

# Effects of Sudden Changes in External Sodium Concentration on Twitch Tension in Isolated Muscle Fibers

SHIGEHIRO NAKAJIMA, YASUKO NAKAJIMA, and  
JOSEPH BASTIAN

From the Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907 and the Physiological Laboratory, Cambridge, England. Dr. Bastian's present address is the Department of Zoology, University of Oklahoma, Norman, Oklahoma 73069.

**ABSTRACT** When  $[Na]_o$  was suddenly introduced to single muscle fibers (*Xenopus* or frog), which had been pretreated with Na-free solution (Tris-substituted), the time-course of twitch recovery was very variable, half-time ranging from less than 1 s to 5 s. The  $[Na]_o$  vs. twitch height relationship was also variable. In small *Xenopus* fibers, decreases of  $[Na]_o$  to 50% increased the twitch, while in large *Xenopus* fibers twitch height remained constant or decreased as  $[Na]_o$  was decreased to 50%. The apparent diffusion constant ( $D'$ ) of  $Na^+$  or  $K^+$ , calculated from the time-course of twitch recovery and the  $[Na]_o$  vs. twitch relation, and from the time-course of the slow repolarization upon sudden reduction of  $[K]_o$  was about  $1-1.5 \times 10^{-6}$  cm<sup>2</sup>/s. This is one order of magnitude smaller than the diffusion constants in an aqueous solution. Even if the tortuosity factor of the T system is taken into account, there remains a substantial discrepancy. Although our value of  $D'$  is subject to various errors, if we accept the value, the twitch recovery is predicted to be either very quick or slow depending upon the variation of  $[Na]_o$ -twitch relation and fiber size. Thus, both quick and slow twitch recoveries can be explained by the diffusion time of  $Na^+$  in the T system, and therefore the results are consistent with the idea that the T system is excitable.

## INTRODUCTION

When the external solution is suddenly changed from normal Ringer to a Na-free (choline-substituted) solution in single amphibian muscle fibers, twitch contraction disappears almost instantly. If, after the fiber has been treated in the Na-free solution, normal Ringer is suddenly reintroduced, the twitch reappears very quickly with a half-time of less than 1 s. This quick recovery of twitch is described in Hodgkin and Horowicz (1960 *b*). While we were repeating their experiments, we found that in some fibers the recovery

was indeed very quick, whereas in large fibers (when Tris-Ringer was used as a Na-free solution) a relatively slow recovery with a half-time of up to 5 s was seen.

There is now strong evidence for the presence of a Na-dependent action potential in the transverse tubular system (Costantin, 1970; Costantin and Taylor, 1971; Bezanilla et al., 1972; Bastian and Nakajima, 1972, 1974). Therefore, it is natural that one would attempt to explain the delay of the twitch recovery by the diffusion time of Na ions entering the transverse tubular system (T system) from outside. In the case of fibers that show a slow twitch recovery, the explanation seems to be plausible. However, in fibers that show a very quick twitch recovery, with a half-time of less than 1 s, the explanation encounters difficulty, since the retardation due to diffusion in the T system has been reported to have a half-time of a few seconds (Hodgkin and Horowicz, 1960 *a*, *b*; Nakajima et al. 1973). Thus, on the face of it, the quick recovery appears to invalidate the idea that the T system produces a Na-dependent action potential.

The experiments reported here were performed in order to solve this question. It will be shown that the somewhat complicated results are still explainable by the diffusion process of ions in the T system. Preliminary accounts were given at Biophysical Society meetings (Nakajima and Bastian, 1972; Nakajima and Nakajima, 1974). The recent papers by Caputo and DiPolo (1973 *a*, *b*) have a close bearing on the present report.

#### METHODS

Single fibers were isolated from *M. flexor brevis digiti V* of *Xenopus laevis*, or from *M. semitendinosus* of *Rana temporaria*. The fibers were stretched to 130% (in several experiments to 115%) of the slack length, and were directly stimulated through external platinum electrodes. Isometric twitch tensions were measured through a piezo-resistive strain-gauge (Pixie 8206, Endevco Div., Becton, Dickinson & Co., Pasadena, Calif.), and recorded with a chart recorder and/or a cathode ray oscilloscope. The intracellular microelectrode technique was a conventional one. The method of exchanging external solutions was the same as described previously (Hodgkin and Horowicz, 1959, 1960 *a*; Nakajima et al., 1973). This method allows solutions to be exchanged very rapidly; with the flow speed used in this study (about 1.5 ml/s), the half-time of potential changes upon sudden changes of  $[Cl]_o$  was about 0.2 s (Nakajima et al., 1973).

Normal Ringer solution contained:  $Na^+$  120;  $Cl^-$  121;  $K^+$  2.5;  $Ca^{++}$  1.8;  $HPO_4^{--}$  2.15;  $H_2PO_4^-$  0.85 mM. Choline Ringer was made by replacing Na of normal Ringer by choline on an equimolar basis. Tris-Ringer is composed of: Tris 125 mM adjusted to pH 7.1 by HCl; KCl 2.5;  $CaCl_2$  1.8 mM. This solution was isosmotic (within 2%) with normal Ringer solution. Solutions with different sodium concentrations were made by mixing normal Ringer with either Tris- or choline-Ringer solution. The hypertonic 270 mM-Na solution contained 270 mM NaCl in place of

115 mM NaCl in normal Ringer. The hypertonic 100 mM-Na solution contained 100 mM NaCl and 170 mM choline chloride instead of the 270 mM NaCl in the above solution. The choline chloride was purified through crystallization from hot absolute ethanol. The fiber radius  $a$ , measured as in Hodgkin and Nakajima (1972 *a*), refers to the value at slack length, unless otherwise stated.

## RESULTS

### *Na Concentration and Action Potential Height*

Fig. 1 illustrates the effects of suddenly changing  $[Na]_o$  on the height of action potentials. Hypertonic solutions were used to prevent muscle contractions. The experiments were done on *Rana temporaria*. The height of the action potential was increased quickly as  $[Na]_o$  was changed from 100 to 270 mM, and was reduced rapidly as 100 mM-Na solution was flushed again. With this stimulus frequency it was impossible to obtain exact time-courses, but an estimate using linear interpolation indicated that the half-time of the effect of Na increase was less than 0.4 s and that of Na decrease was less than 0.5 s (four fibers, 59–75- $\mu$ m radius). At first sight this quick onset of Na effects seems to conflict with the idea that the T system produces a Na-dependent action potential. Indeed, if the T system was excitable, a delay might be expected to occur due to the diffusion of Na ions into and out of the T system. On the other hand, if the T system were not excitable, the action potential, which occurs only in the surface membrane, should change very quickly upon changes in external sodium concentration.

However, the result of Fig. 1 can be reconciled with the idea of an excitable T system if due consideration is given to the length constant of the T system. At the moment of action potential peak, the length constant of the T system becomes so short (due to the drastic increase in Na permeability as well as to the high frequency nature of the wave form) that only events occurring near

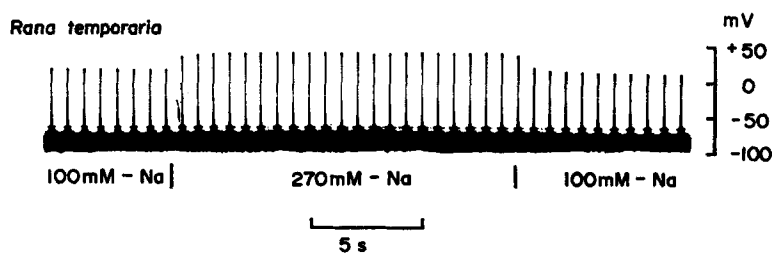


FIGURE 1. Changes in action potential height induced by quick changes of  $[Na]_o$ . Action potentials were recorded by an intracellular microelectrode inserted into an isolated fiber of the semitendinosus muscle of *Rana temporaria*. Twitches were abolished by the hypertonicity. The 100 mM Na solution contained 170 mM choline chloride to keep the osmolarity constant. Fiber radius 69.5  $\mu$ m. Temperature 22.0°C.

the fiber surface would be recorded by a microelectrode. Thus, the result of Fig. 1 is in agreement with either an excitable or nonexcitable transverse tubular membrane.

#### *Effects of Na on Twitch Height*

Fig. 2 illustrates the effects of suddenly changing  $[Na]_o$  on isometric twitch tension. Records A1, A2, and A3 are examples from a single frog fiber ( $a = 60 \mu m$ ). The fiber had been equilibrated in normal Ringer, and twitches had been elicited at 0.5 times/s. When new normal Ringer (referred to as 1.0-Na in the figure) was exchanged with the normal Ringer (A1) which had already been in the bath, there was hardly any change in twitch height, except when the stimulus was delivered in the midst of a flush (arrow in A1).<sup>1</sup>

When Na-free solution (Tris-substituted, referred to as O-Na in the figure) was applied, the twitch disappeared abruptly (A1). This abrupt twitch disappearance was expected, since the surface action potential would suddenly stop firing, resulting in conduction block. After about 1 min in the Na-free solution, normal Ringer was restored, and the fiber started to produce twitch (A1). The time-course of the twitch recovery was very rapid. Records B1 and B2 of Fig. 2 illustrate an example of a *Xenopus* fiber ( $a = 44 \mu m$ ). The procedure of solution changes was the same as in A1; namely, the fiber had been presoaked in Na-free solution for 1 min before normal Ringer was reintroduced. As these two examples show, the twitch recovery was so rapid that it was impossible to follow the exact time-course. An approximate estimate of the recovery time-course can be reconstructed from several runs of solution exchanges (Fig. 2, B3). Each symbol represents the recovery of twitch height in an experimental run like B1 and B2. The abscissa is the time after the start of the flush. As can be seen from B3, the recovery is quite quick; the time to reach 90% of the final level was about 1.3 s, and the half-time of twitch recovery, estimated by a linear extrapolation, was 0.45 s (see footnote 2 for the procedure of extrapolation).

However, this quick recovery of twitch was seen in small to medium-size fibers (say,  $a < 60 \mu m$ ). In larger fibers the recovery (when Tris-Ringer was used) was a slower process. Fig. 3 A shows an example of a large *Xenopus* fiber ( $a = 76.5 \mu m$ ), which showed a half-time of recovery of 4.3 s, and a 90% recovery of 10.6 s. The time-course of twitch recovery was dependent on how long the fiber had been immersed in Na-free solution. The shorter the duration, the quicker the recovery. However, no difference was obtained in the recovery between the treatments for 1 min and for 2 min. Thus, the effect of Na-

<sup>1</sup> When 1.0-Na  $\rightarrow$  1.0-Na exchanges took place, the first twitch after completion of the flush was sometimes slightly reduced. This artifact, described also in Hodgkin and Horowicz (1960 *b*), was pronounced at 115% stretch, but at 130% stretch, which was used in most of the present experiments, it usually caused less than 1% (rarely 1-3%) error in the twitch height.

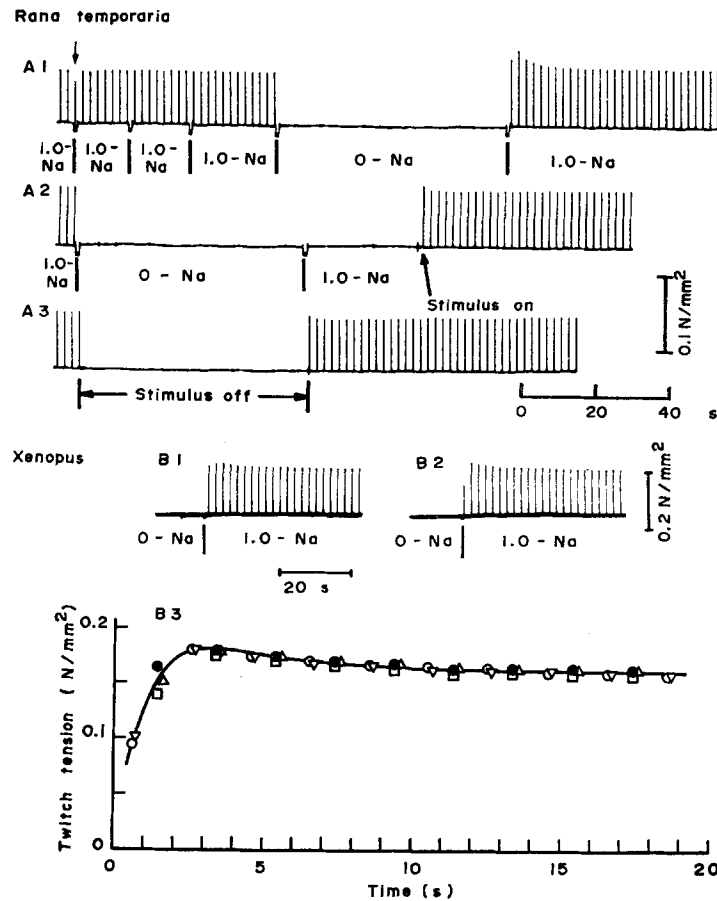


FIGURE 2. Changes in isometric twitch tension produced by changes in  $[Na]_0$ . 1.0-Na is normal Ringer. 0-Na is the Na-free solution (Tris-substituted). On each record, whenever the solution was exchanged, a downward deflection of the tension record is seen. This reflects a stress of the flow exerted on the tension recording system. A1, A2, A3: 60- $\mu$ m radius fiber from *Rana temporaria*. B1, B2, B3: 44- $\mu$ m radius fiber from *Xenopus laevis*. In B1 and B2 the sequence of solution changes was similar to A1; i.e., normal Ringer  $\rightarrow$  Na-free solution (about 1 min)  $\rightarrow$  normal Ringer. Only the last part of the sequence is shown. B3 is the reconstruction of the twitch recovery: each symbol represents a run as in B1 or B2. Abscissa is the time from the beginning of the flushes. In this paper all records displayed are taken with a chart recorder except for B1 and B2 in this figure, which were recorded by cathode ray oscilloscope. The small arrow near the beginning of A1 indicates a small twitch, which occurred in the midst of the flush. Temperature: 22.0  $\sim$  23.0°C.

free solution seemed to be fully developed within 1 min. When choline-Ringer was used (as in Hodgkin and Horowitz, 1960 *b*) instead of Tris-Ringer, the twitch recovery became far more rapid. The difference of choline Ringer and Tris Ringer will be discussed later.

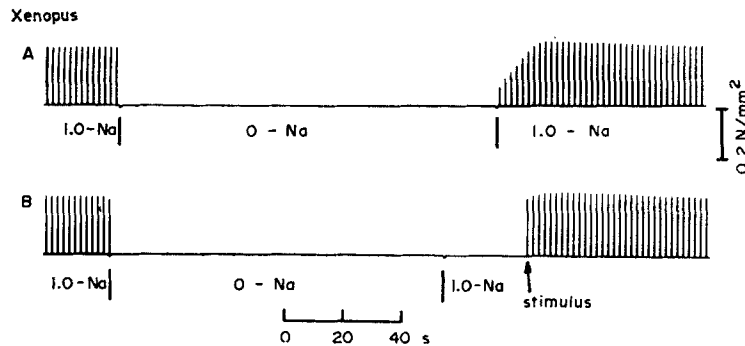


FIGURE 3. Changes in isometric twitch tension in a large *Xenopus* fiber produced by changes in  $[\text{Na}]_o$ . O-Na is the Tris-substituted Na-free solution. In A the duration in O-Na solution was about 2 min. (The recovery was found to be the same whether the duration in O-Na was 1 or 2 min in experiments with other fibers.) In B, the stimulus started 30 s after the exchange O-Na  $\rightarrow$  1.0-Na took place. Radius  $76.5 \mu\text{m}$ . Temperature:  $22.0^\circ\text{C}$ .

#### *Cessation of Stimulus: Staircase*

In the above described experiments, while the muscle was in O-Na solution, the fiber did not produce twitches. This quiescent period itself might have some effects on the subsequent twitch recovery, and therefore it is necessary to distinguish the effects of O-Na solution from the effects of the quiescent period. In Fig. 2 A2, the sequence was: 1.0-Na  $\rightarrow$  O-Na  $\rightarrow$  stimulus stop  $\rightarrow$  1.0-Na (31 s)  $\rightarrow$  stimulus start. In Fig. 2 A3, the stimulus was simply turned off, without solution changes, for about 1 min. In both of these cases, when the stimulus was started, one or two twitches at the beginning were slightly large, but they quickly declined to a steady-state level. We will refer to this transient increase of twitch after the quiescent period as "reverse staircase" in analogy with the situation in heart muscle (Hajdu and Leonard, 1959). Therefore, if we could have measured the twitch recovery without the complication from the reverse staircase phenomenon, the time-course might have been slower than observed.

In Fig. 3 B, the sequence was again: 1.0-Na  $\rightarrow$  O-Na  $\rightarrow$  stimulus stop  $\rightarrow$  1.0-Na (30 s)  $\rightarrow$  stimulus start. In this case the slow recruitment of twitch that was seen in Fig. 3 A was practically absent. Thus, the slow recovery in Fig. 3 A represents a direct result of return to 1.0-Na, and is not due to the start of twitch per se after the quiescent period. In other words, the slow recovery of A is different from the "staircase" phenomenon originally described in heart muscle (Bowditch, 1871).

The staircase phenomenon in its original sense was also observed in isolated single fibers. This occurred after a long rest, or when the stimulus was started for the first time after isolating single fibers. As shown in Fig. 4, the twitch

height first declined (reverse staircase), and then very slowly grew during the continuous low frequency stimulus (compare with the case of whole muscle; Mashima et al. 1962). The staircase tended to occur more markedly in frog fibers than in *Xenopus* fibers and was more evident in small fibers than in large ones. In large *Xenopus* fibers (say  $a > 70 \mu\text{m}$ ), staircase was virtually absent. All our experiments of the twitch recovery were performed after the staircase, when present, had reached practically the plateau level.

### Fatigue

Large *Xenopus* fibers, although showing virtually no staircase phenomenon, developed fatigue fairly quickly at low frequency stimulation. Fig. 5 A1 shows the recovery time-course of twitch of a *Xenopus* fiber, which was recorded 5 min after the beginning of the experiment, i.e., 5 min after starting the low

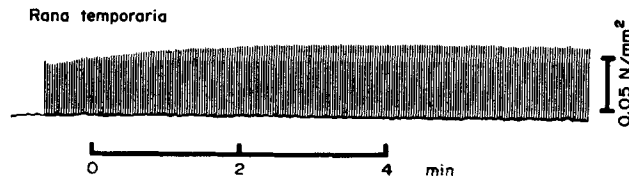


FIGURE 4. Staircase phenomenon in an isolated fiber of *Rana temporaria*. The record shows an initial phase of decline of twitch height (reverse staircase) followed by a progressive increase during the low frequency train of twitches (0.5 times/s). After isolating the fiber, before this experiment was started, only several twitches were evoked. Radius  $60 \mu\text{m}$ . Temperature  $23.0^\circ\text{C}$ .

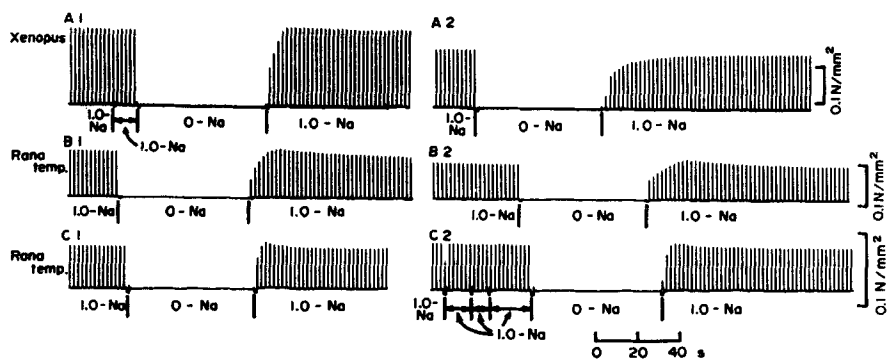


FIGURE 5. Retardation of the twitch recovery by prolonged low frequency stimulation. O-Na is the Tris-substituted Na-free solution. A1, A2: *Xenopus* fiber,  $a = 69 \mu\text{m}$ . B1, B2: *Rana temporaria*,  $a = 84 \mu\text{m}$ . C1, C2: *Rana temporaria*,  $a = 78.5 \mu\text{m}$ . A1, B1, C1 show time-courses of the twitch recovery at an early phase of the experiment (A1 5 min, B1 15 min, and C1 15 min after starting the experiments). A2, B2, C2 are records obtained at a later phase (A2 at 44 min, B2 at 47 min, and C2 at 53 min after the start of experiments). Between the early and late phases, fibers were treated with various concentrations of Na solutions, and were continuously stimulated at 0.5 times/s. Temperature  $22.5^\circ\text{C}$ .

frequency stimulation (the staircase was virtually absent). A2 is a record 39 min after A1 was taken. Between A1 and A2 the fiber was treated with various concentrations of Na solutions, and was continuously stimulated at 0.5 times/s. The twitch height at A2 was reduced to 77% of that at A1. Together with development of the fatigue, the twitch recovery became much slower.

Fatigue and the retardation of the twitch recovery developed less readily in frog fibers. Fig. 5 B and C illustrates two examples of large frog fibers, in which some degree of fatigue (B) or no fatigue (C) developed after repetitive stimulation lasting 32 min (B) and 38 min (C). The twitch recovery was only a little prolonged in both cases. In small fibers (*Xenopus* and *Rana*) both fatigue and the retardation of the twitch recovery hardly developed under similar conditions.

#### *Diameter vs. Twitch Recovery*

In Fig. 6 the relation between fiber radius (in this figure, radius is the value under experimental conditions, i.e., usually under 130%, in several fibers under 115% stretch) and the recovery times of twitch was plotted. The experimental procedure was the same as in Figs. 2 or 3. In fibers that showed the staircase, the recovery times were obtained after the staircase had reached a plateau level. In larger *Xenopus* fibers, since fatigue easily occurred, the values in Fig. 6 were obtained from the first run of the recovery experiment, which was carried out between 5 and 13 min after the start of repetitive stimulation. In a fiber with a very rapid twitch recovery, the present method did not allow an accurate time-course of the recovery to be measured. Thus, in Fig. 6, the recovery times that fell within 1 s (in a few cases 1–1.7 s) were rough estimates by an extrapolation procedure,<sup>2</sup> which would have produced a large error.

In Fig. 6, circles (*Xenopus*) and squares (*Rana*) are the time to reach 90% of the final level, and crosses (+: *Xenopus*, ×: *Rana*), are the half-time of twitch recovery. It is evident that the recovery time becomes slower as fiber size is increased, and its relation is very steep. The recovery in *Rana* fibers is somewhat quicker than that of *Xenopus* fibers.

#### *[Na]<sub>o</sub> vs. Twitch Relation*

In order to analyze the time-course of twitch recovery in terms of the diffusion process of Na ions in the T system, it was essential to know the relationship

<sup>2</sup> The procedure of linear extrapolation was as follows. At  $t = 0$  the twitch height was assumed to be 30% of the final level, since if only the surface action potential occurred, the twitch height was reduced to 30% (Bastian and Nakajima, 1974). We connected this point through the peak of the first twitch recorded after the completion of solution change O-Na → 1.0-Na. In most fibers in which the linear extrapolation was done, the first twitch occurred within  $t = 1.5$  s, and the first twitch was less than 85% of the peak value of twitch. In these cases the extrapolation would have produced an error of less than 40% in estimating the half-time.



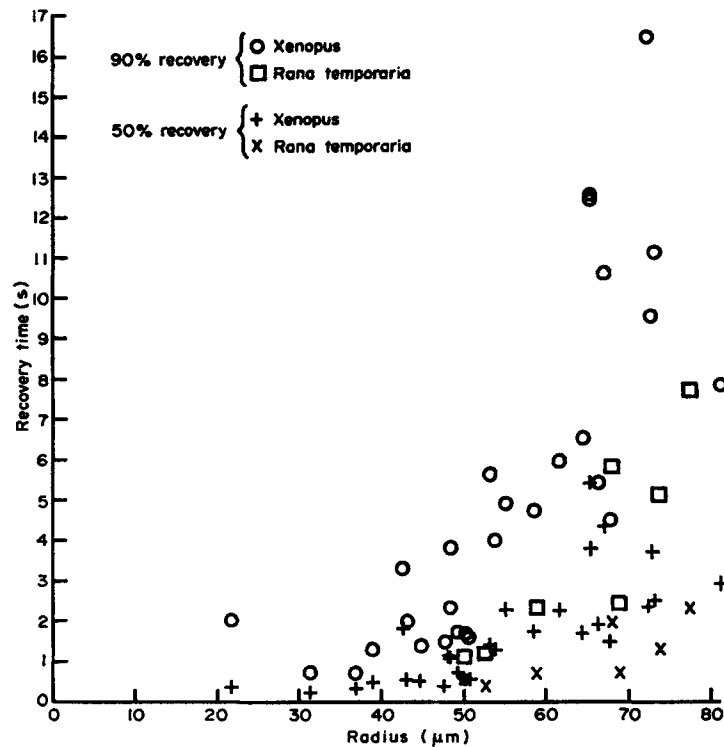


FIGURE 6. Relation between the recovery times of twitch and fiber size. Each value was obtained from the flush sequence like the one shown in Figs. 2 and 3. The duration in O-Na solution was either about 1 min or about 2 min. The Tris-substituted Na-free solution was used. The ordinate is the time for twitch height to reach 90 or 50% of the steady-state level in normal Ringer. The abscissa is the radius under experimental conditions; *i.e.* under 130% stretch (in several fibers 115% stretch). In the preliminary report (Nakajima and Bastian, 1972) fiber diameter was the value at slack length, and the recovery times were in reference to the peak twitch value (the recovery of twitch passes through a peak before going down to a steady value, see Fig. 2): these account for the minor discrepancies of this figure with the preliminary report. Temperature 22.0–24.0°C.

between twitch height and  $[Na]_o$ . In Fig. 7 B twitch height was plotted at varied  $[Na]_o$  in four different fibers. Both  $[Na]_o$  (abscissa) and twitch height (ordinate) were relative values to those at normal Ringer solution. The figure shows that the relation varied greatly from fiber to fiber. Curve 1 is an example of a *Xenopus* fiber ( $a = 53.5 \mu\text{m}$ ), and Fig. 7, A1, A2 illustrates sample records. Curve 1' was taken from a *Rana* fiber  $a = 78.5 \mu\text{m}$ . In these fibers, reduction of  $[Na]_o$  to 0.4 or 0.5 times the normal concentration augmented the twitch height (Mashima and Matsumura, 1962; Grabowski et al., 1972; Caputo and DiPolo, 1973 *b*).

This augmentation was somewhat unexpected. One possible cause is that it is due to a prolongation of action potential at low  $[Na]_o$ . One might also sup-

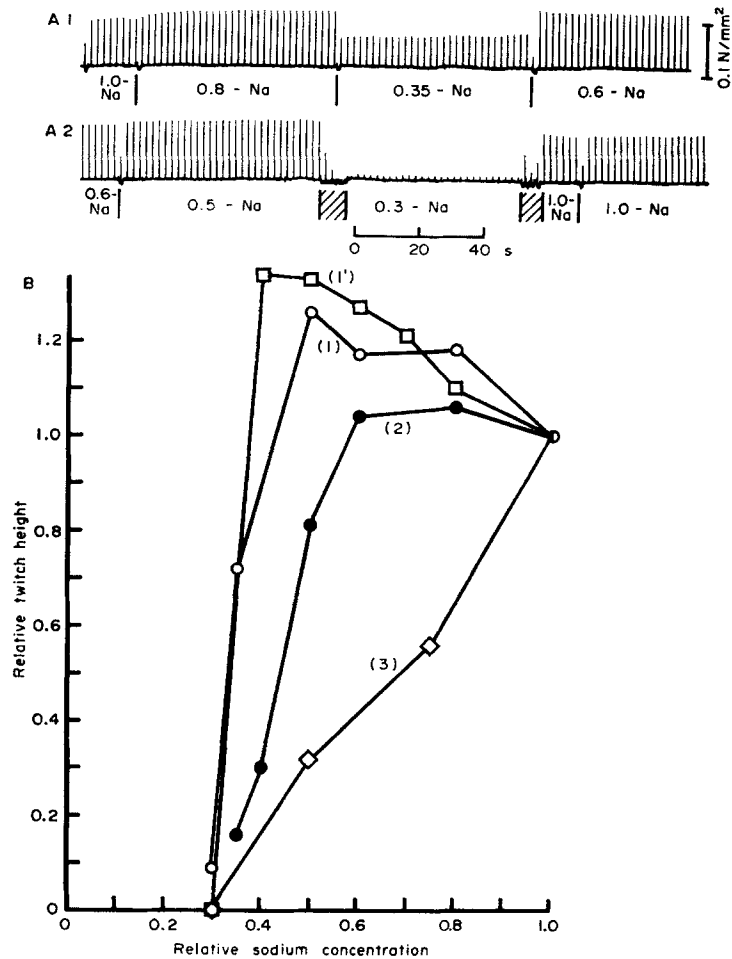


FIGURE 7. (A1 and A2) Effects of varying  $[Na]_o$  on twitch. 1.0-Na is normal Ringer. 0.8-Na means 80% Na, 20% Tris-Ringer solution. The end of A1 is continuous with the beginning of A2. When the solution exchanges 0.5-Na  $\rightarrow$  0.3-Na and 0.3-Na  $\rightarrow$  1.0-Na took place, there was a larger dead space in the exchange system, so that the solution was flushed for a longer time (see the artifact in the record). *Xenopus*;  $a = 53.5 \mu\text{m}$ . (B) Relationship between  $[Na]_o$  vs. twitch. Twitch heights are values at 1 min after solution exchanges. The Na-deficient solutions were made by replacing Na by Tris. Twitch height and sodium concentration are relative values referred to that at normal Ringer. Curve 1: Obtained from records A1 and A2. *Xenopus* fiber,  $a = 53.5 \mu\text{m}$ . Curve 1' *Rana temporaria* fiber,  $a = 78.5 \mu\text{m}$ . Curve 2 *Xenopus* fiber,  $83 \mu\text{m}$ . Curve 3: *Xenopus* fiber,  $a = 74.5 \mu\text{m}$ . Temperature 22.0–24.0°C.

pose that it could be due to an antagonistic action between Na and Ca, like that described in heart muscle (Lüttgau and Niedergerke, 1958). But the latter possibility is unlikely because in skeletal muscle fibers an increase of  $[Ca]_o$  is known to decrease, rather than increase, the twitch height (Caputo and Gimenez, 1967; Frankenhaeuser and Lännergren, 1967).

When  $[\text{Na}]_o$  was reduced to 0.3, the twitch height became very small. In many fibers, the twitch height at  $[\text{Na}]_o = 0.3\text{--}0.5$  was unstable and changed slowly or fluctuated during successive stimulation (see Fig. 7 A1 at  $[\text{Na}]_o = 0.35$ , and Fig. 9 A at  $[\text{Na}]_o = 0.4$  and 0.5). The unstable nature of twitch is understandable because of the steep  $[\text{Na}]_o$  vs. twitch relation over this region. Thus, the twitch height at this region could not be uniquely determined. In plotting Fig. 7 B, we arbitrarily chose the twitch height at 1 min after the change of solution.

Results with two large *Xenopus* fibers are plotted in curve 2 ( $a = 83 \mu\text{m}$ ) and curve 3 ( $a = 74.5 \mu\text{m}$ ) of Fig. 7. It is seen that in these fibers when  $[\text{Na}]_o$  was lowered twitch tension started to decrease at higher values of  $[\text{Na}]_o$ . There was a definite tendency for small *Xenopus* fibers to show a relation like curve 1 and for large *Xenopus* fibers to give a curve like 2 or 3. In the case of frog muscle, even large fibers tended to show a relationship like curve 1. The cause for the differences in the  $[\text{Na}]_o$  vs. twitch relation among fibers of different diameters is not known, and deserves further investigation.

#### *Twitch Recovery as Determined by Ionic Diffusion*

We are now able to explain qualitatively the very steep relationship between radius and the twitch recovery time (Fig. 6). The radius would affect the time-course of twitch recovery in three different ways. First, in large fibers, the diffusion time is longer, the time being proportional to the second power of radius. Second, in small fibers,  $[\text{Na}]_o$  vs. twitch relation is like the one in curve 1 of Fig. 7 B. Thus, when the sodium concentration in the T system attains 0.4–0.5 times the normal level, an almost full recovery of twitch will be reached. On the other hand, in large fibers, the  $[\text{Na}]_o$  vs. twitch relation being of the type of curve 2 or 3, for the full recovery to be attained the solution inside the T system has to be more completely replaced by normal Ringer solution. Third, there would be a moment immediately after the completion of the solution exchange  $0\text{-Na} \rightarrow 1.0\text{-Na}$ , when the surface membrane produces a full-size action potential, whereas the T system, being still devoid of sodium, is largely inexcitable. Under these conditions the T-system membrane will be depolarized by electrotonic spread of the surface action potential, and the fiber will produce a small twitch (about  $\frac{1}{3}$  of normal twitch in 100- $\mu\text{m}$  diameter fibers, Bastian and Nakajima, 1974). The *relative* height of this small twitch would be, at least in theory, larger in the smaller fiber because of a more effective electrotonic spread of depolarization toward the axis. (In the paper by Bastian and Nakajima, 1974, this relationship was not apparent, since the range of fiber size used was not wide enough.) Thus, in small fibers the recovery starts from a relatively higher level, and this will contribute to the rapid recovery.

### Comparison of Choline-Ringer and Tris-Ringer

In four fibers we compared the effects of choline Ringer with those of Tris-Ringer. (In two experiments out of the four, all the solutions contained  $10^{-5}$  g/ml D-tubocurarine: the results were essentially the same as those done without D-tubocurarine.) An example is shown in Fig. 8. To our surprise, the time-course of twitch recovery was quicker when the fiber had been treated by choline Ringer than by Tris-Ringer (compare A and B). This problem was partially solved by observing  $[\text{Na}]_o$  vs. twitch relation. When choline was used to replace Na, the twitch at low  $[\text{Na}]_o$  was far larger than when Tris was used.

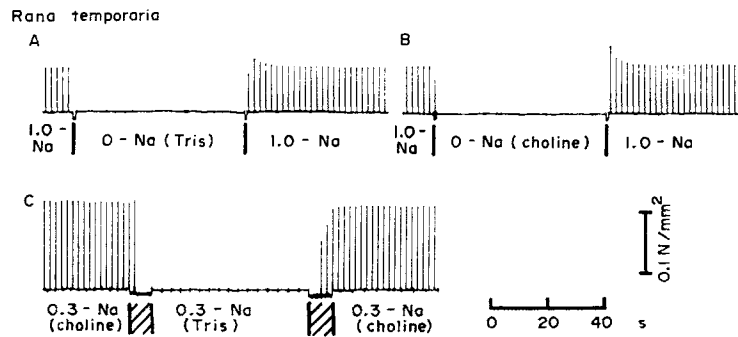


FIGURE 8. Comparison of Tris-chloride and choline chloride. A and B show that when choline is used to replace Na in the O-Na solution, the recovery is quicker. (C) At 30% Na concentration, when Tris was used to replace Na, there was no twitch, whereas with choline a larger than normal twitch was obtained. In C the solutions were exchanged for a longer time because of a larger dead space in the exchange device. The tiny spikes regularly seen in the record C are artifacts caused by the suctioning device.  $a = 60 \mu\text{m}$ .  $23.0^\circ\text{C}$ .

In the example of Fig. 8, the fiber did not produce any twitch at 0.3-Na when Tris was used to substitute for Na, whereas it produced a larger than normal twitch at 0.3-Na when choline was used (Fig. 8 C). Whatever the cause for this twitch augmentation by choline is, this phenomenon seems to offer an adequate explanation for the quickness of the twitch recovery. When the fiber is treated with choline-Ringer, a 30% saturation of the T system with normal Ringer is all that is needed to produce a full recovery. This would be one of the reasons why Hodgkin and Horowicz (1960 *b*), who used choline, observed a very quick twitch recovery.

### Determination of Apparent Diffusion Constant

The above explanation for the very steep relationship between fiber size and twitch recovery can become somewhat more quantitative by calculating the apparent diffusion constant of sodium in the T system. Assuming the shape of

single fiber to be a circular cylinder, if a substance at a concentration  $C_o$ , the concentration being zero previously, is suddenly introduced into the external solution at zero time, the concentration  $C$  of this substance inside the fiber (actually inside the T system) at a distance  $r$  from the axis of the fiber is (Eq. 45 of Hill, 1928; Eq. 5.22 Crank, 1967):

$$\frac{C}{C_o} = 1 - 2 \sum_{n=1}^{\infty} \frac{J_o\left(\frac{r\alpha_n}{a}\right) \exp(-T\alpha_n^2)}{\alpha_n J_1(\alpha_n)}, \quad (1)$$

where

$$T = \frac{D't}{a^2}, \quad (2)$$

and  $a$  is radius,  $t$  is time,  $D'$  is the effective diffusion constant of the substance inside the fiber,  $J_o$  and  $J_1$  are Bessel functions of the first kind, and  $\alpha_n$  is the  $n$ th root of  $J_o(\alpha_n) = 0$ .

The average concentration,  $\bar{C}$ , of the substance inside the fiber divided by  $C_o$  is the degree of saturation, and is given by (Eq. 47 of Hill, 1928):

$$\frac{\bar{C}}{C_o} = 1 - 4 \sum_{n=1}^{\infty} \frac{\exp(-T\alpha_n^2)}{\alpha_n^2}. \quad (3)$$

An easy way to calculate the diffusion constant  $D'$  is to make use of Eq. 3. We have computed  $T$  for round values of  $\bar{C}/C_o$  by numerically solving Eq. 3. The results are tabulated in Table I (the values of  $\bar{C}/C_o$  for round values of  $T$  were given by Hill, 1928).

In the example shown in Fig. 9 A<sub>1</sub>-A<sub>3</sub>, the twitch height at  $[\text{Na}]_o = 0.6$  was 0.93 times the twitch height in normal Ringer. It can be seen from A<sub>1</sub> that when 1.0-Na was suddenly restored, after the fiber had been treated by O-Na solution, the twitch height reached this 0.93 level (the level  $h$  in A<sub>1</sub> and A<sub>2</sub>) at 9.7 s; thus  $t = 9.7$  s. At this moment the average concentration of Na inside

TABLE I  
VALUE OF SATURATION ( $\bar{C}/C_o$ ) vs. NORMALIZED TIME ( $T = D't/a^2$ ).  
FROM EQ. 3 (HILL'S EQ. 47)

$\bar{C}/C_o$	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
$T$	0.00205	0.00470	0.00855	0.0137	0.0202	0.0282	0.0379	0.0495	0.0631
$\bar{C}/C_o$	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95
$T$	0.0790	0.0976	0.1195	0.1453	0.1764	0.2147	0.2643	0.3344	0.4543

Values of  $\bar{C}/C_o$  for round values of  $T$  were given by Hill (1928).

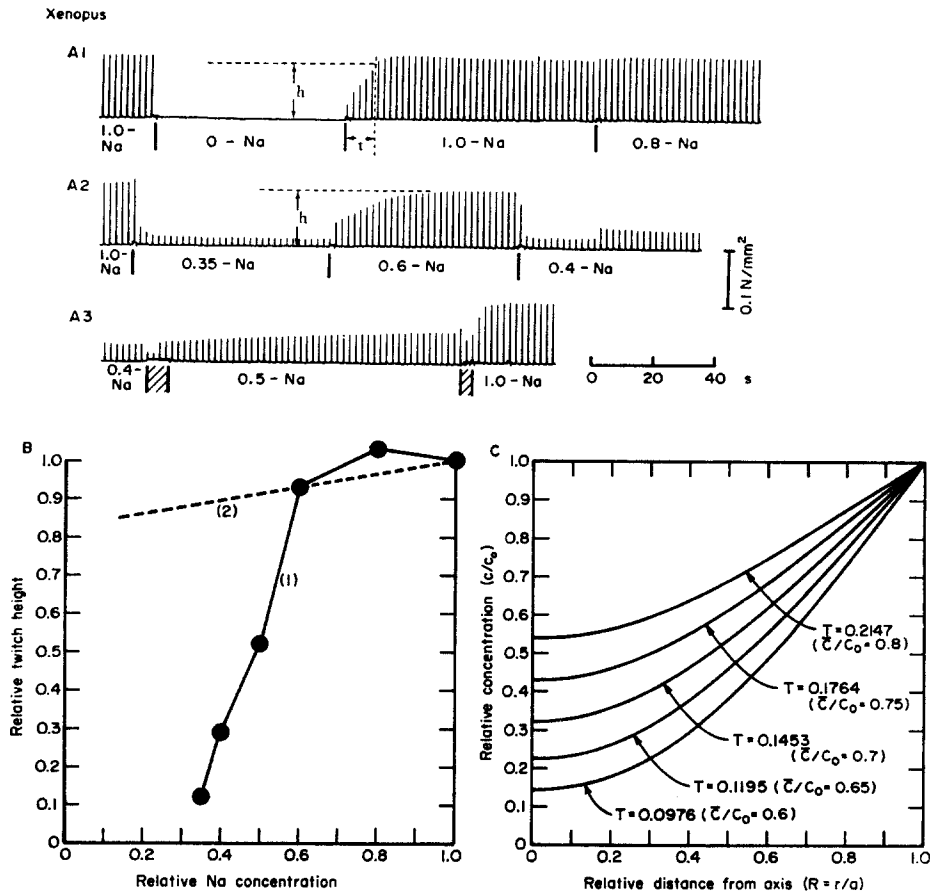


FIGURE 9. (A) experiment to determine  $[\text{Na}]_o$  vs. twitch relation. The end of A1 and A2 are continuous with the beginning of A2 and A3, respectively. In A3 solution exchanges took place for longer times because of larger dead spaces. See text and the legend for Fig. 7 for further explanation. The Na-deficient solutions were made by replacing Na with Tris. *Xenopus* fiber,  $a = 83 \mu\text{m}$ . Temperature =  $22.0^\circ\text{C}$ . (B) curve 1,  $[\text{Na}]_o$  vs. twitch relationship obtained from record A. For curve 2 see text. (C) Profile of the relative concentration of a substance suddenly introduced in the outside at zero time at five different values of  $T$  (corresponding to five different values of  $\bar{C}/C_o$ ), computed from Eq. 1.

the fiber would roughly be 0.6 that of normal Ringer. From Table I,  $\bar{C}/C_o = 0.6$  corresponds to  $T = 0.0976$ . The radius  $a$  of this fiber (when stretched to 130% of the slack length), was  $72.8 \mu\text{m}$ . Putting these values of  $t$ ,  $T$ , and  $a$  into Eq. 2, the diffusion constant is:

$$D' = \frac{(72.8 \times 10^{-4})^2 \times 0.0976}{9.7} = 0.53 \times 10^{-6} \text{ cm}^2/\text{s}.$$

However, the above simple method of obtaining  $D'$  assumed that the relation between  $[\text{Na}]_o$  and the twitch height was linear over a certain range of  $[\text{Na}]_o$ . Since the relationship is not linear, a more involved analysis is necessary. Curve 1 of Fig. 9 B shows the  $[\text{Na}]_o$  vs. twitch height of the fiber shown in Fig. 9 A. The series of curves in Fig. 9 C was computed from Eq. 1, illustrating the profiles of relative concentration ( $C/C_o$ ) inside the fiber at five different values of  $T$ , when the outside concentration is suddenly changed from zero to  $C_o$ , or more generally, when the concentration is changed from  $C_1$  to  $C_o$ , the ordinate being  $(C - C_1)/(C_o - C_1)$ . The abscissa is the relative distance from axis of fiber ( $R = r/a$ ). These values of relative concentration at any  $R$  can be converted into the relative twitch height  $F$  using the  $[\text{Na}]_o$  vs. twitch relationship shown in curve 1 of Fig. 9 B. (The relation in Fig. 9 B is that at steady state. The use of the steady-state relation might cause some errors; see Discussion.) This conversion was done over the whole range of  $R$  with the increment of  $R$  set at 0.01 (in some fibers 0.02) at a fixed value of  $T$ . Now from these local values of twitch height  $F$ , the relative value of average twitch height  $\bar{F}$  of the whole fiber was computed by equation:

$$\bar{F} = 2 \int_0^1 RF \, dR. \quad (4)$$

The factor 2 arises because an annulus between  $R$  and  $R + dR$  is a fraction  $2R \cdot dR$  of the total area. The numerical intergration was done by a subroutine based on Simpsons-Newton's rule.

In the example of Fig. 9 B curve 1,  $\bar{F}$  became 0.65 at  $T = 0.0976$  ( $\bar{C}/C_o = 0.6$ ). Now from Fig. 9 A1, the time for the twitch height to reach the level of 0.65 was determined, and found to be  $t = 6.2$  s. Since  $a = 72.8 \mu\text{m}$ , from Eq 2:

$$D' = \frac{(72.8 \times 10^{-4})^2 \times 0.0976}{6.2} = 0.83 \times 10^{-6} \text{ cm}^2/\text{s}.$$

Table II lists the values of  $D'$  determined in this way.<sup>3</sup> Instead of  $\bar{C}/C_o =$

<sup>3</sup> The simple method of obtaining  $D'$  using Eq. 3 assumed a linear relationship between  $[\text{Na}]_o$  and twitch. In the example discussed, the  $[\text{Na}]_o$  vs. twitch relation was assumed to be line 2 of Fig. 9 B instead of curve 1. We have computed the average twitch  $\bar{F}$  at  $\bar{C}/C_o = 0.6$  with the same program, except now the conversion from  $[\text{Na}]_o$  into twitch height was done according to line 2 instead of curve 1. The average twitch  $\bar{F}$  turned out to be 0.9299 ( $\cong 0.93$ ) as expected. This constitutes a verification of our programs; the procedure numerically validated Eq. 3 from Eq. 1.

Any value of  $\bar{C}/C_o$  could be used to compute  $D'$ . However, if it is too large (say more than 0.8),  $\bar{F}$  becomes close to 1.0, and thus an accurate determination of recovery time  $t$  becomes difficult. If it is too small, say less than 0.6, it is difficult to determine the value of  $t$  precisely in small fibers, in which the recovery is very rapid. Furthermore, at small values of  $\bar{C}/C_o$ , the concentration near the axis becomes so small that action potential would not occur, and thus the value of local twitch height ( $F$ ) cannot be determined precisely. The values in Table II were calculated using  $\bar{C}/C_o = 0.65$ , which was a compromise between the above two requirements. With  $\bar{C}/C_o = 0.65$ ,  $C/C_o$  is less than

TABLE II  
APPARENT DIFFUSION CONSTANT FROM TWITCH RECOVERY

Fiber	$a$	$D'$ (at $\bar{C}/C_o = 0.65$ )	90% Recovery time
<i>Xenopus</i> Group A ( $a \geq 60 \mu\text{m}$ )	$\mu\text{m}$	$10^{-6} \text{ cm}^2/\text{s}$	$\text{s}$
HH-34	72.5	2.2	4.5
HH-35	87	1.81	7.8
HH-36	62.5	1.38	4.7
HH-37	78.5	0.91	11.1
HH-43	83	0.80	9.5
Mean	76.7	1.42	7.5
<i>Xenopus</i> Group B ( $a < 60 \mu\text{m}$ )			
HH-38	51	(2.5)	(1.4)
HH-39	57.5	1.28	(1.6)
HH-40	56	1.75	(1.7)
HH-41	59	0.94	4.9
HH-42	57.5	0.89	4.0
HH-45	53.5	1.32	1.7
HH-46	45.5	0.48	3.3
HH-47	42	(2.0)	(0.7)
Mean	52.8	1.40	2.4
<i>Rana temporaria</i>			
TP-2	77.5	0.79	5.8
TP-3	78.5	1.60	2.4
TP-4	84	0.60	5.1
TP-7	88.5	1.03	7.7
Mean	82.1	1.01	5.3
Mean of total		1.31	

The values in parenthesis were obtained by the linear extrapolation procedure, and thus have a large error. (See footnote 2.) The means include the values in parenthesis. Temperature: 22.0–23.5°C.

0.6, we adopted  $\bar{C}/C_o = 0.65$  in Table II (reasoning in footnote 3). Each value of  $D'$  was obtained by one measurement of twitch recovery bracketed by two sets of measurements of the  $[\text{Na}]_o$  vs. twitch relation. Group A of *Xenopus* are large fibers ( $a \geq 60 \mu\text{m}$ ), all of which showed a slow recovery of twitch, average time to reach 90% level being 7.5 s. The average value of  $D'$  was about

0.35 over the inner 37% of fiber radius (Fig. 9 C). Thus,  $F$  was treated as zero in this region in the case of Fig. 9 B. In other words, a possible small activation from the periphery by passive electrotonic spread was neglected. However, 37% of radius means 14% of the area, and the value of  $F$  in this area would be less than 0.12 ( $F$  at  $C/C_o = 0.35$ ). And neglecting the passive spread would result in an error of  $\bar{F}$  of less than about 2%. With  $C/C_o = 0.70$ , the error is smaller. In Group A of *Xenopus* fibers, we also computed the values of  $D'$  using  $C/C_o = 0.70$ , and it gave  $D' = 1.38 \times 10^{-6} \text{ cm}^2/\text{s}$ .



$1.4 \times 10^{-6}$  cm<sup>2</sup>/s. Group B of *Xenopus* consists of small fibers, most of which showed a rapid recovery. The value of  $D'$  was about  $1.4 \times 10^{-6}$  cm<sup>2</sup>/s. The data of *Rana temporaria* (large fibers only) gave  $D' = 1.0 \times 10^{-6}$  cm<sup>2</sup>/s. The mean of the total 17 fibers was  $1.31 \times 10^{-6}$  cm<sup>2</sup>/s.

#### *Fatigue and $D'$*

As mentioned above, larger *Xenopus* fibers easily fatigued. It was also mentioned that as the fiber fatigued, the twitch recovery upon restoration of Na became slower. As fatigue ensued we also noticed that  $[\text{Na}]_o$  vs. twitch relation gradually changed. Fibers showing a relationship of the type of curve 1 in Fig. 7 B at the beginning, changed into the type of curves 2 or 3 as fatigue developed. Thus, at first, we suspected that the change of  $[\text{Na}]_o$  vs. twitch relation was solely responsible for the development of the slower recovery of twitch.

In three large *Xenopus* fibers,  $D'$  was computed not only at the beginning of the experiment (9–11 min after the start of stimulation), but also well after the fatigue developed (about 20 min after the start of stimulation,  $a$  ranging from 62.5–87  $\mu\text{m}$ ).  $D'$  in these fatigued fibers was decreased to a value about 40% of that at the early stage. In frog muscle, reflecting the fact that fatigue did not develop as easily as in *Xenopus* muscle,  $D'$  decreased only by about 20% under similar conditions (two large frog fibers; radius 77.5 and 78.5  $\mu\text{m}$ ). These results indicate that the change of the  $[\text{Na}]_o$  vs. twitch relationship alone cannot explain the retardation of the twitch recovery that occurred as the muscle fatigued.

#### *Diffusion Constants Obtained from K-Repolarization*

Hodgkin and Horowicz (1960 *a*) and Nakajima et al., (1973) have observed that a sudden reduction of  $[\text{K}]_o$  in single muscle fibers produces a slow repolarization (with a half-time of a few seconds), which probably reflects a wash-out process of potassium ions from the T system. The data in Table I A of Nakajima et al. (1973) shows that the average half-time of repolarization is 2.3 s in the fiber of  $a = 51.5 \mu\text{m}$  ( $a$  at stretched condition = 45.2  $\mu\text{m}$ ), when  $[\text{K}]_o$  was suddenly reduced from 165 to 40 mM in the absence of chloride. The membrane potential  $V$  and  $\log [\text{K}]_o$  is linearly related, (Adrian, 1956; Hodgkin and Horowicz, 1959), and the average value of  $V$  at  $[\text{K}]_o = 165$  mM is 0 mV, and that at  $[\text{K}]_o = 40$  mM is  $-30$  mV (from the original data of Nakajima et al., 1973). Thus, the membrane potential (in millivolts) is:

$$V = 48.75 \log [\text{K}]_o - 108.1. \quad (5)$$

From Eq. 5 the half-time ( $V = -15$  mV) would correspond to the average K concentration in the T system of 81.2 mM. This means that at this moment exchange of 40-K solution by 165-K solution was 67% complete ( $[81.2 - 165]/$

[40 – 165] = 0.67). From Eq. 3 it was computed that  $\bar{C}/C_o$  of 0.67 corresponded to  $T = 0.1293$ , and from these data  $D' = (45.2 \times 10^{-4})^2 \times 0.1293/2.3 = 1.15 \times 10^{-6} \text{ cm}^2/\text{s}$ .

However, the above is a very rough estimation. First, as pointed out by Nakajima et al. (1973), the membrane potential is determined not only by the electromotive force of the T system ( $V_T$ ) but also by the electromotive force of the surface membrane. Thus, immediately upon the reduction of  $[\text{K}]_o$ , there was a quick small component of repolarization, reflecting a situation, where the electromotive force of surface membrane was already fully repolarized to  $-30 \text{ mV}$ , while that of the T system was still at the level of  $0 \text{ mV}$  (since K ions are still outside the T system). This sudden repolarization was followed by a slow repolarization, which reflected the wash-out process of K ions from the T system (see Nakajima et al., 1973, for full explanation). Therefore, the above simple procedure would have underestimated  $t$ , and thus overestimated  $D'$ . Secondly, the conversion of the recordable membrane potential into the average potassium concentration produces an error.

These two factors were taken into consideration in the following computation. First consider the moment when the average concentration of K inside the T system reaches a point of 80% exchange by the 40-K solution ( $T = 0.2147$ ). The profile of concentration is illustrated in Fig. 9 C (in this case the ordinate is  $[\text{C} - 165]/[40 - 165]$ ). Now each value of local concentration  $C$  was converted into local electromotive force,  $V$ , by applying Eq. 5.

Suppose, for the moment, that there is no contribution from the surface membrane, then the membrane potential at the openings of the T system  $V_T$  can be calculated by averaging  $V$ :

$$V_T = 2 \int_0^1 RV \, dR. \quad (6)$$

Eq. 6, which is a simple average of the T-system potential, is an approximation. Parts near the edge will have more effect than the middle. The errors due to this approximation as well as from other sources are discussed in the footnote.<sup>4</sup>

<sup>4</sup> There are several sources of error in the estimation of  $D'$  described here: (a) The ratio of  $G_s:G_T$  was assumed to be 1:7. This value was chosen from the data of low frequency capacity of 100- $\mu\text{m}$  diameter fiber (Hodgkin and Nakajima, 1972 a). If  $G_s$  is assumed to be zero,  $D'$  becomes  $1.26 \times 10^{-6} \text{ cm}^2/\text{s}$  at  $\bar{C}/C_o = 0.7$ . If  $G_s:G_T$  is 1:3,  $D'$  becomes  $0.73 \times 10^{-6} \text{ cm}^2/\text{s}$  at  $\bar{C}/C_o = 0.7$ . (b) It is assumed in Eq. 6 that at the time of 70% or 80% exchange, the membrane at all locations has the same value of conductance per unit area. Actually the membrane near the axis would have a higher conductance (since potassium concentration is higher), and thus contributes more to the overall value of  $V_T$  than the case calculated from Eq. 6. A correction was made in Eq. 6 by introducing a weight factor proportional to the local conductance, the latter being calculated based on the constant field equation (Goldman, 1943; Hodgkin and Katz, 1949). The average value of  $D'$  became  $0.97 \times 10^{-6} \text{ cm}^2/\text{s}$  at  $\bar{C}/C_o = 0.8$  and  $1.24 \times 10^{-6} \text{ cm}^2/\text{s}$  at  $\bar{C}/C_o = 0.7$ . (c) During the course of K repolarization from 0 to  $-30 \text{ mV}$ , inward current would flow through the wall of the tubules, and this would result in a further increase in the conductance of the tubular membrane due to the

TABLE III  
 APPARENT DIFFUSION CONSTANT CALCULATED  
 FROM K-REPOLARIZATION

Fiber	Radius $\mu\text{m}$	$D'$ ( $10^{-6} \text{ cm}^2/\text{s}$ )	
		$\bar{C}/C_o = 0.8$	$\bar{C}/C_o = 0.7$
SF33	45.5	0.54	0.64
SF36	59	1.25	1.49
SF38	57.5	1.14	1.27
SF40	50	0.77	0.78
SF43	56	0.81	1.03
SF44	51.5	1.04	0.96
SF45	47.5	0.87	0.87
SF52	46	0.70	0.66
SF53	50	0.77	0.78
Mean	51.4	0.88	0.94

Values were calculated from *Rana temporaria* data (Nakajima et al., 1973). The changes of solution were: 40-K  $\rightarrow$  165-K  $\rightarrow$  40-K all in Cl-free solutions. The repolarization which follows the last solution exchange was analyzed.

The computation of Eq. 6 was carried out with almost the same program as used in the previous section. In the present example  $V_T$  became  $-20.4$  mV. Now the electromotive force of surface membrane ( $V_s$ ) is already  $-30$  mV. Assuming the ratio of surface membrane conductance  $G_s$  to the input conductance of T system  $G_T$  to be 1:7, the overall membrane potential would be  $-21.6$  mV ( $[-30 \times 1 - 20.4 \times 7]/[1 + 7]$ ). A membrane potential of  $21.6$  mV corresponds to a level of  $8.4$  mV ( $30 - 21.6$ ) depolarized from the final potential at 40 mM-K. From the actual data (the fibers listed in Table I A of Nakajima

anomalous rectification. This factor would contribute to the apparent slowness of the K repolarization (Almers, 1972). In order to precisely assess the contribution of anomalous rectification, we need to know the values of potassium conductance at various driving forces at different values of  $[\text{K}]_o$ . Since this information is lacking, we estimated a rough extent of the contribution as follows. With a driving force of 25 mV in hyperpolarizing direction, the conductance was assumed to increase by a factor of 3.1 (Fig. 14 of Adrian and Freygang, 1962), and the conductance was assumed to be linearly related with the driving force over this range. This weight factor due to anomalous rectification was introduced in addition to the weight factor due to the increase of  $[\text{K}]_o$  discussed above in (b). The computation revealed that the average values of  $D'$  became  $1.20 \times 10^{-6}$  at  $\bar{C}/C_o = 0.8$  and  $1.64 \times 10^{-6} \text{ cm}^2/\text{s}$  at  $\bar{C}/C_o = 0.7$ . (d) Another assumption in Eq. 6 is that each local membrane potential propagates to the openings of the T system without decrement. Under our conditions  $a/\lambda_T$  ( $\lambda_T$  is the length constant of the T system) is about 0.75. With this value of  $a/\lambda_T$ , the electrotonic decrement along the T system from surface to the inside is negligible (Adrian et al., 1969), but the decrement from a location deep inside a fiber to the surface may not be negligible. In order to obtain an idea of how much error would be produced by neglecting the decrement, we have constructed a crude model of the T system under the present conditions using resistors. From this model we could see that the error in  $D'$  caused by neglecting the decrement is of a smaller magnitude than, and in the opposite direction to the error discussed in (b) above.

et al., 1973), the time  $t$  to reach 8.4-mV depolarization was measured, and  $D'$  was calculated from Eq. 2. Table III summarized the results. The average value of  $D'$  was  $0.88 \times 10^{-6}$  cm<sup>2</sup>/s (calculated from 0.8 saturation) or  $0.94 \times 10^{-6}$  cm<sup>2</sup>/s (calculated from 0.7 saturation). However, because various sources of errors discussed in footnote 4 are interrelated with each other in a complicated way, the values of  $D'$  in Table III are regarded as hardly accurate. They are probably an underestimate particularly because of the presence of anomalous rectification in the T system, and the true values of  $D'$  could be  $1.5 \times 10^{-6}$  cm<sup>2</sup>/s or somewhat more.

#### DISCUSSION

##### *Effective Diffusion Constant*

The present study has revealed that the effective diffusion constant of Na and K in the T system is about  $1 \sim 1.5 \times 10^{-6}$  cm<sup>2</sup>/s. This is one order of magnitude less than the diffusion constants of Na or K in aqueous solution. The diffusion constant in aqueous solution of 0.1 M NaCl is  $1.48 \times 10^{-5}$  cm<sup>2</sup>/s, and that of 0.1 M KCl is  $1.84 \times 10^{-5}$  cm<sup>2</sup>/s both at 25°C (Robinson and Stokes, 1959). From these data, assuming that the self-diffusion constant of K and Cl are equal, the self-diffusion constant for Na<sup>+</sup> is  $1.2 \times 10^{-5}$  cm<sup>2</sup>/s, and that of K<sup>+</sup> is  $1.8 \times 10^{-5}$  cm<sup>2</sup>/s, both at 0.1 M and at 25°C (cf. Keynes, 1954). Since the transverse tubules form a network inside the fiber, the tortuous geometry would decrease the apparent diffusion constant by a factor of 2.0–2.5 in the same way as it affects the radial luminal conductance of the T system (Adrian et al., 1969; Schneider, 1970; Barry and Adrian, 1973). Even after this tortuosity factor, together with the effect of longitudinally oriented tubules (Peachey and Schild, 1968), is taken into account, our effective diffusion constant is still smaller than that in the free solution by a factor of about 2.5–6.

Before accepting the conclusion that the apparent  $D'$  is really small, additional sources of error, other than those already mentioned, should be considered: (a) When the action potential changed its size, due to changes in  $[\text{Na}]_o$ , the change of twitch might not have followed immediately. In other words, in calculating  $D'$  from the twitch recovery, the steady-state relation between twitch and  $[\text{Na}]_o$  was used, although the relation might have been slightly different while the fiber was recovering. (b) The finding that when the fiber fatigued, the diffusion constant became less is puzzling. Perhaps this is related with the phenomenon recently reported by Gonzalez-Serratos et al. (1974) that fiber diameter increases by as much as 30% during fatigue. Whatever the causes of the decrease of  $D'$  by fatigue, it could be argued that the values of  $D'$  of large *Xenopus* fibers are less reliable since even in fresh fibers some degree of the unknown causes existed, yielding lower values of  $D'$ . (c) In small fibers the twitch recovery took place so rapidly that nonsystematic

errors in determining the recovery time were large. Also the presence of "negative staircase phenomenon" contributed to the error, but neglecting the latter factor would result in an overestimation of  $D'$ . (d) When NaCl was restored in the external solution, after the fiber had been preloaded with Tris chloride (or choline chloride) since the diffusion constant of Tris is less than that of Na, Tris chloride was unable to move out from the T system as quickly as NaCl entered. Thus, the T system would transiently swell, and would result in an apparent reduction of  $D'$ . (e) In the case of the K-repolarization experiment, since it was performed in Cl-free solution, the T system might have swollen (Girardier et al., 1963; Freygang et al., 1964), and if the swelling was uneven along the T system, it would have produced an apparent reduction of  $D'$ . (f) The value of apparent  $D'$  might become smaller by the presence of a barrier between the fluid and membrane or a delay time for the adsorption of ions on the T-system membrane. This factor seemed to be very small for the surface membrane, because, as illustrated in Fig. 1, the action potential quickly changed as  $[Na]_o$  was altered. Potential changes caused by  $[Cl]_o$  changes take place quickly (Hodgkin and Horowicz, 1960 *a*). Also potassium de- or repolarization takes place quickly in the glycerol-treated fibers (Nakajima et al., 1973).

It is noted that among the above-mentioned sources of errors, *a-d* all apply only to the experiments of twitch recovery, (e) applies only to the K-repolarization experiment, and (f) applies to both types of experiment. Unless these factors are properly evaluated, we cannot say for certain about the real causes for the low values of  $D'$ . Nevertheless, the results suggest a possibility that there are additional factors that contribute to lowering the value of  $D'$ . It could be that ions flow really slowly in the T system, or it could be related with a more complex anatomy of the tubular system. These may be the common causes for the observation of a slow movement of fluorescent dye in the T system (Endo, 1966). Also they might be related to the suggestions of the low values of specific conductance ( $G_L$ ) of the luminal fluid (Schneider, 1970; Hodgkin and Nakajima, 1972 *b*; Valdiosera et al., 1974), or to the data which were interpreted as indicating the presence of access resistance<sup>5</sup> near the openings of the tubules (Peachey and Adrian, 1973; Adrian and Peachey, 1973; Barry and Adrian, 1973).

#### *Excitability of T system*

In spite of the considerable errors and uncertainty involved in deriving the values of  $D'$  in the T system, the puzzling finding that the recovery of twitch

<sup>5</sup> The results of Table II that the values of  $D'$  are not much different between small and large fibers may seem to invalidate the idea that the presence of access resistance is a cause for the smallness of  $D'$ . However, as mentioned, the value of  $D'$  in small fibers is subject to large errors, and therefore the present results are not strong enough to rule out the possibility of the presence of access resistance.

in some fibers is apparently too fast to be explained by the diffusion delay, seems to be resolved. Accepting the value of  $D'$  at  $1.3 \times 10^{-6}$  cm<sup>2</sup>/s, main features of the twitch recovery seem to be explained on the basis of diffusion time, although the actual time-course of the recovery was more complex than we had expected because of the complication involving negative staircase, staircase, and fatigue.

Calculating in the reverse way, with this value of  $D'$ , in the case of a fiber of  $a = 50$   $\mu$ m, having the  $[\text{Na}]_o$  vs. twitch relationship shown in curve 1 or 1' of Fig. 7 B, the 90% recovery is reached in 1.9 or 1.8 s, and thus the half-time would be less than 1 s. If choline chloride, instead of Tris-chloride, is used to substitute for Na, the recovery would be further accelerated. With a fiber of  $a = 80$   $\mu$ m, having the  $[\text{Na}]_o$ -twitch relation of curve 2 or 3 of Fig. 9 B, the 90% recovery becomes 7.3 or 21 s. Thus, the very quick recovery, as well as the slow one, cannot be regarded as invalidating the idea of the excitable T-system membrane. It should be noted, however, that the present results by themselves cannot be regarded as evidence for the excitability of the T system. All that can be said is that the results are not in conflict with the idea that seems to have already been established.

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