Internode Length in the Skin Plexuses of Fish and the Frog

By MARY WHITEAR

(From the Department of Zoology and Comparative Anatomy, University College, London)

SUMMARY

Measurements made in the skin plexuses of teleosts and of Rana have shown that the internode lengths of myelinated fibres may here be shorter than the 'minimum internodal length' previously reported.

INVESTIGATIONS into the anatomy of vertebrate peripheral nerves have included studies of the spacing of the nodes of Ranvier on medullated fibres of various diameters; there is a relationship between internode length and fibre diameter, which was shown by Vizoso and Young (1948) to depend on the time of medullation of the fibre and its subsequent growth. Several workers, whose results are summarized by Thomas and Young (1949), reported that the length of the myelin segment between adjacent nodes has a minimum value, regardless of diameter; this figure, which may be of importance in a consideration of the physical properties of the myelin, is usually given as 200 \( \mu \), though Young (1945) records it as 150 \( \mu \) in the foetal rabbit. The diameters of the fibres concerned were not less than 2 \( \mu \); studies were made on mammals, amphibians, and fishes.

During an investigation of the anatomy of the skin plexus of the minnow, Phoxinus laevis (Whitear, 1953), it was noticed that the myelin segments were considerably shorter than any previously reported, being about 50–60 \( \mu \) in length. The skin was stained by the local subcutaneous injection of methylene blue and subsequently treated with ammonium molybdate, dehydrated and cleared in the usual way. Measurements of internode length and fibre diameter were made with a moving-wire micrometer eyepiece, under the highest convenient magnification. The fibres in the plexus are seldom straight, so that some errors in the measurement of length were unavoidable, although measurements were not made on the more tortuous fibres. To determine the extent of the error, measurements of 28 internodes, on four fibres, were made twice, independently, with the ½-inch objective. The mean of the differences between the 28 pairs of measurements was 0·7 \( \mu \) with a standard error of \( \pm 0·5 \mu \) (\( t = 1·40 \) for 26 degrees of freedom); it follows that there was no significant difference between the two sets of measurements. (Another source of error lies in shrinkage during preparation. Since the fibres cannot be observed until the material has been cleared this error is not measurable, but can be assumed both to be constant and to be insufficient to account for the [Quarterly Journal of Microscopical Science, Vol. 93, part 3, pp. 307-13, Sept. 1952.]
Fig. 1. Diagrams showing the lengths of myelin segments in sub-epidermal plexuses. A–E, minnows; F, a gudgeon. For explanation see text.
large differences between the lengths of these internodes and those reported elsewhere.) The mean of the two sets of twenty-eight measurements was 55.6 μ and the standard deviation of the internode length 16.4 μ. These internodes are those shown in fig. 1, A; measurements made on other minnows are recorded in fig. 1, B, C, D, and E. All the measurements recorded in any one diagram are from the same animal; each vertical column of the histogram represents the length of a single myelin segment, and successive internodes on a single fibre are shown in blocks, the proximal end to the left, the peripheral to the right. Blocks separated by only the width of a single column from the preceding block represent branches of the same fibre, though the point at which the fibre branched is not indicated. The blocks separated by more than the width of a single column each represent a separate fibre from the same preparation. The diameters of the measured fibres ranged from something over 1 μ ('fine' fibres) to about 2 μ ('coarse' fibres); exact measurement was not possible. All the fibres measured in the fish preparations were of the sub-epidermal plexus.

The length of the shortest internodes seen was 20 μ. These were rare on the thicker cutaneous fibres (see fig. 1, A, E) but were more common on the finer fibres; the average internode length on the 'fine' group of fibres was lower than on those of greater diameter, as may be seen by comparing blocks c, d, e, and f in fig. 1, D with fig. 1, A, B, C, and E. In fig. 1, D, blocks a and b represent the terminal internodes of 'coarse' fibres closely adjacent to the 'fine' measured fibres (blocks c to f); fibre e, which is fine, contains two long internodes, which may have arisen by the coalescence of myelin segments across a node (see Thomas and Young, 1949; or Speidel, 1933). There is in any case a wide variation in internode length. The presence of scales may sometimes cause irregularities as the advancing edge of a growing scale will stretch that part of an adjacent nerve fibre which passes round the scale towards the surface; in fig. 1, F, which represents fibres from a gudgeon, Gobio fluviatilis, block h represents such a fibre, the myelin segment at the edge of the scale being considerably longer than the proximal internodes which lie under the scale.

It was apparent that in the sub-dermal plexus the internodes were usually longer than in the sub-epidermal plexus, that is, that internode length decreased as the fibre neared its end; this was confirmed in osmium tetroxide preparations, where it was seen that diameter also decreased towards the periphery. Measurements were not made as it was difficult to identify a single fibre for any distance owing to the crossing of the numerous fibres in these nerve bundles, which are larger than those of the sub-epidermal plexus. In the frog, Rana temporaria, on the other hand, individual fibres may be traced in larger nerve bundles; accordingly, preparations of frog skin were made, by subcutaneous injection of methylene blue, as in the fish. Internode length was measured on fibres approaching and ramifying in the skin of the thigh; representative results are shown in fig. 2, A, B, and C. It will be seen that here, as in the fish, the peripheral internodes are often less than 150 μ in length.
FIG. 2. Diagrams showing the lengths of internodes on fibres entering the dermal plexus of the thigh of frogs. A and B, length of thigh 2·5 cm.; C, length of thigh 1·5 cm.
The various fibres represented cannot be directly compared as only that part of a fibre which was well stained could be studied and the distance of the well-stained segments from the final node is not constant. Though there is considerable variation in internode length along any part of the fibre, it is clear that, on the whole, there is a progressive decrease in the length as the fibre approaches its termination (that is, towards the right of each block in the diagrams); this is correlated with an increased frequency of branching, a fact which does not appear in the figures. The columns at the extreme left of fig. 2, B represent individual internodes from a larger nerve bundle, approaching the skin; here the nodes are farther apart than in the dermis. The statement of Takahashi (1908), that the average length of the internodes on the fibres in the nerves of the leg diminishes towards the periphery, does not refer to the same phenomenon, for his measurements were made at different levels of the nerve trunks in the leg, not in the finer cutaneous branches.

No measurements were made in the larger nerve trunks of the leg. Boycott (1904) measured the internodal lengths found in the sciatic nerves of frogs of various sizes; his figures are quoted and further analysed by Hatai (1910). It is not possible to make direct comparisons of the fibres measured by Boycott and those seen in the skin plexus, unless a detailed analysis of the range of fibre diameters be made. The diameter decreases as the fibre branches, though the various branches are not always of equal calibre. Furthermore, though the coarsest fibres stained were selected for measurement, these were not necessarily the largest fibres present. Only the minimum length of internode is relevant in the present instance; Boycott records none of less than 200 μ in the sciatic nerve. In the dermal plexus of the frog no internodes less than 50 μ in length were seen, but myelin segments of less than 100 μ were common in the branches; these were not necessarily at the extreme end of the medullated part of the fibre, for measurements could not be made beyond the point at which the fibre turned towards the surface.

Young (1944) and Thomas and Young (1949) suggest that a limiting physical condition, such as the stable length of a droplet under surface tension, may be one of the factors determining the minimum length of an internode. Hiscoe (1947) disagrees on the grounds that the myelin is not continuous from one node to the next but is interrupted by the incisures of Schmidt-Lantermann; she also considers that the laminated microstructure of myelin (citing Schmitt and Bear, 1937) precludes the consideration of any simple forces of surface tension. Whether the minimum internodal length of the nerve trunks is taken as 200 μ or as 150 μ, it is evident that the myelin segments in the dermis may be shorter than this. If therefore it is suggested that the minimum length of the myelin segment does depend on surface tension, or on some similar physical factor, it must be shown that the conditions affecting this factor differ in the two environments, to such an extent as to account for the difference in minimum length. If the myelin segment is regarded not as a stable droplet but as a droplet held in dynamic equilibrium by several factors which include the passive (or elastic) limiting pressure of
the neurilemma and the surrounding tissues, its form or length might perhaps be the more readily modified by external factors.

The decrease in the lengths of the internodes as the fibre approaches its end may be partly explained by the fact that the proximal parts of the fibre were medullated first; during growth these proximal segments must have been stretched more than those which were established later, but the decrease in internode length as the fibres pass through the dermis is sudden enough to make it unlikely that such a factor alone is responsible. The fact that growth has taken place may mean that the minimum length in the nerve trunks was in fact less than 150 \( \mu \) when the myelin first appeared; Young (1945) states that in the foetal rabbit the myelin segments on their first appearance were 150 \( \mu \) in length, and that earlier, when the Schwann cells (nuclei?) were fairly regularly spaced at intervals of 100 \( \mu \) or less, no myelin was detectable.

It has also been suggested that the length of the young Schwann cell itself may govern the position of the nodes (Young, 1945; Hiscoe, 1947). If this were so, the difference of minimum internode length in the nerve trunks and in the skin might reflect a difference in the initial periodicity of the Schwann cells, perhaps due to such environmental factors as a restriction in the length of the Schwann cells where two or three come to occupy the limited distance between two points of branching of a fibre; in such a case the myelin segments being produced under their respective influences would also be short. In regenerated nerves of adult rabbits the internodes are all relatively short, ranging from 150 \( \mu \) to 700 \( \mu \) (Vizoso and Young, 1948); although Vizoso and Young speak of 150 \( \mu \) as the lower limit of internodal length in these regenerated nerves, they record a few myelin segments only 100 \( \mu \) long on the finer fibres. In the minnow, where the shortest internodes seen measured 20 \( \mu \), the length of a Schwann cell nucleus was 10 \( \mu \); in photographs of regenerating rabbit nerve published by Holmes and Young (1942) most of the Schwann cell nuclei are longer, about 20 \( \mu \) in length.

It can be concluded that the minimum internodal length is less than that previously reported and that, even when allowance has been made for subsequent growth, the internodes in the dermis may be considerably shorter than those in the nerve trunks.

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