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THE EFFECTS OF STRETCH ON CABLE AND SPIKE PARAMETERS OF SINGLE NERVE FIBRES; SOME IMPLICATIONS FOR THE THEORY OF IMPULSE PROPAGATION

BY L. GOLDMAN*

From the Department of Zoology, University of California, Los Angeles, California, U.S.A.

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The equations of Hodgkin & Huxley (1952) describe the dependence of conduction velocity in non-myelinated fibres on cable and spike parameters. The success of these relations in predicting the form of the spike potential under a variety of conditions (Hodgkin & Huxley, 1952; George & Johnson, 1961; Fitzhugh, 1962) is still, perhaps, the most convincing line of evidence in support of the sodium theory of impulse propagation, a conceptual framework which has strongly shaped our thought on the nature of basic nervous processes, not only for axonal but also for junctional (Takeuchi & Takeuchi, 1960) and integrative (Eccles, 1961; Boistel & Fatt, 1958; Werman & Grundfest, 1961) sites. This treatment requires that when all intrinsic spike, membrane, and axoplasm parameters are constant, conduction velocity must be proportional to the square root of fibre diameter.

However, a number of nerve fibres in annelids (Bullock, Cohen & Faulstick, 1950; Goldman, 1963) and gastropods (Jenkins & Carlson, 1904; Turner, 1951; Goldman, 1961) as well as vertebrate striated muscle fibres (Martin, 1954; Hakansson, 1957) can be stretched far enough to produce considerable decreases in fibre diameter without accompanying changes in conduction velocity. Moreover, extended stretch of the muscle fibres (Wilska & Varjoranta, 1939; Inoue, 1955; Hakansson, 1957) as well as stretch on leech nerve fibres (Dittmar, 1954) have been reported to produce an increase in conduction velocity. Both of these types of results contradict the theoretically predicted relation between velocity and diameter.

Hodgkin (1954) proposed an explanation for this apparent independence of velocity and diameter which would reconcile the observations with the theory and which was based on the postulate that stretch would smooth folds in the membrane such that the true surface area of the fibre would

* Present address: Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, U.S.A.

not change. Hodgkin was able to demonstrate that under such conditions the increase in longitudinal resistance produced by the decreasing diameter would be compensated for by the decreasing surface area/unit length produced on unfolding so that conduction velocity would remain constant. Martin (1954) proposed a similar explanation, but his treatment depended on some rather special assumptions.

However, Goldman (1963) described the relation of diameter to stretch in the earthworm median giant fibre over that same stretch (from $2L_0$, which is twice reference length [L_0], to $4L_0$, which is four times reference length; see Methods), for which spike conduction velocity remained constant. From the diameter–stretch curves it was concluded that from $2L_0$ to $2{\cdot}9L_0$ the fibre volume remained constant while its surface area appeared to increase, while from $2{\cdot}9L_0$ to $4{\cdot}0L_0$ the fibre lost volume sufficient to prevent the further increase in surface area expected on fibre elongation. However, Hodgkin's unfolding model requires that *both* surface area and volume remain constant, or at least that their ratio remains constant. Goldman concluded that the unfolding model is inadequate, at least for the earthworm median giant fibre, to account for the entire range of stretch over which conduction velocity is constant while fibre diameter decreases.

It should be noted that there exists then no empirically supported explanation which will account for the independence of stretch and conduction velocity in excitable fibres, or, indeed, any empirical data which can account for the discrepancy with the Hodgkin–Huxley (1952) equations. Moreover, Goldman (1961, 1963) concluded from an examination of all the available data correlating conduction velocity and fibre diameter in both nerve and muscle fibres that there exists, as yet, no reliable evidence of any sort to support the prediction that velocity will be proportional to square root of fibre diameter. This prediction follows from the statement that a fibre maintains its essential cable organization during excitation. This prediction, then, is crucial to the Hodgkin–Huxley model.

The present experiments are an attempt to test directly the theoretically predicted velocity–diameter relation by observing cable and spike properties of the earthworm median giant fibre as a function of stretch over that range where conduction velocity remains constant.

METHODS

Earthworms, *Lumbricus terrestris*, obtained from a supply house were kept, refrigerated, in moist earth in the laboratory for periods of up to one week.

Isolation of the nerve cord was carried out by the procedure used by Goldman (1963). A median, dorsal incision was made in the body wall of intact, unanaesthetized animals, care being taken not to rupture the very delicate gut. The preparation could then be pinned, ventral side down, to a paraffin dissecting pan and covered with saline solution. Then, under

a dissecting microscope, the gut was gently moved aside, exposing the ventral nerve cord. The dorsal blood vessel was stripped off and several centimetres of nerve cord dissected free from the ventral body wall and transferred to a dish of fresh saline solution. After a standard period of equilibration, the cord's full length under zero tension (reference length), i.e. when visible slack was just taken up, was recorded. Reference lengths were usually about 2–3 cm.

Stretch was applied in the following manner. Both extreme ends of the cord were pinched by self-closing clamps. Each clamp was mounted through a universal joint and a Lucite holder to a brass key, hand polished to ride snugly in a dural keyway. One shaft, threaded with right-hand threads for one-half of its length and left-hand threads for the other half, was fitted through both keys. In this way rotation of the shaft caused the clamps to move smoothly apart or together, and the cord could be stretched to the desired length.

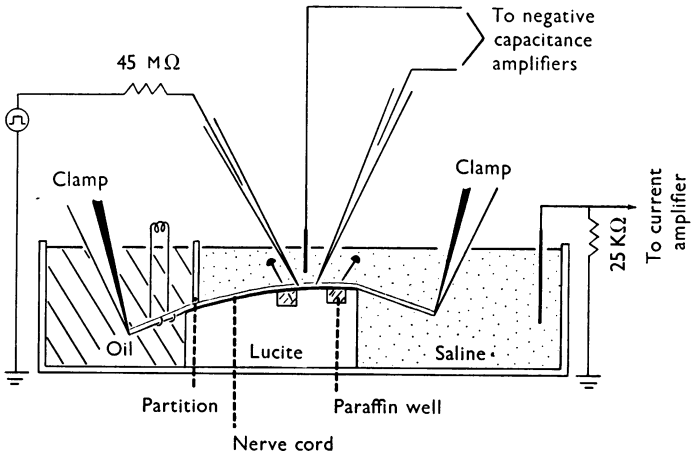

Fig. 1. Arrangement of the apparatus used to measure cable parameters in the earthworm median giant fibre. For description see text.

For micro-electrode recording, the stretched cord was mounted in a chamber consisting of a longer and a shorter saline solution-filled trough, each 8 cm wide and 2·5 cm deep. The two troughs were connected by a channel 2 mm long and only slightly wider than the cord. A Lucite block with a gently convex upper surface was fastened to the floor of the large trough and extended to just inside the small trough. One of the clamps holding the stretched cord extended into each of the troughs. The fibre was threaded through the narrow channel separating these troughs and pressed, ventral side down, firmly over the curved Lucite surface (see Fig. 1). The median giant fibre was then the most superficial nerve fibre in the median dorsal aspect. Firm mounting was essential for micro-electrode penetration owing to the tough connective tissue of the cord, and also because the muscle in the cord will cause the preparation to move back and forth longitudinally during the experiment (Kao & Grundfest, 1957; Wilson, 1961). To eliminate further this movement, vertical wells, whose innermost rims were separated by 4 mm, were drilled in the Lucite block. These wells were filled with paraffin, shaved off flush with the curved surface. The cord was pinned to these paraffin surfaces with fine insect pins, care being taken not to nick or compress the median fibre or to put additional stretch between the pins. In this way, virtually all movement was eliminated, even at low stretch, and electrodes could be maintained *in situ* for many tens of minutes.

After mounting, the channel between the two troughs was sealed with vaseline and the saline solution in the small trough replaced with mineral oil. In this way the fibre could be stimulated with external electrodes in the oil trough. This was found to be useful, as the spike potential amplitude and duration were found to be more reliable indications of a proper, well-seated micro-electrode penetration than the d.c. shift. The d.c. shift was often preceded by several potential steps of different amplitudes which made interpretation difficult. The cable measurements, on the other hand, could be made over a region of the fibre immersed in a large volume of saline solution. All penetrations were confined to that region between the insect pins. There was always a length of at least four space constants between the current-passing internal electrode and the channel on one side and the clamp in the saline chamber on the other side, so that these barriers should have virtually no effect on current distribution in the fibre.

Penetration was performed under visual control with slightly diffused illumination from beneath. In this way the median giant fibre may be readily distinguished (Goldman, 1963). The micro-electrodes were conventional glass pipettes, filled with 3 M-KCl. Tip diameters were *ca.* 0·5 μ, and tip resistances were usually 20–30 MΩ. The fibres were penetrated diagonally from above.

The recording micro-electrode was connected through a chlorided silver wire to the input grid of a high impedance input, low grid current, negative capacitance pre-amplifier (Argonaut). A second chlorided silver wire in the saline trough went to the grid of a second negative capacitance pre-amplifier. A third chlorided wire coupled the saline trough through a 25 KΩ resistor to ground. The outputs of the two single-sided pre-amplifiers were fed differentially into one channel of a Tektronix dual beam oscilloscope. The current-passing micro-electrode was coupled through another chlorided silver wire and a 45 MΩ resistor to one output lead of a Grass stimulus isolation unit which was driven by a Grass stimulator. The other output lead was earthed so that the current-passing circuit was completed through the 25 KΩ resistor to the saline bath. Current was monitored by recording the potential drop across this resistance on the second channel of the oscilloscope. External stimulation (oil trough) was provided by silver-wire electrodes isolated from earth by a second isolation unit driven by a second stimulator. Still photographs were made of the oscilloscope screen.

The cable properties, axoplasm resistance, membrane resistance and membrane capacitance were determined by the square pulse analysis method of Hodgkin & Rushton (1946) as modified by Fatt & Katz (1951). In practice an initial penetration was made with the potential recording electrode, after first compensating for the electrode capacitance with the electrode in saline solution. Then records were made of the spike responses produced by external stimulation. If, on subsequent penetrations, the spike amplitude could not be made to agree to within 5 mV with this initial amplitude the preparation was discarded. Spikes were always produced with single shocks of just-suprathreshold value. The second (current-passing) electrode was then inserted close to the first. The current electrode was made to pass current pulses during insertion. Penetration was indicated by the sudden detection of a signal by the potential-recording electrode. No such signal could be recorded when either micro-electrode was withdrawn from the fibre. Current electrode seating was checked during the course of the experiment by noting the threshold *current*. Records were made at slow sweep speeds to observe the steady-state potential value and at high sweep speeds to observe the rise time. The inter-electrode distance was recorded and the recording electrode withdrawn. The resting membrane potential was measured as the d.c. shift on withdrawal. The fibre was then re-penetrated at a new inter-electrode distance and the response to current pulses again recorded. The difference between the two inter-electrode distances was always at least half a space constant and never much greater than one space constant. Resting membrane potentials always agreed to 2 or 3 mV at the two inter-electrode distances. These distances and fibre diameter were measured with an ocular micrometer.

Time constants were calculated from

$$V = \frac{\sqrt{(r_i r_m)}I}{4}\left\{e^{-X/\lambda}\left[1+\operatorname{erf}\left\{\frac{-X/\lambda}{2\sqrt{(t/\tau)}}+\sqrt{(t/\tau)}\right\}\right]-e^{X/\lambda}\left[1+\operatorname{erf}\left(\frac{-X/\lambda}{2\sqrt{(t/\tau)}}-\sqrt{(t/\tau)}\right)\right]\right\},$$

where V is membrane potential, $\frac{1}{2}\sqrt{(r_i r_m)}$ is the 'effective resistance', I is the current, X is the inter-electrode distance, λ is the space constant, t is time and τ the membrane time constant. This is the Hodgkin–Rushton equation modified to incorporate the new boundary conditions of the Fatt & Katz method. Time constants were evaluated by a convergence routine which was essentially Newton's iteration technique on an IBM 7090 Computer at the U.C.L.A. Computing Facility. To calculate C_M the time constant and R_M were used.

Tip potentials, measured at the end of most experiments, were never greater than 5 mV and usually less than 2 mV. No correction was made for tip potentials.

The saline solution used was Rushton's earthworm perfusion fluid (see Kao & Grundfest, 1957).

Temperature ranged from 15 to 19° C but never varied by more than 1° C during any individual experiment.

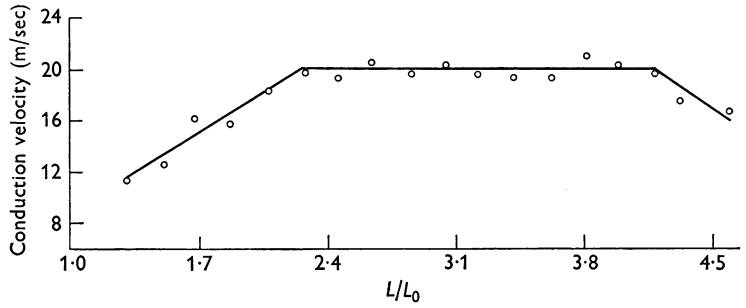

Fig. 2. The relation between the spike conduction velocity and stretch (length relative to reference length $[L/L_0]$ for an individual earthworm median giant fibre. Each point represents a single observation.

RESULTS

According to Goldman's (1963) operational definition of reference length, that range of lengths where conduction velocity is constant is from $2L_0$ to $4L_0$. Velocity declines on either side of this range. These limits are typical, not absolute values, i.e. some fibres on stretch reached the plateau of the stretch–velocity curve before $2L_0$ and some after, also some preparations showed the decline in velocity at high stretch before $4L_0$ and some after. Goldman presented the data pooled from many preparations. Figure 2 shows the relation for an individual preparation between spike conduction velocity in m/sec and stretch as fibre length relative to reference length (L/L_0). Each point represents an individual velocity observation measured by the delay in propagation between two fixed pairs of external recording electrodes. The solid lines were fitted by eye to the points and are very similar in shape to those drawn by Goldman to fit the pooled data. The apparent increase in conduction velocity in the initial

part of the stretch–velocity curve is probably due to uncoiling of the fibre under stretch (Bullock *et al.* 1950; Goldman, 1963).

Resting membrane potential. Figure 3*a* shows the relation between resting membrane potential (E_m) in mV and stretch (L/L_0). Each point was taken from an individual preparation and represents one observation. Each preparation was recorded from at only one degree of stretch. The data shown here were pooled from eighty-six preparations. E_m clearly remains constant under stretch. The mean value is 70·8 mV, inside negative, and the range from 62 to 80 mV. These values are about 5–6 mV greater than those reported for this preparation by Kao & Grundfest (1957), probably because the present observations were made on electrode withdrawal while Kao & Grundfest reported the values observed on initial penetration. The withdrawal method is, perhaps, more reliable as it is easier, using the spike amplitude and duration, to ensure that the electrode is well seated when internal potential is recorded.

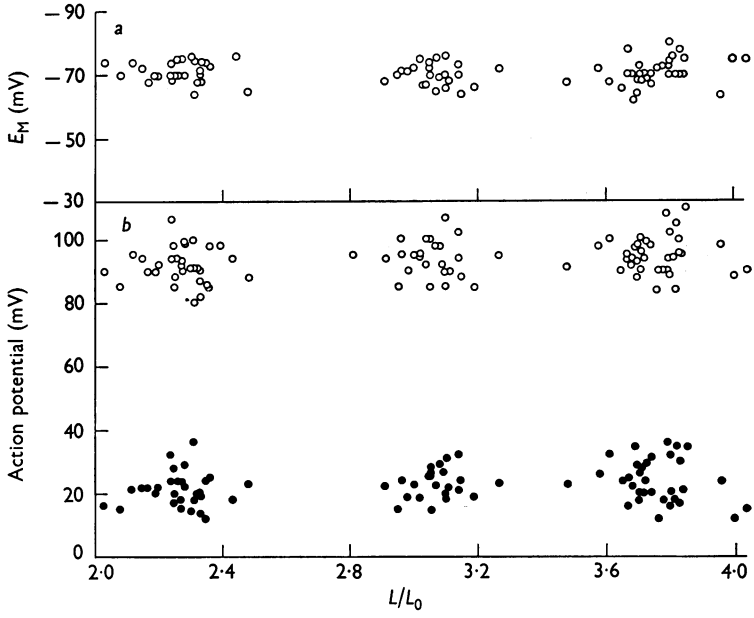

Fig. 3. The relation between: *a*, resting membrane potentials; *b* (open circles), spike peak amplitude; *b* (filled circles), spike overshoot; and stretch (see Fig. 2) in the earthworm median giant fibre. Each point represents a single observation on an individual preparation. These data pooled from: *a*, 86; *b* (open circles), 95; *b* (filled circles), 86 preparations.

Very few micro-electrode observations of any sort were made at stretch less than about $2{\cdot}15L_0$ since even with the mounting and pinning procedure described there was usually still too much longitudinal movement at very

low stretch. Also below $2{\cdot}15L_0$ some preparations were off the flat part of the stretch–velocity curve. Very few observations were made above $3{\cdot}85L_0$. In this way it could be assured that very few, if any, preparations would be on the high-stretch falling part of the stretch–velocity curve. This falling portion probably reflects damage to the preparation (Goldman, 1963).

Spike potential. Figure 3*b* (open circles) shows the relation between the peak amplitude of the spike potential in mV and stretch (L/L_0). Each point was taken from an individual preparation and represents one observation. The data shown here were pooled from ninety-five preparations. There is no change in spike-potential amplitude under stretch. The mean amplitude is 93·3 mV and the range 80–110 mV. These values agree well with those of Kao & Grundfest.

Figure 4*a* is a record of a typical spike response and is similar to those of Kao & Grundfest. As reported by these authors, the spike rose sharply out of the resting potential level, had a falling phase, which was slower than the rising phase, and a depolarizing after-potential.

Overshoot. Figure 3*b* (filled circles) also shows the relation between spike overshoot potential in mV and stretch (L/L_0). It too remains constant under stretch. Again, each point is one observation taken from an individual preparation. The data shown are pooled from eighty-six preparations. The mean value is 22·8 mV and the range from 12 to 36 mV. These overshoot values are about 6 mV lower than those reported by Kao & Grundfest.

Spike rate of rise. Figure 5*a* shows the relation between the maximum rate of rise of the spike in V/sec and stretch (L/L_0). Each point represents one observation taken from one preparation. The data shown here were pooled from eighty-four preparations. Maximum rate of rise of the spike potential is constant under stretch. The mean value is 248 V/sec and the range from 127 to 420 V/sec. Figure 4*b* is a typical record from which measurements of spike rate of rise and decline were taken.

Spike rate of decline. Figure 5*b* shows the relation between the maximum rate of spike decline, taken from the initial linear portion of the repolarization phase (see Fig. 4*b*) and stretch (L/L_0). Each point represents one observation taken from one preparation. The data shown here were pooled from eighty-five preparations. The spike rate of decline remains essentially constant under stretch. The few unusually high values in the $3{\cdot}0L_0$ group were all taken from one lot of animals. Note that the low and high stretch groups agree very well. The mean value is (-67) V/sec and the range from (-44) to (-116) V/sec.

Current–voltage relation. Steady-state current–voltage curves were plotted for eight preparations at a mean length of $2{\cdot}27L_0$ and nine preparations at a mean length of $3{\cdot}71L_0$. In each case the current-passing

electrode was placed very close to the recording electrode, usually one to two diameters away, never further than $0{\cdot}10\,\lambda$. Figure 6 shows some representative curves. Each curve is taken from one preparation and each point represents one observation. Critical depolarizations are not included.

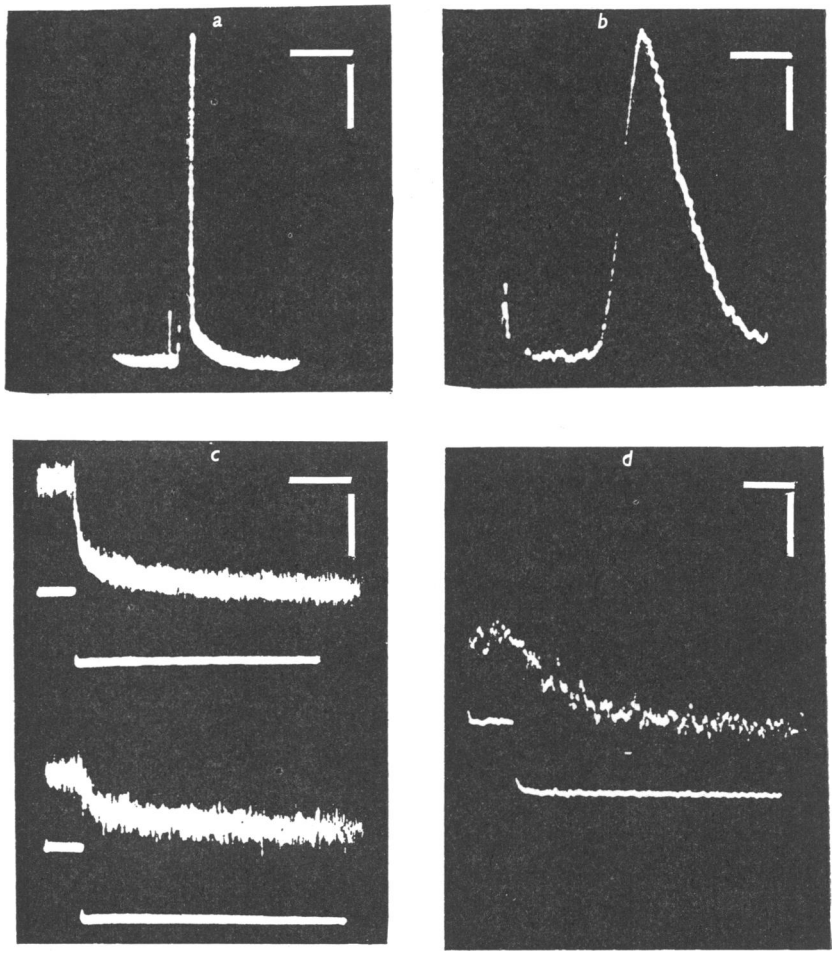

Fig. 4. Records taken from the earthworm median giant fibre of: *a*, a spike response to external stimulation showing after-potentials; *b*, same as *a* with a high sweep speed so that the rates of rise and fall may be observed; *c*, response of the membrane potential to a pulse of current in the electrotonic range delivered $0{\cdot}4\,\lambda$ (upper pair of records) and $1{\cdot}1\,\lambda$ (lower pair) from a current-passing internal electrode; *d*, same as the upper pair of records in *c* at a high sweep speed so that the membrane time constant may be observed. All potential records were made with full capacity compensation. The lower trace of each pair in *c* and *d* is the current record. Vertical scales: *a*, *b*, 20 mV; *c*, *d*, 10 mV, 4×10^{-8} amp. Horizontal scales: *a*, *c*, 10 msec; *b*, *d*, 1 msec.

Threshold current values are indicated by the arrow on the current axis. In every case the curves were smoothly linear for values of current from just below the threshold value, where some rectification is observed, to values of hyperpolarizing current at least as great in absolute value as the depolarizing threshold current. Therefore, at any range of stretch, current equal in value to the threshold current but hyperpolarizing in direction could be passed with assurance that the membrane was passive as required by the square-pulse analysis method. All cable measurements were taken with such hyperpolarizing currents. Figure 4*c* shows the records from a typical experiment made with the recording electrodes proximal (upper pair of traces) and distal (lower pair) to the current-passing electrode. The lower trace of each pair is the current record. Voltage records were made with the electrode capacity compensated.

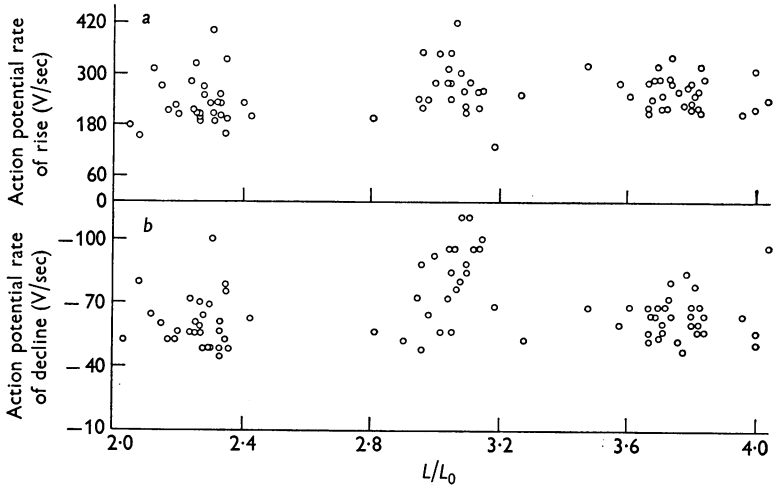

Fig. 5. The relation between: *a*, spike maximum rate of rise; *b*, spike maximum rate of decline and stretch (see Fig. 2) in the earthworm median giant fibre. Each point represents a single observation on an individual preparation. These data pooled from: *a*, 84; *b*, 85 preparations.

Spike threshold. Spike thresholds assayed as critical depolarizations in response to current passed internally were observed on nine preparations at low stretch and ten preparations at high stretch. As described in the current–voltage relation section, the current-passing electrode was always close to the recording electrode relative to λ. In this way results from different preparations could be quantitatively compared. Table 1 summarizes these results. At high stretch, the mean critical depolarization was 23·2 mV and the range was 21·5 to 24·1 mV. At low stretch the mean was 22·9 mV and the range 21·5–24·4 mV. Threshhold, then, remains constant under stretch.

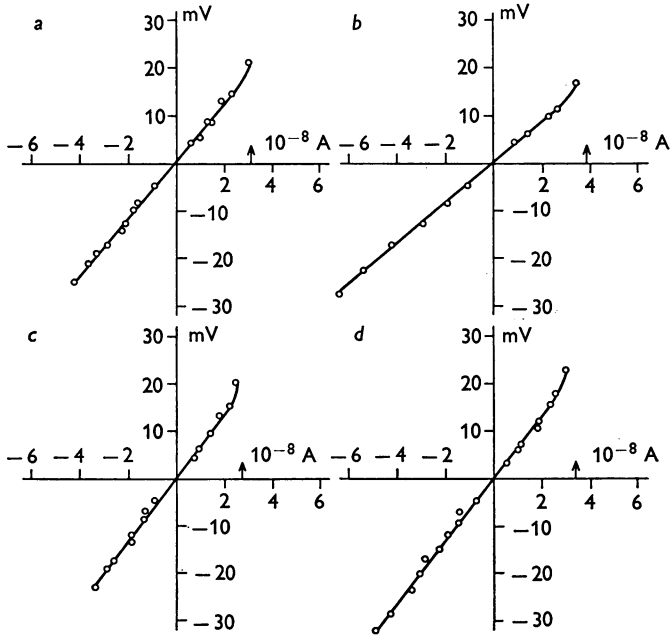

Fig. 6. Current–voltage curves taken from four individual earthworm median giant fibres stretched to: *a*, $2{\cdot}17L_0$; *b*, $2{\cdot}20L_0$; *c*, $3{\cdot}61L_0$; *d*, $3{\cdot}79L_0$. The current-passing electrode was always within $0{\cdot}10\,\lambda$ from the potential-recording electrode. Voltage values were taken from the steady-state region of the potential record. Critical depolarizations are not included. The arrow on the current axis indicates the spike-threshold current.

TABLE 1. Action-potential threshold at low and high stretch

Mean length relative to reference length	Mean critical depolarization (mV)	Total range of variation (%)
2·29	23·2 (9)*	±7
3·74	22·9 (10)	±6

* The numbers in the brackets refer to the number of preparations from which the mean is taken.

Axoplasm resistivity. Figure 7 shows the relation between axoplasm resistivity (R_i) plotted relative to the mean value at low stretch (R_{i0}) and stretch (L/L_0). The points are the means of nineteen preparations at a mean length of $2{\cdot}28L_0$, ten preparations at a mean length of $3{\cdot}03L_0$, and nineteen preparations at a mean length of $3{\cdot}74L_0$. The brackets indicate ±2 S.E. of the mean. R_i clearly falls on stretch. The difference between the means at low and high stretch (23·8 %) is highly significant ($P < 0{\cdot}01$) while the middle stretch group is not significantly different from the low stretch group ($P > 0{\cdot}40$). The absolute values of R_i at the low, middle, and high stretch groups were 206, 187, and 157 Ω cm respec-

tively. The interrupted line in Fig. 5 shows how much R_i must fall at any length if this parameter alone is to compensate for the decrease in diameter and maintain the conduction velocity constant (see Discussion).

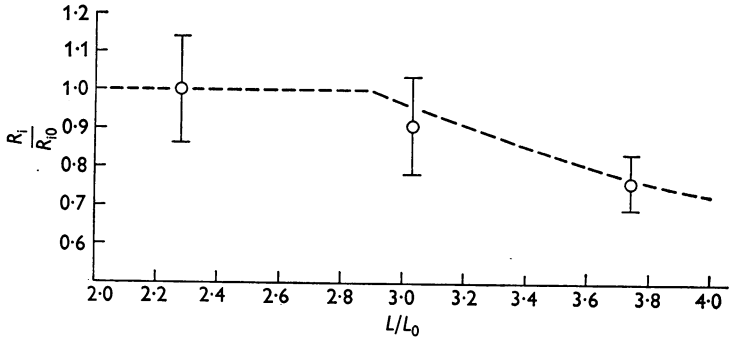

Fig. 7. The relation between axoplasm resistivity (R_i), plotted relative to the value at $2{\cdot}28L_0$ (R_i/R_{i0}) and stretch (see Fig. 2). The points are the means of: 19 ($2{\cdot}28L_0$), 10 ($3{\cdot}03L_0$), and 19 ($3{\cdot}74L_0$) preparations. The brackets indicate ± 2 S.E. of the mean. The $3{\cdot}74L_0$ group is significantly different from the $2{\cdot}28L_0$ group, but the $3{\cdot}03L_0$ is not. Mean absolute values are 206, 187, and 157 Ω cm, respectively. The interrupted curve is the manner in which R_i must change if it alone is to account for the fall in diameter and hence maintain the conduction velocity constant.

For all cable experiments and in fact for all micro-electrode experiments, since spike and cable parameters were observed on the same animals, each preparation was recorded from at only one degree of stretch. In this way, although variance was fairly high, no systematic errors due to ageing or multiple electrode penetrations were introduced. Most cords after being stretched will not return on release (Goldman, 1963), so that all reference values would have to be at low stretch. When it is considered that to take even two sets of observations on a fibre, six micro-electrode penetrations must be made in that single fibre it is clear that the danger of introducing systematic error is real.

Specific membrane resistance. Figure 8*a* shows the relation between specific membrane resistance (R_M) in Ω cm^2 and stretch (L/L_0). Each point represents one observation on one preparation. These data are pooled from forty-eight preparations. R_M remains constant under stretch. The mean value is 12·4 KΩ cm^2 and the range from 6·7 to 21·6 KΩ cm^2.

Membrane capacitance. Figure 8*b* shows the relation between membrane capacitance (C_M) in μF/cm^2 and stretch (L/L_0). Each point represents one observation on one preparation. These data are pooled from forty-seven preparations. C_M remains constant under stretch. The mean of the low stretch group is not significantly different from that of the high stretch group ($P > 0{\cdot}17$). Figure 4*d* shows a typical record from which τ was

measured. The lower trace is the current record. Note that the current rise-time is not instantaneous. This should produce some error in the absolute values of C_M, but the error will depend only on the rise-time of the current-passing electrode and should not be systematic with stretch. The mean C_M value is 0·29 $\mu F/cm^2$ and the range from 0·13 to 0·56 $\mu F/cm^2$.

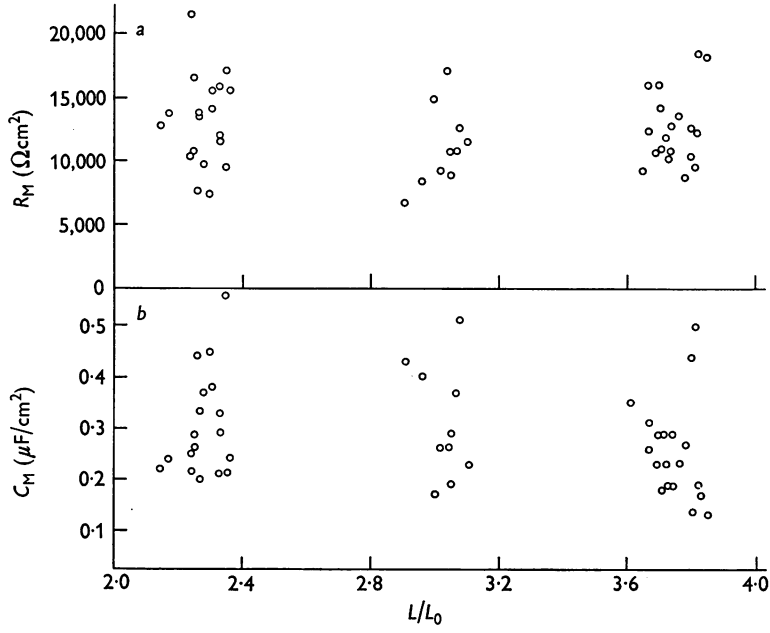

Fig. 8. The relation between: *a*, specific membrane resistance; and *b*, specific membrane capacitance and stretch (see Fig. 2). Each point represents a single observation on an individual preparation. These data are pooled from: *a*, 48; *b*, 47 preparations.

Other properties. At a mean length of 2·28L_0, λ (mean of nineteen preparations) was 4·03 mm with a range of 2·90–6·27 mm. At a mean length of 3·74L_0, λ (mean of nineteen preparations) was 3·76 mm with a range of 3·01–5·47 mm. Time constants ranged from 1·79 to 6·41 msec with a mean of 3·36 msec. The mean diameter at $2L_0$ (mean of ninety-five preparations) was 105 μ.

Summary of results. Summarizing, it is noted that all of the spike parameters: amplitude, overshoot, rate of rise, rate of decline, and threshold, and the intrinsic membrane constants, resistance and capacitance, as well as resting membrane potential all remain constant over that range of stretch where spike conduction velocity is also constant. Only axoplasm resistivity and fibre diameter show dramatic changes in stretch over this region.

DISCUSSION

What process might be envisioned so as to account for the constancy of conduction velocity under stretch on the basis of the empirical observations described above? Goldman (1963) concluded that Hodgkin's 1954 constant surface-area model was in itself not adequate to account for the entire phenomenon. However, from the region $2L_0$ to $2{\cdot}9L_0$ there is no reason why this model could not apply. Figure 9 shows fibre diameter (D) relative to diameter at $2L_0$ (D_0) as a function of stretch (L/L_0). These are Goldman's original data. Each point represents one diameter observation. The data are the pooled observations from twenty preparations. Goldman fitted to the points a straight line of negative slope which was a fair empirical description of the data. The interrupted curve in Fig. 9 shows the manner in which diameter should change under stretch assuming the fibre is a cylinder of constant volume (Goldman, 1963). Note that from $2L_0$ to $2{\cdot}9L_0$ the interrupted curve describes the data well. We may conclude, then, that fibre volume, over this region, remains constant as required by Hodgkin's model. Goldman also showed that over this same region fibre surface area appeared to increase, again a requirement of the unfolding model. It may, at least tentatively, be proposed that from $2L_0$ to $2{\cdot}9L_0$ conduction velocity remains constant under stretch for the reasons proposed by Hodgkin in his constant surface-area model.

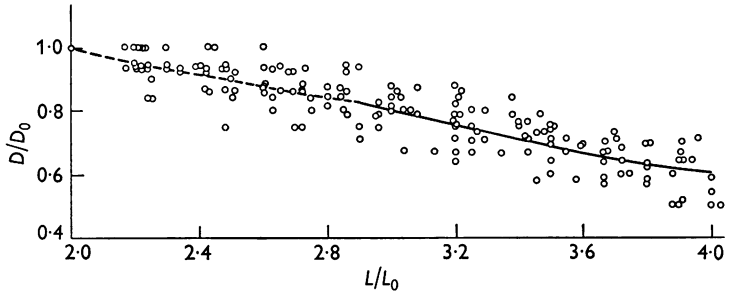

Fig. 9. The relation between fibre diameter (D) plotted relative to D at $2L_0(D/D_0)$ and stretch (see Fig. 2). Each point represents a single diameter observation. These data were pooled from twenty preparations. The interrupted curve shows the manner in which D should change on stretch if fibre volume remains constant. The solid curve shows the manner in which D should change on stretch if fibre surface area remains constant.

The solid curve of Fig. 9 shows how the diameter should change on stretch if surface area were to remain constant. This is the same relation as Goldman's (1963) lower, interrupted curve of Fig. 4, but normalized to $2{\cdot}9L_0$ not $2L_0$. Note that from $2{\cdot}9L_0$ to $4L_0$ this function fits the points well, and recall that this same region is the region where the fibre is losing

enough volume to prevent further increase in surface area. Therefore, we may consider that initially ($2L_0$) the membrane has some sort of slack, perhaps Hodgkin's folds, and under stretch this slack is taken up, volume being maintained. This is the behaviour postulated by Hodgkin. Then, at about $2{\cdot}9L_0$, all the membrane slack is taken up and the fibre is a smooth bored tube. Now, further stretch is incapable of increasing surface area because the membrane itself, an array of bimolecular leaflets, is non-distensible, i.e. it cannot actually increase in area and become thin as proposed by Martin (1954). Stretch beyond $2{\cdot}9L_0$ is taken up by squeezing out just enough volume to maintain the constancy of the surface area, and the fibre at any length greater than $2{\cdot}9L_0$ may be considered as a series of rigid tubes. This behaviour (volume loss, probably no unfolding) cannot be shown to predict constant conduction velocity on Hodgkin's model alone.

Now let us consider what would happen if *all* the volume squeezed out owing to non-distensibility of an unfolded membrane were water, which should pass out readily, and the salts, being slower moving, were left in the fibre. Then from $2{\cdot}9L_0$ to $4L_0$ the salts in the axoplasm would become progressively more concentrated and axoplasm resistivity would decrease.

Let R_1 = axoplasm resistivity (ohm cm),
C_M = membrane capacitance/cm^2,
u = spike conduction velocity,
D = fibre diameter,
L = fibre length,
v = fibre volume,
A = fibre surface area,
V = membrane potential.

Then, by definition

$$A = \pi DL$$

Now, since A is constant we must have

$$D \propto (L)^{-1} \quad \text{(the solid curve of Fig. 8).}$$

But $$v \propto D^2L.$$

So $$v \propto (L)^{-1}.$$

Now, where the activity coefficients for all ions always remain unity, resistivity is approximately inversely proportional to the concentration in a salt solution. And, if we consider that all volume loss is water, then

$$v \propto (\text{concentration})^{-1}.$$

And $$R_1 \propto v.$$

So $$R_1 \propto (L)^{-1}$$

Now, from Hodgkin & Huxley (1952)

$$u = (KD/4R_iC_M)^{\frac{1}{2}},$$

where $$K = (d^2V/dt^2)/\{(dV/dt) + (1/C_M)[\bar{g}_K n^4(V - V_K) + \bar{g}_{Na} m^3 h(V - V_{Na}) + \bar{g}_L(V - V_L)]\}.$$

But, from $2{\cdot}9L_0$ to $4L_0$ there should be no changes in any specific properties of the membrane. Empirically both intrinsic membrane constants and the amplitude and form of the spike did remain constant. Now assuming what is empirically true, that there are no changes in E_m or overshoot, we may write

$$u = K'(D/R_i)^{\frac{1}{2}},$$

or, substituting from above

$$u \propto [(L)^{-1}/(L)^{-1}]^{\frac{1}{2}}.$$

This model, then, predicts that R_i will decrease just enough to compensate for the decreasing diameter under stretch. Indeed, R_i and D were the only parameters empirically observed to change under stretch.

Since, $v \propto R_i$, then v/v_0 against L/L_0 should be exactly congruent with R_i/R_{i0} against L/L_0, where V_0 and R_{i0} are volume and axoplasm resistivity at $2L_0$. The interrupted line in Fig. 7 is actually v/v_0 calculated from the theoretical D/D_0 against L/L_0 curves of Fig. 9. Note the excellent agreement with the observed values. Axoplasm resistivity then changes just in the manner predicted by this model. Also, independently of any model as to how such changes in R_i are produced, it may be noted that the observed changes in R_i are enough to compensate quantitatively for the observed changes in D from $2{\cdot}9L_0$ to $4L_0$ and so maintain the conduction velocity constant. Taking D values from the theoretical curves of Fig. 9 and the R_i values from Fig. 7 we find that the mean values in relative units for $(D/R_i)^{\frac{1}{2}}$ at $2{\cdot}28L_0$, $3{\cdot}03L_0$ and $3{\cdot}74L_0$ are 0·067, 0·066, and 0·064, respectively. Ishiko & Sato (1960) in their experiments on the effects of stretch on cable properties on skeletal muscle fibres unfortunately did not measure R_i.

The behaviour of C_M, R_M, spike rate of rise, amplitude, rate of decline, and threshold under stretch are all just as predicted by the model, i.e. no change in specific membrane properties. Dudel & Trautwein (1954) working on mammalian papillary muscle, and Ishiko (1958) working on frog skeletal muscle also found no change in the form or amplitude of the action-potential under stretch.

If the unstretched fibre is folded, true specific membrane resistance and capacitance should appear to change by about 10% owing to the folding that would be present at $2{\cdot}28L_0$. However, such small differences could not be resolved in parameters of such high variance. Ishiko & Sato (1960) also failed to demonstrate a reliable difference in R_M and C_M under stretch.

On the basis of the model we would expect sufficient water to be lost to concentrate potassium in the axoplasm enough to hyperpolarize the membrane by 5–6 mV at a length of $3{\cdot}75L_0$, were the membrane a *pure* potassium electrode. However, considering the well-known insensitivity of the E_m to small changes in potassium concentration near the normal resting value in a variety of preparations (Curtis & Cole, 1942; Huxley & Stämpfli, 1951; Fatt & Katz, 1953; Grundfest, Kao & Altamirano, 1954; Baker, Hodgkin & Shaw, 1962) it is no surprise that no hyperpolarization was detected. In fact, it might be argued that were this 5–6 mV hyperpolarization expressed, it would mean that potassium had been concentrated a great deal more than predicted. This insensitivity to potassium is probably due to an increase in the resting membrane sodium to potassium permeability ratio $(P_{Na}/P_K)_{rest}$ (Baker *et al.* 1962).

Ling & Gerard (1949) in striated muscle fibres, Dudel & Trautwein (1954) in mammalian papillary muscle and, as long as they were not in the region of irreversible changes and hence damage, Gray & Richie (1954) in myelinated nerve fibres all found that stretch produced no change in E_m. Girardier, Reuben, Brandt & Grundfest (1963) reported hyperpolarization of crayfish muscle fibres on stretch. However, Ishiko (1957, 1958) reported a reversible fall in E_m of about 4 mV, in skeletal muscle fibres on 30 % stretch.

On the basis of a pure sodium electrode, overshoot might be expected to fall by about 5 mV on stretch. For many preparations (Nastuk & Hodgkin, 1950; Draper & Weidmann, 1951; Huxley & Stämpfli, 1951; Baker *et al.* 1962) the overshoot potential does behave like a sodium electrode without postulating any changes in P_{Na}/P_K. However, Coraboeuf & Otsuka (1956) working on guinea-pig cardiac tissue did report a pronounced insensitivity of the overshoot to changes in external sodium. Should there be an active membrane compensation for the increased internal sodium at the peak of the spike, similar to that for the E_m, so that the overshoot is maintained constant, $(P_{Na}/P_K)_{peak}$ would only have to increase by about 20 % to make the change in overshoot non-detectable under the conditions of this experiment. Alternatively, the increased sodium may trigger increased sodium pumping in the resting state. This is similar to the proposal of Brady & Woodbury (1957) that a decreased sodium concentration *ratio* across the membrane may stimulate active pumping. In the perfused axon experiments of Baker *et al.* (1962) increased pumping would have no effect on internal sodium concentration or overshoot. Harris (1954) did observe an increased sodium efflux from the frog skeletal muscle on stretch of 20–30 % which he felt was sufficient to produce an increase in overshoot potential. On the other hand, no change in potassium influx or efflux was observed on 10 % stretch (Harris. 1953).

Dudel & Trautwein (1954), Gray & Richie (1954) (at stretch below that which produced injury) and Ishiko (1957, 1958) all found overshoot to be independent of stretch. This constancy could not be understood by Ishiko considering the results of Harris. However, on the basis of the model proposed here, the increased sodium efflux observed by Harris can be understood as a compensation for the increased sodium concentration produced on water expression and hence the overshoot should remain constant.

The model proposed here may also be expanded to account for the increased conduction velocity of muscle fibres on extended stretch observed by Hakansson (1957), Wilska & Varjoranta (1939) and Inoue (1955), for which there has previously been proposed no physically realistic explanation. All that is required is that the changing $(P_{Na}/P_K)_{rest}$ continues just to compensate for the E_m while the increased sodium pumping gradually begins to *overcompensate* for the overshoot as the diameter continues to decrease. This would provide, through an increased spike amplitude, the increased safety factor proposed by Hakansson (1957) and Ishiko (1958) as a purely formal explanation.

One consequence of the model proposed here would be that the fibre at high stretch would be tolerating, perhaps by means of the structural rigidity of the fully unfolded-non-distensible membrane, a rather high osmotic gradient. If we assume that the axoplasm has the same total osmotic concentration as Rushton's solution and considering the observed volume loss, the concentration gradient at $4{\cdot}0L_0$ should be 0·106 moles or a little more than 2 atm. We may duplicate this gradient by plunging cords, stretched to $2{\cdot}9L_0$, directly without pre-equilibration into Rushton's solution diluted to only 60 % of its normal value. The clamps (see Methods) should be adequate ligatures. The model (all volume loss is water) predicts no rupture or swelling. Four preparations were treated in this way. Diameter was observed always at the same point on the fibre, while average diameter was checked both in normal Rushton's solution and at the end of the experiment in diluted saline solution. In every case after 3 hr there was no sign of swelling or loss of excitability. One experiment was extended to $4\frac{1}{2}$ hr with the same result. Were the fibre diameter able to increase, it should have increased by 18 %, a readily discriminable difference.

That this diluted solution was actually reaching the extracellular space within the cord could be assured as (1) the peripheral nerves at each segment had been cut leaving numerous holes in the sheath of the cord and (2) excitability was lost within 10 min in distilled water and fully recovered again in less than 10 min on return to normal saline solution.

These experiments do not prove that the fibre membrane is fully distended and supporting this gradient, but should swelling have occurred

the model proposed here would have been untenable. It is not unreasonable to believe this fibre could actually tolerate such a gradient. Sections examined in the electron microscope (Goldman & Galey, unpublished observations) revealed the fibre sheaths consist of about fifty densely packed, identical-looking lamellae. Hama (1959) reported up to thirty lamellae in the sheath of the median giant fibre of the earthworm *Eisenia*. If the osmotic gradient were developed across all these layers it would be equivalent to a gradient of 0·001 M-NaCl or about 0·05 atm/layer.

Unfortunately, since bimolecular leaflets are non-distensible, stretch produces no perturbations on the membrane and, although the results presented here are consistent with cable theory, these experiments are no rigorous test of the statement that an axon maintains its cable organization under excitation. However, on the basis of the model proposed here we may conclude that preparations which may be stretched without an accompanying fall in conduction velocity are in no contradiction to the Hodgkin–Huxley theory of excitable membranes.

SUMMARY

1. It was pointed out that a number of nerve and muscle fibres could be stretched far enough to decrease fibre diameter without an accompanying decrease in spike conduction velocity. Also, it was pointed out that there exists, as yet, no reliable empirical confirmation of the velocity proportional to square root of fibre diameter prediction of the Hodgkin–Huxley theory.

2. It was concluded that, for the earthworm median giant fibre, Hodgkin's unfolding model alone was not adequate to account for this apparent independence of velocity and diameter on stretch.

3. Measurements were presented on the effects of stretch on cable (square pulse analysis method) and spike parameters of the earthworm median giant fibre over that range of stretch where conduction velocity was constant (twice reference length [$2L_0$] to four times reference length [$4L_0$]).

4. Resting membrane potential, spike amplitude, rate of rise, rate of decline, overshoot, threshold, specific membrane resistance and capacitance all remained constant under stretch. Axoplasm resistivity decreased on stretch by an amount sufficient to maintain the conduction velocity constant, considering the diameter decrease encountered.

5. It was concluded that from $2L_0$ to $2{\cdot}9L_0$ Hodgkin's unfolding model probably applies. However, for the region $2{\cdot}9L_0$ to $4L_0$, where the fibre loses volume under stretch, a new model was proposed (all the volume loss is water). This new model can quantitatively account for the observed

changes in axoplasm resistivity produced by stretch. It was concluded that with Hodgkin's unfolding model and the model proposed here essentially all of the observations made on the effects of stretch on excitable fibres could be accounted for. This was regarded as being support to a proposal incorporated into the Hodgkin–Huxley theory never independently confirmed, i.e. that axons maintain their essential cable organization under excitation.

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