2. Salicylate reduces the glycosuria and hyperglycaemia induced by cortisone in the normal rat. Cortisone causes deposition of liver glycogen in the adrenalectomized rat while the concurrent administration of salicylate produces not only depletion of existing glycogen but also prevents the deposition of new glycogen by the cortisone.

3. The implications of these effects of salicylate are discussed.

I wish to express my thanks to Miss E. Quilley and Miss M. Sandiford for technical assistance, to Evans Medical Supplies Ltd. for a gift of Hepovite and to the Board of Governors of King's College Hospital for a grant towards the cost of the work.

REFERENCES

- Benedict, S. R. (1911). *J. Amer. med. Ass.* **57**, 1193.
 Ershoff, B. H. (1951). *Proc. Soc. exp. Biol., N.Y.*, **78**, 836.
 Good, C. A., Kramer, H. & Somogyi, M. (1933). *J. biol. Chem.* **100**, 485.
 Harrison, G. A. (1949). *Chemical Methods in Clinical Medicine*, 3rd ed. London: Churchill.
 Hetzel, B. S. & Hine, D. C. (1951). *Lancet*, **2**, 94.
 Ingle, D. J. (1941). *Endocrinology*, **29**, 649.
 Ingle, D. J. (1952). *Proc. Soc. exp. Biol., N.Y.*, **79**, 184.
 Long, C. N. H., Katzin, B. & Fry, E. G. (1940). *Endocrinology*, **26**, 309.
 Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
 Smith, M. J. H., Meade, B. W. & Bornstein, J. (1952). *Biochem. J.* **51**, 18.
 Stetten, D., Welt, J. D., Ingle, D. J. & Morley, E. H. (1951). *J. biol. Chem.* **192**, 817.

Chemical Studies of Peripheral Nerve During Wallerian Degeneration

4. PHOSPHATASES

BY D. M. HOLLINGER, R. J. ROSSITER AND H. UPMALIS

Department of Biochemistry, University of Western Ontario, London, Canada

(Received 25 April 1952)

When a peripheral nerve is cut, that portion of the nerve distal to the point of section soon loses its ability to transmit a nerve impulse. This is associated with a series of changes characteristic of Wallerian degeneration. The axon of each nerve fibre is destroyed, followed by the physical and later by the chemical destruction of the myelin sheath. There is also a cellular proliferation associated with an increase in the concentration of nucleic acid. Accompanying the destruction of the myelin sheath there is a decrease in the concentration of myelin lipids and neurokeratin (see Young, 1942; Abercrombie & Johnson, 1946; Johnson, McNabb & Rossiter, 1949, 1950; Logan, Mannell & Rossiter, 1952, for references).

With the exception of acid phosphomonoesterase, which was studied by Heinzen (1947) and Bodian (1947), chemical determinations of the activity of enzymes in peripheral nerve during Wallerian degeneration have been confined to those enzymes responsible for the destruction or the synthesis of acetylcholine. In this paper, changes in the activity of a number of phosphatases are described. During the degeneration, there is an increase in the rate of hydrolysis of phenylphosphate at pH 4.9 (acid phosphomonoesterase) and of adenosine-5-phosphate at pH 7.5 (5-nucleotidase), a fall in the rate of

hydrolysis of phenylphosphate at pH 9.9 (alkaline phosphomonoesterase) and little change in the rate of hydrolysis of adenosinetriphosphate (ATP) at pH 7.2 in the presence of Ca^{++} (adenosinetriphosphatase).

When the nerve is crushed (axonotmesis) rather than sectioned (neurotmesis), a procedure that interrupts the axons and myelin sheaths, but leaves the connective tissue sheaths intact, it 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. During the phase of degeneration the changes in the activity of the phosphatases are similar to those following nerve section. During the phase of regeneration the myelin lipids and neurokeratin slowly reappear and the concentration of nucleic acid falls (Burt, McNabb & Rossiter, 1950; Logan *et al.* 1952). There is an increase in the activity of alkaline phosphomonoesterase and adenosinetriphosphatase. While the activity of these two enzymes is increasing, the activity of acid phosphomonoesterases and 5-nucleotidase is falling. A preliminary report of some of these experiments has already appeared (Hollinger & Rossiter, 1952).

METHODS

The sciatic nerve on one side of each of a series of fifty-seven cats was either sectioned or crushed high in the thigh. To minimize the possibility of regeneration in the sectioned nerves, the proximal stump was retracted and sutured to the overlying muscle. In only one instance was there functional or post-mortem evidence of regeneration. This animal was excluded from the series. In the crushed nerves there was a progressive return of function. Microscopic examination of a number of animals killed 96 days after crushing showed that myelination had spread throughout the length of the nerves. In these animals function appeared normal. 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 1 to 600 days the animals were killed and the distal degenerating or regenerating segment of nerve was removed. At the same time a similar length of sciatic nerve from the opposite side was removed to serve as a control. Each nerve was cleaned of adherent fatty and epineural connective tissue, rapidly weighed, frozen in liquid nitrogen and ground to a fine powder in a mortar. Homogenates of the required dilution (1:20) were prepared in ice-cold isotonic sodium chloride using an ice-cooled Potter-Elvehjem type glass homogenizer. Because of its high collagen content, the sciatic nerve of the cat is a difficult tissue to homogenize, but good homogenates may be obtained from the frozen powder. Experiments with brain tissue, which can readily be homogenized directly, showed that the freezing had no effect on the enzymes under investigation.

Enzyme determinations

For each determination, 0.2 ml. of the homogenate was taken, except for the determination of adenosinetriphosphatase activity, where 0.1 ml. sufficed. All determinations and the appropriate blanks were run in duplicate.

Phosphomonoesterase activity. This was determined by a modification of the original method of King & Armstrong (1934). Alkaline phosphomonoesterase was determined in carbonate-bicarbonate buffer pH 9.9, as described by King, Haslewood, Delory & Beall (1942) and acid phosphomonoesterase in citrate buffer pH 4.9, as described by Watkinson, Delory, King & Hadow (1944). The reaction mixture contained MgCl_2 in a final concentration of 0.001 M, which is optimal for many phosphatases (Jenner & Kay, 1931). The reaction mixture was incubated for 1 hr. at 37°. The quantity of free phenol liberated from the substrate phenylphosphate (0.004 M) was determined by the method of Folin & Ciocalteu (1927). The results are reported in terms of $\mu\text{g. P}$ liberated/hr./mg. wet weight of nerve, based on the assumption that inorganic phosphate and free phenol were liberated in equimolecular proportions.

5-Nucleotidase activity. This was determined by the method of Reis (1937, 1951). The reaction mixture, buffered with 0.05 M-diethylbarbiturate buffer, pH 7.5, was incubated for 30 min. at 37°. Following Reis (1951), the results are reported in terms of $\mu\text{g. P}$ liberated from 0.00083 M-adenosine-5-monophosphate (Schwartz)/hr./mg. wet weight of nerve.

Adenosinetriphosphatase activity. This was determined by the method of DuBois & Potter (1943). The reaction mixture, which contained 0.01 M- CaCl_2 and was buffered with 0.05 M-

diethylbarbiturate buffer, pH 7.2, was incubated for 15 min. at 37°. The results are reported in terms of $\mu\text{g. P}$ liberated from 0.003 M-ATP (Schwartz)/hr./mg. wet weight of nerve.

Recording of results

Because of the great increase in the wet weight of degenerating and regenerating nerves, we have, as in previous publications (Johnson *et al.* 1949; Burt *et al.* 1950; Logan *et al.* 1952), expressed the results in terms of the wet weight of a similar length of the control 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. To assess the validity of this procedure, the activity of each of the enzymes was determined in both the right and the left sciatic nerve of six cats that were not subjected to any operative procedure. In no instance did the enzyme activity in the right nerve differ significantly from that in the left, the standard error of the mean percentage difference being $\pm 9.1\%$ for the acid phosphomonoesterase, $\pm 6.1\%$ for the alkaline phosphomonoesterase, $\pm 9.0\%$ for the 5-nucleotidase and $\pm 5.7\%$ for the adenosinetriphosphatase.

RESULTS

Acid phosphomonoesterase. Table 1 shows that 16 days after either nerve section or nerve crush there was an increase in the acid phosphomonoesterase activity. After each type of operation the mean increase was of the order of 300%. Fig. 1 shows the time course of the mean change in acid phosphomonoesterase activity for the first 96 days after nerve section. The enzyme activity was greatest 16 days after the operation. As early as 4 days, the mean activity in the degenerating nerve was significantly ($P < 0.01$) greater than that in the control nerves, but 24 and 48 hr. after the operation the difference between the mean activities of the two sides was not statistically significant.

When the nerve was crushed there was also an increase in the mean acid phosphomonoesterase activity (Fig. 2). During the first 96 days the figures were not significantly different from those obtained after nerve section. After 175 days the mean activity in the crushed nerve was still significantly ($P < 0.01$) greater than that of the control nerves. Three animals were kept for 600 days after the operation. In each of them the activity in the crushed nerve was greater than that in the control nerve of the opposite side, but the percentage increase varied so greatly from animal to animal (11–71%) that the mean increase of $37\% \pm 18$ was only of doubtful significance ($P = 0.1$).

Alkaline phosphomonoesterase. Whereas the activity of acid phosphomonoesterase increased after either nerve section or nerve crush, the activity of alkaline phosphomonoesterase decreased. Table 2 shows that by 16 days, after either type of operation, the mean enzyme activity in the degenerating nerves had fallen to less than 50% of the

Table 1. *Acid phosphomonoesterase activity of the sciatic nerve of the cat 16 days after either nerve section or nerve crush*

(Substrate, phenylphosphate. pH 4.9. Incubation time, 1 hr. Temp. 37°.)

Enzyme activity ($\mu\text{g. P/hr./mg. wet wt.}$)			
Normal	After nerve section (16 days)	Increase	Percentage increase
0.57	2.40	1.83	320
0.44	1.39	0.95	217
0.64	1.69	1.05	164
0.32	1.49	1.17	366
0.27	0.97	0.70	260
0.27	1.99	1.72	637
0.41	1.20	0.79	192
Mean ($\pm$ S.E.M.) = 308 ($\pm$ 61)			

Enzyme activity ($\mu\text{g. P/hr./mg. wet wt.}$)			
Normal	After nerve crush (16 days)	Increase	Percentage increase
0.28	0.98	0.70	249
0.30	1.46	1.16	387
0.37	1.24	0.87	234
Mean ($\pm$ S.E.M.) = 290 ($\pm$ 49)

Fig. 1. Mean percentage change in the activity of acid phosphomonoesterase (●-●-●) and alkaline phosphomonoesterase (▲-▲-▲) in the sciatic nerve of the cat after nerve section. Each point is the mean of three or more determinations.

Fig. 2. Mean percentage change in the activity of acid phosphomonoesterase (●-●-●) and alkaline phosphomonoesterase (▲-▲-▲) in the sciatic nerve of the cat after nerve crush. Each point is the mean of three or more determinations.

Table 2. *Alkaline phosphomonoesterase activity of the sciatic nerve of the cat 16 days after either nerve section or nerve crush*

(Substrate, phenylphosphate. pH 9.9. Incubation time, 1 hr. Temp. 37°.)

Enzyme activity ($\mu\text{g. P/hr./mg. wet wt.}$)			
Normal	After nerve section (16 days)	Decrease	Percentage decrease
0.30	0.10	0.20	68
0.49	0.00	0.49	100
0.51	0.11	0.40	79
0.55	0.26	0.29	52
0.25	0.05	0.20	79
0.40	0.14	0.26	66
0.33	0.12	0.21	63
Mean ($\pm$ S.E.M.) = 72 ($\pm$ 6)			

Enzyme activity ($\mu\text{g. P/hr./mg. wet wt.}$)			
Normal	After nerve crush (16 days)	Decrease	Percentage decrease
0.15	0.07	0.08	55
1.06	0.63	0.43	41
0.49	0.08	0.41	83
0.73	0.53	0.20	27
0.23	0.12	0.11	49
0.43	0.09	0.34	79
Mean ($\pm$ S.E.M.) = 56 ($\pm$ 9)			

figure for the control nerves. Fig. 1 shows that after section the mean alkaline phosphomonoesterase activity fell rapidly during the period 4-16 days and was still at this low level after 96 days.

Fig. 2 shows the mean changes in the alkaline phosphomonoesterase activity when the nerve was crushed. The enzyme activity decreased during the first 32 days, as it did after nerve section. It then rose sharply, so that by 96 days the mean activity in the crushed nerves was some 60% greater than that in the controls. After 175 days the activity of the alkaline phosphomonoesterase in the crushed nerve was still high ($P < 0.01$). In each of the three animals kept for 600 days the activity in the crushed nerves was greater than that of the control nerves but, as was found for the acid phosphomonoesterase, the percentage increase varied so much from animal to animal (12-59%) that the mean increase of $30\% \pm 15$ was only of doubtful significance ($P = 0.1$).

Table 3. 5-Nucleotidase activity of the sciatic nerve of the cat 16 days after either nerve section or nerve crush

(Substrate, adenosine-5-phosphate. pH 7.5. Incubation time, 30 min. Temp. 37°.)

Enzyme activity ($\mu\text{g. P/hr./mg. wet wt.}$)			
After nerve section (16 days)			Percentage increase
Normal		Increase	
2.23	3.47	1.24	56
2.77	3.15	0.38	14
3.12	5.90	2.78	89
2.87	3.98	1.11	39
2.15	3.22	1.07	50
3.43	5.44	2.01	59
1.38	1.71	0.33	24

Mean ($\pm$ s.e.m.) = 47 ($\pm$ 9)

Enzyme activity ($\mu\text{g. P/hr./mg. wet wt.}$)			
After nerve crush (16 days)			Percentage increase
Normal		Increase	
1.19	1.91	0.72	61
1.46	2.71	1.25	86
1.71	3.03	1.32	77
3.40	3.23	-0.17	-5
4.15	4.27	0.12	3
2.54	4.73	2.19	86

Mean ($\pm$ s.e.m.) = 51 ($\pm$ 17)

5-Nucleotidase. Table 3 shows that in normal nerves inorganic phosphate was liberated much more rapidly from adenosine-5-phosphate at pH 7.5 than from phenylphosphate at pH 4.9 or 9.9. The mean activity of the 5-nucleotidase also increased 16 days after either nerve section or nerve crush. The activity of this enzyme in the intact nerve was

found to be more variable than that of the non-specific phosphomonoesterases. The increase after the operation was also more irregular.

The time course of the mean change in 5-nucleotidase activity after either type of operation is shown in Fig. 3. In contrast to the findings for the acid phosphomonoesterase, the activity of 5-nucleotidase was greater at 32 days than at 16 days. Because of the great animal to animal variation with this enzyme, the difference between the mean activities of the crushed and sectioned nerves at 96 days is of doubtful significance ($P = 0.1$).

Fig. 3. Mean percentage change in the activity of 5-nucleotidase in the sciatic nerve of the cat after either nerve section ($\blacktriangle$ - $\blacktriangle$ - $\blacktriangle$) or nerve crush ($\bullet$ - $\bullet$ - $\bullet$). Each point is the mean of three or more determinations.

Adenosinetriphosphatase. In normal nerves inorganic phosphate was liberated from ATP more rapidly than from adenosine-5-phosphate (Table 4). There was little change in this activity 16 days after nerve section. Again there was considerable variation from animal to animal for both the intact nerves and the sectioned nerves. On the other hand, when the nerve was crushed there was a consistent increase in activity ($P < 0.01$) 16 days after the operation.

Fig. 4 shows the time course of the change in the rate at which inorganic phosphate was liberated from ATP. With the sectioned nerves the mean increase was at no time statistically significant. After nerve crush the mean activity at 96 days was greater than that at 16 days. In this respect adenosinetriphosphatase resembled alkaline phosphomonoesterase (cf. Fig. 2). By 175 days the increase was less, but the mean activity in the crushed nerves was still significantly ($P < 0.01$) greater than that in the controls.

Table 4. *Adenosinetriphosphatase activity of the sciatic nerve of the cat 16 days after either nerve section or nerve crush*

(Substrate, adenosinetriphosphate (0.003M). pH 7.2. Incubation time 15 min. Temp. 37°.)

Enzyme activity ($\mu\text{g. P/hr./mg. wet wt.}$)			
Normal	After nerve section (16 days)	Increase	Percentage increase
13.1	12.8	-0.3	-2
14.3	14.4	0.1	1
14.8	20.6	5.8	39
15.8	22.1	6.3	40
17.2	15.3	-1.9	-11
14.5	20.1	5.6	39
12.4	14.4	2.0	16
Mean ($\pm$ S.E.M.) = 17 ($\pm$ 9)			

Enzyme activity ($\mu\text{g. P/hr./mg. wet wt.}$)			
Normal	After nerve crush (16 days)	Increase	Percentage increase
2.6	5.3	2.7	104
9.6	14.4	4.8	50
6.6	12.1	5.5	83
13.6	20.8	7.2	53
13.6	20.0	6.4	47
16.8	25.6	8.8	52
Mean ($\pm$ S.E.M.) = 65 ($\pm$ 10)

Fig. 4. Mean percentage change in the activity of adenosinetriphosphatase in the sciatic nerve of the cat after either nerve section (▲-▲-▲) or nerve crush (●-●-●). Each point is the mean of three or more determinations.

DISCUSSION

Acid phosphomonoesterase. The mean acid phosphomonoesterase activity in cat sciatic nerve was only one-third of the value found by McNabb (1951) for the cerebral cortex of the dog, although it was of the same order as that reported by Reis (1951) for human cerebral cortex.

The observed increase in acid phosphomonoesterase activity during Wallerian degeneration confirms the previous findings of Bodian (1947) and Heinzen (1947), but the biphasic curve described by the latter worker was not evident. To relate, as

Fig. 5. Mean percentage change in the activity of acid phosphomonoesterase (●-●-●) and in the concentration of deoxypentose nucleic acid (DNA) (▲-▲-▲) in the sciatic nerve of the cat after nerve section. Each point is the mean of three or more determinations.

Heinzen (1947) has done, the initial increase in acid phosphomonoesterase activity to the chemical destruction of myelin would not appear to be justified, for in cat nerve the chemical, as opposed to the physical, destruction of myelin does not occur until after the first week of degeneration (Johnson *et al.* 1949, 1950). It would be reasonable to associate the increase in acid phosphomonoesterase activity with the increase in the number of Schwann cells and endoneurial cells (chiefly fibrocytes and macrophages) found by Abercrombie & Johnson (1946) in the degenerating sciatic nerve of the rabbit, for the increase takes place at a similar time and is of the same order of magnitude. With the cellular proliferation there is also an increase in both pentose nucleic acid and deoxypentose nucleic acid (Logan *et al.* 1952). Fig. 5 shows that after nerve section the changes in the activity of acid phosphomonoesterase follow the same general pattern as the changes in the concentration of deoxypentose nucleic acid. If the mean quantity of deoxypentose nucleic acid per

diploid cell is constant for any one species (see Davidson, Leslie & White, 1951, for references), the activity of acid phosphomonoesterase per cell does not change greatly during the degeneration. The curves representing the changes with time of the concentration of deoxypentosenucleic acid and of the activity of acid phosphomonoesterase (Fig. 5) are of the same general form as that reported by Abercrombie & Johnson (1946) for the total number of cell nuclei in the degenerating sciatic nerve of the rabbit.

Using histochemical methods, Wolf, Kabat & Newman (1943) detected the presence of an acid phosphatase in the axons of normal peripheral nerve, slight activity in the nuclei of the Schwann cells and endoneurial cells, but none in their cytoplasm and none in the myelin sheath. These results were confirmed by Smith (1948). Heinzen (1947) and Bodian (1947), although they found large increases in the acid phosphomonoesterase activity of degenerating nerve determined chemically, could find no comparable change in histological sections examined by the methods of Gomori (1939). In addition, both Lassek & Beuker (1947) and Smith (1948) reported the disappearance of the strong acid phosphatase reaction of the axon during Wallerian degeneration. This disturbing difference between the results obtained by histochemical and chemical methods may in part be technical, for Bartelmez & Bensley (1947) reported results more in keeping with the chemical findings when fresh, rather than fixed, preparations were used.

Alkaline phosphomonoesterase. We are aware of no previous reports on the chemical determination of alkaline phosphomonoesterase activity in normal or in degenerating and regenerating peripheral nerves. The mean alkaline phosphomonoesterase activity of cat sciatic nerve was of the same order as that found for acid phosphomonoesterase. It was considerably less than that reported by McNabb (1951) for the cerebral cortex of the dog, but it was of the same order as that found by Reis (1951) for the cerebral cortex of man. The fall in alkaline phosphomonoesterase activity during Wallerian degeneration was quite rapid and may be compared to the fall in the activity of the true or specific cholinesterase that occurs in peripheral nerve after nerve section (Sawyer, 1946) and in the superior cervical ganglion after denervation (Sawyer & Hollinshead, 1945; Hard, Peterson & Fox, 1951). Similar changes occur in the enzyme system responsible for the synthesis of acetylcholine (Feldberg, 1943; Nachmansohn, John & Berman, 1946; Banister & Scrase, 1950). It is possible that alkaline phosphomonoesterase, like true cholinesterase, is associated with the axon of a nerve fibre rather than with the sheath tissues.

When the nerve was crushed, rather than

sectioned, there was a remarkable increase in alkaline phosphomonoesterase activity during the regeneration phase (Table 2). At 96 days, when the alkaline phosphatase activity was at its height, functional activity appeared to have been restored. Despite this, it is well known that at this time regeneration is far from complete (Young, 1942) and neither the myelin lipids (Johnson *et al.* 1949) nor the nucleic acids (Logan *et al.* 1952) have returned to normal. It is possible that the increase in alkaline phosphomonoesterase activity is associated with the regeneration process and with remyelination.

Although the intact sciatic nerve of the cat undoubtedly contains alkaline phosphomonoesterase and the activity of this enzyme decreases greatly during Wallerian degeneration, this activity is difficult to detect with the standard histochemical tests. Gomori (1941) stated the belief that the only histochemically detectable site of alkaline phosphatase activity in the nervous system was in the blood vessels, an observation confirmed by Landow, Kabat & Newman (1942) for the sciatic nerve of the cat. That the fall in alkaline phosphomonoesterase activity in degenerating nerve is due to the disappearance of the blood vessels would appear to be unlikely, since Abercrombie & Johnson (1946) reported that the number of blood-vessel nuclei (endothelial and smooth muscle) in the degenerating sciatic nerve of the rabbit actually increases during degeneration, although the increase is small compared with the increase in the number of tubal and endoneurial cells. More recently Newman, Feigin, Wolf & Kabat (1950) reported a slight alkaline phosphatase reaction in the axon of nerve fibres from several species; Marchant (1949) described a slight activity, sporadically distributed in 'streaks', in intact rabbit nerves, and a strong reaction in the nuclei of most cell types and also in the cytoplasm of the Schwann cells and in the myelin debris of nerves undergoing Wallerian degeneration.

It is surprising that histochemical studies indicate that there is a decrease in the activity of acid phosphatase and an increase in the activity of alkaline phosphatase in peripheral nerve during Wallerian degeneration, whereas chemical studies indicate the direct opposite. These differences between the results obtained by chemical and histochemical techniques merit further study.

5-Nucleotidase. Reis (1937) reported the presence of a 5-nucleotidase in brain and peripheral nerve, specific for substrates such as adenosine-5-phosphate and inosine-5-phosphate. Thus the presence of 5-nucleotidase in nerve homogenates will not interfere with the determination of the activity of the non-specific acid or alkaline phosphomonoesterases, but since adenosine-5-phosphate is hydrolysed by acid and alkaline phosphomonoesterase these enzymes, if present in the homogenates in appreciable

concentration, will interfere with the determination of the 5-nucleotidase activity. Tables 1, 2 and 4 show that with normal and degenerating nerve the rate at which inorganic phosphate is produced from phenylphosphate, even at pH 4.9 or 9.9, is considerably less than the rate at which it is produced from adenosine-5-phosphate at pH 7.5. Since the 5-nucleotidase activity was determined at pH 7.5, a pH far from the optimum for either acid or alkaline phosphomonoesterase, the interference due to non-specific phosphomonoesterase activity would be minimal (Reis, 1951). That during nerve degeneration there is a true increase in the activity of a specific 5-nucleotidase is shown by the observation that inorganic phosphate was formed at a greater rate 32 days after the operation than at 16 days (Fig. 3), while at 32 days the rate of hydrolysis of phenylphosphate at pH 4.9 or 9.9 was less than that at 16 days (Fig. 1).

5-Nucleotidase has been demonstrated histochemically in the interstitial substance of the central nervous system and in the axons of peripheral nerve by Newman *et al.* (1950). The activity found in the undegenerated sciatic nerve of the cat is a little greater, and that in the degenerating nerve considerably greater, than the activity reported by Reis (1951) for human brain.

Adenosinetriphosphatase. Adenosinetriphosphatase activity was reported in squid nerve by Libet (1948) and in frog nerve by Sharples, Grundfest & Nachmansohn (1948). The finding that, in homogenates of the sciatic nerve of the cat, inorganic phosphate is liberated from ATP much more rapidly than from phenylphosphate or adenosine-5-phosphate suggests that this tissue also contains an enzyme specific for ATP. The adenosinetriphosphatase activity was determined in the presence of added Ca^{++} but with no added Mg^{++} . Adenosinetriphosphatases vary greatly in their sensitivity to added Mg^{++} and Ca^{++} . It should be noted that Meyerhof & Wilson (1947) found that the addition of Mg^{++} increased the adenosinetriphosphatase activity of brain homogenates, and that a Mg^{++} -sensitive enzyme, capable of liberating inorganic phosphate from ATP, was recently prepared from pig brain by Binkley & Olson (1950). The activity found for the intact cat nerve is only about one-half of that found for rat brain by DuBois & Potter (1943).

Since adenosinetriphosphatases do not liberate inorganic phosphate from phenylphosphate or adenosine-5-phosphate, it is unlikely that the presence of adenosinetriphosphatase in the nerve homogenates will interfere with the determination of the other phosphatases. A more important problem is the possibility that the non-specific phosphomonoesterases and 5-nucleotidase may interfere with the determination of the adenosine-

triphosphatase. Although there is evidence that acid phosphomonoesterase is inactive towards ATP (McLeod & Summerson, 1946; Meister, 1948) purified alkaline phosphomonoesterase has been shown to be active (Liebknecht, 1939; Schmidt & Thannhauser, 1943). Under the conditions of the adenosinetriphosphatase test, the non-specific phosphomonoesterases are at a pH far from optimal, but the production of inorganic phosphate from ATP may be in excess of that caused by the activity of adenosinetriphosphatase alone. This discrepancy may be greater in degenerating and regenerating nerves, where the activity of the non-specific phosphomonoesterases and 5-nucleotidase is increased.

SUMMARY

1. The rate of hydrolysis of phenylphosphate at pH 4.9 (acid phosphomonoesterase), phenylphosphate at pH 9.9 (alkaline phosphomonoesterase), adenosine-5-phosphate at pH 7.5 (5-nucleotidase) and adenosinetriphosphate at pH 7.2 in the presence of Ca^{++} (adenosinetriphosphatase) was determined in the sciatic nerve of the cat at intervals of time from 2 to 96 days after nerve section and from 16 to 600 days after nerve crush.

2. After either nerve section or nerve crush the activity of the acid phosphomonoesterase increased rapidly, reaching a maximum at 16 days after the operation and then decreased slowly. The increase was of the same order and occurred at the same time as the increase in the concentration of deoxypentose-nucleic acid previously reported.

3. After nerve section the activity of the alkaline phosphomonoesterase decreased rapidly during the period 4–16 days after the operation and then remained at a low level until 96 days. After nerve crush the activity decreased during the first 32 days and then increased, so that by 96 and 175 days the activity in the crushed nerves was greater than that in the controls.

4. The activity of the 5-nucleotidase increased after either type of operation, reaching a maximum in 32 days after nerve section and 96 days after nerve crush.

5. The rate of hydrolysis of adenosinetriphosphate did not change after nerve section, but increased after nerve crush, reaching a maximum 96 days after the operation.

6. These results have been discussed in relation to the morphological and histochemical changes known to occur in degenerating and regenerating nerves.

This work was aided by grants from the National Research Council of Canada and the National Mental Health Grants.

REFERENCES

- Abercrombie, M. & Johnson, M. L. (1946). *J. Anat., Lond.*, **80**, 37.
- Banister, J. & Scrase, M. (1950). *J. Physiol.* **111**, 437.
- Bartelmez, G. W. & Bensley, S. H. (1947). *Science*, **106**, 639.
- Binkley, F. & Olson, C. K. (1950). *J. biol. Chem.* **186**, 725.
- Bodian, D. (1947). *Symp. Soc. exp. Biol.* **1**, 163.
- Burt, N. S., McNabb, A. R. & Rossiter, R. J. (1950). *Biochem. J.* **47**, 318.
- Davidson, J. N., Leslie, I. & White, J. C. (1951). *Lancet*, **1**, 1287.
- DuBois, K. P. & Potter, V. R. (1943). *J. biol. Chem.* **150**, 185.
- Feldberg, W. (1943). *J. Physiol.* **101**, 432.
- Folin, O. & Ciocalteu, V. (1927). *J. biol. Chem.* **73**, 627.
- Gomori, G. (1939). *Proc. Soc. exp. Biol., N.Y.*, **42**, 23.
- Gomori, G. (1941). *J. cell. comp. Physiol.* **17**, 71.
- Hard, W. L., Peterson, A. C. & Fox, M. D. (1951). *J. Neuropath.* **10**, 48.
- Heinzen, B. (1947). *Anat. Rec.* **98**, 193.
- Hollinger, D. M. & Rossiter, R. J. (1952). *Fed. Proc.* **11**, 231.
- Jenner, H. D. & Kay, H. D. (1931). *J. biol. Chem.* **93**, 733.
- Johnson, A. C., McNabb, A. R. & Rossiter, R. J. (1949). *Biochem. J.* **45**, 500.
- Johnson, A. C., McNabb, A. R. & Rossiter, R. J. (1950). *Arch. Neurol. Psychiat., Chicago*, **64**, 105.
- King, E. J. & Armstrong, A. R. (1934). *Canad. med. Ass. J.* **31**, 376.
- King, E. J., Haslewood, G. A. D., Delory, G. E. & Beall, D. (1942). *Lancet*, **1**, 207.
- Landow, H., Kabat, E. A. & Newman, W. (1942). *Arch. Neurol. Psychiat., Chicago*, **48**, 518.
- Lassek, A. M. & Bueker, E. D. (1947). *Anat. Rec.* **97**, 395.
- Libet, B. (1948). *Fed. Proc.* **7**, 72.
- Liebkecht, W. L. (1939). *Biochem. Z.* **303**, 96.
- Logan, J. E., Mannell, W. A. & Rossiter, R. J. (1952). *Biochem. J.* **51**, 470.
- McLeod, J. & Summerson, W. H. (1946). *J. biol. Chem.* **165**, 533.
- McNabb, A. R. (1951). *Canad. J. Res. (E)*, **29**, 208.
- Marchant, J. (1949). *J. Anat., Lond.*, **83**, 227.
- Meister, A. (1948). *J. clin. Invest.* **27**, 263.
- Meyerhof, O. & Wilson, J. R. (1947). *Arch. Biochem.* **14**, 71.
- Nachmansohn, D., John, H. M. & Berman, M. (1946). *J. biol. Chem.* **163**, 475.
- Newman, W., Feigin, I., Wolf, A. & Kabat, E. A. (1950). *Amer. J. Path.* **26**, 257.
- Reis, J. L. (1937). *Enzymologia*, **2**, 110.
- Reis, J. L. (1951). *Biochem. J.* **48**, 548.
- Sawyer, C. H. (1946). *Amer. J. Physiol.* **146**, 246.
- Sawyer, C. H. & Hollinshead, W. H. (1945). *J. Neurophysiol.* **8**, 135.
- Schmidt, G. & Thannhauser, S. J. (1943). *J. biol. Chem.* **149**, 369.
- Sharples, W., Grundfest, H. & Nachmansohn, D. (1948). *Fed. Proc.* **7**, 113.
- Smith, W. K. (1948). *Anat. Rec.* **102**, 523.
- Watkinson, J. M., Delory, G. E., King, E. J. & Haddow, A. (1944). *Brit. med. J.* **2**, 492.
- Wolf, A., Kabat, E. A. & Newman, W. (1943). *Amer. J. Path.* **19**, 423.
- Young, J. Z. (1942). *Physiol. Rev.* **22**, 318.

Chemical Studies of Peripheral Nerve During Wallerian Degeneration

5. β -GLUCURONIDASE

BY D. M. HOLLINGER AND R. J. ROSSITER

Department of Biochemistry, University of Western Ontario, London, Canada

(Received 25 April 1952)

In an endeavour to outline more fully the chemical changes that take place in a peripheral nerve after nerve section or nerve crush, measurements have been made of the activity of the enzyme β -glucuronidase.

Previous papers in this series have described the changes that occur in the concentration of lipids (Johnson, McNabb & Rossiter, 1949; Burt, McNabb & Rossiter, 1950) and protein-bound phosphorus compounds (Logan, Mannell & Rossiter, 1952) and in the activity of phosphatases (Hollinger, Rossiter & Upmalis, 1952) in peripheral nerve after either nerve section or nerve crush.

METHODS

The sciatic nerve on one side of each of a series of fifty-six cats was either sectioned or crushed high in the thigh. 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 1 to 600 days the animals were killed and the distal degenerating or regenerating segment of the nerve was removed. At the same time a similar length of nerve of the opposite side was taken to serve as a control. The two pieces of nerve were homogenized in 10 ml. isotonic saline as described by Hollinger *et al.* (1952).