRC, the distance of propagation was less than 200 μ m, while in another in which furrows were transversely induced in a muscle fibre, the distance of propagation was sometimes more than 600 μ m. The direction of propagation was not only longitudinal but also transverse.

The contraction waves were sometimes induced simultaneously in different portions of the muscle fibre. When these waves collided with each other, the propagation was inhibited in the region of collision and the contraction waves were abolished.

The duration for which caffeine-induced contraction waves continued to be evoked was variable, but in most cases it was 2–3 min. After the contraction waves had been abolished, they could be induced again by certain stimuli, such as stretching the fibre or stirring the solution in which the muscle fibre was immersed.

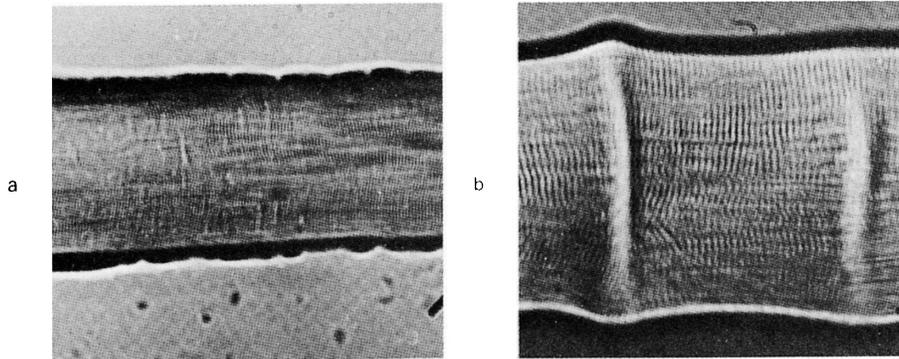


Fig. 38. The periodic minute contractions induced by application of caffeine. *M. sartorius* of Japanese toad. Temperature: 24°C.
 a. Minute contractions of one or two sarcomeres length induced by 1.5 mM caffeine at various portions of the muscle fibre.
 b. Minute contraction waves were induced by 1.5 mM caffeine at various portions of a muscle fibre.
 The propagation distance was short due to decrement of propagation. When the propagation was stopped, the contraction disappeared (Natori *et al.* 1976).

Table 11 shows the propagation velocity, period, wavelength and propagation distance of IRC.

When 120 mM KCl solution containing 1–2 mM caffeine was applied to a muscle fibre which had been isolated after irrigation with 120 mM KCl solution for several hours, contractions were induced which were closely similar to those in muscle fibres immersed in Ringer's solution.

The wavelength, propagation distance, period, *etc.* varied considerably in different specimens, but it seems likely that there was no difference between the normal muscle fibres and depolarized ones. When a depolarized muscle fibre exhibited frequent IRC, they were observed by chance to spread out in a ring-like shape (Fig. 39).



Fig. 39. The ripple-like contractions induced by application of caffeine. *M. sartorius* of Japanese toad. Temperature: 15°C.
 When muscle fibres were immersed in Ringer's solution containing 2 mM caffeine, the contraction waves were propagated from a certain portion in a ring-like shape.
 The cine film was taken in a quasi-dark field produced by adjusting the quantity of light in a stereomicroscope (Natori *et al.* 1976).

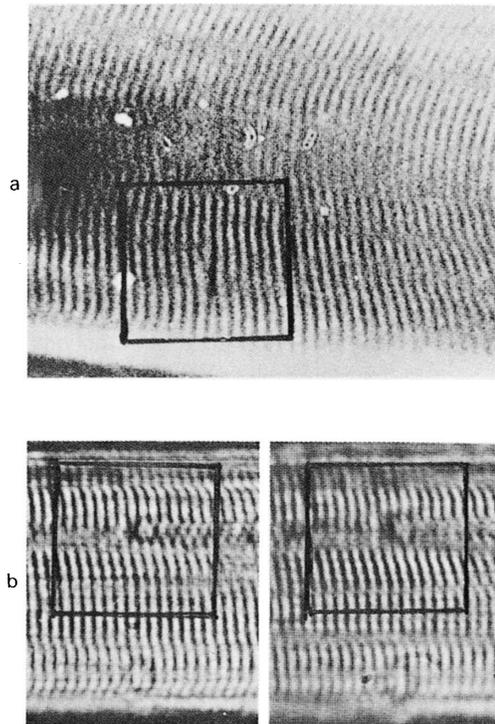


Fig. 40. Minute contractions of one sarcomere length by application of caffeine.

a. *M. sartorius* of Japanese toad. Temperature: 22°C.

Muscle fibres were immersed in Ringer's solution containing 1.5 mM caffeine for a short while and then placed on a glass plate covered with paraffin oil.

A pattern of contraction in which both neighbouring A bands approached each other is seen within the frame.

b. *M. sartorius* of Japanese toad. Temperature: 22°C.

A tiny amount of 10 mM caffeine-Ringer's solution was applied to one end of a skinned fibre in paraffin oil. Ripple-like contractions were evoked at the portion where caffeine was applied. These ripple-like contractions were then propagated. With the lapse of time, minute contractions of one sarcomere length were sometimes observed. Within the frame, both neighbouring A bands are seen to have approached each other (Natori *et al.* 1976).

When normal Ringer's solution or isotonic KCl or K_2SO_4 solution containing 1–1.5 mM caffeine was applied to muscle fibres which were fixed at both their ends at resting length, contractions of one sarcomere were randomly induced here and there in the muscle fibre. This contraction of one sarcomere exhibited a pattern in which the A band on both sides approached the Z-line (Fig. 40), like local activation of striated muscle fibres reported by A.F. Huxley and Taylor (1958)¹²⁾. These contractions were in many cases observed in the range of 1–2 μm , and consequently they were regarded as contractions induced in one myofibril. They often spread in a transverse direction in the muscle fibre and exhibited contractions linear in form (Fig. 41). Contractions of several sarcomeres in the transverse direction of the muscle fibre often exhibited a wrinkle-like form.

Contractions of one sarcomere length were observed when the muscle fibres were previously immersed in isotonic KCl solution for a short time, immediately transferred to a dry glass plate and dragged along its surface to minimize the amount of externally adhering solution, and then bathed in paraffin oil. The amplitude of the contractions induced in various portions of the fibre was increased with increasing caffeine concentration. These contractions were induced in portions previously exhibiting no contracture, and were propagated for 20–30 μm before stopping, thus exhibi-

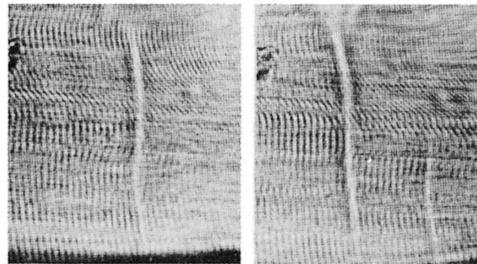


Fig. 41. Linear contractions in skinned muscle fibres.
M. sartorius of Japanese toad. Temperature: 20°C.
 A tiny amount of isotonic K_2SO_4 solution containing 10mM caffeine was applied to one of a skinned portion. The linear contraction was induced in a portion apart from that where caffeine was applied (Natori *et al.* 1976).

ting decrement. The propagation distance of IRC increased with the increasing number of sarcomeres exhibiting simultaneous contractions. In the case of a contraction wave of 10–12 sarcomeres length, it was propagated almost without decrement, unless it collided with another wave. However, caffeine-induced contractions were often observed to have a band-like shape in the transverse direction. Such band-like contraction was a sustained contraction. IRC induced by applying 2mM caffeine to depolarized muscle fibre was inhibited by application of 2–3mM procaine.

Upon immersion in Ringer's solution containing 7.6mM $MnCl_2$ (NaCl 103.3mM, $MnCl_2$ 7.6mM, KCl 2.5mM, $CaCl_2$ 1.8mM, Tris 4.29mM, Amerizol $3 \times 10^{-6}g/l$, pH 7.4, 18°C), caffeine-induced IRC seemed to be slightly inhibited, but the inhibition was too small to be confirmed.

Referring to the effect of caffeine, with skinned fibres isolated from muscle fibres which were immersed in isotonic KCl solution containing caffeine, the rapid cooling contracture (RCC) reported by Sakai (1965)²³ and Sakai and Kurihara (1971)²⁴ was also observed.

IRC induced by application of HCl-quinine was reexamined with KCl solution (140mM KCl, 4mM $MgCl_2$, 2mM EGTA, 10mM Tris-maleate, pH 7.0) containing 1–5mM HCl-quinine. The contractions were found to be similar to the caffeine-induced IRC. IRC was induced with Ca^{++} -free external solution, if the period of immersion was within several minutes.

When a tiny amount of more than 10mM caffeine or HCl-quinine solution was applied to one end of a skinned muscle fibre, it induced contracture at the portion around which periodic contractions were induced. These were gradually propagated to the middle portion of the skinned fibre and IRC was induced.

The process of occurrence of these contractions was similar to that in single muscle fibres (Fig. 40a), in that contractions of one sarcomere length were not propagated

and that the propagation distance was increased with an increase in the number of sarcomeres, as shown in Table 10. With skinned fibres isolated from muscle fibres which had previously been immersed in isotonic KCl solution, these phenomena were found to be similar to those of single muscle fibres described above. Since the speed of propagation, period time and length of the contraction wave varied considerably in different skinned muscle fibres at nearly the same temperature, averaged values are shown in Table 11.

Table 11. IRC in muscle fibres evoked by caffeine.
M. sartorius of Japanese toad.
Temperature, 12–15°C

Speed of propagation ($\mu\text{m}/\text{sec}$)	Periodic time (sec)	Length of contraction wave (μm)	Distance of propagation (μm)
30–150	2–4	25–30	40–64

(Natori *et al.* 1976)

Table 12. IRC in skinned muscle fibres evoked by caffeine.
Temperature, 18–20°C.

Speed of propagation ($\mu\text{m}/\text{sec}$)	Periodic time (sec)	Length of contraction wave (μm)
50–100	1–4	20–30

(Natori *et al.* 1976)

(iii) Galvanic surge-like contraction (GSC) (Natori 1972b, Natori and Ishii 1972d)

The skinned muscle fibre often responded to a direct current of a certain intensity with surge-like contraction waves (GSC). These contraction waves were reported to occur in whole skeletal muscle by Khüne (1860) and Du Bois Reymond (1860), and Hermann (1886) later described the details of this phenomenon under the name of *das galvanische Wogen*⁹⁾.

When a direct current of 0.1–0.2V ($1-3 \times 10^{-5}\text{A}$) was applied to a muscle fibre, on-contraction was evoked and then GSC were induced. The point of origin of the contraction waves was in the vicinity of the anode of the stimulating electrodes and the contractions were propagated toward the cathodic side. The contractions exhibited propagation with decrement and stopped within ca. 1mm. Period time was 0.5–1 sec. If the number of repetitions was large, the contractions were repeated nearly 30 times before stopping, but in most cases the number of repetitions was less than 10.

In the skinned muscle fibre, GSC was similar to that induced in muscle fibres by applying a direct current more than twice as strong as the rheobase of the skinned muscle fibre (4–5 V). When stretched to more than 130% of resting slackened length, GSC was difficult to evoke, but at 110% of resting length, GSC was induced rather markedly. GSC was induced in the interpolar region and the period time was in most cases 0.3–0.5 sec. GSC of skinned muscle fibres was not the generally bead-like type of contraction observed in single muscle fibres, but was in most cases a small contraction with a wavelength of 10–20 μm . The propagation distance was 100–200 μm and the propagation stopped after decrement. When a direct current of over 20 V was transmitted to a skinned muscle fibre which was capable of responding well to a square pulse of short duration with a twitch, a strong on-contraction was induced. This on-contraction continued throughout the period of application of the stimulating current and GSC occurred simultaneously, as shown in Fig. 42. After periodic contractions, a strong off-contraction was induced when the stimulating current was broken. In the skinned muscle fibres prepared from muscle fibres which had previously been immersed in 120 mM KCl or 96 mM K_2SO_4 solution for more than 1 min, GSC was difficult to evoke (Table 13). However, when Ringer's solution containing less than 40 mM K^+ ions was used for immersion, GSC was induced. When the immersion solution contained more than 30 mM K^+ ions, the contraction was not induced at the initiation of current flow, but was induced when it was broken (off-contraction).

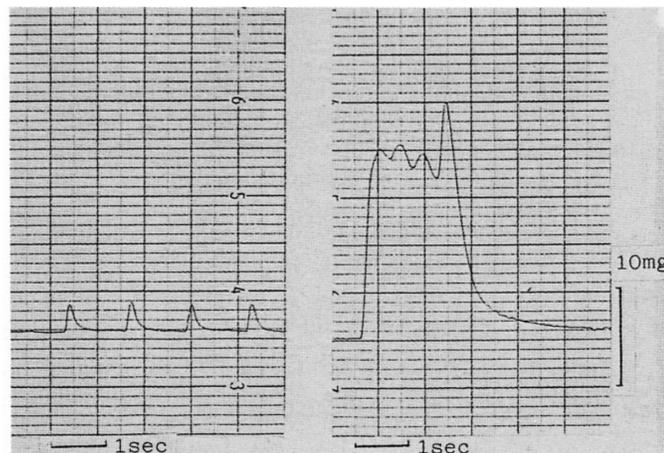


Fig. 42. Galvanic surge and off-contraction.
 Skinned fibre of *m. sartorius* from bullfrog.
 Stimulation: square pulse of 3 msec duration and 20 V intensity was used for twitch, and square pulse of ca. 1.5 sec duration and 30 V intensity was used for on- and off-contraction.
 The curves on left are twitches and the curve on the right demonstrates a large on-contraction occurring simultaneously with small periodic contractions (galvanic surge) and marked off-contraction (Natori 1985c).

Table 13. The relation between the extent of depolarization and GSC in skinned muscle fibres. *M. sartorius* of bullfrog.

K ion concentration in Ringer solution (mM)	Occurrence of local twitch	Occurrence of GSC	Occurrence of break contraction
2.5	+	+	-
10	+	+	-
20	±	+	±
30	-	+	+
40	-	±	+
60	-	-	+
120	-	-	+

(Natori *et al.* 1976)

Table 14. The relation between occurrence of GSC and the ionic composition of the external solution in which muscle fibres were immersed at a temperature +18°C.

Solution	Threshold of GSC (volt)
Ringer	3.8
120mM NaCl	4.1
120mM NaCl + 10mM CaCl ₂	3.3
120mM NaCl + 10mM MnCl ₂	7.8
120mM Choline Cl	5.2
Ringer + 3mM Caffeine	3.4
Ringer + 4 × 10 ⁻⁶ g/ml TTX	4.8

Each value of the threshold of GSC is an average of three measurements.

(Natori *et al.* 1976)

When 10–20mM K-Ringer's solution was used for immersion, the threshold intensity of current for GSC was slightly decreased.

In muscle fibres immersed in Ringer's solution containing 4 × 10⁻⁶ g/ml tetrodotoxin and in skinned fibres isolated from tetrodotoxin-treated muscle fibres, GSC was nearly similar to that in muscle fibres immersed in Ringer's solution. Also, when Na⁺ ions in Ringer's solution were replaced with choline ions, the skinned muscle fibres immersed in it exhibited GSC, although the threshold of direct current was slightly increased. When Ringer's solution containing 7.6mM MnCl₂ was used for immersion and the period of immersion was within 3–5 min, the skinned muscle fibres responded to a square pulse of 5msec duration with local contractions, but not with propagated contraction. When a direct current was applied to this type of skinned muscle fibre, GSC was evoked (Table 14).

When the duration of immersion time was more than 90sec, it became difficult to evoke local contractions by repetitive stimulations (square pulse of 5msec duration).

In the case of responsiveness to repetitive stimulations being lost, this skinned portion of the fibre no longer exhibited GSC. When, however, the sarcolemma of some other portion of the same muscle fibre was peeled and direct current was applied to that portion, GSC was again observed in that portion.

A summarized view of local contraction induced by application of some salt solutions and periodic contractions induced by application of certain solutions and electrical current

When salt solution was applied to a skinned muscle fibre, a local contraction was induced, and this contraction was also induced by distilled water.

Two possible processes can be envisaged for the cause of this contraction, in accordance with the studies on Ca^{++} action reported by Ebashi and others^{1-6,8,17,21}.

One of these is the direct action of Ca^{++} on the contractile protein of the sarcomere, and the other is indirect action through the internal membrane mentioned above, that is, the applied solution causes an immediate change in the distribution of ions in the internal membrane and then Ca^{++} released from the sarcoplasmic reticulum (SR) evokes a contraction of the sarcomere. Alternatively, Ca^{++} of the salt solution evokes Ca^{++} release from the SR and then the contraction process is activated by the mechanism of Ca-induced Ca release discovered by M. Endo (1970)⁹.

In any event, the fundamental mechanism of activation of local contraction induced in skinned muscle fibres could be explained in terms of Ca^{++} action. As mentioned above, the velocity of propagation of the contraction wave evoked by an electrical square pulse in skinned muscle fibres is larger than those of galvanic surge-like contraction (GSC), KCl-induced periodic contraction and caffeine-induced propagated contraction (IRC), and the minimal width of contraction necessary for propagation is at least 40–50 μm in the former, but 10–20 μm in the latter three. This suggests a difference in the mechanism of propagation between the former and the latter.

The propagation of contractions evoked by a square pulse seems to be concerned with an impulse evoked mainly by electrical change in the internal membrane. As to GSC, a dominant factor of propagation may be a depolarization of the terminal cisterna or triadic junction rather than that of the T-tubule. Since the GSC was evoked in a depolarized state as shown in Table 13, a humoral form of conduction is more probable than an electrical one. Although the substance responsible for such humoral conduction has not yet been confirmed, Ca^{++} may play such a role, since Endo reported a stimulating effect of Ca^{++} on the SR. The propagation is especially likely to be a humoral one in rhythmic caffeine contraction, since the contraction was evoked in a depolarized skinned muscle fibre.

When the existence of two possible mechanisms of contraction propagation in skinned muscle fibres is taken into consideration, that is, an electrical mechanism

and a chemical one, together with the fact that the significance of each of them would be different in the various kinds of contractions mentioned above, a number of suggestions could be made with respect to the following question: What is the nature of the intracellular conduction of the impulse triggered by the action potential of twitch or tetanus? and how is the impulse coupled with the release of Ca^{++} from the SR?

5. Properties of various fibres prepared by stepwise treatment of muscle fibres

a. Properties of skinned muscle fibres separated in salt solution (Natori and Sakai 1957b)

The sarcolemma of an intact muscle fibre was peeled in Ringer's solution instead of in oil. Although contracture occurred as soon as the sarcolemma was injured, scattered myofibrils were obtained when fixed isometrically and left for 30 minutes. This specimen was placed in oil and the fluid around the skinned fibre was removed. Hardly any contraction occurred even when a small amount of salt solution was added. However, when 0.5M CaCl_2 was added, a strong contraction was observed.

By application of a large amount of 0.12M KCl solution to the skinned muscle fibre contracted by CaCl_2 relaxation again occurred. Its length became shortest in a solution of pH 5.5 and it readily contracted with ATP.

The elasticity was smaller than in skinned muscle fibres separated in oil, and the linear expansion coefficient became positive within the limits of 10–20°C, the absolute value of the expansion coefficient being about 10^{-4} .

Although contraction occurred at temperatures over 50°C, the maximum contraction rate was 30% which was far smaller than the 70–80% for skinned muscle fibres separated in oil.

These facts indicate that skinned muscle fibres separated in Ringer's solution and in 120mM KCl solution have properties similar to those of glycerinated muscle fibres.

When a single muscle fibre was immersed isometrically in 0.12–0.15M KCl solution for 36–48 hours, the viscoelasticity and contractility tested by applying salt solution to the skinned fibre isolated from the above-treated muscle fibre lay between those for skinned fibres separated in paraffin oil and those for skinned fibres separated in Ringer's solution. When skinned muscle fibres separated in Ringer's solution and in 0.12–0.15M KCl solution were washed with distilled water, and then left at 0–4°C for more than 24 hours, they became similar to actomyosin thread. If the pH was returned to 5.5 from 9.0 and the preparation again left at 0–4°C for over 24 hours, fibres simulating extracted myosin-B were obtained.

b. Short-term glycerol-treated muscle

From the experiments mentioned above, it became clear that there was a possibility of preparing fibres similar to actomyosin thread chemically extracted by separating

the myofibrils from the living muscle fibre without changing their properties.

An experiment was carried out with the object of investigating the position occupied by glycerinated muscle in the preparation steps mentioned above. Bundles of muscle fibres fixed at an isometric length were immersed in 50% glycerol at 0°C for 1,5,10,20,30 minutes and 1,2,5,10,24 and 50 hours, respectively. They were then taken out and their properties examined.

Immersion in 50% glycerol caused a 30–50% reduction in the weight of the muscle fibres, reaching a minimum after 1 hour. Thereafter, the fibres gradually returned to their former weight. The loss of fibre weight was chiefly caused by dehydration (Fig. 43).

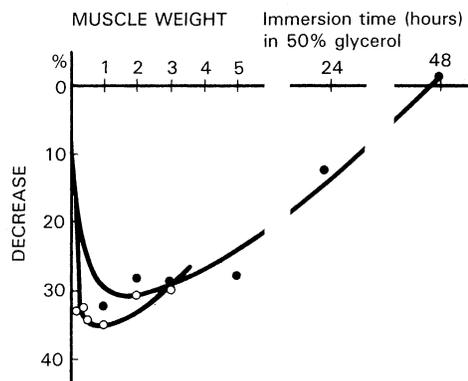


Fig. 43. Change in muscle weight during immersion in 50% glycerol (Natori and Sakai 1957).

When a short-term glycerol-treated muscle was immersed in 0.15M KCl solution, a marked contraction occurred indicating a contraction rate $((L_0 - L)/L_0 \times 100\%)$ of 70–80%, accompanied by a tension of about 100 g/cm² (Fig. 44). Such a contraction became most conspicuous in those fibres immersed in 50% glycerol for 2 hours. The contraction was weak when fibres were immersed for a short or a long period, and especially in the case of over 10 hours, it was weak when only 0.15M KCl solution was used. In one such specimen, ATP-contraction occurred in the presence of MgCl₂. As to the concentration of KCl, the contraction and tension development occurring in 0.15M KCl solution showed maximum values, as shown in Fig. 45. With immersion for more than 20 minutes, the contraction caused by KCl was not so remarkable and relaxation occurred.

c. Reversibility of glycerinated muscle

Glycerinated muscle fibres immersed for only a short period have similar properties to dried muscle fibres, as will be mentioned later. Therefore, if distilled water is slowly supplied, there is a possibility that the properties of the short-term glycerol-treated muscle will reverse back to those of the intact muscle fibre.

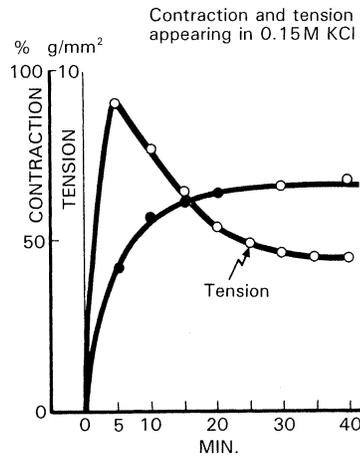


Fig. 44. Contraction rate and tension development of glycerinated muscle immersed in 0.15M KCl solution. Horizontal axis is immersion time in 50% glycerol (Natori and Sakai 1957).

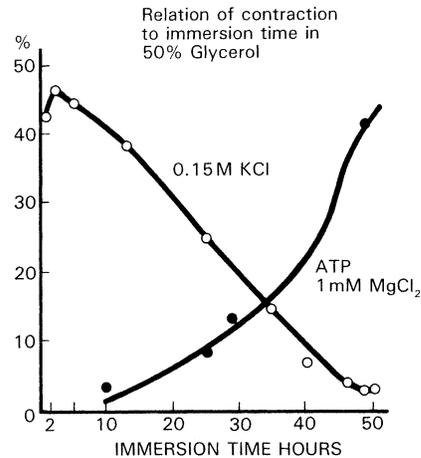


Fig. 45. Relation of contraction rate to immersion time (hours) in 50% glycerol (Natori and Sakai 1957).

Specimens fixed in an isometric condition in 50% glycerol at 0°C were taken out intervals, immersed at once in Ringer's solution at the same temperature and the properties of their single muscle fibres were examined 10–20 minutes later.

When a muscle fibre was immersed in glycerol the properties of the skinned fibre were almost completely reversed compared with those of the intact muscle fibre within 10 minutes. Although skinned muscle fibres indicated a rather poor reversion when the muscle fibre was immersed for over 30 minutes, a twitch (non-propagated) and periodic contraction could sometimes be induced by application of KCl if immersed for no more than 1 hour. After 2 hours, twitch and periodic contraction could no longer be fully induced. However, this transition was gradual and not rapid.

d. Contraction of dried muscle fibres and skinned fibres

When a muscle fibre was dried in air and then immersed in distilled water or salt solution, a marked contraction occurred immediately, the contraction rate reaching a value of 80% ($(L_0 - L)/L_0 \times 100\%$) of the slack length of the muscle fibre (Table 15). Since glycerinated muscle has a certain degree of dehydration, a contraction occurs as in the dry muscle when it is immersed in distilled water or salt solution. In the above-mentioned glycerinated muscle within 10 minutes of treatment, the contraction rate was more or less identical to that of the intact muscle following application of Ringer's solution after drying, as shown in Table 16. Both reversibilities became poor in accordance with the period of immersion in glycerol.

Table 15. The degree of shortening $((L_0-L)/L_0 \times 100\%)$

Living muscle fibre	Short-term glycerinated muscle fibre
78	75
80	78
76	78
79	78
76	76
78	77

(Natori and Sakai 1957)

Table 16. Tension-length relation of skinned muscle fibres separated in Ringer's solution.

Tension (mg)	0	15	30	45	60
d (μ)*	Percentage of elongation				
30	0	17	30	43	52
25	0	21	35	47	56
27	0	15	33	50	60
35	0	18	32	43	55
25	0	20	36	45	56

d*: diameter of the skinned muscle fibre.

(Natori and Sakai 1957)

Summary of stepwise study of glycerinated fibres and skinned muscle fibres

From the above results, if muscle fibres which have been immersed in glycerol for certain periods, ranging from short to long, are placed in stepwise order between living muscle and contractile protein thread (Fig. 1), the properties of fibres showing a gradual step-by-step change may be obtained in accordance with the time of treatment in glycerol. There is at least a possibility that a reversible process will take place in those specimens treated for a short period. In other words, in reviewing the above experiments, we observed the possibility of subdividing the living muscle fibre into various elements and then gradually reconstructing these and reverting their properties back to those of living muscle.

6. Various experiments using dehydrated muscle fibres and skinned fibres

a. Contraction of dehydrated muscle fibres and skinned fibres by application of distilled water (Natori R. and Natori Rb. 1982, Natori 1984)

When a skeletal muscle fibre is immersed in distilled water after drying in air, a strong contraction reaching 20% of the resting length of the muscle fibre develops (Fig. 46) (Natori 1956, Natori and Shibuya 1954). A similar contraction develops when

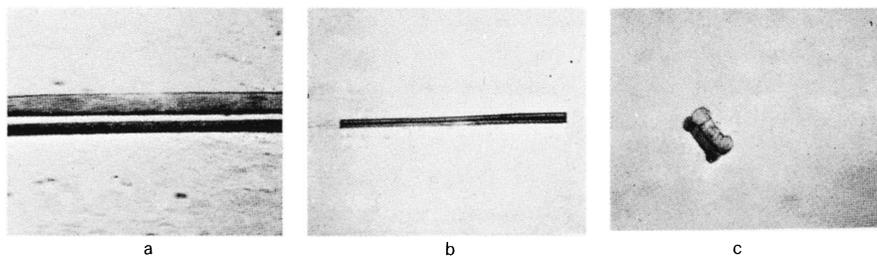


Fig. 46. Contraction of dried muscle fibres induced by immersion in distilled water.
 a. Living muscle fibres (a large bright fibre and a small dark fibre).
 b. Dried muscle fibres (the fibres are cut at appropriate length).
 c. The fibres after addition of distilled water. Severe contraction is observed.
 M. sartorius of Japanese toad (Natori 1956).

a muscle fibre is dehydrated in glycerol and then placed in distilled water.

A single muscle fibre from skeletal muscle, *e.g.* the *m. sartorius* of a bull-frog, was isolated, immersed in paraffin oil and fastened to a tension recorder. When this dehydrated fibre was placed in a relaxing solution (120mM KCl, 4mM MgCl₂, 4mM EGTA, 4mM ATP, 10mM MOPS, pH 7.0) a transient force developed and complete relaxation occurred in about 10 seconds.

A marked force developed when the dehydrated fibre was immersed in distilled water (Fig. 47), and the tension gradually dropped after reaching its peak. This tension, however, did not completely disappear when the fibre was left as it was. It was then placed in a relaxing solution, resulting in immediate relaxation, and the tension curve returned to its baseline.

As shown in Fig. 47, when a skinned muscle fibre in paraffin oil was immersed in turn in relaxing solution, distilled water, and relaxing solution, hardly any transient force was observed and the rise of tension which developed due to immersion in distilled water was slightly slower than that of a dehydrated skinned fibre (Fig. 47b). However, the tension curve of the dehydrated skinned fibre was similar to that of the dehydrated fibre (Fig. 47c).

When a dehydrated fibre was immersed in relaxing solution to which CaCl₂ had been added, the force developed more strongly than it did when the fibre was immersed in distilled water alone (Fig. 47d).

When the fibre was placed in relaxing solution at the time when tension of the dehydrated fibre started to develop in distilled water, relaxation occurred immediately. A force, however, again developed when the fibre was returned to a chamber containing distilled water (Fig. 48a). As indicated in Fig. 48b, relaxation occurred whenever the fibre was immersed in a relaxing solution. On the other hand, a force developed at any stage of relaxation upon immersion in distilled water. Tension recovery by treatment with distilled water during the falling phase was weaker in the early phase of relaxation than it was in the end phase of relaxation.

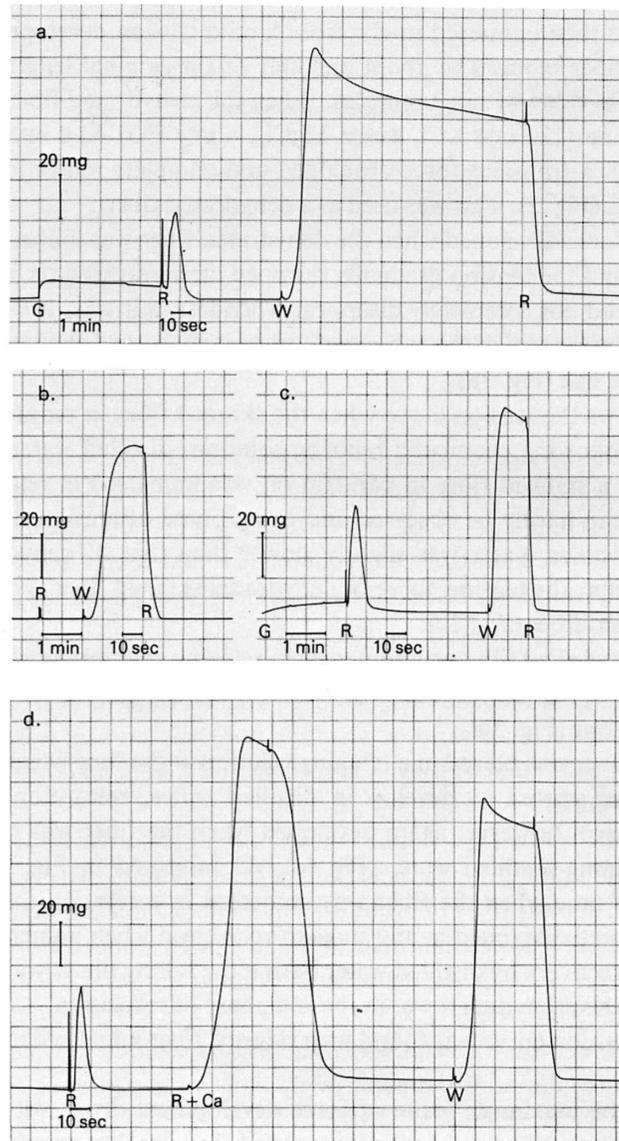


Fig. 47. Time course of tension development in dehydrated and skinned muscle fibres which were transferred from a relaxing solution to distilled water.

a. Dehydrated muscle fibre ($s \approx 80 \times 10^{-6} \text{cm}^2$, $l \approx 5 \text{mm}$) 18°C .

b. Skinned muscle fibre ($s \approx 80 \times 10^{-6} \text{cm}^2$, $l \approx 1.5 \text{mm}$).

c. Skinned muscle fibre ($s \approx 60 \times 10^{-6} \text{cm}^2$, $l \approx 3.2 \text{mm}$).

d. Dehydrated muscle fibre ($s \approx 80 \times 10^{-6} \text{cm}^2$, $l \approx 1.7 \text{mm}$).

W: Immersion in distilled water, R: Immersion in relaxing solution, R-Ca: Immersion in relaxing solution containing 3mM CaCl_2 . M. sartorius of bull-frog (Natori R. and Natori Rb. 1982).

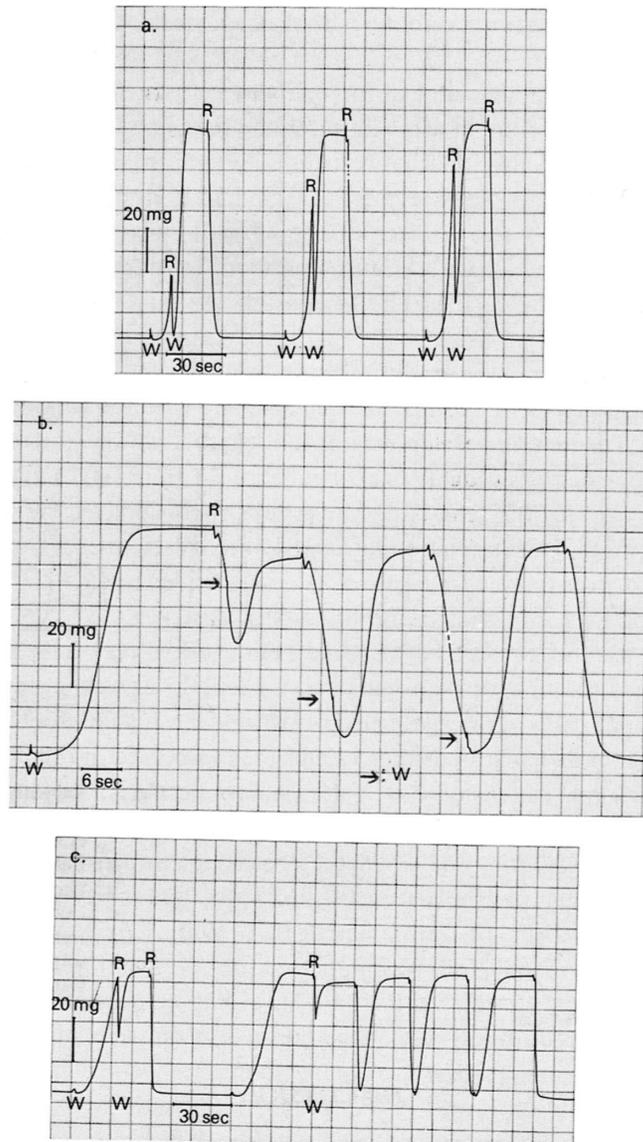


Fig. 48. Tension development in dehydrated and skinned muscle fibres by distilled water and tension diminution by relaxing solution.

a. Relaxing solution was applied to a dehydrated fibre during the rising phase of tension and distilled water was then applied immediately. Temperature: 20°C.

b. Distilled water was applied during the falling phase of tension and the fibre was then replaced in relaxing solution. Temperature: 20°C.

c. Similar recording of tension curve with a skinned fibre of m. sartorius. Temperature: 20°C (Natori R. and Natori Rb. 1982).

A similar tension curve was found with skinned muscle fibre immersed in relaxing solution (Fig. 48c).

Although the maximum value of force development dropped with repetitive immersion of dehydrated fibre in distilled water and then in relaxing solution, the rate of drop was not remarkable. When a relaxing solution containing CaCl_2 was applied to a dehydrated fibre in which repetitive tension had been developed by immersion in distilled water, a very strong force still developed. However, after immersion in the solution containing 2–4mM CaCl_2 followed by immersion in the relaxing solution, there was a gradual drop and the tension curve did not return to its original baseline.

The dehydrated muscle fibre in relaxing solution was stretched or relaxed by changing the distance between the two fasteners and then the fibre was replaced in distilled water. The tension development of the dehydrated fibre as a result of immersion in distilled water was roughly proportional to the fibre length in the range from resting slack length to 50% length, and inversely proportional to the stretching rate, although the length-tension relation differed somewhat from case to case; when the dehydrated fibre was relaxed to less than 50% or extended to over 200% of the original resting length *in situ*, some tension was still observed.

When the distance between both fasteners of the dehydrated fibre, which isometrically maintained the fibre at its original resting length, was changed suddenly to a relaxed length (90–50% of the resting length) during tension development in distilled water, the tension dropped immediately and then increased gradually, but did not reach the peak value of tension which should have been induced in a relaxed fibre of that length (Fig. 49a).

When CaCl_2 solution was applied, tension developed more strongly after sudden relaxation than was the case in distilled water, and re-development of tension was observed below 30% of resting length (Fig. 49b).

When the dehydrated fibre was immersed in ATP solution (4mM ATP, 4mM MgCl_2 , 120mM KCl, 10mM MOPS, pH 7), a marked tension followed by slow relaxation was induced. In this case, relaxation was incomplete, but the tension curve returned following immersion in a relaxing solution.

When ATP solution (4mM ATP, 120mM KCl, 4mM MgCl_2 , 10mM MOPS, pH 7) was applied to a contracting dehydrated fibre which was immersed in distilled water, relaxation occurred immediately at any stage of tension development. However, the tension curve did not return to its original baseline after repetitive application of ATP, and tension re-developed markedly after incomplete relaxation.

b. Slow contraction of skinned muscle fibres and dehydrated muscle fibres (Natori et al. 1985a,b)

A single muscle fibre was dehydrated in pure glycerol, and tension development by subsequent immersion in one of several solutions was recorded.

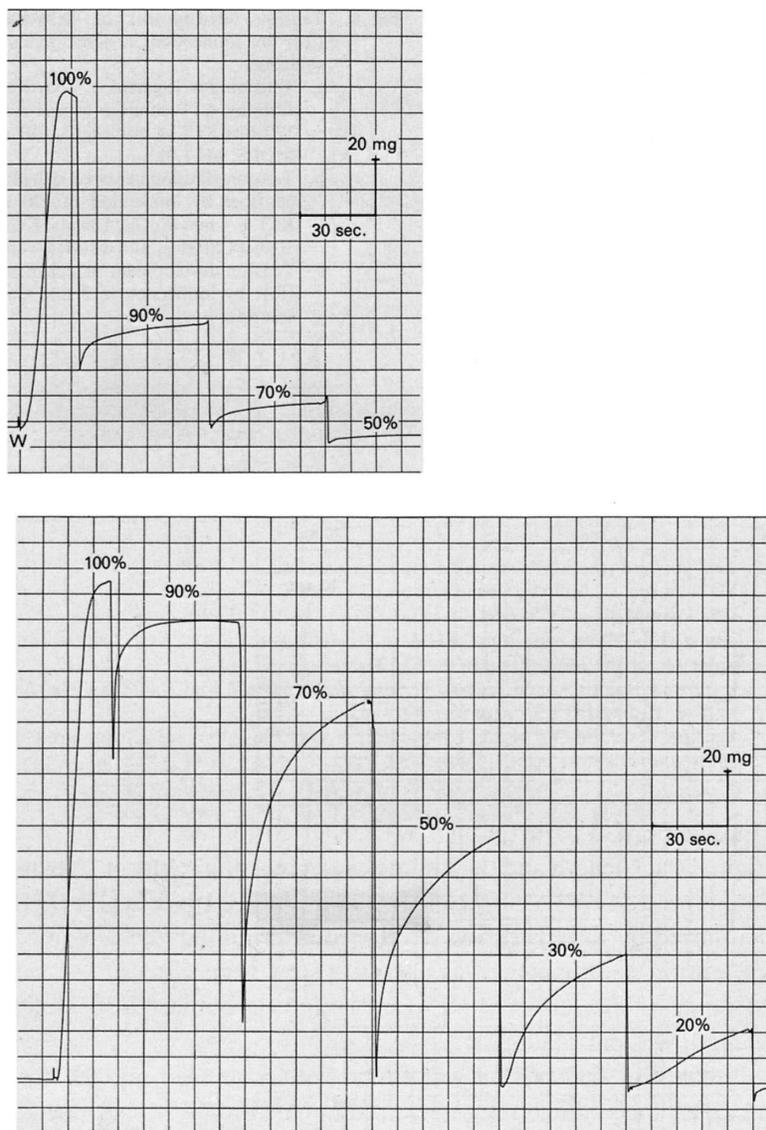


Fig. 49. Tension development after sudden change of fibre length.

Dehydrated fibre of *m. sartorius* from bullfrog.

The distance between the fasteners of the dehydrated fibre was changed suddenly to a certain length (90–20% of original length of fibre) during tension development by immersion in distilled water or 4mM CaCl_2 solution.

a. Tension development by distilled water. Re-development of tension is observed after sudden relaxation of fibre length, but this is not remarkable.

b. Tension development by 4mM CaCl_2 solution.

Tension develops markedly after sudden relaxation and shows further tension development under 30% of the original length of the fibre (Natori Rb. and Natori R. 1983).

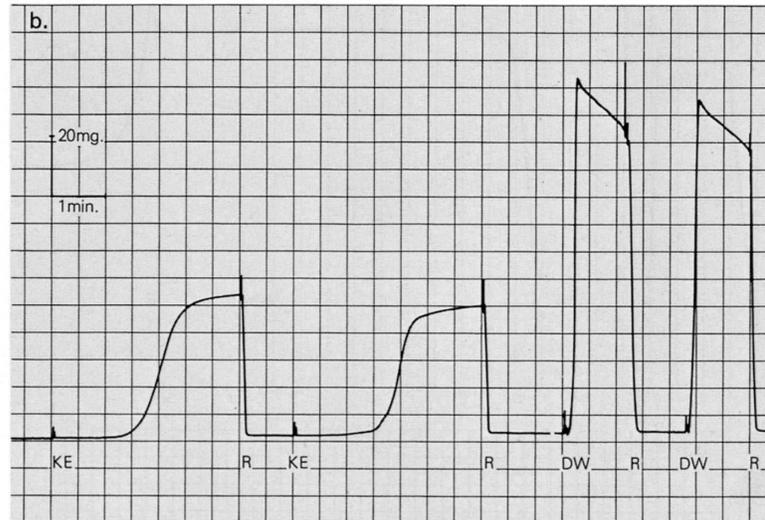


Fig. 50. The slow contraction of skinned muscle fibres induced by application of KE. *M. sartorius* of bullfrog. Temperature, 24–25°C.
 KE: 120mM KCl, 1mM MgCl₂, 4mM EGTA, 10mM PIPES buffer. The pH of KE was adjusted to 7.0 by addition of KOH and the concentration of KCl changed somewhat in order to set the ionic strength at 0.15M.
 R (relaxing solution of *M. Endo*): 77.96mM KCl, 5.2mM MgCl₂, 4.3mM Na₂ ATP, 4mM EGTA, 10mM PIPES buffer.
 Tension development of KE-contraction is very slow and weak compared with DW-contraction (Natori *et al.* 1985a).

A muscle fibre in paraffin oil was transferred to pure glycerol for 2–3 minutes at its original slack length and then immersed in relaxing solution. When this treated fibre was immersed in KE (113mM KCl, 1mM MgCl₂, 4mM EGTA, 10mM PIPES, pH 7.0 adjusted by KOH) tension developed after a long latent period. The rising phase of tension development was also very slow (Fig. 50).

The grade of shortening of KE-contraction was about 40–50% of the original resting length without loading.

A similar slow contraction was found with a skinned muscle fibre. The sarcolemma of a single muscle fibre immersed in paraffin oil was stripped for a length of 1–1.5cm and both ends of the skinned part were fastened to a transducer. As shown in Fig. 50, when a skinned fibre in paraffin oil was transferred to KE-solution, tension developed slowly as in the case of the dehydrated fibre.

The tension produced by application of KE dropped immediately following immersion in relaxing solution, but when dehydrated or skinned muscle fibres were immersed in KE for a period of hours, the peak-tension was maintained without any clear decrease and when the fibres were transferred to a relaxing solution the tension-curve did not return to its original baseline, that is, a marked residual tension was observed.

When a dehydrated fibre was repetitively immersed in KE and relaxing solution, the latency of tension development became more and more shortened, particularly as a result of reduction of the immersion time in relaxing solution.

Both the latency and increasing phase of tension development corresponded to the temperature of KE, but the time course of the tension drop caused by immersion in relaxing solution indicated a slight dependency on the temperature of the solution (Fig. 51).

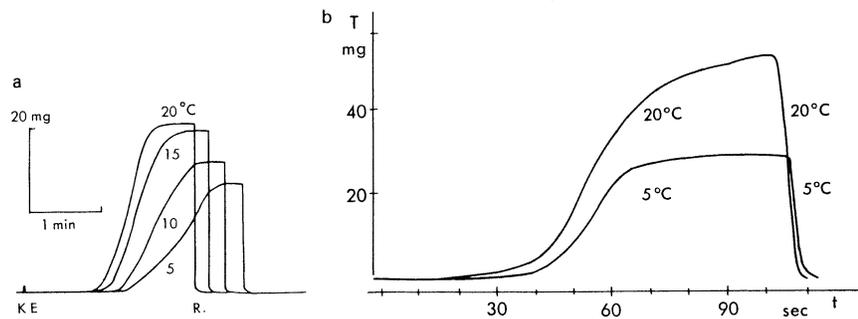


Fig. 51. Temperature dependency of time course of KE-contraction.

a. The relation between temperature of KE and tension development.

In this case, the temperature of R was 20°C. The latency is longer and peak tension is smaller at lower temperature than at higher temperature.

b. A somewhat rapid recording of the tension curve.

Hardly any difference in speed of relaxation is observed with application of R of 5° and 20°C (Natori *et al.* 1985a).

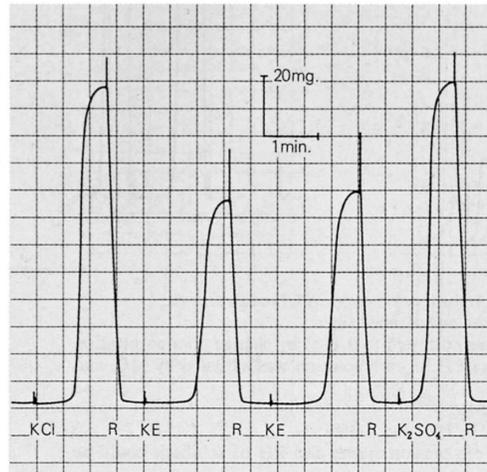


Fig. 52. Comparison of time courses of tension development by application of KE, KCl and K₂SO₄.

KE: pH 7.0, KCl: 120mM, pH 6.6 and K₂SO₄: 96mM, pH 6.5 (Natori *et al.* 1985a).

A comparison was made of time courses of tension development between KE-contraction and the contractions induced by other solutions. KE contraction was the slowest and weakest of these (Fig. 52). The order of speed of contraction and peak value of tension was $KE < KCl < K_2SO_4 < DW$ (distilled water). A very slow contraction was occasionally observed in the tension development induced by immersion in isotonic KCl solution without EGTA, but the latency was shorter and the rising phase of tension development was slightly more rapid than those of KE-contraction with the same sample.

The time course of tension development by immersion in KE depended on the pH of the solution for the same dehydrated and skinned muscle fibres, is shown in Fig. 53.

The speed and peak value of tension development increased nearly in proportion to the pH of the solution.

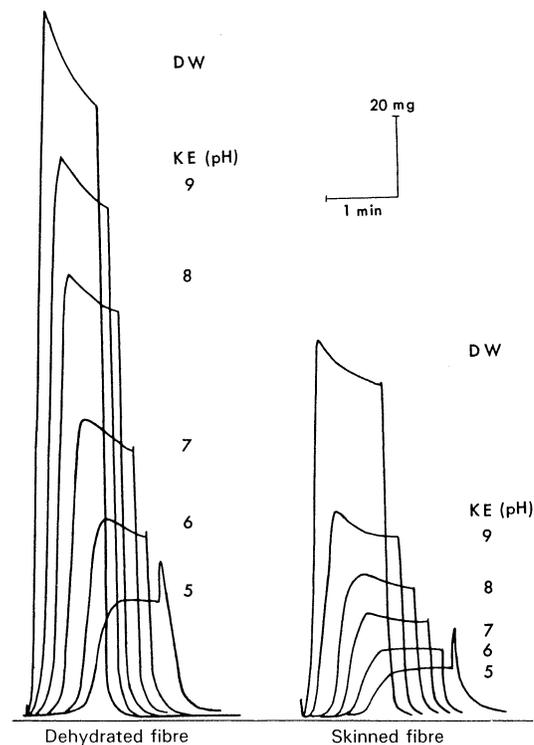


Fig. 53. The influence of pH change upon the tension curve of KE-contraction. KE-contraction of dehydrated and skinned muscle fibres. Ionic strength of each KE was adjusted to 0.15M by change of concentration of KCl (99–124mM), and the pH of each solution was adjusted by HCl and KOH, respectively (Natori *et al.* 1985a).

When a dehydrated or skinned muscle fibre was immersed in KE of pH 5 and then transferred to a container of relaxing solution at pH 7, a marked transient force was observed just before relaxation (Fig. 53), but in the case of contractions produced by application of pH 6–9 these transient forces were not marked.

In the range from the original slack length to 130% the length of the muscle fibre, the latency decreased nearly in inverse proportion and the peak value of tension development increased in proportion to fibre length. The peak tension decreased with the increase of fibre length beyond 130% of its resting length, but the decrease of latency corresponded to fibre length (Fig. 54).

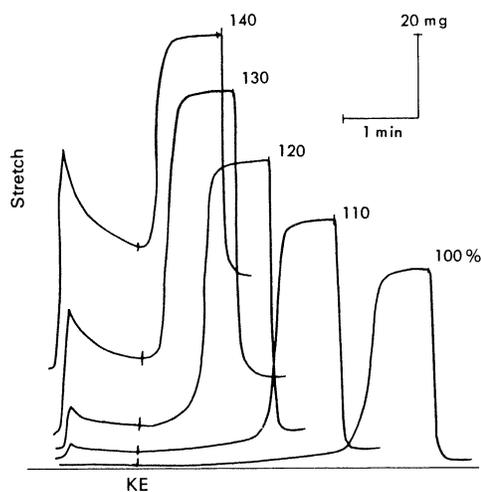


Fig. 54. The influence of stretching of a fibre upon tension curve of KE-contraction. 100% length of fibre is the slack length which is estimated to be the length of the muscle fibre *in situ*. Since the tension curve after stretching did not terminate for less than several minutes, KE was applied to the fibre after 1 minute of stretching (Natori *et al.* 1985b).

We obtained a case of extremely slow tension development in the KE-contraction of skinned muscle fibres. In the case shown in Fig. 55a, a very slight increase of tension was observed for 90 seconds after application of KE solution. A slow and then rapid increase of tension followed a slight increase of tension. The rapid increase of tension then changed to a gradual development of tension and approached a peak of tension. By application of relaxing solution, this tension dropped promptly.

When a skinned muscle fibre in relaxing solution was transferred to an MgE solution (30mM MgCl₂, 25mM KCl, 4mM EGTA, 10mM PIPES, pH 7), a slower development of tension than that in KE-contraction occurred (Fig. 55b).

As shown in Fig. 55c, a clear stepwise increase of tension was often observed in KE- and MgE-contraction.

During the mechanical latent period of the above slow contractions, a flicker in the light intensity of the diffraction line produced by a He-Ne laser beam was detected before tension development (Natori *et al.* 1985b).

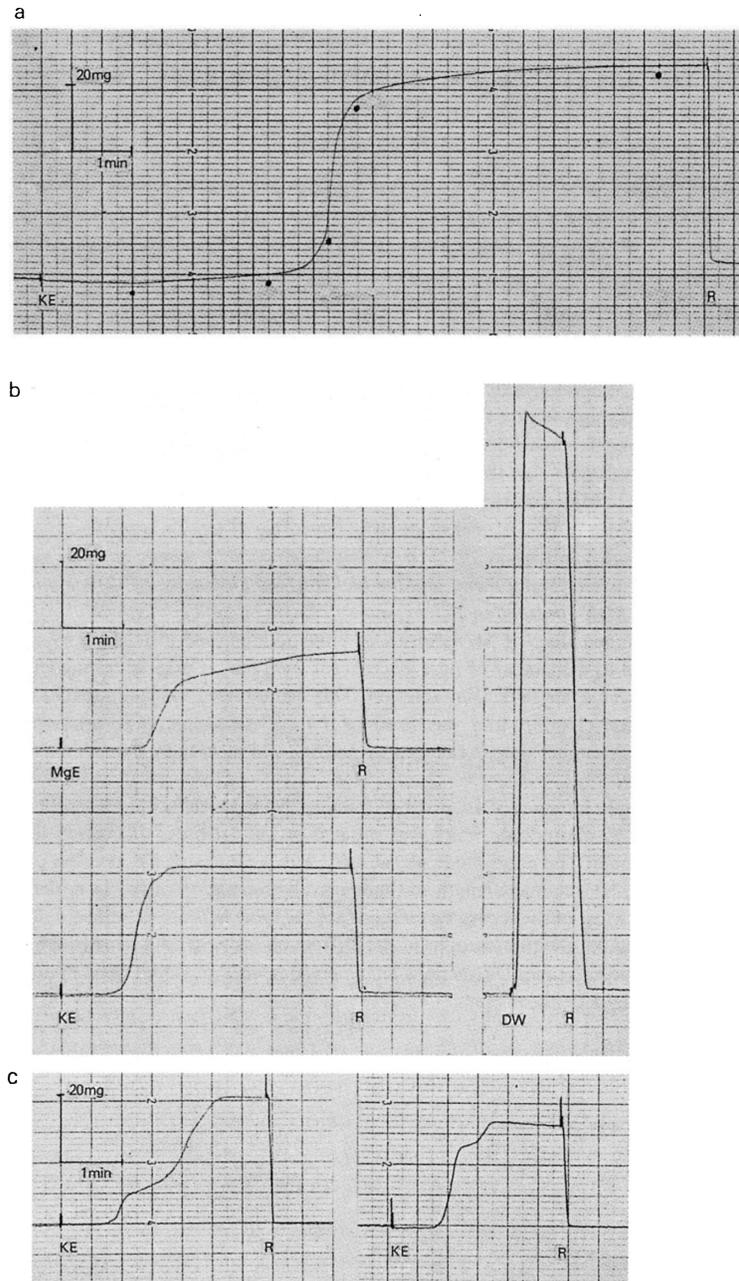


Fig. 55. An extremely slow tension development which was observed in KE-contraction. A skinned fibre of *m. sartorius* from bullfrog (length, ca. 5mm and fibre diameter, ca. $75\mu\text{m}$) was transferred from a chamber containing paraffin oil to another chamber containing KE solution. Temperature, 25°C (Natori *et al.* 1985b).

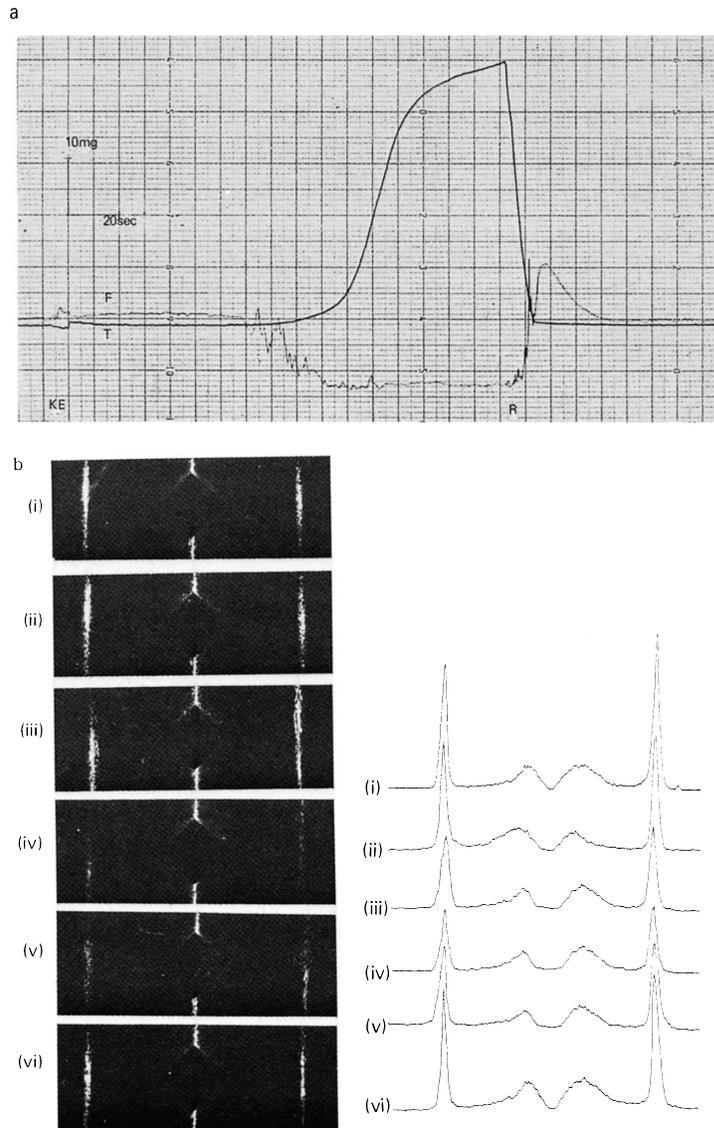


Fig. 56. Tension development and 1st-order diffraction line in KE-contraction.

a. Tension development and light intensity of 1st-order diffraction line. Skinned fibre of *m. sartorius* from bullfrog. Temperature, 20°C.

Diffraction lines are observed during mechanical latency, the rising phase of tension, and after the drop of tension, respectively.

T: tension curve, F: flicker curve of 1st-order diffraction line.

b. Diffraction patterns of a skinned muscle fibre (sarcomere length, 2.5 μm) and densitometry of photographic negatives of diffraction patterns. Temperature, 20°C.

Diffraction line (i) at resting state, (ii) at the beginning of tension development, (iii) at rising phase of tension development, (iv) at the peak of tension, (v) during drop of tension, (vi) at resting state.

Position of the 1st-order diffraction line hardly changed throughout the period of contraction, but the beam scattered slightly during isometrical tension development (Natori *et al.* 1985b).

As shown in Fig. 56, a minute change in the light intensity of the 1st-order diffraction line was evoked during mechanical latency and the flicker in the light intensity started before the development of tension. A large flicker in the intensity was observed at the beginning and in the subsequent rapidly increasing phase of tension development. The diffraction intensity decreased during peak tension. During and after the drop in tension evoked by application of relaxing solution, a flicker in the diffraction intensity was also observed. The intensity then recovered after relaxation.

The total intensity of the 1st-order diffraction line started to decrease during mechanical latency. At the beginning and in the rapidly increasing phase of tension development, the total intensities decreased further and the half-width of the 1st-order diffraction lines became broad. The half-width of the diffraction line at peak tension was about twice that in the relaxed condition. The diffraction pattern returned to its original form after the drop in tension produced by application of relaxing solution. The position of the diffraction lines hardly changed during tension development.

When the sarcomere length was stretched from $2.5\ \mu\text{m}$ to $3.4\ \mu\text{m}$, tension decreased and the duration of latency was reduced markedly. The minute flicker in the diffraction intensity in the stretched fibre which was expected during mechanical latency was indistinct due to shortage of latency. The frequency of flicker during the rising phase of tension was smaller than that of a non-stretched fibre. When the length of the stretched fibre was brought back to the original sarcomere length of $2.5\ \mu\text{m}$, the time course of tension development and the flicker in the diffraction intensity recovered almost to those of the original fibre.

When a skinned muscle fibre was immersed in a relaxing solution containing $0.1\ \mu\text{g/ml}$ trypsin, the resting tension of the skinned fibre was reduced and as the time of immersion increased, the fibre became easily broken by stretching. The light intensity of the 1st-order diffraction line after the drop of tension in KE- or MgE-contraction oscillated periodically. The period of oscillatory change of the diffraction intensity increased with the increase of immersion time in relaxing solution containing $0.1\ \mu\text{g/ml}$ trypsin.

c. Changes in stiffness during KE-contraction

When a skinned muscle fibre was stretched repetitively at the rate of 3–5% of its resting slack length during the time course of KE-contraction, tensions against stretch (stiffness) revealed 3 stages corresponding to the steps of tension development of KE-contraction mentioned above.

As shown in Fig. 57, the stiffness during mechanical latency was the same as that in the resting state and hardly any change in the stiffness was observed during late latency even when a flicker of the 1st-order diffraction line was exhibited. Also, a gradual increase in the stiffness during the period of slowly increasing tension development of KE-contraction (2nd step) was indicated. In the 3rd step of tension development, the stiffness increased, corresponding to the tension height of contraction. Eventually, a marked stiffness was occurred when the fibre was in a rigor state.

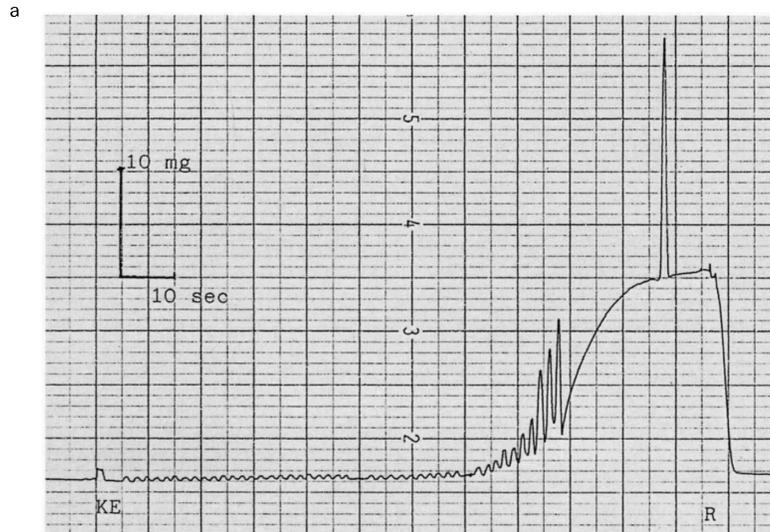
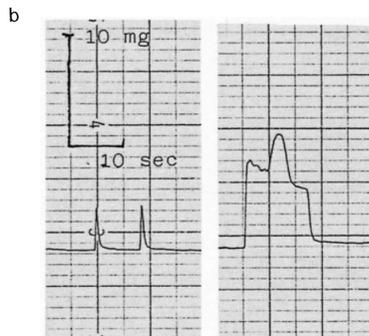


Fig. 57. The change of stiffness of skinned muscle fibres during KE-contraction and tetanus.



a. KE-contraction
Skinned fibre of m. sartorius from Japanese toad. 23°C.
Repetitive stretching during KE-contraction. Stretching rate is from 30 to 31 in scale unit.

b. Twitch (left) and tetanus (right) induced by electrical stimulation.
Skinned fibre of m. sartorius from Japanese toad. 23°C.
Stimulation: 3 msec duration and 10V intensity. Frequency of stimuli: 50/sec. Stretch is applied during tetanus (right). Stretching rate is from 25 to 26 in scale unit. Scale unit 62 corresponds to 5 mm.

As shown in Fig. 57b, tension by stretching during tetanus induced by electrical stimulation was much smaller than that of the rigor state.

When frequent stretching (14 times per sec) was applied to a skinned muscle fibre during KE-contraction, peak tension decreased (Fig. 58) and tension produced by 5% stretching was also smaller than that on the rigor state.

Speculative explanation of the contraction mechanism

A schematic diagram of tension development, stiffness and flicker of the 1st-order diffraction line in KE-contraction is shown in Fig. 59. I postulate that in the contraction process of the sarcomere, the molecular mechanism of tension development in muscle contraction may consist of certain steps which can be distinguished from each other by analysis of the time course of tension development during contraction.

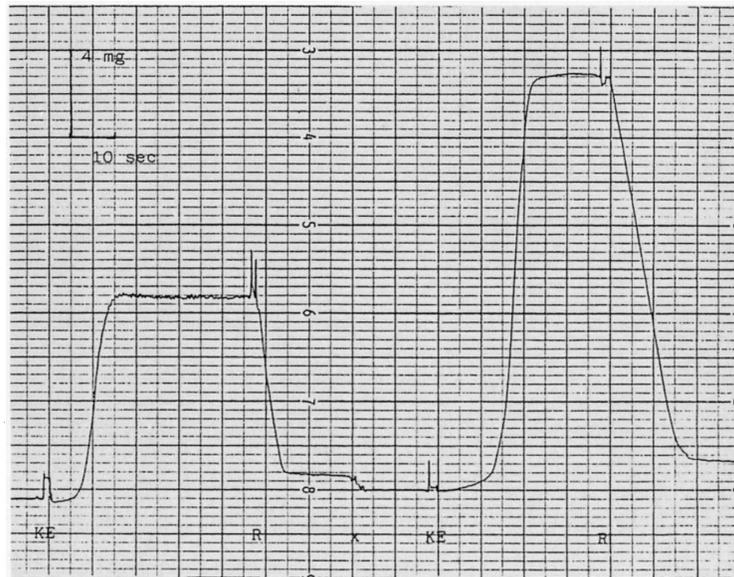


Fig. 58. Left: Frequent stretching during tension development of KE-contraction. Skinned fibre of *m. sartorius* from Japanese toad. Frequent stretching (14/sec). Stretching rate is from 50 to 55 in scale unit. Right: control.

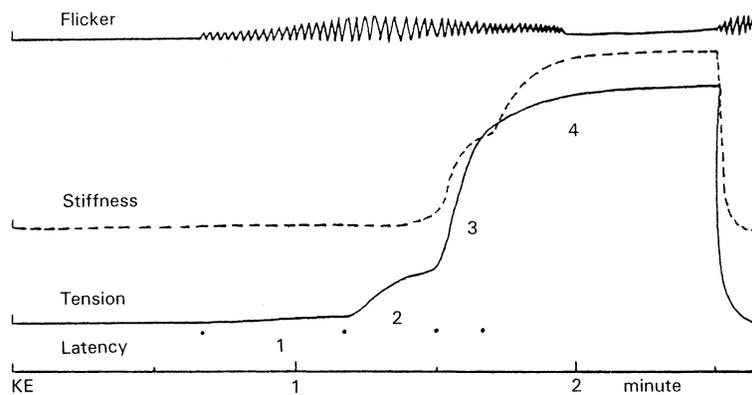


Fig. 59. A schematic diagram of time course of KE-contraction. (i) Tension curve, (ii) Flicker of 1st-order diffraction line, (iii) Stiffness measured by stretching.

I speculate that the 1st step of tension development is a partial approach of the thick and thin filaments after true mechanical latency, and that this process may be evoked by a slight increase in the flexibility of the thin filaments and the van der Waals force between the thick and thin filaments. The 2nd step is an attraction between the end portion of the actin molecule and the myosin head, this change perhaps

being induced by electrostatic force between the actin dipole and the ionized myosin head. The 3rd step is the formation of an actin and myosin bridge, while the 4th step may be a state of completion of cross-bridges.

According to comparison between the change of stiffness at each step of tension development in KE-contraction and that of tetanus induced by electrical stimulation, it is supposed that a twitch corresponds to the beginning of the 2nd step while tetanus corresponds to the early stage of the 3rd step in KE-contraction. At least, cross-bridge formation may not be completed during tetanus and the formation of cross-bridges may be a transient change.

Y. Umazume found that, when an electrical current of 30–50 V/cm was applied to both ends of an over-stretched skinned muscle fibre with a sarcomere length of more than $8\mu\text{m}$, a change in intensity of light transmission through the skinned fibre was induced.

The birefringence was decreased for half an I band on one side of the Z line and was increased for half the band on the other side. Almost the same effect was observed in a ghost skinned fibre from which myosin had been extracted by immersion in high-concentration KCl solution (Umazume and Fujime 1975)²⁵.

From these results and analysis of the diffraction patterns of over-stretched skinned muscle fibres, Umazume and Fujime supposed that the dipole property of an actin filament was similar to that of extracted actin (Osawa *et al.* 1972)²⁰. These reports suggested that the thin filament tended to be more flexible than in the resting state during the first step of KE-contraction, and support the above speculation regarding the first step of KE-contraction.

From the above evidence I postulate that the thin filaments change their thread form from straight to wavy under the influence of various factors, e.g. the composition of the fluid surrounding the filaments, temperature, etc.

With regard to the mechanism of muscle contraction, at present, the sliding of thick and thin filaments proposed by A.F. Huxley¹⁰⁻¹² and H.E. Huxley^{13,14} may still be phenomenally acceptable. The sliding process may explain twitch, tetanus and various other type of muscle contraction. Some reports published recently have proposed some possible mechanism on sliding process, however, I believe that it is still necessary to obtain and store many detailed results on the molecular changes occurring during contraction in order to explain the molecular mechanism of contraction.

The above explanation of the time course of KE-contraction is only a provisional one, but I have ventured to describe it as one facet of my recent findings against the historical background of my previous research.

RETROSPECT

During a lifetime of work in the field of muscle physiology, I have had the good fortune to have been able to conduct my research in my own way. Professor Seizaburo Uramoto, my teacher, gave me many opportunities for carrying out my research plan. I graduated from Jikeikai School of Medicine in 1936 and soon afterwards entered the Department of Physiology of my Alma Mater under the direction of Professor Uramoto, where I held in succession the positions of Assistant (1936–1939), Full-time Lecturer (1939–1945), Assistant Professor (1945–1949) and finally the post of Professor as the successor to Professor Uramoto in 1949. I retired from the position of Professor in 1977 and at present I am Professor Emeritus of our School of Medicine.

Half a century has already gone by since I entered study life, and so I recently felt the need to collect and arrange my investigations with skinned muscle fibres, particularly since in the past I mainly contributed my work to the Jikeikai Medical Journal, which is not a journal specialising in physiology. I hope this summarized report will serve as a useful introduction to my studies.

Needless to say, my research is still in progress and many problems remain to be elucidated. However, the properties of skinned muscle fibres isolated mechanically and the contraction responses which they exhibit have, for the most part, been demonstrated at least qualitatively from the viewpoint of “decomposition and reconstruction” mentioned in the preliminary remarks.

It would be an honour for me if this paper were to prove useful as a reference for future studies on muscle physiology.

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