J. Anat. (1978), **127**, 1, pp. 101–115
With 5 figures
Printed in Great Britain

A quantitative study of peripheral nerve fibres in the mouse following the administration of drugs

1. Age changes in untreated CBA mice from 3 to 21 months of age

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(*Accepted* 12 *August* 1977)

INTRODUCTION

This is the first of what we hope will be a series of papers whose initial objective is the establishment of a method for the quantitative analysis of certain parameters of peripheral nerves and whose second objective is the evaluation of the alterations occurring as the result of the administration of various drugs over extended periods. Particular attention has been given to a study of the effect of the prolonged administration of anti-leprosy drugs, which have to be given throughout the greater part of the life span of certain patients affected with this disease. The mouse was chosen for our study as it is possible to reproduce leprosy in this rodent by the intravenous injection of *Myco. leprae* and because the normal life span of the CBA mouse is usually not more than 2½ years (Rees, 1976, personal communication), allowing the effects of the drug to be determined within a reasonable time. Before the effects of drug administration can be assessed critically, however, it is necessary to validate the method chosen by establishing the changes which take place in mouse peripheral nerves as the animals get older.

Our quantitative study is concerned with those parameters considered the most important for testing the functional activity of myelinated axons, for we wished to match our observations against those which have been made previously. The majority of workers in this field have measured the diameters of the fibres and made histograms of their size and frequency distribution; however, cross section areas of fibres have been used in our study for two reasons. Firstly, areas are more easily and accurately measured with the Quantimet 720 Image Analyser (QTM), an instrument which we have used because it has the advantage of speed, accuracy and repeatability over methods customarily used for this purpose. Secondly, area measurements are completely independent of any assumptions concerning the circularity or otherwise of transversely sectioned nerve fibres.

Duncan (1934), Fernand & Young (1951), Wendell-Smith & Williams (1959), Williams & Wendell-Smith (1960), Donovan (1967), Williams & Wendell-Smith (1971), Berthold & Carlstedt (1973) and others have used manual methods, measuring two diameters of the fibre and taking their mean value. For our purposes such methods would be so time-consuming that too few of the total nerve fibre profiles could be measured. In addition, these manual methods suffer from the disadvantage that an element of selectivity and bias is difficult to avoid.

Recently Dunn, O'Leary & Kumley (1975) used a PDP 11 computer in their analysis of the spatial distributions and nerve fibre sizes of large numbers of individual myelinated fibres in the horizontal ampullary nerve of a fish. They claimed

that their method resulted in a large saving of time which they used to increase the number of fibres examined, so increasing the statistical validity of their results. In our laboratory the photography, the measurements with the Quantimet Image Analyser, and the calculation of results, for three whole sciatic nerves occupied, at the most, 12 working hours.

The QTM Image Analysing Computer is a modular system designed to provide numerical data from microscopical specimens and to carry out pattern analyses at very high speed and with low systematic error. In addition, images can be provided for the vidicon scanner by a whole series of imput peripherals, such as microscopes, epidiascopes, ciné and slide projectors. Numerical data, including measurements of area, perimeter, projection and Feret diameters are generated in the central processer modules and passed to the output peripherals for transfer either to paper tape or to the operating registers of a large programmable calculator. Detailed descriptions of the instrument are provided by Fisher (1971) and Bradbury (1977).

MATERIALS AND METHODS

Animals

Twenty one normal adult CBA female mice were used in this experiment. Throughout the whole period of the experiment the animals were maintained under comparable controlled environmental conditions. The mice were aged between 3 and 21 months with an average adult weight of 21 g. The left sciatic nerves of each of three mice were taken at 3, 6, 9, 12, 15, 18 and 21 months.

Preparation of material for electron microscopy

The animals were anaesthetized by means of an intraperitoneal injection of Avertin (tribromoethanol), 0·1 ml/g body weight. The left sciatic nerve from its emergence from the pelvis to the knee was excised, placed on card, and fixed by immersion in buffered glutaraldehyde according to the method of Hall & Gregson (1971).

After 20 minutes in fixative a segment approximately 1·5 mm long was removed from a point just distal to the branches to semitendinosus and biceps femoris muscles under a dissecting microscope (Morris, Hudson & Weddell, 1972). Every care was taken to ensure that each step in the processing sequence was standardized, so that any change introduced by the preparative techniques would be, as far as possible, similar throughout the whole series of animals.

Electron microscopy

The sections were examined with a Siemens Elmiskop 102 microscope operated at 80 kV and at a film magnification of $\times 1000$. Micrographs were taken on Ilford SP332 film. Approximately 20 films were exposed for each sciatic nerve, covering its entire area. The microscope magnification was calibrated and checked at each session by using a replica diffraction grating with 2160 lines/mm.

Presentation of micrographs for quantitative analysis

In view of the complex nature of the micrograph fields, which included not only myelinated nerve fibres, but also unmyelinated fibres, connective tissue elements and blood vessels, it was not possible at the start of the project to analyse them

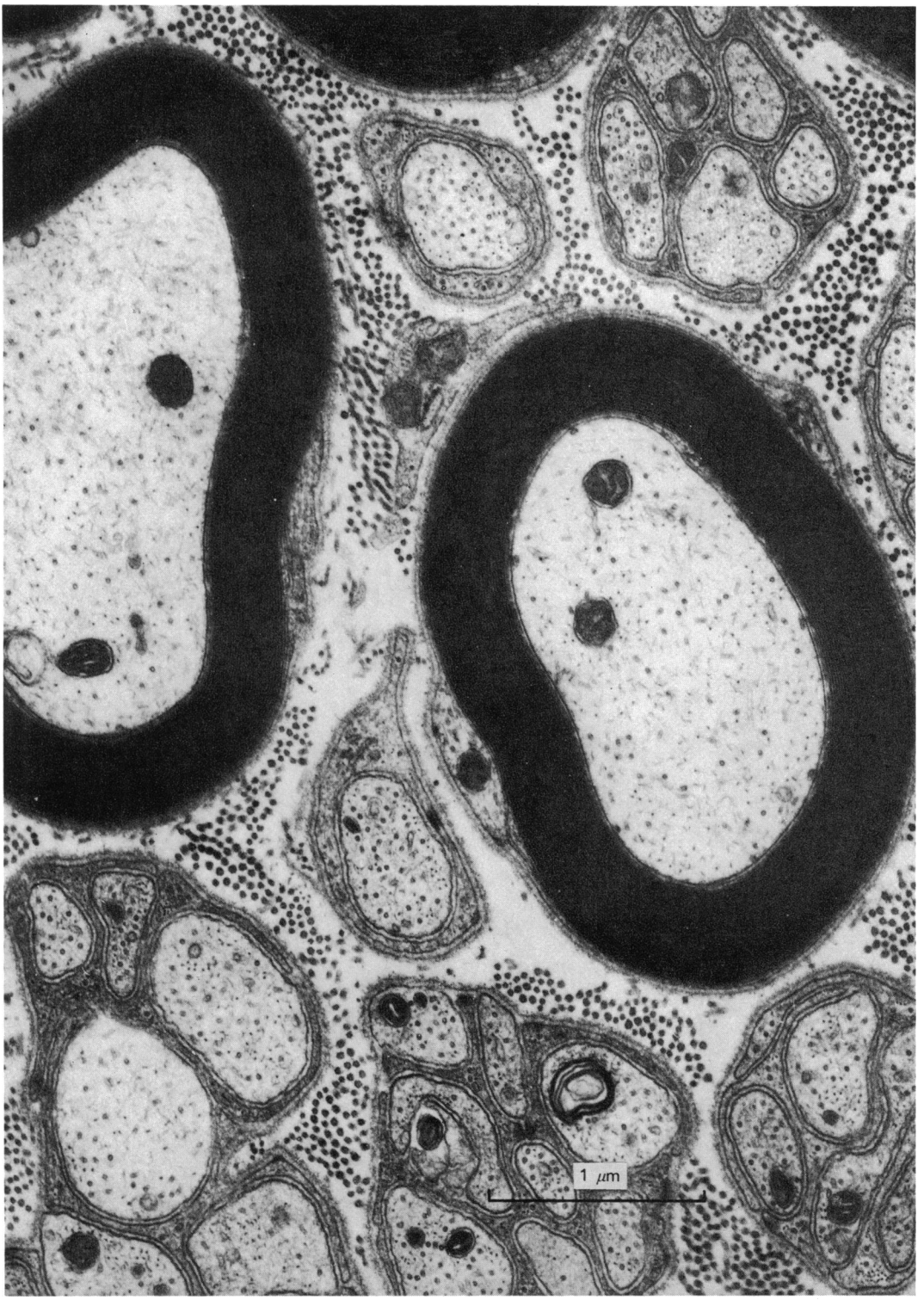

Fig. 1. An electron micrograph of a young nerve (6 months) in transverse section to illustrate the quality of the general morphology obtained for quantitative study. Note the microtubules in the cross section of an axon. Original micrograph ×37500.

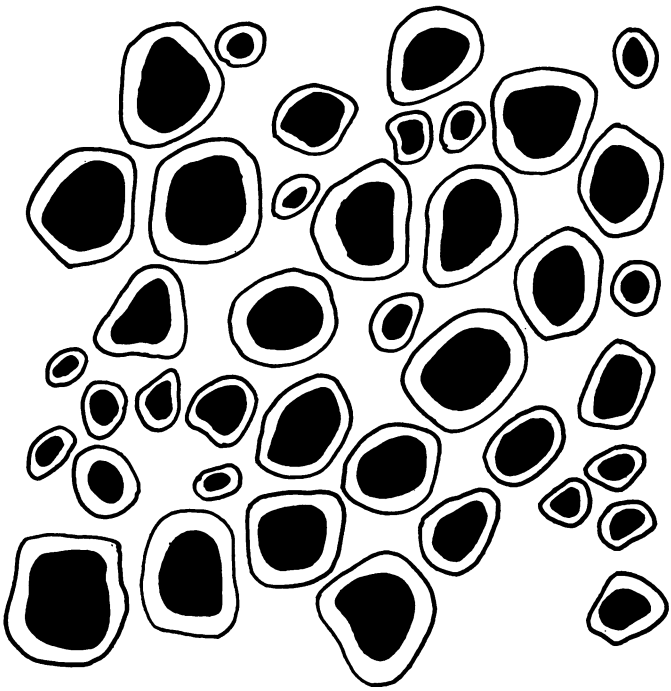

Fig. 2. Part of a typical tracing of myelin sheaths and axons (solid black) obtained from the original EM negative. Note that all other elements (which would interfere with the QTM analysis) have been excluded.

directly on the QTM 720. This problem arose because the machine cannot discriminate between the nerve elements, which are relevant to the study, and those irrelevant components which have a similar 'grey level' of optical density. To overcome this difficulty an interactive technique was adopted. The original micrographs were enlarged to a final magnification of $\times 3000$ and traced in India ink directly on to typing paper, outlining the myelin sheath and axon of each myelinated fibre (Fig. 2). However, only those fibres with an uncomplicated myelin sheath were traced so that measurements were not made on fibres showing (*a*) wrinkled myelin sheaths indicative of damage during the preparation of the material, (*b*) clefts of Schmidt–Lanterman, (*c*) nodes of Ranvier, (*d*) paranodes and (*e*) myelinated fibres cut through a Schwann cell nucleus. The exclusion of all these categories meant that only around 75 % of the total number of myelinated fibres present in the nerve's cross section were included in the final quantitative analysis.

Each tracing was placed in a wall holder in front of the vidicon scanner of the Image Analysing Computer, which was programmed to measure the area, perimeter, horizontal and vertical Feret diameters of each traced fibre in turn, the results being presented in digital form both on punched tape and on a teletype print-out. By a simple change in the machine programme these data could be obtained for either the total fibre or for the axon drawn within the outline of the myelin sheath.

Reduction of the raw data, and calculation of basic statistical parameters was carried out by running the tapes through a Hewlett Packard tape-reader coupled to a 9810 calculator. In the present work only the area and perimeter values were

used; the other parameters measured are intended for more detailed analysis of possible shape changes at a later date.

Counts of myelinated nerve fibres

Montages were made from the electron micrographs of whole cross sections of the nerves. From each montage counts were made of the myelinated nerve fibres. The fibres were marked with a felt tip pen as they were counted in order to avoid repetition or omission, and a hand tally counter was used to record the numbers.

A separate count was made of degenerating and regenerating nerve fibres, and of fibres in which the plane of section passed through a specialized region, e.g. a node of Ranvier.

Statistical methods

The statistical methods used in analysing the large quantity of data obtained from the study included:

Davies' test for logarithmic distribution

Because of the skew distributions shown by the axon and total fibre area/size histograms (see Figs. 3, 4) it seemed likely that they had a logarithmic distribution. Davies' test was applied and found to give positive values in each case, a sign that the data were indeed logarithmically distributed. This implied that a geometric mean rather than the arithmetic mean (Langley, 1968) should be used to compare the difference in cross sectional areas between the various ages, and, in later studies, between the normal and treated animals.

One-way analysis of variance (A.O.V.)

This was used to obtain an F value (variance ratio) which allows a test for the equality or otherwise of the geometrical mean values of the fibre and axon diameters.

Regression analysis

Power regression equations were calculated for myelin sheath thickness against axon diameters. Power regressions of the form $y = ax^b$ were used rather than linear regressions as the former consistently gave much higher values for the correlation coefficient r. The analysis of variance was performed according to the methods developed by Zar (1975).

RESULTS

As can be seen from Figure 1, which represents an electron micrograph of a nerve in transverse section, the preservation of the material used in this study matches that of previous workers (e.g. Morris *et al.*) and therefore may be regarded as satisfactory.

In particular, the majority of the myelinated fibre profiles showed regular lamellae of myelin and Schwann cell coverings in which the cytoplasmic organelles, including neurotubules, appeared undistorted. In any quantitative study shrinkage must be regarded as a major artefact. We believe that in the present material shrinkage, although present (as indeed it must be in any fixed and dehydrated material) is minimal and consistent. In particular, there is no evidence, in any of our material, of the separation of the myelin from the axon, which is a well known phenomenon when excessive shrinkage is present in nerves prepared for electron

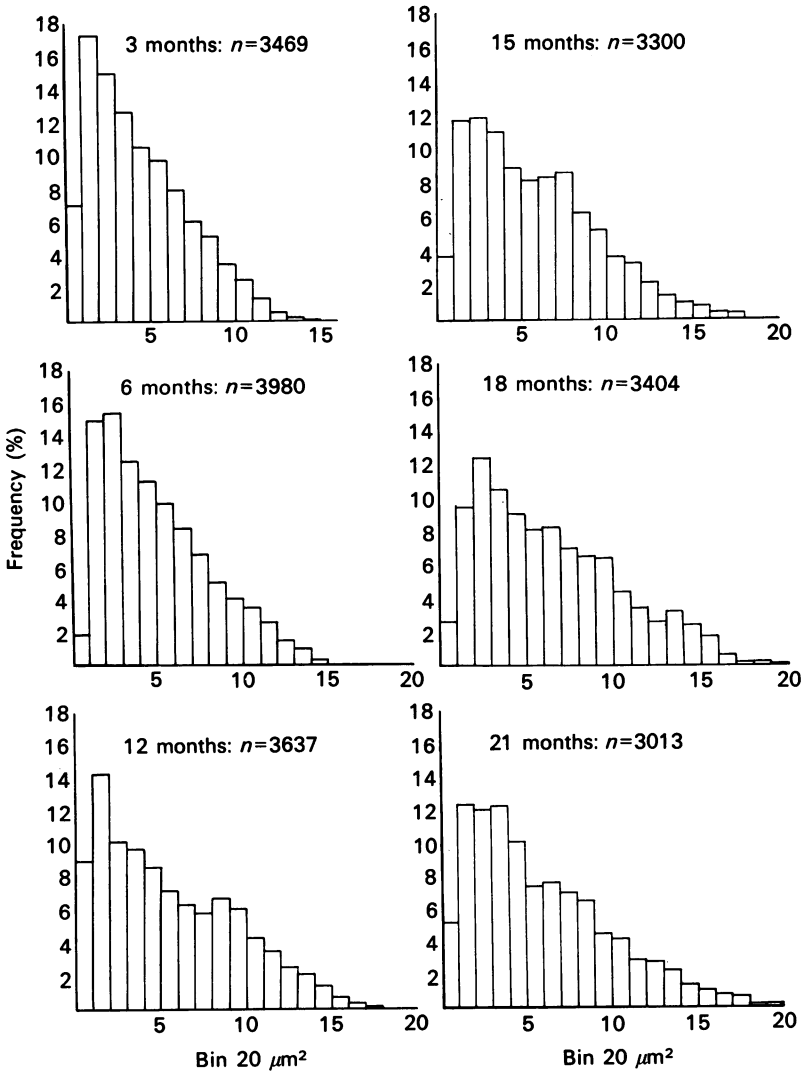

Fig. 3. Frequency distribution histograms of nerve fibre areas of animals at selected ages.

microscopy. Furthermore, the erythrocytes in our sections generally appeared undistorted and of appropriate diameter.

In addition, the morphological features of the unmyelinated fibres, blood vessels, and elements of the perineurium, were in all cases consistent with the well documented morphology at the ultrastructural level of these tissues. We feel, therefore, that the quantitative results are not invalidated by processing artefacts.

Quantitative results

Fibre area sizing

The histograms of the frequencies of nerves of different cross sectional areas in animals at various selected ages are shown in Figure 3; the full numerical data, expressed as percentage frequencies, are given in Table 1. From the Figure it can be

Table 1. *Percentage frequency of fibre areas*

Bin number	Age						
	3 months	6 months	9 months	12 months	15 months	18 months	21 months
1	7·0	2·0	5·7	9·1	4·0	2·7	5·1
2	17·0	15·0	14·9	14·3	12·0	9·5	12·3
3	12·8	15·5	12·4	10·2	12·0	12·4	12·0
4	10·9	12·5	12·9	9·7	11·1	10·2	12·2
5	9·8	11·4	10·5	8·6	9·2	9·1	10·0
6	8·0	9·9	9·5	7·2	8·3	8·2	7·3
7	6·1	8·4	8·2	6·4	8·5	8·3	7·5
8	5·2	6·8	6·6	6·8	8·9	7·0	6·9
9	3·5	5·1	5·7	6·7	6·4	6·5	6·4
10	2·5	4·1	3·9	6·1	5·5	6·4	4·4
11	1·3	3·5	4·2	4·4	3·9	4·5	4·1
12	1·0	2·7	2·8	3·5	3·4	3·4	2·8
13	0·2	1·5	2·6	2·5	2·3	2·6	2·7
14	0·1	0·3	1·5	2·1	1·5	3·3	2·2
15	—	1·0	1·0	1·4	1·1	2·5	1·3
16	—	—	0·3	1·0	0·9	1·7	1·0
17	—	—	0·1	0·3	0·5	0·6	1·0
18	—	—	0·1	0·1	0·4	0·3	0·1
19	—	—	—	0·1	0·1	0·2	0·1
20	—	—	—	—	—	0·1	0·1
21	—	—	—	—	—	0·1	—
22	—	—	—	—	—	0·1	0·1

Bin width = 20 μm^2.

Table 2. *Geometric means of fibre area sizes*

(All values are given in μm^2)

Age	Animal			Grouped mean
	A	B	C	
3 months	66·968	65·063	98·161	76·731
6 months	77·182	91·740	81·882	83·601
9 months	91·462	94·863	89·668	91·998
12 months	73·969	95·729	92·908	87·535
15 months	115·433	90·605	100·427	102·155
18 months	95·070	107·424	115·192	105·835
21 months	97·330	77·095	112·428	95·618

$F = 1{\cdot}976$; $F = 0{\cdot}05_{(2)}$ 6, 14 = 3·50.

seen that the size distributions of nerve fibre areas for all age groups is markedly skewed to the right, with the mode appearing in the small fibres having areas ranging between 40 and 80 μm^2. A very much lower peak (possibly representing a second population of larger fibres) is seen from 12 months onwards, with the location of the secondary mode ranging from 160 to 200 μm^2 at 12 months, 160 μm^2 at 15 months, and possibly a very slight peak at 260 μm^2 at 18 months.

The marked skewness suggests that the distribution of the values follows a logarithmic pattern. Application of the test devised by Davies (Langley, 1968) shows this to be the case, and rather than use a logarithmic transformation of the data

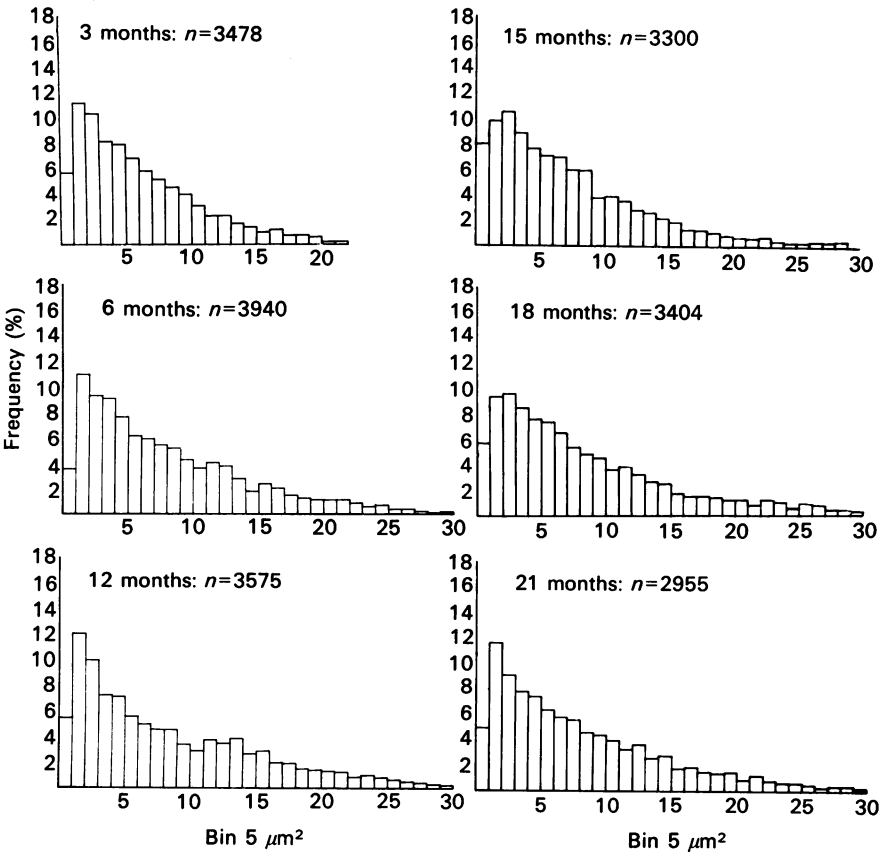

Fig. 4. Frequency distribution histograms of axon areas of animals at selected ages.

(which would render direct visual comparisons of the histogram shape with data of other workers difficult), it seemed appropriate to accept the skewness but use geometric means for comparison purposes.

The histograms and data in Figure 3 and Table 1 represent the combined figures from 3 representative animals in each age group. Examination of the geometric means for the fibre sizes for each individual in the age groups (Table 2) by a one-way A.O.V. gave a calculated F ratio of 1·97. This figure is below the critical F value for the appropriate degrees of freedom and a P of 0·05, so that no significant difference can be shown statistically between the geometric means of fibre areas at the different ages.

From Figure 3 it can be seen that there is a gradual increase in the maximum fibre area from 200 μm^2 at 3 months to 400 μm^2 in the oldest animals, but as there are more smaller fibres in the nerves of young animals, this explains the apparent statistical identity of the geometric means for all age groups.

Axon area sizing

The axon area sizing histograms (Fig. 4), and the numerical data (Table 3) for the axons, show no significant pattern differences between the animals of different age groups. Indeed, the only notable difference is that in animals of 3 months the maximum size is 110 μm^2, whereas at all other subsequent ages the maximum is

Table 3. *Percentage frequency of axon areas*

Bin number	Age: 3 months	6 months	9 months	12 months	15 months	18 months	21 months
1	5·7	3·7	4·3	5·4	7·9	5·7	6·0
2	13·3	11·0	10·9	12·1	9·7	9·31	11·7
3	11·3	9·4	8·1	10·0	10·4	9·6	9·2
4	14·5	9·2	7·0	7·2	8·8	8·5	7·9
5	8·3	7·7	7·9	7·1	7·7	7·6	7·4
6	8·1	6·3	6·4	5·6	7·1	7·3	6·4
7	6·9	6·0	5·9	5·0	6·9	6·5	5·8
8	5·9	5·5	5·4	4·7	6·1	5·4	5·7
9	5·2	5·3	4·8	4·6	5·9	4·8	4·6
10	4·6	4·4	4·7	3·4	3·8	4·5	4·4
11	4·0	3·7	4·0	3·0	3·9	3·5	4·0
12	3·2	4·2	3·3	3·9	3·5	3·9	3·4
13	2·4	3·8	3·5	3·5	2·8	3·2	3·8
14	2·3	2·8	2·6	3·9	2·7	2·7	2·7
15	1·7	2·0	2·9	2·8	2·0	2·5	2·8
16	1·4	2·4	2·2	3·0	1·9	1·7	1·8
17	1·1	2·2	2·3	2·0	1·2	1·5	1·9
18	1·3	1·5	1·6	2·0	1·2	1·5	1·5
19	0·7	1·3	1·7	1·6	1·1	1·4	1·5
20	0·7	1·2	1·4	1·5	0·7	1·3	1·6
21	0·6	1·1	1·4	1·4	0·7	1·2	0·9
22	0·3	1·2	1·4	1·3	0·6	0·9	1·3
23	0·2	1·0	1·0	0·9	0·7	1·1	0·8
24	0·1	0·7	1·2	1·0	0·4	1·0	0·7
25	0·1	0·7	1·0	0·8	0·3	0·5	0·7
26	0·1	0·4	1·0	0·7	0·3	0·9	0·6
27	0·1	0·4	0·6	0·6	0·4	0·8	0·3
28	0·1	0·3	0·3	0·4	0·4	0·5	0·5
29	—	0·2	0·6	0·4	0·4	0·3	0·4
30	—	0·2	0·3	0·3	0·3	0·3	0·3

Bin width = 5 μm^2.

Table 4. *Geometric means of axon area sizes*

(All values are given in μm^2)

Age	Animal: A	B	C	Grouped mean
3 months	22·523	23·745	31·355	25·874
6 months	31·074	32·734	32·419	32·075
9 months	34·914	33·988	32·390	33·760
12 months	28·450	33·419	32·540	31·469
15 months	34·748	25·340	32·710	30·932
18 months	31·217	31·695	36·136	33·016
21 months	39·046	32·939	28·230	33·405

$F = 1{\cdot}6211$; $F = 0{\cdot}05_{(2)}$ 6, 14 = 3·50.

150 μm^2. The highest peak appears, as might be expected, in the smaller axons, with a mode between 10 and 20 μm^2. No second larger population peak occurs in any age group.

As with the fibre areas, the axon sizing histograms show the typical skewness of a logarithmic distribution. The geometric means for the axon areas in each of three animals at each age are given in Table 4; from a one-way A.O.V. the calculated F ratio was found to be 1·62, well below the critical F value for the number of degrees of freedom and a P of 0·05. This may be interpreted as showing statistically that no significant differences exist between these mean values.

Relationship of myelin sheath thickness to axon diameter

For each nerve fibre profile measured on the QTM 720, data were obtained directly for fibre area and axon area. These measurements enabled us to calculate a 'notional diameter' for both axon and fibre for each profile. (The sizing histograms of fibres and axons were carried out on area measurements which are, of course, shape-independent.) The results were plotted in the form of scatter diagrams of twice the sheath thickness against axon diameter, parameters chosen to allow comparisons with the results given in previous publications (e.g. Sunderland & Roche, 1958; Williams & Wendell-Smith, 1971). The calculation of 'notional diameters,' however, requires the assumption that the cross sectional profiles are circular. This is only an approximation, as some fibres deviate quite markedly from circularity. It seems reasonable, however, to make this assumption, which allows the calculation of a representative thickness of myelin sheaths, as the error thus introduced may be assumed to be constant for all animals, either normal or drug treated, and because the numbers of fibres measured was large.

To test this a simulation experiment was performed; two drawings were made on squared paper to represent an axon and its myelin sheath. In the first of these the profiles were made exactly circular and concentric, whilst in the second they were made irregular. The areas of 'axon' and 'sheath' were then determined both manually (by counting the squares) and with the QTM 720, and the respective notional diameters and 'sheath thicknesses' calculated. Comparison of the results from the two methods showed that the maximum error introduced even by a marked departure from circularity was of the order of 3 %. It is thus felt that the concept of 'notional diameter' calculated as outlined previously is of value in allowing a reasonable approximation to be made of the myelin sheath thickness.

The scatter diagrams are all very similar in shape, so only the data for 6 months and 18 months are presented as Figure 5. It is clear that there is a marked relationship between the sheath thickness and axon diameter. Up to a certain sheath thickness (6 μm in younger animals and 7·5 μm in older animals) there is a gradual and marked increase in the thickness of the myelin with increasing axon diameter. Above this value sheath thickness continues to increase at a much slower rate in the young animal even though the axon may reach a diameter of 12 μm. In the older animal, however, the increased slope of the regression line shows that there is considerable growth in thickness of the myelin sheath still taking place.

The regression lines were calculated from the data, together with the correlation coefficient r. Calculation showed that the highest values of r (0·84 and 0·76 for the 18 and 6 months old animals respectively) were obtained with the power regression characterized by the equation

$$y = ax^b.$$

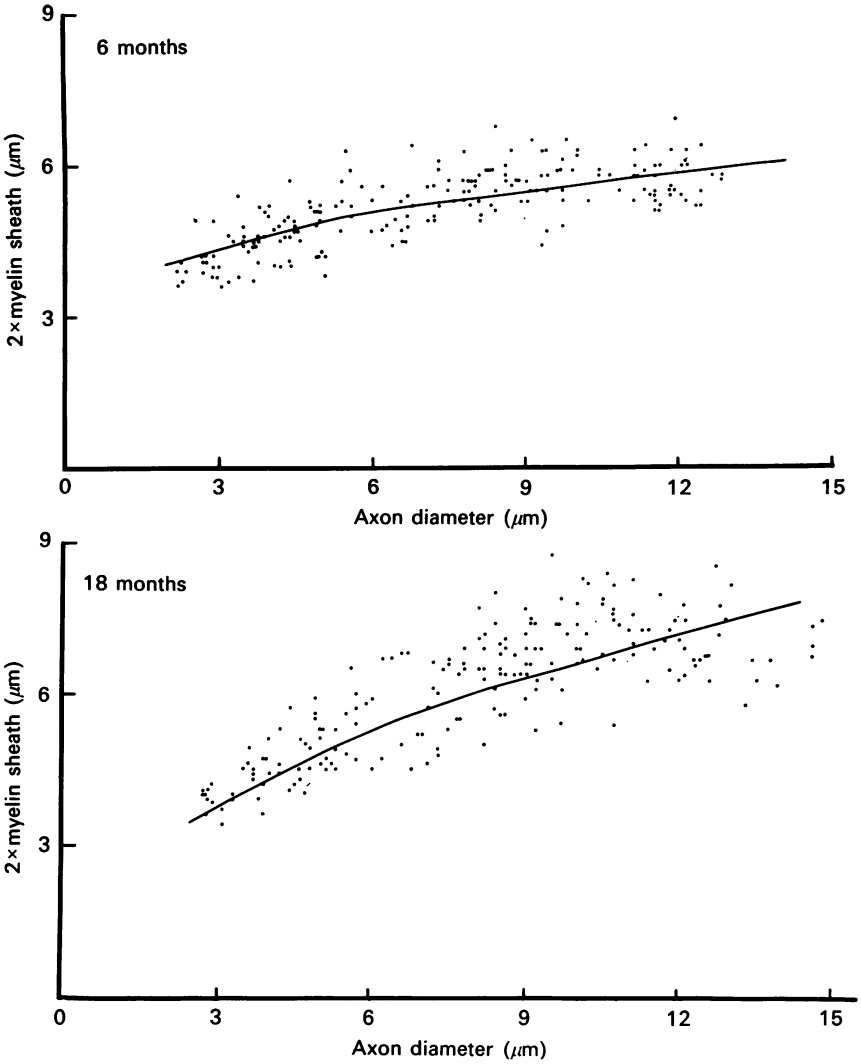

Fig. 5. Scatter diagram (6 and 18 months animals) of relationship between myelin sheath thickness and axon diameter. Power regression lines calculated from the expressions $y=1{\cdot}0x^{0{\cdot}4}$ (6 months) and $y=1{\cdot}22x^{0{\cdot}6}$ (18 months) have been superimposed.

It can be seen that when the 18 months old animal is compared with that of 6 months, the scatter of the points increases with age and the slope of the regression line also increases.

Numbers of myelinated nerve fibres

Counts of the total number of intact, degenerating and regenerating nerve fibres were performed on montages of electron micrographs, as described under Materials and Methods. Detailed results are presented in Table 5 for the animals of various ages.

The total number of normal myelinated fibres only varies by 261 amongst the whole range of ages, so that there is a marked homogeneity amongst all the nerves in this respect.

Table 5. *Myelinated fibre counts*

Age of animals	No. of myelinated fibres	No. of degenerating fibres	No. of regenerating fibres
3A	2351	2	0
3B	2237	0	0
6A	2210	2	0
6B	2247	0	0
9A	2187	2	0
9B	2260	1	0
12A	2368	4	3
12B	2241	0	2
15A	2401	12	7
15B	2458	16	10
18A	2276	27	15
18B	2344	22	14
21A	2209	59	86
21B	2288	24	36

A and B denote duplicate counts on different animals of the same age.

Table 5 shows that degeneration and regeneration are not significant until the animal is 15 months old. After this age, the numbers of both degenerating and regenerating fibres increase, suggesting some correlation with the ageing process.

DISCUSSION

The major object of our studies (of which this is the first to be reported) is to quantify the changes which occur in peripheral nerve fibres following the administration of drugs to CBA mice. Before this could be done it was essential to establish that the method was satisfactory, and to determine the changes which might be expected to occur as the result of the ageing process in normal nerves.

In any quantitative study of myelinated nerve fibres (especially if a figure for the total number of fibres is required) it is necessary to consider the level of section of nerve fibre presented for analysis. Mixed nerves have posed problems in this respect because of the variability of the levels at which branches leave main nerve trunks. Such variability may give wide differences in total myelinated nerve fibre counts, depending on whether the section plane is above or below the point at which a branch arises. For this reason much previous work has been restricted to studies on either purely sensory or motor nerve bundles before they start branching.

In the present study, however, as the results (see Table 5) show, at the level chosen there is no significant variation in the total myelinated fibre count in normal animals of different ages. For quantitative studies it thus seems valid to use the sciatic nerve (taken at a known level in respect of branches leaving it), which contains both sensory and motor fibres. The similarity of the total myelinated fibre counts also very strongly suggests that absolute number of fibres is not a good age-related character for quantification. This conclusion is supported by the findings of Birren & Wall (1956). Other workers, however, disagree; for example, Thomas (1956) finds that there is a clear increase in numbers with age, whilst exactly the contrary

view, i.e. that there is a decrease in myelinated fibre number, is put forward by Samorajski (1974); the level at which they examined their nerve bundles, however, is not given.

Fibre diameter, on the other hand, seems to be a much better parameter for showing changes in the make-up of nerve fibre bundles, once the animal is mature. The majority of previous authors have suggested that the myelin sheath ceases to increase in thickness when the ratio of the diameters of axon to total fibre reaches its optimum, which is about 0·6 μm, and after this time the diameter appears to remain constant. In normal adult animals therefore, total fibre diameter has been regarded as a valid parameter for classifying nerve fibres in relation to their conduction velocities and thus, in an approximate way, to their functions. This view has been adopted in the present work, with the substitution of the fibre area for the diametral measurement, because the former falls more directly within the capabilities of the QTM 720.

It is well known that before maturity, changes do occur in the total fibre diameter and that the myelin sheath increases in thickness, but relatively little appears to be known about the changes of axon diameter with increasing age. It seems that, although the measurements reported here are given as areas rather than as diameters, there is no appreciable increase in maximal size with age. Even at 6 months of age some axons have reached the maximum size found in animals of 21 months.

In any quantitative study care must be taken with the preparation of the material. This is true not only with regard to obvious sources of error such as fixation artefacts and shrinkage, but also in respect of the evaluation of the results and in the extent to which generalizations can be made from a limited number of observations. Even with the assistance of the automated image analyser, it is still a considerable task to acquire numerical data from a large number of fibres, especially if several age groups are to be studied with extensive duplication of animals.

In the present work these restrictions led to the use of only three animals in each age group chosen. It might be argued that conclusions based on combining geometrical mean values from analysis of only three animals are not wholly valid. From the data, however, it seems that general trends do appear, even though an occasional animal in one or other of the age groups may seem to be significantly different from the rest. Such individual variations are only to be expected, even amongst littermates of inbred laboratory mice. The fact that consistent trends do appear in the plots suggests, therefore, that the sampling procedure and number of replicates are sufficient for the purpose. This may well be because in the present work every measurable myelinated fibre in the transverse section of the nerve was taken, whereas with most previous work only a fraction of the total was quantified.

The numerical data obtained from the nerves by the use of the Quantimet 720 has given an objective picture of the size changes consequent upon ageing. In particular, the distinctive skew to the right in the histograms of axon and fibre area sizing is present at all ages; this accords with the findings of Birren & Wall and Samorajski, although these workers were reporting their manual measurements in terms of diameters rather than areas. A relative increase in the number of large myelinated fibres in the older animals, presenting as a second modality in the histograms (see, for example, Fig. 3), is also in agreement with Samorajski's findings. It thus seems clear that a transverse section of sciatic nerve at any age contains a relatively large proportion of small fibres but that, as the animals' age increases, more and more larger myelinated fibres appear. The pattern of the histograms in

young animals is unimodal, but as the number of large fibres increases, a suggestion of a second modality in the histogram appears.

One of the most often quoted measurements to be found in the literature is the relationship of twice the thickness of the myelin sheath to the axon diameter. Most authors agree that at a certain stage of development this ratio assumes a maximum value, suggesting that there is no further increase in myelin sheath thickness or in axon diameter.

For the sural nerve of the rabbit this maximum was reached at 8 weeks of age; but in the nerve to the medial head of gastrocnemius this value was not attained until the animals had reached maturity (Williams & Wendell-Smith, 1971).

In the present paper the measurements of myelin thickness and of axon and fibre diameter are expressed as scatter plots. In general, the shape of these plots resembles markedly those given by previous authors, showing a flattening with increase in both axon and total fibre diameter. Although there is considerable scatter and variability it was considered worthwhile to fit a regression line by the 'least-squares fit' method. Originally a linear regression was applied, in order to allow comparison with the findings of other workers (Williams & Wendell-Smith, 1960, 1971). In the younger animals (up to 9 months of age) such a line fitted quite well, as measured by the value of r, the correlation coefficient. In older animals however, it was found that a better fit was obtained by using a power regression. This suggests that in the younger animals the myelin is increasing linearly with the increase in diameter. However, at 12 months and after, the scatter plots do level off in a similar manner to those reported on by Williams & Wendell-Smith (1971), and hence the better fit of a power regression for the older animals. This suggests a levelling off in the rate of myelin production with age. Nevertheless, it is not certain that, even for these animals, a power regression is the 'best' fit; visual inspection suggests that the curve could well be asymptotic, and hence a better fit, as measured by an increase in the correlation coefficient, might be given by the use of an asymptotic regression.

Results for the relationship of axon diameters to myelin thickness are similar to these for the total fibre diameter/myelin thickness. Previous work on the axon myelin relationship has shown that the thickness of the myelin sheath varies among axons of the same diameter (indicated, if not always commented on, by Schmidt & Bear, 1937; Sanders, 1947; Sunderland & Roche, 1958; Williams & Wendell-Smith, 1971). The present study strongly supports these observations. Sunderland & Roche also found that the myelin sheath thickness was greater in the larger axons, but because of the great variation they concluded it was not possible to predict with any certainty the amount of myelin that would surround an axon of any particular diameter. Again, this is in accord with our findings, where the amount of myelin increases with age. There may be some variation in myelin thickness along the length of an internode, but the use of the large numbers of fibres in the present study should average this out and demonstrate the general trend.

The continued but diminishing increase in the myelin sheath thickness in the ageing animals indicates that the Schwann cell continues to produce myelin after maturity.

The patterns demonstrated throughout the greater part of the CBA mouse life span serve to provide a base line for the evaluation of changes which are to be reported in a subsequent paper. A similar technique may also prove of value for quantifying changes taking place in human peripheral neuropathies.

SUMMARY

This study is concerned with the quantification of changes which occur in peripheral nerves of normal mice from the onset of maturity to old age.

The parameters chosen were total fibre area and axon area. Size distributions of these were expressed in the form of histograms.

From the area data notional fibre and axon diameters were calculated, together with the thickness of the myelin sheath. The notional diameters were contrasted with the comparable myelin sheath thicknesses in the form of scatter diagrams.

These results are intended to provide a reference scale for subsequent assessment of changes induced by drugs administered throughout life. They are in general accord with previous observations, but were obtained quickly, and with less margins of error, by using a Quantimet 720.

This work was supported by a grant from LEPRA to A. G. M. Weddell which is gratefully acknowledged.

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