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Repetitive Electrical and Mechanical Responses of an Isolated Single Muscle Fibre to Prolonged Electrical Currents[1],[2]

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(Wih 8 Text-figures)

The mechanical response of a single muscle fibre has been studied by many investigators. A recent investigation demonstrated that the fresh preparation of an isolated frog muscle fibre shows repetitive contractions in response to prolonged direct stimulation (Tamasige, 1953). On the other hand, repetitive firing of action potential in response to prolonged current has also been found in the crustacean muscle (Fatt and Katz, 1953), in the squid axon (Frankenhaeuser and Hodgkin, 1957) and in the frog muscle (Hisada and Miyamoto, 1961).

In the frog muscle, the frequency of the repetitive contraction is about 10 cycles/sec. (Tamasige, 1953), but that of the repetition of the action potential is higher than that of the contraction; about ten action potentials will correspond to one contraction (Hisada and Miyamoto, 1961). Recent reports on the correlation between the membrane potential and the mechanical response (Hökansson, 1957, in the frog muscle and Orkand, 1962, in the crustacean muscle) give no solution for this discrepancy between the frequencies of mechanical and electrical repetitive responses.

In order to get a reasonable answer to the problem, the correlation between mechanical and electrical repetitive responses to prolonged stimulation was studied in the present investigation on an isolated single muscle fibre of the frog by simultaneous recording of contraction and action potential. Moreover, for the study of the correlation between them, it is fundamentally important to know the nature of the firing of the action potential. In the present investigation, a detailed analysis of the nature of electrical excitability to prolonged current stimulation was also attempted in the frog muscle fibre in the normal Ringer's fluid or low-calcium solutions.

Material and Methods

     Single muscle fibres were isolated, free of any injury along the entire length, from the iliofibralis muscle of the frog *Rana japonica*.   The Ringer's solution used had the following ionic composition, expressed in m.mol/l: Na, 126.4; K, 2.0; Ca, 1.3; Cl, 127.6; H_2PO_4, 2.0; HCO_3, 1.4.   The reduced calcium-Ringer's solution was made by mixing the isotonic calcium-free Ringer with the normal Ringer at various volume ratio.   All solutions were buffered with isotonic $NaHCO_3$ solution to pH$=7.2$.   All experiments were carried out at room temperature, 18°–20°C.

     Stimulation and recording apparatus: The recording and stimulating apparatus for the mechanical response was the same as that described by Tamasige (1951, 1953), except the special arrangement described below (see Fig. 1).   In order to secure simultaneous recording together with the electrical and mechanical response on a two beam cathode-ray oscilloscope, the movement of a small stained glass hook hung through the tendon
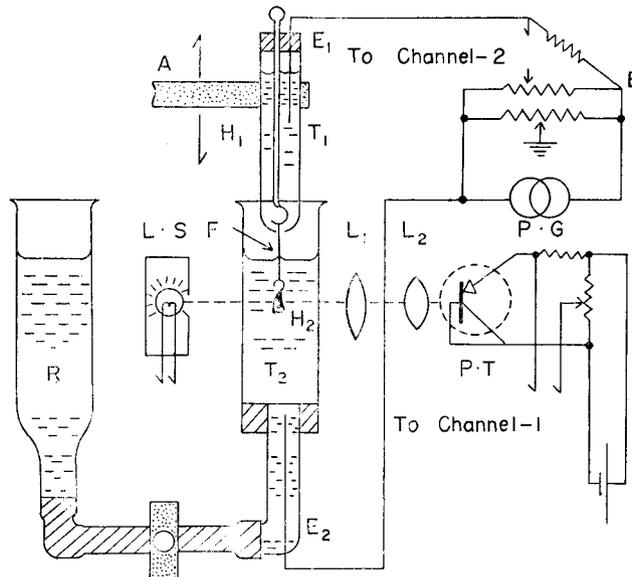

Fig. 1   Diagram of the experimental arrangement.   A: Adjustable stand; B: Weatstone brdige used in order to achieve two-fold usage of a couple of electrodes for the electrical response recording and the stimulation; E_1, E_2: Non-polarized electrodes; F: Single muscle fibre; H_1: Glass hook to suspend the muscle fibre; H_2: Small stained glass hook; L_1, L_2: A couple of convex lenses; L.S: Light source; P.G: Pulse generator; P.T: Photo-transistor; R: Reservoir for Ringer's solution; T_1, T_2: Upper and lower glass vessels.   The upper part of the fibre was drawn up for a length of about 4–5 mm Ringer's solution in which an Ag-AgCl electrode was set up.   The other end hung through the air into Ringer's solution in lower vessel in which a second electrode was set up.   Another explanation is to be seen in the text.

attached to the lower end of the fibre was focused through a coupled convex lens and projected on a photo-transistor, whose current change was amplified and displayed on one beam of the oscilloscope. The double usage of coupled electrodes for external stimulation and potential recording was achieved by placing the muscle fibre together with the electrodes in one arm of a Wheatstone bridge. This caused less damage to the fibre and reduced the stimulus artifact. The method was in principle identical with that used in a previous investigation (Hisada and Miyamoto, 1961). Stimulus currents of various intensities were applied through the coupled electrodes to the fibre and from the same electrodes the electrical response of the fibre was transmitted to an amplifier and displayed on the other beam of the oscilloscope.

Results

Propagated response: By application of a brief direct current of just above threshold intensity a fresh preparation of the muscle fibre produced a diphasic action potential having fully propagated nature and a successive contraction after a reaction time. The moment of initiation of the response varied according to the intensity of stimulus as was found also in previous investigations (Tamasige, 1953; Hisada and Miyamoto, 1961). In the propagated response which appeared in the fresh fibre to the brief current stimulation, the maximum height, the duration and the maximum rate of contraction were 4.2 mm, 100 msec. and 13.5 cm/sec., respectively.

Local response: With advance of time, the action potential of the fibre in response to the brief current showed gradual transition from the diphasic shape to the monophasic one, and the amplitude of the action potential was slightly decreased. The change of the action potential shapes indicated that the propagation of the excitation from the site of its origin to the other parts of the fibre was becoming less complete. The local action potential was followed by a local contraction whose reaction time between the application of stimulation and the initiation of contraction was more prolonged than that of the propagated response (see Fig. 2, No. 6).

Intensity-reaction time relationship: The time required for the initiation of mechanical and electrical response after application of the stimulus current was measured as the reaction time and plotted against the intensity of the stimulation (see Fig. 3). The curves expressing this intensity-reaction time relationship are of hyperbolic shape, for both mechanical and electrical response. The time delay between the initiation of action potential and the contraction did not depend much upon the intensity of the stimulation but upon the physiological condition of the fibre. The obtained time delay between these two responses was 5.2 msec. in the response on the fresh fibre and 7.5 msec. on the stale fibre, 15 and 45 min. after preparation respectively.

Response to prolonged current stimulation: When the fibre was fresh, the repetition of the action potentials and that of the contractions appeared in the responses to the prolonged current stimulation (durations of 200–500 msec. were

applied in the present experiment).   The frequency of the action potential firing
in response to the stimulus at the intensity of the threshold level was less than 10
c.p.s., the repetitive twitches of normal size evoked by the train of action potential
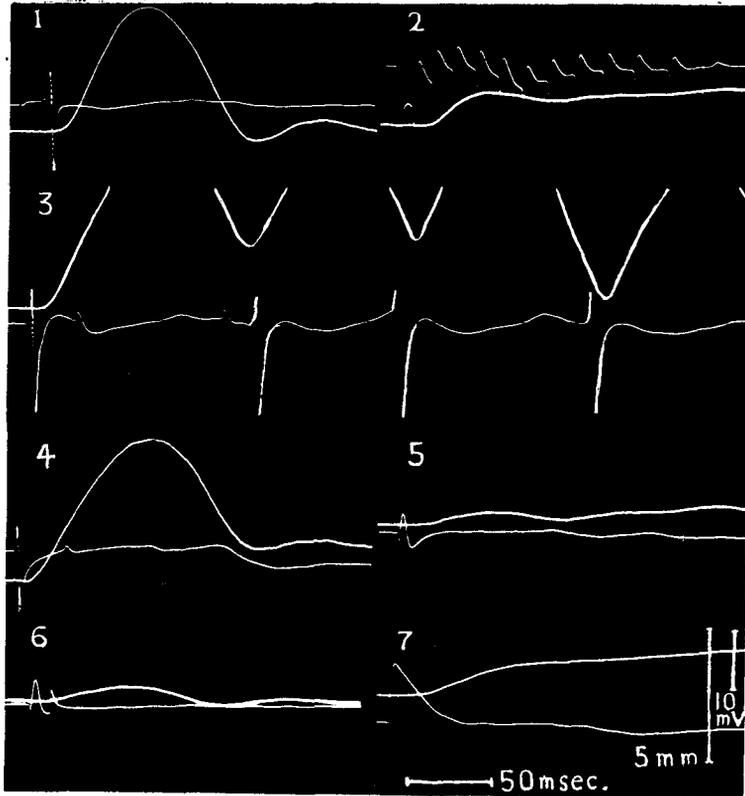

Fig. 2.   Various types of mechanical and electrical responses to electrical stimulation of
an isolated single muscle fibre of the frog, *Rana japonica*.   1. Propagated response,   10
min. after preparation.   Stimulation: 150 mV, 0.2 msec.   2. Repetitive firing of electrical
responses and slowly rising mechanical response,   15 min. after preparation.   Stimulation:
250 mV, 500 msec.   3. Repetitive mechanical and electrical responses of a fresh fibre produced
by constant current of just threshold intensity,   15 min. after perparation.   Stimulation:
90 mV, 500 msec.   4. Propagated response and successvie cathodic shortening produced by a
strong and prolonged current, 30 min. after preparation.   Stimulation: 700 mV,   500 msec.
5.   Repetitive local contraction and action potential, 45 min. after preparation.   Stimula-
tion: 300 mV, 500 msec.   6. Local contraction and action potential produced by single
pulse stimulus, 45 min. after preparation.   Stimulation: 500 mV, 10 msec.   7. Cathodic
shortening and local action potential produced by a prolonged currnet, 60 min. after
preparation.   Stimulation: 500 mV, 500 msec.

were observed, and here, each action potential clearly corresponded to each contraction (see Fig. 2, No. 3). As the stimulus intensity increased, the frequency of the repeated action potential became higher, thus the summation of various degrees was often reflected in the mechanical responses (see Fig. 2, No. 2 and Fig. 4).
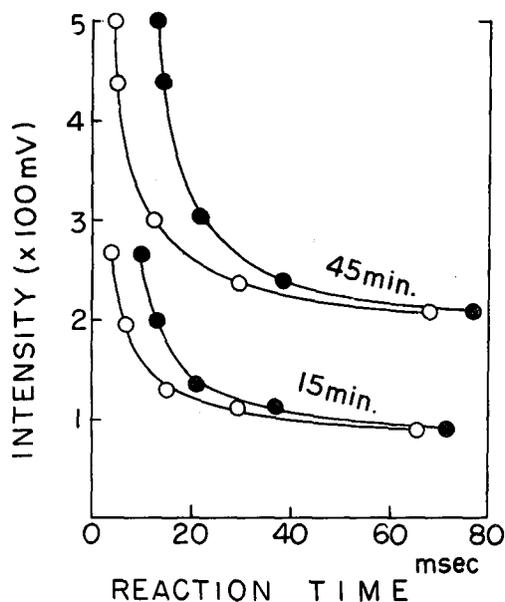

Fig. 3. The intensity-reaction time relationship for the action potential and contraction. Open spots: reaction time for action potential. Black spots: reaction time for contraction. Numerals along the curves indicate the time at which the determinations were made. The time delay between the initiation of action potential and that of the contraction is not dependent upon the stimulus intensity.

In the stale fibre, the repetitive action potential of monophasic shape and the corresponding repetition of contractions were also observed (see Fig. 2, No. 5). With the advance of time after preparation, the frequency and the amplitude of the repeated action potential gradually decreased. The minimum frequency of the action potentials was 6 c.p.s., 60 min. after preparation. In the more stale fibre, one or two hours after preparation, no electrical response was found in response to application of prolonged current, except the initial one having a small amplitude and long duration. The contractions, however, showed successive increase as an increment of the stimulus intensity (see Fig. 2, No. 7). This type of contraction corresponds to the cathodic shortening described by Tamasige (1953). The two series of photographic records of the responses to the prolonged currents and the
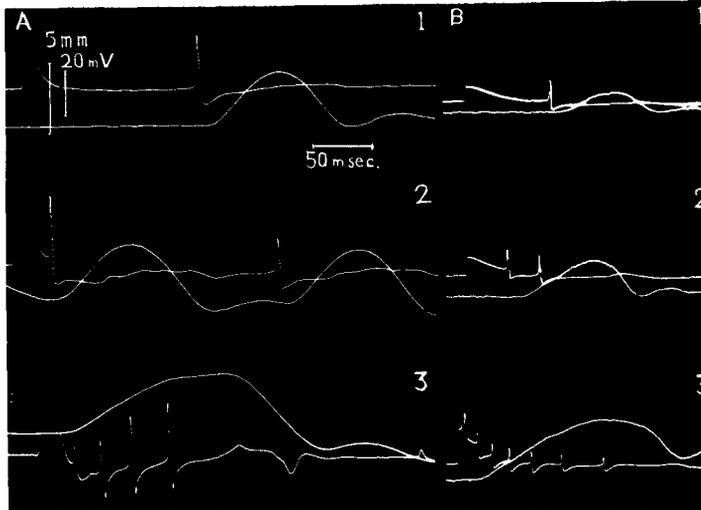
Fig. 4.   Two series of responses which appeared on the fibres under the different
physiological conditions.   A: A series of the propagated responses of a fresh fibre, 15 min.
after preparation.   Stimulation: 90 mV in (1), 110 mV in (2) and 140 mV in (3) respectively.
B: Another series of the response of aged fibre, 40 min. after preparation.   Stimulation: 100
mV in (1), 110 mV in (2) and 180 mv in (3) respectively.   Note the gradual increase in
the frequency of the action potential and in the height of contraction, which become pro-
gressively remarkable as the stimulus intensity increases.
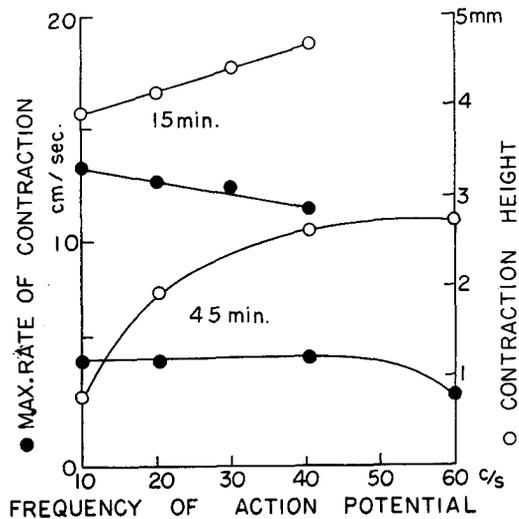

Fig. 5.   The contraction height and the maximum rate of contraction plotted against the
frequency of the action potential.   They were plotted from the photographic records of
Fig. 4.

relationship between the action potential frequency and the mechanical response are also shown in Figs. 4 and 5, respectively. The maximum rise of contraction in correspondence to the repetitive action potentials having frequency of 40 c.p.s. was 14.5 cm/sec.

In the present experiment, the successive change from propagated response to the local one was recorded by application of strong and prolonged current from the start on the fresh fibre, where the period of the repetition of the action potentials was much shorter than that of the contraction (about ten action potentials corresponded to one contraction). Here, there was no summation in the size of the first or the second contraction, and the frequency of the mechanical response appeared as if it had no relation to that of the action potential. A possible explanation of this type of response may be that after an all-or-none type contraction was produced by the first action potential, the successive firing did not exert any effects on the previous exciting contraction, because the relaxation was not allowed due to the strong and prolonged current which was constantly applied to the muscle fibre. So this special case dose not prove the existence of the refractory period of the contraction to the firing of the action potentials.

Analysis of the nature of the repetition of the action potentials in the normal Ringer's fluid or in lower calcium solutions: A detailed analysis was made of the nature of the electrical excitability to prolonged current stimulation. The photographic records of the action potential spikes which appeared in responses to the prolonged stimulations of various intensities are shown in Fig. 6, A. A fresh fibre produced only one action potential to the stimulus of intensity just above the threshold even if the duration was prolonged (200 msec.). With the increase of the stimulus intensity, the second and the third action potentials appeared, the repetition of action potentials became more frequent and the intervals between these successive spikes narrower. There was a progressive decrease in size of the action potential with time during application of stimulus currents. The rate of increase in the time interval between spikes and the rate of decrease in their amplitude were closely related with the stimulus intensity; both rates were plotted against the number of action potential (see Fig. 7, A). As already reported (Hisada and Miyamoto, 1961), the muscle fibre in calcium-poor solution tended to produce repetitive action potentials in response to weak stimulation. The photographic records of the firing taken from the fibre in Ringer's solutions at various concentration of calcium were shown in Fig. 6, B. The rate of decrease in the amplitude and that of increase in the reaction time of each spike were also plotted against the number of action potentials (see Fig. 7, B). The electrical excitability of the fibre in response to the same stimulus in Ringer's solution of various calcium concentrations was very like to that of the fibre to the stimuli of increasing intensities in the normal Ringer's solution, in respect to the rate of increase in the frequency and other general features of the response. In order to express the quantitative change in the excitability of the fibre with decrease in

calcium concentration in Ringer's solution, the frequencies of the action potentials in each solution were plotted against the stimulus intensity (see Fig. 8). The effect of the reduction of calcium on the electrical excitability became distinct with the increased intensity of the stimulus.
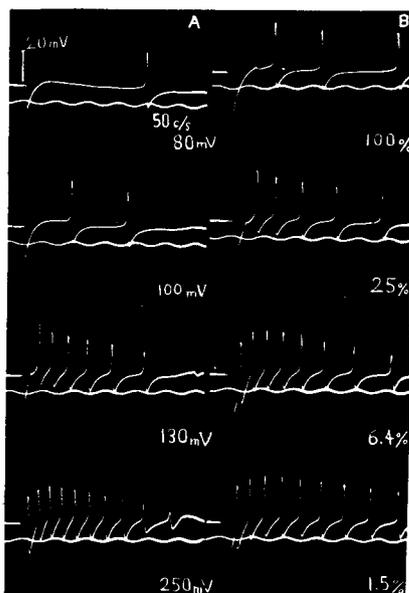

Fig. 6.

Fig. 7.

Fig. 6. Two series of the repetitive action potentials which appeared in responses to prolonged current stimulation. A: A series of responses in the normal Ringer's solution. Numerals on each photograph indicate the stimulus intensity. B: Another series of responses in Ringer's solution of various calcium concentrations to the constant current stimulation, in which the normal concentration of the clacium is expressed as 100%. The decrease in calcium and the increase in stimulus intensity seem to exert the same effects on the muscle membrane.

Fig. 7. The changes of the reaction time and of the amplitudes of the action potentials, which are plotted against the number of action potentials in which they occurred. A: relationship plotted in the normal Ringer's solution, and B: plotted in calcium-poor Ringer's solutions. Numerals along the curves are the same as those in Fig. 6. Open spots: Amplitude corresponding to the right ordinate. Black spots: Reaction time corresponding to the left ordinate.
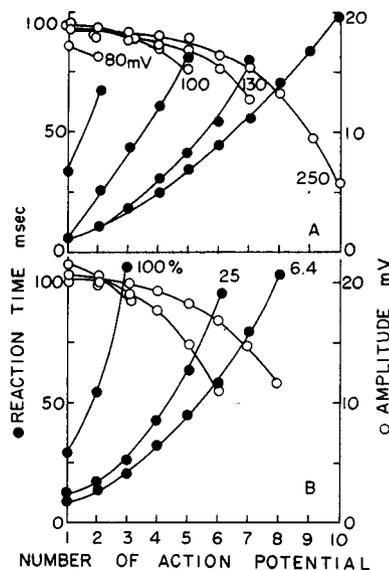
In solution lacking calcium the fibre elicited vigorous repetitive action potentials, but they ceased within a few minutes. The same results have already been reported by Tamasige in the mechanical responses of the frog muscle fibre (1953), and in electrical responses of many excitable tissues (Frankenhaeuser in the forg nerve, 1957; Hisada and Miyamoto, 1961 in the frog muscle etc.).
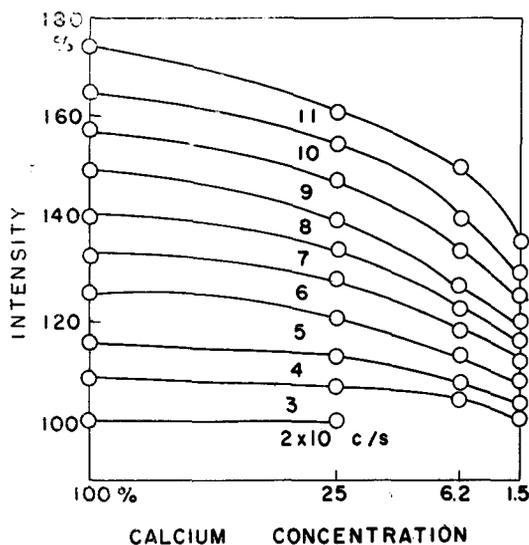
Fig. 8. The relationship between the stimulus intensity and the calcium concentration in Ringer's solution. The calcium concentration in the normal Ringer's solution together with the stimulus intensity of just threshold are expressed as 100%. Numerals along the curves indicate the frequency of action potentials. The effects of reduction of calcium on the muscle membrane become higher as the stimulus intensity increases.

Discussion

The various types of mechanical responses recorded in the present investigation were in good accordances with those already described by Tamasige (1953) in their general features. With advance of time after the preparation, the propagated twitch which appeared in the fresh fibre gradually changed to local contraction in which the maximum rate of contraction and the contraction height of the fibre slightly decreased. This time change in the contraction was closely related with the change in the action potential shapes, from the diphasic shape of large amplitude to the small monophasic one, which seems to indicate incomplete propagation in the stale fibre.

It is above stated that the hyperbolic curve of the relationship between the reaction time of the action potential and the intensity of stimulation can be expressed by Weiss's formula and that the latency is decreased to almost nil when the stimulus intensity is suprathreshold (Hisada and Miyamoto, 1961); this fact has clearly been confirmed in the present investigation.

The time delay required for initiation of the contraction after occurrence of the electrical response seemed not to depend much upon the stimulus intensity, but upon the physiological condition of the fibre as effected by passage of time

after preparation. This fact should be considered since the propagation of the electrical response becomes less complete with time. The minimum time delay for the elicitation of the mechanical response obtained here to the stimulation of suprathreshold intensity was 5.2 msec., which shows good agreement with those reported by Sandow (1952), Tamasige (1953) and Hôkansson (1957).

The rate of increase in the summation of the mechanical response was generally proportional to the frequency of the repetitive action potential, especially in the aged fibre. A reasonable explanation for the relation between the rate and the frequency is as follows. After the initial contractile displacement was produced by the first action potential, the part of the fibre neighbouring to the displaced place was brought up to the surface of the solution, where the current density is the highest, and at the new site of the fibre exposed to the densed current the second action potential was elicited. The displacements of successive parts along the fibre continued one after another until all the remaining parts were exposed to the stimulus current, and as the result of this successive process, the summations of various degrees were caused appear in the mechanical response, because the velocity of the contractile displacement was extremely smaller compared to the reaction time of the action potential under these stimulating currents of suprathreshold intensity.

Analyzing the present result of observation of the correlation between the repetitive contractions and the repetitive action potentials in response to prolonged current stimulation, it may be concluded that (1) the period of the former is closely related with that of the latter; (2) between the two frequencies, one to one relation is maintained when the period of the latter is less than 10 c.p.s; and (3) there is no evidence for the existence of the refractory period of contraction. The muscle fibre has the tendency to produce repetitive action potentials in calcium-poor Ringer's solution in response to prolonged current stimulation (Hisada and Miyamoto, 1961). The effects of calcium reduction on the electrical excitability of the muscle membrane appear in parallel with the effects of increase in the stimulus intensity. Analysis of the results obtained from the present and previous experiments indicates that a decrease in concentration of calcium ions on the surface of muscle membrane caused by the cathodic current of stimulation is directly linked with a transient increase in the permeability of the membrane in the mechanism of elicitation of the action potential. The progressive increase in the time interval between each spike respectively and the decrease in their amplitude in a train of action potentials will reflect the time delay corresponding to the recovering process of the movement of calcium ions toward the membrane and the time change in the membrane permeability. The increase in the threshold at the cathode caused by the prolonged current has already been reported by Takeuchi and Tasaki (1942), and it may be explained by the fact that the calcium recovery and permeability recovery are inhibited by the prolonged cathodic current. Tamasige reported an increase (35%) of the membrane resistance with a

tenfold increase of calcium concentration (1951). These facts present the actual evidence for the gradual increase in the permeability of the membrane and in the excitability to electrical stimuli in calcium-poor solutions. It is also concluded that complete calcium lack in the Ringer's solution brings about irreversible increase in the membrane permeability, and that it results in electrical inexcitability of the muscle fibre.

Summary

1. Simultaneous recording of the mechanical and electrical response was achieved on isolated single muscle fibre of the frog, *Rana Japonica*.

2. The time delay between the initiation of action potential and that of the contraction did not depend upon the intensity of stimulation but upon the physiological condition of the muscle fibre.

3. With a prolonged current stimulation, the muscle fibre showed repetitive mechanical and electrical responses, whose frequencies depended directly upon the stimulus intensity.

4. The frequency of the repetitive contraction was closely related with that of the action potentials; and there was one to one relation between them when the frequency of the latter was less than 10 c.p.s.

5. Mechanical summation occurred in response to the prolonged current stimulation of suprathreshold intensity and the maximum rate of contraction was 14.5 cm/sec. in the fresh fibre. There was no evidence for the existence of the refractory period in the contraction.

6. The rate of increase in the height of mechanical summation was proportional to the frequency of action potentials. It was more remarkable in the aged fibre than in the fresh.

7. In calcium-poor Ringer's solution, the muscle fibre produced repetitive action potentials of higher frequency in response to prolonged electrical stimulation than in the normal Ringer's solution; the role of calcium ions in the mechanism of elicitation of the action potential was discussed.

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