

The Effect of Stretch on the Conduction Velocity of Single Nerve Fibers in *Aplysia*

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It seems well established (Gasser and Grundfest, '39; Hursh, '39; Tasaki, Ishii and Ito, '44; Berry, Grundfest and Hinsey, '44) that in vertebrate myelinated fibers, conduction velocity is a linear function of fiber diameter.

For non-myelinated fibers, Offner, Weinberg and Young ('40) and Hodgkin and Huxley ('52), proposed mathematical relationships, derived analytically, which were very similar and required conduction velocity in non-myelinated axons to be dependent on membrane resistance and capacitance, axoplasm resistance, and fiber diameter. Both these analytical approaches predicted that conduction velocity in non-myelinated fibers would be proportional to the square root of fiber diameter when all intrinsic membrane parameters were constant. Rushton ('51) felt this same relationship was implicit in the cable equation.

Pumphrey and Young ('38) working on *Loligo* and *Sepia* seemed to supply empirical confirmation of the velocity proportional to square root relation, but the very careful work of Hodes ('53) showed that in the third order giant of *Loligo*, conduction velocity is a linear function of fiber diameter. Gasser ('50, '55) using the method of reconstruction found the linear relation also held in non-myelinated fibers of mammalian peripheral nerves. Adey ('51) also found a linear relationship in the giant axons of the oligochete, *Megascolex*. However, Nicol and Whitteridge ('55) felt that some of their data on the median giant of the polychete, *Myxicola*, could be best described by the square root relation between velocity and fiber diameter. Their data, however, showed so much scatter that describing it by any such simple relationship is perhaps unjustified. Berkowitz ('55), using indirect methods, did find some empirical support for the

square root relationship from the non-myelinated fibers of the lamprey, *Entosphenus*.

This work, as well as the work of Gasser and Erlanger ('27); Blair and Erlanger ('33); Zotterman ('37); and Eccles, Granit and Young ('32) is all in agreement that, not considering the shape of the function, conduction velocity is definitely a positive function of fiber diameter.

Bullock, Cohen and Faulstick ('50) measured the conduction velocity of the median giant of *Lumbricus* while holding the animal stretched at various lengths. With appropriate magnification and careful arrangement of background illumination, they were able to observe directly this fiber while recording from it. The unstretched fiber was coiled within the ventral cord. As the animal was stretched the fiber uncoiled and the velocity appeared to increase, without any changes in fiber diameter. However, when the fiber had been pulled out straight, further stretching reduced the fiber diameter up to one half its original value, but had no effect on conduction velocity. Martin ('54) found also in frog muscle that changes in length from 67% to 122% of reference length produced no changes in conduction velocity. Martin, using Offner, Weinberg and Young's equation, and Hodgkin ('54) proceeding directly from the cable equation, independently proposed explanations of these surprising findings based on the postulate that the stretch would be taken up by the smoothing of submicroscopic folds. Both these explanations required that the area of the axon membrane would not change during stretching of the axon.

The present experiments extend the findings of Bullock, Cohen and Faulstick to another group of animals, and demonstrate the phenomenon on a preparation

which, at least in certain respects, seems more favorable for quantitative study.

MATERIALS AND METHODS

All this work was done on 20 specimens of the tectobranch, *Aplysia californica*. In each case, either the left or the right pleuro-visceral connective and its ganglia was removed from the animal, and its length under zero tension was recorded. The connective was then clamped just posterior to the pleural and anterior to the visceral ganglia. The clamps were arranged so that the distance between them could be varied by small increments. Conduction velocity was recorded in terms of delay between two fixed pairs of silver wire macro-recording electrodes, separated by about 1.5–2.0 cm. Signals were led off to an AC coupled amplifier and dual beam oscilloscope. Delays were measured between the peaks of the two diphasic spikes from photographs of the oscilloscope screen. Figure 1 is typical of the records from which velocity measurements were taken. Square wave stimuli of 0.2 msec. duration were delivered from a pair of silver wire stimulating electrodes whose poles were separated by about 2 mm. The stimulating electrodes were never placed within 0.3 cm of the recording electrodes closest to them. The length of these connectives at zero tension ranged from 2.0 to 3.5 cm. Wet weight ranged from 125 to 275 gm.

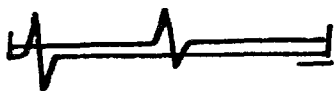


Fig. 1 Record of R3 recorded at 0.3 and 2.3 cm from the stimulating electrodes. The stimulus artifact is at the left. The length of the connective is 3.5 cm (71% stretch). Calibration, 200 μ V, 5 msec.

RESULTS

The lowest threshold response of the right pleuro-visceral connective to electrical stimulation on that connective is a relatively fast conducting, low amplitude, all or none spike (R1). When the connective was clamped at a length just sufficient to prevent visible slack, but with no stretch on it (zero tension), R1 measured 200 μ V in amplitude. In this and in all other fibers studied, the spike amplitude in-

creased by several fold as the connective was stretched out. This is probably due to a decrease in the amount of shunting tissue between the active membrane and the recording electrodes. By raising the stimulus intensity, a second, slower conducting, larger (400 μ V at zero tension), all or none spike (R2) is observed. The lowest threshold response of the left pleuro-visceral connective to electrical stimulation on that connective is another all or none spike (R3).

The conduction velocity was measured for R1, R2, and R3 as a function of length of the connective. The results of all measurements on any one of the three fibers are plotted on the same graph. For each specimen velocities were recorded for each length of the connective. As the length of any connective increased from the unstretched length, the first few velocity measurements would each be greater than the preceding, these values increasing with increasing length to a maximum value. Velocity would not differ from this value appreciably at any length of the connective greater than that at which this maximum value was first reached, regardless of how far the connective was stretched. For each specimen the mean maximum value velocity (the average of velocity measurements obtained at all lengths greater than that at which maximum value velocity is first observed) was calculated and all velocities from that specimen plotted as per cent difference from this value. Lengths are plotted as per cent increase in length over resting length (i.e., per cent stretch). Resting length is the recorded length at zero tension. The results are plotted in figures 2(R1), 3(R2), 4(R3); the pooled measurements of 12, 7 and 6 specimens, respectively.

These data may be represented by the intersection of two straight lines, one of positive, and the other of zero slope. In R1 and R2, velocity is independent of stretch from 40% to 325% stretch, in R3 from 70% to 425%.

When the conduction velocity of a fiber was measured at constant per cent stretch, as a function of time (fig. 5), the variability in conduction velocity over a period of time which was twice as long as that

of a typical stretch experiment, was as great as that in figures 2, 3 and 4, indicating that any deviation of points from a straight line in figures 2, 3 and 4 is within the experimental limits of reproducibility and not a systematic function of stretch.

In each case, the fiber conducted until the connective broke. These curves were not reversible, as releasing tension at any high per cent stretch would cause a decrease in the length of only a few per cent.

The mean absolute velocities, calculated by averaging the mean maximum value velocities (flat part of the stretch-velocity curve) for each specimen for which there is data on that fiber, are recorded in table 1. Temperature varied between 17.5 and 20°C.

CONCLUSIONS

Tauc ('57), using *Aplysia depilans*, stimulated the largest cell in the right visceral ganglion with intracellular electrodes and recorded extracellularly on the right pleuro-visceral connective. He showed that the largest spike from the right pleuro-visceral connective was produced by a process from the largest cell in the right visceral ganglion, and hence, presumably from the largest fiber in the connective.

The discrepancy which R1 and R2 exhibit with respect to the usual conduction velocity relationship (a positive function of fiber diameter) may be ascribed to differences in intrinsic membrane and axoplasm constants. This points to the possibility of error inherent in an approach which tries to compare velocities in different fibers by assuming that intrinsic characteristics are constant.

If these fibers behave like the median giant of the earthworm, then the rising portion of the stretch-velocity curves is due to uncoiling of the fibers, reducing the

actual conducting distance between the fixed recording electrodes, and producing an apparent increase in velocity. In fact, the nervous portions of the connectives at zero stretch are tubes twisted into helices lying within a cylinder of connective tissue which, when viewed histologically, forms a well defined capsule surrounding the nervous tissue proper.

The adaptive value of this portion of the curve to an animal which can change its body length by a factor of two is obvious. The author has never observed these connectives stretched more than 70% when in the animal. If the uncoiling hypothesis is correct, then the total conducting time between the pleural and visceral ganglia should not change over the rising portion of the curve, and in nature should remain constant.

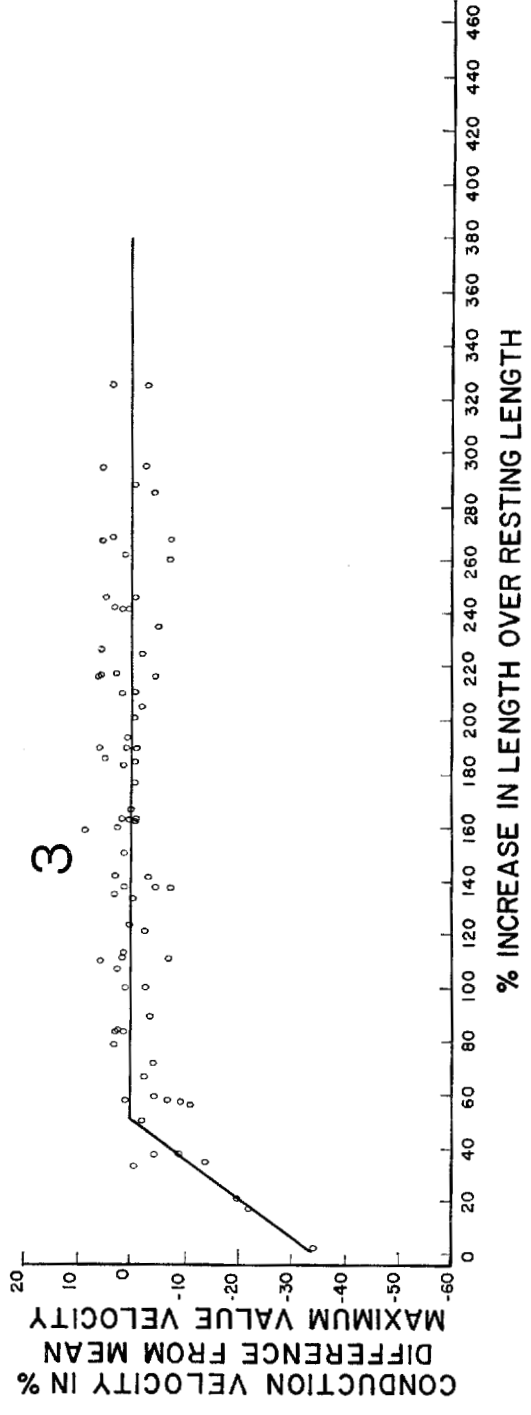
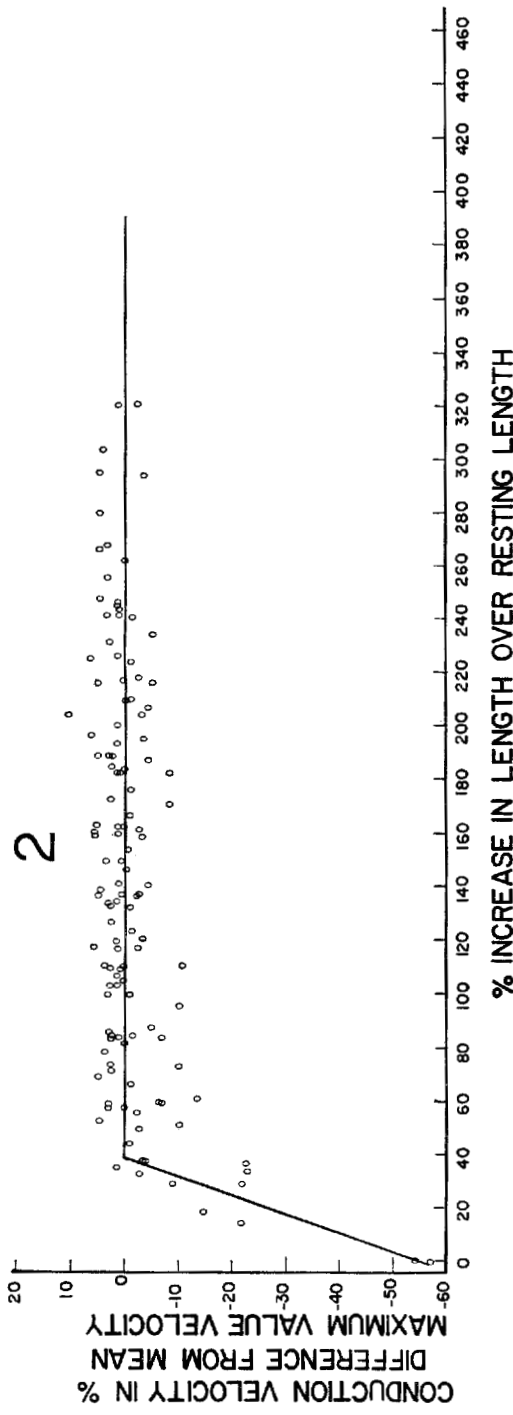
If we regard the axon as a cylinder of uniform diameter, then, since the membrane is very thin compared to the axon diameter, the axoplasm volume (V) will be given by

$$V = \pi r^2 L$$

where r is the axon radius and L , its length. For R1, if the uncoiling hypothesis is correct, L is changing only when the fiber is being stretched from 40% to 325%. At 40% stretch, let $L = L_1$. Then at 325% stretch, $L = L_2 = 3.03 L_1$. If there were no diameter changes over this range of stretch, then the axoplasm volume would have to increase 3.03 times. Similarly for R2, the volume would have to increase 3.03 and for R3 3.09 times. As an increase in axoplasm volume is unlikely the diameter must have decreased as the fiber was stretched from L_1 to L_2 . It is likely, then, that these fibers behave like the median giant of *Lumbricus*, and that over the flat portion of the stretch-velocity curve diameter is decreasing, and conduc-

TABLE 1

Fiber	Mean maximum value velocities averaged for all specimens used	Range in mean maximum value velocity among different specimens	Number of specimens data collected from
	cm/sec.	cm/sec.	
R1	91	75-108	12
R2	50	43-55	7
R3	114	98-136	6



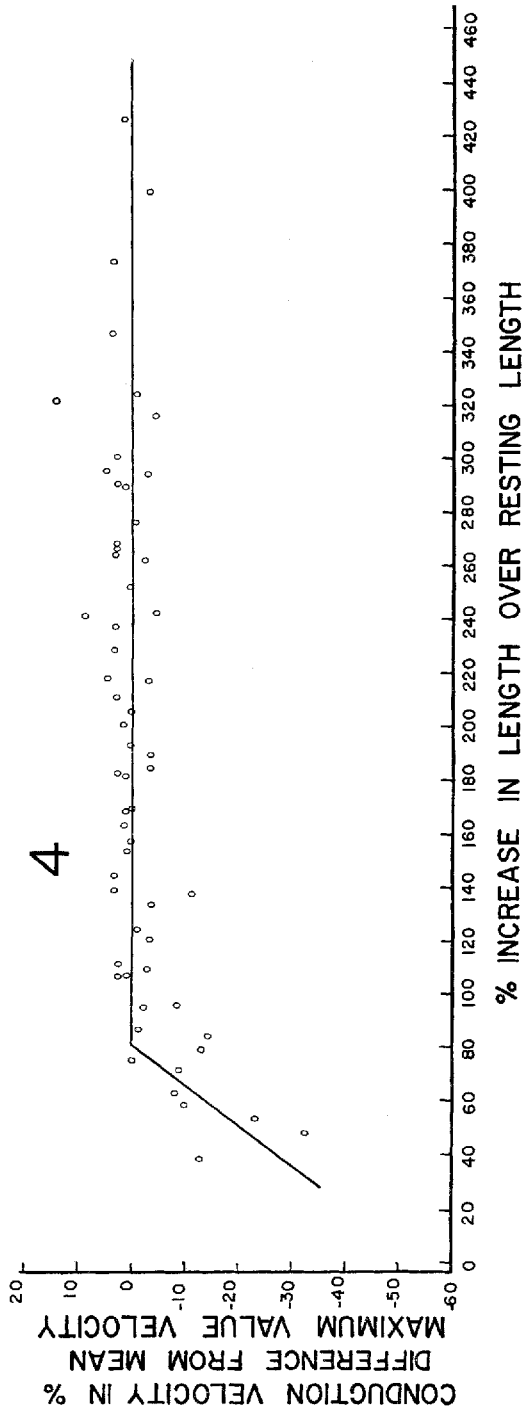


Fig. 2 Plot of conduction velocity in per cent difference from the solid line of zero slope (see text) vs. length of the connective in per cent increase in length over resting length for the R1 response of the right pleural-visceral connective in *Aplysia californica*. Each circle represents a single velocity measurement. Data collected from 12 specimens. Eighty-five per cent of the circles between 40 and 320% stretch fall within $\pm 5\%$ of the solid line of zero slope.

Fig. 3 Plot of conduction velocity in per cent difference from the solid line of zero slope (see text) vs. length of the connective in per cent increase in length over resting length for the R2 response of the right pleural-visceral connective in *Aplysia californica*. Each circle represents a single velocity measurement. Data collected from 7 specimens. Eighty-six per cent of the circles between 40 and 320% stretch fall within $\pm 5\%$ of the solid line of zero slope.

Fig. 4 Plot of conduction velocity in per cent difference from the solid line of zero slope (see text) vs. length of the connective in per cent increase in length over resting length for the R3 response of the left pleural-visceral connective in *Aplysia californica*. Each circle represents a single velocity measurement. Data collected from 6 specimens. Ninety-two per cent of the circles between 80 and 420% stretch fall within $\pm 5\%$ of the solid line of zero slope.

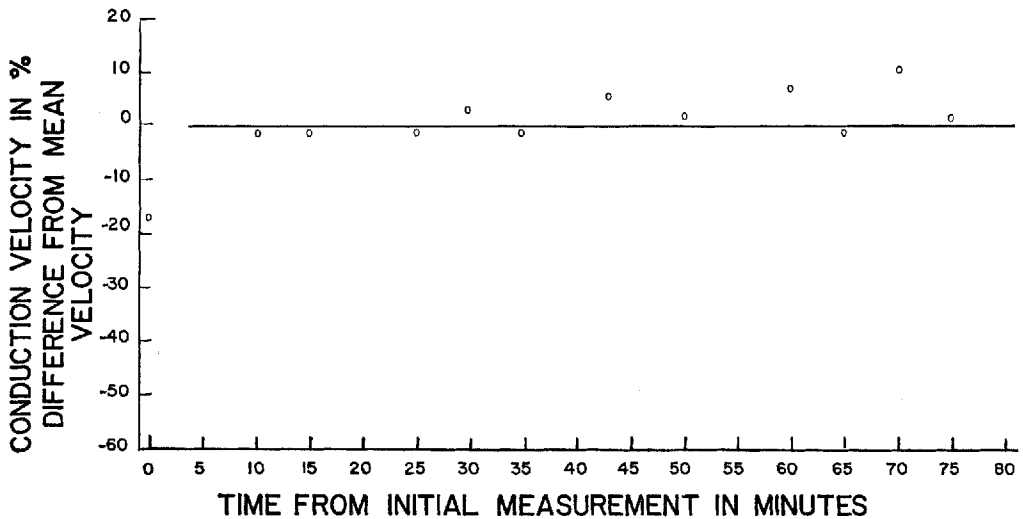


Fig. 5 Plot of conduction velocity in per cent difference from the solid line vs. time for the R1 response of the right pleural-visceral connective in *Aplysia californica*. Each circle represents one velocity measurement. These data were taken from a single preparation held at 70% stretch.

tion velocity is independent of fiber diameter.

In Martin's unfolding model

$$r \propto L^{-0.5}$$

then, if $r = r_1$ where $L = L_1$, and $r = r_2$ where $L = L_2$, and since L_2 is approximately equal to $3L_1$

$$r_2 = 3^{-0.5}r_1.$$

For a cylinder of uniform diameter

$$A = 2\pi rL$$

Where A is the surface area. In Martin's model surface area is constant over that range of length where velocity is constant. If we assume at L_2 , i. e., the point at which the fiber breaks, all the membrane folds have been pulled out straight and the axon is a smooth bored tube, then

$$A = 2\pi r_2 L_2 \text{ or } A = 2\pi 3^{-0.5} r_1 L_1$$

The apparent area at L_1 (A_1) is given by

$$A_1 = 2\pi r_1 L_1$$

Then, at L_1 A_1 is only 58% of A , i.e., 42% of the membrane would be occupied in foldings.

Martin noted that capacitance per unit apparent area and conductance per unit apparent area would both be functions of fiber length over that range of length in which the fiber membrane was unfolding.

In Martin's view conduction velocity would appear to be independent of fiber length and hence diameter because the length changes were inducing changes in capacitance and conductance which would exactly compensate for one another. Certainly his model and the equation upon which it is based require measurements of membrane capacitance and conductance as functions of stretch for full confirmation. Until this is done the observations of Bullock, Cohen and Faulstick and those presented here will not be capable of reconciliation with that large body of data which establish velocity as a positive function of diameter.

SUMMARY

1. Measurements were made on the effects of stretch on two fibers in the right and one fiber in the left pleuro-visceral connective.

2. It was possible to change the length of these connectives 300% to 350% without a change in conduction velocity.

3. It is expected that over this range conduction velocity is not a function of fiber diameter in these three fibers.

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ADDENDUM

Since this paper went to press the work of Turner ('51) on the pedal nerves of the slug *Ariolimax* was called to my attention. Turner was able to stretch these nerves to twice their resting length without a change in conduction velocity of the fastest response. The amount of folding that Turner observed histologically in the sheath of the unstretched largest fibers is not sufficient to account for the increased length on the basis of Martin's model, supplying direct evidence on at least this one preparation that the constant surface area model is inadequate to explain the observed velocity-diameter relationship.

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