(3) *Extraction of nucleic acids*

Fig. 1 shows that the absorption at 268.5 μ . of a TCA extract of cat sciatic nerve was not significantly greater when the extraction time was increased from 15 to 30 min. Similar results were obtained with white matter and grey matter of dog brain. However, after prolonged heating with TCA (2 hr.) a slight increase in the absorption at 268.5 μ . was observed. When the residue remaining after a 15 min.

extraction with TCA was extracted for a further 105 min., the extract had a slight absorption at 268 μ ., but the maximum absorption was at 274–275 μ ., presumably due to protein degradation products.

In the standard 15 min. extract of brain or nerve there was no suggestion of a secondary peak at 274–276 μ . as was observed with 6% (w/v) HClO_4 . It is felt that interference due to protein degradation products in samples heated in 5% (w/v) TCA for no longer than 15 min. is minimal.

Chemical Studies of Peripheral Nerve During Wallerian Degeneration

3. NUCLEIC ACIDS AND OTHER PROTEIN-BOUND PHOSPHORUS COMPOUNDS

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This paper describes changes in the concentration of nucleic acids and other protein-bound phosphorus compounds in the sciatic nerve of the cat after nerve section or nerve crush. If a nerve is cut (neurotmesis) that portion of the nerve peripheral to the point of section undergoes the familiar series of changes known as Wallerian degeneration. If the nerve is crushed, a procedure that interrupts the axons and myelin sheaths but leaves the connective tissue sheaths intact (axonotmesis), the nerve degenerates as before. After a short latent period the degenerating peripheral segment is re-innervated by axon tips from the intact central stump. There is thus a regeneration superimposed upon the degeneration. In previous publications from this laboratory changes in the concentration of lipids after nerve section or nerve crush have been described (Johnson, McNabb & Rossiter, 1949; Burt, McNabb & Rossiter, 1950).

While this work was in progress, papers by Bodian & Dziewiatkowski (1950) and Samuels *et al.* (1951) appeared giving some figures for protein-bound phosphorus compounds in peripheral nerve during Wallerian degeneration. By using the method of ultraviolet absorption described in the preceding paper (Logan, Mannell & Rossiter, 1952), a more reliable estimate of the concentration of total nucleic acid in a degenerating peripheral nerve has been obtained. In addition, the changes that occur in the concentration of deoxypentose-nucleic acid (DNA), pentosenucleic acid (PNA), 'phosphoprotein' (PP) and 'inositide P' after nerve section or nerve crush have been described for the first time. The figures given for 'inositide P' probably represent the phosphorus of the inositol-containing trypsin-resistant lipid-protein complex

described by Folch & Le Baron (1951) and thought by Dr Jordi Folch to be part of the 'neurokeratin' of Kühne & Chittenden (1889).

METHODS

The right sciatic nerves of fifty-two cats were either sectioned or crushed at the level of the greater trochanter of the femur. Details of the operations have already been described (Johnson *et al.* 1949; Burt *et al.* 1950). No attempt was made to control the age, sex or weight of the animals. After intervals of time varying from 2 to 600 days the animals were killed, and the segment of nerve distal to the site of the operation was removed. At the same time, a similar length of left sciatic nerve was removed to serve as a control. Each nerve was cleaned of adherent fatty and epineural connective tissue, weighed, and the phosphorus compounds were estimated as described in the preceding paper (Logan *et al.* 1952). The nucleic acids were estimated by ultraviolet absorption.

Because of the great increase in the wet weight of degenerating and regenerating nerves, the results have all been expressed in terms of the fresh weight of a similar length of the intact nerve of the opposite side. This is equivalent to expressing the results in terms of the fresh weight of the nerve before it had been sectioned or crushed, i.e. at zero time. All analyses were done in duplicate.

RESULTS

Nerve section

Table 1 shows that 16 days after nerve section there was an increase in the concentration of acid-soluble P and protein-bound P, i.e. phosphorus remaining after the removal of acid-soluble P and lipid P, and a decrease in the concentration of lipid P and total P. Table 2 shows that the increase in the concentration of protein-bound P was due to an increase (by a

factor of 3 to 4) in the concentration of nucleic acid. The concentration of both DNA and PNA increased, but the increase in the concentration of PNA was greater than that of DNA, causing a rise in the PNA/DNA ratio. Neither PP nor 'inositide P' contributed to the rise in the protein-bound P. After 16 days the mean concentration of PP was less than that in the control nerves. The mean concentration of 'inositide P' was also less than that in the control nerves, although the difference is not

Figs. 1-5 show the changes in the concentration of the various phosphorus compounds at different times after nerve section. The concentration of acid-soluble P (Fig. 1) increased, reaching a maximum at 8-16 days and then decreased to values well below those of the control nerves. As was found in the previous study (Johnson *et al.* 1949), the concentration of lipid (Fig. 2) did not change greatly during the first 8 days and then decreased rapidly during the period 8-32 days.

Table 1. *Phosphorus compounds in sciatic nerve of the cat 16 days after nerve section or nerve crush*

(Results are expressed as mg. P/100 g. wet tissue. No. of animals is stated in parentheses under each result. *P* gives the significance of the difference between values for experimental and control nerves.)

Estimation	Control		16 days after nerve section			16 days after nerve crush		
	Mean	S.E.M.	Mean	S.E.M.	<i>P</i>	Mean	S.E.M.	<i>P</i>
Acid-soluble P	52.2 ± (42)	0.7	62.1 ± (6)	2.1	<0.01	64.9 ± (5)	2.5	<0.01
Lipid P	310 ± (50)	5.6	156 ± (6)	20	<0.01	149 ± (5)	14	<0.01
Protein-bound P	19.4 ± (52)	0.5	41.7 ± (6)	1.2	<0.01	41.5 ± (5)	2.2	<0.01
Total P (by addition)	380.8 ± (42)	5.8	259.7 ± (6)	33.8	<0.01	254.9 ± (5)	11.7	<0.01

Table 2. *Protein-bound P in sciatic nerve of the cat 16 days after nerve section or nerve crush*

(Results are expressed as mg. P/100 g. wet tissue. No. of animals is stated in parentheses under each result. *P* gives the significance of the difference between values for experimental and control nerves.)

Estimation	Control		16 days after nerve section			16 days after nerve crush		
	Mean	S.E.M.	Mean	S.E.M.	<i>P</i>	Mean	S.E.M.	<i>P</i>
Total protein-bound P	19.4 ± (52)	0.5	41.7 ± (6)	1.2	<0.01	41.5 ± (5)	2.2	<0.01
Total nucleic acid	8.7 ± (52)	0.2	32.9 ± (6)	1.1	<0.01	34.5 ± (5)	2.3	<0.01
DNA	4.8 ± (52)	0.1	14.4 ± (6)	0.9	<0.01	13.4 ± (5)	0.5	<0.01
PNA	3.9 ± (52)	0.1	18.5 ± (6)	0.8	<0.01	21.1 ± (5)	2.4	<0.01
PP	1.8 ± (51)	0.1	1.0 ± (6)	0.1	<0.01	1.2 ± (5)	0.1	<0.01
'Inositide P'	9.0 ± (51)	0.4	7.7 ± (6)	0.8	<0.3	5.8 ± (5)	1.2	<0.02
PNA/DNA ratio	0.9 (52)		1.3 (6)			1.6 (5)		

significant statistically ($P=0.2-0.3$). This is no doubt due to the lack of precision in the 'inositide P' determination. 'Inositide P' is obtained by difference from three individual determinations. After 32 days, however, the mean concentration of 'inositide P' in the degenerating nerves was very much less than that in the control nerves and the difference is statistically significant. The standard errors given in Tables 1 and 2 indicate the degree of reproducibility to be expected with different animals.

Fig. 3 shows that the concentration of protein-bound P rose to a maximum at 16 days and then fell slowly throughout the course of the degeneration. The rise, which was apparent after only 2 days of degeneration, was due to an increase in the concentration of nucleic acid. There was a steady fall in the concentration of PP. The concentration of 'inositide P', like the concentration of lipid P, did not change greatly in the first 8 days and then fell sharply in the period 8-32 days.

Fig. 1. Acid-soluble P of cat sciatic nerve after nerve section (▲—▲) or nerve crush (●—●). Each point represents the mean for three or more animals.

Fig. 2. Lipid P of cat sciatic nerve after nerve section (●—●) or nerve crush (▲—▲). Each point represents the mean for three or more animals.

Fig. 3. Protein-bound P of cat sciatic nerve after nerve section. ●—●, total protein-bound P; ▲—▲, nucleic acid P; ■—■, 'inositide P'; ▼—▼, PP. Each point represents the mean for three or more animals.

Fig. 4 gives the concentrations of DNA and PNA. Both nucleic acids increased in concentration, but PNA increased more rapidly than DNA, so that by 32 days the PNA/DNA ratio had increased from 0.9 to 2.0 (Fig. 5). After 32 days the PNA/DNA ratio fell gradually.

Fig. 4. Nucleic acids of cat sciatic nerve after nerve section. ●—●, total nucleic acid; ■—■, DNA; ▲—▲, PNA. Each point represents the mean for three or more animals.

Fig. 5. Ratio of PNA to DNA in cat sciatic nerve after nerve section. Each point represents the mean for three or more animals.

Nerve crush

Tables 1 and 2 show that 16 days after nerve crush the phosphorus compounds changed in the same direction and to the same extent as after nerve section. None of the figures for the crushed nerves is significantly different from that for the corresponding substance in the sectioned nerves.

By 96 days signs of regeneration began to appear in the crushed nerves. The concentration of acid-soluble P (Fig. 1) and lipid P (Fig. 2) was greater than that in the sectioned nerves ($P < 0.01$, in both instances). With much longer periods of regenera-

tion the concentration of total P and lipid P continued to increase (Fig. 6), and the concentration of protein-bound P continued to fall. Fig. 7 shows that the fall in protein-bound P was due to a fall in the concentration of nucleic acid. By 96 days the concentration of both PP and 'inositide P' had commenced to increase, even although the concentration

ably less than that in the control nerves and the concentration of nucleic acid, especially of DNA, was greater. In four additional animals the nerves were crushed and then allowed to regenerate for 550–600 days after the operation. Even after this length of time the concentration of lipid P and 'inositide P' was still less than that in the control nerves of the opposite side and the concentration of nucleic acid was still greater.

Fig. 6. Total P (●—●) and lipid P (▲—▲) of cat sciatic nerve after nerve crush. Each point represents the mean for three or more animals.

Fig. 7. Protein-bound P of cat sciatic nerve after nerve crush. ●—●, total protein-bound P; ▲—▲, nucleic acid P; ■—■, 'inositide P'; ▼—▼, PP. Each point represents the mean for three or more animals.

of protein-bound P was still falling. Both DNA and PNA contributed to the fall in the concentration of nucleic acid, but the PNA decreased more rapidly than the DNA, so that the PNA/DNA ratio, which in the period 8–32 days was greatly in excess of the figure for the control nerves, fell steadily, reaching a value below that of the controls by 250 days (Fig. 8).

After 250 days, at a time when the acid-soluble P and PP had returned to normal, the concentration of both lipid P and 'inositide P' was still consider-

Fig. 8. Ratio of PNA to DNA in cat sciatic nerve after nerve crush. Each point represents the mean of three or more animals.

DISCUSSION

When a peripheral nerve is sectioned or crushed that portion of the nerve distal to the site of the lesion degenerates. The axon disintegrates and the myelin sheath at first fragments and ultimately is destroyed. The Schwann cells proliferate and the endoneurium and neurilemma thicken to form a Schwann tube, which is able to receive a growing axon tip from the intact central stump. The degeneration is, in a sense, a preparation for regeneration.

The sharp fall in the concentration of lipid P during the period 8–32 days after either type of operation is most likely the result of the destruction of the myelin sheath (Johnson, McNabb & Rossiter, 1950). The finding that the 'inositide P' also disappears at this time is of interest. If this fraction represents the lipid-protein complex of Folch & Le Baron (1951) and is part of the classical 'neurokeratin', the observation provides good evidence that 'neurokeratin' is a constituent of the myelin sheath of a peripheral nerve fibre. This view is strengthened by the further finding that during the deposition of myelin in nerves regenerating after a crush, the 'inositide P' returns to the nerve at the same time as the lipid P.

The rise in the concentration of nucleic acid, reaching a peak 16–32 days after nerve section or

nerve crush, is probably accounted for by the increase in the number of Schwann cells and endoneurial cells (chiefly fibrocytes and macrophages) that takes place during this time (Young, 1942; Abercrombie & Johnson, 1946). If, as has been suggested, the mean quantity of DNA per diploid cell is constant for any one species (see Davidson, Leslie & White, 1951, for references), it is apparent that the total amount of DNA in a nerve is an index of the number of cell nuclei present. The curve representing the change in the concentration of DNA with time (Fig. 4) is of the same general form as that reported by Abercrombie & Johnson (1946) for the change in the total number of cell nuclei in the degenerating sciatic nerve of the rabbit. Quantitatively the curves are different, but this is not surprising when the many differences between the two sets of experiments are taken into consideration. Our experiments were with cats, not rabbits, and the chemical determination gives an estimate of the number of cell nuclei throughout the whole length of the nerve rather than the number at one selected level. Also it is now known that the extent of the increase in the total number of cell nuclei in a peripheral nerve during Wallerian degeneration depends upon the size and the degree of myelination of the constituent fibres (Joseph, 1947, 1948; Thomas, 1948).

The concentration of PNA gives an indication of the total mass of cytoplasm within the nerve. During the period of cellular proliferation the PNA/DNA ratio, i.e. the concentration of PNA per cell, is considerably greater than that in the control nerves. At this time an increase in the amount of Schwann cell cytoplasm can be demonstrated histologically (Young, 1942). There is also an increase in the concentration of acid-soluble P.

The increase in the concentration of nucleic acid is statistically significant as early as 2 days after the operation, yet even at 8 days, when the concentration of nucleic acid is greatly in excess of that in the control nerves, the concentration of lipid P and 'inositide P' is not significantly decreased. This would indicate that the cellular proliferation commences well in advance of the chemical degradation of the myelin sheath, although, with the degeneration of the axon in the first few days after nerve section, there is a physical destruction of the myelin sheath, with segmentation and ovoid formation (Young, 1945; Johnson *et al.* 1950).

In the sectioned nerves there was no post-mortem or functional evidence of regeneration 96 days after the operation, and in other animals similarly treated this absence of regeneration was confirmed microscopically. In the crushed nerves, on the other hand, myelination had spread throughout the length of the nerve and function appeared normal. Yet even after 600 days, neither the lipid P

nor the 'inositide P' had returned to normal. At this time the regenerating nerves still contained more nucleic acid than the control nerves of the opposite side. This finding is of interest in view of the slow restitution of fibre pattern (Gutman & Sanders, 1943; Sanders, 1948) and full physiological activity (Berry, Grundfest & Hinsey, 1944; Sanders & Whitteridge, 1946; Erlanger & Schoepfle, 1946) in regenerating nerves.

The figures for the concentration of phosphorus compounds in the control nerves agree well with those reported in the previous paper (Logan *et al.* 1952) for a smaller series of normal cat sciatic nerves.

May (1930), Bodian & Dziewiatkowski (1950) and Samuels *et al.* (1951) have studied the distribution of phosphorus compounds in peripheral nerves during Wallerian degeneration. Comparison of our results with those of May (1930) is of little value because of the great differences in the analytical techniques employed. Bodian & Dziewiatkowski (1950) reported a fall in the concentration of lipid P and a slight increase (of the order of 18%) in the concentration of protein-bound P 7–23 days after section in monkey nerves. Because the 'inositide P' accounts for such a high percentage of the protein-bound P of the control nerves and the concentration of 'inositide P' falls while that of nucleic acid rises during Wallerian degeneration, the percentage increase in the concentration of nucleic acid would be much greater than that of the protein-bound P. In our experiments the protein-bound P of cat sciatic nerve increased by 116% in 16 days, whereas the concentration of nucleic acid increased by 280%. Samuels *et al.* (1951), using the method of Schneider (1945), also reported an increase in the concentration of nucleic acid (112%) in guinea-pig nerves 16 days after section. For the reasons given by Logan *et al.* (1952), the figures of Samuels *et al.* (1951) for nucleic acid, especially those for the control nerves, are probably too high.

SUMMARY

1. The concentration of acid-soluble phosphorus, lipid phosphorus, protein-bound phosphorus, total nucleic acid, deoxypentosenucleic acid (DNA), pentosenucleic acid (PNA), phosphoprotein (PP) and 'inositide phosphorus' was determined in the sciatic nerve of the cat at intervals of time from 2 to 96 days after nerve section (neurotmesis) and 8–600 days after nerve crush (axonotmesis).

2. The acid-soluble phosphorus increased to a maximum at 8–16 days after either nerve section or nerve crush, returning to values not significantly different from those of the control nerves by 32 days. The acid-soluble phosphorus then remained normal in the crushed nerves, but fell to lower values in the sectioned nerves.

3. The lipid phosphorus changed little during the first 8 days and then decreased rapidly during the period 8–32 days after either nerve section or nerve crush. In the sectioned nerves the lipid phosphorus fell still further, whereas in the crushed nerves it had commenced to increase by 96 days and continued to increase throughout the period of regeneration.

4. The concentration of total nucleic acid increased after either nerve section or nerve crush, reaching a maximum in 16 days. Thereafter the concentration of nucleic acid fell in both the sectioned and crushed nerves. After each type of operation the PNA increased more rapidly than the DNA, so that there was an increase in the PNA/DNA ratio.

5. The PP fell throughout the whole course of the degeneration in the sectioned nerves. In the crushed nerves it had commenced to rise by 96 days, and by

250 days it approximated the concentration in the control nerves.

6. The 'inositide phosphorus' followed a course similar to that of the lipid phosphorus. After either type of operation there was little change during the first 8 days, followed by a rapid fall during the period 8–32 days. In the sectioned nerves the 'inositide phosphorus' fell still further, whereas in the crushed nerves it had commenced to increase by 96 days and continued to rise during the entire period of study.

7. The increase in the concentration of nucleic acid and the decrease in the concentration of lipid phosphorus and 'inositide phosphorus' still persisted 600 days after nerve crush.

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A Study of the Amino-acid Complexes Formed by Metals of Group II of the Periodic Classification

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Albert (1950) published a study of the formation of complexes between a number of metals and α -amino-acids containing only two ionizing groups. The mathematical treatment of the results from potentiometric titrations enabled the stabilities of the metal complexes to be calculated. Using his potentiometric method all the metals of Group II of the periodic classification were studied in their relation

to glycine, its derivatives and other α -amino-acids with only two ionizing groups. In this paper an attempt has been made to study the effect on complex formation of (a) the nature of the metal, and (b) substitution in the amino-acid. An attempt was also made to correlate the results obtained by potentiometry with the solubility studies of Monk (1951*a*, *b*).