ABSENCE OF CELL MULTIPLICATION DURING DEGENERATION OF NON-MYELINATED NERVES

By J. JOSEPH, *Department of Anatomy, University College, London*

INTRODUCTION

One of the most remarkable features of the degeneration which follows severance of a myelinated nerve is that the multiplication of the nuclei of Schwann occurs not only in the neighbourhood of the lesion but throughout the whole extent of the peripheral stump. Abercrombie & Johnson (1946) have recently examined this multiplication quantitively in the sciatic nerve of the rabbit and have found that the increase occurs between the 4th and 25th days and produces a Schwann cell population thirteen times that of the normal nerve. Information about the agency which produces this nuclear multiplication might provide us with valuable clues as to the nature of cell proliferation.

Few previous studies have dealt with the degeneration of non-myelinated nerve. Tuckett (1896) observed that when non-myelinated fibres are cut off from their nerve cell and degeneration is shown by loss of irritability, 'the nuclei and walls of the fibres seem to be quite unchanged and as normal as ever'. Other writers had different impressions. Ranson (1912) stated that 'by the 19th day after degeneration of non-myelinated nerve the nuclei of the neurilemma have greatly increased in number', and Cajal (1928) described a thickening and proliferation of the cells surrounding the non-myelinated nerve-fibres. The only other reference found to histological changes was the work of Machida (1929) who showed a picture of a longitudinal section showing 'proliferation of the neurilemma', but who stated in the text that 'the neurilemma was essentially unchanged'. Tuckett's excellent paper is open to the criticism that it is now known that the nerve he used contained a large number of finely myelinated nerve-fibres, an error due to the stage of technical advance reached at that time.

The present paper describes an attempt to determine the quantitive changes in the cell population of a non-myelinated nerve which has been interrupted.

MATERIAL AND METHOD

The nerve used was the anterior mesenteric of the rabbit, which has been shown by Simpson & Young (1946) to consist of a number of bundles containing only a few (total less than ten) small myelinated fibres. The nerve was crushed with smooth-tipped forceps, or cut, just distal to the anterior mesenteric ganglion. After 21 days the ganglion and nerve were removed. These constituted two specimens each about 1 cm. long, one consisting of the ganglion and proximal part of the nerve and the other of the distal part of the nerve. The specimens were fixed in Bodian's fluid containing 15 c.c. formaldehyde, 5 c.c. acetic acid

and 80 c.c. of 80% alcohol, and were embedded in paraffin. Transverse sections at 5 μ of each end of each specimen were cut, and four slides of each end were prepared. One slide was stained for axons by Bodian's method, one by Bodian and Mallory's stain, one by Bodian and Masson's stain and one with haematoxylin and eosin. An equal number of normal ganglia and nerves were prepared in a similar way. Longitudinal sections of both the interrupted and normal nerves were cut at 7 μ and used in the case of the interrupted nerves chiefly to determine the existence of normal axons proximal to the interruption and their absence beyond. Sections showing at least three satisfactory nerve bundles stained with haematoxylin and eosin, in each specimen, were used for counting. Photographs of these sections were taken at a magnification of $\times$ 350, and the number of nuclei in each of the three nerve bundles was counted. No attempt was made to define the type of cell to which the nuclei belonged. The area of each nerve bundle was measured with a planimeter, the outline followed being that of the perineurium from which the nerve bundle had invariably shrunk away to a greater or less extent. The number of nuclei in each nerve bundle was expressed as so many per 10,000 μ^2.

RESULTS

Table 1 shows the counts for three nerve bundles in each of five normal nerves, and Table 2 shows those for a similar number of bundles in nerves which had been interrupted.

Table 1

Serial no. of rabbit	No. of nerve bundle	Nuclear count	Area in 10,000 μ^2	Nuclei per 10,000 μ^2
49	1	40	0·56	72·06
	2	24	0·33	72·14
	3	68	0·91	74·55
47	1	81	1·46	56·04
	2	24	0·31	81·14
	3	24	0·30	80·00
72	1	164	2·81	58·34
	2	38	0·74	51·53
	3	143	2·00	71·50
48	1	26	0·38	68·24
	2	144	2·59	55·53
	3	46	0·52	88·51
110	1	46	0·51	89·91
	2	94	0·75	125·17
	3	72	0·87	82·43

Mean = 74·95.
Standard deviation = $\pm$ 18·24.

The nerve in rabbits 13, 19, 12 and 23 was interrupted by crushing and that in rabbit 27 by cutting.

The Bodian staining showed that a number of axons had regenerated but these were very fine at the levels studied. This should not affect the results but in confirmation a further experiment is being performed in which regeneration is completely prevented.

The estimates of the number of nuclei per 10,000 μ^2 both in the normal and damaged nerves vary considerably in different animals. These differences can be accounted for by factors such as slight obliquity of the sections, and variation in the degree of shrinkage of the nerve bundles, besides some normal variation in the size of the nuclei and their density along the length of the nerve. On the whole, variation within the limits shown in the Tables is to be expected in counts of this type.

Comparison of the means and standard deviations of the two sets of results allows us, in spite of the variation, to assert that the nuclear population of a non-myelinated nerve 21 days after it has been interrupted is not greater, by a factor of two, than in the normal nerve. Probably there has been no cell multiplication at all, certainly it has not affected every cell in the nerve. This is in very striking contrast to the multiplication by eight times which occurs during degeneration of myelinated nerves.

Table 2

Serial no. of rabbit	No. of nerve bundles	Nuclear count	Area in 10,000 μ^2	Nuclei per 10,000 μ^2
13	1	95	1·38	68·81
	2	124	1·80	69·04
	3	228	4·36	52·30
19	1	119	2·26	52·68
	2	90	1·35	66·48
	3	8	0·10	80·00
12	1	226	2·04	110·74
	2	45	0·73	61·25
	3	96	1·16	83·00
23	1	60	0·91	66·02
	2	51	0·81	62·68
	3	41	0·79	52·14
27	1	128	1·33	96·00
	2	41	0·75	54·99
	3	66	1·59	41·39

Mean = 75·56.
Standard deviation = ± 18·12.

DISCUSSION

The exact histological details of the structure of mammalian non-myelinated nerve have not yet been decided. One of the main problems is whether or not there is a complete absence of myelin between the neurilemma and the axon. The fullest description that I have found is that given by Nageotte (1932). He describes an axon surrounded by a 'sheath of Schwann' which has a 'membrane of Schwann' outside it, and states that there is no myelin sheath. He considers that the sheath is syncytial and is irregularly studded with elongated nuclei and that the membrane in cross-section forms a multitubular structure with an axon in each tube. The cells of this sheath (cells of Remak) are usually regarded as Schwann cells. Ranson (1911) described what he called a 'halo' round the axons of non-myelinated nerve bundles in spinal nerves, and

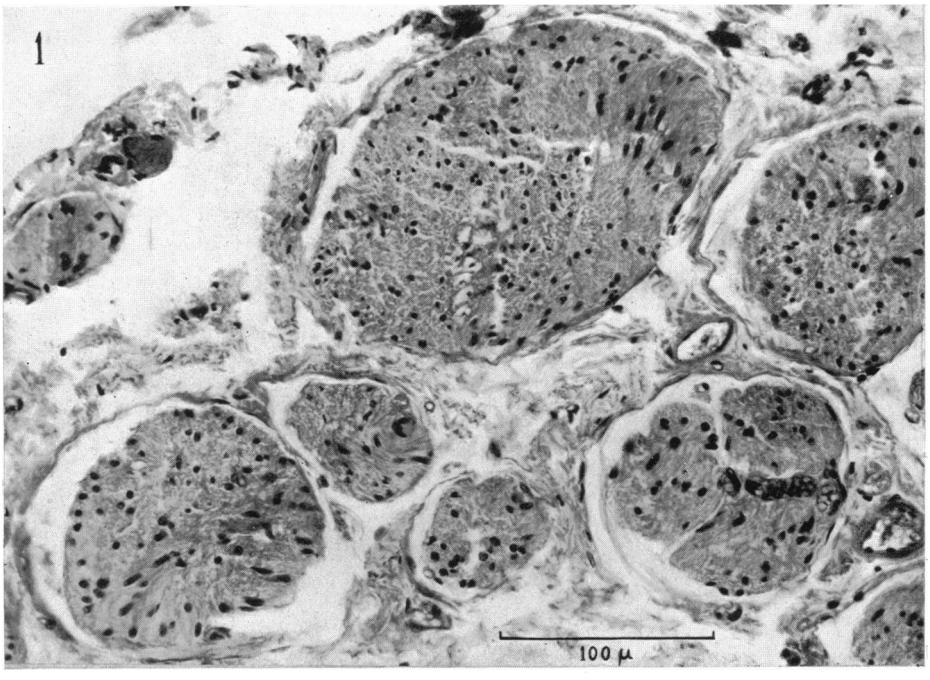
suggested that the 'halo' may be a definite sheath apart from the neurilemma. This sheath may be a layer of fatty molecules around the axon, i.e. a myelin sheath which contains too little fat to be demonstrable after treatment with osmium tetroxide (Schmitt & Bear, 1939). Ranson, however, could find no trace of nodes of Ranvier. The terminology is confusing but comparison with the structure of a myelinated nerve will help to clarify the position. In the myelinated nerve it is important to distinguish between the Schwann cell outside the myelin sheath and the neurilemma outside the Schwann cell. On the other hand, in the non-myelinated nerve both these layers are called the neurilemma ('sheath of Schwann' of Nageotte) and the outer layer the endoneurium ('membrane of Schwann' of Nageotte). Nageotte's description of the 'membrane of Schwann' correponds to what Gaskell (1886) and Ranson (1911) both call the background of 'connective tissue' in which non-myelinated axons appear. The nuclei within the perineurium bounding a non-myelinated nerve bundle are either those belonging to the neurilemma (these may or may not be equivalent to the Schwann cell nuclei of a myelinated nerve) or connective tissue nuclei or blood-vessel nuclei. No differentiation between these nuclei was attempted in the present work.

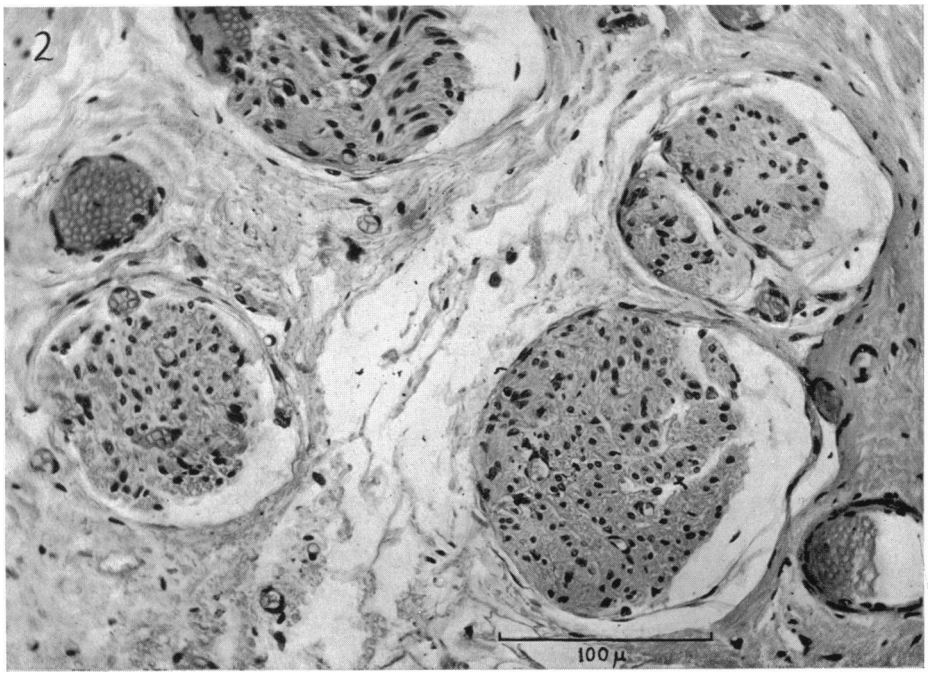
Abercrombie & Johnson (1946) have studied the changes in nuclear density in the degenerated sciatic nerve of a rabbit at varying intervals after interruption of the nerve. They point out that the maximum increase is after 25 days, the tubal nuclei, i.e. Schwann cell nuclei, having then increased to thirteen times and the endoneural nuclei, i.e. all the others, to four times their original number, the overall increase being 8·4 times. The difference between their results and those in this paper is very striking. There are several possible explanations for this difference. The absence of myelin leads to two obvious differences between the process of degeneration in non-myelinated and myelinated nerves. In the first place the space left within the sheath is small when the small non-myelinated fibres degenerate. Secondly, there are no myelin remains to provide a chemical stimulus to division of the cells. The failure of the cells of degenerating non-myelinated nerve to divide suggests that one of these two factors is responsible for causing the multiplication in myelinated nerves. It must not be forgotten, however, that the nature and origin of the nuclei in both cases is still somewhat uncertain and we cannot be sure that they are strictly comparable. Denny-Brown (1946) has recently claimed that many of the cells normally regarded as Schwann cells are a special type of 'neural fibroblast'. Further work on the nature of the nuclei in all types of nerve is needed, but for the present we may consider the cells in non-myelinated nerve essentially similar to Schwann cells.

SUMMARY

1. The anterior mesenteric nerve of the rabbit, consisting almost wholly of non-myelinated nerve-fibres, was found to show $75{\cdot}56 \pm 18{\cdot}12$ nuclei per $10{,}000\,\mu^2$ in transverse sections $5\,\mu$ thick.

2. Twenty-one days after the fibres of this nerve had been interrupted by





JOSEPH—CELL MULTIPLICATION IN DEGENERATED NERVES

crushing or severance the nuclear population in the distal stump was 74·95 ± 18·24.

3. Evidently the cell population has changed little, if at all, in contrast to the multiplication by more than eight times which occurs in the nuclei of myelinated nerve during degeneration.

4. The differences between the two types of nerve are (*a*) the lack of myelin, and (*b*) the smaller space within the neurilemma of the non-myelinated nerve. The cell multiplication in a myelinated nerve must therefore be due either to the effect of the break-down products of myelin or to the physical collapse of the large axons.

I wish to thank Professor J. Z. Young for many helpful suggestions and for reading and criticizing the manuscript; Mr J. Armstrong for technical assistance; and Mr E. G. Reeve, of the Social Survey, for advice on statistics.

REFERENCES

ABERCROMBIE, M. & JOHNSON, M. L. (1946). Quantitative histology of Wallerian degeneration. 1. Nuclear population in rabbit sciatic nerve. *J. Anat., Lond.*, **80**, 37–50.

CAJAL, S. RAMÓN Y. (1928). *Degeneration and regeneration of the nervous system.* London: Oxford University Press.

DENNY-BROWN, D. E. (1946). Importance of neural fibroblasts in the regeneration of nerve. *Arch. Neurol. Psychiat., Chicago*, **55**, 171–215.

GASKELL, W. H. (1886). On the structure, distribution and function of the nerves which innervate the visceral and vascular systems. *J. Physiol.* **7**, 1–80.

MACHIDA, K. (1929). Observations on the degeneration and regeneration of post ganglionic nerve fibres. *Johns Hopk. Hosp. Bull.* **45**, 247–263.

NAGEOTTE, J. (1932). In W. Penfield, *Cytology and Cellular Pathology of the nervous system*, 1. New York: Hoeber.

RANSON, S. W. (1911). Non-medullated nerve fibres in the spinal nerves. *Amer. J. Anat.* **12**, 67–87.

RANSON, S. W. (1912). Degeneration and regeneration of nerve fibres. *J. comp. Neurol.* **22**, 487–537.

SCHMITT, F. O. & BEAR, R. S. (1939). The ultrastructure of the nerve axon sheath. *Biol. Rev.* **14**, 27–50.

SIMPSON, S. A. & YOUNG, J. Z. (1946). Regeneration of fibre diameter after cross unions of visceral and somatic nerves. *J. Anat., Lond.*, **79**, 48–65.

TUCKETT, L. (1896). On the structure and degeneration of non-medullated nerve fibres. *J. Physiol.* **19**, 267–311.

EXPLANATION OF PLATE

Fig. 1. Transverse section of normal anterior mesenteric nerve of rabbit. Sections 5 μ thick. Stained haematoxylin and eosin. (Rabbit 47.)

Fig. 2. Transverse section of anterior mesenteric nerve of rabbit distal to crush performed 21 days previously. Sections 5 μ thick. Stained haematoxylin and eosin. (Rabbit 12.)