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Fibre numbers and sizes in the inferior alveolar nerve of the cat

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INTRODUCTION

The inferior alveolar nerve of the cat has been examined both electrophysiologically (Pfaffman, 1939; Hannam, 1969; Matthews & Holland, 1975) and histologically (Windle, 1926; Brashear, 1936; Thomas, 1946; Mohiuddin, 1951; Kizior, Cuozzo & Bowman, 1968). Because they survive dissection more readily and provide a stronger signal, larger fibres tend to dominate electrophysiological recordings, and we must turn to histological techniques for a reliable description of the A delta (1–6 μm) and C (non-myelinated) fibre components. Previous reports show A delta fibres making up as little as 28% of the myelinated total. Windle (1926) reported that 17% of the total fibre count was non-myelinated, while Brashear (1936) reported 22%, Thomas (1946) 10·9% and Mohiuddin (1955) 11%. Spinal nerves on the other hand contain as many as 6 times more non-myelinated than myelinated fibres, and cutaneous nerves 4 or 5 times more (Iggo, 1973). Recent work on the intradental nerves of cats (Johnsen & Karlsson, 1974; Beasley, 1977) and marmosets (Buetlmann, Karlsson & Edie, 1973) have shown their fibres to be almost exclusively in the A delta and C ranges, with up to 9 times as many non-myelinated as myelinated fibres. Early studies on the intradental innervation of the cat (Brookhart, Livingston & Haughen, 1953) failed to demonstrate any C fibres. Since the inferior alveolar contains many fibres from the mental region and the periodontal tissues as well as from the pulp, one would expect it to have a larger population of small fibres than has previously been described.

It seems reasonable to suggest that technical limitations have prevented accurate assessment of the narrow fibre composition of the inferior alveolar nerve in the past. While silver impregnation techniques such as that used by Windle (1926) almost certainly underestimate the non-myelinated component, routine histological techniques may also fail to show up many of the small myelinated fibres. Biedenbach, Bauerman & Brown (1975), using light microscopical examination of 1 μm plastic sections, found counts for the lingual nerve of the cat almost double those quoted in earlier studies. Hughes & Wassle (1976) report a similar 'increase' in their study on the optic nerve. Many factors may be responsible for these higher counts. Improved fixation, a reduced leaching of myelin components in plastic embedding procedures, and higher resolution from thinner sections are evident possibilities. There is also less possibility of over estimating diameter measurements in thinner sections (Dyck *et al.* 1968). While the use of 1 μm plastic-embedded sections no doubt increases the accuracy of counts, it could well be that low power electron

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microscopy would enable even more narrow, lightly myelinated fibres to be seen. Using low power electron microscopy McAlear, Camougis & Thibodeau (1961) found counts 60 % higher than those reported in previous studies using light microscopy. None of these studies, however, compared counts obtained by light microscopy with counts from the same specimen using electron microscopy.

Young & King (1973), using electron microscopical techniques, showed that 40 % of the fibres present in the sensory root of the trigeminal nerve of a baboon were non-myelinated. No similar survey has been carried out on the inferior alveolar nerve of the cat. According to Windle (1926) there are considerable differences in the composition of different branches of the trigeminal nerve. Thus he found only 17 % of the fibres of the inferior alveolar nerve were non-myelinated but 60 % were non-myelinated in the lacrimal branch of the ophthalmic. Biedenbach *et al.* (1975) reported marked differences between the lingual and ethmoidal nerves, and suggested that each nerve needed to be examined individually.

This paper reports observations on the full myelinated nerve spectrum, and the proportion of non-myelinated fibres present, in the inferior alveolar nerve of the cat. In addition, the myelinated spectrum obtained by light microscopy of one major branch has been compared with that of an adjacent ultrathin section examined by electron microscopy.

METHOD

Specimen preparation

A young adult cat anaesthetized with sodium pentobarbitone was perfused with a 2·5 % solution of glutaraldehyde in 0·1 M phosphate buffer for 30 minutes. The inferior alveolar nerve was dissected out of the mandible and post-fixed in 2 % osmium tetroxide in distilled water for 2 hours. The nerve was stained *en bloc* with uranyl acetate and embedded in Epon. The block was trimmed so as to present a cross section of the nerve at the cutting face. A 1 μm section of the complete nerve was cut, mounted on a glass slide, and stained with toluidine blue (Fig. 1). The block was retrimmed to present two of the larger bundles, A in Figure 1 (assumed from the study of De Lange, Hannam & Matthews (1969) to be the mental nerve) and C in a form suitable for thin sectioning. An ultrathin section was cut and mounted on a 1 × 3 mm slot grid supported with a formvar film. This was the largest section that could be accommodated without obscuring part of the nerve. The section was stained with uranyl acetate and lead acetate.

Light microscopy

The 1 μm section was examined in a Zeiss photomicroscope and reproduced as a photographic montage at a magnification of × 200 on 35 mm Kodak high contrast black and white copy film. Each of the bundles (labelled A–I in Fig. 1) was printed at × 10 photographic magnification, giving a total magnification of × 2000. The largest bundle, A, was divided into three concentric zones of equal width. Each nerve fibre in each zone of A, and in all the other bundles, was traced onto clear acetate sheets, and measurements were made from these sheets using the Quantimet 720 (Cambridge Imanco, 40 Robert Pitt Drive, Monsey, N.Y. 10952) image analysis system.

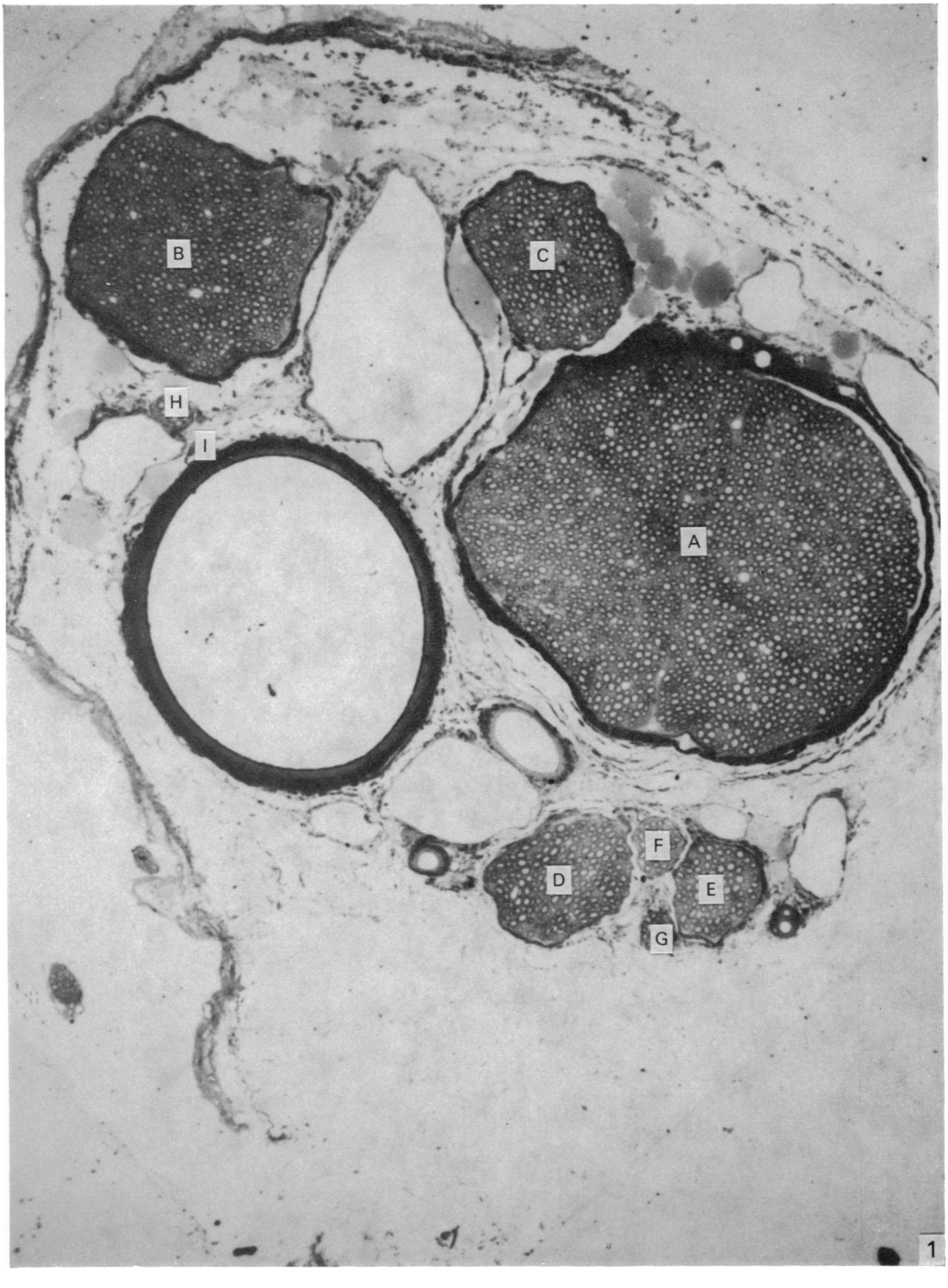
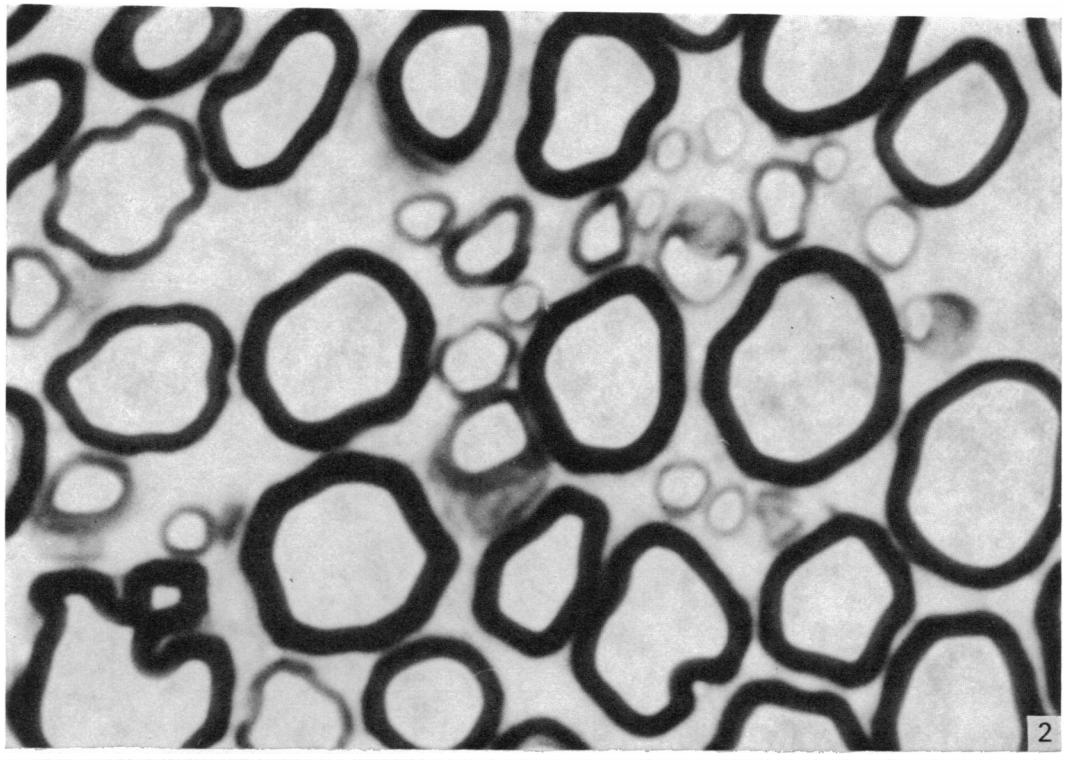


Fig. 1. A photomicrograph of a toluidine blue-stained 1 μm section of the inferior alveolar nerve of the cat. $\times 75$. The letters relate to the fibre counts listed in Table 1.



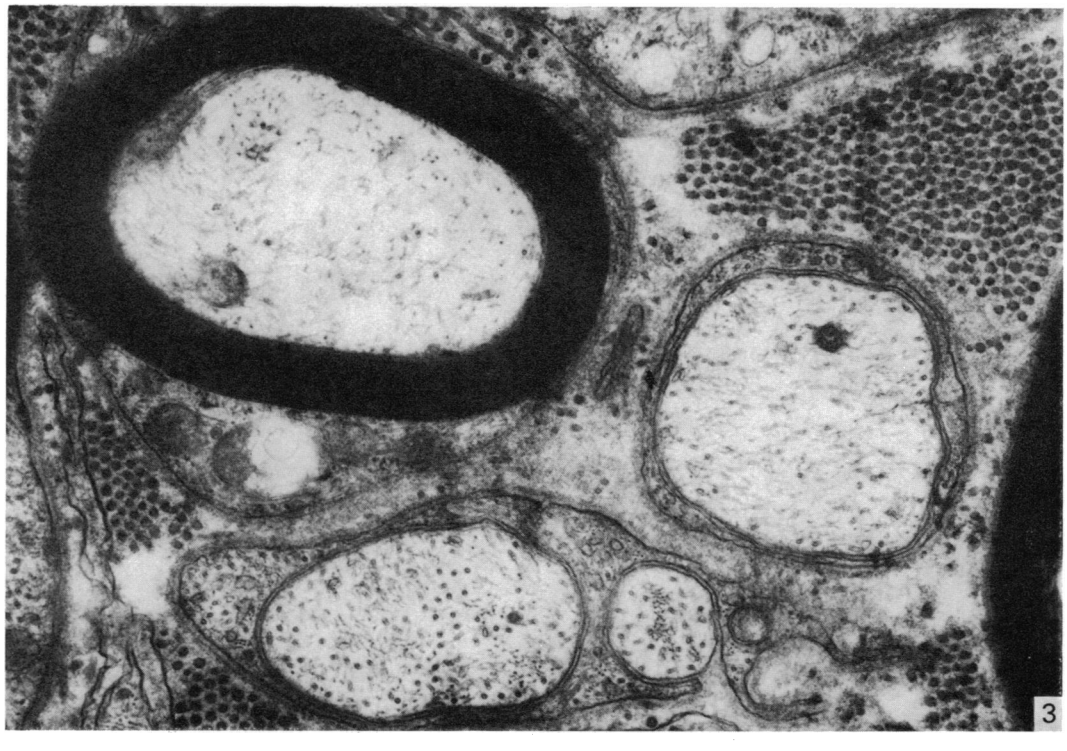


Fig. 2. A light micrograph of part of the 1 μm section at higher power. $\times$ 2000.

Fig. 3. An electron micrograph showing a small myelinated and several non-myelinated axons from bundle A. $\times$ 24000.

Table 1. *The number of myelinated fibres present in individual bundles of the inferior alveolar nerve*

Nerve bundle	No. of fibres
A	4244
B	1413
C	609
D	317
E	157
F	50
G	32
H	33
I	1
Total	6856

Electron microscopy

The ultrathin section of bundles A and C were examined in a Siemens 101 electron microscope at $\times 2400$. Montage electron micrographs were prepared of the entire bundles. In all, 600 micrographs were taken at standard exposures, with overlap between adjacent areas to ensure full coverage. Each micrograph was printed at $\times 3$ photographic magnification, giving a total magnification of 7200. The montage of A was assembled, and when complete was 13 feet in diameter. This montage was divided into three concentric zones of equal width, and the myelinated and non-myelinated fibres in each area were traced. The number of axons per Schwann cell was also recorded. The montage of bundle C was used merely to count the incidence of non-myelinated axons in a second bundle.

Diameter measurements

In material fixed in this manner myelinated nerves usually have an irregular outline (Fig. 2); they are rarely circular. To compensate for this the diameter was calculated as the diameter of the circle having the same area as the fibre. The area of each nerve fibre was measured from the tracings using a Quantimet image analysis system linked to an epidiascope (Sturgess, Berry & Heil, 1972). The equivalent diameters were computed automatically by a Wang 600 PTS calculator attached to the Quantimet system. The error involved in making the measurement is less than 1 % when measuring a profile of known area. The Quantimet system allowed rapid and accurate measurement. In all more than 15000 nerve profiles were examined in this study.

RESULTS

Qualitative observations

Fixation, despite the large size of the specimen, was good. The myelinated nerves were usually irregular in outline (Fig. 2). Most of them were intact, only a small proportion showing any distortion or disruption of the lamellar arrangement. In the 1 μm section the narrower, more lightly myelinated fibres were difficult to distinguish from fine capillaries, and their outline was often not sharp (Fig. 2). The smaller myelinated fibres often occurred in columns wedged between larger fibres. The non-myelinated fibres rarely occurred in groups of more than three.

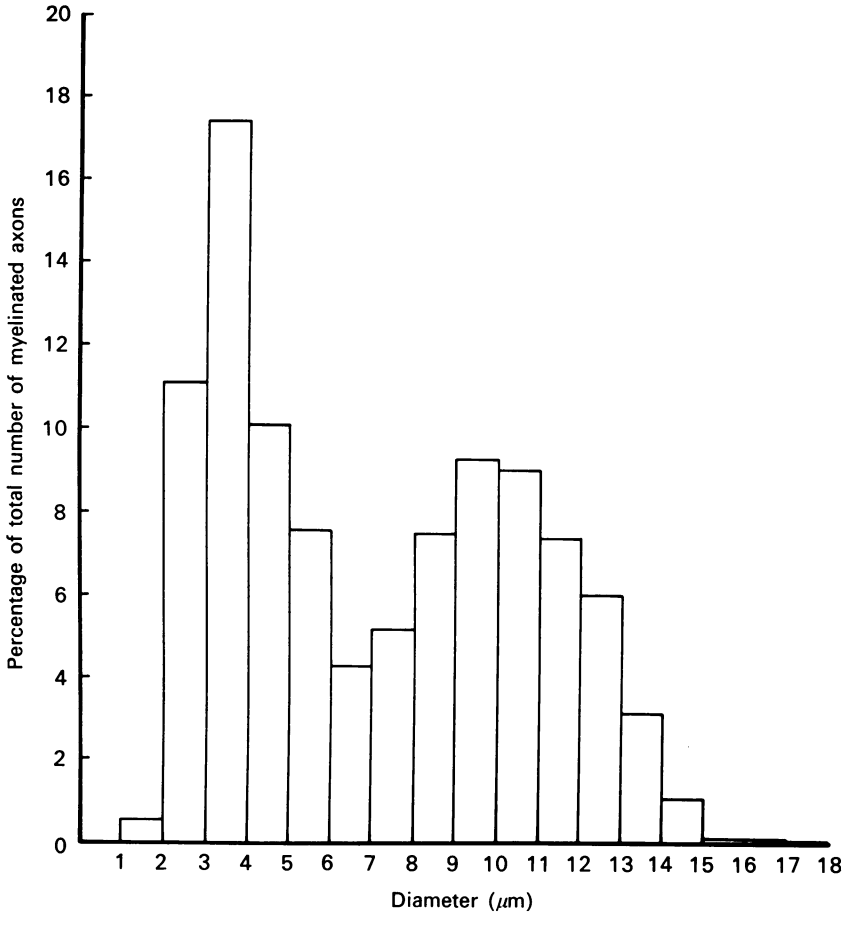


Fig. 4. A histogram showing the size distribution of myelinated fibres in the whole inferior alveolar nerve.

Fibre numbers

Myelinated fibres

The number of fibres found in each bundle by light microscopy is listed in Table 1. The total number of myelinated fibres was 6856. In bundle A the count was 4212 by electron microscopy, 4244 by light microscopy.

Non-myelinated fibres

In bundle A there were 4470 non-myelinated fibres, making 51·4% of the total. In bundle C, 809 of the fibres, i.e. 57·5% were non-myelinated. If the incidence of non-myelinated fibres in the other bundles is the mean of these two then the total number of axons in the inferior alveolar nerve at this point may be estimated as 13750. There were between 1 and 12 axons per Schwann cell (mean 2·8).

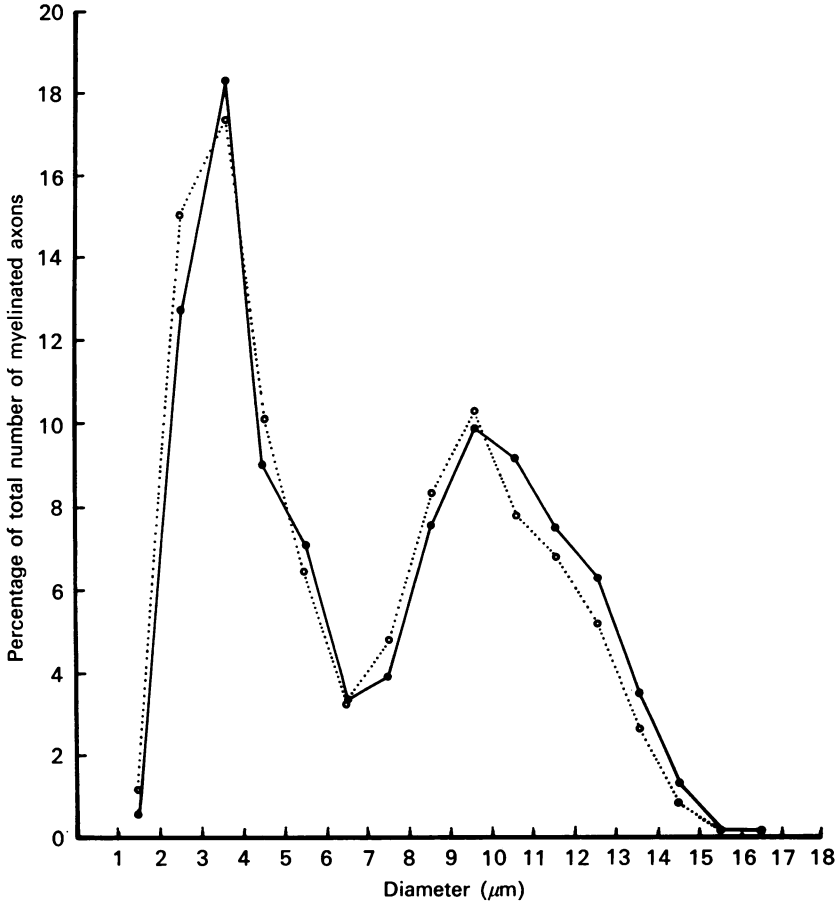


Fig. 5. Histograms of the size distribution in bundle A measured by light microscopy (solid line) and electron microscopy (broken line).

Size distribution

Myelinated fibres

The fibre size distribution of the myelinated fibres in the whole nerve is shown in Figure 4. The distribution is bimodal, with peaks at 3–4 and 9–10 μm. The fibre spectrum of bundle A obtained by light microscopy is shown as a solid line in Figure 5, that obtained by electron microscopy as a broken line. In all the bundles the distribution was very similar. The first and largest mode was always at 3–4 μm. The second mode was at either 9–10 μm or 10–11 μm, except for bundle C where it lay at 7–8 μm. No myelinated fibres larger than 18 μm or smaller than 1 μm were found.

Non-myelinated fibres

The non-myelinated fibre size distribution for bundle A is shown in Figure 6. The modal diameter is 0·6–0·7μm and over 99% of the fibres are within the range 0·2–1·7 μm.

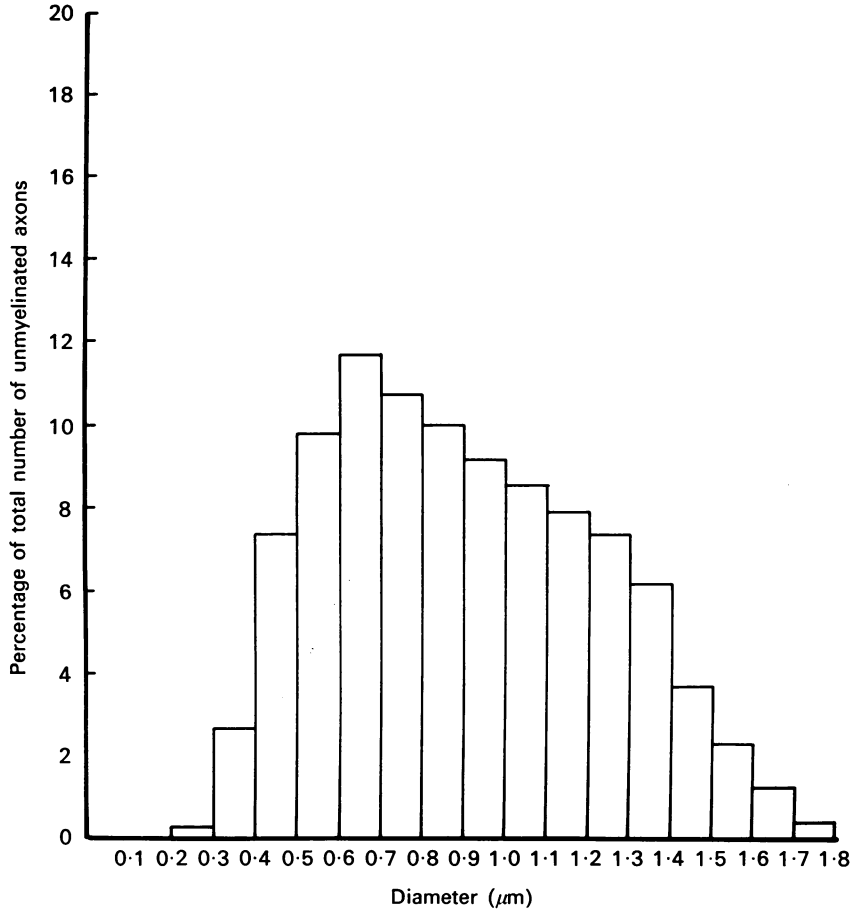


Fig. 6. Histogram showing the size distribution of non-myelinated axons in bundle A.

Regional variations within one bundle

Myelinated

The size distribution was very similar for all the three zones into which bundle A was divided, whether measured by light or electron microscopy, and no difference from the overall distribution (Fig. 4) was discernible.

Non-myelinated

The size distribution of the non-myelinated fibres was similar in all three areas, although there were relatively fewer (45 % of the total) in the core than in the periphery (55 %). There were also relatively fewer Schwann cells in the core, each containing an average of 4·1 axons. In the periphery each Schwann cell contained 2·8 axons. The incidence of Schwann cells containing only one axon was similar in both the core (32·9 %) and the periphery (33·7 %).

DISCUSSION

The total number of myelinated fibres is considerably higher than in most other reports on this species. Kizior *et al.* (1968) reported an average of 2909. Thomas' (1946) highest figure was 5199. Mohiuddin (1951) found as many as 6202 fibres in specimens somewhat older than the one examined in this study. Comparison of the distribution of fibre diameters reveals that the increased count is largely accounted for by smaller fibres less than 6 μm in diameter. Mohiuddin shows a similar bimodal distribution, but in his specimen the peak at about 4 μm is similar in height to the peak at 9–10 μm. In this present study the earlier peak is twice the height of the later one – a distribution similar to other peripheral nerves, such as the human saphenous (Boyd & Davey, 1968). Comparison of the numbers and distributions obtained by light microscopy of a 1 μm toluidine blue-stained section with those found by electron microscopy would suggest that the light microscopy of plastic-embedded material gives a good assessment of the myelinated fibre population. The difference in total numbers is small enough ($< 1\%$) to be due to observer error and would, in part, be due to the inclusion of some small capillaries in the light microscopical survey. In terms of effort it is considerably easier to use 10 light micrographs as opposed to several hundred electron micrographs.

The incidence of non-myelinated fibres is more than double the highest figure found in previous studies. It agrees with the finding on the sensory root of the baboon by Young & King (1973), but is still a relatively low proportion when compared to spinal and cutaneous nerves. It is difficult in light microscopical preparations to determine the number of axons in each Remak bundle, although all the axons included in this study were within the theoretical resolution of the light microscope. Windle (1926) did recognize the difficulty, and it is interesting to note that if you adjust his figures by allowing three axons for each non-myelinated nerve he counted, this figure becomes 51 %, and so is very close to the value obtained in the present study.

The regional variations within the bundle examined electron microscopically were not great. There appeared to be fewer non-myelinated fibres in the core, and each Schwann cell carried more axons. The size distribution for both myelinated and non-myelinated fibres showed no variations. There was also no appreciable difference between the spectra of the different bundles. This is somewhat surprising in view of the findings of De Lange *et al.* (1969) in the dog. It may be due to the more central position of this specimen, and perhaps also to a species difference.

SUMMARY

Previous studies with the light microscope of paraffin-embedded, metal-impregnated sections have probably underestimated the narrow fibre components of the inferior alveolar nerve in the cat. In this study the inferior alveolar nerve of a young adult cat was fixed *in situ* with glutaraldehyde, post-fixed in osmium and embedded in plastic. A 1 μm section of the whole nerve, and an ultrathin section of its largest bundle were cut. Montage micrographs were made from both sections, and from tracings the number and size distribution of the nerve fibres were measured. The entire nerve contained 6856 myelinated nerves whose diameters were distributed bimodally with peaks at 3–4 and 9–10 μm. This total is higher than all previously published counts of this nerve, the increase being due to the detection of a greater

number of narrower myelinated fibres. In the bundle examined electron microscopically 51·4% of the fibres counted were non-myelinated, four times as many as found in previous studies. The number and distribution of myelinated fibres found by electron microscopy was very similar to that found by light microscopy. The size distribution of myelinated nerves was similar in all the bundles examined. Apart from a lower incidence of non-myelinated fibres, the population of the core of the larger bundle was similar to that of the periphery.

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