PROPAGATION SPEED IN MYELINATED NERVE

I. EXPERIMENTAL DEPENDENCE ON EXTERNAL Na^+ AND ON TEMPERATURE

W. L. HARDY

From the Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle, Washington 98195. Dr. Hardy's present address is the Department of Physiology, Boston University School of Medicine, Boston, Massachusetts 02118.

ABSTRACT Conduction speed (θ) in single myelinated *Rana pipiens* sciatic nerve fibers has been precisely measured using intracellular recording and on-line digital computer techniques. The dependence of relative speed on external Na concentration at 15°C has been found to be $\ln(\theta_1/\theta_2) = 0.524\ (\pm 0.018)\ \ln\ ([Na^+]_1/[Na^+]_2) + 0.003$. Thus θ has very close to a square root dependence on $[Na^+]_0$ for these fibers. This experimental finding is not in complete agreement with a theoretical prediction based on a solution of the Hodgkin-Huxley (H.H.) equations. The effect of small temperature variations around 15°C on θ has also been measured for *Rana* fibers in Ringer's solution. θ has close to an exponential dependence on T and a Q_{10} of 2.95 has been estimated.

INTRODUCTION

The sodium hypothesis, formulated by Hodgkin, Huxley, and Katz, predicts that a reduction in the $[Na^+]$ gradient across the membrane of an excitable cell will reduce inward flowing I_{Na} during activity and cause a decrease in the rate of spread of activity along the membrane. While this prediction is readily confirmed qualitatively, a detailed quantitative test on single nerve cells has never been published. In one approximate measurement reported by Hodgkin and Katz (1949), the conduction speed of a squid giant axon bathed in a solution containing half the normal $[Na^+]_0$ was found to be reduced to approximately 0.7 of normal. (NaCl in artificial seawater was partially replaced by dextrose.)

Colquhoun and Ritchie (1972), in a recent paper, report that the relative conduction speed of the compound action potential (AP) of nonmyelinated (*C*) fibers of the rabbit cervical vagus varies approximately with the logarithm of the relative $[Na^+]_0$.

Conduction speed measurements are described here for propagation in single myelinated sciatic nerve fibers of the frog, *R. pipiens*, bathed in solutions of various $[Na^+]_0$. A second paper (Hardy, 1973) describes the predicted effect of $[Na^+]_0$ on

speed, calculated from a complete numerical solution of the H.H. equations, as modified by Dodge (1961, 1963), for *R. pipiens*.

METHODS

Preparation

Sciatic nerves 6–7 cm long were dissected from large *R. pipiens* frogs and desheathed. A bundle of fibers was mounted in a constant temperature Lucite perfusion chamber, and the nerve was stimulated at distances 3–4 cm from the intracellular recording site. The extracellular compound AP was simultaneously recorded from the distal end of the nerve bundle. Fibers were not identified as being either motor or sensory.

Stimulation Parameters

The nerve was stimulated at a constant rate of 2.6 impulses per second during periods of speed measurement. The duty cycle was usually 12 or 24 s of stimulation every 50 s. Stimulus pulse duration was 200 μs and amplitude was approximately 10% suprathreshold. The amplitude had to be varied (generally increased) by less than 10% during the course of any one set of measurements on a given single fiber.

Stimulus pulses were delivered through two identical stimulus isolation transformers alternately to each of two pairs of silver wire stimulus electrodes spaced 0.25 cm apart; this is just greater than the internodal separation of the largest *Rana* fibers. Similar pairs of mho-shaped transverse wires were spaced along the entire length of the nerve, partially surrounding and suspending it in the perfusion fluid. The nerve bundle was held down in this framework by six small Lucite feet fitted to project down between the wires and just contact the upper surface of the bundle.

The two cathodes of the pairs of stimulus electrodes were usually 1 cm apart; in a few experiments a 0.75 or 1.25 cm separation was used. The two stimulus cathodes were located at least 2 and 3 cm from the intracellular recording site. The pairs of stimulus electrodes were not changed during an experiment with a given fiber. Uncertainty in the measured separation of the cathodes ($\pm$0.05 cm) would affect the determination of absolute conduction speed but would not affect the measuring of ratios of speeds for a fiber bathed in different solutions; only the inverse ratios of conduction times are needed.

Recording Techniques

Electrical activity was measured with hand-pulled glass microelectrodes inserted into single fibers. A neutralized capacity input amplifier was used to improve frequency response. The nerve bundle at the recording site was raised and held just at the surface of the perfusion fluid to permit continuous recording during solution flow. The compound AP was monitored at the distal end of the nerve by means of a glass suction electrode. The common ground for intracellular and extracellular recording was a silver wire electrode located between the stimulating and recording sites. Conduction time was measured as the difference in arrival time at the single intracellular recording electrode of APs propagating from the two proximally located stimulus sites. Only the properties of the nerve fiber in the space between the stimulus electrodes determine the calculated conduction speed; nonuniform ion concentrations or temperature variations outside of the interstimulus region cannot have affected the determination of relative speed. Also, damage at the recording site is unlikely to have affected recorded speed.

On-Line Computer Technique

Extensive use was made of a small on-line computer (LINC) to perform the experiment, gather, analyze, display, and store the data. The stimulus pulse was generated by the computer through a D-to-A converter and the stimulus site was selected by computer-controlled pairs of fast relays. Thus, the site of stimulation, the stimulus rate, duration, and intensity were all precisely controlled and these parameters and others were stored by the computer with the recorded data. To reduce noise in the data, four or eight APs generated at each stimulus site were averaged. Up to 32 APs were averaged in noisy records.

At its maximum A-to-D conversion rate, the shortest sample and store interval of the classic LINC is 32 μs, an interval much too long for an adequate resolution of the rising phase of an AP, even at 15°C. An 8 μs sample interval was simulated by combining four sets of data, each set obtained with a precise 8 μs offset from the previous. Averaging of data was done after the interleaving process. Thus, for any single record from which conduction speed was determined, generally 32 or 64 but occasionally up to as many as 256 individual APs were combined. The data presented here represent approximately ¼ million individual APs.

Conduction Time Calculations

Conduction time was estimated by three different computer methods:

(*a*) An on-line determination was made within a second of data acquisition so that optimally timed solution changes could be made. The LINC located the center of area of the middle three-quarters on the graphs of the rising phase of the APs arising from the two stimulus sites. The difference in time of occurrence of these was the estimated conduction time between the stimulus sites. This time was immediately displayed by the computer on a CRT along with other numerical parameters and plots of the averaged data. Conduction time measured in this way was generally consistent (to ± 8 μs) with that found by two slower but slightly more dependable methods: occasionally, however, it was in error as could be visually judged by the location of the point displayed by the computer for the center of area. The error could be attributed to a nonsteady base-line voltage just before an AP; this resulted in the computer mislocating where the rising phase began.

(*b*) All reported conduction times were reevaluated using an interactive computer program. Pairs of displayed APs were brought to superposition by manually adjusting two potentiometers which shifted one AP in the x and y directions. The magnitude of the x axis shift gives the conduction time directly. The method is simple, accurate, and requires only about 5 s per record (including acquisition and restorage of data on magnetic tape).

(*c*) Some of the conduction times were verified by means of a computer cross-correlation program. The lag required to obtain the maximum cross-correlation between pairs of APs gave a conduction time which differed, at most, by ± 8 μs from that obtained by the visually controlled superposition method.

Perfusion Solutions

$[Na^+]_o$ was varied in experimental solutions by replacing NaCl by choline Cl, tetramethylammonium Cl (TMCl), or Na_2SO_4. Since conduction through an axon depends, in part, on the resistance of both the surrounding and internal circuit elements, it is essential that the resistance of these elements remain unchanged during perfusion with solutions of various $[Na^+]_o$. Solutions with a higher $[Na^+]$ generally also have a higher conductivity. They were mixed with a solution similar to frog Ringer's but with NaCl totally replaced by sucrose. Relative tonicity of final solutions differed by less than $\pm 4\%$ (measured on an Advanced

TABLE I

COMPONENTS AND PROPERTIES OF EXPERIMENTAL SOLUTIONS

Solution	1 *a*	2 *a*	3 *a*	4 *a*	5 *a*	6 *a*	1 *b*	2 *b*	3 *b*	4 *b*	5 *b*	6 *b*
Relative [Na^+]	1	¾	½	¼	<3%	5/4	1	¾	½	¼	<3%	5/4
Na^+	102.2	77.3	52.5	27.6	2.7	127.9	110.0	83.2	56.3	29.5	2.7	137.3
$Choline^+$	0	29.3	58.7	88.0	117.4	0	0	0	0	0	0	0
TMA^+	0	0	0	0	0	0	0	29.3	58.7	88.0	117.4	0
Cl^-	106.6	110.0	114.6	119.0	123.5	6.1	113.4	115.9	118.4	120.9	123.5	6.1
$SO_4^=$	0	0	0	0	0	62.6	0	0	0	0	0	68.3
Sucrose	35.8	26.8	17.9	9.0	0	47.0	20	15	10	5	0	30
Mean conductance $g \times 10^6$ mho/cm	875	874	879	879	885	865	979	971	971	974	977	980
Mean pH	7.22	7.23	7.27	7.30	7.32	7.30	7.32	7.27	7.32	7.35	7.40	7.31

A phosphate buffer (1.5 mM/liter) was used to maintain solution pH at 7.3 ± 0.1. K^+ and Ca^{++} concentrations were 2.5 and 1.8 mM/liter respectively, in all solutions. Free Ca^{++} would likely have been somewhat less, particularly in solutions where the principal anion was $SO_4^=$.

osmometer [Advanced Instruments, Inc., Needham Heights, Mass.]) and conductivities differed by less than ±2% (measured with a conductivity cell and bridge, Table I). The concentrations of all constituents in each solution were calculated after the conductivity equalizing process.

Choline Cl (Eastman Kodak Co.) was not recrystallized, but it was dessicated. Stock solutions made with different batches of choline varied in conductivity by less than 3%. When choline completely replaced the Na of frog Ringer's ("Chol-Ringer's"), the solution was found to have a conductance slightly less than TMA-Ringer's. Thus, in experiments using TMA-Ringer's, less sucrose solution had to be added to the higher [Na^+] solutions to equalize conductivities.

Na^+ Activity

Estimations of ionic activities in mixed solutions cannot, as yet, be made with great precision. The Na^+ activity coefficient γ_{Na} in most of the NaCl solutions used in my experiments was about 0.78 ±0.08 at 15°C (estimated from data for pure NaCl solutions, Robinson and Stokes [1965] Appendix 8.9).

Reduction of [Na^+] in a pure NaCl solution by simple dilution with water results in an increase in γ_{Na}. However, the replacement of NaCl by choline Cl or TMACl in the solutions probably limits this increase in γ_{Na}. If the variation of γ_{Na} caused by the replacement of

Na^+ by choline$^+$ or TMA^+ is no greater than that which occurs when NaCl is replaced by HCl at a constant total ionic concentration, then over the range of $[Na^+]_0$ used, γ_{Na} for the various solutions should have varied due to the partial replacement of Na^+ by choline$^+$ or TMA^+ by no more than 2 or 3% (Robinson and Stokes, 1965; Fig. 15.1).

A greater variation in γ_{Na} would be expected for the Na_2SO_4 solution. Baker et al. (1962 *a*) used Na_2SO_4 and K_2SO_4 solutions in internally perfused squid giant axons and measured both Na^+ and K^+ activity coefficients using ion-selective glass electrodes. Later (Baker et al., 1962 *b*) these authors returned to using Cl^- solutions because of uncertainties in the activity measurements). γ_{Na} in the SO_4 solutions used in my experiments before addition of the sucrose solution (see below) is probably about 0.70 $\pm$0.05. This estimate is based on the activity of Na^+ in Na_2SO_4 solutions taken from the data of Baker et al. (1962 *a*) and an estimate of the activity coefficient of Na_2SO_4 of a 0.1 M solution taken from the data of Eggars et al. (1964) and Robinson and Stokes (1965).

An additional variation in γ_{Na} may be expected to result from the dilution of the higher $[Na^+]_0$ solutions with the sucrose solution (Table I). There is little information available concerning effects on activities resulting from a partial dilution of ions by sucrose molecules. Chandler and Hodgkin (1965) measured the Na^+ activity of an artificial seawater solution in which sucrose partially replaced NaCl (at constant total molarity). Replacement of approximately one-third of the NaCl by sucrose increased γ_{Na} by about 8.5%. Simple dilution by the addition of one-third more water to a NaCl solution of similar initial concentration would be expected to increase γ_{Na} by just over 4% (Robinson and Stokes, 1965). From these figures, I estimate that the addition of sucrose to the solution I used may have increased γ_{Na} by at most 5%. The Na_2SO_4 solutions had to be diluted with sucrose solutions to a greater extent than the others because of their initial higher conductivity; this probably would increase γ_{Na} of these solutions from 0.70 to perhaps 0.73 or 0.74.

Ca^{++} Concentration

Hodgkin and Horowicz (1959) have noted that the concentration of ionized Ca in a sulfate solution is very limited. The dissociation constant of $CaSO_4$ at 25°C is only 5.3 mM and is only slightly larger at lower temperatures (Robinson and Stokes, 1965). The concentration of free Ca^{++} in the sulfate solutions I used may be estimated at about 0.2 mM/liter provided the added sucrose does not affect the ionization of Ca. Such a low $[Ca^{++}]_0$ would be expected to result in increased excitability of axons and thus increased conduction speed (see Discussion).

The activity of Ca^{++} also may not be constant in the different NaCl solutions. Moore (1968) and Butler (1968) found, using cation sensitive electrodes, that in mixed NaCl and $CaCl_2$ solutions, the $CaCl_2$ activity coefficient decreases when NaCl concentration is increased. Replacement of Na^+ by choline$^+$ or TMA^+ ions rather than the simple reduction of the NaCl concentration might be expected to modify any increase in Ca^{++} activity, but the variation of γ_{Ca}, although expected to be small, nevertheless is uncertain.

Perfusion System

Solutions were gravity fed from six oxygenated flasks through a drip system designed to preclude cross-contamination. The nerve chamber volume was 1.2 ml and solution flow rate 0.5 ml/min. Approximately 35 s were required for a dye-marked solution to pass from the intracellular recording site to beyond the two stimulus sites. The sequence of solution changes was partially randomized; however an axon was usually first impaled while in the standard Na solution.

Temperature Control

Since conduction speed is very temperature sensitive (see Fig. 5), temperature was closely controlled and was monitored. All solutions and the nerve chamber itself were maintained at 15.0 ±0.5°C. Variation of temperature along a nerve fiber was less than ±0.1°C.

RESULTS

Electrodes of extremely high resistance were found to be most satisfactory for prolonged impalements. These were not chosen for low tip potentials and therefore

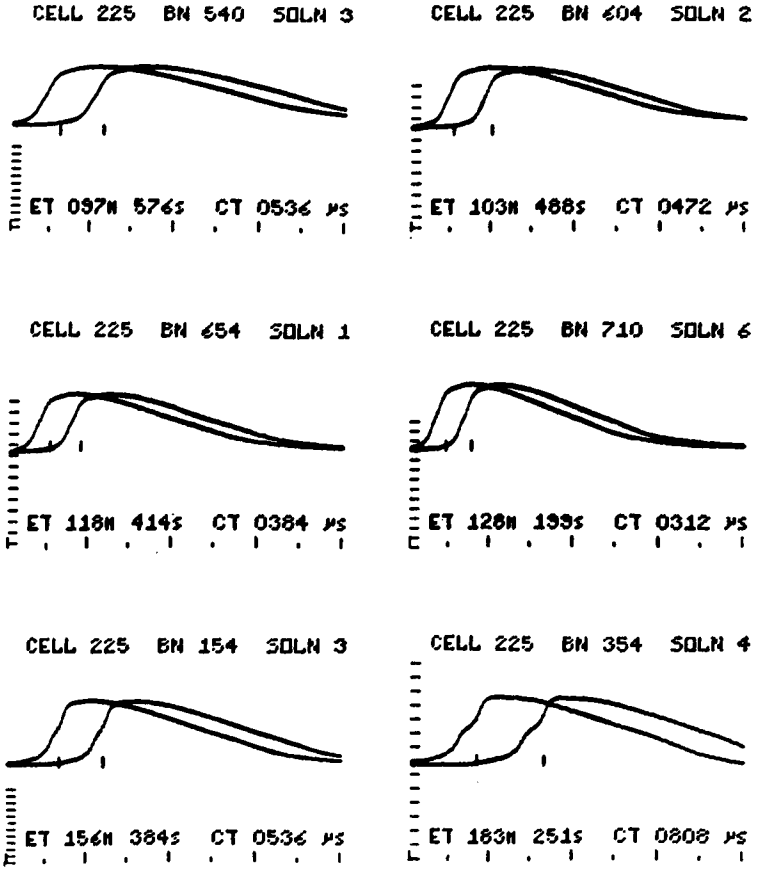

FIGURE 1 Examples of experimental records photographed directly from the computer display. Each record shows the membrane potential for a 4 ms period a few (2 or 3) milliseconds after stimulating the nerve at each of two locations. Each graph is the composite of 32 separate APs. The ordinate scale is 10 mV (calibrated separately for each record). The lower right-hand set of numbers is the calculated conduction time between the two stimulus sites (1 cm apart). The two lower left sets of numbers are the elapsed time from the beginning of the experiment (M, minutes) and since the last solution change (S, seconds). Solution 1 is 110 mM/liter $[Na^+]_o$. The others: 3 is ½, 2 is ¾, 6 is ⅝, and 4 is ¼ the concentration of solution 1.

absolute values of potential are not certain. In most successful impalements, resting potentials (RPs) were not large initially and generally did not improve with time. Most recorded values were in the range of -10 to -30 mV; the largest was -68 mV. A positive DC potential ($+10$ to $+30$ mV) was frequently found on penetrating a fiber and often it persisted. Likely the tip of the electrode rested inside the myelin sheath and not in the axoplasm. In all cases, however, only positive-going monophasic APs were recorded with the microelectrode. Splitting of APs, as described by Woodbury (1952), was frequently seen. APs generally were 20–60 mV in height initially, although a few were over 110 mV and others were under 5 mV (Fig. 1). APs which initially were large (>60 mV) often deteriorated in 10–30 min to a height of 10 mV or less, presumably due to membrane damage by the electrode. APs which initially were small generally maintained their size more dependably. Conduction time calculated by the computer was invariant when APs suddenly deteriorated from 20 mV or more to less than 0.5 mV.

Conduction speed, measured in 116 cells bathed in normal $[Na^+]_o$ at 15°C, ranged from 5 to 38.5 m/s. A histogram of the speeds shows a bimodal distribution with maxima at 17 and 27 m/s (Fig. 2) (see Discussion). 42 fibers were held long enough to permit more than two solution changes. Nine cells were held for an hour or more and one was held for 5½ h.

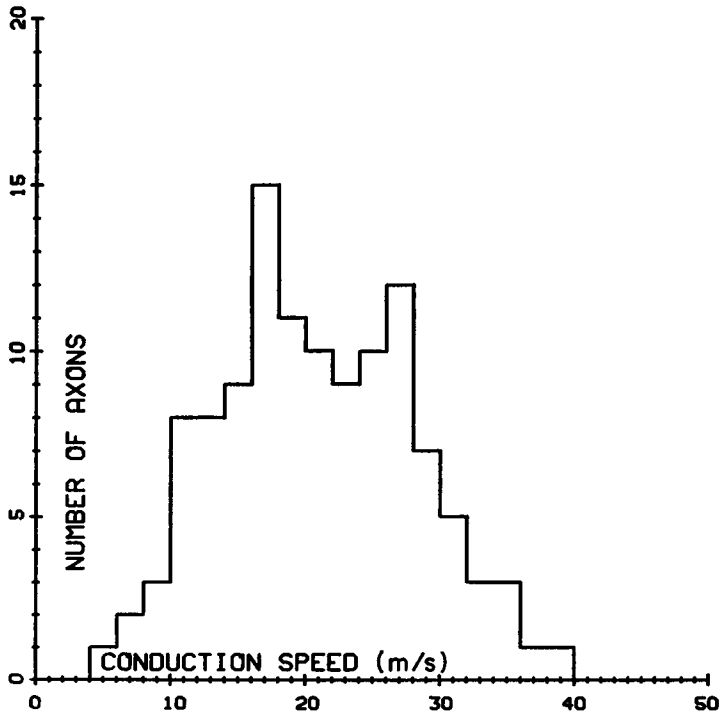

FIGURE 2 Conduction speed histogram of *R. pipiens* sciatic nerve fibers in normal $[Na^+]_o$ at 15°C.

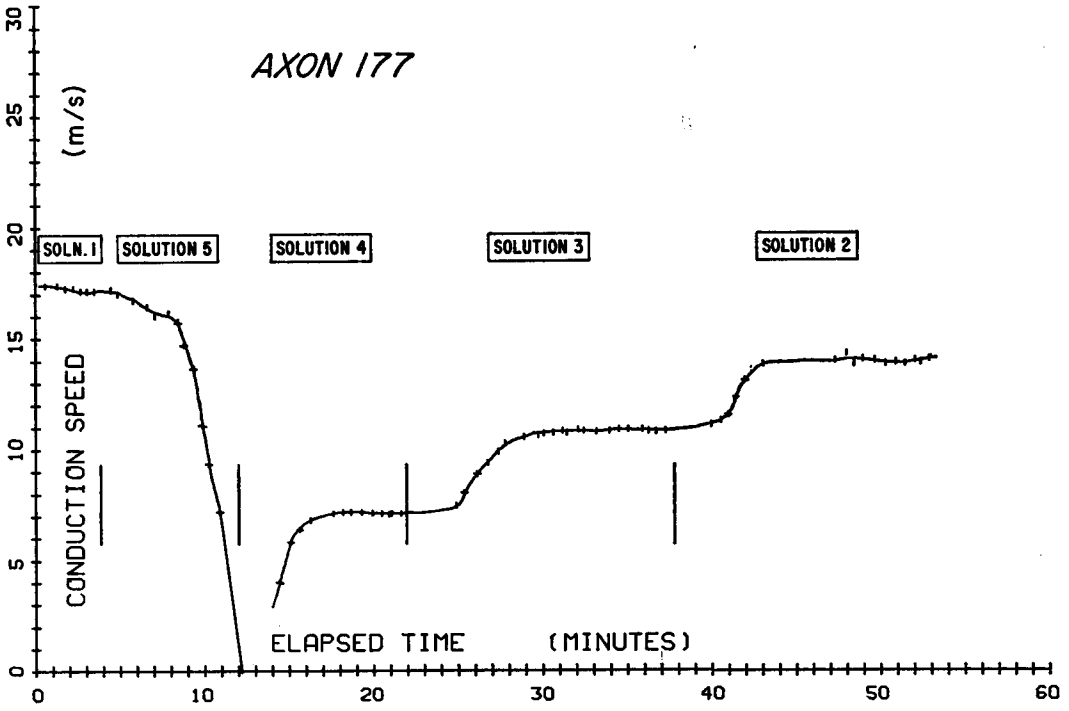

FIGURE 3 Conduction speed of a single *R. pipiens* sciatic nerve fiber at 15°C. Bars indicate start of solution changes. Solutions, given in Table I, have the following approximate relative $[Na^+]_o$: solution 1, 1; solution 5, $\frac{1}{20}$; solution 4, $\frac{1}{4}$; solution 3, $\frac{1}{2}$; solution 2, $\frac{3}{4}$.

After a solution change, a change in conduction speed generally appeared after 1–3 min. This time is consistent with the time constant for washout of Na^+ from the extracellular space (Hurlbut, 1965). The time required for conduction speed to reach a new steady state varied widely for different fibers; it was never less than 2 min. Conduction always blocked within 3 min after a change to solution 5 *a* or 5 *b* ($[Na^+]_o$ = 2.7 mM/liter). Single fiber excitability generally, but not always, could be detected after a return to a solution of higher $[Na^+]$ (Fig. 3); the compound AP always recovered. Cells were frequently lost during solution changes, probably because of momentary changes in the solution level as the flow rate changed. Continuous changes of conduction speed with elapsed time frequently occurred during perfusion with a given solution and often persisted for periods of 20 min or more. These long duration changes were observed principally after changes to or from solution 4 (one-fourth normal $[Na^+]_o$). Both long-term reductions (Fig. 4 *a*, slope −0.14 m/s per min) and long-term increases (Fig. 4 *b*, slope 0.13 m/s per min) in speed with time were observed.

Diphasic transients in conduction speed after solution changes were also occasionally observed. For instance, after an increase in $[Na^+]_o$, the increase in speed was

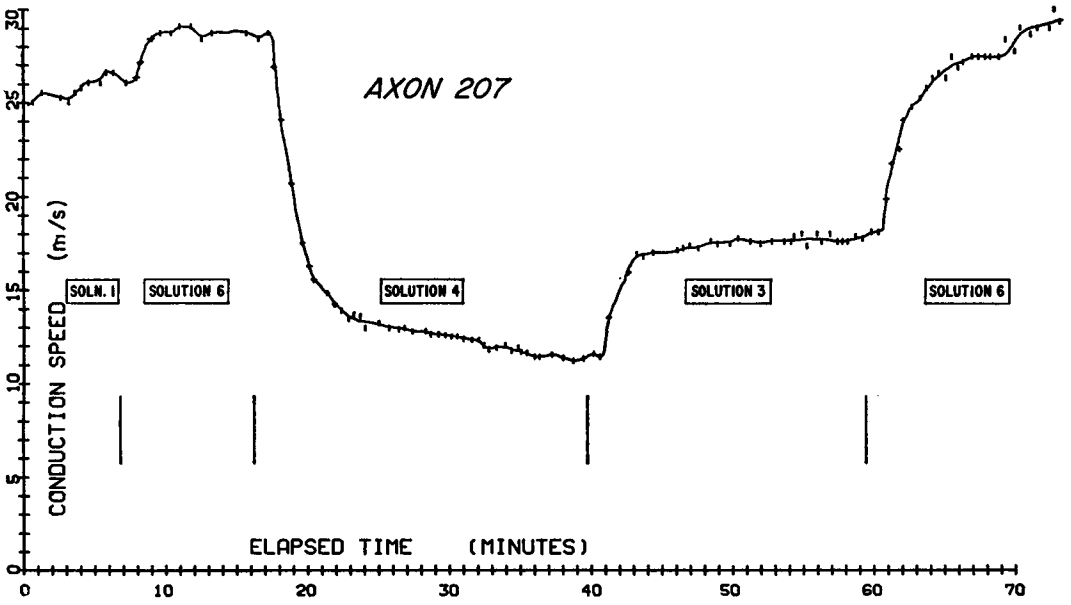

FIGURE 4 *a* Conduction speed of cell 207 at 15°C as a function of elapsed time during changes in $[Na^+]_o$. Slope of the decline in speed in solution 4 is about −0.14 m/s per min. Relative $[Na^+]_o$ are: solution 1, 1; solution 6, ¾; solution 4, ¼; solution 3, ½.

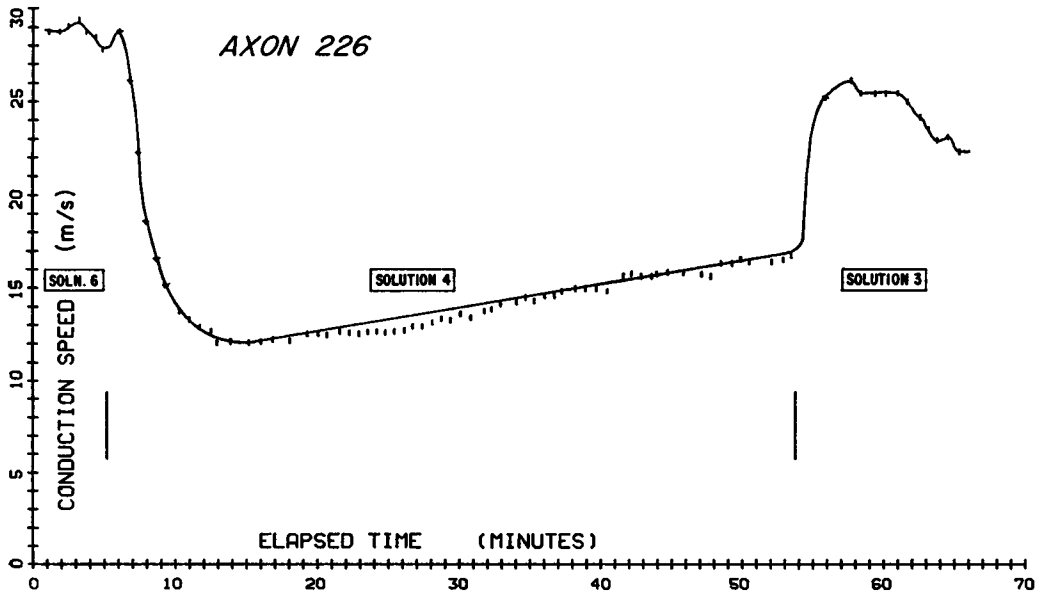

FIGURE 4 *b* Conduction speed of cell 226 at 15°C. Slope of the increase in speed in solution 4 is about 0.13 m/s per min. Relative $[Na^+]_o$ are: solution 6, ¾; solution 4, ¼; solution 3, ½.

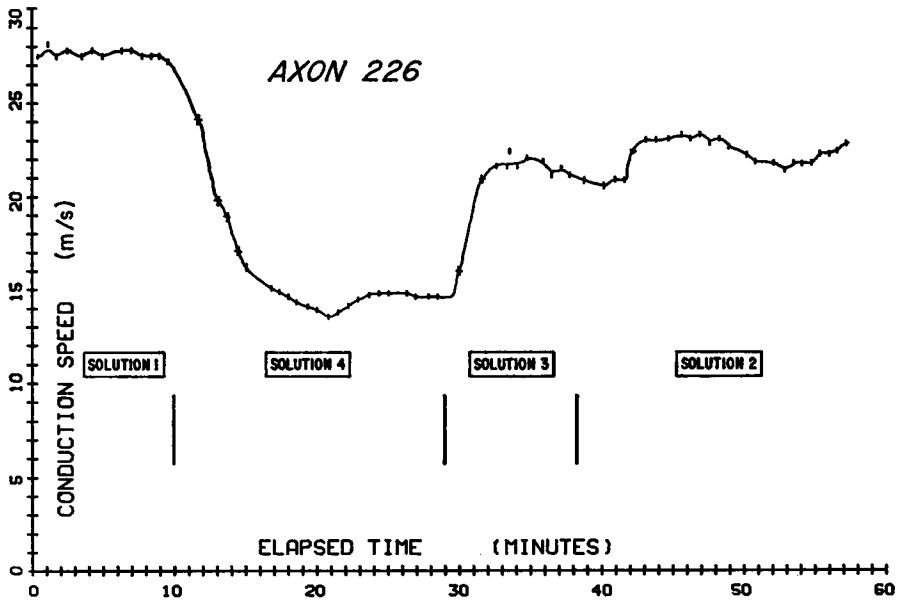

FIGURE 4 *c* Conduction speed of cell 226 at 15°C. This represents a slightly earlier period than shown in Fig. 4 *b*. Relative $[Na^+]_0$ are: solution 1, 1; solution 4, ¼; solution 3, ½; solution 2, ¾.

occasionally followed after a few minutes by a slight slowing (Fig. 4 *c*). The converse effect, an initial slowing followed by a slight rise in speed after a lowering of $[Na^+]_0$, was only clearly identified once (Fig. 4 *c*).

Tests were run to detect other possible factors which might confound the conduction speed measurements. Rapid stimulation for prolonged periods might be expected to alter intracellular ionic concentrations and thus affect propagation speed. One axon in normal $[Na^+]_0$ was stimulated for 5 min at 78/s, 20 times the standard rate. The alteration in speed (a 6% increase) was no greater than the background variations of speed seen both before and after the test period. Changes of other stimulus parameters (pulse intensity and pulse duration) were found to produce insignificant (less than 5%) changes in conduction speed.

Variations of conduction speed were definitely obtained when the flow rates of the perfusing solutions were altered. However, these variations could be completely accounted for by the observed changes in the temperature of the solution flowing past the axon. The temperature of the perfusate declined slightly at higher flow rates.

Effect of Temperature on Conduction Speed

At the usual flow rates the temperature control system held the perfusion solution temperatures to within ±0.5°C of the set value. In order to obtain a factor to correct speeds for the small departures from 15°C, conduction speed was measured as a

function of temperature in the region of 15°C. Conduction speed θ and perfusing solution temperature T were simultaneously measured at approximately 50 sec intervals while the temperature was gradually lowered to 11.5°C and then returned to 15°C. The descending temperature phase lasted 15 min and the ascending phase lasted 21 min (Fig. 5). A linear regression analysis was made of $\ln(\theta)$ as a function of temperature. This analysis gives a Q_{10} of 2.95 $\pm$1.03 (N = 44). Four additional somewhat less detailed evaluations of the Q_{10} of conduction speed for other fibers gave values ranging from 2.42 to 8.90. The latter measurements were made in solutions of various [Na^+], were made over a very limited temperature range (1 or 2°C), and were all made toward the end of the principal experiment. There is some preliminary evidence that the Q_{10} for conduction speed of fibers in solutions of lower $[Na^+]_0$ is higher than for fibers in normal $[Na^+]_0$.

A Q_{10} of 2.95 was used to correct the data to 15°C for the analysis of the variation of conduction speed with $[Na^+]_0$. I estimate that if this value of Q_{10} is incorrect by as much as 50%, the maximum resulting error in the corrected speeds would be 6%. Furthermore, since temperature variations were not correlated with particular solutions, they would not likely introduce a systematic error into the calculated effect of $[Na^+]_0$ on θ.

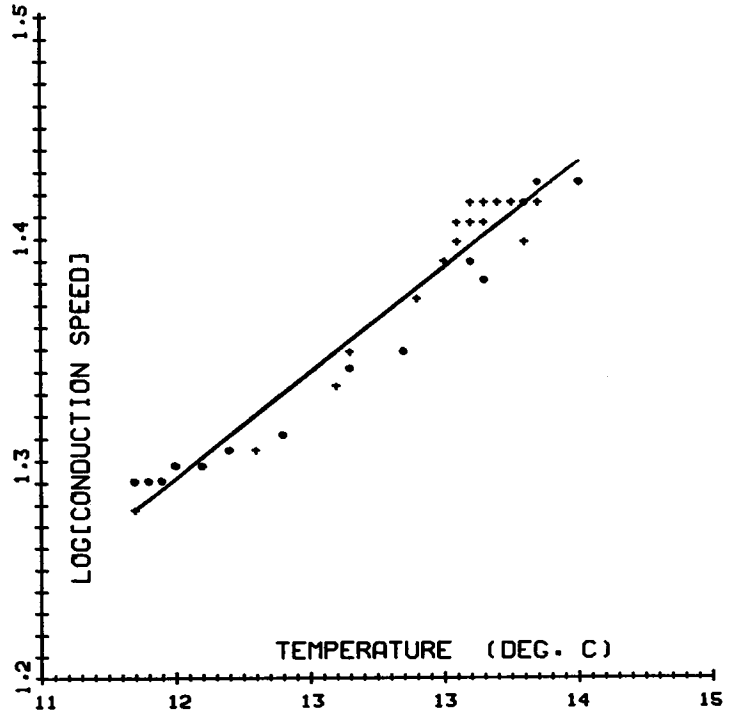

FIGURE 5 Variation in θ of a single myelinated fiber resulting from cooling (●) then warming (+) the bathing solution. The regression line gives a Q_{10} of 2.95.

Variation of Speed with Na

The experimental data for the dependence of conduction speed on $[Na^+]_0$ for different fibers were grouped into two sets for initial analysis, depending on whether the principal Na^+ substitute during an experiment was choline or TMA^+. Since the fibers recorded from have a wide range of absolute speeds, depending on geometrical and possibly other cell properties, the only meaningful way to combine data for statistical analysis is to use the ratios of speeds (θ_1/θ_2) for a given fiber in different solutions. A preliminary analysis showed that $\ln(\theta_x/\theta_1)$ varies linearly with $\ln([Na^+]_x/[Na^+]_1)$, where $[Na^+]_1$ is the standard external $[Na^+]_1$ (solution 1 *a* or 1 *b*). A more extensive comparison of all the data can be made by taking ratios of speeds for all available pairs of solutions, even though this introduces some redundancy.

Figs. 6 *a* and 6 *b* show the effect on conduction speed resulting from a partial replacement of NaCl by choline Cl and by TMACl, respectively. (Note that log scales are used on both axes.) The data were fitted using a regression analysis program and the slopes and intercepts from this analysis are compiled in Table II. There is little doubt that a power relationship, $\theta_1/\theta_2 = ([Na^+]_1/[Na^+]_2)^m$, fits the data very well.

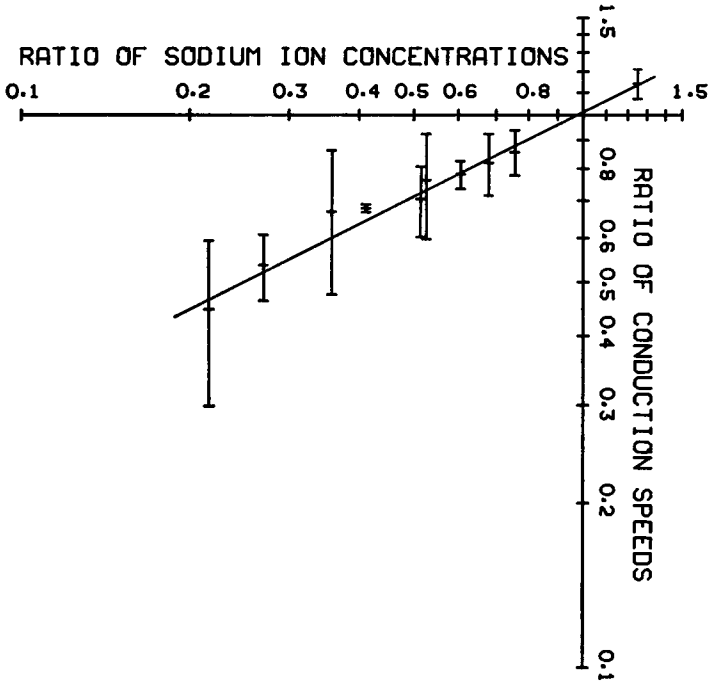

FIGURE 6 *a* The effect on relative conduction speed resulting from partial replacement of external NaCl by choline Cl. Temperature 15°C. Bars indicate ±SE. The least squares regression line is shown.

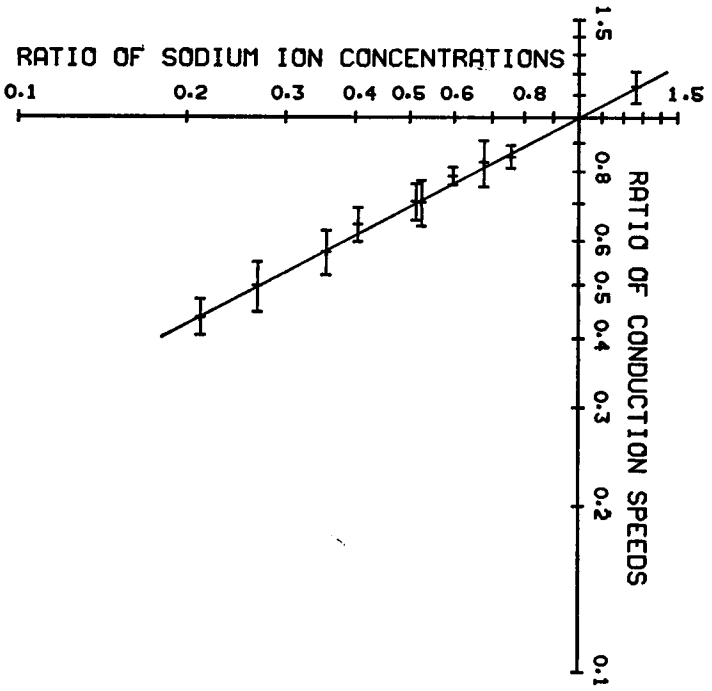

FIGURE 6 *b* The effect on relative conduction speed resulting from partial replacement of external NaCl with TMACl. Temperature 15°C.

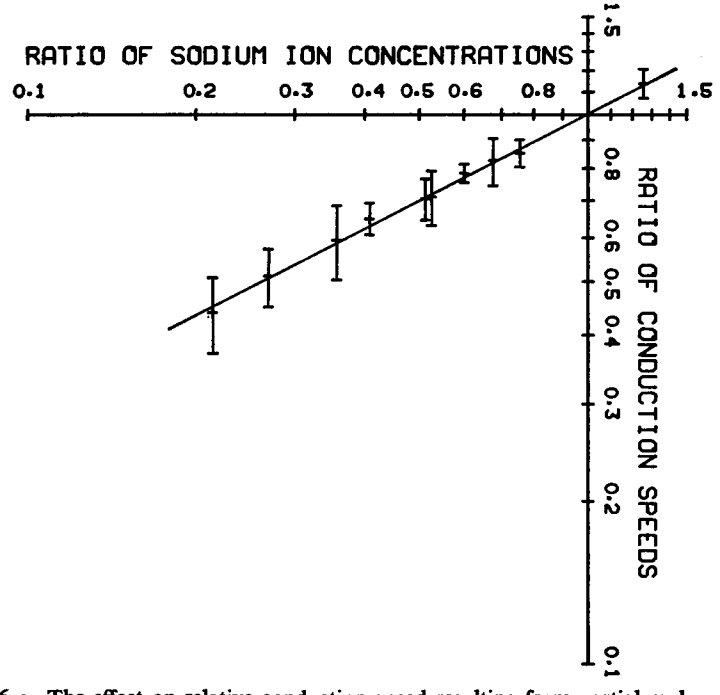

FIGURE 6 *c* The effect on relative conduction speed resulting from partial replacement of external NaCl with choline Cl or TMACl. Temperature 15°C.

TABLE II

COMPILED RESULTS FROM REGRESSION ANALYSIS

$\ln(\theta_1/\theta_2) = \ln([Na^+]_1/[Na^+]_2)m + b.$

Solution	No. of ratios N	Correlation coefficient r	Slope and standard error of slope $m \pm SE$	Ordinate intercept b
Choline	34	0.907	0.502 ± 0.042	0.011
TMA	88	0.956	0.534 ± 0.018	0.002
Combined	122	0.937	0.524 ± 0.018	0.003

More scatter is apparent in the data derived using choline Cl replacement of NaCl than using TMACl replacement, but there is little indication that the dependence of conduction speed on $[Na^+]$ differs significantly between the two experimental conditions. The slopes of the two curves (0.502 using choline replacement and 0.534 using TMA replacement) differ by less than the standard error of their mean (0.046). The two sets of data have been combined in Table II and are shown in Fig. 6 *c*. Regression analysis of the combined data gives $m = 0.524 \pm 0.018$ ($n = 122$). The coefficient of correlation of $\ln(\theta_1/\theta_2)$ with $\ln([Na^+]_1/[Na^+]_2)$ is 0.937. I conclude that conduction speed in *R. pipiens* myelinated nerve fibers has close to a square root dependence on the external Na^+ concentration.

DISCUSSION

Solution Effects

There is little indication that the ratios of conduction speeds obtained where one solution is Na_2SO_4 departs significantly from the plotted regression curve despite the expected lower γ_{Na} (and γ_{Ca}) in sulfate solutions. If γ_{Na} for the Na_2SO_4 solution is lower than for the NaCl solutions, points on the graphs for speed ratios where the denominator was obtained using the Na_2SO_4 solution should be above the regression line. Just over half of such data points on the three graphs were above the regression line; however, only a single point was at a distance greater than a standard error. The uppermost point plotted on each of the three graphs was from data in which the numerator of the ratio was for the Na_2SO_4 solution. These points are almost exactly centered on the regression lines.

A reduction of $[Ca^{++}]_0$ from 1.8 to 0.2 mM/liter can be represented by a −18 mV shift in both τ_m and τ_h (Hille, 1968). A theoretical study (Hardy, 1969) has shown that a −5 mV shift of τ_m, τ_h, and τ_n would be expected to increase θ by about 1.5 m/s (independent of $[Na^+]_0$) while a +5 mV shift would decrease θ by about 2.0 m/s. Thus a −18° mV shift might be expected to produce a 4.5–5 m/s increase in speed for a "standard axon" in a Na_2SO_4 solution over and above the increase due to the greater $[Na^+]_0$. No evidence of such an increase in speed can be seen in the experimental data (Fig. 6). The experimental results using the Na_2SO_4 solution

do not depart significantly from the regression line, but one could argue that since all three parameters (τ_m, τ_h, τ_n) were shifted in the theoretical study, the conditions are not comparable. Hille (1968) and Moore (1971) both found that τ_n for myelinated nerve in normal $[Na^+]_0$ is relatively independent of $[Ca^{++}]_0$. In contrast, Brismar and Frankenhauser (1972) report that in high $[K^+]_0$ low $[Na^+]_0$ solutions an e-fold increase in $[Ca^{++}]_0$ produces a 3 mV positive shift of n_∞ along the potential axis. In either case, however, it is unlikely that small changes in τ_n will affect θ. Hardy (1969) has shown in a theoretical study that θ for *R. Pipiens* is insensitive to variations in g_K. Setting $\bar{g}_K = 0$ results in only a 2% increase in θ relative to the normal case in which g_K follows its usual time-course.

Fiber Size Spectrum

Conversion factors relating propagation speed to fiber diameter for amphibian sciatic myelinated fibers have been estimated or can be obtained from the data of the following: Tasaki et al. (1943), bullfrog 2.4 m/s per μm; Frankenhaueser and Waltman (1959), single 30 μm *Xenopus laevis* fiber 2.5 m/s per μm; Hutchinson et al. (1970), *X. laevis* 1.8–2.6 m/s per μm. The largest fibers in *R. pipiens* sciatic nerve are about 15 μm in diameter (Gasser and Erlanger, 1927). The fastest speed I recorded (15°C, normal $[Na^+]_0$) was 38.5 m/s. If this were a 15 μm fiber, the conversion factor for it would be 2.6 m/s per μm. The slowest speed recorded (15°C, normal $[Na^+]_0$) was 5 m/s indicating, if the same conversion factor holds, a 2 or 3 μm diameter fiber. Since this fiber was held for over 30 min with a microelectrode of 160. MΩ resistance, it is apparent that even fibers this small can tolerate intracellular microelectrodes.

Gasser and Erlanger (1927) have shown that the spectrum of diameters of *R. pipiens* peroneal nerve fibers is bimodally distributed with maxima at 7.5 and 10.5 μm. Speeds calculated (using a factor of 2.6 m/s per μm) for these peaks would be 19.5 and 27 m/s, in reasonable agreement with the location of the two maxima at 17 and 27 m/s on the speed spectrum (Fig. 2). A comparison of the areas under the two maxima in the spectrum of Erlanger and Gasser and the speed spectrum shows the expected sampling bias; large fibers are relatively more likely to be impaled successfully.

Transients in Conduction Speed

The data of Huxley and Stämpfli (1951) show that the height of both the AP and the RP of frog sciatic nerve fibers undergo low amplitude transients (a few millivolts per hour) when measured in normal frog Ringer's solution after various solution changes. The transients they observed in the AP height (but not in the RP) are likely to be related to the speed transients observed here. The simulation study cited above has shown that conduction speed is relatively insensitive to the potassium conduc-

tion system and to RP. Decreasing the magnitude of the RP by 10 mV (from −75 to −65 mV) causes less than a 2% increase in θ.

Variations of $[Na^+]$ in the axoplasm under the nodal membrane or within the nodal membrane after changes in $[Na^+]$ in the perfusing solution could cause transients in both θ and the height of the AP. Any net shift in axoplasm $[Na^+]$ resulting from influx occurring during activity is unlikely to be significant at the stimulus rate used (averaging less than 1 impulse/s). Asano and Hurlbut (1958, Table 2) stimulated one member of paired *R. pipiens* sciatic nerve bundles at rates of 50–100/s for 2–3 h at 20°C; they found a net increase of $[Na^+]_i$ of only about 8% relative to the unstimulated nerve of the pair. These investigators also found a virtually identical relative decline of the compound AP for a nerve stimulated after an initial 2 h soaking period in Ringer's solution or in a solution in which half the NaCl was replaced by choline Cl. However, there is no doubt that reducing $[Na^+]_0$ does result in a reduction in $[Na^+]_i$. Hurlbut (1963) has shown that both $[Na^+]_i$ and Na^+ efflux are proportional to $[Na^+]_0$ for unstimulated frog sciatic axons (20°C). The time constant for exchange of Na^+ between axoplasm and extracellular fluid is relatively long (100 min) compared with the time fibers were kept in low $[Na^+]$ solutions in my experiment and compared with the durations observed for most, but not all, of the conduction speed transients. Thus, variations in average axoplasm $[Na^+]$ after changes in $[Na^+]_0$ is not likely the principal cause of the conduction speed transients. Nevertheless, there could be localized regions in or near the inner surface of the excitable membrane in which $[Na^+]_i$ is more directly dependent on $[Na^+]_0$.

Connelly (1959) measured both the O_2 consumption and the time-course of the height of the compound AP in frog nerves stimulated at a high rate in normal $[Na^+]_0$. He observed an almost linear decline in the AP with time to about 60% of normal in a period of 1 h. Recovery required several hours. The onset of the rising O_2 uptake had a time constant of 5–8 min, a period that may be related to the diffusion time of metabolites from the internode. Hurlbut (1963) and Segal (1970) have estimated similar times for Na^+ diffusion in the internode assuming that the axoplasm diffusion constant for Na^+ is about the same as that for a free solution. It is likely that some of the transients in conduction speed observed here are related to the pumped efflux of Na^+ when $[Na^+]_0$ is relatively low and to diffusion between the immediate subnodal membrane axoplasm and the internodal axoplasm. The effects of $[Na^+]$ on conduction speed will be discussed in more detail in the following paper.

I am indebted to Dr. J. Walter Woodbury and Dr. Bertil Hille for support and guidance during the course of this work.

Most of the research is from a Ph.D. thesis and was supported by National Institutes of Health

training grants GM-00739 and GM-00260 and by a National Institutes of Health research grant NS-01752 to Dr. Woodbury.
I was also supported by U. S. Public Health Service postdoctoral grant NS-45361.
Computer services were supported by U. S. Public Health Service grant RR-00374 to Dr. T. H. Kehl.

Received for publication 8 October 1972 and in revised form 21 May 1973.

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