The Change in Birefringence of the Activated Crayfish Single Muscle Fibre[1),2)]

By

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(*With 8 Text-figures*)

It has been generally recognized that the cross striation pattern of skeletal muscles was due to two zones different in the refractive index, and that the so called dark bands were anisotropic and of higher refractive index, while the light bands were isotropic and of lower refractive index. Contrary to the popular view of the muscle structure, Washio, Kakiuchi and Tamasige, 1961, provided the following evidence from their observation under a polarizing microscope on the single muscle fibre isolated from hind leg of the frog. They explained that the light bands in the striation pattern of the muscle were uniaxial birefringent bodies while the dark bands contained a light-absorbing structure which was a transversely arranged network of inter-myofibrillar connective filaments.

In the present paper, their explanation on the structure of muscles is again supported by the results obtained from the crayfish muscle investigation. And the mechanism of muscle contraction is more clarified by elucidation of the contraction propagating along the crayfish muscle fibre.

Material and Methods

All experiments were carried out on single muscle fibre isolated free of injury from dorsal extensor muscles of the crayfish, *Procambarus clarkii*. Myofibril bundles and single myofibrils were prepared with Natori's method (1949).

Most observations on the features or the change in birefringence of single muscle fibres under various conditions were performed with a polarizing microscope, Nikon POH. And a phase contrast-interference microscope (Nikon S-IP) was used for ultimately fine and low contrast objects, i.e. single myofibrils. The light source was a low voltage in candescent lamp (21V, 150W).

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Chemical stimulation was applied with chloroform. Most electrical stimulations were applied by an electronic stimulator (Nihon Koden MSE-2). And in the special experiment, electric stimuli were applied from an audio frequency oscillator (National RC-2K).

The schematic representation of the experimental arrangement for electrical stimulation is given in Fig. 1.

The principles on which the present work were based was described in a previous paper (Washio, *et al.* 1961). All experiments were carried out at room temperature.

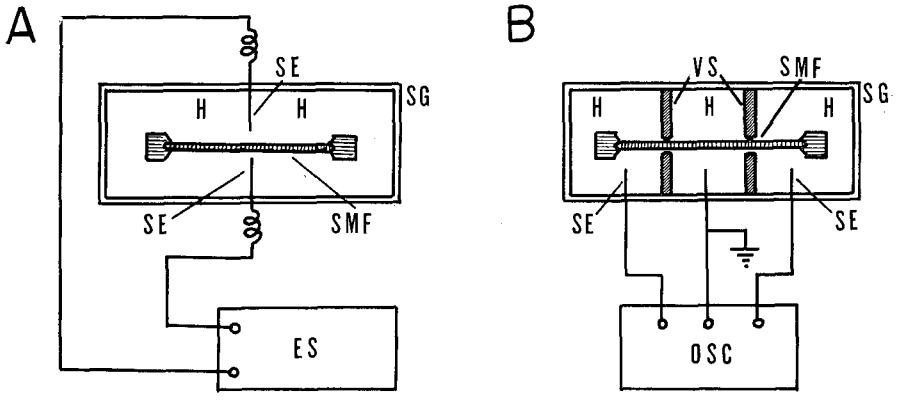

Fig. 1 Schematic diagram of experimental arrangement for studies on contraction wave along an isolated single muscle fibre of the crayfish. H: Harreveld's solution, OSC: Audio frequency oscillator RC-2K, SE: Stimulating electrode of Ag-AgCl type, SMF: Single crayfish muscle fibre, SG: Slide glass, ES: Electronic stimulator, MSE-2, and VS: Vaseline septum.

Results

Structure of muscle fibres: In order to inspect the difference in the striation pattern of a muscle fibre viewed under a polarizing microscope or under an ordinary light microscope, microphotographs of the muscle fibre taken with the two kinds of microscope were taken and compared (Fig. 2, A and B). And the two microphotographs were superimposed with strict spacial coincidence (Fig. 2, C).

The records apparently showed that no reversal between the dark bands and the light bands of the muscle fibre was found from the microphotographs; the light bands looked still bright and the dark bands were unchanged under both polarizing and light microscope. This result agreed with that obtained from the frog skeletal muscle fibres by Washio, *et al.* 1961, and supported their finding on the muscle structure that light bands of the muscle consist of the uniaxial birefringent body while dark bands contain light-absorbing structure.

Myofibrils: A bundle of myofibrils separated longitudinally from a single muscle fibre in the transparent paraffin oil showed a striation pattern similar to that of a single muscle fibre. Strong birefringence remained in myofibrils over

the entire length. Further, myofibrils had contractility to both chemical and electrical stimuli. Application of a small amount of sodium chloride crystals evoked the local contraction around the applied site. Electrical stimuli produced continued contraction of the myofibrils over the entire length to which the stimulating electrodes were connected (Fig. 3).

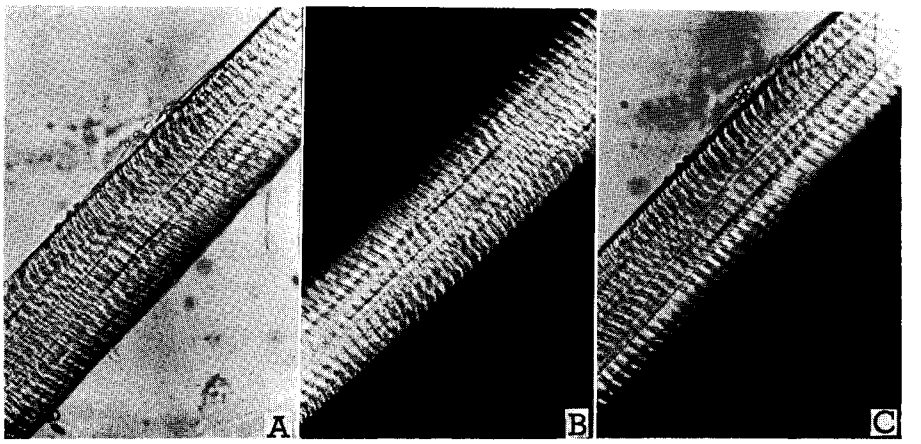

Fig. 2 A single crayfish muscle fibre under two kinds of microscopes. A) under an ordinary light microscope, B) under a polarizing microscope. C) superposition of A on B. Scale division, 10μ.

Single myofibrils were able to be isolated from a single muscle fibre which swelled to some extent in the hypotonic solution (the ionic concentration of the solution was 1/25 Harreveld's solution) (Fig. 4). Observation on the single myofibril was carried out under the phase contrast and interference microscope. The single myofibril preparation was of 0.5μ in diameter and of uniaxial structure over the entire length. However, observation under the polarizing microscope was impossible because of the very fineness of the preparation even at the highest magnification of the microscope.

Effects of hypotonic solution: Effects of hypotonic solution on the volume, striation pattern and birefringence of the crayfish muscle fibre were studied. Hypotonic solutions were made by dilution of the Harreveld's solution with distilled water and buffered by $NaHCO_3$ to a pH of 7.2. For the sake of convenience the concentrations were expressed relative to that of the Harreveld's solution, such as 1/25 Harreveld, and 1/100 Harreveld. The contractility of the muscle fibres treated with hypotonic solution was inspected by application of chloroform or electrical stimuli through the extracellular electrodes. Immersion in 1/5 Harreveld caused no change in the striation pattern, in the volume and in the activity of the muscle fibre even an hour after immersion. In 1/25 Harreveld, the muscle fibre began to swell, the striation pattern became gradually obscure, but it still remained two

hours after treatment (Fig. 5). In 1/50 Harreveld the striation pattern disappeared completely within half an hour. In the case of the frog muscle fibre, the striation pattern disappeared rapidly in 1/20 Ringer (Washio *et al*, 1961). The effect of hypotonic solution was more remarkable on the frog muscle than on the crayfish muscle. Immersion of muscle preparations in 1/100 Harreveled for 20 min. caused the striation pattern to vanish completely. The swollen muscle fibres which lost the striation pattern still exhibited strong birefringence and maintained contractility to electrical, chemical and mechanical stimulation. In the electrical stimulation, muscle fibres treated with 1/100 Harreveld had 2.5 times higher threshold than that of the control.

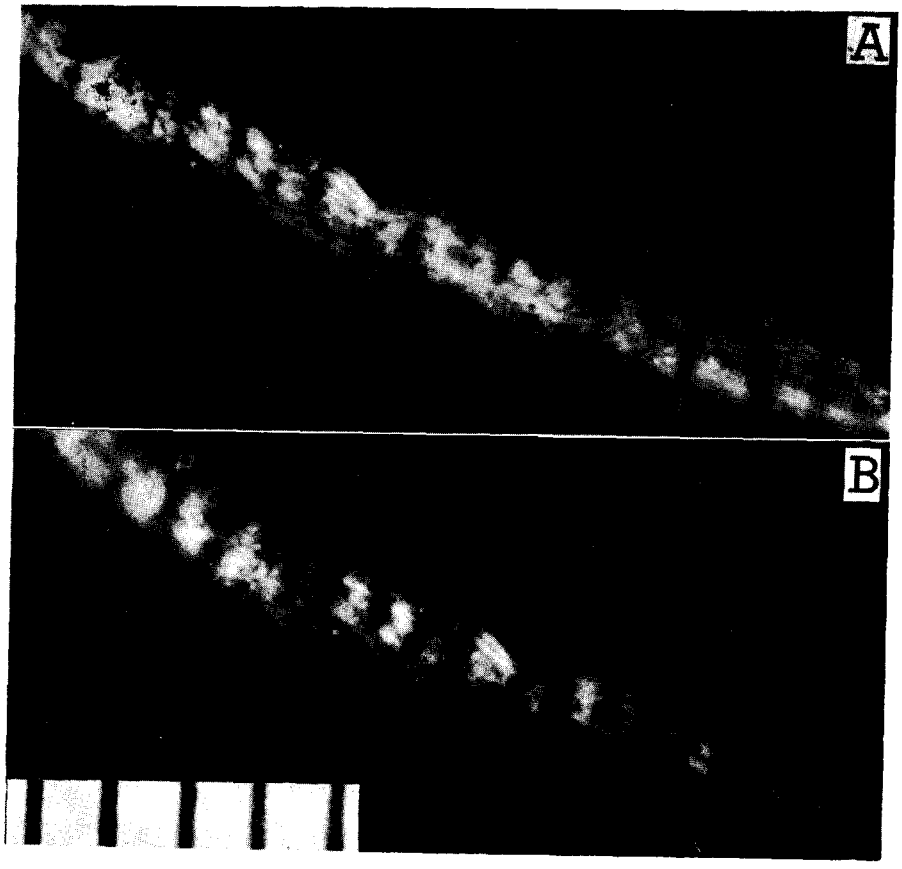

Fig. 3 An activated bundle of myofibrils. A: resting stage, B: during continued contraction by electric stimulation. Scale division, 10μ.

Muscle elongation: By elongation of muscle preparation the width of the light bands was considerably broadened whereas the dark bands changed slightly.

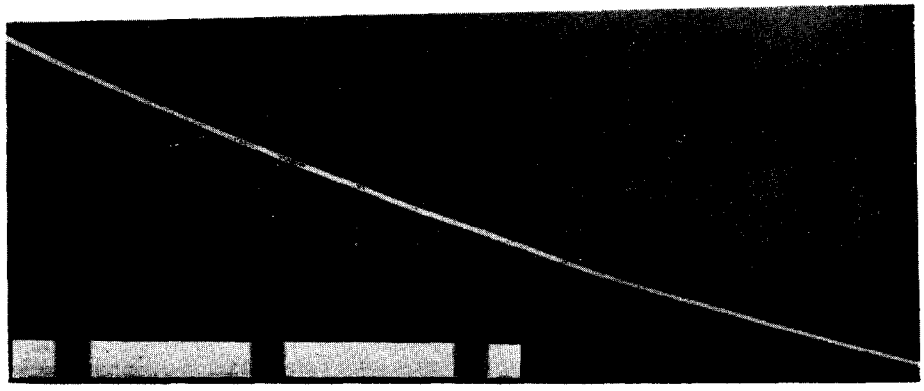

Fig. 4 A single myofibril isolated from a swollen muscle fibre in transparent paraffin oil. Scale division, 10μ.

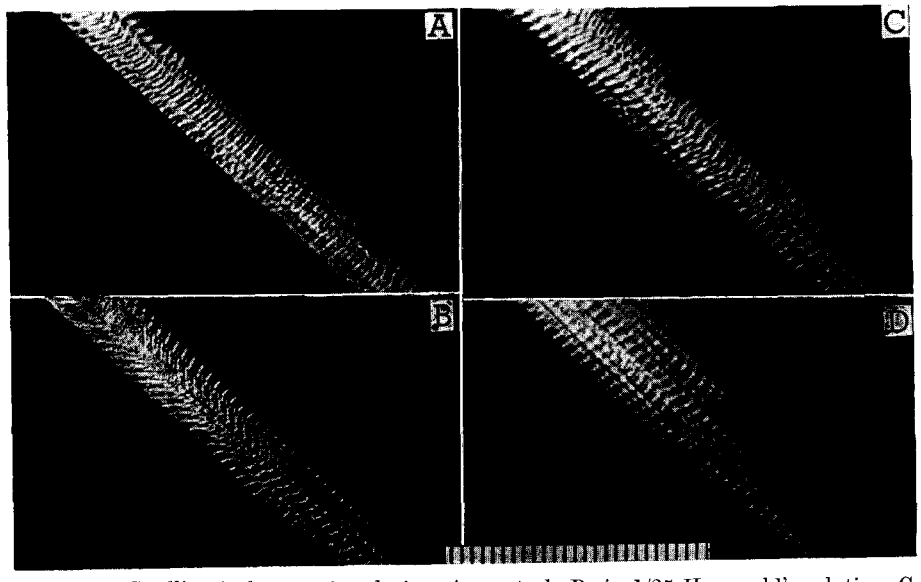

Fig. 5 Swelling in hypotonic solution. A: control, B: in 1/25 Harreveld's solution, C: in 1/50 Harreveld's solution, D: in 1/100 Harreveld's solution. B,C and D were photographed just 10 seconds after immersion in hypotonic solutions, respectively. Scale division, 10μ.

This result was in accordance with that obtained by other scholars (Huxley and Niedergerke, 1958, Washio *et al.*, 1961). The change in birefringence of the muscle fibre during passive stretch was not clearly detectable as in the reports by Fischer, 1936, Buchthal and Knappeis, 1938, and Washio *et al.*, 1961.

Muscle fibre contraction: Chloroform produced reversible contraction of

the crayfish muscle. Complete vanishing of the striation pattern of the muscle fibre was observed by immersing it in the saturated chloroform-Harreveld's solution, and at the same time an irreversible contracture occurred during the preparation. The contracture produced in 0.005% chloroform-Harreveld's solution is shown in Fig. 6, where the light bands shortened as much as about 30% of their initial length and the drak bands remained unchanged.

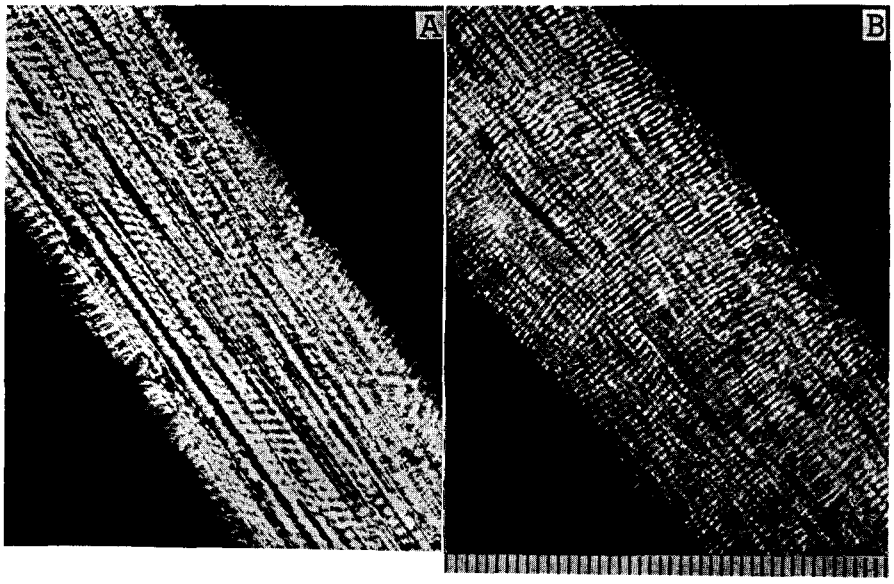

Fig. 6 Contracture produced by chloroform. A: resting state, B: contracted state in 0.005% chloroform-Harreveld's solution. Scale division, 10μ.

Single induction shock caused local shortening but no propagating contraction was observed in the crayfish muscle. By application of repetitive stimuli (50 c/s) continued contraction and decrease in the birefringence were observed over the entire length of the muscle fibre; the light bands shortened as much as approximately 10%, but the dark bands did not (Fig. 7, A and C).

Contraction waves: Contraction waves propagating along the muscle fibre were recorded, when polarized light microphotograph were taken of the change in birefringence along the fibre. A single muscle fibre was placed vertically between the stimulating electrodes facing each other along the long axis of a slide glass at distance of 2 mm (Fig. 1, A). In response to repetitive stimuli, the local contraction of the muscle fibre grew up to the continuous contraction. In the present observation, however, at an early stage of application of stimuli (square pulses, pulse width: 1.5 msec; pulse height: 6 V; and frequency: 80 c/s), contraction waves took place at the nearest position to the electrodes in the muscle fibre and propagated along the fibre to both ends. Fig. 7, A is a microphotograph showing a resting

muscle fibre in Harreveld's solution bath. Fig. 7, B shows contraction waves propagated along the fibre at the early stage of stimulation. The contraction waves were recorded as the dark cross arcs propagating toward both ends of the fibre. The continued contraction was also observed over the entire length of the muscle fibre.

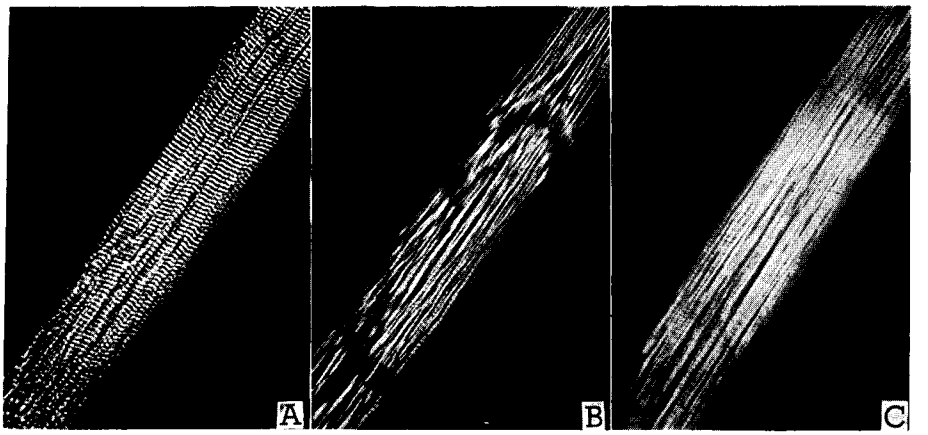

Fig. 7 Propagation of contraction wave. The change in birefringence at the front of contraction wave which spreads along the muscle fibre under the polarizing microscope. A: in the resting state, B: in the early stage of tetanic contraction by electrical stimulation. The contraction wave propagating along the fibre was recorded as dark cross arcs. C: in tetanic contraction following the stage of the photograph B.

Fig. 7, C (the photograph was taken 2 sec. after Fig. 7, B) shows the continued contraction of the muscle fibre after propagation of the contraction wave. The birefringence of the muscle fibre was decreased and the length of sarcomere was shortened to 70% of its initial length. In addition, an interesting phenomenon was observed for the first time. The contraction waves were produced only by alternating current stimulation of just subthreshold intensity. Sustained contraction waves were recorded by application of repetitive stimuli for this purpose, under special conditions of pulse frequency and intensity (Fig. 8).

As shown in Fig. 1, B, the electrodes in the Harreveld's solution pools in which the both ends of the fibre were immersed, were connected to the stimulator (Audio frequency oscillator, National RC-2K) and the center pool was grounded. When the alternating current was applied through the stimulating pools, both parts of the muscle fibre in stimulating pools were alternately activated by cathodic current. The excitation was initiated just inside of the vaseline septum and propagated along the fibre. These contraction waves propagating alternately from the stimulating pools intercept each other at the center of the fibre and produce a piling up of the wave for a while. The position of piling up of the wave depended on the frequency of the stimuli and on the propagation velocity of the contraction wave. The piling up wave was observed as a decrease in birefringence under the polarizing

microscope (the dark portion of the fibre in Fig. 8).

The decrease in birefringence continued for about a second after the stimulation, and after the decrease stopped the muscle fibre maintained a state of rest even when stimuli were applied. The relation between the intensity and the frequency of the alternating current stimulus had a very critical point.

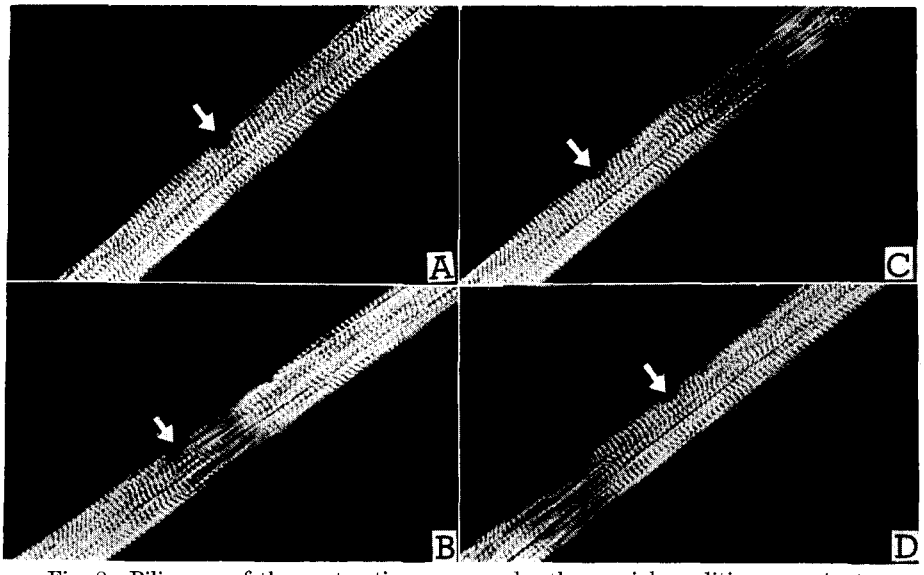

Fig. 8 Piling up of the contraction wave under the special conditions; see text.

From the appearence of the piling up of the wave the propagation velocity of contraction waves was measurable. For instance, the length of the fibre was 13 mm, the frequency of the stimuli was 130 c/s, the intensity of the stimuli was 7.7V, where the exact threshold stimulus intensity was 7.75 V. Fig. 8, A shows a polarized light microphotograph of a single muscle preparation and a black stimulated mark is seen on the muscle fibre. In Fig. 8, B, the decrease in birefringence (the drak portion of the fibre) was recorded in the vicinity of the stimulated position mark. The band width of the striation pattern decreased also at the contracted portion. Fig. 8, C and D are the records obtained from stimulation of 147 c/s and 113 c/s respectively. In these records, each dark portion of decrease in birefringence (contraction wave) deviated as long as about 200μ in the opposite direction from the stimulated position mark.

Assuming that propagation velocity of contraction waves under these conditions was equal and that time required for application of stimuli to initiate a contraction wave was constant in each case, the propagation velocity of the contraction waves was estimated roughly as

$$V = 4 / | T_1 - T_2 |$$

where V: propagation velocity of the contraction wave: D: distance between the marked point and the position of the dark portion: T_1: the period of the alternative stimulus current which produced the dark portion at the stimulated mark, and T_2: the period of the alternative stimulus current which produced the dark portion at any place in the muscle fibre ($T_1 \neq T_2$).

From the records in Fig. 8, the propagation velocity V=0.8 m/s was obtained.

Discussion

Since Brücke's study in the nineteenth century on the structure of a striated muscle, the striation pattern of the muscle has been recognized as constitution of two zones different in the refractive index; dark bands and light bands have been generally accepted as anisotropic bands and isotropic bands, respectively. Against the explanation of the striation pattern mentioned above, Washio *et al.* presented the evidence in their polarized microscopy on the frog skeletal muscles: no dark-light reversal of the striation pattern was observed under any microscopes; and the length of light bands was remarkably changed by either active contraction or passive elongation of muscle fibre, while no change of the length occurred in dark bands. They concluded that the light bands were uniaxial birefringent bodies whereas the dark bands contained a light-absorbing structure which was regarded as a transversely arranged network of inter-myofibrillar connective fine filaments.

There are no fundamental difference in the striation pattern between the crayfish muscles and the frog muscles from optical investigation. In comparison between the frog and crayfish muscles in detail, however, the sarcomere length of crayfish muscles was three times larger than that of the frog, and the surface of the former was rougher than the latter.

Effects of hypotonic solution on the crayfish muscle were small compared with those on the frog muscles.

By application of an electrical single shock, no twitch of the crayfish muscle was evoked over the entire length but a local contraction was produced only at the site where stimuli were applied. In the crayfish muscle, however, propagating contraction waves similar to the twitch that is generally seen in the frog skeletal muscle were observed under the polarizing microscope; there may be a close relationship between the contraction wave observed here and the twitch in frog muscles. above all, in the points of propagation over the entire length and velocity as they appeared under the polarizing microscope.

And the contraction wave developed by subthreshold alternative current stimulation accompanied no profound contraction, but it looked like an elastic wave (Fig. 8). The change in birefringence may imply some disorder of the submicroscopical arrangement of the contractile elements of the muscle fibre. These contraction waves observed here are analogous to the twitch in the frog muscle. The propagation velocity of the contraction wave was approximately 80 cm/s from

the result in Fig. 8, the value was remarkably larger than that of conduction velocity of action potentials (20 cm/s) of crustacean muscle fibre (Katz and Kuffler, 1946, Fatt and Katz, 1953). The measuring of the propagation velocity of the contraction wave employed in this study was apparently less accurate than that of the action potential.

Summary

1. A single muscle fibre isolated from dorsal extensor muscle of crayfish under various physiological conditions was investigated under both the polarizing and ordinary light microscopes.

2. The results obtained from the present studies agreed generally with those obtained from the investigation on the frog muscle (Washio *et al.*, 1961) and supported adequately their explanation of the muscle structure.

3. Contraction waves of the crayfish muscle were recorded, and their propagation velocity was measured at 0.8 m/s, average.

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