J. Physiol. (1970), **208**, *pp.* 279–289
With 5 text-figures
Printed in Great Britain

CONDUCTION VELOCITY IN MYELINATED NERVE FIBRES OF *XENOPUS LAEVIS*

BY N. A. HUTCHINSON, Z. J. KOLES AND R. S. SMITH

From the Neurophysiology Laboratory, Department of Surgery, University of Alberta, Edmonton, Alberta, Canada

(*Received 1 July 1969*)

SUMMARY

1. The relationship between conduction velocity and nerve diameter in single myelinated nerve fibres from *Xenopus laevis* was measured and was found to be linear.

2. The relationship between conduction velocity and temperature in nerve fibres of various diameters was measured over the range 15–30° C and was found to be linear for each fibre.

3. The slope of the conduction velocity–temperature relationship was directly proportional to the diameter of the nerve fibre.

4. The form of the conduction velocity–temperature relationship was determined by numerical solution of the Frankenhaeuser–Huxley equations for the case of a propagated action potential. The computation predicted that conduction velocity is a linear function of temperature over the range studied.

INTRODUCTION

Conduction velocity in myelinated nerve fibres may be related systematically to geometrical properties of the fibres such as internodal length (Rushton, 1951) or fibre diameter (Hursh, 1939; Rushton, 1951). The relationship between conduction velocity and nerve diameter has been determined experimentally for the frog (Tasaki, Ishii & Ito, 1943). This relationship is rectilinear and passes through the origin. Computer simulation, using the Frankenhaeuser–Huxley (1964) equations, of the dependence of conduction velocity on nerve diameter (Goldman & Albus, 1968) shows that the form of the relationship agrees well with experimental findings. It should be kept in mind, however, that while the properties of the nodal membrane used in the calculation by Goldman & Albus (1968) were derived entirely for fibres from *X. laevis*, the actual relationship between fibre diameter and conduction velocity is unknown for this animal.

In addition to being dependent on geometrical properties of the fibres, conduction velocity will be affected by any environmental factors which

affect the activity of the nodal membrane. Among poikilothermous animals, temperature variation is probably the commonest and most significant environmental factor causing changes in conduction velocity. The temperature dependence of conduction velocity has been very little studied in the Amphibia (Tasaki & Fugita, 1948; Frankenhaeuser & Waltman, 1959) and has not been computed although there is sufficient information available to do so (Frankenhaeuser & Huxley, 1964;Frankenhaeuser & Moore, 1963; Frankenhaeuser, 1965).

In the work reported here, we have determined experimentally the relationship between conduction velocity and fibre diameter in the anuran *X. laevis*. The temperature dependence of conduction velocity in nerve fibres of various diameters has been measured and we have verified that the form of the temperature dependence is as predicted by the Frankenhaeuser–Huxley equations.

METHODS

Measurement of conduction velocity and fibre diameters

Adult female specimens of *X. laevis* were used throughout. (The female is approximately twice the size of a male of comparable age.) A length of sciatic and peroneal nerve was dissected out under Ringer solution and desheathed. Except when the conduction velocity of the fastest component in the whole nerve was being measured, the nerve was cut down to either one nerve fibre or two fibres of dissimilar diameter at a site about 1 cm from the distal end of the nerve. The nerve was transferred to a Perspex chamber containing four pools of Ringer solution separated by three narrow partitions. Vaseline seals were placed across the nerve at the three partitions to produce electrical separation of the pools of Ringer solution. The two seals on the proximal (sciatic) part of the nerve were about 2 mm in width while the third seal was about 0·5 mm wide and was placed directly across the dissected nerve fibre(s). Thus the nerve could be stimulated electrically at the two proximal seals and longitudinal action currents could be recorded from the dissected fibre(s) at the distal seal. The conduction time between the two stimulation sites was obtained by subtraction. In some experiments the conduction time was measured in a similar way over two shorter adjacent lengths of nerve, in these cases one extra partition was added to the chamber to act as the additional stimulating site. The fastest component in the whole nerve was measured from a nerve which was not cut down distally but was subsequently examined histologically at the recording site (Hursh, 1939).

The nerve was stimulated with 200 μsec voltage pulses of about twice threshold amplitude delivered to the pools of Ringer solution via silver–silver chloride electrodes. Action currents were led off across the distal seal with silver–silver chloride electrodes and were amplified with an a.c. coupled amplifier with a band width (3 db down) of 40 Hz–40 kHz.

Conduction time was measured using the delayed time base feature of the Tektronix 565 oscilloscope. One beam of the oscilloscope displayed the stimulus pulse. The start of the second beam which recorded the action current was delayed from the start of the first beam until the first peak of the biphasic signal intersected the rising phase of the stimulus pulse. The delay time was read directly off the oscilloscope control. Subtraction of two such measurements for different stimulating sites gave the conduction time between the sites. When recording from the whole nerve

the delay time to 25% of the amplitude of the first peak of the action current was used. Individual delay measurements were estimated to be subject to about ±50 μsec error.

Conduction distance was determined by cutting the nerve at the cathodal side of each seal across which the stimulating pulses had been applied. The segment of nerve was gently dragged straight on a piece of card and its length measured with vernier calipers. Staining the segments of nerve with osmic acid while on the card showed that the course of the individual fibres was essentially straight. The conduction distances of 35–40 mm were measured with better than 2% accuracy.

The recording chamber was heated or cooled by water which flowed through channels in its walls. The temperature of the Ringer solution in the pool across which the conduction velocity was measured was detected to the nearest 0·5° C with a thermistor thermometer. In those experiments in which temperature was varied the temperature changes were slow (0·5–1° C/min) and continuous. Measurements of conduction velocity were made over several cycles of increasing and decreasing temperatures in all such experiments.

Immediately following the measurement of conduction time the dissected fibre(s) under the recording seal was transferred to a microscope slide, and, while immersed in Ringer solution, its diameter was measured at four places using a microscope with a filar micrometer eyepiece and a 40 × objective. The mean of the four measurements was taken as the nerve diameter.

Nerves for histological examination were stained in 1% osmium tetroxide in a phosphate buffer (pH 7·4) and embedded in an epoxy resin. Cross-sections about 1 μm thick were cut with glass knives on an ultramicrotome and were examined in a light microscope. Nerve diameters were measured, with a filar micrometer ocular, as the means of two diameters taken at right angles.

The Ringer solution used for the entire procedure had the composition (mM): NaCl 112, $NaHCO_3$ 2·5, KCl 2·5, $CaCl_2$ 2·0.

Computational methods

The equation system to be solved for a propagated action potential in a myelinated nerve fibre has been given in full by Goldman & Albus (1968). The computations were carried out on an IBM 360/67 computer, using a 4th order Runge–Kutta numerial integration technique.

The model fibre consisted of twenty-one nodes (node 0 to nodes ±10) and twenty internodes. At nodes ±10 the exterior of the fibre was shorted to its interior. The voltage changes between the axis cylinder and the exterior of the fibre following a brief (10 μsec) stimulating current pulse to node 0 were computed at each node of Ranvier and at nine evenly spaced points in each internode. Stable computations could be performed using 2·5 μsec time increments in the numerical integration. One computation was carried out in this manner at 20° C. Conduction velocity was computed from the time required for the 50 mV level of the leading edge of the action potential to propagate from node 3 to node 4. Using this computation as a standard, the calculation was repeated using fewer spatial points and larger time increments. It was found that, if the internode were divided into five segments, the computation remained stable when the integration time steps were increased to 7·5 μsec; the conduction velocity then differed, owing to a slight change in the shape of the action potential, by 2½% from the original computation. Since this latter procedure gave considerable savings in computer time and since experimental values used in the computation and our measurements of conduction velocity did not approach 2% accuracy, we considered that the modified computation was acceptable.

Computations were carried out at 15, 20, 25, 30 and 35° C for a fibre with an out-

side diameter (D) of 15 μm. The stimulating pulse was kept at the same amplitude for all temperatures; the shape of the potential changes at node 1 was of no consequence in the calculation of conduction velocity. The distance between nodes was taken as $100D$ and the ratio between axis cylinder diameter (d) and the total fibre diameter was taken as $d/D = 0{\cdot}7$. The temperature coefficients of the rate constants and permeability constants in the Frankenhaeuser–Huxley equations were taken from Frankenhaeuser & Moore (1963) and Frankenhaeuser (1965).

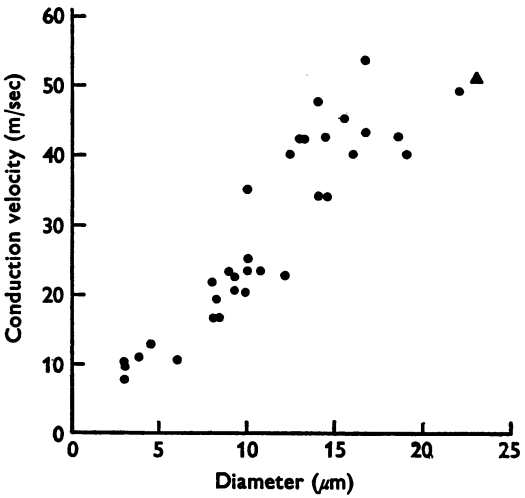

Fig. 1. Conduction velocity related to nerve fibre diameter at 23° C. Filled circles represent measurements made on single isolated fibres. The triangle represents the maximum conduction velocity in a whole nerve trunk plotted against the diameter of the largest nerve fibre as determined by histological section.

RESULTS

Conduction velocity–diameter relationship

Fig. 1 shows the results of thirty-three measurements of conduction velocity made on single nerve fibres at 23° C. Included in the plot is a determination, indicated by the triangle, of the fastest conduction velocity in the whole nerve plotted against the diameter of the largest fibre at the recording site, as measured from sections of the nerve. The results might appear to demonstrate reasonably the linear relationship between conduction velocity and diameter. If the points were fitted with a straight line by the method of least squares, than a slope of 2·6 (m/sec)/μm was obtained and the line intersected the ordinate very close to the origin (Table 1, Row 1).

Close inspection of the results, however, indicated that they might be subject to systematic error. The points of Fig. 1 show a marked increase in scatter for fibres with diameters greater than about 10 μm. There were

larger errors involved in the measurement of conduction velocity of larger fibres, and, as will be seen below, the larger temperature dependence of conduction velocity in the larger fibres could contribute to the error. However, if the point representing the fastest conduction velocity in the whole nerve is joined to the origin by a straight line it will be seen, first, that the line is a reasonable fit to the data points obtained for the smaller fibres, and secondly, that all the scatter in the points representing larger fibres is above this line. That is, the distribution of points in Fig. 1 suggests that in some cases the measurements of nerve diameter were less than would be expected from the corresponding conduction velocities.

Cross-sections of the proximal and distal parts of the nerve trunk showed that the range of nerve diameters was similar at both sites; thus there is good reason to believe that the estimate of the fastest conduction velocity in the whole nerve was matched with the correct nerve diameter. In the nerve examined diameters ranged from 2 to 24 μm with a modal class of 12–14 μm. The sections also showed that proximally 41 % of the fibres had diameters greater than the modal value while distally only 34 % of the fibres were larger than the mode. An absence of many of the largest fibres from the distal part of the nerve was particularly noticeable: proximally 13 % of the fibres were larger than 18 μm in diameter while distally only 5 % were larger than 18 μm.

Loss of a proportion of large diameter fibres in the distal part of the nerve trunk might be explained by preferential distribution of the larger fibres to end-organs in the proximal part of the limb, and/or thinning of the nerve fibres. The distribution of points in Fig. 1 is consistent with the idea that a proportion of the larger nerve fibres thinned between that portion of the nerve trunk over which conduction velocity was measured and the site at which fibre diameter was measured.

If it be assumed that thinning of the fibres occurred at sites where they branched, then a possible consequence of branching within the main nerve trunk would be innervation of different muscles by the separate branches. We tested for an axon reflex by stimulating the distal end of the peroneal nerve and observing proximal muscle groups for contraction. In six experiments the extensor tarsi anterior was consistently found to show the axon reflex, and moreover the contraction was fast indicating that large nerve fibres (Smith & Lannergren, 1968) were indeed branching within the nerve trunk.

From the evidence presented thus far, we conclude that some of the larger nerve fibres in the sciatic-peroneal nerve trunk thin by branching as they course distally. The branches of motor nerve fibres may innervate separate muscles. None of the stem fibres in the proximal part of the sciatic nerve was found to be greater than 24 μm in diameter. Thus, some

but not all of the points of Fig. 1 are likely to be representative of the relationship between conduction velocity and nerve fibre diameter. Since the validity of some of the measurements relating conduction velocity to fibre diameter was questionable, some arbitrary, but reasonable, estimates of the ideal relationship between the variables were made as a guide to further experiments. The measurements of the faster conduction velocities in single nerve fibres were most suspect; if these were eliminated, then a straight line of slope 1·8 (m/sec)/μm (Table 1, Row 2) fitted the points representing conduction velocity in the eighteen smallest nerve fibres of Fig. 1. If the point representing the greatest conduction velocity in the whole nerve were included then the best fitting straight line had a slope of 2·0 (m/sec)/μm (Table 1, Row 3). A slope of about 2 (m/sec)/μm at room temperature is in good agreement with the values given by Tasaki *et al.* (1943). Fibres showing this agreement between their diameters and conduction velocities were selected for examining the temperature dependence of conduction velocity.

TABLE 1. Coefficients and statistical measures pertaining to the lines fitted, by the least squares method, to experimental values. Lines are referred to in the text by row numbers 1 to 7. n, number of data points, a and b, coefficients in the equation $y = ax+b$. RMS dev., the root mean square deviation of the values y about the fitted line. r, correlation coefficient between the variables x and y

Row no.	n	a	b	RMS dev.	r
1	33	2·6 (m/sec)/μm	−0·8 m/sec	5·9 m/sec	0·91
2	18	1·8 (m/sec)/μm	3·7 m/sec	2·0 m/sec	0·93
3	19	2·0 (m/sec)/μm	2·2 m/sec	2·1 m/sec	0·97
4	6	2·0 (m/sec)/μm	2·5 m/sec	2·0 m/sec	0·99
5	6	0·06 (m/sec)/° C per μm	0·13 (m/sec)/° C	0·10 (m/sec)/° C	0·98
6	6	0·63 (m/sec)/μm	−1·6 m/sec	1·4 m/sec	0·96
7	4	1·97 (m/sec)/° C	−19·5 m/sec	0·53 m/sec	0·999

Conduction velocity–diameter–temperature relationship in selected fibres

Six nerve fibres were selected from somewhat shorter lengths of nerve than used before in order to avoid, as far as possible, any progressive tendency for nerve fibres to branch in the distal part of the nerve trunk. Conduction velocities were required not to differ by more than 10 % over two adjacent 2 cm lengths of nerve, and at room temperature (23° C) the conduction velocity for any given nerve diameter was required not to differ by more than 5 m/sec from a line of slope 2·0 (m/sec)/μm. Conduction velocities were determined over a range of temperatures, the minimal range being 15–30° C.

The relationship between conduction velocity and temperature was linear over the range studied (Fig. 2*A*). If the conduction velocities at 23° C, as read from the best fitting straight lines to the measured values, were plotted against fibre diameter, a straight line was obtained with a slope of 2·0 (m/sec)/μm and an intercept of 2·5 m/sec on the ordinate (Table 1, Row 4). When the temperature dependence of conduction velocity (in (m/sec)/° C) was plotted against nerve diameter (Fig. 2*B*) then the results were well fitted by a straight line (Table 1, Row 5). That is, the temperature dependence of conduction velocity was linearly related to nerve diameter. This result is expected, for if, as shown above, conduction velocity (θ) is linearly related to both the diameter of the nerve (D) and to temperature (T) then $\theta \propto (D+C_1)(T+C_2)$ where C_1 and C_2 are constants. It then follows that $\partial\theta/\partial T \propto D+C_1$.

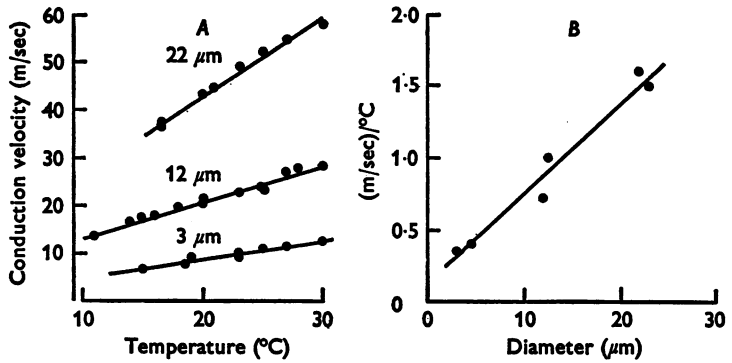

Fig. 2. *A*. Conduction velocity in three isolated nerve fibres of diameters 22, 12 and 3 μm as a function of temperature.

B. The slope of the conduction velocity–temperature relationship of six single nerve fibres as a function of fibre diameter. The line was fitted to the points by a least-squares method (Table 1, row 5).

In addition it was noted that the intercepts which the conduction velocity–temperature curves (Fig. 2*A*) made on the ordinate were also scaled with nerve diameter (Table 1, Row 6). Using the values given in Table 1, Rows 5 and 6, an approximate empirical expression for the relationship between conduction velocity, fibre diameter and temperature over the range 15–30° C is

$$\theta = D(0{\cdot}06T + 0{\cdot}6),$$

where θ is the conduction velocity in m/sec, D is the nerve diameter in μm and T the temperature in ° C. The constants have the appropriate dimensions (Table 1).

Computed conduction velocity–temperature relationship

The theoretical basis for the conduction velocity–diameter relationship in myelinated nerve is well established (Rushton, 1951; Goldman & Albus, 1968), but the relationship to be expected between conduction velocity and temperature is not known. It is usual to express the variation of conduction velocity with temperature in terms of a Q_{10} (Gasser, 1931; Tasaki & Fugita, 1948; Paintal, 1965) which implies that the relationship is exponential. In view of the fact that our experimental findings were that conduction velocity increases linearly with temperature over the range studied, we felt it necessary to determine whether this is a consequence of the known physical events taking place during nerve conduction.

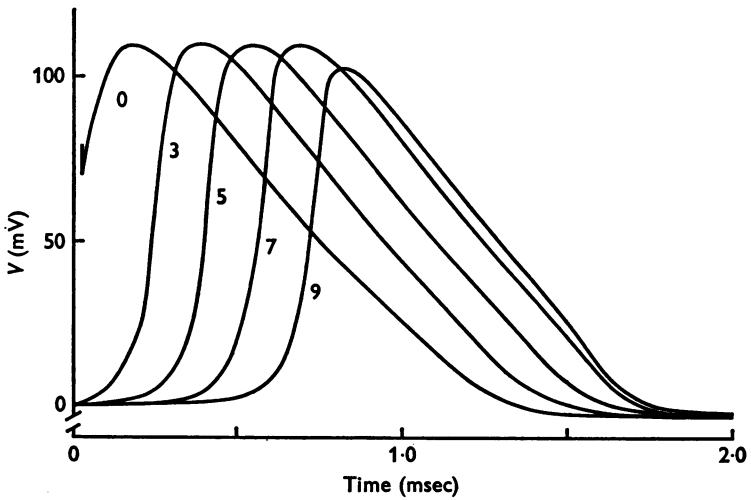

Fig. 3. Computed propagated action potential at 20° C. Curves show the membrane potential changes at nodes 0, 3, 5, 7 and 9 in a model fibre which is short-circuited at node 10. Fibre diameter 15 μm.

Computed propagated action potentials (at 20° C) at nodes 0, 3, 5, 7 and 9 in a model fibre which has the inside of the axis cylinder shorted to the outside of the fibre at node 10 are shown in Fig. 3. It is seen that propagation proceeded through to node 9 even though this node was loaded by the short circuit at node 10. The amplitude of the action potential at node 9 was decreased but the membrane potential changes of all nodes up to node 8 and the conduction velocity were unaffected by the short-circuited end.

Segments of action potential computed for a 15 μm diameter fibre at 15, 20, 25 and 30° C are shown in Fig. 4. A dramatic alteration in duration

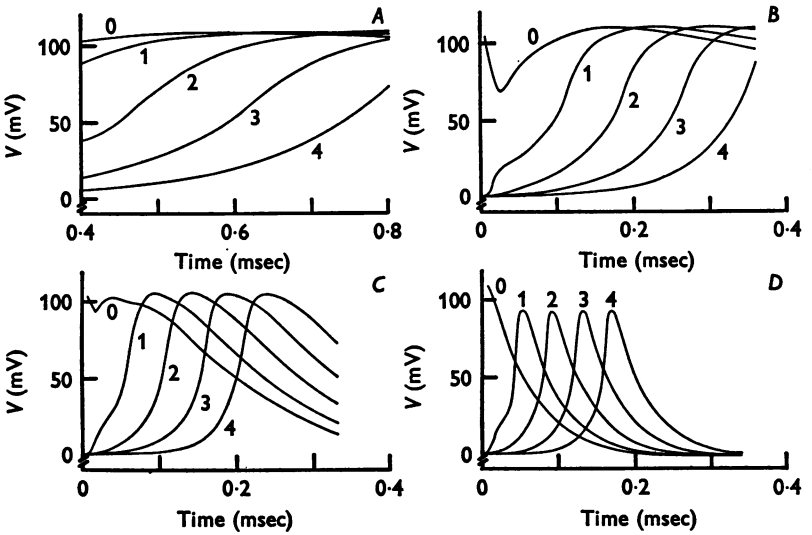

Fig. 4. Segments of action potentials computed for a 15 μm diameter nerve at *A*, 15° C; *B*, 20° C; *C*, 25° C and *D*, 30° C. Conduction velocities were calculated using the delay time between 50 mV levels of the leading edges of the action potentials at nodes 3 and 4.

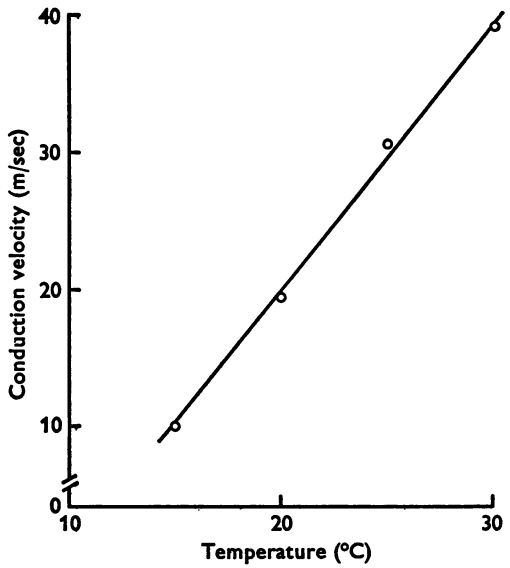

Fig. 5. Conduction velocity as a function of temperature obtained from computed responses as shown in Fig. 3. The best fitting straight line was calculated by a least-squares method (Table 1, row 7).

of the action potential with changing temperature is predicted; this has been previously noted at low temperatures by Frankenhaeuser (1965). The model fibre failed to conduct an impulse at 35° C.

Conduction velocities between nodes 3 and 4 at the four temperatures are plotted in Fig. 5 and the coefficients for the fitted line are given in Table 1, Row 7. While differences between the computed and experimental relationships do exist (these will be considered in the Discussion), the form of the relationship in each case is similar, that is, conduction velocity is a linear function of temperature over the range 15–30° C. We conclude that the linear form of the experimental observations was not likely to have been the result of any particular rate, nor direction of change, of temperature.

DISCUSSION

It is likely that the properties, described in the Results, of myelinated nerve in *X. laevis* are generally applicable to myelinated nerve fibres in other vertebrates. That conduction velocity is a linear function of nerve fibre diameter is generally accepted on both theoretical (Rushton, 1951; Goldman & Albus, 1968) and experimental grounds (Hursh, 1939; Tasaki *et al.* 1943). Existing experimental results suggest that the conduction velocity–temperature relationship is also generally linear over the major part of the physiological range. In *Xenopus* in particular, Frankenhaeuser & Waltman (1959) state that the temperature dependence of conduction velocity is about 2 (m/sec)/° C over the range 10–30° C. The results shown by Paintal (1965) and by Franz & Iggo (1968) indicate that for myelinated nerves in the cat, the relationship is also linear. As pointed out in the Results, the linearity of the conduction velocity–diameter (θ–D) relationship and the conduction velocity–temperature (θ–T) relationship leads to the conclusion that the temperature dependence of conduction velocity, i.e. the slope of the θ–T curve, is proportional to fibre diameter; that this is so in the cat can be derived from the results of Paintal (1965) and Franz & Iggo (1968). These workers have shown that, if the conduction velocities of myelinated nerve fibres from the cat were normalized to their conduction velocities at 37° C, then all nerve fibres, whether fast or slowly conducting, had identical curves of normalized conduction velocity against temperature. The normalizing procedure was equivalent to scaling out the effects of nerve diameter, hence the data support the idea that the temperature dependence of conduction velocity is scaled with nerve diameter.

The two predictions, that for myelinated nerve fibres of *X. laevis* conduction velocity is a linear function of diameter (Goldman & Albus, 1968) and that conduction velocity is linearly related to temperature over the range 15–30° C, are experimentally substantiated. However, the slope of

the computed θ–D curve does not match that which is measured for fibres from *X. laevis*; the slope and position of the computed θ–T relationship are also slightly in error. These discrepancies indicated that a correction should be made to the values used in the computation that would result in rotation of the θ–D curve about the origin and translation of the θ–T curve. It is to be expected that a modification of the passive properties of the model system would produce the major part of the required transformation.

This work was supported by the Medical Research Council of Canada. N.A. Hutchinson received personal support from a Medical Research Council Fellowship. Z. J. Koles was supported by a scholarship from the National Research Council of Canada. The authors would like to thank those responsible in the Department of Electrical Engineering and the Surgical Medical Research Institute, University of Alberta for providing space and facilities to conduct this investigation.

REFERENCES

FRANKENHAEUSER, B. (1965). Computed action potential in nerve from *Xenopus laevis*. *J. Physiol.* **180**, 780–787.

FRANKENHAEUSER, B. & HUXLEY, A. F. (1964). The action potential in the myelinated nerve fibre of *Xenopus laevis* as computed on the basis of voltage clamp data. *J. Physiol.* **171**, 302–325.

FRANKENHAEUSER, B. & MOORE, L. E. (1963). The effect of temperature on the sodium and potassium permeability changes in myelinated nerve fibres of *Xenopus laevis*. *J. Physiol.* **169**, 431–437.

FRANKENHAEUSER, B. & WALTMAN, B. (1959). Membrane resistance and conduction velocity of large myelinated nerve fibres from *Xenopus laevis*. *J. Physiol.* **148**, 677–682.

FRANZ, D. N. & IGGO, A. (1968). Conduction failure in myelinated and non-myelinated axons at low temperatures. *J. Physiol.* **199**, 319–345.

GASSER, H. S. (1931). Nerve activity as modified by temperature changes. *J. Neurophysiol.* **11**, 311–315.

GOLDMAN, L. & ALBUS, J. S. (1968). Computation of impulse conduction in myelinated fibres; theoretical basis of the velocity–diameter relation. *Biophys. J.* **8**, 596–607.

HURSH, J. B. (1939). Conduction velocity and diameter of nerve fibres. *Am. J. Physiol.* **127**, 131–139.

PAINTAL, A. S. (1965). Effects of temperature on conduction in single vagal and saphenous myelinated nerve fibres of the cat. *J. Physiol.* **180**, 20–49.

RUSHTON, W. A. H. (1951). A theory of the effects of fibre size in medullated nerve. *J. Physiol.* **115**, 101–122.

SMITH, R. S. & LANNERGREN, J. (1968). Types of motor units in the skeletal muscle of *Xenopus laevis*. *Nature, Lond.* **217**, 281–283.

TASAKI, I. & FUGITA, M. (1948). Action currents of single nerve fibres as modified by temperature changes. *J. Neurophysiol.* **11**, 311–315.

TASAKI, I., ISHII, K. & ITO, H. (1943). On the relation between the conduction rate, the fibre diameter and the internodal distance of the medullated nerve fibre. *Jap. J. med. Sci. Biophys.* **9**, 189–199.