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Nutritional Deficiency and Wallerian Degeneration in the Rat

1. Effect of Protein Depletion on the Concentration of Nucleic Acid and Phospholipid in Intact and Sectioned Nerves

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If a peripheral nerve is cut, that portion of the nerve distal to the point of section undergoes a series of changes characteristic of Wallerian degeneration. Logan, Mannell & Rossiter (1952*a*) showed that in the distal segment of the sciatic nerve of the cat degenerating after nerve section there is an increase in the concentration of nucleic acid. This change, which is probably related to the cellular proliferation known to occur in Wallerian degeneration, involves both pentosenucleic acid (PNA) and deoxypentosenucleic acid (DNA), but the change is disproportionate, so that there is an increase in the PNA:DNA ratio. Johnson, McNabb & Rossiter (1949) showed that there is also a later decrease in the concentration of myelin lipid, related to the destruction of the myelin sheath. Mannell (1952) reported similar changes in the sciatic nerve of the rat, although for this species all the processes are considerably speeded up. By measuring the respective changes in the concentrations of nucleic acid and phospholipid, a rough quantitative estimate may be obtained both of the total number of cells and of the amount of myelin material present in a given portion of the nerve.

In this paper is described the effect of protein depletion on (*a*) the concentrations of nucleic acid and phospholipid in the intact sciatic nerve of the rat, and (*b*) the changes that occur in the concentrations of these substances during Wallerian degeneration. The experiments were undertaken with the hope that protein depletion might alter the cellular reaction in the degenerating nerve. If protein deficiency were found to alter the cellularity of the degenerating peripheral segment, it should be possible to put to the direct experimental test the hypothesis of Marinesco (cited by Ramon y Cajal, 1928) that Schwann cells appearing in the nerve during Wallerian degeneration provide the enzymes that are responsible for the destruction of the myelin sheath.

The situation in peripheral nerve is complicated by the finding of Mannell & Rossiter (1952, 1954) that the sciatic nerves of younger, and hence smaller, animals contain a greater concentration of both nucleic acid and phospholipid than the nerves of older, larger animals. In addition, the changes associated with Wallerian degeneration take place more rapidly in the nerves of smaller, younger animals. It is not known whether these effects are due to the age or to the body-weight of the animal. Since protein-depleted rats are considerably smaller than adequately nourished controls of the same age, it was thought advisable to study two groups of control animals, one of the same weight and one of the same age as the animals of the experimental group. These control animals, supplied with unrestricted quantities of a diet containing sufficient protein, are referred to as 'weight controls' and 'age controls' respectively. In addition, a third group of control animals was supplied with the sufficient diet, but the daily intake was restricted in quantity so that the mean body-weight of the animals remained the same as that of the animals of the protein-deficient group. Since these animals were maintained at the same body-weight as those of the protein-deficient group and since they were also of the same age, they are referred to as 'weight and age controls'.

A preliminary report of these experiments has already appeared (Mannell, 1953).

METHODS

Animals. The animals used were all male rats of the Sprague-Dawley strain.

Experimental plan. The experiment was run in two parts. In the first part, thirty-four rats, whose mean initial body-weight was 311 ± 3 g (mean $\pm$ S.E.M.), were divided into two groups. One group was supplied with unrestricted quantities of a diet deficient in protein for 69 days. The other group, referred to as 'age controls', received unrestricted quantities of a diet containing adequate protein. Details of the diets are given in Table 1. In addition, a further group of sixteen rats, whose initial body-weight was 128 ± 2 g, was supplied with the diet containing adequate protein. These animals were killed after 16 days, at a time when their mean body-weight was the same as that of the protein-depleted group. They thus formed 'weight controls'.

In the second part of the experiment thirteen rats of initial body-weight 315 ± 7 g were supplied with the diet containing adequate protein. Five of the animals received the diet in unrestricted amounts and the remaining eight, the 'weight and age controls', were supplied with such quantities of the diet that their curve of mean body-weight

followed closely that of the protein-depleted animals described above. This part of the experiment enabled the effect of a diet restricted in calories, but containing adequate protein, to be compared with the effect of a protein-deficient diet that brought about the same fall in body-weight. The mean weight changes of the animals of the first part of the experiment are shown in Fig. 1*a* and those of the animals of the second part are shown in Fig. 1*b*. It will be noted that before the animals were killed the mean body-weight of the protein-depleted group had decreased by more than 25 %.

Table 1. *Percentage composition of diets*

	Control diet	Diet deficient in protein		Control diet	Diet deficient in protein
Salt mixture*	4	4	Maize starch	48	68
Vitamin mixture†	1	1	Cod-liver oil‡	0·1	0·1
Non-nutritive cellulose	5	5	Maize oil	3·9	3·9
Brewer's yeast	2	2	Water	16	16
Casein	20	—			

*Salt mixture:

Calcium phosphate, monobasic (%)	13·58	Potassium phosphate, dibasic (%)	23·98
Calcium lactate (%)	32·70	Sodium phosphate, monobasic (%)	8·72
Ferric citrate (%)	2·97	Sodium chloride (%)	4·35
Magnesium sulphate (%)	13·70		

†Vitamin mixture:

Thiamine (mg)	50	Nicotinic acid (mg)	100
Riboflavin (mg)	25	Choline (g)	7·5
Pyridoxin (mg)	20	Powdered sugar to (g)	100
Pantothenic acid (mg)	100		

‡1·0 g contained 3000 i.u. vitamin A and 400 i.u. vitamin D.

Nerve section. The sciatic nerve of one side of each of the animals was sectioned at a high level in the thigh. The operation was performed under ether anaesthesia using full aseptic precautions. To minimize the possibility of regeneration, the two cut ends were separated as far as possible with a muscle mass. After allowing 8 days for the nerve to degenerate, the animal was killed and the distal degenerating segment was removed for analysis. In none of the animals was there post-mortem evidence of regeneration in the distal segment. A similar length of the sciatic nerve of the opposite side was taken to serve as a control. Each nerve was rapidly cleaned of adherent fatty and epineural connective tissue and weighed on a torsion balance. The nerve was then homogenized in 3 ml. ethanol in a Potter & Elvehjem (1936) type glass homogenizer.

Analytical methods. The lipids were removed from the homogenate by extracting twice with 5 ml. ethanol, three times with 5 ml. of a 3:1 ethanol-ether mixture heated to boiling for 3 min, and once with 5 ml. ether. The acid-soluble compounds were removed with cold 10 % trichloroacetic acid and the nucleic acids were removed by extraction with 5 % trichloroacetic acid for 15 min at 90° according to the method of Schneider (1945). Total phosphorus was determined in the combined lipid extract by the method of King (1932). The factor of 25 was used to convert lipid phosphorus to phospholipid. Total nucleic acid was determined in the hot trichloroacetic-acid

extract by the ultraviolet absorption method described by Logan, Mannell & Rossiter (1952*b*).

Recording of results. Because of the great increase in the wet weight of degenerating nerves, the results have been expressed in terms of the fresh weight of a similar length of the intact nerve of the opposite side. This method of recording results, which has been used in previous papers from this laboratory, is equivalent to expressing the results for the degenerating nerve in terms of the fresh weight of a similar nerve that has not been sectioned.

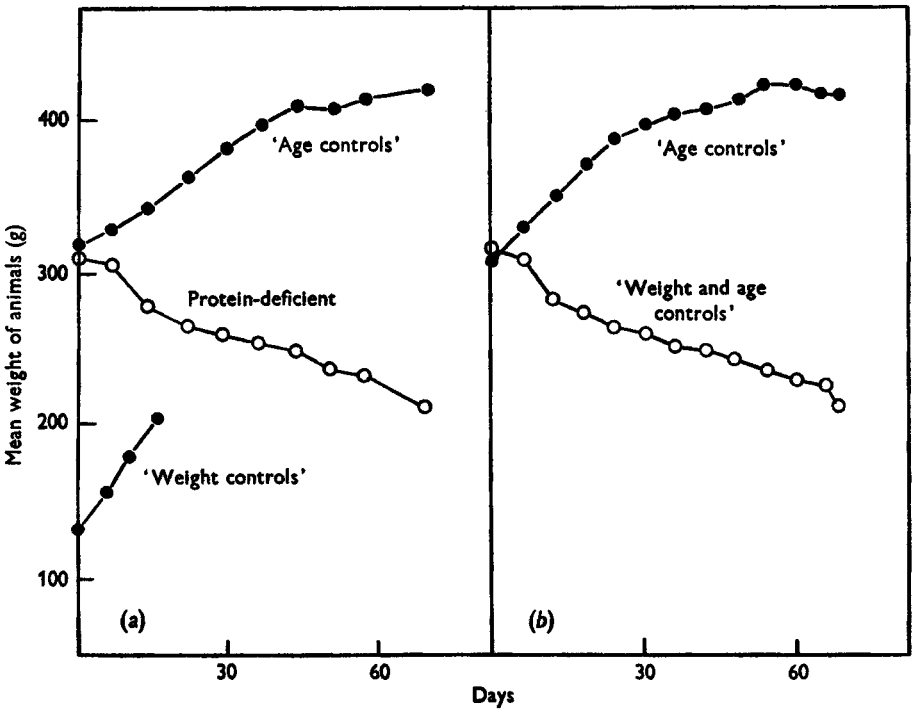

Fig. 1. Mean body-weight of rats used in first part (*a*) and second part (*b*) of experiment.

RESULTS

Intact nerves

Nerve weight. For the control rats there was a highly significant ($P<0{\cdot}001$) correlation between the weight of the animal and the wet weight of the sciatic nerve per unit length. Fig. 2 shows the calculated regression line of nerve weight on body-weight ($y=0{\cdot}11x+2$) and the standard error of the estimate of nerve weight from body-weight for all the animals receiving unrestricted quantities of diet containing adequate protein. In the same figure are shown points for the protein-depleted animals (open circles) and points for the 'weight and age' controls, i.e. the rats receiving a restricted calorie intake (closed circles). For a given animal weight, the mean weight of the nerves from the protein-depleted or calorie-deficient rats was

significantly ($P < 0{\cdot}001$) greater than the mean weight of the nerves from animals receiving unrestricted quantities of the diet containing adequate protein.

That protein depletion causes an increase in the ratio of nerve weight to body-weight is shown further in Table 2, where it can be seen that the mean nerve weight of the protein-deficient animals was significantly ($P < 0{\cdot}001$) greater than that of the adequately nourished 'weight controls'. However, Table 2 also shows that protein depletion causes some fall in nerve weight, for the mean weight of the nerves of the protein-deficient animals was significantly ($P < 0{\cdot}001$) less than that of the 'age

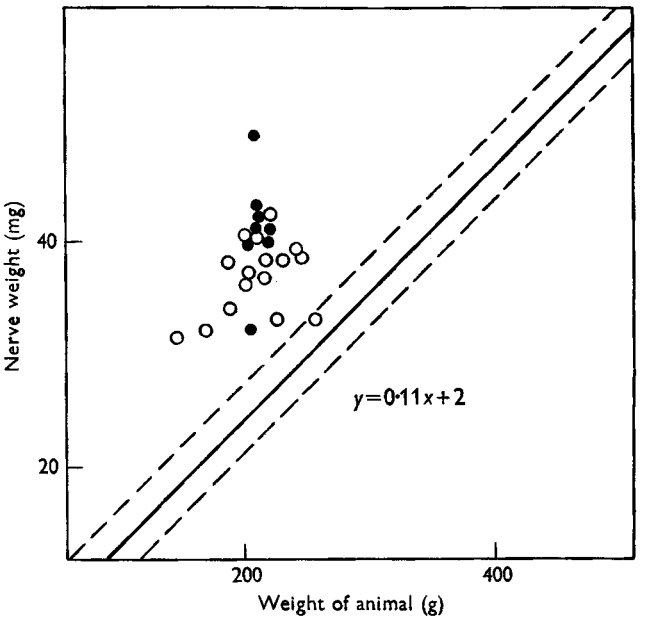

Fig. 2. Relation between weight of sciatic nerve and body-weight of rats maintained on an adequate diet. The solid line represents the calculated regression line and the broken lines show the standard error of the estimate of nerve weight from body-weight. Open circles (○), protein-depleted rats; closed circles (●), 'weight and age controls', i.e. rats receiving a restricted calorie intake.

controls'. Restricting intake of a diet containing adequate protein, although it causes an increase in the ratio of nerve weight to body-weight (Fig. 2), also causes some decrease in nerve weight. This is evident from Table 3, which shows that the mean weight of the nerves from the animals whose daily intake was restricted was significantly ($P < 0{\cdot}001$) less than that of the animals receiving an unrestricted intake.

Nucleic acid. Mannell & Rossiter (1954) have shown that there is a significant negative correlation between the concentration of nucleic acid in the sciatic nerve of the rat and the age (or weight) of the animal. This is again demonstrated by the findings reported in Table 2. The mean concentration of nucleic acid in nerves from the younger 'weight controls' was significantly ($P < 0{\cdot}001$, not shown in table) greater than that in the nerves from the 'age controls'.

Table 2 also shows that, although the mean concentration of nucleic acid in nerves from the protein-deficient rats was significantly ($P < 0{\cdot}001$) less than that in nerves

from 'weight controls', it did not differ significantly from that in nerves from the 'age controls'. These findings suggested the possibility that in protein depletion the age of the animal, rather than the weight, might be a factor in determining the concentration of nucleic acid in the sciatic nerve. The same generalization may apply to rats receiving a diet deficient in total calories, for it can be seen from Table 3 that there was no significant difference between the concentration of nucleic acid in the nerves of the 'weight and age controls' receiving a restricted intake of an adequate diet, and that in the 'age controls' receiving unrestricted amounts of the same diet.

Table 2. *Mean values with their standard errors for effect of protein deficiency on the weight and the concentration of total nucleic acid and phospholipid of the intact sciatic nerve of the rat*

(Figures in parentheses indicate the number of animals in each group)

	Protein-deficient diet (*a*)	Control diet: 'Age controls' (*b*)	Significance of difference (*a*) − (*b*) (*P**)	Control diet: 'Weight controls' (*c*)	Significance of difference (*a*) − (*c*) (*P**)
Age of rat (days) ...	160	160		60	
Body-weight (g) ...	212 ± 7	419 ± 8		205 ± 5	
Nerve weight (mg)	37 ± 0·8 (17)	49 ± 0·8 (17)	< 0·001	24 ± 0·6 (16)	< 0·001
Nucleic acid (mg P/100 g wet weight)	11·3 ± 0·27 (17)	11·7 ± 0·21 (16)	> 0·2	19·3 ± 0·57 (16)	< 0·001
Phospholipid (mg/100 mg wet weight)	8·15 ± 0·12 (17)	7·66 ± 0·13 (17)	< 0·01	8·14 ± 0·17 (16)	1

* *P* = probability that a mean difference at least as great as the observed mean difference would have arisen by random sampling from a homogeneous population.

Table 3. *Effect of restricting the dietary intake on the weight and the concentration of total nucleic acid and phospholipid of the intact sciatic nerve of the rat*

(Figures in parentheses indicate the number of animals in each group)

	'Weight and age controls' (restricted intake)	'Age controls' (unrestricted intake)	
Age of rat (days) ...	160	160	
Body-weight (g) ...	215 ± 2	421 ± 17	
	Mean ± S.E.M.	Mean ± S.E.M.	*P*
Nerve weight (mg)	41 ± 2 (8)	56 ± 0·1 (5)	< 0·001
Nucleic acid (mg P/100 g wet weight)	9·8 ± 0·28 (8)	9·5 ± 0·32 (5)	> 0·4
Phospholipid (mg/100 mg wet weight)	8·58 ± 0·27 (8)	7·43 ± 0·21 (5)	< 0·01

Evidence supporting the above supposition, that the age of the animal rather than the body-weight determines the concentration of nucleic acid in the sciatic nerve, is presented in Fig. 3*a* and *b*. Fig. 3*a* shows the relation between the concentration of

nucleic acid in the sciatic nerve and body-weight for all of the animals receiving unrestricted amounts of the diet containing adequate protein. The solid line is the calculated regression line ($y = -0{\cdot}035x + 26$), and the dotted line represents the standard error of the estimate of the concentration of nucleic acid from body-weight. Fig. 3*b* shows similar data, but in this instance the concentration of nucleic acid in the sciatic nerve has been related to the age of the animal rather than to its body-weight. The calculated regression equation for the concentration of nucleic acid on age is $y = -0{\cdot}081x + 24$.

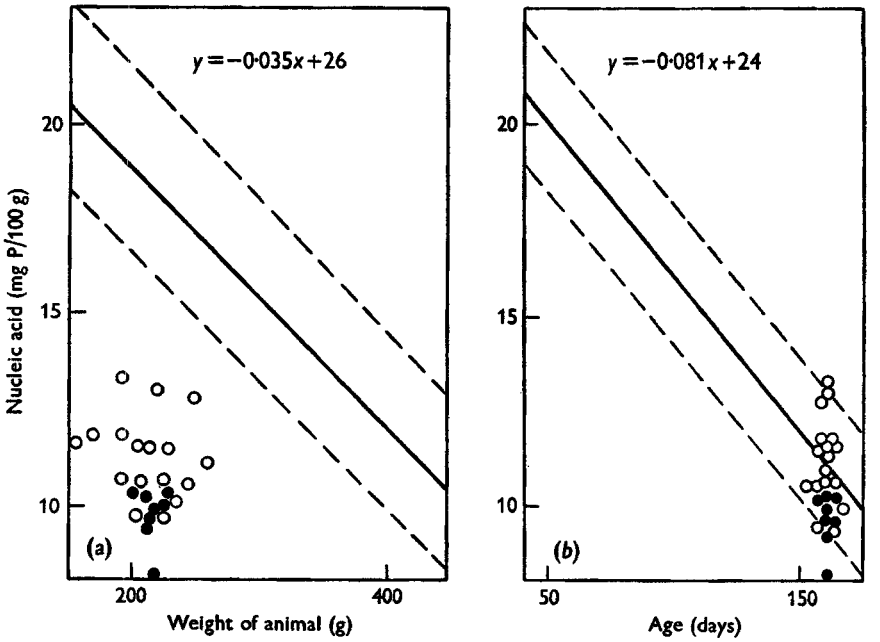

Fig. 3. Relation between the concentration of total nucleic acid in the sciatic nerve and either the body-weight (*a*) or the age (*b*) of rats maintained on an adequate diet. The solid lines represent the calculated regression lines and the broken lines show the standard error of the estimate of nucleic-acid concentration from either body-weight or from age. Open circles (○), protein-depleted rats; closed circles (●), 'weight and age controls', i.e. rats receiving a restricted calorie intake.

In Fig. 3*a* and *b* are also shown points for the protein-depleted animals (open circles) and points for the 'weight and age controls' receiving a restricted calorie intake (closed circles). For a given animal weight (Fig. 3*a*), the concentration of nucleic acid in the nerves from either the protein-depleted or the calorie-deficient animals was much less than that in nerves from the adequately nourished controls. On the other hand, for a given age (Fig. 3*b*), the concentration of nucleic acid in the nerves from either the protein-depleted or calorie-deficient animals was not significantly different from that in nerves from the controls receiving an adequate diet.

Phospholipid. Mannell & Rossiter (1954) also reported that, similarly to the findings for nucleic acid, there was a significant negative correlation between the concentration of phospholipid in the sciatic nerve and the age (or weight) of the animal. In confirmation of this report is the observation (Table 2) that the mean concentration of phospho-

lipid in nerves from younger 'weight controls' was significantly ($P<0{\cdot}01$, not shown in table) greater than that in nerves from the 'age controls'. However, in protein deficiency or calorie deficiency, the findings for the phospholipid were in sharp contrast to those for nucleic acid. Table 2 shows that the mean concentration in nerves from the protein-depleted animals was significantly ($P<0{\cdot}01$) greater than that in nerves from the 'age controls', but did not differ significantly from that in nerves from the 'weight controls'. Also the mean concentration of phospholipid in the nerves from animals receiving a restricted dietary intake ('weight and age controls') was significantly ($P<0{\cdot}01$) greater than that in nerves from animals receiving unrestricted quantities of the same diet (Table 3).

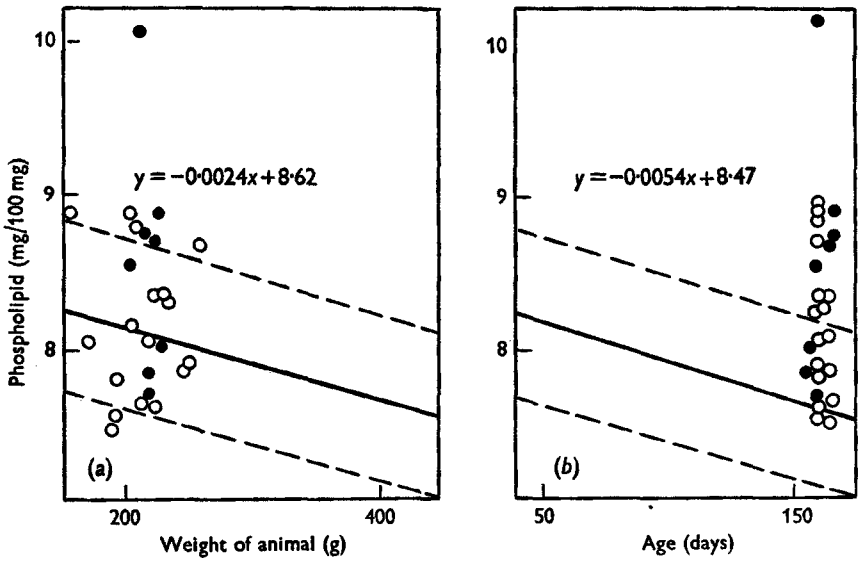

Fig. 4. Relation between the concentration of phospholipid in the sciatic nerve and either the body-weight (*a*) or the age (*b*) of rats maintained on an adequate diet. The solid lines represent the calculated regression lines and the broken lines show the standard error of the estimate of phospholipid concentration from either body-weight or from age. Open circles (○), protein-depleted rats; closed circles (●), 'weight and age controls', i.e. rats receiving a restricted calorie intake.

The above findings would suggest that, unlike the concentration of nucleic acid which is a characteristic of the age of the animal and not of the body-weight, the concentration of phospholipid is determined by the body-weight of the animal and not by the age. Fig. 4*a* and *b* illustrates this supposition. Fig. 4*a* shows the relation between the concentration of phospholipid in the sciatic nerve and body-weight (regression equation: $y=-0{\cdot}0024x+8{\cdot}62$). Fig. 4*b* shows similar data for age (regression equation: $y=-0{\cdot}0054x+8{\cdot}47$). Each of the regression lines was calculated from the results obtained for all the animals receiving unrestricted quantities of the diet containing adequate protein.

In Fig. 4*a* and *b* are also shown the points for the protein-depleted animals (open circles) and the points for the 'weight and age controls' receiving a restricted calorie intake (closed circles). For a given animal weight (Fig. 4*a*) the concentration of phospholipid in the sciatic nerves from either the protein-depleted or the calorie-

deficient animals did not differ significantly from that in the nerves from the well-nourished controls. But for a given age (Fig. 4*b*) the mean concentration of phospholipid in the nerves of either the protein-depleted or the calorie-deficient animals was greater than that in nerves from the controls receiving the adequate diet.

The difference between the findings for nucleic acid (Fig. 3*a*, *b*) and those for phospholipid (Fig. 4*a*, *b*) is apparent. Regardless of weight changes induced by the provision of a diet deficient either in protein or in total calories, the concentration of nucleic acid in the sciatic nerve is determined by the age of the animal. On the other hand, in animals subjected to the same deficiencies, the concentration of phospholipid in the sciatic nerve would appear to be determined by the weight of the animal, regardless of the age.

Wallerian degeneration

Nucleic acid. When the sciatic nerve of the rat is sectioned there is an increase in the concentration of nucleic acid in the distal degenerating segment (Mannell, 1952). Mannell & Rossiter (1954) found that, like the concentration of nucleic acid, the increase in the concentration of nucleic acid during the first 8 days after nerve section is greater in nerves from younger rats. This is again demonstrated in the experiments reported in Table 4, where it can be seen that the mean increase in the concentration of nucleic acid in the nerves from the 'weight controls' was significantly ($P<0.01$, not shown in table) greater than that in the nerves from the older 'age controls'. Table 4 also shows that the mean increase in nerves from the protein-depleted animals

Table 4. *Effect of protein depletion on the increase in the concentration of total nucleic acid and the decrease in the concentration of phospholipid in the sciatic nerve of the rat 8 days after nerve section.*

(Figures in parentheses indicate the number of animals used to obtain the mean difference)

	Protein-deficient diet	Control diet			
		'Age controls'		'Weight controls'	
Age of rat (days) ...	160	160		60	
Body-weight (g) ...	212 ± 7	419 ± 8		205 ± 5	
	Mean ± S.E.M.	Mean ± S.E.M.	*P*	Mean ± S.E.M.	*P*
Increase in nucleic acid (mg P/100 g wet weight)	22·8 ± 1·29 (26)	25·0 ± 0·98 (21)	> 0·1	32·5 ± 2·65 (24)	< 0·01
Decrease in phospholipid (mg/100 mg wet weight)	0·97 ± 0·22 (26)	1·18 ± 0·28 (23)	> 0·5	3·24 ± 0·35 (24)	< 0·001

was not significantly different from that in nerves from the 'age controls', but that it was significantly ($P<0.01$) less than that in the nerves from the 'weight controls'. Thus the age of the animal rather than its body-weight determined the rate of increase in the concentration of nucleic acid during the first 8 days after nerve section. The same was true when the weight of the animal was reduced by restricting the calorie intake, for Table 5 shows that the mean increase in the concentration of nucleic acid in the nerves from the 'weight and age controls' did not differ significantly from that in nerves from the 'age controls'.

Phospholipid. After nerve section there is a decrease in the concentration of phospholipid in the peripheral degenerating segment of nerve (Mannell, 1952). This decrease is more rapid in nerves from younger animals (Mannell & Rossiter, 1954), a point illustrated by the finding reported in Table 4 that the decrease in the concentration of phospholipid during the first 8 days after nerve section in nerves from the 'weight controls' was significantly ($P < 0.001$, not shown in table) greater than that in nerves

Table 5. *Effect of restricting the dietary intake on the increase in concentration of total nucleic acid and the decrease in concentration of phospholipid in the sciatic nerve of the rat 8 days after nerve section*

(Figures in parentheses indicate the number of animals used to obtain the mean difference)

	'Weight and age controls' (restricted intake)	'Age controls' (unrestricted intake)	
Age of rat (days) ...	160	160	
Body-weight (g) ...	215 ± 2 g	421 ± 17	
	Mean ± S.E.M.	Mean ± S.E.M.	*P*
Increase in nucleic acid (mg P/100 g wet weight)	22·4 ± 5·24 (16)	19·8 ± 1·29 (10)	> 0·6
Decrease in phospholipid (mg/100 mg wet weight)	0·81 ± 0·40 (16)	1·33 ± 0·43 (10)	> 0·3

from older 'age controls'. Table 4 also shows that the mean decrease in the concentration of phospholipid in nerves from the protein-deficient animals was not significantly different from that in nerves from the 'age controls', but that it was significantly ($P < 0.001$) less than that in nerves from the 'weight controls'. Restricting the dietary intake had no significant effect on the decrease in concentration of phospholipid (Table 5). The age of the animal, rather than the body-weight, would thus appear to determine the rate at which the concentration of nucleic acid increases and the concentration of phospholipid decreases during Wallerian degeneration.

DISCUSSION

These results indicate that, although the concentration of nucleic acid in the sciatic nerves of protein-depleted rats is less than that in nerves of animals of the same weight receiving a diet containing adequate protein, the concentration is similar to that in nerves of control animals of the same age. Also, restricting the intake of a diet containing adequate protein, although it causes great changes in the body-weight of the animals, causes little change in the concentration of nucleic acid in the sciatic nerve. On the other hand, the concentration of phospholipid in nerves of protein-depleted rats does not differ significantly from that in nerves from animals of the same weight, but is greater than that in nerves of animals of the same age. Restricting the calorie intake causes an increase in the concentration of phospholipid in the nerve. Thus, regardless of changes in body-weight brought about by a deficient intake of either protein or total calories, the concentration of nucleic acid in the sciatic nerve would appear to be related to the age of the animal rather than to its body-weight, and the

concentration of phospholipid would appear to be related to the body-weight of the animal rather than to its age.

We are aware of no reports in the literature on the effect of protein depletion on the chemical composition of peripheral nerve. Comparison with control animals of the same age shows that protein depletion does not affect the concentration of nucleic acid, but it causes an increase in the concentration of phospholipid. This is in contrast to the effect of protein depletion on other organs, such as the liver, where there is a decrease in the concentration of nucleic acid and also a decrease in the concentration of phospholipid. Addis, Poo & Lew (1936) found that a fast of 7 days caused a loss of 40% of the protein from the liver of the rat. This is associated with a decrease in the content of pentosenucleic acid with no change in the content of deoxypentosenucleic acid (Davidson & Waymouth, 1944). Kosterlitz (1947) reported that a diet deficient in protein caused a decrease in the concentration of both phospholipid and pentosenucleic acid, expressed in terms of wet weight, and Thomson, Heagy, Hutchison & Davidson (1953) reported a similar decrease in pentosenucleic acid, expressed in terms of units of deoxypentosenucleic acid. Protein deficiency also causes a decrease in the concentration of pentosenucleic acid in muscle (Mandel, Jacob & Mandel, 1949), kidney (Mandel, Jacob & Mandel, 1950*a*) and spleen (Jacob, Mandel & Mandel, 1951). There is no similar change in brain (Mandel *et al.* 1950*a*, *b*), but it is well known that the chemical composition of the central nervous system is not readily altered by dietary means.

The increase in the concentration of nucleic acid that occurs in peripheral nerve after nerve section is not greatly modified by protein deprivation. This finding indicates that protein depletion can have little effect on the development of the cellular reaction in the degenerating segment of nerve. The results thus provide no evidence, either for or against, the hypothesis of Marinesco (cited by Ramon y Cajal, 1928) that the Schwann cells appearing in the nerve during Wallerian degeneration are a source of the enzymes responsible for the destruction of the lipids of the myelin sheath. The finding that protein depletion has little effect on the cellular reaction in a degenerating nerve is in contrast to the reported effects of protein deficiency on the formation and activity of white blood cells (Wright & Skeggs, 1946; Guggenheim & Buechler, 1949; Kornberg, 1946, for white cell formation; and Mills & Cottingham, 1943; Wissler, 1947; Guggenheim & Buechler, 1948, for their phagocytic activity). In this regard, it should be noted that Drabkin (1947) found that the rise in the concentration of pentosenucleic acid in rat liver, regenerating after partial hepatectomy, was not affected by a diet deficient in protein.

SUMMARY

1. The concentrations of nucleic acid and phospholipid were determined in the intact and degenerating (8 days after nerve section) sciatic nerves of a series of protein-depleted rats. The values were compared with those obtained for (*a*) animals of the same age that received a diet containing adequate protein ('age controls'), (*b*) younger animals receiving the same adequate diet that were killed when they had attained a body-weight similar to that of the protein-depleted animals ('weight controls'), and

(*c*) animals of the same age as the protein-depleted animals that were maintained on the diet containing adequate protein, but restricted in quantity so that their body-weight was similar to that of the protein-depleted animals ('weight and age controls').

2. For the intact nerves, the mean concentration of nucleic acid in the protein-depleted animals was less than that in the 'weight controls', but did not differ significantly from that in either the 'age controls' or the 'weight and age controls'. On the other hand, the mean concentration of phospholipid in the protein-depleted animals did not differ significantly from that in either the 'weight controls' or the 'weight and age controls', but was greater than that in the 'age controls'.

3. Despite changes in body-weight brought about by protein depletion or a deficiency of total calories, the concentration of nucleic acid in the intact sciatic nerve of the rat thus appeared to be a characteristic of the age of the animal (and not of the body-weight) and the concentration of phospholipid appeared to be a characteristic of the body-weight (and not of the age).

4. The increase in the concentration of nucleic acid and the decrease in the concentration of phospholipid that occurs after nerve section took place more slowly in nerves from protein-depleted animals than in nerves from the 'weight controls', but at a rate similar to that found for the 'age controls' and the 'weight and age controls'.

5. The experiments provide no evidence either for or against the hypothesis that in Wallerian degeneration proliferating Schwann cells provide enzymes that destroy the myelin sheath.

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