CHANGES IN NUCLEAR POPULATION FOLLOWING TWENTY-ONE DAYS' DEGENERATION IN A NERVE CONSISTING OF SMALL MYELINATED FIBRES

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INTRODUCTION

There is a remarkable difference between the eightfold increase in nuclear population at the end of 25 days' degeneration in a typical somatic nerve as shown by Abercrombie & Johnson (1946), and the absence of cell multiplication after a similar period in a non-myelinated nerve as shown by Joseph (1947). It was thought that an investigation into nuclear changes which occur following degeneration in a nerve consisting of small fibres might help to elucidate the causes of this difference and also shed some light on the nature of the stimulus or stimuli which cause cell multiplication. This paper deals with a study of the nuclear changes in a nerve consisting almost entirely of fibres less than 6μ in diameter. Thomas (1948), in an independent investigation, has studied the changes in nerves consisting of fibres of larger size.

MATERIAL AND METHOD

The nerve chosen was the greater splanchnic nerve of the rabbit. Simpson & Young (1945) showed that only 4 % of the fibres of this nerve are larger than 6μ in diameter. The left nerve was cut with fine sharp scissors just where it emerges below the diaphragm, as it lies beside the abdominal aorta. The intact right nerve was used for comparison. An attempt was made to carry out a similar operation on the right side, but this was found to be impossible owing to the difficulty of avoiding a fatal pneumothorax. In addition, the liver tore very readily in attempting to expose the site of operation and caused very troublesome bleeding. Since, therefore, all the interruptions were made on the left splanchnic nerve it was necessary to investigate any possible difference between the right and left nerves in normal rabbits. In eight experimental rabbits 21 days after operation, both right and left nerves were removed, and each nerve was divided into a proximal and distal piece. Similarly, in 7 normal rabbits four specimens were obtained, two from each side. Each specimen was 1–2 cm. long. They were fixed in Bodian's fluid containing 15 ml. formaldehyde, 5 ml. acetic acid and 80 ml. of 80 % methyl alcohol. Transverse sections at 5μ of each end of all four specimens were cut and two slides of each were prepared. One slide was stained for axons by Bodian's method, and one with haematoxylin and eosin. The absence of normal axons in the sections of the Bodian-stained slide confirmed that the nerve had been completely interrupted. Photographs at a magnification of $\times 350$ of sections stained with haematoxylin and eosin from two different levels from each side of each rabbit were used for counting nuclei.

RESULTS

Table 1 shows the number of nuclei at two levels in the right and left nerves of 7 normal rabbits. Frequently, the greater splanchnic nerve in the rabbit consists of more than one nerve bundle (Pl. 1, fig. 1). Where this occurred, the nuclei in all the bundles were counted and added together. The nuclei of all cells within the perineurium were counted. The variability which is obvious in these nuclear counts may be due to differences between rabbits, between the different sides of the same rabbit, between the different levels of the same nerve, or may be due to these factors acting together. It is important that one should be able to isolate the variability due to these different factors and reach a decision as to the relative significance of each. The method for reaching this decision is the analysis of variance discussed by Fisher (1946). The Table resulting from this analysis is given in an Appendix (Table 3), and shows that

Table 1

Serial number of rabbit	Level	Number of nuclei per transverse section at 5μ	
		Right (normal)	Left (normal)
184	Proximal	295	452
	Distal	430	540
337	Proximal	279	249
	Distal	266	337
338	Proximal	421	458
	Distal	508	379
348	Proximal	271	260
	Distal	325	303
350	Proximal	324	308
	Distal	318	366
380	Proximal	304	258
	Distal	272	266
422	Proximal	335	257
	Distal	357	213

variations due to differences between the two sides and different levels are not significant at the 5 % level. On the other hand, the variation between individual rabbits is clearly significant, as might be expected.

Table 2 shows the number of nuclei at two levels in the right and left nerves in 8 rabbits, in which the left nerve was cut and allowed to degenerate for 21 days (Pl. 1, fig. 2). The two levels at which the nuclear counts were made were 5 mm. or more from the site of the cut.

The greater splanchnic nerve consists largely of preganglionic fibres. In many of the nerves ganglion cells occur somewhere along their course. Where they occur distal to the cut the axons of these cells do not degenerate. This results in some uninterrupted fibres being sometimes found in the distal part of the cut nerve. There are never more than a few of these fibres and their presence cannot influence the results.

Abercrombie (1946) showed that the length of nucleus has to be considered in comparing the number of nuclei in the transverse sections of two specimens.

Consequently, two normal nerves were cut in longitudinal section at 7μ and stained with haematoxylin and eosin. Photographs of suitable sections were taken at a magnification of $\times$500 and the length of 300 nuclei in each nerve was measured. The mean length was 12·60μ in one and 12·92μ in the other. Two degenerated specimens were treated in the same way. The mean nuclear length was 12·71μ in one and 13·91μ in the other. The difference in nuclear length between the normal and degenerated nerves is so small that it can be ignored.

The nuclear counts in Table 2 were subjected to an analysis of variance in the same way as were the counts in the normal rabbits. The Table resulting from the analysis is given in an appendix (Table 4) and shows that, as before, there is no significant variation at different levels. The variation between rabbits is still significant, as one might expect. In estimating the variation between the sides the effect caused by differences between rabbits is taken out, and in the experimental animals, unlike the normal animals, the remaining variation between the sides is highly significant. Consequently, it can be concluded that there takes place a definite increase in nuclei after 21 days' degeneration in the greater splanchnic nerve of the rabbit.

Table 2

Serial number of rabbit	Level	Number of nuclei per transverse section at 5μ: Right (normal)	Left (after 21 days' degeneration)
128	Proximal	369	547
	Distal	435	627
129	Proximal	305	351
	Distal	265	451
174	Proximal	320	361
	Distal	417	334
175	Proximal	312	600
	Distal	397	600
176	Proximal	234	362
	Distal	244	295
351	Proximal	444	447
	Distal	413	618
383	Proximal	430	665
	Distal	493	580
384	Proximal	242	321
	Distal	272	325

In order to obtain some estimation of the extent of this increase the mean percentage difference in the number of nuclei between the right nerve and the left nerve of the 8 experimental rabbits was calculated and found to be 34%.

It was thought that some attempt should be made to determine what types of nuclei had increased in number. It is assumed that the structure of a nerve bundle of a myelinated nerve consisting of small fibres is similar to that of any other myelinated nerve. Thus the nuclei seen in the transverse section of the greater splanchnic nerve can belong to Schwann cells, connective tissue cells, macrophages or cells associated with blood vessels, endothelial and smooth muscle cells. Of these cells only the Schwann cell is inside the neurilemma. Careful examination of the nuclei in the preparations used for counting failed to show which cells had increased in number. There is usually an obvious increase in the number of blood vessels which results in an increase of blood vessel nuclei from about 5 to 25, but could not account for an increase of 34 % in the total nuclei. The increase is therefore due, to a large extent, to an increase in Schwann cells, fibroblasts or macrophages, or all of these.

Abercrombie & Johnson (1946), when studying the nuclei in the sciatic nerve of the rabbit after 25 days' regeneration, divided them into two groups, intratubal and extratubal, and showed that the former multiplied thirteen times and the latter four times. Such a classification in a nerve consisting of small myelinated fibres is impossible since after 21 days' degeneration it is too difficult to determine whether a nucleus is inside or outside the nerve fibre tube. Moreover, since macrophages enter the neurilemmal tubes, especially the large ones, a classification of this kind does not indicate precisely the increase in each type of cell.

In specimens of the greater splanchnic nerve following 6–12 days' degeneration, it is possible to distinguish two types of nuclei, apart from the blood-vessel nuclei, and further study of these nerves may indicate more clearly the changes in number of each type of nucleus.

DISCUSSION

Although Langley (1909), Langley & Orbeli (1911), Ranson & Billingsley (1918), and others, studied degeneration in preganglionic nerves of the sympathetic nervous system, nerves similar to the greater splanchnic nerve of the rabbit in fibre size, they made no special observations about the changes in nuclear population in these nerves. The figures of Table 2 show that there is a striking difference between the increase in nuclei during degeneration in a nerve consisting of small myelinated fibres and one consisting of large fibres. The increase in nuclei in the latter type of nerve is due almost entirely to an increase in macrophages and Schwann cells. Observations regarding macrophages in relation to degenerating small myelinated fibres were made by Weddell & Glees (1941), who showed that there were no cells containing particles in the region of these small fibres following vital dye injections.

When describing degeneration in small myelinated fibres, Nageotte (1932) suggested that the axon and myelin autolyse and 'simply disappear'. He stated that the Schwann cell has the power of phagocytosis in these nerves. If, however, the space within the neurilemma is large enough to receive immigrant cells, then macrophages enter the sheath and phagocytose the debris within it, and in these circumstances the Schwann cells multiply and line the neurilemmal sheath but do not act as phagocytes. Cajal (1928) maintained more definitely that the phagocytes are derived from outside the neurilemmal sheath of the nerve fibre, and that the Schwann cells, although they may have some effect on the degeneration of the myelin and axon, do not participate in the removal of the products of degeneration. That there is an increase of only 34 % in the nuclei of the greater splanchnic nerve after 21 days' degeneration suggests that only a small number of macrophages is required to deal with the degenerated myelin and axon and also that there is practically no increase in Schwann cells.

The size of the neurilemmal tube is therefore evidently a factor in determining the increase in the Schwann cells. Cajal (1928) pointed out that the multiplication of Schwann cells, which begins about the fourth day of degeneration and ends in the third week, is closely related to the shrinking away of the myelin sheath from the Schwann cells. This shrinking results in increased space within the nerve fibre so that the Schwann cell can enlarge and divide. Thus there is probably a relationship between the size of the nerve fibre and the multiplication of Schwann cells, the smaller the nerve fibre the smaller the increase. The different results obtained following degeneration in nerves of different sizes support this.

In order to explain the multiplication of the Schwann cells in the peripheral stump of a degenerated nerve some writers have maintained that this increase is connected with preparation of the peripheral stump for regeneration (Howell & Huber, 1892; Kirk & Lewis, 1917). In the spinal cord, where multiplication of cells did not take place following degeneration, regeneration apparently did not occur (Halliburton, 1907). Regeneration, however, readily takes place both in non-myelinated nerve where there is practically no increase in nuclei after degeneration, and in the greater splanchnic nerve where there is an increase of the order of 34 %. It is unlikely that proliferation of the Schwann cells in degenerating nerve fibres plays as important a role in regeneration as earlier writers suggest.

It can be seen from the results of this paper, and those of Abercrombie & Johnson (1946) and Thomas (1948), that there is a considerable difference in the increase in nuclear population at the end of similar periods of degeneration in different peripheral nerves. If it is assumed that the basic structure of the nerve fibres is the same, the main difference between them is the size of the nerve fibres. The results show that the larger the nerve fibres the greater is the increase in the number of nuclei. This difference is almost certainly associated with the greater space within the sheath following degeneration and an increased quantity of the products of degeneration, especially those of the myelin sheath, in large fibres.

SUMMARY

1. In the greater splanchnic nerve of the rabbit, consisting of myelinated fibres of which about 96 % are 6μ in diameter or smaller, the number of nuclei in a transverse section 5μ thick shows no significant difference between the right and left nerves and between two levels in the same nerve. There is a significant difference between the nerves of different rabbits.

2. After 21 days' degeneration in this nerve there is a significant increase in the number of nuclei in a transverse section 5μ thick amounting to 34 %,

which, it is suggested, is due to a slight increase in all the cells, Schwann cells, macrophages and those of the blood vessels.

3. This is compared with the increase seen in nerves consisting of myelinated fibres of greater size, for example, the nerve to the medial head of gastrocnemius which shows a fourteenfold increase, the sciatic nerve which shows an eightfold increase, and the sural nerve which shows a fivefold increase, and a non-myelinated nerve such as the anterior mesenteric nerve which does not appear to show a significant increase.

4. The reasons for the increase in nuclei in Wallerian degeneration are discussed, and it is suggested that the larger the diameter of the nerve fibres and the greater the quantity of the products of degeneration, the greater is the increase.

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APPENDIX

Table 3

Source of variation		Sum of squares	Degrees of freedom	Mean square	Variance ratio	Probability level
	Sides (S)	54·3214	1	54·3214	—	—
Main effects:	Rabbits (R)	126833·8571	6	21138·9761	—	—
	Levels (L)	6572·8929	1	6572·8929	—	—
	S × R	33009·4286	6	5501·5714	—	—
Interactions:	S × L	150·8928	1	150·8928	—	—
	L × R	10754·8571	6	1792·4761	—	—
	S × R × L	10823·8572	6	1803·9762	—	—
	Total	188200·1071	27			

Since none of the interactions is significant one may pool and then we have

	Sides	54·3214	1	54·3214	0·019	N.S.
Main effects:	Rabbits	126833·8571	6	21138·9761	7·337	< 0·001
	Levels	6572·8929	1	6572·8929	2·281	N.S.
	Residual	54739·0357	19	2881·0018	—	—
	Total	188200·1071	27			

Table 4

Source of variation		Sum of squares	Degrees of freedom	Mean square	Variance ratio	Probability level
	Sides (S)	111864·5	1	111864·5	—	—
Main effects:	Rabbits (R)	286296·0	7	40899·4	—	—
	Levels (L)	6498·0	1	6498·0	—	—
	S × R	45631·0	7	6518·7	—	—
Interactions:	S × L	338·0	1	338·0	—	—
	L × R	8884·5	7	1269·2	—	—
	S × R × L	27589·5	7	3941·4	—	—
	Total	487101·5	31			

Since the interactions are non-significant they may be pooled

	Sides	111864·5	1	111864·5	29·85	< 0·001
Main effects:	Rabbits	286296·0	7	40899·4	10·91	< 0·001
	Levels	6498·0	1	6498·0	1·73	N.S.
	Residual	82443	22	3747·4	—	—
	Total	487101·5	31			

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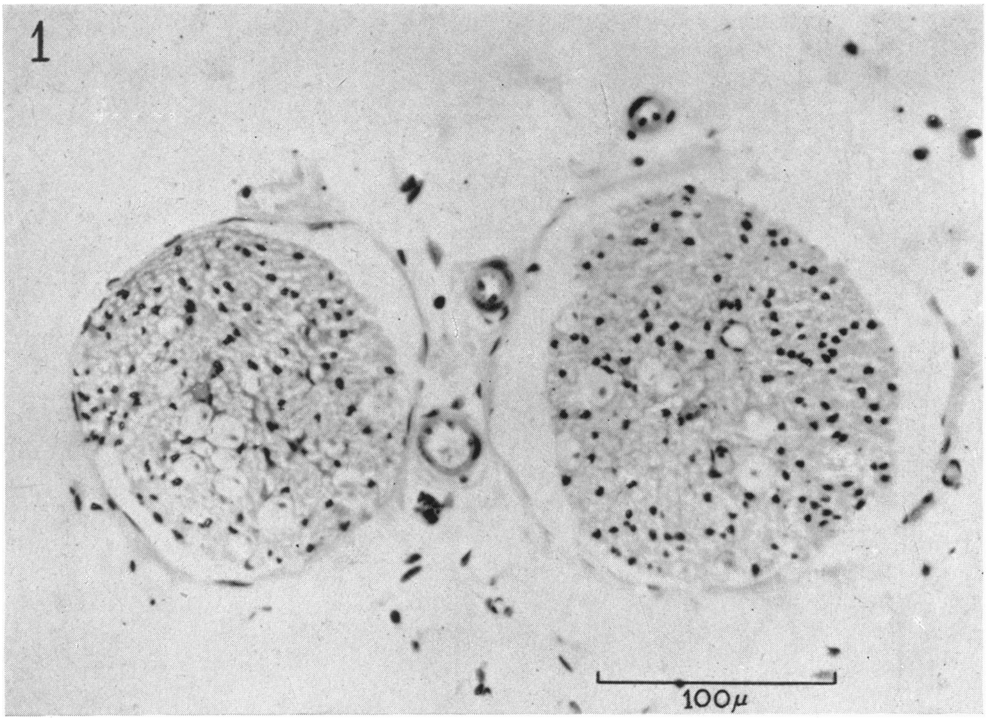
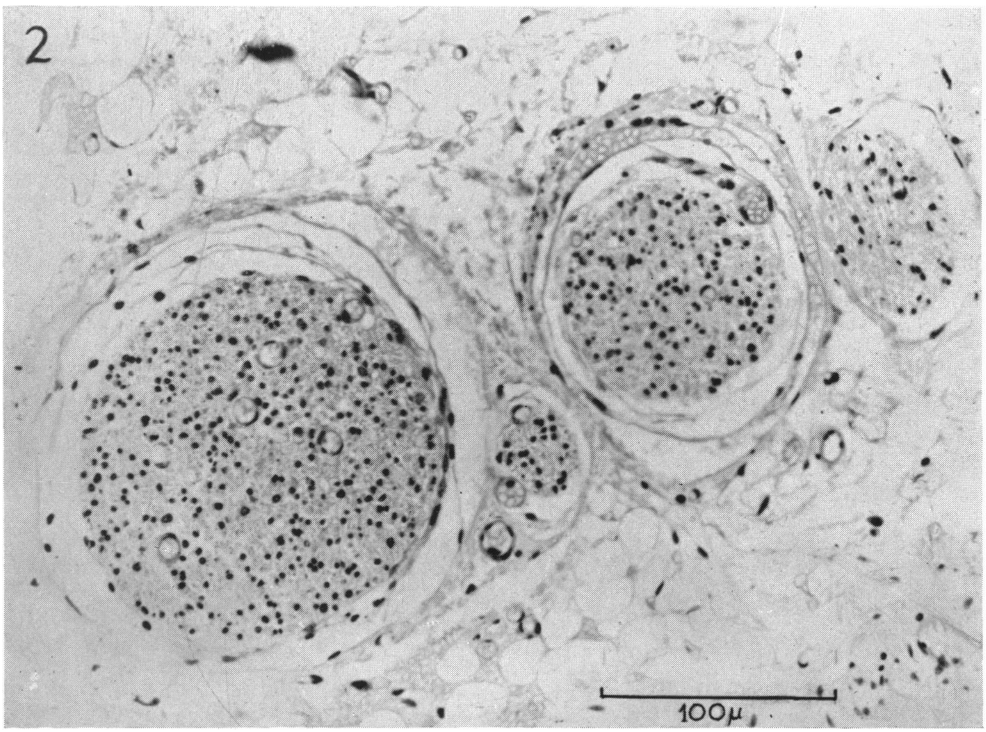
EXPLANATION OF PLATE

Fig. 1. Transverse section of normal greater splanchnic nerve of rabbit. Sections 5μ thick. Stained haematoxylin and eosin. (Rabbit 129.)

Fig. 2. Transverse section of greater splanchnic nerve of rabbit distal to cut made 21 days previously. Sections 5μ thick. Stained haematoxylin and eosin. (Rabbit 175.)





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