

CONDUCTION VELOCITY AND DIAMETER OF NERVE FIBERS

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Previous studies of the relationship between the size of nerve fibers and the velocity of conduction of impulses have resulted in the bringing forward of three formulations:

1. $V = kD$ (Gasser and Erlanger, 1927; Gasser, 1934)
2. $V = kD^2$ (Blair and Erlanger, 1933)
3. $V = kD^{0.5}$ (Pumphrey and Young, 1938)

At the time at which the present studies were started only the first two proposals had been made, and it was hoped that by the utilization of an experimental method different from those used for the measurements that constitute the basis of the original derivation, new evidence would be acquired which would throw the balance toward one or the other of the two formulas. The method employed depends upon the generally accepted assumption that in any given nerve the largest fiber conducts at the highest velocity. The procedure in accord with the method is to select nerves with different maximal velocities, measure the velocities, and then prepare histological sections of the nerve in which measurements of the diameter of the largest fiber can be made.

A similar method was used by Pumphrey and Young for the small fibers in their nerves. The velocities in the large axons were determined individually and the diameters were measured directly in the fresh state. Squid nerves were used exclusively in their experiments, and this fact must be kept in mind when their data are compared with the data to be presented later, all of which were obtained from medullated vertebrate nerve.

METHOD. In order to obtain valid velocity readings it is essential that the fibers be in the best possible condition. Precaution is especially necessary when small fibers are concerned, as they are more susceptible to injury than large fibers, and if injured they acquire a velocity rating that is too low. The method is a favorable one from the standpoint of the conservation of fibers, as dissection is limited to freeing the nerves of adherent fascia, and it is not necessary to use small strands. Excised nerves of the cat were mounted on silver-silver chloride electrodes in a moist

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chamber, and velocity readings were made as soon as the nerve could be brought into equilibrium with the temperature in the chamber which was maintained at 37.5°C. After the measurements had been made, the nerve was immediately taken out and fixed. Thus distortion of the fibers, which is often seen in nerves that have been subjected to long periods of stimulation, was held to a minimum. Krebs-Ringer solution was used to moisten the nerve during the experiment, and the atmosphere in the observation chamber was 5 per cent CO₂ in O₂.

Measurement of velocity. Condenser discharges controlled by a thyatron were used for stimulation, and the shock strengths were adjusted to give a maximal response of the group of fibers yielding the first elevation in the action potential of the nerve stimulated. Oscillographic records of the action potential were made at five or more distances of conduction, and then the shock-response times were measured on enlarged projections of the records. Because of the utilization period of the shock and the possibility of spread of the stimulus, no single shock-response time can be depended upon for the calculation of the velocity. But the series of times, plotted against the distances, yields a straight line graph the slope of which gives the velocity freed from these sources of error.

High amplification was used for recording. In nerves containing large fibers it was possible, by reducing the strength of shock, to stimulate single fibers and record from them. In view of this fact the conclusion is warranted that the first deviation of the action potential from the baseline in the records made for velocity measurements could be referred to the single largest fiber in the nerve. When small fibers were being studied it was not possible in nerves of the sizes employed to record single spikes with certainty, because of interference by the noise level. Arbitrarily therefore it was assumed, when the maximal velocities were 20 m.p.s. or less, that the first deviation of the action potential should be referred to the three largest fibers instead of to the single largest fiber. Procedure on this basis introduced at most only a small error, since the average diameter of the three largest fibers was ordinarily only a few tenths of a micron less than the diameter of the largest fiber.

Measurement of fiber diameter. When the nerves were removed from the moist chamber they were tied to glass rods to prevent a change in length, and fixed in either 1 per cent osmic acid or 10 per cent formalin. In the latter case they were subsequently stained according to the Kultschitzki technique. The osmic acid method was satisfactory for nerves in which large fibers were to be measured and it had the advantage of being less time-consuming. For nerves presenting small fibers for measurement the second method was preferable. The size measurements, however, were found not to be significantly different when a portion of a nerve prepared in one way was compared with another portion of the same nerve

prepared in the other way. The sections were made after embedding in paraffin, and the diameters of the fibers outside of the myelin sheath were measured with the aid of an ocular micrometer.

Sources of error. 1. *Tapering or branching of the nerve fibers.* The nerves used for embedding were cut into three pieces, and the pieces placed side by side in the paraffin block. Thus each of the finished slides contained in the serial sections samples of the middle segment of the nerve and of the two end segments; and the juxtaposition of the samples made it convenient to determine whether the "largest fiber" tapered or branched in the course of the preparation upon which the velocity measurement had been made. As was to be expected from the straight-line character of the conduction-time conduction-distance curves, no evidence of tapering or branching was found in any of the preparations. It should be stated, however, that the only means of identifying the largest fiber in widely separated nerve regions was by its size, and the measurements reported here establish only the fact that the largest fiber in the peripheral segment of the preparation was not smaller than the largest fiber in the middle and proximal segments.

2. *Random variation of the fiber diameter.* In order to investigate the magnitude of the possible variation in size in the course of the fibers, due to the technique of preparation or other causes, measurements were made of 100 consecutive 6 μ paraffin sections of a saphenous nerve stained with osmic acid. By following one of the small bundles which made up the nerve, it was possible to make sure that the several measurements were all made on the same fiber. The distribution of the measured diameters is shown in figure 1. From the data it can be shown that the standard deviation of a single measurement of a 6.5 μ fiber (modal value) is ± 0.47 μ . As the slides, prepared from the nerves on which the velocity measurements had been made, contained six sections each from three portions of the nerve and as the largest fiber was measured in each of the sections, the error of measurement would be less than that for a single measurement.

If this variation in diameter is brought about by the histological preparation and is not a characteristic of the living fiber, it must occur during the fixation and not in the dehydration process, since Duncan (1934) has shown a comparable diameter alteration along the length of nerve fibers fixed in osmic acid and teased in glycerine.

3. *Differential distortion of large and small fibers.* If nerve fibers of all sizes are not affected in the same manner in the course of histological preparation, the true form of the curve relating velocity and nerve diameter will not be revealed. It was, therefore, necessary to show either that distortion does not occur during the preparation of the nerves, or that if it does occur, it affects fibers of different diameters in a proportional manner.

Donaldson and Hoke (1905) reported that in nine experiments on the

sciatic nerve of the rat there was an average increase in nerve diameter of 0.3 per cent in the course of the steps from the fresh to the paraffin embedded state. They measured the diameter of the entire nerve trunk, as they believed it was impossible to tease out single fibers without stretching them and altering their reactions to histological reagents. Lopicque and Desoille (1927) measured the ranges of variation in the diameter of the largest fibers found at the several levels of the frog sciatic nerve. The range at a given level was the same whether the diameters were measured on fresh teased fibers or on the paraffin cross-sections of fixed specimens.

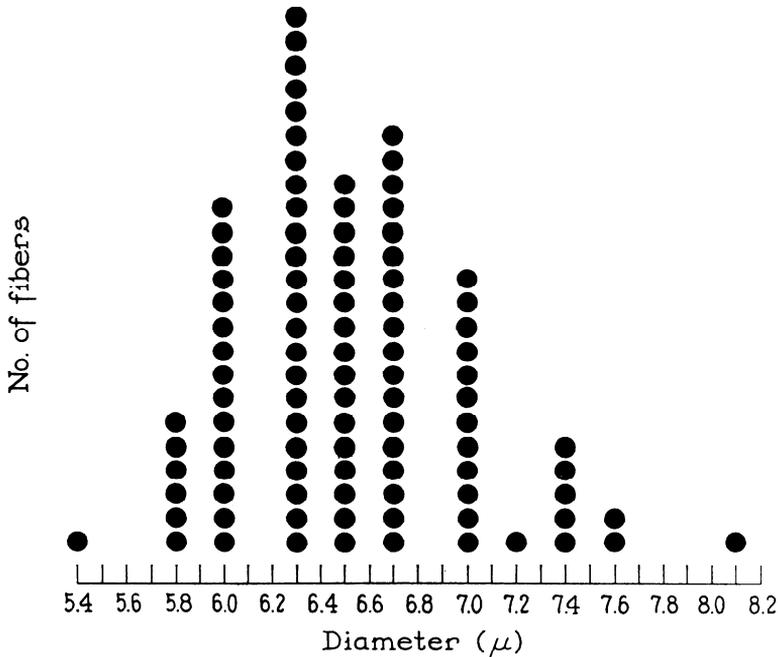


Fig. 1. Distribution of the diameter measurements on a single fiber followed through 100 six-mu serial sections.

Duncan (1934) also examined the question of shrinkage and concluded that the diameters of the living fibers were retained in the mounted cross-sections.

Evidence to the contrary has been presented by Arnell (1936) who measured the fiber diameters in fresh frozen sections and in paraffin sections of the same nerve. Comparison of his tables II and XII shows that a shrinkage of 20 to 30 per cent occurred during the histological preparation. Our experience is in agreement with that of Arnell in showing that shrinkage is produced.

Since the difficulties of dealing with single fibers were recognized, small

spinal root bundles having relatively little connective tissue were used as experimental material. Bundles measuring 0.1 to 0.05 mm. in diameter were attached with ceresin to a glass slide. The slide was ruled at right angles to the course of the nerve, so that measurements could be repeated at exactly the same locus. Diameters at eight points along each bundle were measured by means of a binocular microscope fitted with an ocular micrometer. Fixation in osmic acid caused an average (36 nerve bundles) increase in diameter of 2.2 ± 0.5 per cent. Fixation and dehydration caused an average decrease in the fresh nerve bundle diameter of 23.6 ± 1.2 per cent.

The interpretation of these results was made somewhat ambiguous by the fact that although the spinal root bundles do not have the thick outer connective tissue sheath found in nerve trunks, there is connective tissue between the individual nerve fibers. Doubt, therefore, existed regarding the proportion of the total shrinkage due to connective tissue as compared with that due to nerve fibers.

Inasmuch as fixation in osmic acid did not change the root bundle diameter, it was thought probable that the nerve fiber was not distorted by this treatment. Nerve fibers were, therefore, teased out from a spinal root bundle which had been fixed in osmic acid. Measurements of the nerve diameters were made before and after dehydration. Twenty measurements were made for each diameter and eleven nerve fibers, ranging in size from 22.5 to 10.2 μ , were examined. The shrinkage found varied from 8.2 to 12.9 per cent, with an average value of 10.1 ± 0.16 per cent. There was no correlation between the percentage of shrinkage and the size.

These data considered in relation to the nerve bundle experiments show that a considerable proportion of the shrinkage found during dehydration is due to connective tissue. Shrinkage of the fibers occurs, however, but without indication of its being differential.

Nerves used in the investigation. The maximal velocity of conduction and the diameter of the largest fiber were measured in the following nerves of the cat:

NERVES	MAXIMAL VELOCITIES
	<i>m. p. sec.</i>
Peroneus.....	108-111
Suralis.....	69- 86
Saphenous.....	65- 82
Vagus.....	67- 73
Cervical sympathetic.....	29- 56
Hypogastric.....	11- 41

RESULTS. The data obtained from these nerves are presented in figure 2. The points seem to fit a straight line more readily than any other

graph. The line which has been drawn is the calculated regression line. It has a slope of 6.0, which means that the velocity in meters per second can be obtained by multiplying the diameter in micra by this factor. The broken lines represent the velocity as a function of the square and the square root of the diameter respectively, assuming that a 13.8 μ fiber conducts at a velocity of 78 meters per second. It is quite apparent that the data are not compatible with either of these two assumptions; and they would be still more incompatible if the square and the square-root

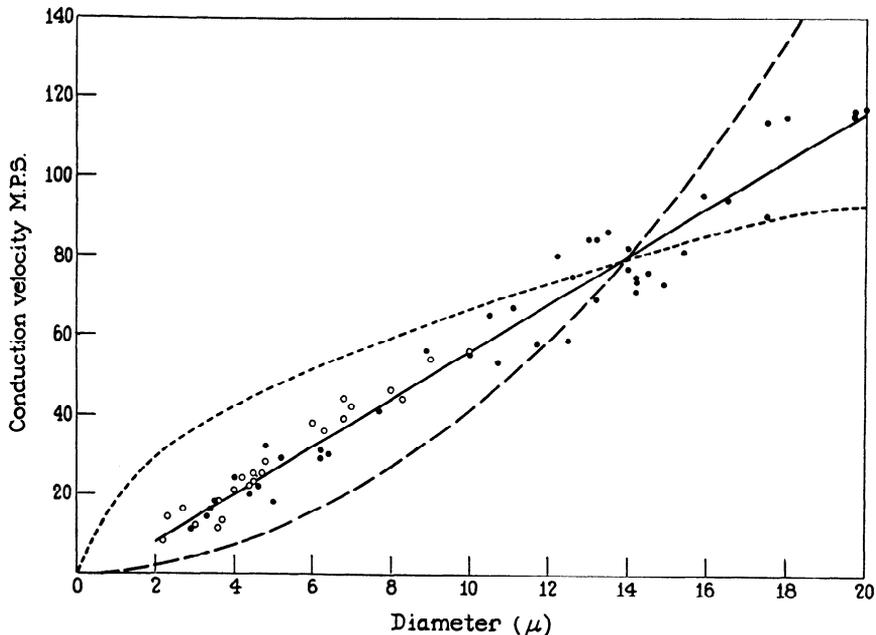


Fig. 2. The fastest conduction rate plotted against the diameter of the largest fiber for nerves of different velocities. The dots are data on adult cats; circles, data on kittens. The dotted and dashed lines represent the velocities calculated as the square root and square functions of the diameter.

curves were drawn to include the points representing the extremes of velocity.

The diameters of the constituent fibers in any particular nerve are known to be smaller in young animals than in the adult (Boycott, 1904; Duncan, 1934). Young animals, therefore, provide an additional source of nerves in which the largest nerve fibers are of small caliber. On these nerves two questions may be studied. Is the conduction velocity in immature nerves proportional to the diameter and is the factor of proportionality the same as that holding for adult fibers?

Both questions may be answered in the affirmative. The circles in

figure 2 present data obtained from the nerve trunks of kittens. It is obvious that they lie very close to the regression line drawn for the cat fibers. The exact slope of the latter is 6.04. The regression line drawn for both the kitten and cat fibers has a slope of 6.00.

DISCUSSION. Correlation of the conduction velocity with the outside diameter of the fiber means that the velocity may be determined by any

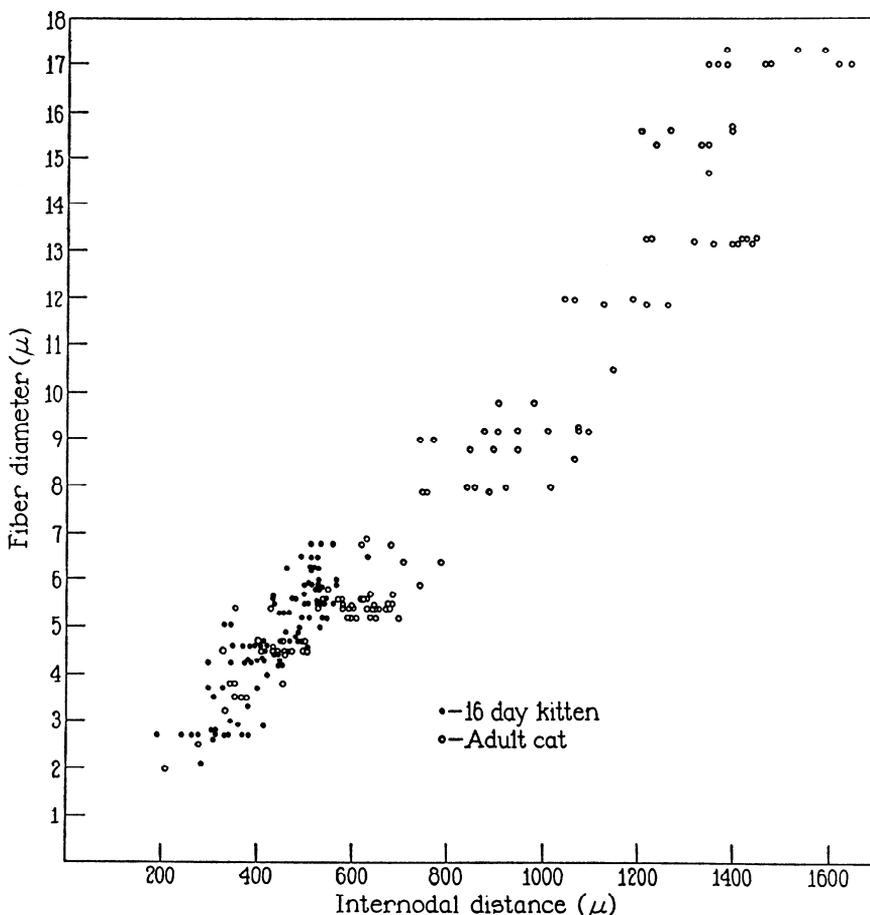


Fig. 3. The internodal distance plotted against nerve fiber diameters of a 16-day-old kitten and an adult cat.

dimension in linear relation to the diameter. The axon diameter is the dimension demanding first consideration. According to Donaldson and Hoke, the ratio of the axon diameter to the total diameter is constant. On the other hand, Arnell (1936) and Schmitt and Baer (1937) find a systematic deviation in the ratio dependent upon the fiber size. The scatter in

the data, however, is too great to permit their use for or against an hypothesis proposing control of the velocity by the axon diameter.

Another dimension to be considered is the length of the internodal segments. The evidence that these lengths are proportional to the fiber diameter has recently been cited by Zotterman (1937). Before the internodal lengths could be considered, however, it would be necessary to show that the lengths are the same in kitten and in cat fibers of the same diameter, inasmuch as kitten and cat fibers of the same size conduct impulses at the same velocity (fig. 2). The point has been especially examined because Boycott in 1904 published data showing that in frog fibers of a given size the internodes are longer in mature frogs than they are in young frogs.

The fibers of the peroneal nerve were teased in a 50 per cent glycerine solution, after fixation with osmic acid according to a method described by Takahashi (1908). The data are presented in figure 3. The dots represent kitten fibers and the circles cat fibers. In the region where the diameters overlap there is very little difference between the internodal lengths for the two ages. The relationship defined by the curve, therefore, is consistent with the substitution of internodal length for the fiber diameter in arguments about the control of velocity.

SUMMARY

The conduction velocities in the fastest fibers in various nerve trunks of the cat and the kitten were correlated with the outside diameters of the largest fibers found in those trunks. The limits of the range of velocities measured were 8 and 117 meters per second.

The best curve relating velocity and diameter is a straight line. It holds equally well in all parts of the range of velocities. Curves drawn in accord with the hypothesis that the velocity varies as the square or square root of the diameter vary widely from the observed points.

Comparative data relating internodal length to fiber diameter are plotted for nerve fibers from cats and kittens.

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